NO HOMOCHIRALITY – NO LIFE

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Abstract

Life is made up from biomolecules with one-handed structure. L-dominant exclusive choice of enantiomeric type in optical amino acid isomers raises important issues concerning life science. This report discusses why it is essential for life to be made up of homochiral molecule. A mechanism that selects homochiral molecules lies in enzyme stereoselectivity, and therefore the studying of it will be important to solve a problem on origin of life and an enigma of life's birth. This report concludes there is no life without homochirality.

(Keywords)
Origin of homochirality, Enzyme enantioselectivity, High level of organization, Kaname-isi,
No homochirality – no life

1. Importance of optical isomeric form in biomolecules

A life body can be classified into organ, tissue and cell according to size. Further segmentation comes to the macroscopic structures from subcellular organelle as cell membrane, nucleus or mitochondria to microstructural body as ribosome or microtubule. Additional fragmentation only ends in a subdivision of biomacromolecule. We can thus say that life is a highly organized macromolecular assembly. Protein and nucleic acid of biogenic macromolecules are the most important. Protein or nucleic acid is produced through dehydrated polycondensation reaction between L-amino acids or nucleotides. Their biomolecules provide ultraprecise stereostructure. For example, enzyme protein consists of one or more protein chains. A protein chain will be in the range of 50 to 2000 amino acid residues, giving primary amino acid sequence, and even secondary structure as right-handed α- helix and/or β-pleated sheet that are connected to each other through hydrogen bonding occurring between NH and CO groups in the chain (Fig. 1). A single protein chain may contain multiple secondary structures. In addition to hydrogen bond, other interactions between the side chains of amino acids such as ionic interactions, van der Waals dispersion forces and sulphur bridges fold into higher stereostructure to exhibit a catalytic activity. The elaborate stereostructure of enzyme conducts the catalytic reaction with substrate. However, if there is the slightest change in the stereostructure, the enzyme cannot immediately react with the substrate. Transient fluctuation of environment surrounding enzyme, for example, pH of aqueous solution, changes its surface charge state to cause a small change of the stereostructure and then lead to a decrease in the enzyme activity. Thus there is the most optimal environment in enzyme

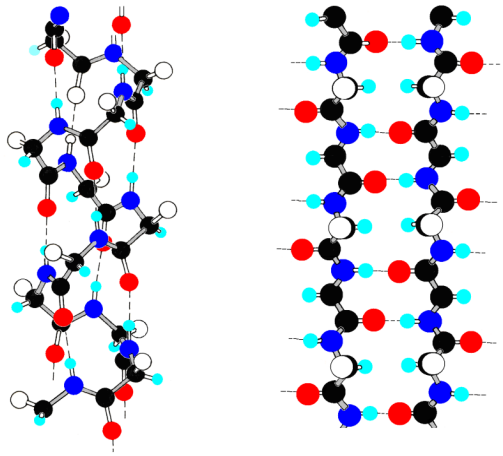

Fig. 1 Right-handed α- helix and β-pleated sheet structures formed by poly-L-peptide

reaction, in which enzyme provides stereospecificity together with substrate specificity. As a result protein is made up from L-amino acids, leading to have homochiral structure. On the other hand, deoxyribonucleic acid (DNA) has antiparallel right-handed double helical structure, which shows consequences in base- pairing and base- stacking by hydrogen bonds that occur between complementary nucleotide base pairs, that is, cytosine-guanine and adenine-thymine. L-amino acid and D-deoxyribose (Fig. 2) are essentially responsible for the right-handed helical structure of protein and DNA. The homochirality in an optical isomeric type of amino acid or sugar, which is used as parts in

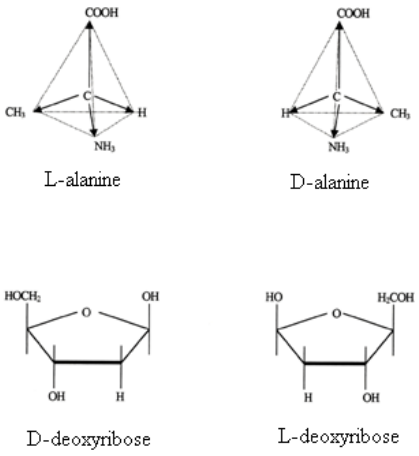

Fig. 2 Stereochemical structure of alanine and deoxyribose

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building this spiral structure, is absolutely required.

