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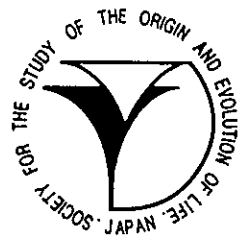

The Society for the Study of the Origin
and Evolution of Life
JAPAN

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。

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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

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Viva Origino

VOL.27 (No.4)

December 1999

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

目　　次

Catalytic Hydrolysis of *p*-Nitrophenyl Phosphate by Rare Earth Elements

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(Received April 13, 1999; Accepted May 30, 1999)

Catalytic action of rare earth elements (REEs) to hydrolyze phosphomonoester bonds was confirmed. Namely, among the all elements examined, the activity of Ce(IV) was the most highest, and the activities of Sm and Ho followed Ce(IV). Under the ordinary condition (37 ℃-1hr), Ce(IV) could hydrolyze 0.28 mmole of *p*-NPP per 1 mmole Ce(IV). The magnitude of this activity was about 1/70000 that of standard alkaline phosphatase. Moreover, it was found also that the activity did not change at different pH, while that of the enzyme was completely missing at pH 5.4. The kinetic analysis of this reaction showed that the km value of Ce(IV) (0.405 mM) was close to that of enzyme (0.630 mM), and that the reaction rate (0.28 umole/umol.hr^{-1}) was retained until at least 16 hr when total hydrolyzed *p*-NPP reached more than 2.5 umole/umol Ce(IV). From these results we concluded that the reaction is catalyzed by Ce(IV).

Key words: Rare earth element (REE), Ce(IV), Phosphomonoester compounds, Hydrolysis, Primitive sea

Viva Origino 27 (1999) 189－196

希土類元素のリン酸エステル切断作用

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1. はじめに

われわれは希土類元素がその呼称に反して地球上での存在量も高く、海水中においても江上博士の唱える生物学的臨界濃度 [1] に近い濃度で溶存していること、また、これら元素がもつ特異的な物理化学的性質に着眼し、希土類元素と生物との相互作用の有無[2,3]や生命の起源・化学進化との関連[4,5]を明らかにするための実験的研究に従事してきた。また一方、われわれは酵素活性等の生物的活性を持たない蛋白質、例えばポリグリシンやゼラチンに放射線を照射することによりアルカリフォスファターゼ類似活性が誘導されることに興味を持ち、誘導の機構やこの活性をさらに高める物質を検索する過程で、希土類元素それ自身が強いフォスファターゼ類似活性を持つことを見出した。希土類元素がDNA、RNA[5] および 3-,5-cyclic AMP[6]に対してリン酸エステル切断作用を持つことはすでに小宮山らによっても報告されている。彼らは希土類元素がそれぞれによって特異性が異なることを利用して、核酸化合物類に対する制限酵素として作用させる可能性について検討を進めており、遺伝子操作技術の面からも着目されているところである。われわれが見た酵素類似活性は上記とは異なり、パラニトロフェニルリン酸(*p*-NPP)を基質として作用する末端に存在するリン酸エステルに対する切断作用であり、この現象は化学進化の段階におけるリン酸エステル化合物の無生物的合成とその蓄積および核酸等の合成に関連して非常に興味深い現象と考えられる。そこでわれわれは「化学進化及び生命の起源」の観点から、希土類元素のリン酸エステル切断作用をさらに深く追求することにした。

本報ではこの切断作用が希土類元素に特有のものかどうか、また希土類元素の中でもどの元素が最も活性が高いのか等を調べるとともに切断作用のカイネティックスをも調べ、この現象の「生命の起源・化学進化」における意義を明らかにしたいと考える。

2. 実験方法

2-1 薬品

Kirkegaard&Perry Laboratories Inc. から市販されているアルカリフォスファターゼ活性測定用キットを用いた。 その他の希土類元素を主とする試薬類としてはSigma 社または和光純薬社より購入した reagent 級の硫酸塩または塩化物を用いた。

1-2 リン酸エステル切断活性の測定

反応は 4 M のジエチルアミン緩衝液中に 5mg の *p*-NPP を含む基質溶液 5 ml に 0.2 M に調整した各種 REE 溶液 10 μl を加え、通常 37 ℃において 1 時間培養を行い、*p*-NPP の加水分解によって生じたパラニトロフェニルによる 410 nm の吸光度を切断に対する指標として用いた。

3. 結果

1) 各希土類元素のリン酸エステル切断作用

図 1 に Sc と Y を含む 17 種類の希土類元素の 0℃, 37℃および 80℃におけるリン酸エステル切断作用を示す。希土類元素の中では各温度条件において Ce(IV) が最も切断活性が高く、Sm, Ho がそれに続く。37℃における Ce(IV) によるリン酸エステル切断の反応速度は 0.28 μmol/μmol・hr^{-1} となり、アルカ

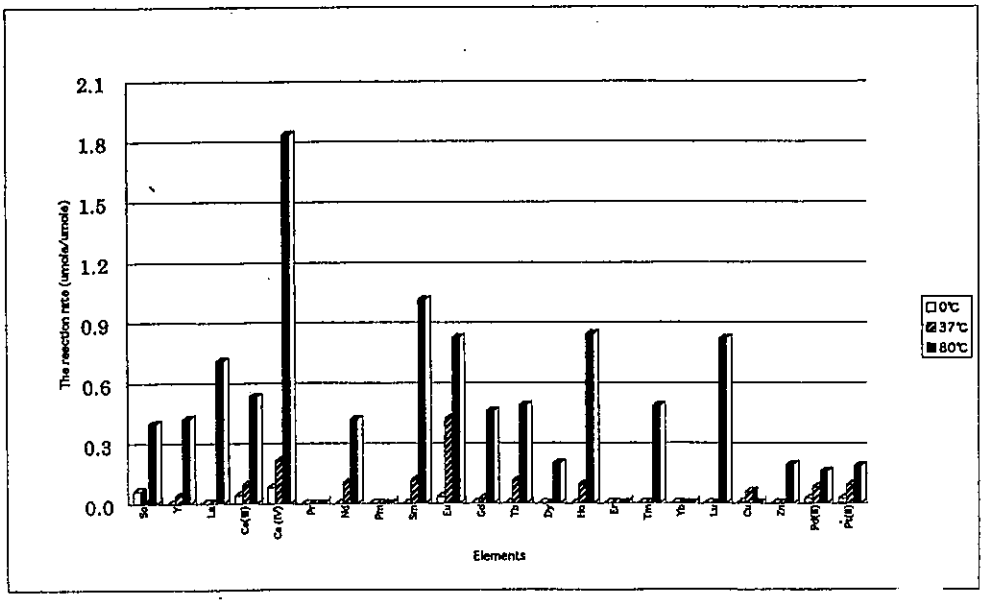

Fig. 1 Dephosphorylating activity of rare earth and other elements.

リフォスファターゼの 1.82×10^4 μmol/μmol・hr^{-1} と比べると約 1/70000 の速さであった。Pt や Zn 等の金属元素類でも切断活性は認められるが、希土類元素のそれに比べると弱く、37℃ 以下の低温条件では殆ど観測されない。

2) 切断活性のカイネテイックス

希土類元素によるリン酸エステル切断作用が通常の化学反応なのか触媒作用なのかを明らかにするために，反応の基質濃度依存性と反応の時間経過（図 2 および図 3）を調べた。

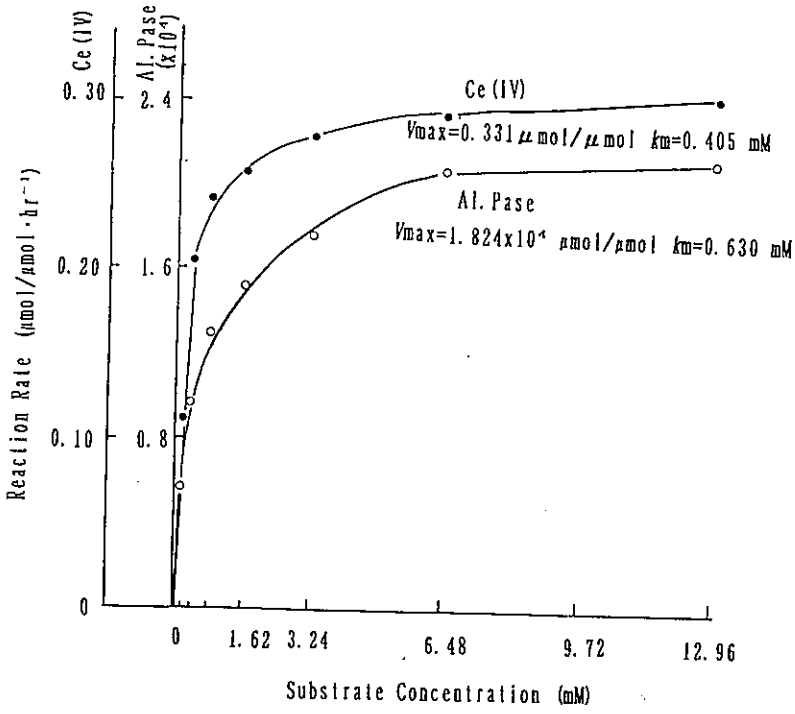

Fig. 2 Vmax and km of Ce(IV) to hydrolyze phosphmonoester bonds.

