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Origin of amino acid homochirality: Relationship with the RNA world and origin of tRNA aminoacylation

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Abstract

The origin of homochirality of L-amino acids has long been a mystery. Aminoacylation of tRNA might have provided chiral selectivity, since it is the first process encountered by amino acids and RNA. An RNA minihelix (progenitor of the modern tRNA) was aminoacylated by an aminoacyl phosphate oligonucleotide that exhibited a clear preference for L- as opposed to D-amino acids. A mirror-image RNA system with L-ribose exhibited the opposite selectivity, i.e., it exhibited an apparent preference for the D-amino acid. The selectivity for L-amino acids is based on the stereochemistry of RNA. The side chain of D-amino acids is located much closer to the terminal adenosine of the minihelix, causing them collide and interfere during the amino acid-transfer step. These results suggest that the putative RNA world that preceded the protein theatre determined the homochirality of L-amino acids through tRNA aminoacylation.

Keywords: Homochirality; Amino acids; tRNA; RNA world; Minihelix; Aminoacylation; Stereochemistry; Origin of life

1. Introduction

The biological system is mostly composed of homochiral molecules. The most well known examples of homochirality is the fact that natural proteins are composed of L-amino acids, whereas nucleic acids (RNA or DNA) are composed of D-sugars. The reason for this phenomenon continues to be a mystery, although more than 50 years have passed since the DNA double helix model was proposed (Watson and Crick, 1953), which led to the completion of human genome sequencing (International Human Genome Sequencing Consortium, 2004). No satisfactory explanations have been provided regarding the origin of the homochirality of the biological system. Proteins are ubiquitous molecules and are assembled from approximately 20 L-amino acids except in certain limited cases where D-amino acids play an essential role. Why is it so?

While considering this question, we should revisit the experiments that involved the synthesis of biomolecules such as amino acids. The most memorable one was performed by Urey and Miller in 1953 (Miller, 1953): it was a discharge experiment carried out in a putative primitive atmosphere that contained H_2, CH_4, NH_3 and H_2O (although the validity of the use of these components remains controversial), and the synthesis of several amino acids was observed. The most abundant amino acids among the constituents of natural proteins were Ala and Gly, followed by Asp and Val (interestingly, these 4 amino acids are located at the bottom column of the genetic code table). However, they did not detect any chiral preferences for any of the amino acids produced. An extreme theory known as "directed panspermia" states that the biomolecules and biosystem originated from outer space (Crick and Orgel, 1973). In fact, amino acids (Kvenvolden et al., 1970) and also a nucleobase (Stoks and Schwartz, 1979) were found in meteorites. However, the "directed panspermia" merely attributed the origin of the biomolecules to a place in the universe other than the Earth, and it cannot provide any insight into the origination of life or the homochirality of the biomolecules.

Thus far, several physical and chemical/biochemical explanations have been performed to determine the origin of L-amino acids in the biological systems. The effect of parity violation in weak interaction has been suggested, as observed in the β-decay of nucleus (Hegstrom, 1987). However, based on this parity violation effect, the enrichment of the L-enantiomer would merely be 10^{-11}. In addition, the polarized synchrotron radiation from neutron stars might have caused preordained molecular homochirarity (Bonner, 1996). It has also been proposed that enantiomeric enrichment might have occurred in an interstellar

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environment (Chyba and Sagan, 1992; Chyba et al., 1990; Oró, 1961).

However, although none of these possibilities can be completely denied, biological homochirality should be considered in terms of the evolution of life on the Earth (Bailey, 1998). I will focus on the origin of the homochirality of amino acids based on the aminoacylation of primitive tRNA. It is definitely the first step where RNA encounters amino acids not only in the current biological system and but also possibly in the prebiotic stage. I will present recent experimental evidence that strongly attributes the homochirality of amino acids to the aminoacylation of RNA in the RNA world.

2. RNA world and the origin of aminoacylation

The protein biosynthetic system involves 2 major processes—the aminoacylation of tRNA by aminoacyl-tRNA synthetases (aaRSs) and peptide bond formation on the ribosome. However, in the modern system, the players are very sophisticated and a protein is used for generation of a protein, leading us to the following question: how was the first protein produced at such a primitive stage? This is the so-called a contemporary version of the classic chicken-or-egg conundrum: that is, whether the nucleic acids originated first or the proteins. However, the discoveries that RNA not only carries genetic information but can also function as a catalyst (Kruger et al., 1982; Guerrier-Takada et al., 1983) gave rise to the "RNA world" hypothesis (Gilbert, 1986). The "RNA world" is believed to have been followed by the "protein world" in which proteins might have taken over RNAs' catalytic roles. In the course of transition from the "RNA world" to the "protein theatre", the aminoacylation of primitive tRNA would have been a critical step. Therefore, it is possible that a primitive aminoacylation system would have appeared in the RNA world.