It is enzyme stereospecificity that could make differential activity between enantiomeric substrate molecules. The most conspicuous instance of it is a pharmacological effect of thalidomide (Fig. 3). Thalidomide has R type and S type in optical isomeric type because it has a chiral carbon. While the R type has a positive effect of sedative activity, the S type teratogenesis that causes phocomelia, quite the contrary and enormous negative property as compared with the R type. A single mistake that marketed this racemate later brought about a terrible tragedy. (The later research indicated the R type was racemated into the S type in body.) [1] We can have an immediate experience with the difference between physiological actions on DL-enantiomeric amino acids. All foods include free amino acids. Examples of free amino acid – rich foods include seaweed high in gulutamic acid, aspartic acid and proline, histidine high in fish and dried bonito, or alanine, glycine, serine and proline high in crab, squid and sea urchin. As every amino acid has its own taste, food with special flavor can be created by combining one or more amino acids. [2] For L type of human essential amino acids (threonine, methionine, leucine, valine, isoleucine, phenylalanine, lysine and tryptophan), we note a bitter taste in the mouth except sweet threonine. Generally speaking, non-essential amino acids have sweet or umami. For example, L-gulutamate has umami along with sour. The taste of amino acid is different between enantiomeric type, too. It is nice for practical experiment to lick both D and L types of 19 biological amino acids in addition to glycine with your tongue. Glycine without chiral carbon has the simplest structure of them, but is sweet just as its name suggests. While L-valine and L-leucine have bitter taste, their D type has sweetness. In aromatic amino acids such as phenylalanine, thyrosine and tryptophan, L type is bitter or tasteless, but D type generally has very strong sweet taste. Surprisingly, D-tryptophan is particularly thirty five times sweeter than sucrose. D type is sweet but L type bitter in other neutral α-amino acids, except D-methionine is tasteless. In imino amino acids as proline as well as acidic amino acids, L type is sweet but D type is almost tasteless. Acidic L-amino acids also has umami, and so used as seasoning. Interestingly, D-aspartic acid has no umami, but β-amido-D-aspartic acid is sweet such as neutral amino acids. Amino acids thus have umami, sweet or acerbity according to their stereostructures.

As an aside, there is said to be interspecies difference in sweet sensitivity. For aspartame as an artificial sweetener or stevia, Stevia rebaudiana plant leaves known as a South American herb that has been used as a sweetener by the Guarani Indians of Paraguay for hundreds of years with a delicious and refreshing taste that can be 30 times sweeter than sugar, human and monkey can sense a strong sweet taste, but rat cannot sense it. For monellin, a sweet protein which was discovered in 1969 in the fruit of the West African shrub known as serendipity berry (Dioscoreophyllum cumminsii) and can elicit a flavor

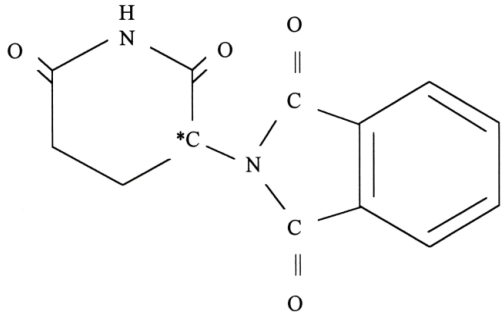

Fig. 3 Thalidomide. *C is a chiral carbon.

approximately 100,000 times sweeter than sugar, old world monkey as crab-eating monkey can sense sweet, but new world monkey cannot.

Five tastes of salty, sour, bitter and umami in addition to sweet are determined by a high or low affinity of a molecule for a species-specific gustatory receptor. Their relation can be just explained by host-guest interaction, that is, three point interaction theory. D and L type in a mirror image relationship have the difference in spatial configuration among residues bonding with chiral carbon, and therefore the antipodes make a difference in affinity with gustatory receptor to allow us to perceive different tastes. This indicates the receptor structure itself on the surface of gustatory cell on our tongue is chiral moiety and consequently, provides different interactions between L and D type. As shown in Fig. 4, in four L-amino acid side chains put as A, B, C, D, and D-amino acid side chains as A, B, C′ , D, when A, B and C residues of L-amino acid respectively bind with α, β, and γ sites on the receptor surface to produce a taste sense. Any gustation doesn’ t occurs or entirely-different one is given in D-amino acid. Since Pasteur’s discovery that yeast ferments to use d-tartrate all up but cannot ferment l-tartrate [3], Van’t Hoff and Le Bell independently developed the tetrahedral structural theory of carbon [4]. Although the three point interaction theory has not yet been perfected, many researchers have applied it to understand a mechanism of enzyme stereospecificity. When we study the physiological activity of biology-related substances, we cannot neglect problems on enantioselectivity since the thalidomide case. Optical resolution technology holds great promise for the future to elucidate the mechanism of the enantioselectivity.