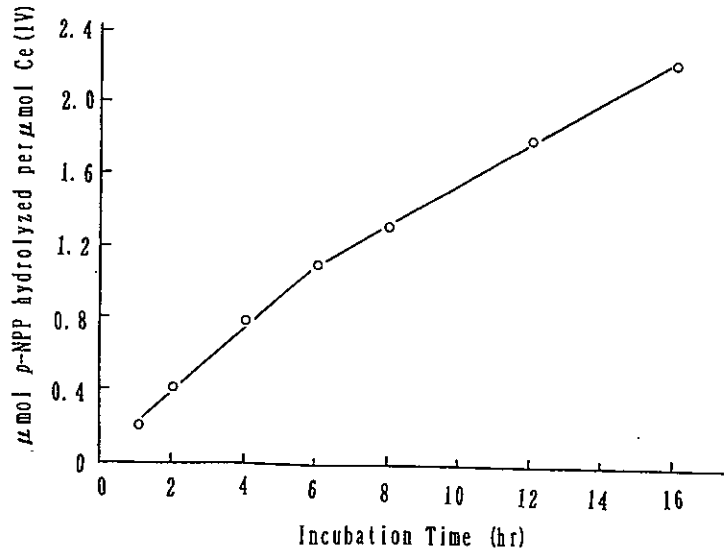

Fig. 3 Time course for the dephospholyrating activity of Ce(IV).

図2より、Ce(IV)による切断作用のVmaxとKmは0.331 μmol/μmol・hr^{-1}および0.405 mMとなり、一方アルカリフォスファターゼによるそれらは$1.824x10^4$ μmol/μmol・hr^{-1} (Vmax)および0.630 mM (Km)となった。この様にKm値は両者でよく近似することが示され、また一方、反応の時間経過も少なくとも6時間までは0.28 μmol/μmol・hr^{-1}の反応速度が保たれ、16時間後には総切断モル数も2.4 μmol/μmolを越えるまで反応が進んだところから、この反応は触媒的に進行するものと判断された（図3）。

3) 切断活性のpH依存性

図4は反応速度に及ぼすpHの影響を示す。5.4から11.0迄のpH変化に対して酵素の場合には著しい依存性が観測されたが、Ce(IV)ではさほど著しい活性の低下は見られない。

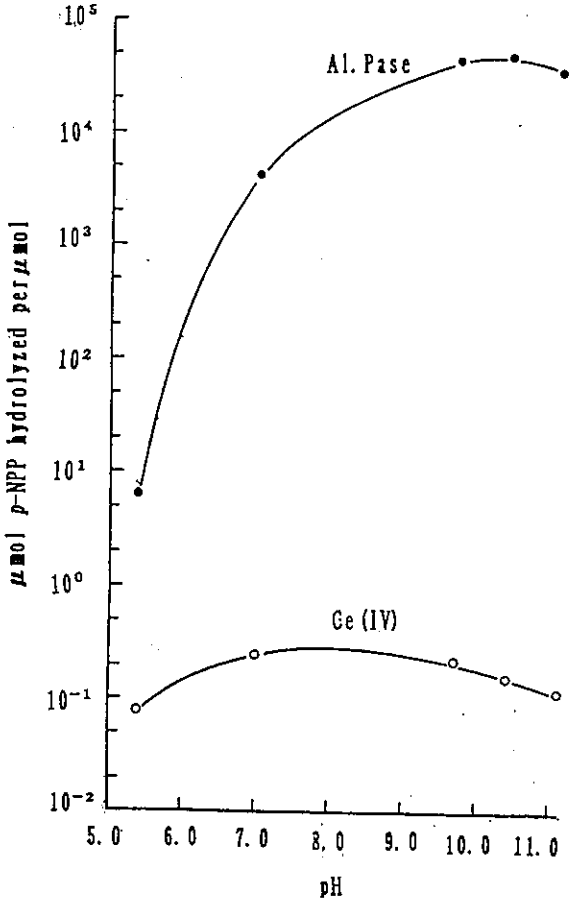

Fig. 4. pH-dependency of the hydrolyzing activity (μmol/μmol・hr^{-1}) by Ce(IV) and alkaline phosphatase.

討論

希土類元素による強い末端リン酸エステル切断作用が確かめられた。この作用は触媒的に進行し、温度に対して指数関数的に高くなる。希土類元素は原始海洋においても現在の海洋中におけると同様大量に存在したと考えられるから、化学進化の段階において各種のリン酸モノエステル化合物の生成に、従って核酸類の無生物的合成に対して抑制的に作用したに違いない。しかしながら、今日、生物は地球上に存在し、それは化学進化と生物進化を経てここまでに到達したものであることは疑いもない。このことから、原始地球上にあっては希土類元素のリン酸エステル切断作用を阻害する物理的または化学的機構があったと考えざるを得ない。人工海水中や粘土質（モンモリロナイト）共存下で同様な反応を試みたが、いずれの条件においても、僅かに（3～5%）反応の促進が観測されこそすれ、抑制は観察されなかった。また、アミノ酸や塩基類、ヌクレオシドオ類、でんぷんの共存下では何らの促進も抑制もみられなかった。しかしながら、アルブミンやゼラチン等の蛋白質共存下では顕著な抑制が観測されたので、現在、その詳細な機構に関する研究に着手しつつある。

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熱水環境での二酸化炭素の固定
- 蟻酸、酢酸の生成 -

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要旨

熱水環境で二酸化炭素の固定を試みた。高温部と低温部を伴う循環型フローリアクターを建設し、そこで二酸化炭素と水の混合物を循環させた。高温部に酸化銅と酸化亜鉛を配置し、この混合物をそこで接触させたとき、蟻酸と酢酸の生成が認められた。最大収量は高温部の温度が約1000°Cのときに得られた。ただし、圧力は標準大気圧のままであった。この実験事実は、化学進化を通して糖、脂質が出現するとしたとき、それらの構成分子となる蟻酸が原始海洋での熱水噴出孔近傍で生成され得ることを示唆する。

Viva Origino 27 (1999) 197－208

Fixation of Carbon Dioxide in Hydrothermal Environments: Synthesis of Formic and Acetic Acids

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ABSTRACT

Fixation of carbon dioxide in a simulated hydrothermal environment was attempted, in which the mixture of carbon dioxide and water was repeatedly circulated in the closed circuit containing both hot and cold regions. When the mixture was made contact to the surface of metal oxides such as copper/zinc oxides maintained at high temperatures somewhere in the circuit, a considerable amount of both formic and acetic acids was made. The best yields were obtained when the temperature of the surface of the metal oxides was maintained at around 1000°C while under the condition of normal atmospheric pressure. This observation suggests the possibility that submarine hydrothermal vents in the ocean on the primitive earth may have functioned as a source of providing formic acid as a basic building block of both sugars and lipids prebiotically.

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1. INTRODUCTION

Prebiotic synthesis of formaldehyde must have been a major evolutionary step towards making the larger organic molecules including sugars and lipids prebiotically (McCollom et al., 1999). Although electric discharge in the primitive

atmosphere containing water and carbon dioxide molecules must certainly have assisted in synthesizing those carbonylic acids (Schlesinger & Miller, 1983), their evolutionary capability could further be enhanced if there has already been available an organization to take advantage of. One such ready-made organization could have been submarine hydrothermal vents (Corliss et al, 1979; Edmond et al, 1982; Shock, 1996). The presence of submarine hydrothermal vents in the primitive ocean could have provided further evolutionary opportunity of making oligomers from formaldehyde if the latter was also available in the neighborhood (Ingmanson, 1997). It thus becomes of interest to see whether formaldehyde and its derivatives can be synthesized in the vicinity of those vents from the more basic building blocks of water and carbon dioxide molecules (Ferris, 1992; Schulte & Shock, 1993). In particular, carbon dioxide molecules were thought to abundantly enter into the primitive ocean through the hot vents from magmas smeared out of the mantle core.

Submarine hydrothermal vents could definitely have provided those environments that may help oligomerize monomers if available in their neighborhood (Matsuno, 1997; Imai et al, 1999a, b). Of particular interest from the perspective of prebiotic evolution would be abiotic synthesis of protocells or micells from lipids. Unless protective protocells were available, the likelihood of further evolution of oligopeptides and oligonucleotides, if ever appeared spontaneously, could extremely be limited. In fact, once formaldehyde becomes available, the Fischer-Tropsch type reaction can help synthesize lipids in some hydrothermal environments (McCollom et al, 1999). Furthermore, if the concentration of lipids thus synthesized is high enough in their aqueous solution, micells could quite easily be formed through their hydrophobic and hydrophilic interactions. We shall thus examine a possibility of synthesizing formaldehyde from carbon dioxide and water molecules in hydrothermal environments.

2. MATERIALS AND METHODS

We constructed a flow reactor simulating an extremely hot region of a hydrothermal vent in which the gas mixture of carbon dioxide and water molecules come to contact with hot surfaces of metal oxides. Serpentinization of olivine at hot temperatures is a possibility in the actual geological situation to be simulated (Berndt et al, 1996). A rough sketch of our flow reactor is depicted in Fig. 1. Carbon dioxide was supplied at the rate of 400 ml/min from its pressurized gas cylinder, and pure water was circulated at the rate of 1 ml/min. Both were mixed in the circuit made of a quartz tube whose inner diameter was 6.0mm. The mixture of carbon dioxide and water was heated with use of an electric furnace. The highest temperature attainable was 1100°C. The mixture of copper and zinc oxides, each 1g in its weight, was placed on the inner wall of the tube located inside the furnace. The outgoing flow from the furnace was fed into a 200 ml flask containing 100 ml pure water maintained at 0°C in temperature. Exhaustion of gaseous carbon dioxide was

expelled from the flask. The aqueous solution of the products was repeatedly circulated in the closed circuit with use of a peristaltic pump, and its small portion was sampled at every fixed interval for identification of the products.