In the present biological system, tRNAs play a crucial role in the translation of genetic information. The first step is tRNA aminoacylation in which each amino acid is attached specifically to the terminal adenosine of its corresponding tRNA by means of a cognate aaRS (Schimmel, 1987). In general, the aminoacylation of tRNA by aaRS occurs via the 2 following consecutive reactions:

$$\text{aa} + \text{ATP} + \text{aaRS} \rightarrow \text{aa-AMP} * \text{aaRS} + \text{PPi}$$

$$\text{aa-AMP} * \text{aaRS} + \text{tRNA} \rightarrow \text{aa-tRNA} + \text{AMP} + \text{aaRS}$$

where an aminoacyl adenylate is formed as an intermediate and then the activated aminoacyl group is transferred from the adenylate to the 3′-end of the tRNA to form aa-tRNA.

The L-shaped three-dimensional structure of tRNA (Robertus et al., 1974; Kim et al., 1974) has distinct functions corresponding to its 2 helical arms. The RNA oligonucleotide helices that recapitulate the acceptor stems of tRNAs retain their native functions in aminoacylation (Francklyn and Schimmel, 1989; Frugier et al., 1994; Martinis and Schimmel, 1997; Musier-Forsyth and Schimmel, 1999) and peptide bond-formation assays (Sardesai et al., 1999) suggesting that this portion (known as "minihelix") might have existed as a part of the ancient

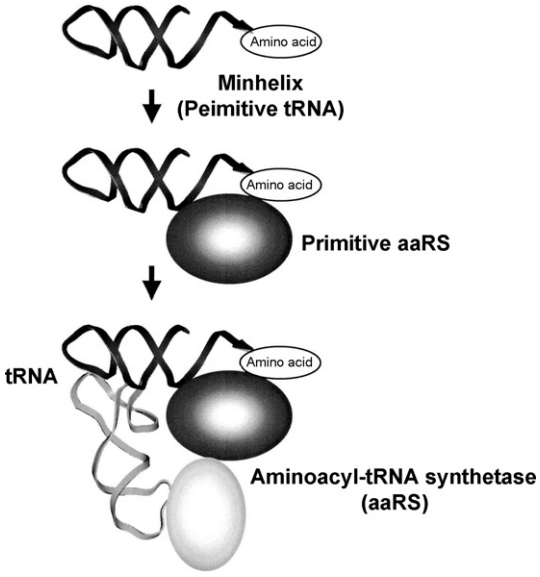

Fig. 1. Possible evolutionary processes of tRNA aminoacylation. Minihelix recapitulates the sequence and function of the tRNA acceptor arm and may represent the ancestral core of tRNA.

tRNA (Schimmel et al., 1993) (Fig. 1). Minihelix and the corresponding minimal primordial aaRS are believed to have evolved to the present-day molecules by adding another arm of tRNA and the corresponding aaRS moiety (Schimmel and Ribas de Pouplana, 1995; Schimmel et al., 1993) (Fig. 1). Thus, although tRNA aminoacylation is conducted by aaRSs, these proteins themselves are the products of tRNA aminoacylation-based translation. As mentioned above, the aaRSs synthesize a high-energy aminoacyl adenylate as the first step in the aminoacylation of tRNA in the present-day system (Schimmel, 1987), and it should be emphasized that aminoacyl adenylate is formed under prebiotic conditions (Paecht-Horowitz and Katchalsky, 1973), and also by ribozyme catalysis (Kumar and Yarus, 2001).

In the RNA world, let us suppose that the existence of aminoacyl phosphate oligonucleotide is a priori. It has been shown that oligonucleotides can be synthesized prebiotically from mononucleotides (Lohrmann et al., 1980). Therefore, the prebiotic formation of aminoacyl adenylate convinces us regarding the formation of aminoacyl phosphate oligonucleotides. How was the primitive tRNA (minihelix) aminoacylated?

3. Chiral-selective aminoacylation

In order to investigate the chiral selection of amino acids in terms of the origin of aminoacylation of tRNA, the possibility of non-enzymatic aminoacylation of an RNA minihelix was investigated (Tamura and Schimmel, 2004) (Fig. 2). Inspired by the contemporary systems that use aminoacyl phosphate (mononucleotide) adenylates as intermediates for aminoacyl tRNA synthesis (Berg, 1961; Tamura and Alexander, 2004; Tamura and Schimmel, 2003), an aminoacyl phosphate oligonucleotide and the universal CCA sequence at the 3′-end of the minihelix were designed to hybridize to a bridging oligonucleotide, thereby bringing the activated amino acid in close proximity to the amino acid-attachment site (Tamura and Schimmel, 2004)