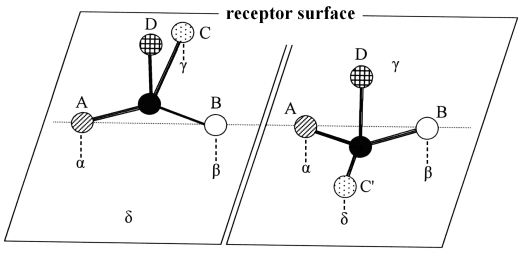

Fig. 4 Binding site with L- and D-amino acid side chains and gustation receptors

As described above, the biological importance of enantiomeric type can be sufficiently understood. Physiological difference on enantiomers reflects that life is made up from biomolecules with one-handed structure. In other words, life itself can be said to be homochiral existence. Here two questions will naturally occur. One is why life must be made up of homochiral molecule, and the other is why amino acids and sugars as biochiral molecules must be L type and D-type, respectively. We discuss on these problems in the below some chapters.

2. The necessity of homochiral molecules for birth of life

We can answer about the first problem with comparative ease from the following two standpoints. One lies in a synthetic efficiency of polypeptide. L-amino acid and its antipode, D-amino acid, have the same physicochemical properties. Now imagine a dipeptide synthesized from two L- and/or D-amino acids. As a dipeptide has two chiral carbons, it has four isomers. Both L-L (dipeptide composed of two L- amino acids; Dipeptide is henceforth expressed in this abbreviated notation) and D-D dipeptides have the same physicalchemistry because they are in a mirror-image relationship to each other. The same goes for L-D and D-L, too. However, the former group (L-L, D-D) and the latter one (L-D, D-L) merely have a diastereomeric relationship with different physicalchemistry. Therefore, they may be considered as different molecules from one another. These relations are illustrated in Fig. 5, in which two enantiomers are shown in continuous line and four diastreomers in dotted line.

When a dipeptide is synthesized from amino acids including L and/or D form, compounds with four different enantiomeric forms are produced. As the number of amino acid increases in a polypeptide such as tripetide, tetrapeptide, pentapeptide---, the number of isomer correspondingly increases at an exponential rate as 8, 16, 32 ---. Let's think about an artificially-synthesized polypeptide from 100 amino acid racemates. It has combinations of 2^{100}, and therefore it means that one of their polypeptides is picked to select the only right L-polypeptide. If we think in terms of all biological amino acids (glycine and 19 amino acids with chiral carbon), its combination is $(20+19)^{100}=39^{100}\sim10^{160}$, astronomical number! If an enantiomeric type of these amino acids is either L or D, its combination is $20^{100}\sim10^{131}$. This combination number decreased to 10^{-29}-fold of the former to give drastic decrease. As understood from this result, when we artificially synthesize L-polypeptide from 100 amino acids, we will use only L type instead of racemate to synthesize. Early life might also have taken the same strategy.

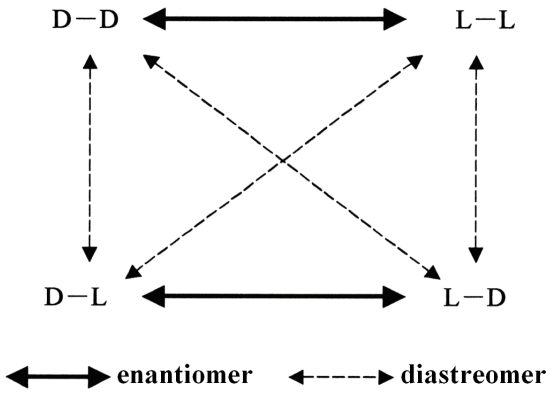

Fig.5 Enantiostructural relationship of a dipeptide composed of two L- and/or D-amino acids