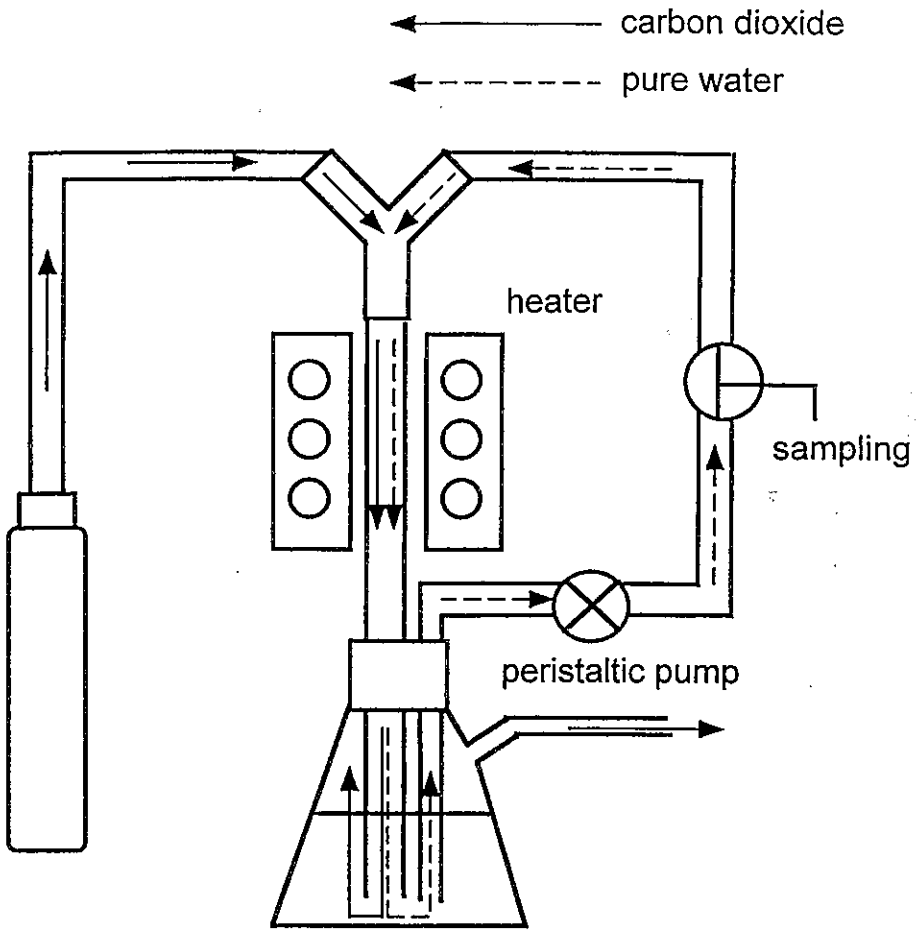

Fig. 1: A schematic representation of a flow reactor simulating a hydrothermal environment.

3. RESULTS AND DISCUSSION

3.1 IR Spectrum

We sampled a specimen when the furnace reached 1000°C, in which the temperature was increased linearly in time at the rate of 33.3°C/min. The specimen of its volume 1ml was absorbed in 0.5 M sodium carbonate 100ml, and it was then dried in a desiccator. The dried sample 0.001g mixed with KBr 0.099g was used for its IR absorption spectrum. The result is shown in Fig. 2, indicating the presence of O-H and C=O bonding. These absorptions are characteristic to carbonylic acids.

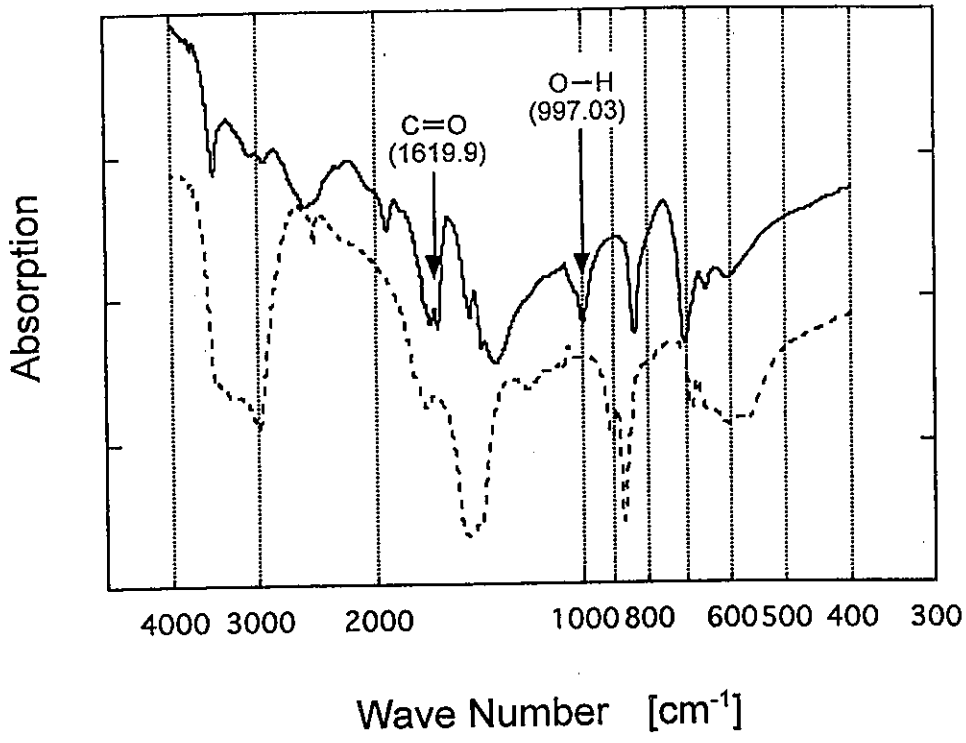

Fig. 2: An IR spectrum of the products (solid line), in which the background spectrum (broken line) is also presented.

3.2 NMR Spectrum

In order to identify the products more precisely, we measured the NMR spectrum. The dried sample prepared by following the method depicted in the previous section was dissolved in 10 μ g/ml DSS in D_2O 1ml. This solution sample was used for

identification of its ^1H NMR spectrum. The sample obtained when the furnace temperature reached 1000°C as raising it at the rate of 33.3°C was examined, and the measured result is shown in Fig. 3. Standards of formic acid, water, ethanol and acetic acid were also displayed. Their comparison clearly demonstrates that both formic and acetic acids were formed in the reactor. Those products prior to reaching 1000°C were also identified as demonstrated in Fig. 4, in which the quantities were measured in terms of their concentrations in the sampled specimen. The synthesis of both formic and acetic acids was identified at the furnace temperature above roughly 800°C.

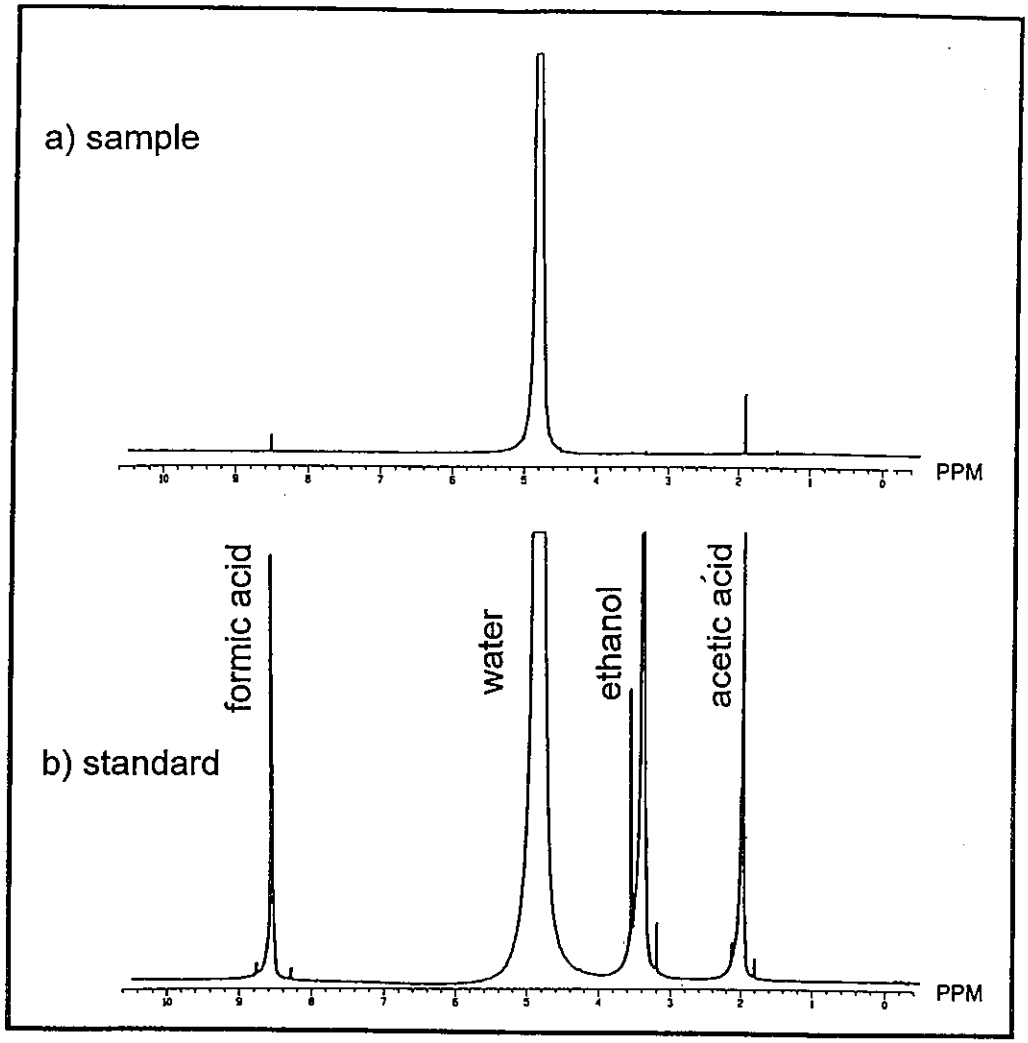

Fig. 3: ^1H NMR spectrum of the products (sample), in which standards of formic acid, water, ethanol and acetic acid are also demonstrated.

