ISSN－0910－4003
CODEN: VIORE 6

Viva Origino

VOL. 25 (No. 4)

December 1997

The Society for the Study of the Origin
and Evolution of Life
JAPAN

生命の起原および進化学会 会則

地球上における生命の起原を科学的に解明することと，生物進化の攻究により，生命体の本質を明らかにしようとする。本学会は，関係諸分野の英知を集め，互の連繋によって新しい型の総合科学を確立・発展させることにより，上記の目的達成を期するものである。

第一条　本学会は，生命の起原および進化学会（Society for the Study of the Origin and Evolution of Life-Japan, SSOEL-Japan）という。

第二条　本学会は，会員の生命の起原および進化の研究の発展と，日本における当該研究の開拓・推進をはかり，関連学(協)会および，多くの人々の当該研究に対する理解を深め，もって学術・文化の発展に寄与するものとする。

第三条　本学会は，前条の目的達成のため次の事業をおこなう。

1．研究発表会・学術講演会の開催

2．学会誌等の出版物の刊行

3．其の他前条の目的達成のため必要な事業

第四条　本学会は前条の事業をおこなうため事務局をおく。

第五条　本学会の会員は，正会員と賛助会員とし，入会手続きは別途定める。

第五条の２　正会員は，第二条に示す研究に従事する個人で，学会が承認したものとする。

第五条の３　賛助会員は，本学会の目的に賛同し，その事業を援助する個人または個体で学会が承認したものとする。

第六条　会員は，別途定められた会費等の費用を前納しなければならない。定められた期間以上これらを滞納した場合は，会員の資格は消失するものとする。

第七条　会員は，本学会のおこなう事業に参加し，本学会発刊の学会誌 Viva Origino その他印刷物の配布をうけることができる。

第八条　本学会は，会長１名，副会長１～２名および学会運営委員（以下委員と略す）を若干名，会計監査２名をおくものとする。

第九条　委員および会計監査は，正会員の互選による。選出された委員は学会運営委員会（以下委員会と略す）を構成し，学会運営の任にあたる。

第十条　会長・副会長は委員会が正会員の中から選出する。

第十一条　会長・副会長・委員・会計監査の任期は２年とする。

第十二条　委員会は，学会運営および学会事業をおこなうため，委員長１名，常任委員若干名を選出し，学会運営常任委員会（以下常任委員会と略す）を構成し，その任にあたらせるものとする。

第十三条　会長は学会を代表し，学会運営は委員長が総括の任にあたる。

第十四条　常任委員会は，必要なとき委員会を招集し，本学会に関する諸事項を審議・決定する。

第十五条　常任委員会は，正会員の中から専門委員を委嘱し，本学会に関する諸事項を諮問することができる。

第十六条　委員会において，本学会員として不適当と決議されたものは，会員の資格を消失するものとする。

第十七条　会員の退会届け者および会員資格消失者については，常任委員会は退会手続きをとるものとする。

第十八条　本学会は，年１回定期総会を開き，必要なときは臨時総会を開くものとする。

第十九条　本学会会則の改正は，会員の⅔以上の出席の総会において⅔以上の同意を要する。

学会入会手続きに関する付則

1．入会申し込み書に必要事項を記入し，常任委員会へ提出のうえ会員資格の承認をうける。

2．会員としての資格を承認されたものは，すみやかに所定の入会金，会費（１年分），学会誌購読料（１年分）を事務局へ納入する。

3．上記費用の納入されたものについて，常任委員会は入会手続きをとり，会員として登録する。

4．本学会の入会は推薦によりおこない，委員会で承認する。

会費その他に関する付則

1．入会金（正会員のみ）　1,000円

2．会費

正会員　年額　5,000円

賛助会員　年額（１口）　10,000円

3．学生のための入会金・会費

正会員で，大学または大学院あるいはこれに準じる学校に在学する学生は，在学証明書の添付により，次の特典を与えるものとする。

入会金 500円，会費（年額） 2,500 円

4．学会誌 Viva Origino 購読料　年額 5,000 円

但し，会員には無料配布とする。

5．会費その他の費用の納入の猶予期限は１年以内とする。

6．会費払込振替口座

（加入者名）生命の起源および進化学会

（口座番号）大阪８－3673

Viva Origino

VOL. 25 (No. 4)

December 1997

The Society for the Study of the Origin
and Evolution of Life
JAPAN

目　　次

トリプトファナーゼにおけるD-トリプトファンに対する活性部位の独立性についての考察

島田秋彦[1]，中村以正[2]

[1]筑波大学応用生物化学系（〒305　つくば市天王台１－１－１）
[2]相模女子大学食物学科（〒208　神奈川県相模原市文京２－１－２）

立体特異性が厳格な酵素として知られているトリプトファナーゼは，通常の条件ではL-トリプトファンに対してのみ活性を示し，D-トリプトファンには全く不活性であるが，高濃度リン酸水素二アンモニウム溶液中ではD-トリプトファンに対しても活性を示すようになる。L-トリプトファンの分解反応で，D-トリプトファンを阻害剤として用いて阻害反応のキネティックスを行った実験は，D-トリプトファンに対する活性部位はL-トリプトファンに対する活性部位から独立している可能性が強いことを示唆した。このことを明らかにするためには，L-トリプトファンの活性に対しては影響しないが，D-トリプトファンのみに対する活性阻害剤の存在が不可欠である。そこでそのような阻害剤にD-ヒスチジンが該当することがわかった。一連の実験よりL-トリプトファンに対する活性部位とD-トリプトファンに対する活性部位が独立して存在していることが強く示唆された。

Viva Origino 25(1997)261－270

Consideration on Independence of Specific Active Site for D-tryptophan in Tryptophanase

Akihiko Shimada[1] and Isei Nakamura[2]

[1]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

[2]Department of Food and Nutrition, Sagami Woman's University, Sagamihara, Kanagawa 228, Japan

(Received September 18, 1997; Accepted November 15,1997)

ABSTRACT

Tryptophanase is known as an enzyme with rigid stereospecificity, having the activity only for L - tryptophan under ordinary condition. However, it becomes active to D - tryptophan in highly concentrated diammoniumhydrogen phosphate solution. Inhibition reaction, in which D-tryptophan was used as an inhibitor when L-tryptophan was degraded by tryptophanase, was studied in terms of kinetics. The results suggested that an active site for D - tryptophan might be independent of that for L - tryptophan. Such the site can be confirmed when using an inhibitor which inhibits only the activity for D - tryptophan but doesn't influence on that for L - tryptophan. D - histidine was known to be compatible with this demand, that is, D - histidine inhibits only the activity for D - tryptophan. This result suggests that the activity for D - tryptophan may be independent of that for L - tryptophan.

KEY WORDS: Tryptophanase, D - tryptophan, D - histidine, Independent active site, Kinetics

Abbreviations: TPase: tryptophanase, L - Trp: L - tryptophan, D - Trp: D - tryptophan, L - His: L - histidine, D - His: D - histidine, DAP: diammoniumhydrogen phosphate, PLP: pyridoxal 5′-phosphate.

INTRODUCTION

Chiral environment in early earth is considered to have been racemic, judging from amino acids chemically synthesized from primordial molecules. However, L - amino acids are dominant in contemporary biological world. The origin of homochirality has intrigued scientists ever since *Pasteur's* discovery of the optical activity of biomatters. The cause of the amino acid homochirality has been discussed from the viewpoint of physics and chemistry (Bonner, 1995), but there is still no general consensus. On the other hand, enzyme plays an important role that selects amino acid optical isomers. It is reasonable to suppose that enzyme should be key of the elucidation of this question. However, there are few discussions about chiral homogeneity on the basis of enzymology. This is because the information on selection mechanism for amino acid optical isomers on enzyme has not been obtained. The first step for studying a selection mechanism is characterize an active site for L - and D - amino acid.

We have studied the stereospecificity of enzyme up to date. We showed that the very rigid stereospecificity of an enzyme could reversibly change with varying environment in the previous report. Tryptophanase (TPase) is known as a pyridoxal 5′ - phosphate enzyme with strict stereospecificity. Diammoniumhydrogen phosphate (DAP) could reversibly change the stereospecificity of TPase (Shimada and Nakamura, 1992). DAP acted on TPase as an activator below 3.1 M, and as an uncompetitive inhibitor over 3.1 M. The reaction was maximal at 3.1 M (Shimada *et al.*, 1996). The catalytic efficiency for D - tryptophan (D - Trp) was 0.3 % of that for L-tryptophan (L - Trp). Reaction pathway showed that the active site for D-Trp was divided into binding site and catalytic site (Shimada *et al.*, 1997 [1]). In addition, this reaction made it possible to compare the active site for D-Trp with that for L - Trp. Inhibition reaction using D - Trp as an inhibitor suggested that the binding site for D - Trp should be apart from that for L - Trp at 3.1 M concentration of DAP, because D - Trp inhibited TPase uncompetitively (Shimada *et al.*, 1997 [2]). This allows to guess that catalytic sites for L - and D - Trp may be also independent each other. A particular inhibitor which has no influence on an active site for L - Trp and competitively inhibits an active site for

D - Trp is needed to confirm it. We have looked for such an inhibitor. This report describes D - histidine (D - His) is an inhibitor suitable to our objective, together with kinetic analyses of inhibition reaction.

MATERIALS AND METHODS

Inhibitor, enzyme and reagents

Both D - Trp and D - His were purchased from Peptide Inst. Inc. (Osaka). Crude tryptophanase was purchased from Sigma Chem. Co. (St. Louis, USA), fractionated by a pH gradient provided with an isoelectric focusing apparatus, BIORAD ROTOFOR CELL (BioRad, California) to be separated from other contaminants prior to experiment. This purified procedure was repeated twice. The tryptophanase solution was prepared in 72 μg/ml of protein concentration, exhibiting a specific activity of 14 μmol/min/mg and Km of 0.33 mM with L-tryptophan at 37 ℃ and pH 8.3, and was a single band on SDS-polyacrylamide gel electrophoresis. Other reagents were obtained from Wako Pure Chem. Co. Ltd. (Osaka). All chemicals were reagent grades. All glasswares were washed by soaking more than 3 days in a special detergent, CLEAN 99CL (Clean Chemical Co. Ltd., Osaka), thoroughly rinsed in deionized and distilled water, and then dried in an oven.

Kinetics of inhibition reaction

Reaction mixtures were prepared in combinations of various amounts of DAP (0, 3.1 M), D - Trp (245, 294, 490, 980 μM) or L - Trp (245, 490, 980 μM) as substrate and D - His (0, 0.65, 1.3 mM) as an inhibitor with fixed concentrations of pyridoxal 5′ - phosphate (PLP) (380 μM) and TPase. Volume of the reaction mixture was adjusted to 10 ml with Briton - Robinson wide range buffer (pH 8.3). The reaction was initiated by adding 50 μl of a solution containing 0.05 units of TPase, and mixing immediately. TPase reacted with D-Trp at 37 ℃ for 4 h or with L-Trp at 37 ℃ for 0.5 h, and indole released from tryptophan degradation increased linearly with time. *n*-butanol was added in the reaction mixture to terminate the reaction and indole was then extracted. The indole was purified from other products by centrifugation, and then colored with Ehrlich's reagents for 30 min at 60 ℃.

Initial velocities, which was calculated from indole spectroscopically determined at $\lambda = 570$ nm, represented indole formed during one minute after the addition of 1 mg of TPase. Different combination was assayed in triplicate. All kinetic data were analyzed by averaging the initial velocities and generating Lineweaver-Burk plots of the averages.

RESULTS

Effect of D - His on L -Trp degradation reaction

It is generally known that D - His doesn't inhibit TPase (Snell, 1975). It is studied whether or not D - His influences on TPase in the reaction of L - Trp degradation in the absence or presence of DAP. D - His has no effect on TPase in the absence of DAP of 3.1 M concentration (Fig.1, ○ and ●). The Km and Vm of TPase in the absence of DAP are 0.33 mM and 14 units/mg, respectively. These values show that D - His doesn't influence on TPase at all regardless of the presence of DAP. Vm decreased by about 10 units/mg in the presence of DAP. The decrease of Vm is occurred not by D - His but by DAP

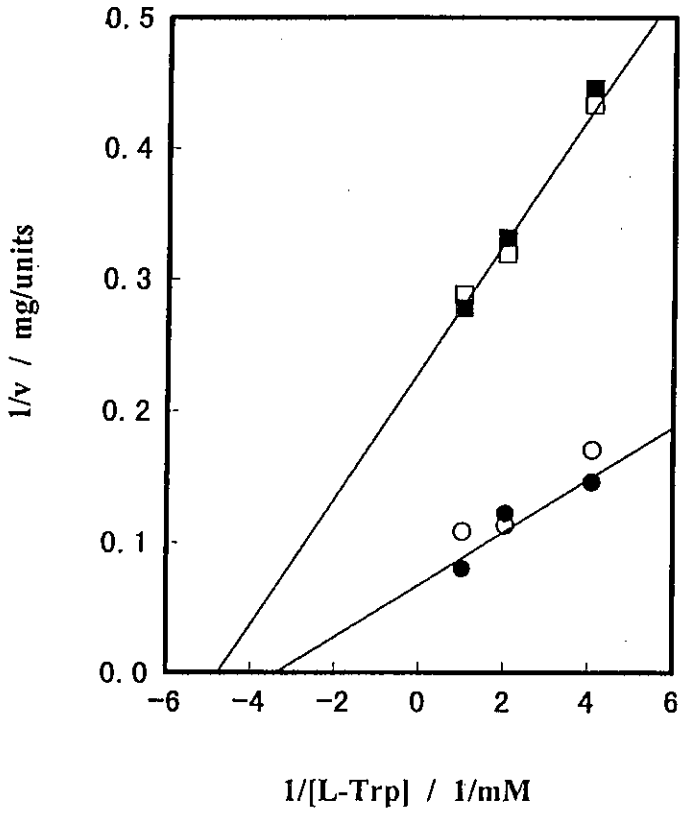

Fig.1 Effect of D-His on TPase in the L-Trp degradation reaction

○: 0 mM D-His + 0 M DAP, ●:1.3 mM D-His + 0 M DAP, □: 0 mM D-His + 3.1 M DAP, ■: 1.3 mM D-His + 3.1 M DAP

because Lineweaver - Burk plots in Fig. 1 is similar to the plots obtained in the absence of D - His as described previously (Shishido, 1995).

Inhibitory action of D - His for D - Trp degradation reaction

D - Trp degradation reaction is maximal at the DAP concentration of 3.1 M. Vm determined from Fig. 2 is about 1 % of the Vm of L - Trp degradation, agreeing with the previous data (Shimada *et al.*, 1997 [1]). Initial velocities decrease with increasing D - His, that is, D - His inhibits TPase in the presence of 3.1 M DAP. Three linear lines intersect each other at a point on the ordinate at 1 / [D - Trp] = 0. D - His acts on TPase as a competitive inhibitor in the reaction of D - Trp degradation.