In the past time, Pasteur thought there was something like symmetry-breaking pressure in space. Today, it is not believed but no one could deny the concept of matters' automotility to higher organization, or evolving matters. Various subnuclear particles are born, repeating constant alignment and realignment in this universe to produce atoms and subsequently elements. Once again, different elements mutually repeat the meeting and parting to yield molecules, whose movement conducts to form organic molecules, and proceeds to chemical evolution possible, and ultimately connects to the birth of life. Matters born in this universe can evolve to obtain new property and function by raising a level of their own organization under natural pressure. Matters will be destined to organize to higher level in this universe. The most highest organized matter system was born in early earth about forty billion years ago. This ultimate highly-organized system was life, a living matter system with dynamically self-preserving and self-propagating property. Of course, it goes without saying that environmental conditions such as temperature, fluid, moisture and sustainable reductive atmosphere was furnished to produce it. Unlike Earth, Mars environment is insufficient to produce life. However, Mars was rich in water four billion years ago. It was once a beautiful water-filled planet like Earth, but today was all gone to pot because water evaporated 3.5 billion years ago. Our plant has too many favorable conditions for life compared to Mars. Self-preservation and self-propagation are the most biotic features of matter's evolution. After early life was born and obtained full adaptation to global environment, it increased the level of the matter's evolution to proceed to a stage of spontaneous life evolution, which created new life with higher organization. Human reaches the highest state of the evolution, regarded as historical existence with the highest precise organization as the matter's evolution that the evolution of cosmic elementary particles starts 13.7 billion years ago, and continues to chemical and biological evolution.

In this context, a key word for essential requirement under which life is generated from abiotic matters is matter's highly-organization. For example, we here think about the relationship between polyamino acids or nucleic acids with higher-order structure. L-amino acid or D-sugar can be regarded as basic parts to assemble these macromolecules. As described above, uniform enantiotype such as L-amino acid or D-sugar is necessary to make higher organized macromolecule.

If some D-amino acids invade L-dominant amino acid world, its stereostructure is known to become unstable [6]. When a polyamino acid chain is synthesized from racemic amino acids mixed with L and D form, the enantiomerically mosaic polypeptide becomes unstable, and can neither elongate nor make a highly precise organized structure. Since life is a very highly-organized system, the first strategy is to unify an enantiotype of enantiomers as components. However, natural world will be impossible to achieve it. Therefore, we should think the way to high organization from D- and L-mixtures. It is indeed possible to make a highly organized macromolecule from DL-racemate. A very sophisticated mechanism which can differentiate D-type from L-type and also deliver the right enantiomeric type to the right place, is necessary to produce it. It may be difficult to imagine a system, which can recognize and choose L- or D-type, is naturally generated. However, enzyme plays the role in real life world. When did a mechanism of this enzyme stereospecificity occur? In other words, it means to ask whether the mechanism was generated during abiotic or biotic evolution. Although the present study cannot give this question a clear answer, we can at least say the mechanism of enzyme enantioselectivity might had been already driven by early life because enzyme should have already existed at its birth. We can thus think it was completed during abiotic period. Primitive environment of early earth was probably filled with racemic mixtures of DL-racemates, and so the mechanism was absolutely essential to select one-handed molecule and make higher organized structure. The emergence of the mechanism is a watershed that proceeds to biotic era from abiotic one. The moment of its appearance become a starting point for life evolution. It is quite right to say that life is a highly-organized homochiral system with homochiral structure and it cannot be constructed until homochiral components are supplied by the mechanism of the enantioselectivity.

3. What is the origin of homochirality?

We can adequately understand through the discussion thus far that homochiral parts selected by enantioselectivity are necessary to make homochiral structure, and a wide variety of sophisticated compilation of it is life. Therefore life itself is homochiral. The main question that begs to be asked is; Why was L-amino acid exclusively chosen as biological component? Since L- and D-enantiomer have the same physicochemical property, any problem is nothing even if early protein selects D-amino acid instead of L-amino acid. Nevertheless life forces through exclusive use of L-amino acid. There must lie some deeper ground for life's exclusive selection of L-amino acid, though our present knowledge cannot give any answer to it. What we can say for sure is that something bad is going to happen when L and D are mixed.