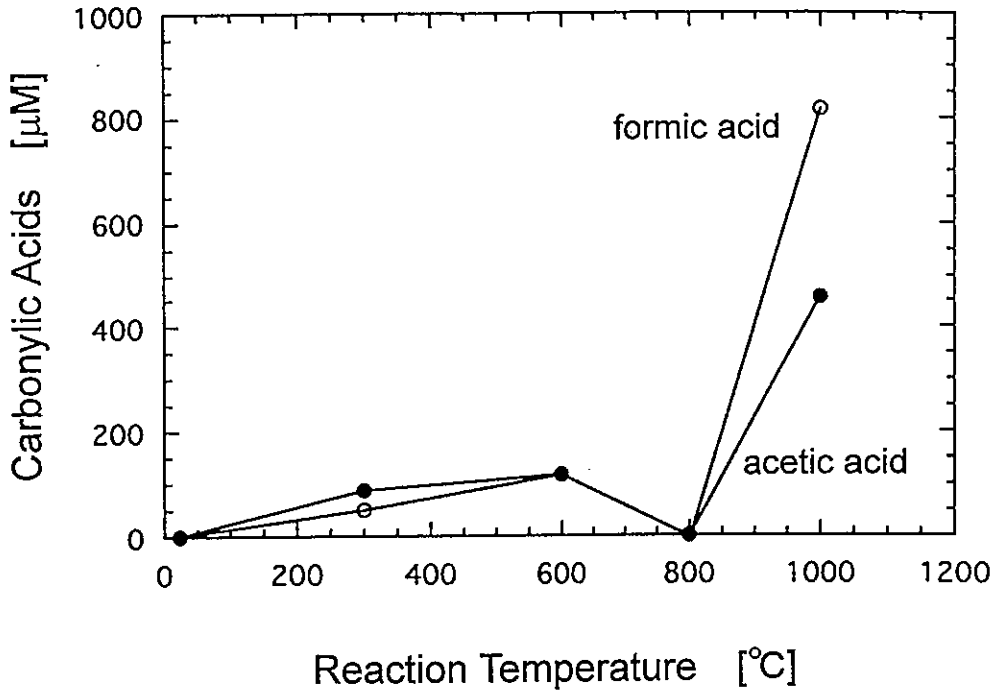

Fig. 4 Temperature dependence of the products, in which the temperature on the surface of metal oxides placed on the inner wall of the quartz tube was referred to.

In order to see the temperature dependence of the products, we then started the circulation of the mixture of both carbon dioxide and water after the furnace temperature reached the designated one. When the furnace temperature was maintained at 1100°C, no identifiable amount of the products was detected. The best yields were obtained when the temperature was at about 1000°C.

The time course of the products at the constant furnace temperature 1000°C is presented in Fig. 5, while the temperature during the first 30 minutes was increased linearly at the rate of 33.3°C/min. The products increased during the first 120 minutes, while they decreased or were decomposed after that.

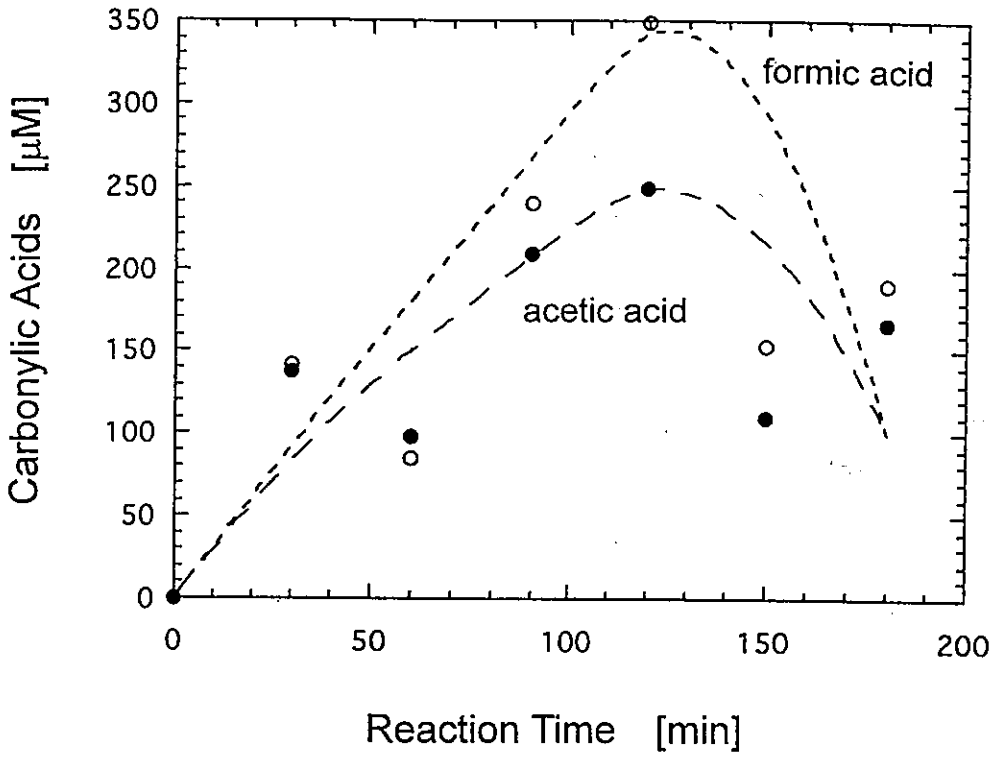

Fig. 5: Time development of the products, in which the temperature of the surface of metal oxides in the furnace was raised from room temperature at the rate of 33.3°C/min during the first 30 minutes. Once it reached 1000°C, the temperature was maintained at that value since then.

These results, when combined together, demonstrate that formic and acetic acids were synthesized from carbon dioxide and water molecules when they came to contact with hot surfaces of metal oxides. Although the best yields of carbonylic acids were obtained when the temperature of the hot surfaces of metal oxides was set around 1000°C, the pressure was maintained at normal atmospheric conditions. If the pressure is increased as in the situation of the actual submarine hydrothermal vents near magmas, the likelihood of having the lower temperature for the best yields of carbonylic acids from carbon dioxide and water may be expected.

4. CONCLUDING REMARKS

The present experimental demonstration of synthesizing carbonylic acids from carbon dioxide and water molecules in hydrothermal environments comes to provide us with a unitary perspective towards chemical evolution. Although synthesis of small organic molecules can be ubiquitous even on the cosmological scale, the transition from prebiotic to biotic evolution has been quite limited, with the only known example having proceeded on the planet Earth so far. This may imply that the transition could significantly have been enhanced if some organization to rely upon was also available in addition to ubiquitous small organic molecules. One such candidate might have been submarine hydrothermal vents and their vicinities in the ocean on the primitive earth. Despite that those hot vents can easily decompose small organic molecules available in their neighborhood, it can also be the very same hot vents which could further assist in synthesizing small organic molecules if the locales of relevant chemical reactions are properly and naturally chosen in hydrothermal environments.

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AB INITIO GB STUDY OF PREBIOTIC SYNTHESIS OF PURINE PRECURSORS FROM AQUEOUS HYDROGEN CYANIDE: RELATIVE ENERGIES OF HCN POLYMERS IN AQUEOUS SOLUTION

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Abstract

Ab initio MO GB calculation including the continuum model of solvent effect using generalized Born formula has been applied to the HCN polymers, $(HCN)_n$ (n=1-5), and the relative energies of the polymers in aqueous solution were compared. The relative energy of the dimer is comparable to twice the HCN energy, while the relative energies of trimers, tetramers and pentamers decrease rapidly as the degree of polymerization increases. Although reaction mechanisms and barrier heights were not examined for the polymerization reactions, the remarkable increase of stability of the polymers suggests that the polymerization of HCN is plausible in aqueous hydrogen cyanide of prebiotic condition, even if the polymerization reactions are not the major reaction processes.

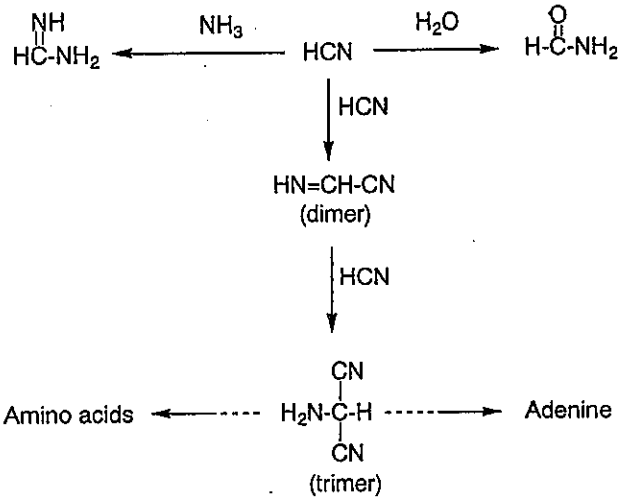
Key words: ab initio GB study, prebiotic synthesis, HCN polymerization

Viva Origino 27 (1999) 209−218

INTRODUCTION

HCN is one of the most plausible starting materials for prebiotic synthesis on the early Earth [1,2]. In dilute aqueous solution, HCN oligomerizes to produce a complex range of products including amino acids and nucleic acid bases [2-11]. Sanchez et al. [7] have shown that two modes of destruction of HCN compete in alkaline solution, hydrolysis to formamide and formic acid and polymerization (Scheme 1). The formation of amino acids includes many steps starting by dimerization of HCN, while the formation of precursors of nucleic acid bases includes the reaction between the trimer and aminomethylenimine which can be produced from HCN and NH_3. It has been considered that the trimer and tetramer play key roles in the formation of adenine [5-7].