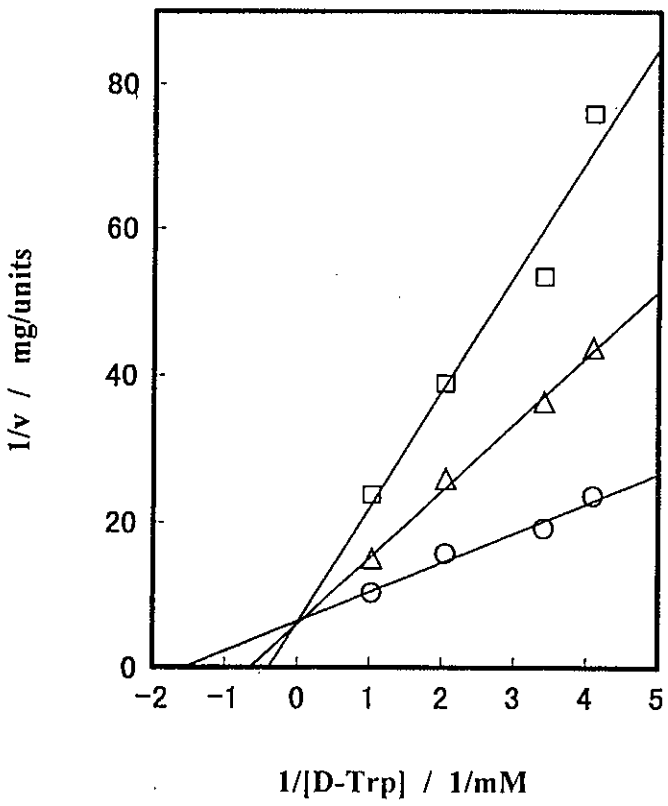

Fig.2 D-His competitively inhibiting TPase in the D-Trp degradation reaction

○ : 0 mM D-His, △ : 0.65 mM D-His, □ : 1.3 mM D-His

DISCUSSION

One of the most powerful analytical methods to characterize an active site of enzyme is to study inhibition reaction in terms of kinetics

by utilizing an inhibitor corresponding to the enzyme. The experiment where D - Trp was used as an inhibitor suggested that the binding site for D -Trp should become apart from that for L - Trp through steric structural change of TPase at the DAP concentration of 3.1 M. The next problem was whether the catalytic site for D - Trp was independent of that for L - Trp. For the purpose of its elucidation, we find out D - His, which doesn't belong to a group of dozens of inhibitors related to TPase. D - His binds at the active site for D- Trp and competitively inhibits D - Trp degradation without influencing on the reaction with L - Trp. This indicates that TPase has another active site for D - Trp in addition to an active site for L - Trp. TPase probably has a variable fragment part corresponding to environmental change, and steric structural change in the presence of DAP allows the fragment to expose to D - Trp degradation. Fig. 3 is a proposed scheme of the active site for D - Trp. The catalytic site for D- Trp is hidden under ordinary condition, though the binding site for D - Trp overlaps the binding site for L - Trp each other. The catalytic site for D - Trp emerges apart from the active site for L - Trp at DAP concentration of 3.1 M. D - His provides a valid evidence supporting that the

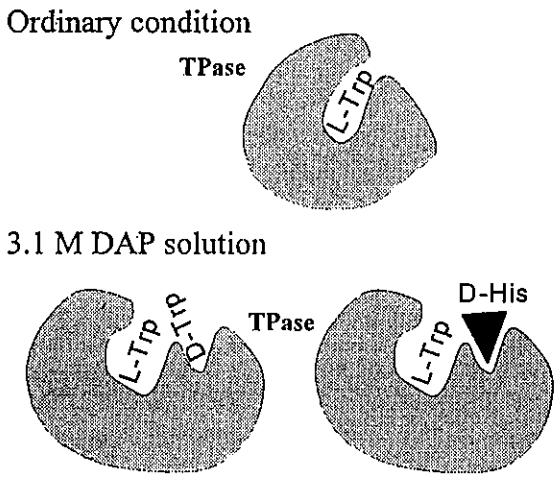

Fig.3 Possible active site for D - Trp independent of that for L - Trp at 3.1 M DAP concentration and competitive inhibition of D - His

active site for D - Trp works independently of that for L- Trp.

It is enzyme that serves as stereochemical selection filter. If there is no enzyme, L-amino acid - dominant asymmetry will not be maintained in today's biological world. But primitive enzymes in early metabolism should be different from extant enzymes. Amino acids dissolved in primitive sea might have been scarce as well as racemic. They might have metabolic pathway to utilize a small amount of L - and D - amino acids. If it is true, reaction pathway which can manage to metabolize D type amino acids may partially survive in today's biological metabolism. Although extant enzymes have made up L - amino acid - dominant world, while there are a few enzymes, for example, D - amino acid oxidase or racemase, active to D - amino acids. Moreover, free D - amino acids are distributed in sea water or crustaceans, too. Approximately 10 % of the free amino acids in sea water are D - amino acid (Zagon et al., 1994). High contents of free D- amino acids in the tissues of crustaceans is reported elsewhere, although the physiological role of D - amino acids remains unclear (Okuma et al., 1995). What does the unexpected wide distribution of D - amino acids signify for homochiral biological world ? Of course, its answer has not so far been supplied, but if a hypothetical explanation is permitted, it is to be a vestige of early metabolism under racemic environment on some four billion years ago earth. It is also of interest that it is applied to the active site for D - Trp of TPase. In this context, it is possible to speculate that originally TPase had domain consisting of two active sites for L - and D - Trp, and fragment corresponding to environmental change, that is, the active site for L - Trp, evolved. Many studies insist that chiral homogeneity was occurred by non - biological cause. Our results provide that active site corresponding to each type of enantiomers exists independently. This implies that homochirality has been formed together with enzyme evolution. A viewpoint from enzymology is also indispensable to the study of the origin of homochirality. The key to solve the question why an active site for L type develops more than one for D type appears to be in the interaction of the enzyme with environment around it.

REFERENCES

Bonner, W (1995). Chirality and life, *Origins of Life*, **25**, 175 - 190.

Okuma E., Fujita E., Amano H., Noda H. and Abe H. (1995). Distribution of Free D - amino acids in the tissue of crustaceans, *Fisheries Science*, **6**, 157 - 160.

Shimada A. and Nakamura I. (1992). Degradation of D-tryptophan by tryptophanase under high salt concentration. *Viva Origino*, **20**, 147 - 162.

Shimada A., Shishido, H. and Nakamura, I. (1996). Tryptophanase-catalysed degradation of D-tryptophan in highly concentrated diammonium hydrogen phosphate solution, *Amino Acids*, **11**, 83 - 89.

Shimada, A., Kogure, H., Shishido, H. and Nakamura, I. (1997) [1]. Reaction pathway of tryptophanase degrading D-tryptophan, *Amino Acids*, **12**, 379 - 383.

Shimada A., Kogure H. and Nakamura I. (1997) [2]. Kinetic investigation of active site for D-tryptophan on tryptophanase, *Viva Origino*, **25**, 121 - 130.

Shishido H. (1995). Expression mechanism of activity for tryptophanase - catalysed D - tryptophan degradation, Master dissertation in Environmental Science, pp.29 - 33.

Snell E. E. (1975). Tryptophanase: structure, catalytic activities, and mechanism of action. *Adv. Enzymol.* **42**, 287 - 333.

Zagon J., Dehne L. and Bogl K. (1994). D - amino acids in organisms and food, *Nutrition Research*, **14**, 445 - 463.

生命の起原への逆問題的アプローチ

飯田一浩

ＮＥＣ基礎研究所
〒３０５　つくば市御幸が丘３４番地

Abstract

生命の基本的システム構造を同定するために，起原の問題を逆問題として捉え直す．生命は他の自然現象と比べてモデル化しにくい．その理由は，複雑すぎて単純なモデルとのギャップが大きく，さらには自然発生などシステム自体が生成消滅する現象を含むからである．我々はまず，記述可能な局所的生命現象の数々を，非平衡統計力学の１パラメタとしてモデル化し，そこから逆向きにシステム構造を同定する．さらに，システムの生成消滅は，特定の条件，即ち生命が満たすべき必要条件群を満足する部分系が検出されることと見なして記述する．この方策により，生命の基本的システム構造が物理化学的に記述可能になるだけでなく，得られたシステムを解析することで，生命をもたらした自然法則についても具体的に考察できるようになる．

Viva Origino 25(1997)271－288

An Inverse Problem Approach to the Origins of Life

Kazuhiro Iida

Fundamental Research Laboratories, NEC Corporation,
34 Miyukigaoka, Tsukuba, Ibaraki, 305 Japan

(Received September 20, 1997; Accepted November 25,1997)

Abstract

A method for identifying the essential system of life is described. The system of life is extremely difficult to model in terms of physics because of the large complexity gap between elegant theories of physics and complicated real biology. Since we must accept the existance of this gap, we start by modeling actual biological transitions, then reconstruct the system from these models. The emergence of life, that is, the generation of the system itself- can also be formulated in our method as the discovery of a partial system that satisfies the physical criteria of life. This method also allows us to discuss initial conditions and natural laws for the origins of life by analyzing the parameters of an identified system.

Keywords: Inverse problems, system identification, non-equilibrium statistical mechanics

1. INTRODUCTION

Science is based upon a combination of theory and experiment. However, theories on the origins of life seems to be underdeveloped. Conducting experiments is the most convincing approach in this field: synthesis of RNA molecules has been duplicated, and the catalytic activity of these molecules has been shown in several experiments, which have provided a picture of the RNA world.[1] In contrast, theory can explain only the superficial features of organisms. This difference is because of three problems in theoretical approaches: (1) laws of nature are ignored, (2) the gap in complexity between pure theories and real biology is large, and (3) no formula has been derived that explains the emergence of life.

In most models, the laws of nature have been ignored, including artificial life simulations.[2,3] Fontana and Buss [4] simulated chemical reactions in a prebiotic soup by assuming the λ-calculus of Lisp as chemical seeds, and considered virtual chemical reactions through the textual rewriting of the calculus. Based on this model, they found that stable rewriting cycles developed automatically from the simulated soup, even when they set the initial conditions randomly. This result suggests that the initial conditions may not be important for the origins of life. However, is this conclusion correct? The premise "if nature approves it" is inevitably required to reach this conclusion. The local environment, especially thermal conditions, restricts chemical reactions. Simulations that ignore these kinds of limitations in nature can be explained in many ways.

There is a large gap in complexity between theory and real organisms. The Schrödinger equation [5] is one of the most elegant models in physics, but nobody uses this equation to explain life. A living organism certainly consists of quantum particles and atoms that obey this equation, but all the complex boundary conditions and thousands of statistical phenomena would have to be figured out to arrive at a model of the organism. Although researchers are aware of this unavoidable gap, they use unnatural variables, such as the λ-calculus, to model the system, since there is no appropriate formula for a prebiotic world. However, since such oversimplification breaks the link between the model and the real world, researchers are forced to reexamine nature. Nonetheless, the gap still exists, and at the end of many reiterations, they choose tiny phenomena such as B-Z reactions.[6]

Another gap exists. Brilliant theories of physics are by no means sufficient for modeling the emergence and the evolution of life taht is, the creation-annihilation of the system itself. A conventional model of water motion may generate an unexpected change of a smooth flow into turbulence. However, it never generates an even greater unexpected change such as the appearance of bacteria in the water because everything is well defined in such a model. It is assumed that nothing outside of the model occurs. Matsuno [7] and Rösslor [8] are searching for another system description framework based on interaction or observation. With such a framework, the system can be described by observing it from its smallest parts. For example, a molecule or a cell can interact with its surroundings only in a limited space-time region, and can change its state only through limited interactions. Researchers are interested in this sequence of local interactions and state changes, because a new system can emerge from the sequence; however, the sequence is hard to describe.

Is there a solution to these formidable problems? In what follows, we describe a method that overcomes the three difficulties inherent in theoretical approaches that seek to model the essential system for life. The key concepts behind this method are given first, then detailed protocols are explained.

2. THREE CONCEPTS FOR MODELING LIFE

Key concepts for solving the above problems are (1) the introduction of a physical-chemical reality into the model, (2) the integration of biological evidence to obtain the model, and (3) the introduction of an observation process into the model to emulate the emergence of the system.

2.1 Introduction of physical-chemical reality into the model

Physical-chemical reality is especially important in modeling the prebiotic world because initial life cannot be considered without the constraints of nature, for example, the laws of thermodynamics. Since, the basic body of *mycoplasma*, the smallest living cell, is only 100 nm (10^{-7} m) in diameter,[9] initial life must be between 10 nm and 100 nm in size. This is much smaller than the width of the smallest mask pattern (350 nm) of advanced MPUs in our computers.

For describing this extremely small physical system, non-equilibrium statistical mechanics [10,11] is probably the best framework, because life is apparently non-equilibrated statistical phenomena. This framework combines the microdynamics of chemical reactions with a highly ordered system.

2.2 Integration of biological evidence to obtain the model

Since the complexity gap will last forever, we should start describing peripheral but real transitions in an organism rather than searching for the fundamental model of life. To do this, we should first write the models of actual biophysical transitions in an organism which are the results of the complex organizing effect of nature on the essential system of life. Then, we should go back to the essential system by eliminating the complexity in those models. Writing the peripheral models is much easier than making a model of complete life. For example, we can model enzymatic reactions based on the evidence from molecular biology, that is, what amount of substrates is delivered, how they are catalyzed, and which products are transferred to the outside. Some research groups have actually started modeling such local biophysical transitions.[12,23] All these individual transitions can be modeled into fluxes j's in non-equilibrium statistical mechanics.

2.3 Introduction of an observation process into the model

The creation and annihilation of the system itself can be described by introducing an observation process into the model. Although everything is determined in a mechanical model of the world, which part of the world we will observe is not determined. Using this indeterminacy, we can create and annihilate a new part of the whole system, what we call a *subsystem*. God knows everything, but we human beings observe only part of the world. We detect living things by putting a template "life" on the observed portion of the system. Since this subsystem is determined by

the relationships between the constant template "life" and the changing world, a new subsystem can be generated by the alteration of the world. Notice that not only the model of the world but also the template can be written down in a conventional formula in physics. Consequently, we can model the creation and annihilation of systems without giving up the advantages of conventional analytical techniques by introducing the template.

This leads us to the question, "what is the template of 'life' "? Life is an ambiguous concept which seems impossible to formulate. Of course, there is no physical definition of life- if the template represents the definition, we can not use the template, because the physical definition is our target. However there is no need to abandon our enterprise. Our goal is to approximate the essential system of life by integrating the necessary physical conditions for life. What we should do is translate the consensus of biologists into physical models to obtain the hypothetical template "life." Approximation is the basic way of science, so the subsystem detected by the template is only an imitation, but it meets all the necessary conditions for the described life. The subsystem will quickly approach the essential system of life as our knowledge of life progresses.

These are the key concepts for solving the three problems. In the following, we discuss the protocols for fulfilling the concepts.

3. SYSTEM IDENTIFICATION STRATEGY

3.1 Approximation cycle

Let us first summarize our strategy. Our target is to identify the essential system for life as a statistical quantity $\rho(t)$. The ρ (we use t only when it is required) is the density in non-equilibrium statistical mechanics. We sometimes say "system ρ," because this quantity enables us to estimate all the physical values obtained from the system so that we can predict the future behavior of the system (see the next subsection). We search for a ρ that satisfies the necessary physical conditions of life. The ρ is obtained through the following approximation cycle (see Fig. 1).

We first write the template for life and use it as the criterion for searching for a life-like subsystem. This template can be obtained by translating the characteristics of life into physical constraints. For example, life has certain characteristics: it is a stable dynamic system, it has negative entropy import,[20] and it increases its free-energy content. These characteristics can easily be translated into physical constraints.