The above discussion has thought of molecular level. When discussing individual level, this is a whole different ball game. We want you to discuss the following future love story. You will travel in space to land on a planet in no distant future. Suppose you find a living creature there, when you land on the ground. While it has the same feature and function as earth's life, an optical isomeric form of amino acid is D-type, or the opposite of earth's enantiomer. D-life evolves on this planet because it independently takes separate evolutionary paths from ours. That is stating, in other words, that L-life on earth lives in L-world and D-life on the planet in D-world. Please here imagine what it would be like if L-earthman contacts with D-girl on the planet. L-man fortunately meets a beautiful D-girl there. Of course, she has the same feature, morphology and function as earthman except that she is made up of D-amino acids. They will soon fall in love at first sight. They promise to love each other and always have such a fun time together. Before long, they want to marry. However, reality is cruel. It is important to note that L-man is composed of the opposite enantiomer against her. Some big troubles occur, involved in reproduction, food and health. First of all, they can't have any child due to impaired fertility problems arising from enantiomeric incompatibility, though both love and newlywed life can be shared immediately. A short time later, another inescapable fatal problem will happen. He must eat D-food on the planet after L-human runs out of L-foods carrying from the Earth. However, D-food is not metabolized as nutrition. He becomes reduced to skin and bone day by day. He will need to return to the earth as early as possible. Consequently, their romance will end in the result that they proceed straight into disaster. Even if food problem is solved, unfortunate calamity will visit. As D-bacterial pathogen in D-world doesn't act on L-living organism, D-world is like bioclean room for L-living organism. This means that L-organism in D-world dies of only old age or cancer. Because, even if D-organism suffer from killing disease, L-organism are as fine as can be, healthy L-organism can easily wipe out the deadly ill D-organism. The only result that their contact brings about will be to end in disaster. L-organism threatens the existence of D-organism, and L-world becomes antagonistic against D-world (Fig. 6).

As above description, L-world and D-world is not mix at all like oil and water in the level of highly-organized life, while the physicochemical

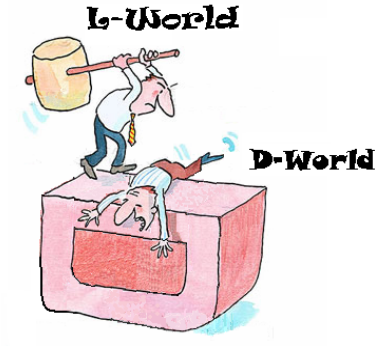

Fig.6 Threat of L-world against D-world on D-planet

character of L- molecule is quite equal to that of D-molecule at low molecule level. Although we distinguish monomeric L- and D-amino acid in optical rotation, life recognizes clearly them by use of enzyme stereospecificity. Why life made the exclusive selection of L-type is a great mystery. If L-system is more stable than D-system on earth, the emergence of L-organism is inevitable consequence. On the other hand, if both systems have same stability, it is accident. A new law and concept connecting monomeric amino acid and macromolecular polypeptide, will become essential to answer this question (Fig. 7). Biological molecules will go through various steps until life are created as a final product. Figure 7 draws up organization levels for the birth of life from racemic primitive earth without animate matters with several steps such as selectivity mechanism, exclusive system, automatism to higher function and organization, indicating the significance of chiral homogeneity to launch the origin of life. Perhaps the problem on origin of homochirality will not be solved until the mechanism of the enzyme stereospecificity are elucidated.

A final goal of spectacular matter's evolution is to create living matter, and so the study for origins of life aims to find a starting line in the magnificent historical drama that retraces the present life's path. However, at the same time, there should be another historical process preceding biotic evolution. Abiotic chemical evolution is that process, merely reviewed as a partial historical scene of matter's evolution. Studies of chemical evolution have demonstrated various biological organic compounds can be artificially produced through model experiment. Amino acids can be synthesized with great ease [7]. As a result, no matter how much they are synthesized, no one can generate life. Abiotic synthesis produces racemic antipodes in the absence of special catalyst or chiral reaction field, and thereby any macromolecule with highly-organized structure cannot be synthesized. Even assuming arguendo that enantiometrically-mixed oligopeptide is produced by accidental synthesis, it will be perfectly impossible to make the oligopeptide grow into polypeptide with longer-chain. Life demands strict discrimination even at lower molecular level as well as higher-order