Recent development of molecular orbital (MO) theory including solvent effect [12] enables us to analyze the chemical reactions in solution quantum chemically and to elucidate the prebiotic synthesis of aqueous hydrogen cyanide. Ab initio generalized Born (GB) method [13-15], which has been developed as a continuum model of solvent effect using generalized Born formula, is an appropriate tool for this purpose, since it is easily employed to determine the molecular structure and energy of a molecule in aqueous solution.



Scheme 1

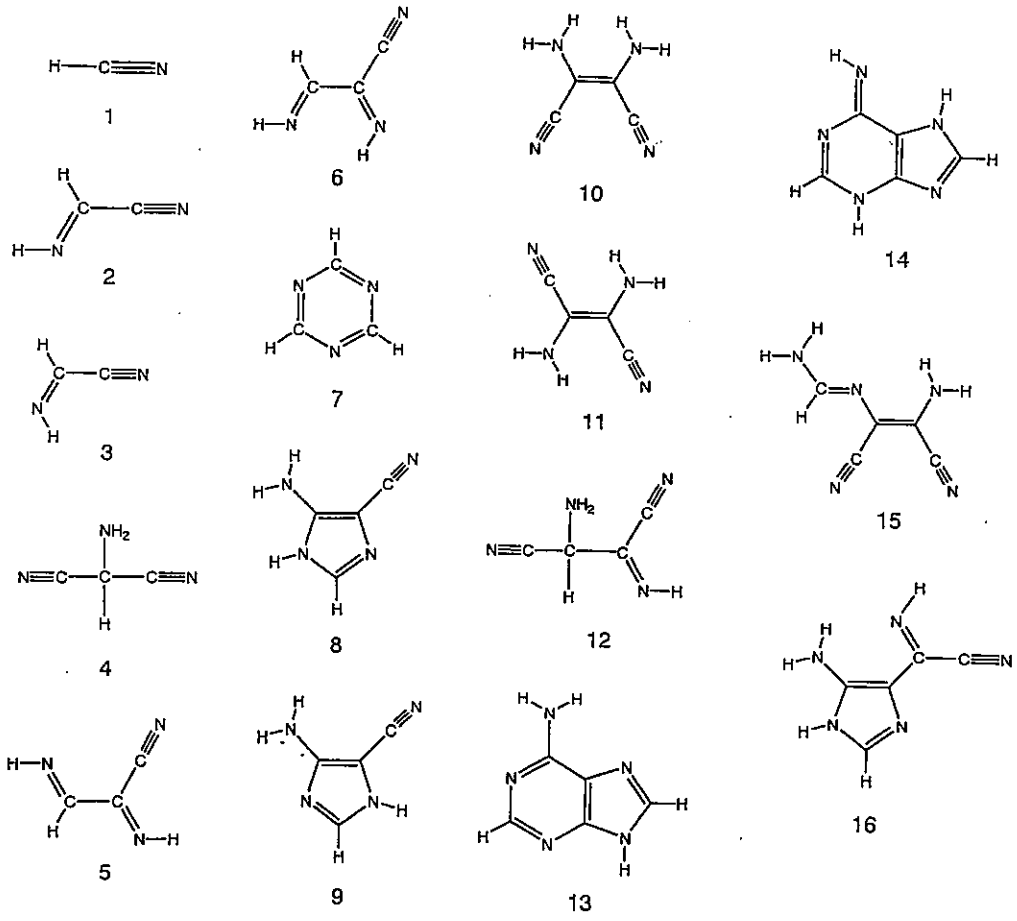


Figure 1. HCN polymers, $(HCN)_n$ (n=1-5).

In this study, the relative energies of the HCN polymers, $(HCN)_n$ (n=1-5), in aqueous solution were calculated, and the possibility of the polymerization of HCN and the formation of purin precursors from aqueous hydrogen cyanide was examined.

METHOD

Ab initio restricted Hartree-Fock (HF) GB calculations[13-15] were performed

to determine the molecular structure and energy of HCN and its polymers in aqueous solution. For the molecules **5-16**, planar geometries were assumed. Moller-Plesset second-order perturbation (MP2) calculations were also performed using the geometries determined by HF calculations. In the ab initio GB calculation, the dielectric constant ε =78.5 was used to represent the aqueous solution. Basis sets[16,17] and diffuse and polarization functions[18] were taken from literature.

In the present study, relative energies are compared among polymers with different molecular sizes. In general, relative energies of molecules with different molecular sizes are not uniquely defined. The relative energy of $(HCN)_n$ was defined by,

$$E[(HCN)_n] - n\,E[HCN] \qquad (1)$$

which is the heat of reaction for the formation of $(HCN)_n$ from HCN. Table 1 shows the relative energies of selected polymers calculated at different levels of theory. Although the relative energies depend on the methods and basis sets, similar trends are observed in energy variations for successive polymerization steps in all methods.

Table 2 shows the relative energies among three isomers of the HCN trimers, which have the same molecular formula and different bonding schemes. The inclusion of polarization functions in the basis sets stabilizes ring structures. The energy of 1,3,5-triazine, **7**, relative to three HCN molecules can be estimated from Tables 1 and 2: 24.9, 44.3, 37.0, 41.3, and 41.6 kcal/mol by HF/3-21G, HF/6-31G**, 6-31++G**, MP2/6-31++G**//3-21G, and MP2/6-31++G**//HF6-31++G** calculations, respectively. The HF/6-31G** and MP2 energies agree well with the experimental value of 43.5 kcal/mol [19].

The MP2/6-31++G**//HF/3-21G calculations give the relative energies which are comparable to those of MP2/6-31++G**//HF/6-31++G** calculations. In the present study, the relative energies of polymers **1-16** in Fig. 1 were calculated by the MP2/6-31++G** method using the geometry optimized by the HF/3-21G method.

All calculations were carried out using our ABINIT program, in which the solvent effect using the GB formula has been incorporated, on the HP-J210 and DEC-500a workstations.

Table 1. Relative energies (in kcal/mol) of HCN polymers[a)]

	HCN	dimer **2**	trimer **4**	tetramer **8**	pentamer **13**
HF/3-21G	0.0	-0.5	-24.6	-57.6	-86.3
HF/6-31G**	0.0	-4.3	-21.4	-55.4	-93.8
HF/6-31++G**	0.0	-2.3	-16.0	-49.2	-85.3
MP2/6-31++G** //HF/3-21G	0.0	-3.8	-21.7	-65.1	-111.2
MP2/6-31++G** //HF/6-31++G**	0.0	-4.0	-22.2	-65.5	-111.8

a) The energy of $(HCN)_n$ is the relative energy to n times of the HCN energy.

Table 2. Relative energies (kcal/mol) of HCN trimers

	AMN **4**	trans-ethane-diimine **5**	1,3,5-triazine **7**
HF/3-21G	0.0	20.4	-0.3
HF/6-31G**	0.0	9.7	-19.9
HF/6-31++G**	0.0	9.3	-21.0
MP2/6-31++G** //HF/3-21G[a)]	0.0	10.1	-19.6
MP2/6-31++G** //HF/6-31++G**[b)]	0.0	9.9	-19.4

a) MP2/6-31++G** calculation for the HF/3-21G structure.

b) MP2/6-31++G** calculation for the HF/6-31++G** structure.

RESULTS

Figure 2 shows the relative energies of HCN polymers. The dimers can be derived by addition of CN^- at the C≡N carbon atom in HCN; the trans iminoacetonitrile (IAN), **2**, is slightly more stable than the cis isomer, **3**. In the dimers, the C=N carbon atom and the C≡N carbon atom may be attacked by CN^- to give trimers; the former gives aminomalononitrile (AMN), **4**, and the latter