We next describe individual biophysical transitions, such as potassium ion flow, in the form of flux $j(t)$. Then, we integrate them into ρ. After reducing the formula,

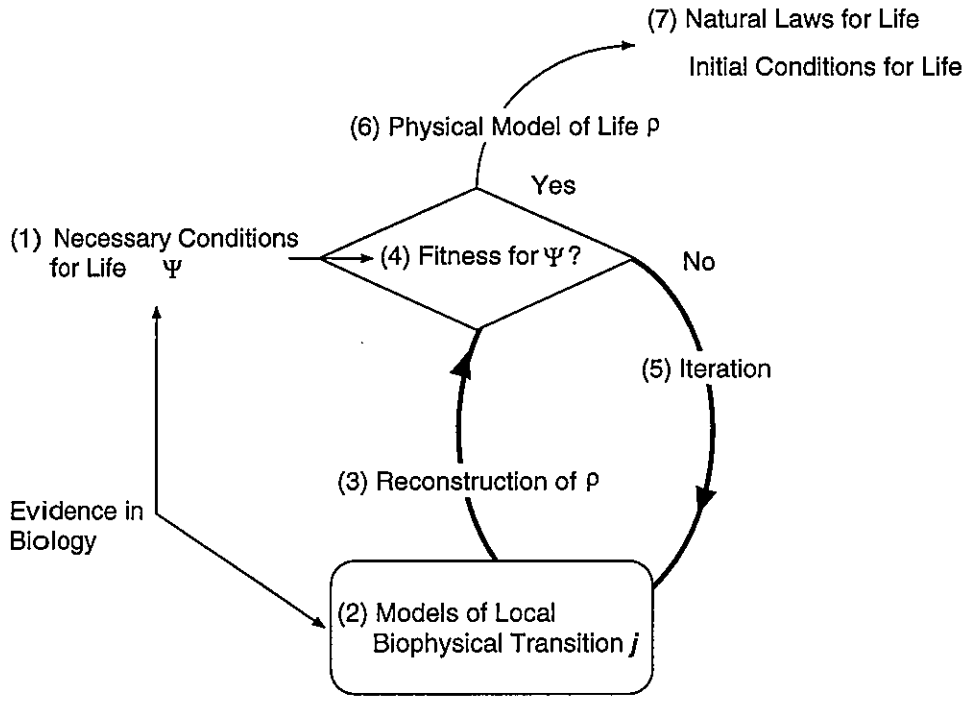

Fig. 1 Strategy for approximating the system of life

we test whether the ρ meets the template. If it fails, we continue to refine the model of j's and integrate them into a new ρ. At the end of this approximation cycle, a life-like subsystem will be detected.

Finally, we study both the essential materials and natural principles for life. The obtained subsystem includes the principles hidden behind the behavior of an organism. We can therefore expect new knowledge on the principles for life baed on the analysis of the physical parameters of the subsystem.

3.2 Prediction of system behavior from the density

Using density $\rho(t)$, we can predict the future behavior of the system, since the expectation value, $\langle A(t) \rangle$, of any physical quantity A, can be obtained. Suppose a system consists of N classical particles. The states of this system can be determined by the set of positions r_n and momenta p_n of each particle $\gamma \equiv \{ r_1, r_2, ..., r_N, p_1, p_2, ..., p_N \}$ (small n indicates particle number n =1, 2, 3,$\cdots$, N). The physical quantity A is a function of r_n's, and p_n's, for example, the kinetic energy $\sum_n^N p_n^2/2m$. The expectation value of A is obtained by summing up the

product of A and density ρ, over all the state index γ's; that is,

$$\langle A(t) \rangle = \int d\gamma \;\; A(\gamma) \; \rho(\gamma, t) \; . \tag{1}$$

(see Appendix A for quantum systems). This relation also holds in a system with heterogeneous particles, and even in a system with chemical reactions,[13] For simplicity, we choose a system with N identical particles in the following sections.

4. RECONSTRUCTING DENSITY FROM FLUXES

The most important step in our strategy is the reconstruction of ρ from the models of the flux j's. This step is based on the following discussion.

4.1 Theoretical basis

Life is apparently in a non-equilibrated and non-stationary state. The ρ of such a system cannot be obtained by using the conventional method of statistical mechanics,[14] because equilibrium is assumed in the classical framework. Therefore, the density of a non-equilibrated system is approximated on the assumption that the system is equilibrated locally. The approximated density, called *quasi-equilibrium density*, (ρ_q) , is calculated by

$$\rho_q(\gamma, t) \;\; = \;\; \frac{1}{Z} \, e^{-f(\gamma,t)}, \quad Z = \int d\gamma' \; e^{-f(\gamma',t)}, \tag{2}$$

$$f(\gamma, t) \;\; = \;\; \int d^3r \;\; \beta(\boldsymbol{r}, t) \{ \; H(\boldsymbol{r}, t; \gamma) - \mu(\boldsymbol{r}, t) \; n(\boldsymbol{r}, t; \gamma) \; \}. \tag{3}$$

The $H(\boldsymbol{r}, t; \gamma)$ is the density of energy; $n(\boldsymbol{r}, t; \gamma)$ is that of particles; $\beta(\boldsymbol{r}, t)$ and $\mu(\boldsymbol{r}, t)$ are respectively the multipliers representing the inverse of the temperature and chemical potential (see Appendix B for quantum systems). We can determine $\beta(\boldsymbol{r}, t)$ and $\mu(\boldsymbol{r}, t)$ by the Lagrange method,[15] assuming that entropy peaks at equilibrium. The ρ_q violates the Liouville equation (see Appendix C), with which the true density must comply.

Zubarev [16] showed the following relationship between $\hat{\rho}_q$ and the true density $\hat{\rho}$ for a non-equilibrated and non-stationary quantum system:

$$\hat{\rho}(t) \;\; = \;\; \lim \, \varepsilon \to +0 \;\; \varepsilon \int_{-\infty}^{0} dt' \;\; e^{\varepsilon t'} \; e^{iLt'} \; \hat{\rho}_q(t + t') \tag{4}$$

(the hat $\hat{}$ indicates an operator). This relation enables us to obtain the true density from the quasi-equilibrium density. The ε is a tiny positive number that is introduced to break the symmetry of time so that $\hat{\rho}$ satisfies the Liouville equation in the limit $\varepsilon \to +0$, after taking conventional thermodynamic limit $V \to +\infty$. The L is the Liouville operator. Equation (4) also holds in classical systems.

4.2 Reconstructing the density in classical systems

The true density ρ can be obtained from Zubarev's relation, Equation (4), and from the continuity equations of the energy and particles (see Appendix D). Suppose that the density of energy flux $\boldsymbol{j}_H(\boldsymbol{r})$ and that of particle flux $\boldsymbol{j}_n(\boldsymbol{r})$ are given in the functions of $\boldsymbol{r}_1,\ldots,\boldsymbol{r}_N$, $\boldsymbol{p}_1,\ldots,\boldsymbol{p}_N$. Then, $H(\boldsymbol{r},t;\gamma)$ and $n(\boldsymbol{r},t;\gamma)$ in identity (3) can be calculated by

$$H(\boldsymbol{r},t;\gamma) = H(\boldsymbol{r},0;\gamma) - \int_0^t dt' \ \nabla\cdot\boldsymbol{j}_H(\boldsymbol{r},t';\gamma), \tag{5}$$

$$n(\boldsymbol{r},t;\gamma) = n(\boldsymbol{r},0;\gamma) - \int_0^t dt' \ \nabla\cdot\boldsymbol{j}_n(\boldsymbol{r},t';\gamma), \tag{6}$$

based on the continuity equations where $H(\boldsymbol{r},0;\gamma)$ and $n(\boldsymbol{r},0;\gamma)$ are the initial values at $t=0$. Putting these parameters into Equation (3), we have $\rho_q(\gamma,t)$, and by Equation (4), we reach the true density ρ (also see Appendix E).

4.3 Reconstructing the density in quantum systems

There is a possibility that quantum effects led to life. It may sound strange that we are going to discuss a quantum system for the model of life, but as we mentioned above, life must be an extremely small system of 10 nm to 100 nm in diameter, whose behavior is better described with statistical mechanics. Moreover, there is evidence that primitive photosynthetic bacteria use quantum effects in photo-excitation.[17] These facts indicate that we also need the construction method in a quantum system.

However, Zubarev's relationship is hard to use because operators $\hat{\boldsymbol{j}}_H$ and $\hat{\boldsymbol{j}}_n$, which are the quantum counterparts of $\boldsymbol{j}_H$ and $\boldsymbol{j}_n$, are too complicated for modeling biophysical transitions. Therefore, we calculate another parameter $P(\vec{n})$ instead of operator $\hat{\rho}$. The $P(\vec{n})$'s are positive numbers such that $\sum_{\vec{n}} P(\vec{n}) = 1$; $\vec{n}$ is the index of the state. If we assume a system of N identical particles, the status of the system can be determined by $\vec{n} \equiv \{\ n(1), n(2), ..., n(S)\ \}$, which is the sequence of the number $n(s)$ of particles occupying each eigenstate s (=1, 2, 3, .., S) of a single particle system.

Note that the density is expanded into [18]

$$\hat{\rho} = \sum_{\vec{n}} P(\vec{n})\ |\vec{n}\rangle\langle\vec{n}|\ . \tag{7}$$

Identity (7) tells us that we obtain $\hat{\rho}$ by calculating $P(\vec{n})$ where the eigenkets $|\vec{n}\rangle$ are given. The $|\vec{n}\rangle$ can be prepared from the eigenkets $|s\rangle$ of the single particle system, which are easy to handle.[19] Considering that $\hat{\rho}_q$ can also be expanded into

$$\hat{\rho}_q = \sum_{\vec{n}} P_q(\vec{n})\ |\vec{n}\rangle\langle\vec{n}|, \tag{8}$$

and that its basis vectors are identical to those of $\hat{\rho}$, we know that $e^{iLt'}$ in Equation (4) affects nothing on $P_q(\vec{n})$. Then, we have

$$P(\vec{n},t) \;=\; \lim\ \varepsilon \to +0\ \ \varepsilon \int_{-\infty}^{0} dt'\ e^{\varepsilon t'}\ P_q(\vec{n},t+t'). \tag{9}$$

The $P_q(\vec{n})$ is calculated from two parameters: the flux $j(r,t;s)$ of the particle in the s-th state, and the eigenenergy $E(s)$ of the single particle system (see Appendix F).

5. DESCRIBING EMERGENCE OF LIFE

5.1 Modeling observation

We found that the emergence of a system can be described by introducing an observation process into the model. But how can an observation process be formulated in the above framework? Suppose that the physical criteria for life are the constraints on the behavior of the system, and that a real-valued functional $\Psi[\ r;\ \rho\ ]$ - what we call a *detector* - represents the constraints (see the next subsection for the formula of Ψ). Then, the domain D of the life-like subsystem we are searching for can be given formally as

$$D \;=\; \{\ r \mid \Psi[r;\rho\ ] > 0\ \} \tag{10}$$

(notice that ρ is in the input parameters of Ψ).

If D is determined by the observation, the density of the life-like subsystem, ρ_D, and the expectation value $\langle A\rangle_D$ obtained from the subsystem, are given by

$$\rho_D \;=\; \psi_D\ \rho, \tag{11}$$

$$\psi_D \;=\; C\ \delta(r - r_D), \quad (\ r_D \in D\ ), \tag{12}$$

$$\langle A\rangle_D \;=\; \int d\gamma\ A\ \rho_D, \tag{13}$$

where C is a normalization factor and $\delta(\cdot)$ is the Dirac delta function.

5.2. Formula of the detector

Detector Ψ can be formulated, for example, by

$$\Psi \;=\; 1 - \prod_j \left(1 - \prod_k u(x_k)^{a(j,k)}\right)_j , \tag{14}$$

when we arrange the criteria for life in *conjunctive normal form* logic. This is where $u(\cdot)$ denotes the Heaviside step function,

$$u(x) \;=\; \begin{cases} 1 & \cdots \quad x > 0\ , \\ 0 & \cdots \quad x < 0\ , \end{cases} \tag{15}$$

where x_k is the functional representing the k-th physical criterion and $a(j,k)$ ($\in \{0,1\}$) is the exponent that determines the logic.

We call this kind of logical formula, such as

$$(A_1 \textit{ and } A_2 \textit{ and } \cdots) \textit{ or } (A_3 \textit{ and } \cdots) \textit{ or } \cdots , \tag{16}$$

that is, "A_1: stable dynamic system" and "A_2: negative entropy import," or "A_3: increase in the free-energy content" is life, as a conjunctive normal form. The truth-value ($\in \{0,1\}$) of the conjunctive normal form logic is obtained by

$$1 \quad -\prod_j \left(1 - \prod_k T(A_k)^{a(j,k)}\right)_j , \tag{17}$$

where $T(A_k)$ indicates the truth-value of the k-th literal expression A_k and $a(j,k)$'s are the exponents (in the above case, $a(1,1)=1$, $a(1,2)=1$, $a(1,3)=0$, $a(2,1)=0$, $a(2,2)=0$, $a(2,2)=1$).

The Heaviside Step function $u(\cdot)$ is substituted for $T(\cdot)$ in identity (15). Functional x_k is, for example, the $\langle \dot{F}(r;\rho) \rangle^\tau$ that represents the criterion "the increase in the free-energy content," that is,

$$\langle \dot{F}(r;\rho) \rangle^\tau > 0 , \tag{18}$$

where $\dot{F}$ is the time derivative of free-energy density and the average is taken for τ, which is the estimate of the characteristic time for local equilibrium.

6. DISCUSSION

1) Going upstream to the initial conditions for life

We still have few ideas on the initial conditions and the laws of nature that led to life. All organisms on earth are made up of organic polymers of carbon compounds. Moreover, these compounds have identical chirality, all L or all R. Why has only carbon been selected among hundreds of elements? Why have monomers of single chirality been polymerized into macromolecules in spite of the fact that only a racemic mixture is synthesized under experimental conditions? Are these properties prerequisites for life? To answer these questions, we should learn about the characteristics of the particles that make up the system, and also about the interactions between the particles.

By analyzing the parameters of the life-like subsystem ρ_D, we attain the characteristics and the interactions. Notice that mass m and chemical potential μ in Equation (3) indicate the independent features of the particles. The permissible range of these parameters will be determined from the physical constraints for life.

Furthermore, the interaction potential M ($M(r,t;\gamma)$) between the particles can be obtained formally as

$$M = H - (K + V), \tag{19}$$

where the density of external potential V ($V(r,t;\gamma)$) is given. The K is the density of kinetic energy $K(r,t;\gamma) = \sum_n^N \delta(r - r_n)\, p_n^2/2m$ (see Appendix G for quantum systems).

The formula of interaction potential M is completely phenomenological, but it may provide us with the inherent laws of nature behind the action of an organism. After mathematical simplification, we compare the formula with those of every known interaction, such as Coulomb potential.

Of course, these interactions drawn from modern organisms are not necessarily required at the beginning of life. Cairns-Smith [21] posed the "Genetic take over" hypothesis. It states that the first living form was a mineral crystal, which automatically duplicated its crystalline arrangement. The replicating mechanism of the mineral species was then taken over by organic polymers, and finally, modern carbonic life appeared. According to the hypothesis, the interactions that supported the initial mechanism must have been lost. However, our analysis on ρ_D still has significance because the essential system of a modern organism and that of the initial life should be identical. Notice that the criteria for life are obtained from our "present" knowledge, and also notice that the prebiotic reactions become "life" only when they meet the criteria. Therefore the configuration of M can be utilized to identify the prebiotic reactions which led to life.

2) Explanation of system behavior

The formula of the life-like subsystem ρ_D is completely different from our common image of life. In what term of the formula can we find properties of life, such as vitality, autonomy, and self-preservation? Is it appropriate to call a subsystem self-preservative? Since our formula is completely mechanical, composed of well-defined terms, there must not be any room for interpreting system behavior. However, we have to remember that we can find vitality and autonomy in an organism because we have the concepts corresponding to the specific behavior. These properties of life exist only when we observe the behavior through these concepts. With the same logic, it is reasonable to accept subsystem self-preservation if the subsystem meets our physical criterion representing "self-preservation" that corresponds to our concept.