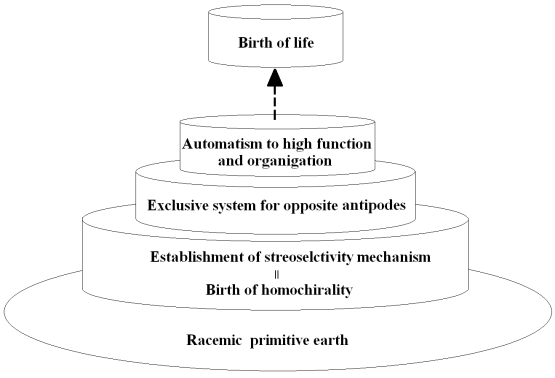

Fig.7 To the birth of life from chiral homogeneity

structure. Whatever be the enantiomeric situation, all basic biological components such as amino acid and sugar must be completely homochiral to synthesize life body with ultraprecise stereostructure. For the synthesis of life, all monomeric molecules should be supplied kept through one-handed selection mechanism. Although many bioorganic compounds as amino acids or sugars are reported by abiotic synthesis through complicate experiments, it is more difficult to find how to select, increase and organize homochiral molecules. When we want to trace a process of life's birth to its origin along a single street, our first big challenge we cannot get by with avoiding is how chiral homogeneity occurs. We cannot cut to the knot of it until we open the heavy gate leading to solve this enigma.

4. How to define life

Unless a life is defined with scientific accuracy, nobody can determine as a matter of course when life was born. Although we discuss the origin of homochirality without defining life, we should discuss about it after defining life. Thus we try to define it again. How was early life born and what kinds of features did it have? In fact, it is very hard to answer their questions. For example, think about the idea that DNA is life. If we admit it, a main research theme of chemical evolution is to study a process of natural synthesis of DNA. However, nobody has succeeded in abiotic experimental synthesis of DNA or creating self-multiplication system without help of enzyme. How about RNA? Since discovery of autocatalytic RNA, many researchers have discussed RNA world, but natural synthesis or self-multiplication system of RNA still hasn't succeeded in as is the case with DNA. They will probably conclude DNA or RNA is not life. On the other hand, how about viroid or virus? Perhaps most of researchers say it is life with the exception of a very tiny minority [9]. Where does this difference come from? Our view of life agrees to recognize that life needs more complex and dynamic substance system than DNA and RNA to make itself fulfill its self-preserving and self-renewal properties. It is estimated that minimal cell size of life require at least a diameter of 200 nm to regulate their properties [10]. A certain cell size is necessary for inanimate material to evolve to life. However, element essential for life is not satisfied only by the size. The difficulties arising in defining life results from another cause. There are different cognitive abilities for life to different people because they cannot be non-quantifiable. For example, a feeling of complexity, precision or dynamics are quite different from person to person. When we observe a computer circuit of CPU or LSI memory, we think it is very complex and precise. However, no one says *E. coli* is more complex. The perfect adjective to describe is only something called 'simple', 'easy' or 'lower' even though it just has more precise and advanced organized structure than the computer chip! There is another good example that the cognitive ability for life depends on people. Japanese people believe that

spirits dwell in all objects. Fig. 8 is Kaname-isi in Kashima-jingu Shrine, on which Kashima god sits neatly as the home. People in old times believed animate thing dwelt even in a little stone in the way as well as divine Kaname-isi. How do we today think about it? Is this an issue which we can afford to laugh it off as a joke? Next we think about antemortem and postmortem change. When a man dies, chemical analysis of his body will possibly give equivalent results before and after his death. Nevertheless, we judge without the slightest hesitation that he is alive before his death or dead after his death. Why can we judge so? The reason is that the judgment of life and death is independent on the absence or presence of material. We regard life not as existing materials itself but as attributes derived from material system. When the material system cannot depict the attributes, life becomes inanimate or dead. The material system must have the capability that can built highly-organized structure from homochiral molecular components. If it lacks the capability, we cannot accept it as life. It is considered a total of 150 million organism species has been born on this earth while over 4 billion years have passed since birth of life. The number of extant species is about 1.75 million, and gene sequence-known species about fifty thousand. Additionally, organic compounds related to them is ten million, and enzymes over one hundred thousand (Known enzymes is no more than about 5000.) [11]. If we sweepingly divide biological metabolic activity into two groups, we can separate into basic and inherent metabolism. All species share the former basic metabolism to maintain vital activity, while each species has the latter inherent metabolism to metabolize specific compounds for environmental adaptation. If both metabolisms are compared to a tree, the former is like trunk, and the latter like peripheral branches and leaves. However, since the inherent metabolism is specific for the organism, it is significant for the study of environmental adaptation or species diversity to elucidate its metabolic pathway. Since basic metabolisms are common to all living organisms, they are primitive. In early life the most primitive metabolism of the basic metabolisms would be used on early earth. The origin of life is estimated to date back to more than 3.8 billion years ago on the basis of microfossil data. As today's life passes through the stage of amazing adaptive