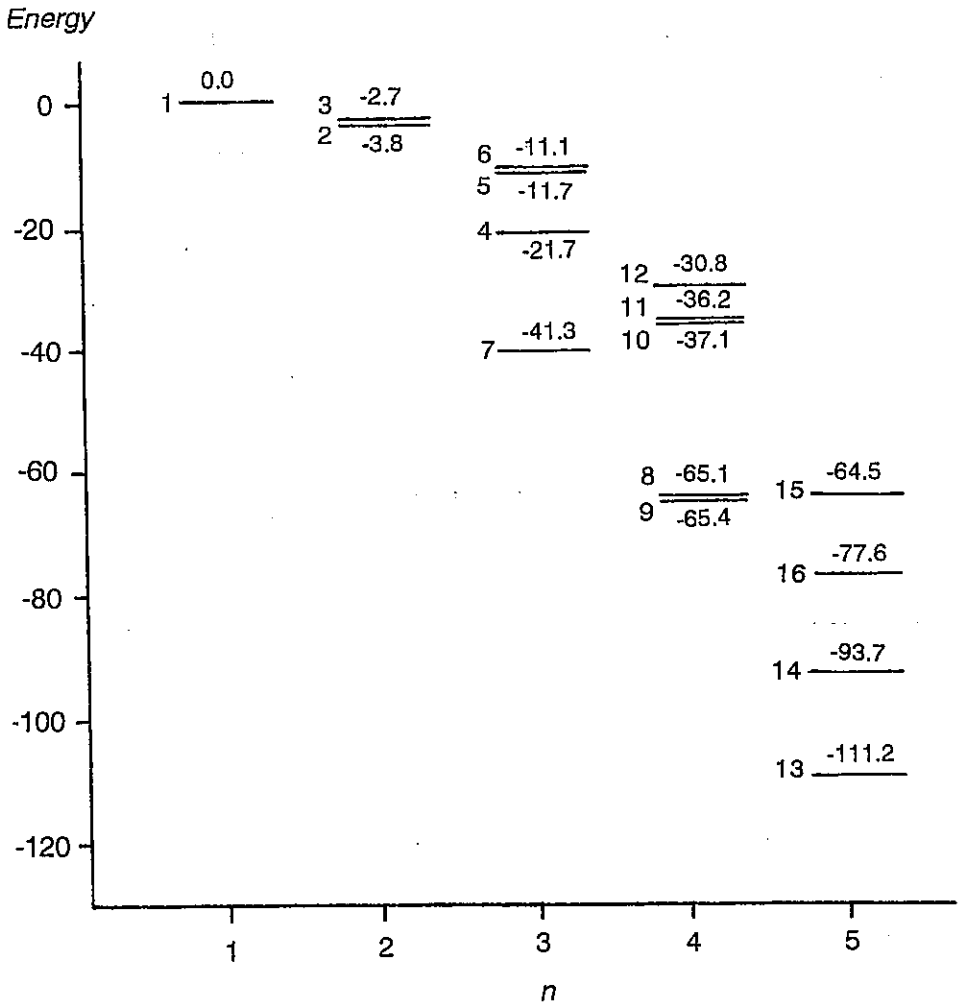


Figure 2. Relative energies (in kcal/mol) of the polymers, $(HCN)_n$ (n=1-5) in aqueous solution calculated by MP2/6-31++G**//HF/3-21G method. The numbering of molecules is shown in Fig. 1.

gives ethanediimines, **5** or **6**. AMN which has been recognized as an important species in prebiotic synthesis, is most stable among these three trimers. Although 1,3,5-triazine, **7**, is more stable than AMN, its formation requires a high activation energy; one-step reaction from three HCN molecules requires more than 70 kcal/mol of activation energy [20]. 1,3,5-Triazine may not be an important species in the formation of adenine.

Five isomers, **8-12**, were considered for the tetramer. Diaminomaleonitrile (cis-tetramer), **10**, and diaminofumaronitrile (trans-tetramer), **11**, can be derived from the reaction of AMN with HCN. The cis tetramer is slightly more stable than the trans tetramer. The stability of the cis isomer may be due to a large dipole moment which is stabilized in aqueous solution. 4-Aminoimidazole-5-carbonitrile (AICN), **8**, is derived by the reaction of AMN and formamidine or from **10** photochemically [6]. The two imidazoles **8** and **9** have similar energies.

Four isomers **13-16** were considered for the pentamer. Adenine is the most stable of these isomers. Pentamers **15** and **16** have been considered as important intermediates in the formation of adenine [9].

The relative energies are estimated from the equation (1). The dimers have the relative energies similar to the monomer. As the polymerization proceeds, the relative energies of polymers become low and the stabilization increases rapidly.

DISCUSSION

Energy profile and reaction mechanism should be examined to analyze a chemical reaction. However, there are numerous plausible reaction paths in the formation of adenine by polymerization of HCN, and it may be practically impossible to examine all of them.

We have studied the mechanism of dimerization of HCN in aqueous solution [21]. The dimerization of HCN proceeds in three steps, formation of the CN^- ion from HCN and H_2O, addition of CN^- to HCN, and the formation of the dimer from the adduct by protonation. For these processes, the transition states were isolated and the activation energies were determined. The second step, the addition reaction between HCN and CN^-, is the rate-determining step and the activation energy (26 kcal/mol) is not large. It was also shown that the dimerization reaction is highly accelerated by NH_3 dissolved in water because the concentration of the CN^- anion increases and the association of NH_4^+ cation with

HCN catalyzes the reaction of HCN and CN^-.

The reactions for the formation of trimers, tetramers and pentamers are much more complex than the dimerization reactions, and it is practically impossible to address many plausible reaction paths. Also a number of reactions which are unrelated to the formation of precursors of nucleic acid bases occur in aqueous solution. The relative energies of the polymers are thus one possible criteria to estimate the easiness of prebiotic synthesis from aqueous hydrogen cyanide. As is seen from Fig.2, stability of the polymers increases as the polymerization proceeds. Adenine is very stable and the formation of adenine from aqueous cyanide is expected strongly.

Only the relative energies have been compared in this study. Two points may be considered for more reliable discussion on the formation of adenine: (1) taking into account the reactions more favorable than the polymerization and (2) estimation of activation energies in every steps of the polymerization. For the reaction of HCN in aqueous solution, the hydrolysis of HCN to formamide and formic acid may proceed efficiently more than the dimerization of HCN. Also many reactions are expected to compete with the trimerization and further steps. However, stable reaction products can be accumulated in a long time provided that HCN is supplied continuously, and the existence of the reaction processes other than the polymerization is not a fatal factor for the prebiotic synthesis of purine precursors.

The activation energy of the dimerization reaction of HCN is reduced substantially by acid or base catalysis[21]. In aqueous solution of prebiotic system, such catalytic action is strongly expected and the polymerization reactions are expected to proceed with a low activation energies. This is expected especially in the system in which the reaction product is lower in energy than the reactant, as in the case of present polymerization of HCN. The present theoretical results support the prebiotic synthesis of purine precursors from aqueous hydrogen cyanide.

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Racemization and isomerization at Asp-105 and Asp-106 in human αA-crystallin

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Abstract

Previously, we found two racemized and isomerized aspartic residues (D-β-Asp) of αA-crystallin (Asp-58, Asp-151) in aged human lenses. The ratios of racemization and isomerization of both Asp residues increased during aging. In order to find the individual isomers of Asp residues of αA-crystallin, we treated the protein with trypsin, then separated the resulting peptides using RP-HPLC and determined the D/L ratio of the Asp-containing peptides after identification of the tryptic peptides. In the present study, we checked whether other Asp residues racemized and isomerized and we succeeded in finding new racemized and isomerized Asp residues (Asp-105 and Asp-106 residues) of αA-crystallin by changing the conditions of RP-HPLC separation. After treatment of αA-crystallin with trypsin, three peaks of T13 peptide, which

Viva Origino 27 (1999) 219−230

included both Asp-105 and Asp-106, were obtained from each age sample. According to mass spectrography and amino acid sequence analysis, it was revealed that the first peak contained isomerized Asp-106(β-Asp-106), the second one contained isomerized Asp-105(β-Asp-105), and the third one contained normal α-linkage at both Asp residues. Isomerization of Asp-105 residue remarkably increased compared with that of Asp-106 residue during aging. The D/L ratio of β-Asp-106 increased with age, while that of β-Asp-105 increased until 33 year old and then slightly decreased in the samples of 80 years old. The results indicate that two new D-β-Asp residues, Asp-105 and Asp-106, were observed in αA-crystallin from lenses of three different age, fetal, 33-year-old, and 80-year old and those post-translational modifications of Asp residues are related to aging process.

Key words : post-translational modification, α-crystallin, racemization, isomerization, D-aspartic acid, β-asparatic acid, lens , aging.

1. Introduction

α-Crystallin is one of major protein in eye lens and since there is low turnover of lens proteins, α-crystallin undergoes various post-translational modifications during aging, such as deamidation (1, 2), racemization and isomerization (3,4), C-terminus truncation (5-9) N-terminus truncation (10), phosphorylation (11), oxidation (12, 3, 4), acetylation of Lys-70 (13), and crosslinking in intramolecular disulfide bonding (14, 15). Most of these

modifications are age dependent and cause extensive heterogeneity of α-crystallin.

It has been considered that D-aspartic acid residues (D-Asp) and β-Asp of polypeptides are formed by racemization and isomerization during the natural aging process. Previously, we found two D- β -Asp residues of αA-crystallin (Asp-58, Asp-151) (4) and two D-β -Asp residues of αB-crystallin (Asp-36, Asp-62) (3) in aged human lenses. Site-specific racemization of the Asp-151 residue of αA-crystallin has also been observed in bovine lens (16), UV-irradiated young rat lens (17), aged and X-ray irradiated mouse lens (18,19), rabbit lens, and those from aged horses (our unpublished observation) despite the difference of amino acid sequences among these species. These results suggested that the configuration of Asp-151 residue in αA-crystallins stereochemically labile. In the present study, our purpose is to find other racemized and isomerized Asp residues in αA-crystallin obtained from human lenses of various ages.