3) Independent observation

The independence of the observation from the whole system is very important. The role of detector Ψ is different from that of our cognition, since our cognition

inevitably depends on the whole system. We are included in the world, and observe the world actively interacting with our surroundings. These actions are too difficult to formulate. Moreover, if we try to add the model of our cognition to the formula of the world, we arrive at the dead end of recursion such that we see the world in which we are seeing. To avoid this messy recursion, every element of Ψ should be independent of the whole system, that is,

1. Symbol Ψ must be removed from the formulas of fluxes j and density ρ,
2. The domain D and density ρ_D of the subsystem should be eliminated in the formulas of Ψ.

4) Characteristic length of the initial system

Which biological phenomenon should we focus on to prepare the physical criteria? In the first place, we should classify the phenomena into several orders of magnitude according to their characteristic lengths. For example, metabolism and reproduction should be classified into completely different orders. Metabolism has the characteristic length of chemical reactions, which is about the mean free path of an ion, 10^{-9} - 10^{-8}m. In contrast, reproduction requires a space larger than 10^{-7} m in diameter (the diameter of *mycoplasma* basic body was considered). This fact indicates that the two phenomena should be formulated differently in statistical physics.

The characteristic length of the initial life probably equals that of metabolism, because metabolism is clearly lower in order than other criteria such as reproduction, and can be detected first. Although it is hard to define the concept "metabolism," it can be approximated with closely related phenomena such as negative entropy import given by

$$\langle \nabla \cdot j_s(r;\rho) \rangle^{\tau} > 0, \tag{20}$$

where j_s denotes the density of entropy flux.

5) Search using constraints of nature

The constraints of nature may seem a real hindrance, but in fact, they are favorable because they help us constrict search space. Consider that you should test myriad combinations of parameters to ensure your conclusion is unexceptionable. The constraints of nature strongly restrict permissible combinations of parameters for life. The same merit for natural constraints has been discussed in the field of brain science,[22] where there are difficulties similar to ours.

The above framework enables us to write down the essential system of life, which has never been attained. What we should do next is to systematically accumulate the properties of life and translate them into physical constraints. This is a simple

but feasible approach to identifying the essential system.

Acknowledgements

I would like to thank Toshihiko Arimitsu of the Institute of Physics at the University of Tsukuba for his valuable comments.

REFERENCES

1. Ferris J. P. ed., *RNA and the Origins of Life*, Special Issue, *Origins Life Evol. Biosphere*, Vol. 23, 1993.
2. Langton, C. G. ed., *Artificial Life*, Santa Fe Institute Studies in the Science of Complexity, Proc. Vol. 6, Redwood City, CA, Addison-Wesley, 1989.
3. Langton, C. G. and Shimohara K. eds., *Artificial Life V*, Proc. of the fifth International Workshop on the Synthesis and Simulation of Living Systems, MIT Press, Cambridge, MA, 1997.
4. Fontana, W. and Buss, L. W. (1994), "The Arrival of the Fittest: Toward a Theory of Biological Organization," *Bulletin of Mathematical Biology* 56:1-64.
5. Shankar, R., *Principles of Quantum Mechanics*, seventh printing, Plenum Press, New York, 1988.
6. Belouzov, B. P. in *Sbornik Referatov po Radiacioni Medicine*, Medgiz Moskow, 1959.
7. Matsuno K., *Protobiology: Physical Basis of Biology*, pp. 31-55, CRC Press, Boca Raton, FL, 1989.
8. Rössler, O. E. (1994), "Micro constructivism," *Physica D* 75:438-448.
9. Sasaki, S., *Mycoplasma*, p.11, Tokai University Press, Tokyo, 1980 (in Japanese).
10. Zubarev, D. , Morozov, V. and Röpke, G., *Statistical Mechanics of Nonequilibrium Process*, Akademie Verlag, New York, 1996.
11. Grandy, W.T., *Foundations of Statistical Mechanics*, Vol. II: Nonequilibrium Phenomena, D. Reidel Publishing Company, Dordrecht, 1988.
12. Ohtake, H., Yako, T. , Tsuji, T., Kato, J., Kuroda, A., and Kaneko, M. (1997), "An Approach to Molecular Artificial Life: Bacterial Intelligent Behavior and its Computer Model." In: Langton, C. G. and Shimohara K. eds., *Artificial Life V*, Proc. of the 5-th International Workshop on the Synthesis and Simulation of Living Systems, pp. 395-401, MIT Press, Cambridge, MA, 1997.
13. Zubarev, D. , Morozov, V. and Röpke, G., *Statistical Mechanics of Nonequilibrium Process*, pp. 165-172, Akademie Verlag, New York, 1996.

14. Grandy, W. T., *Foundations of Statistical Mechanics*, Vol. I: Equilibrium Theory, D. Reidel Publishing Company, Dordrecht, 1988.
15. Avriel, M., *Nonlinear Programming: Analysis and Method*, pp. 9-26, Prentice-Hall, Engelwood Cliffs, NJ, 1976.
16. Zubarev, D. N. and Kalashinikov, V. P. (1970), "Construction of Statistical Operators for Nonequilibrium Process," *Theoretical and Mathematical Physics* 3:126-134.
17. Feher, G., Allen, J., Okamura, M. Y., and Rees, D. C. (1989), "Structure and Function of Bacterial Photo Synthetic Reaction Centers," *Nature* 339:111-116.
18. Grandy, W. T., *Foundations of Statistical Mechanics*, Vol. I: Equilibrium Theory, pp. 84-88, D. Reidel Publishing Company, Dordrecht, 1988.
19. ibid. pp. 101-103.
20. Aoki, I. (1997), "Introduction to Entropy Physiology," *Seibutsu-Butsuri* 37: 106-110 (in Japanese).
21. Cairns-Smith, A. G., *Seven Clues to the Origin of Life*, pp. 107-113, Cambridge University Press, Cambridge, 1985.
22. Valiant, L. G., *Circuits of the Mind*, pp. 9-24, Oxford University Press, New York, 1994.
23. Bray, D. and Bourret, R. B. (1995), "Computer analysis of the binding reactions leading to a transmembrane receptor-linked multiprotein complex involved in bacterial chemotaxis," *Mol. Biol. Cell* 6:1367-1380.

APPENDIX A

Expectation value in quantum systems

We use operators in place of real valued indexes and use trace (Tr) in place of integration, such that

$$\langle A(t) \rangle = Tr(\ \hat{A}\ \hat{\rho}(t)\ ). \tag{21}$$

The $\hat{A}$ is a function of basic operators $\hat{\boldsymbol{r}}$ and $\hat{\boldsymbol{p}}$.

APPENDIX B

Quasi-equilibrium statistical operators in quantum systems

In the case of quantum systems,

$$\hat{\rho}_q(t) = \frac{1}{Z}\, e^{-\hat{f}(t)}, \quad Z = Tr(\ e^{-\hat{f}(t)}\ ), \tag{22}$$

$$\hat{f}(t) = \int d^3r\ \ \beta(\boldsymbol{r},t)\{\ \hat{H}(\boldsymbol{r},t) - \mu(\boldsymbol{r},t)\ \hat{n}(\boldsymbol{r},t)\ \}. \tag{23}$$

The two operators $\hat{H}(\boldsymbol{r},t)$ and $\hat{n}(\boldsymbol{r},t)$ are the respective counterparts of energy density and particle density.

APPENDIX C

The Liouville equation

The Liouville equation,

$$\frac{\partial \rho}{\partial t} + iL\rho = 0, \tag{24}$$

is derived from the continuity equation and the Liouville theorem stating the invariance of phase volume. The operator L, called the Liouville operator, is shown as using Poisson brakets (k is the index of dimension)

$$iL\,(\cdot) \;=\; \sum_k \left(\frac{\partial \cdot}{\partial q_k}\frac{\partial H}{\partial p_k} - \frac{\partial \cdot}{\partial p_k}\frac{\partial H}{\partial q_k} \right). \tag{25}$$

A commutator is substituted for Poisson brakets such that $iL(\cdot) = (1/i\hbar)\,[\,\cdot\,, H\,]$ in quantum systems.

APPENDIX D

The continuity equations

Because the particle number and energy are conserved,

$$\frac{\partial\, H(\boldsymbol{r},t)}{\partial t} \;+\; \nabla \cdot j_H(\boldsymbol{r},t) = 0, \tag{26}$$

$$\frac{\partial\, n(\boldsymbol{r},t)}{\partial t} \;+\; \nabla \cdot j_n(\boldsymbol{r},t) = 0 \tag{27}$$

hold. The $H(\boldsymbol{r},t)$ and $n(\boldsymbol{r},t)$ are the densities of energy and particles. The $j_H(\boldsymbol{r},t)$ and $j_n(\boldsymbol{r},t)$ indicate the respective densities of energy flux and particle flux.

APPENDIX E

Reconstruction method in classical systems

The transformation,

$$e^{t'iL}\rho_q \;=\; \exp\{\, t'iL\;\rho_q\,\}, \tag{28}$$

$$iL\;\rho_q \;=\; -\rho_q\,\{\;iLf(\gamma)\;\}, \tag{29}$$

$$iL\;f(\gamma) \;=\; \int d^3r\;\beta(\boldsymbol{r},t)\,[\;\{iL\;H(\boldsymbol{r},t;\gamma)\} - \mu(\boldsymbol{r},t)\{iL\;n(\boldsymbol{r},t;\gamma)\}\;]\;, \tag{30}$$

helps us obtain the statistical operator.

APPENDIX F

The P in the quasi-equilibrium statistical operator.

$$P_q(\vec{n},t) = \frac{1}{Z}\, e^{-f(\vec{n},t)}, \quad Z = \sum_{\vec{n}} e^{-f(\vec{n},t)}, \tag{31}$$

$$f(\vec{n},t) = \int d^3r \;\; \beta(r,t)\{\; H(r,t;\vec{n}) - \mu(r,t)\; n(r,t;\vec{n})\;\}. \tag{32}$$

Using the continuity equations, we obtain $H(r,t;\vec{n})$ and $n(r,t;\vec{n})$ from the initial density of particle $n(r,0;s)$ at t=0 and the density of particle flux $j(r,t;s)$. We can calculate the density of energy flux $j_H(r,t;\vec{n})$ as $\sum_{s=1}^{S} E(s)\; j(r,t;s)$.

APPENDIX G

Parameters of interaction potential

In the quantum system, the parameters required for obtaining interaction potential M are

$$H(\vec{n}) = \sum_{s=1}^{S} E(s)\; n(s), \tag{33}$$

$$n(s) = \int dr^3\; n(r;s), \tag{34}$$

$$K(\vec{n}) = \sum_{s=1}^{S}\sum_{n=1}^{N} \frac{p_n^2}{2m}\; \delta(s-s_n), \tag{35}$$

$$V(\vec{n}) = \sum_{s=1}^{S}\sum_{n=1}^{N} V(r_n,p_n)\; \delta(s-s_n). \tag{36}$$

The s_n indicates the single particle eigenstate of the n-th particle.

海底熱水孔を擬したフローリアクターでのオリゴペプチド自己触媒生成

今井栄一，本多元，羽鳥晋由，松野孝一郎

長岡技術科学大学・生物系
（〒940-2188　長岡市上富岡町 1603-1）

要旨

海底熱水孔を擬したフローリアクターを用いてグリシンからそのオリゴマーを生成することを試みた．フローリアクターの内容物についての運転初期条件は純水とグリシンのみであった．この状況下で，グリシンの二量体，三量体が得られた．その生成初期段階では，生成物は時間の経過と共に指数関数的に増加して行った．この事実は，ここでのオリゴペプチドの生成が自己触媒的であることを示唆する．

Viva Origino 25(1997)289－296

Autocatalytic Oligopeptide Synthesis in a Flow Reactor Simulating Submarine Hydrothermal Vents

Ei-ichi Imai, Hajime Honda, Kuniyuki Hatori and Koichiro Matsuno*

Department of BioEngineering
Nagaoka University of Technology
Nagaoka 940-2188, Japan

(Received September 30, 1997; Accepted December 4, 1997)

ABSTRACT

A flow reactor simulating submarine hydrothermal vents was employed for examining an oligopeptide synthesis from glycine alone in its aqueous solution without having recourse to either condensing agents, templates or even metallic ions. Initial buildup of the yields of both di- and tri-glycine was found to exponentially increase with the elapse of time, indicating that the oligopeptide synthesis could be autocatalytic.

* To whom correspondence should be addressed

Key words: Autocatalysis, Flow reactor, Hydrothermal vents, Oligopeptides

1. INTRODUCTION

Submarine hydrothermal vents in the Archaean ocean (Kändler, 1993) could have been thought most likely locales for making prebiotic oligomerization or polymerization of small organic molecules on the primitive earth (Corliss et al, 1978; Edmond et al, 1982). Constant thermodynamic gradients available between hot springs from

hydrothermal vents and cold water surrounding them could have driven various synthetic reactions. This scenario has called our attention to constructing a flow reactor simulating submarine hydrothermal vents in the laboratory and examining a likelihood of synthesizing oligomers from monomers. In particular, we observed that oligopeptides were synthesized from monomeric glycine in the flow reactor even in the absence of condensing agents, templates and metallic ions (Matsuno, 1997). In this article, we report that oligomerization of glycine proceeding in the flow reactor could be autocatalytic.

2. A FLOW REACTOR SIMULATING HYDROTHERMAL VENTS

An essence of the flow reactor, whose design principle and details are found in our previous report (Matsuno, 1997), is to circulate the fluid in the unidirectional manner; from the high-temperature, high-pressure chamber through a nozzle into the low-temperature, high-pressure chamber connected further downward to a long needle pipe for depressurization down to normal atmospheric pressure for sampling the specimen, then through a pump for pressurization with the pressurized fluid back again into the high-temperature, high-pressure chamber through a pipe.

All the materials for constructing the flow reactor including the chambers and pipes were made of anti-corrosion stainless steel. The volume of the high-temperature, high-pressure chamber was 15ml. The nozzle connecting the high-temperature, high-pressure chamber to the low-temperature, high-pressure one was 50mm long with its diameter 100μm. The 250mm-long low-temperature, high-pressure chamber with its diameter 20mm was immersed in a water bath of volume 1.3l contacting a cooling pipe carrying coolant at -20°C. The water in the bath was constantly stirred, and the cooling system was controlled so as to maintain the temperature of the outer surface of the chamber at the downstream end at 0°C. The fluid flown out of the low-temperature, high-pressure chamber was fed into a 1m-long pipe of its diameter 100μm through which the fluid loses its pressure down to normal atmospheric pressure. Sampling of the specimen was accomplished there at every fixed time interval. The fluid stored in the depressurized vessel was then pressurized and again fed into the high-temperature, high-pressure chamber. The flow rate of the fluid in the closed circuit was realized somewhere between 8-12ml/min so as to maintain the pressure of the high-temperature, high pressure chamber at 23.0Mpa, that is only slightly above the pressure of the critical point of water, 22.1MPa .