Fig.8 Animate Kaname Stone in Kashima Shrine, the home of gods and refuge to the spirits.

divergence with various features and functions, we are amazed at their rich diversity at the sight of its spectacular beauty for a quick moment. However, we immediately notice that all species and generations are under common strict principles to keep them alive. They help enormously in order for a system, which promotes the production, sustention and development of highly-organized structure, to function adequately. They is shown in the below 1~3.

1. Preparation of homochiral molecules as biological components

L-amino acids and D-sugars are basic components producing highly-organized enzymes or nucleic acids. It is impossible to make a regular structure without them.

2. Continuous exclusion of the opposite enantiomer

An enantiomer must be always homochiral status. However, actual metabolism frequently produces the opposite enantiomer. Therefore a mechanism to exclude it becomes necessary. The best example is O_2-requiring D-amino acid oxidase. Since the enzyme makes specific degradation to D-amino acids, it is one of members to constitute the exclusion system of D-amino acid. The raison d'etre of this enzyme is to wipe out waste D-amino acid. Hydrogen peroxide into which D-amino acid is degraded by the enzyme is subsequently redegraded into O_2 and H_2O by catalase. Since the enzyme demands O_2, D-amino acid oxidase will be emerged after the appearance of atmospheric oxygen, which might revolutionize the metabolic system of early life. D-aspartate is known to exist in animal baby's tissue and amnion liquid right after birth [12]. According to Haeckel's law that ontogenesis repeats phylogeny, this fact indicates that D-amino acid is utilized in early metabolism. After O_2 appearance, D-amino acid would become probably unnecessary life to obtain higher metabolic efficiency. So D-amino acid oxidase would use D-amino acid as an oxygen acceptor for biological defense mechanism against O_2. It values the annihilation mechanism of superoxide more than D-amino acid degradation. D-amino acid oxidaseperfectly obliterated all traces of energy-inefficient early life with the assistance of O_2.

3. Continuous restructuring towards a higher level

Life is still evolving without cease to organize its structure to a higher-level. DNA, whose mutation is largely responsible for biological evolution to create higher-organized structure, has no physiological activity by itself. In collaboration with other biological molecules, DNA exercises a property that spontaneously drives life towards higher-level organization with more advanced functionalization in an efficient way, for example, a sort of hypercycle mechanism.

This report have discussed about no homochirality – no life. Life was not born on racemic early earth until a mechanism of stereoselectivity was

incorporated into catalytically active early polypeptides through spontaneous abiotic process. Since the mechanism is essential for life, it has been handed down from generation to generation. It goes without saying that present enzymes inherit the mechanism from their ancestors. Therefore the study of enzyme stereospecificity will play a key role to a problem on origin of life and an enigma of life's birth. We defined life as a matter system with highly-organized structure that implements the above three items. Even though a small stone you pick up in the side of a road on your way to walking is meager, you would not find it difficult to feel it is alive if it has such a high level of organization system. The time of birth of life is when the enantioselectivity that ensures the stability of the matter system is first appeared. The present report has remained mum on why life makes exclusive selection of L type of amino acid antipodes. Recent study demonstrates even if an enzyme has absolute enantioselectivity on L-amino acid, it becomes active to D-amino acid according to solution condition [13]. This indicates enzyme stereospecificity is indeed flexible. Life seems to select L type under its control as a matter of mere expediency because L-exclusive choice is simply too much advantageous to contemporary environment. The elucidation of its mechanism will be important to answer the question.

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