2. Materials and methods

Fetal lenses and a 33-year-old (young lens) and the lenses from subjects with a mean age of 80 years (aged lenses)were homogenized in buffer (100 mM Na_2SO_4 and 1 mM EDTA/50 mM Tris-HCl buffer, pH 7.4). The proteins were fractionated into water-soluble and water-insoluble fractions by centrifugation at 15000 g for 20 min at 4°C. The water-soluble fractions were applied to Superdex 200 (2.6 X 120 mm, Pharmacia, Uppsala, Sweden), equilibrated with the same buffer, and the α-crystallin fraction was collected. α-Crystallin was digested with trypsin for 20 h at 37°C in 0.1 M Tris-HCl buffer (pH 7.6) with 20 mM $CaCl_2$ at an enzyme-to-substrate ratio of 1:50 (mol/mol). The resulting tryptic (T)

peptides were separated by RP-HPLC (LC-10A, Shimadzu, Kyoto, Japan) using a C18 column (TSK gel-ODS-80 TM, 4.6 x 250 mm, Tosoh, Tokyo, Japan) as previously described (Fujii et al., 1994b) except for the modified gradient, 0-35% acetonitrile in 250 minutes. All glassware was baked at 500°C for 3 h. The peptides were hydrolyzed with gas-phase 6 N HCl in vacuo at 108°C for 7 h. After hydrolysis, the samples were derivatized with *o*-phtalaldehyde (OPA) and *n-tert*-butyloxycarbonyl-L-cysteine (Boc-L-Cys) to form diastereoisomers. The determination of the D/L ratio of amino acids was performed by RP-HPLC with a C18 column (Nova-Pak ODS, 3.9 x 300 mm, Nihon Waters, Tokyo, Japan) using fluorescence detection (344 nm excitation wavelength and 433 nm emission wavelength) according to Fujii et al (3). Amino acid sequences were determined using Edman degradation on a pulsed-liquid protein sequencer equipped with an on-line phenylthiohydantoin (PTH) amino acid analyzer (Applied Biosystems 476A/120A, Foster City, CA, U.S.A). Mass spectra analysis of the tryptic peptides was performed by a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Kompact MALDI IV, Shimadzu Corporation). The MALDI-TOF equipment was operated with a nitrogen laser at a wavelength of 337 nm and ion acceleration voltage of 20 kV. The data were collected in reflection mode as signals of positive ions. As a matrix, 2,5-dihydroxybenzoic acid (DHBA, 10 mg) was dissolved in 1 ml of a 0.1% solution mixture containing trifluoroacetic acid and acetonitrile at a ratio of 2:1 (vol:vol). The sample peptide (0.5 ml) was added to an equal volume (0.5 ml) of the matrix solution on the plate and then dried. Each sample was present at a level of a few pmols per spot.

3.Results

Based on the amino acid sequence of αA-crystallin, 20 tryptic peptides (T1 to T20) were expected. As we previously reported (4), T6 (Asp-58 containing peptide) and T18 (Asp-151 containing peptide) peptides were separated into two peaks on RP-HPLC due to the difference between the α-and β-linkage of Asp-58 and Asp-151, respectively. In addition, we found two racemized and isomerized Asp residues, (Asp-105 and Asp-106), of αA-crystallin obtained from lenses of three different age. Figure 1 is a part of the elution profile (about

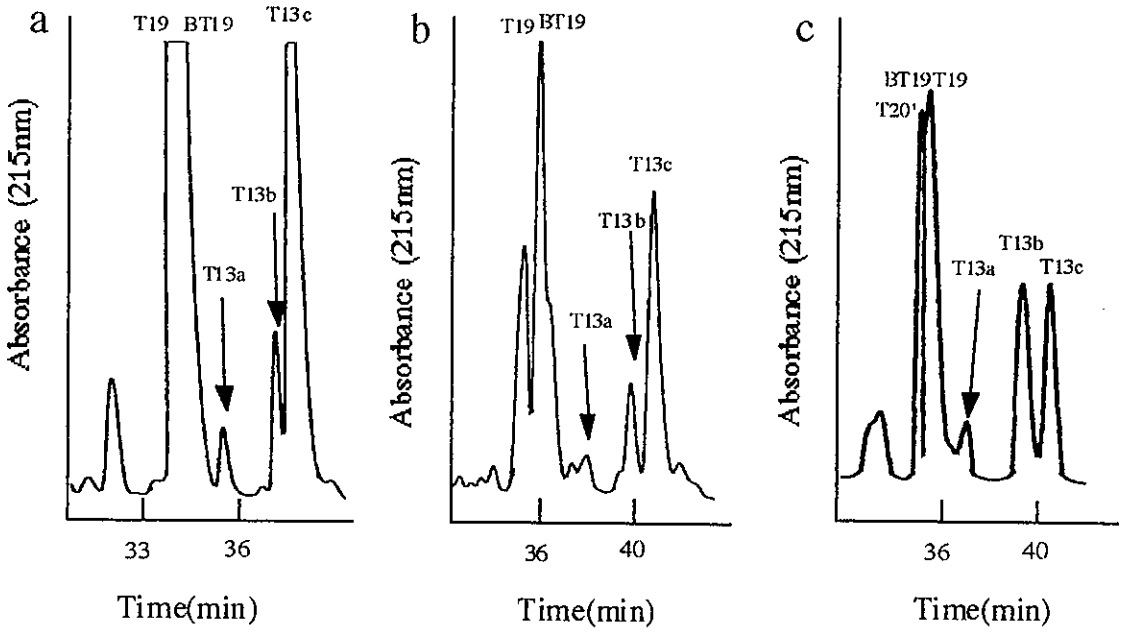

Fig. 1. Portion (10-11%) of elution profile of tryptic peptides of αA-crystallin from fetal lenses(a), a 33-year-old lens(b), and lenses from subjects with a mean age of 80(c). Tryptic peptides were separated by reverse-phase HPLC using a C18 column (TSK gel-ODS-80TM, 4.6 x 250 mm, Tosoh, Tokyo) with a gradient of 0-40% acetonitrile in the presence of 0.1% trifluoroacetic acid at a flow rate of 0.8 ml/min, with monitoring at 215 nm. The peaks were identified on the bases of mass spectrometry and sequence analysis.

10-11% acetonitrile/DW) of the tryptic peptides in α-crystallins obtained from the fetal (a) young(33-year-old) lens (b) and aged(80-year-old) lenses (c). The peaks were assigned on the basis of mass spectrometry (MS) and amino acid sequence analysis. The symbols at each peak correspond to the tryptic fragment. Both Asp-105 and Asp-106 residues were present in the T13 peptide, which was separated into three peaks on RP-HPLC for all three age.

Table I TOF-MS and sequence analysis of T13 ($QD^{105}D^{106}HGYISR$) peptides of αA-crystallin from human lens.

age	Peak	Tryptic Peptide	Observed Sequence	M+H⁺ Observed	M+H⁺ Theoretical	Molar ratio of total T13 peptides
fetal	T13a	T13β(Asp106)	QD	1092.0	1091.0	3%
	T13b	T13β(Asp105)	Q	1091.7	1091.0	11%
	T13c	T13α	QDDHGYISR	1091.7	1091.0	86%
33-year-old	T13a	T13β(Asp106)	QD	1091.3	1091.0	5%
	T13b	T13β(Asp105)	Q	1091.5	1091.0	22%
	T13c	T13α	QDDHGYISR	1092.1	1091.0	73%
80-year-old	T13a	T13β(Asp106)	QD	1091.2	1091.0	4%
		T19	AIPVSR	645.4	643.1	
		BT19	EEKPAV	1194.6*	1140.8	
	T13b	T13β(Asp105)	Q	1091.9	1091.0	42%
		βB2(112-119)	ENPNFTGK	932*	906.4	
		βs(141-146)	ELPNYR	788.6	792.7	
	T13c	T13α	QDDHGYISR	1091.9	1091.0	54%

The molar ratio were calculated based on the area of peaks and amino acid sequence in the peaks.

*It considered to add Na(+23) to theoretical mass value.

The MS analysis of these T13 peptides (T13a-c) revealed 1091 Da which was consistent with the theoretical mass (Table I). In previous studies, we distinguished the α- and β-linkage of Asp residues by sequence analysis because the β -linkage of the Asp residue is resistant to Edman degradation. Sequence analysis of T13a gave the sequence QD^{105}, which indicates β -linkage of Asp-106 (T13 β-Asp-106); the sequence analysis of T13b was only Q^{104}, which indicates at least the β -linkage of Asp-105 (T13 β -Asp-105); and the sequence analysis of T13c was $QD^{105}D^{106}$HGYISR, which indicates normal α-linkages of both Asp-105 and Asp-106 (T13α). The molar ratios of those T13 epimers were calculated by a combination of the areas of peaks of RP-HPLC and amino acid sequence analysis (Table I).

In all three age subjects, the relative amounts of the T13 epimers were in the same order, T13α > T13 (β -Asp-105) > T13 (β -Asp-106) (T13c>T13b>T13a) and T13 (β -Asp-106) was always under 10% of T13α in lenses of all three age (Table II). The relative amount of the peptides containing isomerized Asp-105 residues, T13 (β -Asp-105) to T13α increased with age (0.13 in fetal, 0.30 in the young lens and 0.77 in the aged lenses)(Table II), while T13 (β-Asp-106) kept almost the same ratio of total T13 peptides (Table I).

The D/L ratios of Asp residues in those T13 epimers were determined. As shown in Table II, the D/L ratios of Asp residues of T13 (β-Asp-105) and T13 (β -Asp-106) were higher than that of the Asp residue of T13α in 33 and 80 year old lenses. This result, showing D/L ratios of β-Asp to be higher than that of α-Asp-residues, is consistent with

Table II Characterization of T13 epimers of αA-crystallin in three different age.

age	Peptide	Linkage of Asp	β/α	D/L of Asp
fetal	T13a	β(Asp106)	0.04	0.032
	T13b	β(Asp105)*	0.13	0.031
	T13c	α	-	0.027
33-year-old	T13a	β(Asp106)	0.07	0.183
	T13b	β(Asp105)*	0.30	0.228
	T13c	α	-	0.058
80-year-old	T13a	β(Asp106)	0.08	0.498
	T13b	β(Asp105)*	0.77	0.167**
	T13c	α	-	0.084

The D/L ratios of Asp residues were measured in triplicate.
The ratios of β-linkage to α-linkage (β/α) were calculated based on the area of peaks and amino acid sequence in the peaks.
*We couldn't determined whether Asp-106 were isomerized or not.
**The D/L ratio of Asp-105 residue was underestimated because of coexsitence of Asn-113 in βB2-crystallin and Asn-144 in βs crystallin.

previous reports (4,9). During aging, the D/L ratios of β-Asp-106, α–Asp-105 and α–Asp-106 increase. On the other hand, the D/L ratios of β -Asp-105 increased with age betweer fetal and 33-year-old, then seemed to decrease slightly between 33-year-old and 80-year-old.