3. OLIGOMERIZATION OF GLYCINE IN THE FLOW REACTOR

We prepared 100mM L-glycine solution dissolved into pure water and maintained the total volume of the circulating fluid on the level of 500ml. When the temperature of the injecting jet stream from the nozzle connected to the high-temperature, high-pressure chamber was set at 250°C, the result on the time course of the products, di- and tri-glycine, is displayed in Fig. 1, in which the measurement of the yields was accomplished by comparing the HPLC peak area of the product to a similar one of the corresponding standard specimen provided independently. The initial buildup of the products, di- and tri-glycine, was found to exponentially increase with the elapse of time. Similarly, even when the temperature of the injecting jet stream from the nozzle was increased up to 300°C, the initial exponential buildup of the products, di- and tri-glycine,

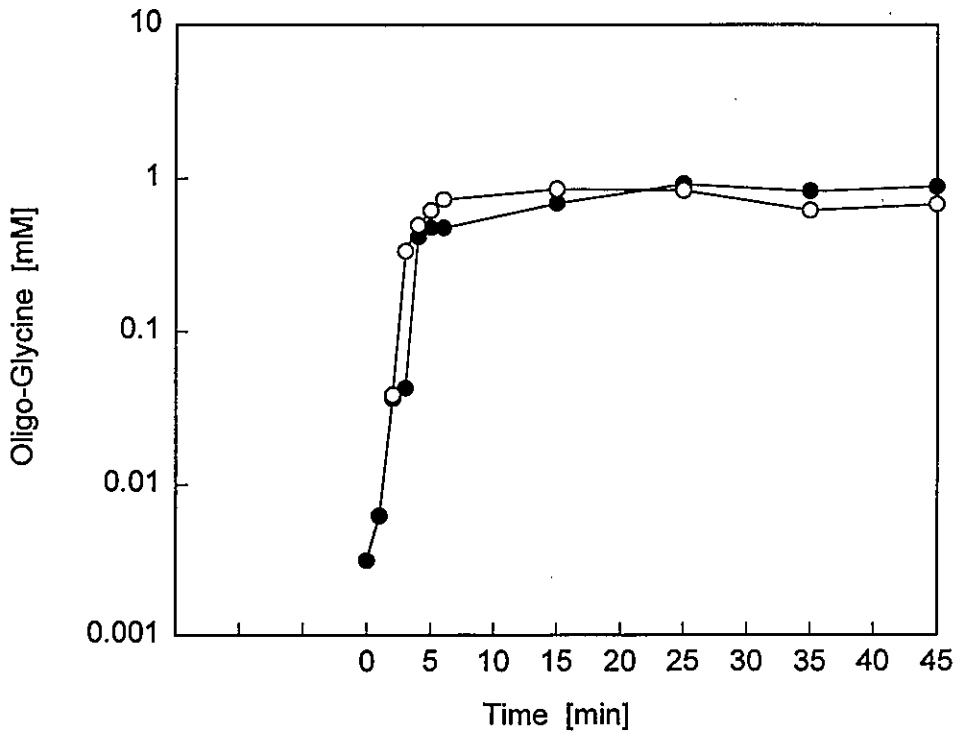

Fig. 1 Time course of the yields of di-glycine (solid circles) and tri-glycine (open circles). The temperature of the high-temperature, high-pressure chamber was set at 250°C.

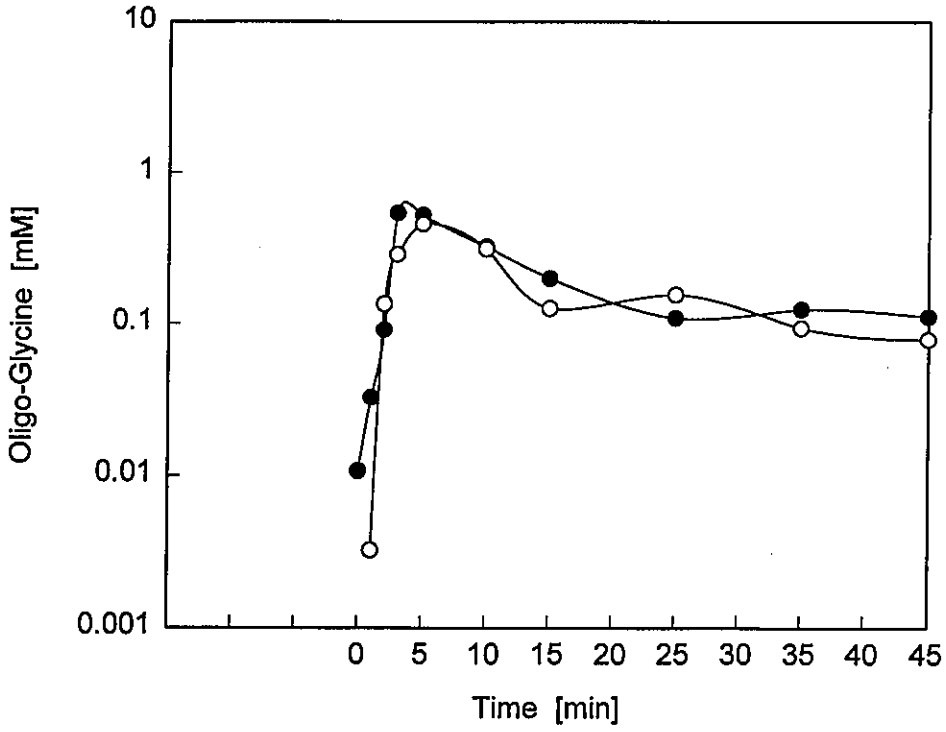

Fig. 2 Time course of the yields of di-glycine (solid circles) and tri-glycine (open circles). The temperature of the high-temperature, high-pressure chamber was set at 300°C.

was confirmed as indicated in Fig. 2. The exponential increase of the product with time, though at least initially, indicates that the production process could be autocatalytic in the sense that the product could enhance further production of the product of a similar kind.

4. DISCUSSION

Prebiotic oligomerization and polymerization could be autocatalytic because of their intrinsic evolutionary capability of selective retention and amplification (Matsuno, 1982; 1989). Furthermore, if there are available monomers sufficiently reactive, an occurrence of autocatalytic polymerization out of these monomers could be quite likely

(Kauffman, 1986; 1993). At issue could be how to prepare such sufficiently reactive monomers without being entrapped by a thermodynamic equilibrium in one way or another. Our present observation of autocatalytic oligomerization out of glycine indicates an instance such that submarine hydrothermal vents could provide various monomers available in their neighborhood with sufficient energy to activate them with the aid of the resulting thermodynamic gradients extending from a hot spring at the center toward cold water in the periphery. The presence of constant thermodynamic gradients prevents reacting monomers available there from approaching their thermodynamic equilibrium. Further functional capability of submarine hydrothermal vents for the sake of prebiotic evolution remains to be seen.

REFERENCES

Corliss, J. B., Dymond, J., Gordon, L. I., Edmond, J. M., von Herzen, R. P., Ballard, R. D., Green, K. K., Williams, D., Bainbridge, A., Crane, K. & van Andel, T. H. Submarine thermal springs on the Galapagos rift. *Science* **203**, 1073-1083 (1979).

Edmond, J. M., Von Damm, K. L., McDuff, R. E. & Measures, C. I. Chemistry of hot springs on the East Pacific rise and their effluent dispersal. *Nature* **297**, 187-191 (1982).

Kändler, O. The early diversification of life. In: *Early Life on Earth* (Bergtson, S. ed.) Nobel Symposium **84**, Columbia University Press, New York, pp. 152-160 (1993).

Kauffman, S. A. Autocatalytic sets of proteins. *J. Theor. Biol.* **119**, 1-24 (1986).

Kauffman, S. A. *The Origin of Order: Self-Organization and Selection in Evolution,* Oxford University Press, New York (1993).

Matsuno, K.. Natural self-organization of polynucleotides and polypeptides in protobiogenesis: appearance of a protohypercycle. *BioSystems* **15**, 1-11 (1982).

Matsuno, K. *Protobiology: Physical Basis of Biology*, CRC Press, Boca Raton Florida (1989).

Matsuno, K. A design principle of a flow reactor simulating prebiotic evolution. *Viva Origino* **25**, 191-204 (1997).

Self-Assembly of Nano-Particles from Thermal Heterocomplex Molecules of Amino Acid

Ken-ichi Tsuchizawa, Shigeru Sakurazawa[¶],
Kuniyuki Hatori, Hajime Honda
and Koichiro Matsuno

Department of BioEngineering
Nagaoka University of Technology
Nagaoka 940-2188, Japan

[¶]National Institute of Bioscience and Human-Technology
Tsukuba 305-0046, Japan

(Received September 30, 1997; Accepted December 4, 1997)

ABSTRACT

Microsphere phase separated from an aqueous suspension of thermal hetero complex molecules of amino acid dissolved into the suspension when the pH value was increased. The dissolved suspension again precipitated further aggregates of those complex molecules when the pH was subsequentry lowered. When thermal heterocomplex molecules from aspartic acid and proline were the case, the particles phase separated from the dissolved suspension were found to have their diameter of order of a hundred nano-meter.

Precipitation of nano-particles expected during the process of increasing and decreasing the pH value of the suspension adds to a candidate of material self-assembly in chemical evolution.

Key words : amino acid, nano particle, self assembly

Viva Origino 25(1997)297−308

アミノ酸熱重合物が形成する ナノスケール分子集合体

土澤 健一, 櫻沢 繁[¶], 羽鳥 晋由, 本多 元, 松野 孝一郎

長岡技術科学大学・生物系
(〒940-2188 長岡市上富岡町 1603-1)

[¶]通商産業省・工業技術院・生命工学工業技術研究所
(〒305-0046 つくば市東 1-1)

1. はじめに

化学進化の過程で生成した低分子有機化合物，特にアミノ酸分子は熱重縮合反応によりアミノ酸熱重合物を生成する。アミノ酸熱重合物が水中で自己集積して形成した構造物としてはマイクロスフィア (1〜6), マリグラヌール, マリソーム (7〜10) などが報告されている。これらは系に含まれる成分の自発的な集合により出現する数ミクロン程度の大きさを有した構造物である。

以前，我々はアスパラギン酸とプロリンから生成するアミノ酸熱重合物からマイクロスフェアを形成し，それを塩基性溶液中に置くことでアミノ酸熱重合物の内部溶出と拡散に伴った殻状マイクロカプセルの出現機構を明らかにした (11, 12)。また，カプセル表面を電子顕微鏡で観察するとさらに微細な粒子が集積していることを確認した。これはアミノ酸熱重合物がマイクロスケールの構造物を形成するのみならずさらに小さな構造物を形成することを示唆している。

今回，我々はアミノ酸熱重合物から構成されるマイクロスフェアがpH変化過程で溶解−析出を行うとき，新たに直径90nm〜300nmの大きさを有する構造物「ナノ粒子」が形成されることを見いだした。本論文ではアミノ酸熱重合物からナノ粒子が形成される過程の詳細を明らかし起源への寄与に言及する。

2. 実験

2-1. 試薬

本研究で使用した試薬は和光純薬の試薬特級，生化学用または精密分析用を用いた。水はガラス蒸留，イオン交換樹脂 (オルガノ G-5A)，純水製造装置 (Millipore Milli-Q SP) を経たものを用いた。

2-2. アミノ酸熱重合物の作成

L-アスパラギン酸 (100mmol) とL-プロリン (100mmol) の混合物をPYREXビー

カーに入れ窒素置換し，イナートオーブン（yamato NDO-450N）を用いて，200℃で3時間加熱反応させた。その後，250mlの水を加えて20分間の煮沸処理を行い氷浴中で冷却した。この操作により析出した沈殿物（微小球）を濾過し，水で洗浄を3回繰り返した。洗浄後の沈殿物をデシケーター中で減圧乾燥し実験試料とした。この試料はクロマトグラフィーにおいて単一ピークを与える分子量約4000の高分子量体であった(11)。以下，このアミノ酸熱集合物をDP1と称する。

2-3. アミノ酸熱重合物の析出条件

DP1を濃度0～20mg/mℓとなるように100，140，200mM KOHに溶解した。この塩基性溶液1mℓと0.1N HCl 1mℓを混合し，析出した沈澱物濃度を測定した。また，DP1塩基性溶液と酸混合後の懸濁液のpHを測定した。pH測定はpHメーター（Horiba pH meter F-12）を用いた。

2-4. アミノ酸熱重合物のpH変化に伴う溶解-析出とpH依存性

DP1を水に分散させて懸濁液（1mg/mℓ）とした。懸濁液80mℓを用意し撹拌しながら2M KOH 5μℓを30秒間隔で入れ一定のpH（pH9.0，10.0，11.0，12.0）まで変化させたときの濁度を測定した。濁度測定には分光光度計（島津製作所 UV-1200）を使用し波長600nmの吸光度を濁度指標とした。各pHに調製された塩基性溶液に対しては2N HCl 5μℓを30秒間隔で入れpH1.0に達するまでの濁度測定を行った。

2-5. ナノ粒子析出と塩の効果

DP1を0，10，100mM KClに分散させて懸濁液（1mg/mℓ）とした。懸濁液80mℓを撹拌しながら2M KOH 5μℓを30秒間隔で入れてpH9.0に調製した。pH9.0に調製した溶液には2N HCl 5μℓをpH1.0に達するまで30秒間隔で入れた。

2-6. ナノ粒子の電子顕微鏡観察

実験2-5で得られた粒子は遠心分離（4000rpm，5min）して上清を除去した。沈澱物の水分を，80%，90%，95%エタノール水溶液（4℃）を用いて段階的にアルコールに置換した。さらに100%エタノール（室温）で10分間静置した後，t-ブチルアルコールに置換しスライドガラス上にのせ20℃で凍結乾燥して沈殿物から完全に水分を除去した。凍結乾燥は恒温槽（Yamato Lo-Temp Chamber IN61）中で行った。

乾燥試料にはイオンスパッタリングデバイス（日本電子株式会社 JFC-1500)を用いて100Åの厚さで金蒸着した。この蒸着試料について走査型電子顕微鏡（日本電子株式会社 JSM-6301F）を用いて観察を行った。

2-7. 粒径測定

電子顕微鏡写真像を，スキャナー（EPSON GT-9000）でコンピューター（Apple Co.，Macintosh Performa 5320）に取り込み，画像解析ソフト NIHImage ver1.62を用いて粒径測定を行った。それぞれの条件で180個の粒子について測定した。

2-8.ゲル濾過クロマトグラフィー,イオン交換クロマトグラフィー

DP1を水に分散させて懸濁液（3mg/mℓ）とした。 懸濁液80mℓを撹拌しながら2M

KOH 5μℓを30秒間隔で入れて各pH (pH9.0, 10.0, 11.0, 12.0) に調製した。各塩基性溶液 (pH9.0, 10.0, 11.0, 12.0) に対して2N HCl 5μℓを30秒間隔で入れpH6.0とpH5.0の2種類の試料を調製した。

pH5.0に調製した塩基性処理済みのDP1を，そのDP1の分子の大きさについてゲル濾過クロマトグラフィー （カラム：生化学工業　GCL-90　φ10×450，溶出液：50mM Succinic acid/NaOH [pH5.0]，流速：0.18mℓ/min，試料量：200μℓ）を用いて分析した。

pH6.0に調製した塩基性処理済みのDP1を，そのDP1の電荷について陰イオン交換クロマトグラフィー（カラム：Pharmacia DEAE Sephacel φ12×120，溶出液：20mM Bis-Tris/HCl [pH6.0]，流速：0.46mℓ/min，試料量：400μℓ）を用いて分析した。試料の溶出にはKClによる塩濃度勾配溶出法を用いた。

2種類のクロマトグラフィー共にフローセルを装着した分光光度計（HITACHI U-1100 spectro photometer）によって波長309.5nmの吸光度 (13) でDP1を検出し，11秒間隔で自動測定した。自動測定の制御とデータ記録はコンピュータ(NEC PC9801) を用いた。

2-9. 赤外吸収スペクトル測定

実験2-8でpH5.0に調製したDP1をデシケーター中で減圧乾燥させた試料を用いて，FT-IR (日本電子株式会社 JIR-7000) によって赤外吸収スペクトルを測定した。測定にはKBr法を用い，積算回数は50回とした。