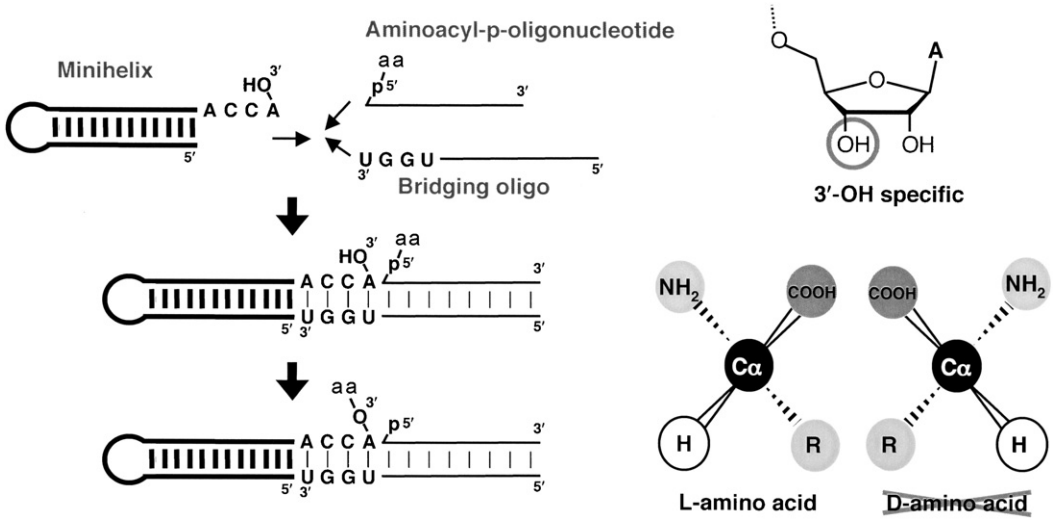

Fig. 2. Scheme for aminoacylation of an RNA minihelix with an aminoacyl phosphate oligonucleotide. This system has the 3′-OH specificity for amino acids attachment, and aminoacylation occurs with a clear preference for L- as opposed to D-amino acids.

(Fig. 2). The rationale was that since the free energy of aminoacyl phosphates hydrolysis is approximately 3 kcal mol^{-1} greater than that of the aminoacyl esters (aminoacyl-tRNAs) (Carpenter, 1960), aminoacylation would be spontaneous.

This system aminoacylated the minihelix at the 3′-end and the charging was hydroxyl-specific (Tamura and Schimmel, 2004) (Fig. 2). The minihelix substrates terminating in 2′-deoxyadenosine (dA) were aminoacylated; however, aminoacylation was not detected in the case of substrates that terminated 3′-dA (Tamura and Schimmel, 2004). Thus, the aminoacylation system exhibited a high preference for 3′-OH as the site for aminoacylation (Tamura and Schimmel, 2004) (Fig. 2). The ribozyme that aminoacylates tRNA also has 3′-OH as the aminoacylation site (Saito and Suga, 2001). In addition, in the non-enzymatic, template-directed ligation of oligonucleotides, a strong preference for 3′-5′-linkages has been indicated (Rohatgi et al., 1996). The preference for 3′-OH in the aminoacylation of an RNA minihelix is also conceivable from the extended Watson–Crick duplex. The CCA end of an RNA minihelix and the aminoacyl phosphate (deoxyribo)oligonucleotide adaptors assemble the A-form double helix containing the bridging (ribo)oligonucleotides (Arnott et al., 1986). In this conformation, the position of the aminoacyl residue that is linked to the 5′-phosphate of the oligonucleotide adaptors is closer to the 3′-OH than to the 2′-OH of the terminal adenosine.

The high preference for the 3′-OH suggested that due to the structurally constrained spatial positioning of the aminoacyl phosphate, chiral selectivity of the amino acid could be observed. In fact, this conformation confirmed the chiral-selective aminoacylation of an RNA minihelix (Tamura and Schimmel, 2004) (Fig. 2). The formation of an L-Ala-minihelix was preferred over that of a D-Ala-minihelix in a ratio of approximately 4:1; this preference is not limited to the case of Ala. The corroboration stems from the experiment that involved RNA components with opposite chirality. In the case of the "artificial" RNA molecules with the L-ribose configuration, "experiment in a mirror world", the formation of a D-Ala-minihelix was preferred over that of an L-Ala-minihelix. The ratio of an L-Ala-minihelix to a D-Ala-minihelix was the reciprocal of the value that was determined for the same when RNA composed of D-ribose was used (Tamura and Schimmel, 2004).

A possible explanation for the higher aminoacylation efficiency in the case of L- as opposed to that of D-aminoacyl phosphate oligonucleotides is the inherent difference in the stability of hydrolysis of the 2 substrates. However, on comparing the spontaneous hydrolysis of the 2 nucleotides under the conditions used for the aminoacylation reactions, no significant difference was observed (Tamura and Schimmel, 2004).