In aged lenses, there were two peptides containing the Asn residue, βB2 (E^{112}NPNFTGK^{119}) and βs (E^{141}LPNYR^{146}) in the same fraction of T13 (β-Asp-105)(peak T13b), which comprised 20-30% of T13 (β-Asp-105) which may result in the underestimation of D/L ratios of β-Asp-105 because we could not distinguish asparagine (Asn) and Asp by the method of determination of D/L amino acids. Unfortunately, we did not have a sufficient sample for rechromatography and further analysis of the fraction. We could not also determine whether Asp-105 or Asp-106 was the main contributor to the high D/L ratio and whether Asp-106 was isomerized in T13 (β-Asp-105)(peak T13b) because we could not separate those two Asp residues even using RP-HPLC separation after treatment of endoproteinase AspN.

4. Discussion

In aged human lens, we reported the isomerization of Asp-58 (20% of total Asp-58) and Asp-151 (50% of total Asp-151) of αA-crystallin (4) and Asp-151 (37% of total Asp-151) of truncated αA-crystallin (9). The relative molar ratios of isomerization found in aged human lenses are higher than other modifications, such as acetylation of Lys-70 (5%), oxidation of Cys residues (<10%) and phosphorylation (20% at Ser-122) and as high as truncation (50% at Ser-173), deamidation (30% at Gln-50, 50% at Asn-101) in aged human lens. In this study, we found new racemized and isomerized residues at Asp-105 and Asp-106 of αA-crystallin and the ratios of β-linkage were also high in T13 (β-Asp-105), with 22% in the young lens and 42% of the total in the aged lenses (Table I). In a previous report (4), we showed that β/α linkages of Asp-151 and Asp-58 increased with age; the β-contents of Asp-151 and Asp-58 in αA-crystallin of 11 months old lens were about 5%, while the residues of αA-crystallin of 80 year old lens were 50 % and 20 %, respectively. In this study, we found the isomerization of Asp-105 and Asp-106 even in fetal at levels of 11% and 3%,

respectively. During aging, the molar ratio of T13 (β-Asp-105)(peak T13b) increased in lenses from older subjects and the amount of increase was almost the same as the decrease of T13α(peak T13c) (Table I).

Now we clarified that four specific Asp residues (Asp-58, Asp-151, Asp-105, Asp-106) of αA-crystallin were simultaneously isomerized and racemized during aging. It is suggested that the configuration of those four Asp residues in αA-crystallin is stereochemically labile, which may cause the local structural change around the Asp residues, resulting in the conformational change of monomeric αA-crystallin. Although the ratios of racemization of Asp-105 and Asp-106 were lower than those of Asp-58 and Asp-151, the ratio of isomerization of Asp-105 was as high as those of other isomerized Asp residues, and those isomers may effect on the three-dimensional structure of αA-crystallin. Subsequently, those changes may produce an effect on surface hydrophobicity leading to the formation of large aggregates (HMW) and affect the transparency in old human lenses. Further studies are being focused on the racemization and isomerization of those specific Asp residues of αA-crystallin in samples from subjects of various ages.

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ISSOL'99を振り返って

楓　千佳

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私は今年の７月に開催されたISSOL'99に参加した。初めての学会・初めての海外・初めての飛行機と初めてづくしの私には、何もかもが新鮮で貴重な体験だった。その中でも心に残ったいくつかについて、書いてみようと思う。

まずはじめに、多くの人と話し合えた機会として印象に残っているのは、自分も発表したポスターセッションだ。ドリンク片手に発表者に気軽に話しかけられる形式だった。英語に自信のない私は紙とペンを持って質問にのぞんだ。どんな質問にも喜んで答えてくれる発表者のみなさんの姿勢を素晴らしいと感じた。自分の発表の時は、緊張しながらも、たくさんの人の話を聞くことができ、また私の話を根気強く丁寧に聞いてもらえた。今までの私にとって自分の研究室以外の人は紙の上の存在だった。ISSOLの発表は他の研究者の方々の存在が生き生きとしたものに感じられた、貴重な時間だった。

また、外国の学生と親しく話す事もできた。特に私の心に残ったのは、同室のマリアさんだった。人への接し方がとてもあたたかく親切で明るい人だった。夜中に二人でお互いのポスターを持ち出して遅くまで話し合った。発表の内容、今後の研究で調べたいことなど話しているうちにあっと言う間に時間は過ぎた。彼女は三人のお子さんを育てながら大学に通っているそうである。大学で学ぶことも学会で発表することも、とても楽しんでいる様子だった。私の周りには、子どもを育てながら学校に通う人はいない。きっと私には想像もつかない大変さがあるのだろうと思う。にもかかわらず彼女は親切で、しかも新しいことを学ぶ意欲を失わない。そんな彼女の姿勢を見習いたいと思った。

その他には、大学の中には予想もつかないことが多くて印象深かった。建物の作りが美しく、緑が多く、気をつけてみるとさりげなく車椅子用のスロープが整備されていた。個人的にとても興味深かったのが、大学の中のホールで頻繁に演奏会が開かれていることだった。室内楽からオーケストラ、クラシックだけでなくジャズ・ゴスペルなど、規模もジャンルも幅広く、チケットの値段も手頃で、聴きに行きたいと思わせる催しが多かった。音楽に興味のある私にはとてもうらやましかった。芸術系の学部があるからなのかもしれないが、大学の懐の深さのようなものを感じた。

私がISSOL'99に参加することができ、その結果このような貴重な経験ができたのもTravel Grantをいただけたおかげです。この場をお借りして、心から厚くお礼申し上げます。また、学会中お世話になった方々、先生方をはじめ研究室のみなさんに、心から感謝しています。ありがとうございました。

1999年「生命の起原」夏の学校開催される

「生命の起源」夏の学校が新潟県長岡市にある NTT 宿泊施設「蒼柴荘」で開催されました．蒼柴荘は 1997 年の第22回学術講演会が開かれた長岡グランドホテルに近い，長岡の繁華街を川一つ隔てた格好の場所にあります．例年7月下旬に開催している夏の学校ですが，今年は ISSOL や参加予定大学の事情もあって8月9日(月)～11日(水)の3日間にずれ込みました．今年の長岡はフェーン現象のため例年になく高温で，暑いこの地で熱い「夏の学校」が繰り広げられました．

参加校は筑波大学，横浜国立大学，東京理科大学と当番校の長岡技術科学大学の4校と，講師として石神正浩先生(元大阪府立大学教授)にお出でいただき，総勢 23 人で行いました． 講演数は 23 題に及び，発表者は参加された先生からの助言から得るものも多かったと思います．今回で7回目となる夏の学校でしたが，大学院生はもちろん研究室に配属されたばかりの学部生にも発表の機会を与え，お互いに切磋琢磨して研究活動に結び付けていこうとする趣旨は健在です．

石神先生には遠路はるばる起こしいただき，学生の講演に対して助言をいただきました．また「遺伝暗号の起源」と題してご講演をいただきました．この場をお借りしまして改めて御礼申し上げます．

夏の学校の運営費に「生命の起原および進化学会」から援助を頂いたことに感謝いたします．

夏の学校の終わりとともに，長岡の異常な暑さも終わりを告げました．

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新刊書のお知らせ

"Advances in Biochirality"
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自然界における対掌体の問題は生物学や生命活動にとって重要な問題であるがその起源や成り立ちについては未だにわかっていない。自然界のタンパク質やペプチド中のアミノ酸はL-体、DNAやRNA中の糖はD-体という一方の対掌体から成り立っているのは必然なのか偶然なのか？（因みにタンパク質中のD-アミノ酸は最近、見つかっているが、L-体の糖は見つかっていない。）今日の生物界における高い光学異性体の選択は原始地球上（あるいは別の惑星？）で生まれ、どの様にして化学進化の過程で片手構造の世界が確立するに至ったのか？本書は昨年イタリアのModena大学G.Payli教授が開催した"Biological Homochirality"の国際会議での講演を基にして化学、物理学、トポロジー、古生物学、生物学、宇宙科学、薬学など様々な分野の第一線の研究者がこの難問を解くべく果敢に挑戦したhomochilarityに関する学際的な興味深い本である。研究対象は分子レベルの問題から巻き貝や植物の蔓の巻き方というマクロなレベルまで右と左の多彩な世界に迫っており、知的好奇心をそそられる。
編者のModena大学G.Payli教授自身も生命の起原及び進化学会会員であるので、会員は30%割引である。購入は直接Elsevier Scienceへお申し込みください。

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われらが学会（SSOEL-Japan）と京都大学が1998年3月に合同で開催した国際会議（ICRROEL)での講演の内、放射線に関連する講演のみを纏め、各講演者が本としての出版の為に加筆、補充したものがこの度、下記の通り出版のはこびとなりました。ご教室・研究室でのゼミや教科書として是非ご利用下さい。購入方法」等について出版社に問い合わせたところ下記の返事が帰ってきております。ご参照下さい（文責：赤星）。

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本の紹介文

「原始地球上に存在した放射線が、化学進化や生命の起源および進化の過程で果たした役割は極めて大きかったはずである。しかし、実験設備の技術的な限界から、その実態に実験的に迫ることは、最近まで非常に困難であった。本書は、世界の第一線の研究者達が、近年の急速な技術発展をうけて、生命の起源と進化における放射線の役割に挑んだ最新の成果である。」

Role of Radiation in the Origin and Evolution of Life

Contents

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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）．

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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

〈1998、1999年度役員〉

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Contents

ARTICLES

NEWS and VIEWS