3. 実験結果と考察

3-1. アミノ酸熱重合物の析出とpH依存性

Fig. 1 にDP1初期濃度とナノ粒子析出量の関係を示した。ナノ粒子析出量はDP1初期濃度に依存した。DP1初期濃度が増加するとナノ粒子析出量も増加する傾向があった。さらに，ナノ粒子析出量はDP1を溶解しているKOH濃度にも依存した。これは，DP1の100mM KOH溶液では初期濃度が4mg/mℓ以上，200mM KOH溶液では初期濃度が7mg/mℓ以上のものよりナノ粒子が析出していることから説明できる。

また，Fig. 2 にはDP1濃度とpHの関係を示した。塩基性溶液中のDP1の濃度が増加するとpHは低くなった。これは，DP1が水中で陰電荷を帯びた高分子電解質として扱われてきた事実と一致する (13)。

ナノ粒子の析出するDP1初期濃度と，そのときの塩基性溶液のpHについて比較すると，ナノ粒子はpH8～pH10程度の塩基性溶液に酸が加えられることにより析出することがわかった。

3-2. pH変化過程での微小球の溶解とナノ粒子形成

DP1から構成された微小球懸濁液は，水中でpH3.5～3.6 （室温） の酸性懸濁液であった。この懸濁液にKOHを加えていくと，Fig. 3 に示したようにpH8付近から微小球の溶解が起きた。

pH9以上で微小球は完全に溶解し，DP1の塩基性溶液となった。この溶液を再び酸性にしていくとpH3.8以下からDP1の集積によりナノ粒子が形成した。ナノ粒子の形成は塩基性溶液のpHに依存しておりpH9.0，pH10.0のDP1溶液からは析出を確認し

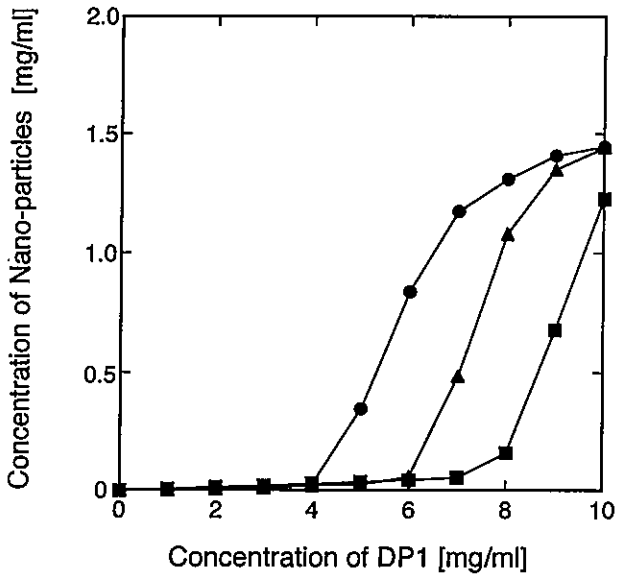

Fig.1 Precipitation of nano-particles. The concentration of nano-particles are plotted as a function of the concentration of DP1. Initial concentration of KOH: 100mM(●),140mM(▲), 200mM(■).

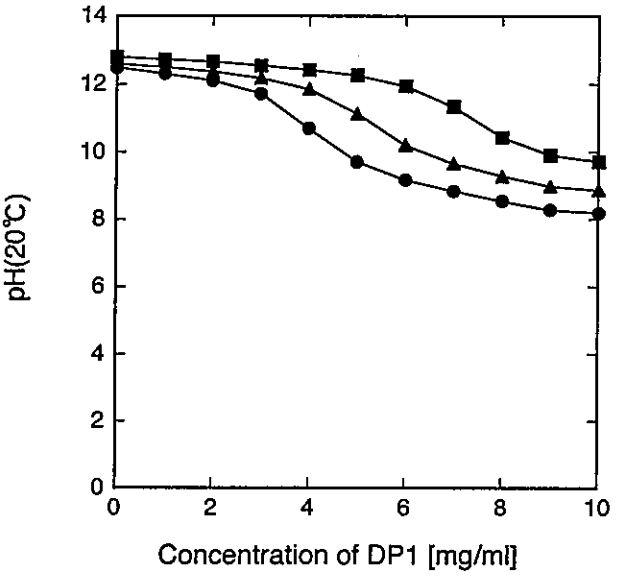

Fig.2 pH of DP1 alkaline solution. Initial concentration of KOH: 100mM(●), 140mM(▲), 200mM(■).

たが，pH11.0，pH12.0のDP1溶液からは析出が認められなかった。これよりDP1の自己集積に関与する分子構造は，pH10からpH11の間で変化していることが判明した。

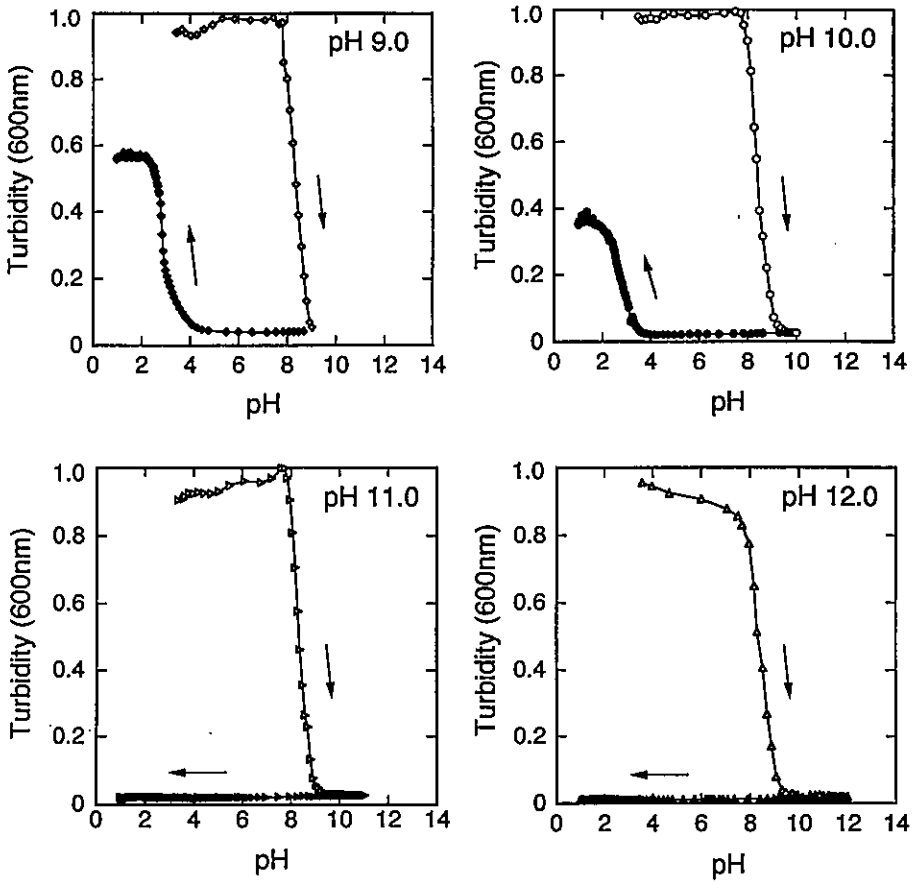

Fig.3 pH-dependent precipitation of nano-particles.
The arrow shows the direction of pH changes.

3-3. 塩溶液中でのナノ粒子形成

Fig. 4 に0～100mM KCl 中でpH変化による微小球の溶解-析出を行って得たナノ粒子の電子顕微鏡写真を示した。pH変化後の溶液は中和塩の生成により3mM KCl溶液となった。

KCl溶液中で調製されたDP1懸濁液にpH変化が起きると，析出するナノ粒子粒径は0mM KClのときは90nmであるが，300mM KClのときは300nmであるように大きくなる傾向が認められた。これは，KCl濃度が高くなると溶解したDP1の溶解度が減少し，集積に関与する部位を伴った分子同士の親和力が強められるためと推測される。

3-4. 塩基処理による分子構造の変化

Fig. 5, Fig. 6 には塩基処理したDP1のゲル濾過クロマトグラフィー，陰イオン交

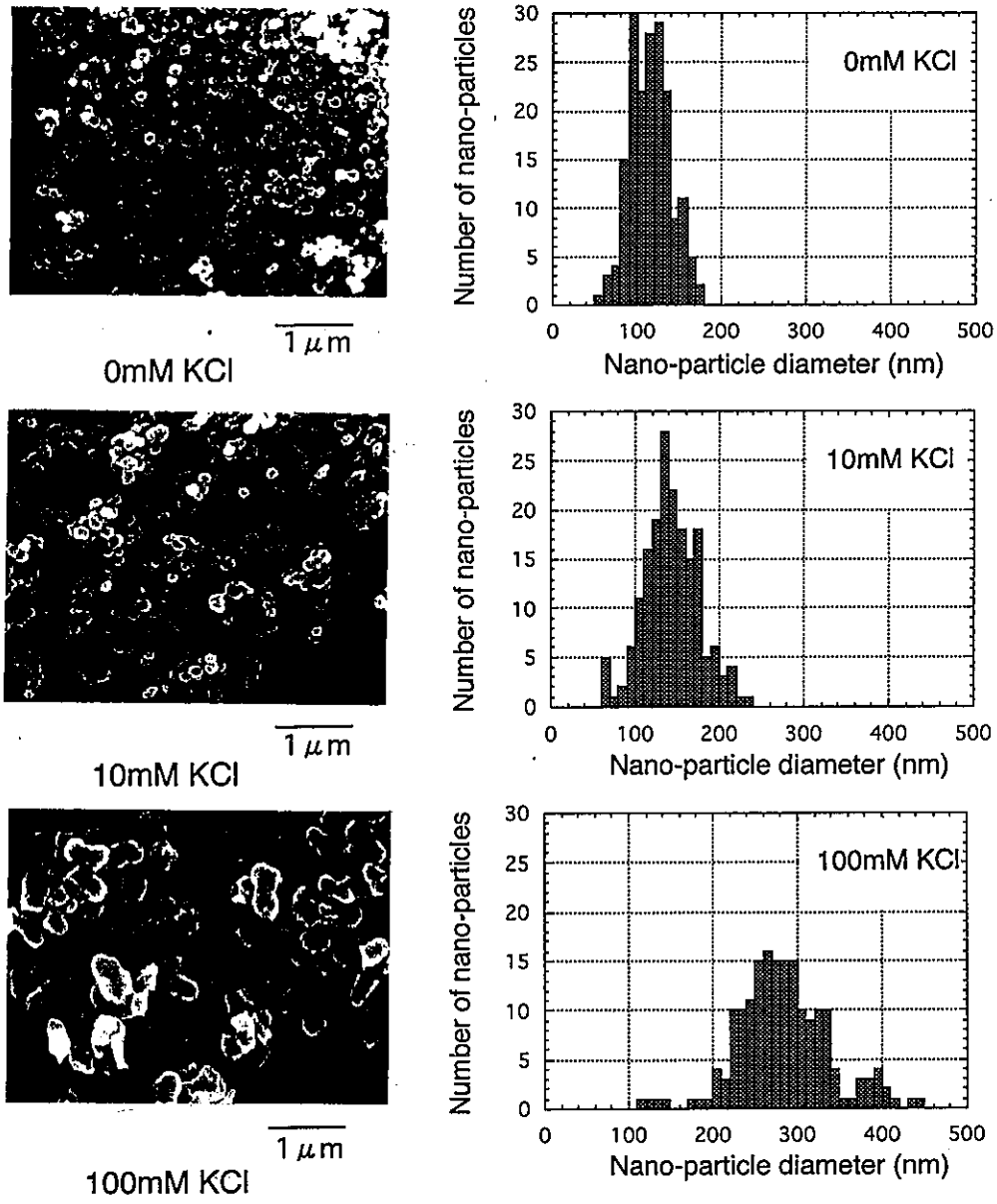

Fig.4 Scanning electron microscope image of dried nano-particles.

換クロマトグラフィー による溶出パターンを示した。

Fig. 5 に示したゲル濾過クロマトグラフィーの溶出時間から，塩基処理においてpH12の変化を経たDP1分子の方がpH9の変化を経たものより大きな分子構造をとりうることが判明した。また，Fig.6に示したイオン交換クロマトグラフィーの溶出時間から，強い塩基処理を受けた分子は陰イオン性を増大していることがわかった。

赤外吸収スペクトルの測定結果からDP1はpH9.0からpH12.0へと強い塩基処理を

受けるに従い，イミド結合に由来するピークが減少しアミドⅡ（3300cm^{-1}, 1730cm^{-1}, 1550cm^{-1}）に由来する吸収が増大することがわかった。以上の結果からアスパラギン酸とプロリンから構成されるDP1分子にはアスパラギン酸無水物の構造が存在し，これが塩基処理による部分加水分解に伴ってカルボキシル基を有した分子へと変化していくことが明らかとなった。2種類のクロマトグラフィーによるDP1分子構造の大きさの変化と陰イオン性の変化は赤外吸収スペクトルの結果と一致した。

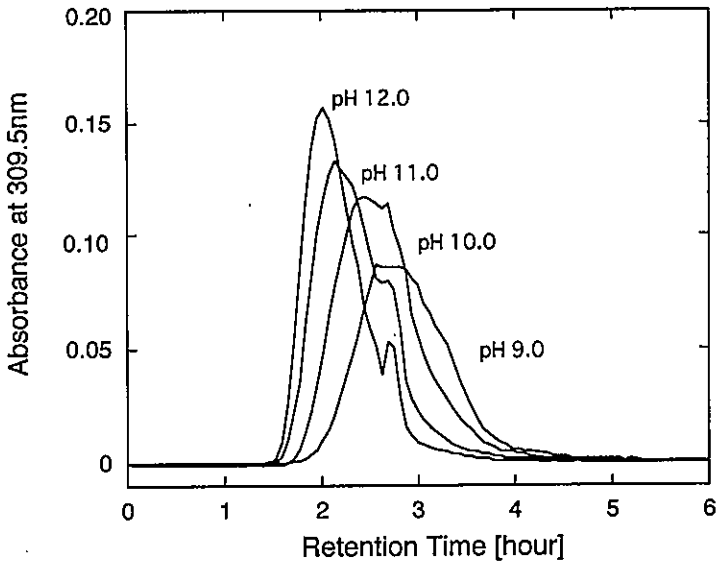

Fig.5 Retention patterns of alkaline-treated DP1 by gel chromatography.

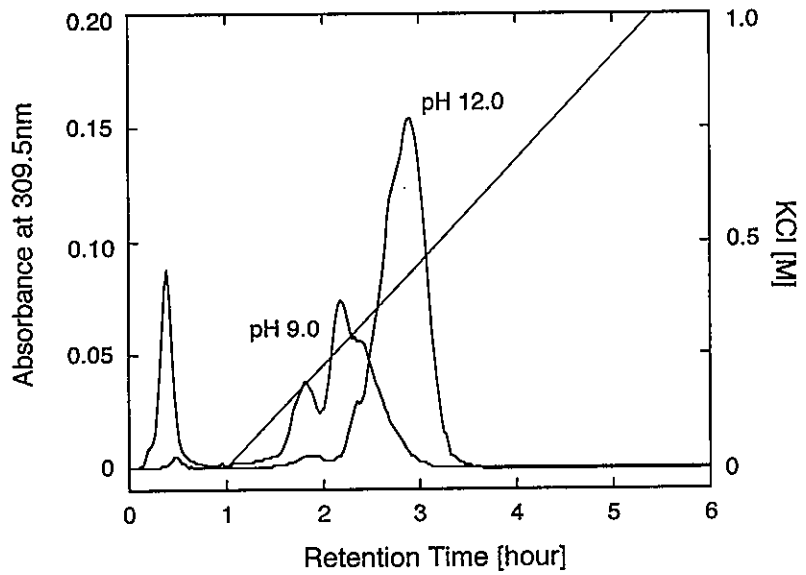

Fig.6 Retention patterns of alkaline-treated DP1 by anion exchange chromatograpy.

4. ナノ粒子の進化過程への意義

我々はアミノ酸熱重合物から形成される構造物として，これまで知られていた数ミクロンの構造物とは大きさが異なるナノ粒子が水中のpH変化から形成されることを明らかにした。巨視的な構造物が確認されるためには構成分子に自己集積的な性質が認められること，さらに自己集積的な性質を伴った構成分子が巨視的な構造物を形成するまでの過程が確かに存在することの二点が必要である。アミノ酸熱重合物の特徴的な性質は，熱溶液や塩基性溶液中で溶解し無秩序状態をとるが，熱溶液の冷却や塩基性溶液の酸性化に伴って再集積することである。さらに，溶液中の電解質はアミノ酸熱重合物の溶解度を減少させる効果があり粒子径を増大させた。

構造物「ナノ粒子」として特徴が現れるのはその大きさである。アミノ酸熱重合物から形成された構造物の機能としては，pH4.5～pH6.5で塩基性アミノ酸を吸着する(14) ことが報告されている。ナノ粒子はマイクロスフェアに比べて大きな吸着面積を有しており，構造物の大きさは物質の選択的吸着量に影響を与える。

水圏で酸性から塩基性のようなpHの変化を伴う過程や塩濃度変遷が海洋生成時に行われた証拠を検証する手段はなく，それは推測に委ねるしかない。ある推測に従えば海洋生成時の水は0.5N程度のHCl水溶液であり，HCl水溶液は地球表面上の玄武岩状岩石と接触することによりNa^+, K^+, Ca^{2+}, Mg^{2+}等の無機イオンを溶出して中和し，さらに生成した粘土鉱物とH^+のイオン交換反応で海水のpHはアルカリ性側に移行した可能性があるとしている (15)。

我々はアミノ酸熱重合物をpH10.0程度の塩基性溶液とし，それをpH3.8以下の酸性溶液にすることでナノ粒子の形成を確認した。Bergeronらはテトラペプチドを含むpH7.7の弱塩基性溶液をpH2.7の酸性溶液に変化させて直径数マイクロメートルの構造物 (16) を確認している。これらの結果はポリアミノ酸がpH変化に依存して自己集積することを示す。

さらに，水中でのポリペプチド生成が期待されるものとして熱水鉱床を模擬した非平衡開放系における環境下でのアミノ酸重合反応 (17) がある。アミノ酸熱重合物からも数十ナノメートルから数ミクロンの大きさを有する構造物が確認されたことから，水中で自己会合性を示すペプチドの連続生成が起こるとすれば，その生成物は温度，圧力，pH，電解質濃度の影響を受けて多様な自己集積を示す可能性が出て来る。

5. 謝辞

本研究を遂行するにあたり，石森　網行氏，今井　栄一氏（長岡技術科学大学）には電子顕微鏡撮影に関して技術的な助力を得ました。深く謝意を表します。

6. 文献

(1) Fox, S. W., Harada, K. & Vegotsky, A. “Thermal Polymerization of Amino Acids and a Theory of Biochemical Origins” *Experientia* **15**, 81-84 (1959)

(2) Fox, S. W. “A New View of the Synthesis of life” *Q. J. Fla. Acad. Sci.* **31**,1-15 (1968)

(3) Fox, S. W. “A Theory of a Macromolecular and Cellular Origins” *Nature* **205**,

328- 340 (1965)

(4) Fox, S. W. "How did Life Begin?" *Sci. Technol.* No.74, 51-61 (1968)

(5) Fox, S. W., Nakashima, T. "Fractionation and Characterization of an Amidated Thermal I : I : I -Proteinoid" *Biochim. Biophys. Acta.* **140**, 155-167 (1967)

(6) Fox, S. W. & Harada, K. "Thermal polycondensation of α-amino acids" *A laboratory Manual of Analytical Methods of Protein Chemistry.* Pergamon Press. 129-151 (1966)

(7) Yanagawa, H., Kobayashi,Y. & Egami, F. "Characterization of Marigranules and Marisomes, Organized Particles with Elastin-like Structure" *J . Biochem.* **85**, 855-869 (1980)

(8) Yanagawa, H. & Egami, F. "Formation of organized particles, Marigranules and Marisomes, from amino acid in a modified sea medium" *BioSystems.* **12**, 147 -154 (1980)

(9) Yanagara, H. & Kojima, K. "Thermophilic Microsphere of Peptide-Like Polymers and Silicates Formed at 250℃" *J. Biochem.* **97**, 1521-1524 (1985)

(10) Yanagawa, H., Kojima, K. & Ogawa, Y. "Recent advances in the study of origin of life" *Viva Origino* **14**, 26-40 (1986)

(11) Sakurazawa, S., Imai, E., Honda, H. & Matsuno, K. "Microcapsule formation in self-assembly of thermal hetero complex molecules from amino acids" *Colloid. Polym. Sci.* **274**, 899-903 (1996)

(12) Sakurazawa, S., Ishimori, T., Honda, H. & Matsuno, K. "Diffusion controlles formation of husk-like microcapsules" *Colloid. Polym. Sci.* **275**, 502-505 (1997)

(13) Honda, H., Maesawa, M., Imai, E. & Matsuno, K. "Molecular Mechanism for the Formation Process of Microspherical Struntures from Heated Amino Acids"*Viva Origino* **22**, 71-80 (1994)

(14) Honda, H., Sakurazawa, S., Kimura, H., Imai, E. & Matsuno, K. "Adsorption of Monomers on Microspherical Structures of Thermal Heterocomplex Molecules from Amino Acids" *Origins of life and Evol. Biosphere* **25**, 443-445 (1995)

(15) 北野 康：原始地球の生成 (2) "海洋の生成と進化" . 化学総説**30** 「物質の進化」, 95-107 (1980)

(16) Bergeron, R. J., Phanstiel, Ⅳ O., Yao, G. W., Milstein, S. & Weimar, W. R. "Macromolecular Self-Assembly of Diketopiperazine Tetrapeptide" *J. Am.*

Chem. Soc. **116**, 8479-8484 (1994)

(17) Matsuno, K. "A Design Principle of a Flow Reactor Simulation Prebiotic Evolution" *Viva Origino* **25**,191-204 (1997)

京都大学国際会議(ICRROEL)
第２３回生命の起原・進化学会 合同会議へご参加の皆さん

当日のスケジュール表とプログラム（仮）をお届けします。
ご覧になって、脱落や参加できない日に出番が当たっている等の問題がありましたらお知らせ下さい。皆様からのアブストラクトの到着後にもう一度チェックしますので、出来るだけご要望に沿うようにします。
特に座長に指名された方（当方で勝手に決めさせて頂きました）は日付のご確認をよろしく。

国内各委員会及び総会の日程は下記の予定です。

お見落としなく、ご出席下さい。

編集委員会： Sunday 1 March (16:00~17:00) at the ROOM CATTLEYA (4th floor).
運営委員会： Monday 2 March (17:00~18:00) at the ROOM CATTLEYA (4th floor).
学会総会： Tuesday 3 March (12:00~13:00) at the Main Hall (2nd floor).

学生アルバイト

は申込者全員が採用されることになりました。
国内旅費は追加が可能かも知れません。大学・研究所等の職員で所属機関からの旅費を使う予定の無い方は至急追加の申請をして下さい。受け付け順にリスト（現在約１０名）に追加していきます。（何処まで可能かは不明ですが）。アルバイト、旅費両方とも採択が決まり次第、改めて湯浅運営委員長から提出書類とお願いが届けられます。

注意！
アブストラクトの締め切りは１月２０日（必着）です。

関西空港からホテルへの無料シャトルバスは

３月１日、１３：００、１５：００、１７：００分頃に出発します。

Conference Schedule

	Sunday 1 March 1998	Monday 2 March 1998	Tuesday 3 March 1998	Wednesday 4 March 1998	Thursday 5 March 1998
9:00 am		Opening Ceremony			
9:30 am		O-1	O-9	O-13	O-21
10:00 am		O-2	O-10	O-14	O-22
10:30 am		Coffee Break	Coffee Break	Coffee Break	Coffee Break
11:00 am		O-3	O-11	O-15	O-23
11:30 am		O-4	O-12	O-16	O-24
12:00 am		Lunch	Lunch	Lunch	Lunch
12:30 pm		Time	Time	Time	Time
13:00 pm	Bus from Kanku	O-5	Excursion to	O-17	O-25
13:30 pm		O-6	Castle Kishi-	O-18	O-26
14:00 pm		O-7	wada, Danjiri-	O-19	O-27
14:30 pm		O-8	kaikan and	O-20	O-28
15:00 pm	Bus from Kanku	Coffee Break	Kuboso-	Coffee Break	Coffee Break
15:30 pm		Poster Presentation	Museum	Poster Presentation	Poster Presentation
16:00 pm		From 2-P-1		From 4-P-1	From 5-P-1
16:30 pm		To 2-P-20		To 4-P-20	To 5-P-20
17:00 pm	Registration	Free		Free	On the bus to
17:30 pm					「Haya」
18:00 pm	Welcome-		Conference-		Farewell-
18:30 pm	Reception		Banquet		Reception
19:00 pm	at the hotel		at the Hotel		at Haya
19:30 pm	Site(Takoyaki		site		(Restaurant in
20:00 pm	Sushi-Party)[a]				Sakai-City)[b]

a) sponsored by Prof. Y. Maeda, the director of KUR b) sponsored by SSOEL-Japan

SCIENTIFIC PROGRAM (Tentative)

The scientific program will consist of Oral Sessions (O) and Poster sessions (P).

Monday, 2 March 1998

9:00~9:30 Opening Ceremony

Remarks: Akaboshi M. (Chairman)
Navarro-Gonzalez R. (Intern'l Executive Committee)
Ferris J. P. (Intern'l Advisory Board)
Harada K. (SSOEL-Japan)

CHEMICAL EVOLUTION (Extraterrestrial)

9:30~10:30 (Chairpersons:Navarro-Gonzalez R. and Saito T.)

O-1. Draganic. I. G.:Radiation chemical approaches to chemical evolution processes on earth and beyond.

O-2. Raulin F.: Radiation prebiotic synthesis in the solar system.

10:30~11:00 Coffee Break

11:00~12:00 (Chairpersons: Noda H. and Kawamura K.)

O-3. Rafaelo Navarro-Gonzalez: Corona and lightning chemistry of the primitive atmosphere.

O-4. Ferris J. P.:Photochemical formation of aerosols on Titan.

CHEMICAL EVOLUTION (Terrestrial)

13:00~14:00 (Chairpersons: Ferris J. P. and Mita H.)

O-5. Brack A.: Delivery of extraterrestrial organics to the primitive Earth: UV processing of amino acids in Earth orbit.

O-6. Collins C. H.: Irradiation promoted production of organic precursor species in inorganic solids on the prebiotic Earth.

14:00~15:00 (Chairpersons: Greenberg J. M. and Matsuno K.)

O-7. Negron-Mendoza A.: Radiation-induced reactions in compounds of biochemical relevance.

O-8. Sofija V.: Biogenic compounds observed in radiation chemical simulation experiments.

15:00~17:00 Coffee Break and Poster Session

2-P-1 Absolute asymmetric synnthesis of metal complex compounds achieved by the first order asymmetric transformation.
Kato T., (Dept. Industrial Chem., Ashikaga Univ.)

2-P-2 A flow reactor for prebiotic synthesis simulating hydrothermal vents.
Imai E., Honda H., Hatori, K. and Matsuno K. (Dept. BioEngin. Nagaoka Univ. of Technol.)

2-P-3 Autocatalytic synthesis of oligopeptides in a flow reactor.
Maejima A., Imai E., Honda H. Hatori K. and Matsuno K. (Dept. BioEngin., Nagaoka Univ. of Technol.)

2-P-4 Formation of amino acids from simulated planetary atmospheres by radiation.
Masuda H[1]., Ushio K.[1], Kaneko T[1]., Kobayashi K[1]. and Saito T[2]. ([1]Yokohama Natl. Univ. and [2]ICRR, Tokyo Univ.)

2-P-5 Degradation of oligopeptides by contact grow discharge electrolysis.
Munegumi T. (Oyama Natl. College of Technol.)

2-P-6 The organic-inorganic interactions as a geochemical origin of life.
Nakajima S.and Yakushiji H.

2-P-7 The polymerization of amino acid on the silica surface.
Yakushiji H. and Nakajima S.

2-P-8 Kinetic analysis of hydrothermal reactions: Monitoring of ATP hydrolysis by flow tube reactor.
Kawamura K., (Dept. Appl. Chem., Osaka Prefect. Univ.)

2-P-9 Hydrolysis of polynucleotides in aqueous solution at elevated temperatures.
Kawamura K., Kameyama N. And Matsumoto O., (Dept. Appl. Chem., Osaka Prefect. Univ.)

2-P-10 α-amino acid formed by N^+ implantation into CH_3COONa.
Wang X.

2-P-11 Radiolysis of carbonates and related organic systems in 3.3-3.1 Ga.
Albarran G.

2-P-12 Quantum mechanical investigations of fullerene, photoactive and organometalic molecules, complexes, supramolecules, supermolecules and design of molecular devices for the electronically genome regulation.
Tamulis. A

2-P-13 Radiation induced stable fullerene anion radical.
Hase H. (Res. Reactor Inst., Kyoto Univ.)

2-P-14 Experimental studies of chemical evolution made by using nuclear disintegration and nuclear radiations.
Akaboshi M., Kawai K., Tanaka Y and Kawamoto K., (Res. Reactor Inst., Kyoto Univ.)

2-P-15 Basiuk V.: Survivability of small biomolecules at high temperatures.

Tuesday, 3 March 1998

9:30~10:30 (Chairpersons: Yuasa S. and Nakajima S.)

O-9. Ramos-Bernal S.: Surface chemical reactions during the irradiation of solid: Prebiotic relevance

O-10. Hiraoka K.: Chemical evolution on the dust grains in the dark clouds: Experimental approach.

10:30~11:00 Coffee Break

11:00~12:00 (Chairpersons: Brack A. and Munegumi T.)

O-11. Zengliang Yu.: Role of low energy ions in the chemical origin of life.

O-12. Harada K.: Versatile chemical reactions of carbon compounds by using water molecule under high energy conditions.

13:00~1800 Excursion to Castle Kishiwada, Danjiri-Kaikan and Kuboso Museum.

Wednesday, 4 March 1998

9:30~10:30 (Chairpersons: Draganic I. G. and Hiraoka K.)

O-13. Miyagawa S, Sawaoka A. and Kobayasi K.: Studies on mechanisms of amino acid formation in plasma by optical emission spectroscopy.

O-14. Lahav N.: The possible role of radiation in biogenesis:A system approach.

10:30~11:00 Coffee Break

11:00~12:00 (Chairpersons: Lahav N. and Nakamura H.)

O-15. Roessler K.: Non-equilibrium processes in prebiotic chemical evolution.

O-16. Ertem. G.: The prebiotic synthesis of RNA oligomers and their uses as template.

RADIATION AND ORIGIN OF CHIRALITY

13:00~14:00 (Chairpersons: Merwiz O. and Hase H.)

O-17. Goldanskii V. I.:Radiation as the advantage factor in the formation of enantiometric excess in prebiotic world.

O-18. Thiemann W.:New concepts on the role of physical parameters inducing homochirality for evolution of biosphere.

14:00~15:00 (Chairpersons: Thiemann W. and Nakagawa K.)

O-19. Wang Wenqing: Radiation in the origin of homochirality

O-20. Greenberg J. M.: Ultraviolet processes leading to prebiotic and chiral organics in interstellar dust.

15:00~17:00 Coffee Break and Poster Session

4-P-1 Independence of active site for D-tryptophan on tryptophanase.
Shimada A., Kouda S. and Nakamura I. (Dept. Chem., Univ. of Tsukuba)

4-P-2 A point of contact between chemistry and biology: The roles of carbohydrate chains in the histry of life.
Hirabayashi J., (Facul. Pharmaceut. Chem., Teikyo Univ.)

4-P-3 Molecular diversity and evolution of the galectin gene family in *C. Elegans.*
Hirabayashi J., (Facul. Pharmaceut. Sci., Teikyo Univ.)

4-P-4 Origin and evolution of endogenous double-stranded RNA in plants.
Koga R. And Fukuhara T. (Facul. Agr., Tokyo Univ., of Agr. Technol.)

4-P-5 Role of terminal base-pair of acceptor stem and CCA sequence of tRNA in aminoacetylation activity.
Hasegawa T[1]and Tamura K[2]. ([1]Facul. Sci. Yamagata Univ. and [2]Inst. Phys. Chem. Res.)

4-P-6 Formation and templete-directed oligomerization of 2'-5' linked RNA.
Sawai H., Totuka S., Yamamoto K. and Ozaki H., (Facul. Eng., Gunnma Univ.

4-P-7 Roles of inorganic phosphorus compounds in life: Molecular recognition and hydrolysis of polyphosphates by natural enzymes.
Yoza N., Facul. Sci. Kyuushu Univ.)

4-P-8 Kinetic analysis of the poly(C) templete-directed synthesis of pligoguanylates in aqueous solution at elevated temperatures.
Umehara M. And Kawamura k., (Dept. Appl. Chem., Osaka Prefect. Univ.)

4-P-9 Molecular evolution of aminoacyl tRNA synthetase and origin of genetic code.
Ishigami M[1]., Ihara T[1]., Shinoda H[2] and Nakano Y[2]. ([1]College Arts and Sci., [2]Dept. Appl. Biol. Chem., Univ. of Osaka Prefecture)

4-P-10 Glutamyl tRNA synthetase of high level halophylic archaebacterium *Haloferax volcanii.*
Shinoda Y[1]., Ihara H[2]., Nakano Y[1] and Ishigami M[2]. ([1]Dept. Appl. Biol. Chem.. and [2]College Arts and Sci., Univ. of Osaka Prefecture)

4-P-13 The origin of ribonuclease P RNA, as viewed from poly-tRNA theory.
Suzuki T., Ohnishi K. and Yanagawa H.:

4-P-14 Origin and molecular evolution of prokaryotic cell-division genes.
Hokari S and Ohnishi, K.

4-P-14 Poly-tRNA-mediated origin of mRNAs and genetic codes.
Ohnishi K and Yanagawa H

Thursday, 5 March 1998

9:30~10:30 (Chairpersons: Goldanskii V. and Harada K.)

O-21. Merwitz, O.: The role of isotope effects in radiation-induced selection processes.

O-22. Nakagawa K., Mochida T., Okamoto T., Saijoh S., Ueji S., Amakawa T. Yamada T. and Onuki H.: Search for asymmetric reaction of amino acids by circularly polarized radiation using a polarizing undulator at the electrotechnical laboratory.

10:30~11:00 Coffee Break

ENERGEITICS FOR CHEMICAL EVOLUTION

11:00~12:00 (Chairpersons: Zengliang Yu. and Mita H.)

O-23. Matsuno K.: Contribution of cosmic ray, radiation, lightning and geothermal heat to prebiotic synthesis on the primitive Earth.

O-24. Saito. T and Kobayashi K.: Energetics for chemical evolution.

BOIOLOGICAL EVOLUTION

13:00~14:00 (Chairpersons: Nagano K. and Ishida M.)

O-25. Nair. C. K. K.: DNA repair and evolutionary conservation of stress response genes in Archaebacteria.

O-26. Yamamoto K.: Induction of macromutations with radiation

14:00~15:00 (Chairpersons: Fukuda I. and Ishimoto M.)

O-27. Miyazaki T. and Watanabe M.: Tunneling reaction in γ-irradiated mammalian cells and their model system at 295 K.

O-28. Shimada A. Activity for D-tryptophan on γ-irradiated tryptophanase.

15:00~17:00 Coffee Break and Poster Session

5-P-1 Accumulation of energy for development in starfish eggs.
Shirai H. And Kuroiwa Y. (Ubhimado Marine Lab., Okayama Univ.,)

5-P-2 Significance of phospholipid bilayer in orogin and evolution of the cells.
Nakamura H. (Konan University)

5-P-3 RAPD analysis of local populations of a mayfly species, *Epeorus ikanonis.*
Kanayama H., Takemon Y., Tanida K., Baek S., Ishigami M. and Kato M.,
(College of Integr. Arts and Sci, Osaka Prefect. Univ.).

5-P-4 Structue and molecular evolution of a satellite DNA isolated from *Sillago japonica.*
Matsunaga, K., Ishigami M. and Kato M. (College of Integr. Arts and Sci, Osaka Prefect.

Univ.).

5-P-5 Sequence polymorphisms of *Sillago japonica* EcoRI family satellite DNA.
Takeda M., Ishigami M. and Kato M. (College of Integr. Arts and Sci, Osaka Prefect. Univ.).

5-P-6 Effect of rare earth elements on morphogenesis of *Dictyostelium discodium* under the presence of various metal ions.
Sumino T., Takada J., Kawamoto K., Tanaka Y., Kawai K. and Akaboshi M., (Res. Reactor Inst. Kyoto Univ.)

5-P-7 Macro-cellular structure of acidothermophilic archaebacterium *Thermoplasma.*
Yamagishi A[1]., Oshima T[1]. and Takahashi G[2]. ([1]Tokyo Univ. of Pharmacy. and [2]Hirosaki Univ.)

5-P-8 Molecular view of microbial diversity and evolution in hot water environments.
Takai K., Nunoura T. and Sako Y. (Facul. Agr., Kyoto Univ.)

5-P-9 Origin, as inverse problem of statistical physics
Iida K. (Fundamental Res. Lab., NEC)

4-P-10 SNS hypothesis on the origu\in of the genetic code
Ikehara K. And Yoshida S, (Facul. Sci, Nara women's Univ.)

4-P-11 A possible.evolutionary pathway of the genetic code deduced from the SNS hypothesis.
Ikehara K. (Facul. Sci., Nara women's Univ.)

5-P-12 Organic compounds of the condensed water in the MIR space station.
Mita H., Shimoyama A., Nakano T. and Nagaoka S. (Dept. Chem., Univ. Tsukuba)

5-P-13 A hypothesis that advent of memberane phospholipid with either enantiometric glycerophosphate backbone caused the divergence of Archaea and Bacteria.
Koga Y., Nishihara M., Kyuragi T. and Sone N. (Dept. Chem., Univ. Occup. Environ. Health)

5-P-14 Fujii N., Momose Y., Ishii N. and Kodama T.: Modification of α A-crystallin obtained from aged and X-ray irradiated lenses.

☆ 学会誌 Viva Origino 投稿規定

Ⅰ．論文の種類

投稿は，以下の区分１～３のいずれかに分類する（Ⅲ－４参照）．

１． Review：解説または総説．

２． Article：オリジナルな研究結果の報告．

３． News and Views：

ａ）研究報告，解説，総説に対するコメント．

ｂ）研究に対するプリンシプル，アイデア，意見．

ｃ）国内外の関係学会報告．

ｄ）教育・研究体制に関する意見．

ｅ）その他．

Ⅱ．論文の体裁

１．使用言語は日本語または英語とする．

２．Review および Aricle については，本文が英文の場合は和文要旨を，また本文が和文の場合は英文の要旨を添える．

３．著者名の下に所属機関の名称・所在地・郵便番号を付記する．

４．引用文献は，引用順に肩つきの通し番号で表示し，本文末尾に引用文献表を付してまとめる．雑誌の省略表示は，Chemical Abstract 等に採用されている標準表示の様式に従う．

５．図表および写真は下記の基準によって準備する．

ａ）図および写真には Fig. 1, Fig. 2 等，また表には Table 1, Table 2 等の通し番号をつける．

ｂ）原図は黒インクで明確に墨入れし，そのまま写真製版できる仕上がりとする．写真はプリントした陽画とする．

６．単位と記号は，国際的に慣用されているものを用いる．単位は CGS (MKS) 系または SI 系を原則とし，両者を混用しない．

７．術語および略語は，IUPAC－IUB の勧告を基準とする．化合物名等で英語表記がよいと判断されるものは，英語表示とする．その他は一般に関係学会誌等で使用されているものにならう．

８．和・英文とも原稿作成にあたっては，それぞれの手引きを参照のこと．

Ⅲ．論文の提出と受理

１．原稿原本のほかにコピー１部を添えて Viva Origino 編集委員会事務局（以下，事務局という）に提出する．

２．投稿受理日は，原稿が事務局に到着した日とする．ただしレフェリーの指摘による訂正などで，再提出がいちじるしく遅れる場合には，再提出日を受理日とすることがある．

３．採否は，事務局が依頼したレフェリーの審査を経て決定する．

４．投稿区分はレフェリーの意見を参照の上，事務局が承諾を得て決定する．

Ⅳ．投稿の資格

１．著者は，生命の起原および進化学会の会員であるか，あるいは会員の紹介を経ることが望ましい．

２．会員以外の著者に原稿を依頼することができる．

Ⅴ．校正

校正は，事務局が形式の統一等に関して校正した後，著者の責任において行う．校正段階での新たな変更等は，技術的な理由から原則として認めない．

Ⅵ．掲載経費の負担

製版・トレース等，別途の費用が必要な場合の実費は，著者が負担する．

Ⅶ．別刷

著者は，校正に同封した申込用紙により別刷を有料で申込むことができる．

☆ 写真製版英文原稿作成の手引き

英文原稿は原寸大の写真製版（和文要旨を除く）とするので，以下の規定による．

１．タイプの文字は elite 12ピッチ，シングル・スペースとし，鮮明に印字する．

２．厚手のタイプ用紙を用い，横14cm×縦21cmの枠内に収める．

３．第１ページに表題，著者名，所属機関等を，この順序に記す．

ア）表題は大文字とし，９行目から始める．

イ）表題のあと，４行あけて著者名を記す．

ウ）著者名のあと，１行あけて著者の所属と所在地（郵便番号付記）を英文で記す．

エ）所在地のあと，４行あけて ABSTRACT を記す．

オ）１行あけて KEY WORDS (10語以内) を記す．

４．原寸大の図表は所定の位置に貼る．縮尺を要する図表は別紙に記し，本文には相当する空白を設け，空白中央に図表番号を鉛筆で指示する．

５．見出しは，区切りの大きいものから順に下記ア)～ウ)の通りとする．各見出しのゴチ指定，改行等については，既刊の実例にならう．

ア）ORIGIN OF LIFE ・・・のごとく，全部大文字

とし，左端から記す．見出しの上を2行あけ，下を1行あける．

イ）Origin of life ・・・のごとく，最初の1文字のみ大文字とする．見出しの上を1行あけ，下を1行あける．

ウ）文節の最初に記し，文頭を下げない（インデントなし）．Origin of life. のごとくアンダーラインを引き，ピリオドを打ち，行を変えずに文章を続ける．

6．各ページとも，タイプ枠外の右上隅に第一著者名とページを鉛筆で記す．この記入は整理のためであり，印刷されない．

7．別に和文要旨を添える．要旨冒頭に和文表題，著者名，所属機関，その所在地（郵便番号を付記）を，この順序に記す．要旨本文の長さはなるべく400字以内とする．

☆ 写真製版和文原稿作成の手引き

和文原稿も英文原稿同様直接写真製版が可能な原稿をワープロを用いて作成することが望ましい．

1．文字は24ドット以上の明朝体とする．

2．厚手の用紙を用い，横17cm×縦25cmの枠内に1行40文字，40行を印字する．すべての文字および，図，表，写真は80%に縮小されて印刷される．

3．第1ページに，表題，著者名，所属機関とその所在地（郵便番号付記）をこの順に記す．

ア）表題は4倍角文字とし，4行目から始める．文字の大きさが変えられない場合はそのまま（全角）の文字を使う．

イ）表題のあと，4行あけて，著者名を記す．

ウ）著者名のあと1行あけて，著者名の，所属とその所在地（郵便番号付記）を記す．

エ）所在地のあと，4行あけて，本文を記す．

4．見出しは，区切りの大きいものから順に下記ア）～ウ）の通りとする．

ア）1，2，3，・・・

イ）1－1，1－2，・・・，2－1，2－2，・・・

ウ）a），b），c），・・・

各見出しのゴチ指定，改行等は既刊の実例にならう．

5．図，表，写真は所定の位置に貼る．図，表，写真の番号，表題，説明は和文原稿の場合にも英文で記すことが望ましく，そのまま写真製版出来るよう図，写真の下，表の上および下に記す．

6．和文原稿の場合には英文要旨をつける．

英文要旨冒頭には，表題，著者名，所属機関，その所在地（郵便番号付記）をこの順で記す．続いて，4行あけた後，ABSTRACT, KEY WORDS （10語以内）を記す．

7．英文要旨は英文原稿作成の手引きを参考にして記す．

8．英文要旨は表題から KEY WORDS まで含めて1頁以内に納める．

☆ 和文原稿作成の手引き

1．原稿は400字詰め原稿用紙に横書きで記す．ワープロを使用の場合は，25字×16行とする．

2．第1ページに和文表題，著者名，所属機関，その所在地（郵便番号を付記）を，この順序に記す．第2ページには英文要旨を記す．第3ページ以下に本文を記す．

3．見出しは，区切りの大きいものから順に下記ア）～ウ）の通りとする．各見出しのゴチ指定，改行等は，既刊の実例にならう．

ア）1，2，3，・・・

イ）1－1，1－2，・・・，2－1，2－1，・・・

ウ）a），b），c），・・・

4．図，写真および表は別紙とし，原稿中にはそれぞれの挿入箇所を指定する．

5．和文原稿の場合にも，図，写真および表の表題および説明文は英文で記すことが望ましい．

6．英文要旨冒頭には，表題，著者名，所属機関，その所在地（郵便番号を付記）を，この順序で記す．

7．英文要旨の後に KEY WORDS （10語以内）を記す．（日本語でのキーワードは不必要．）

生命の起原および進化学会

学会本部事務局　〒560　豊中市待兼山町1－16
大阪大学大学院理学研究科生物科学専攻内
Tel: 06-850-5823(call-in), Fax: 06-850-5817
E-mail: syuasa@bio.sci.osaka-u.ac.jp
責任者　湯浅　精二

経理部事務局　〒940-21　長岡市上富岡町
長岡技術科学大学生物系
Tel: 0258-46-6000(Ex.4518), Fax: 0258-46-8163
E-mail: kmatsuno@voscc.nagaokaut.ac.jp
責任者　松野孝一郎

編集事務局　〒590-04　大阪府泉南郡熊取町野田1010
京都大学原子炉実験所
Tel: 0724-51-2491, Fax: 0724-51-2630
E-mail: akaboshi@HL.rri.kyoto-u.ac.jp
責任者　赤星　光彦

Viva Origino　25巻4号

1997年12月20日　印刷
1997年12月25日　発行

編集者　〒590　大阪府泉南郡熊取町野田1010　京都大学原子炉実験所内
生命の起原および進化学会編集部
印刷者　〒594　大阪府和泉市池上町460－33
和泉出版印刷㈱　TEL 0725-45-2360　FAX 0725-45-6398
発行者及び出版者　〒560　豊中市待兼山町1-16　大阪大学大学院理学研究科生物科学専攻内
生命の起原および進化学会事務局
責任者　湯浅　精二

Contents

ARTICLES

NEWS and VIEWS