The approximate 4-fold difference between the preferences for one enantiomer over the other corresponds to a difference of approximately 0.8 kcal mol^{-1} energy. If repeated several times, this difference in energy is sufficient to confer an advantage to the aminoacylation with one stereoisomer over the other.

4. Perturbation of the reaction

In the aminoacylation reaction, the oxygen in the 3′-OH of the terminal adenosine in the minihelix attacks the carbonyl carbon of the aminoacyl phosphate linkage. The approach of a nucleophile (Nu) to the carbonyl carbon can be described by the Bürgi-Dunitz angle that is defined as the Nu-C=O angle of approximately 105° (Bürgi et al., 1974).

In the original reaction, the amino acids were attached to the 5′-phosphate group of 5′-p-dT_6dA_2 and the dT at the 5′-end of the oligonucleotide was base paired with the A of the bridging oligo (5′-$U_2A_6UG_2U$) in a Watson–Crick manner of base pairing (Tamura and Schimmel, 2004) (Fig. 3). The helix conformation, particularly near the amino-acid attachment site, could be an important factor in considering the mechanism of the aminoacylation system. Considering this, dT-A was substituted with a wobble base pair (dT-G) (Tamura and Schimmel, 2006) (Fig. 3). Surprisingly, this substitution sharply reduced the yield of the L-Ala-minihelix, without altering the production of the D-Ala-minihelix (Tamura and Schimmel, 2006) (Fig. 3).

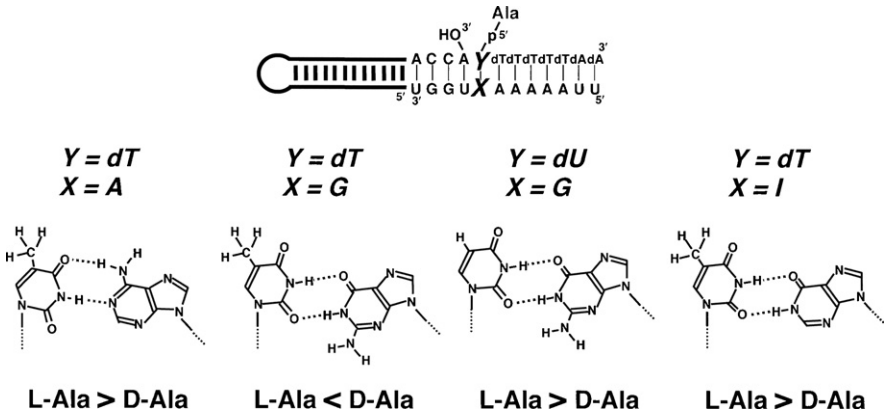

Fig. 3. The comparison of the aminoacylation using Watson–Crick (dT-A) and wobble base pairing (dT-G, dU-G, dT-I) at the position closest to the amino acid-attachment site. The inequalities at the bottom show the chiral-preferences of the amino acids in each reaction.

Introduction of dT-G instead of dT-A rendered the helix distorted, and CH_3 in the thymine shifts its position toward the outside of the helix (due to the wobble base pairing) (Fig. 4). The decrease in the L-Ala aminoacylation of the minihelix suggests a potential clash of the CH_3 of dT with the CH_3 of L-Ala (Tamura and Schimmel, 2006) (Fig. 5). In contrast, the CH_3 of the D-Ala is believed to be located at a great distance from the CH_3 of the thymine of dT-G (Fig. 5). These positions are consistent with the preference of the L-Ala for the original reaction (Tamura and Schimmel, 2004), which ensures that the CH_3 of D-Ala is located much closer to the terminal adenosine of the minihelix, possibly causing some clash between them during the amino acid-transfer step (Fig. 5). The ablation of the CH_3 of the ring due to the substitution of dU-G with a dT-G pair yielded a construct in which the chiral preference for L-Ala was retained (Tamura and Schimmel, 2006) (Fig. 3). These results are consistent with the idea regarding the clash between the ring and the L-amino acid methyl groups (Fig. 5).

Next, in order to investigate the role of the NH_2 of guanine in a wobble base pairing (dT-G) closest to the amino acid-attachment site, inosine (I) was used instead of guanine at the wobble pairing position (dT-I) (Tamura and Schimmel, 2006) (Fig. 3). The aminoacylation of the minihelix with L-Ala was more efficient than with D-Ala (Tamura and Schimmel, 2006) (Fig. 3); this result was the same as the original reaction (Tamura and Schimmel, 2004). The distortion of the helix caused by dT-G and dT-I are believed to be identical, and the positions of the CH_3 of the thymine in dT-G and dT-I should be the same, suggesting that Ala is positioned differently in the case of dT-G and dT-I (Tamura and Schimmel, 2006). Possibly, the CH_3 of Ala is positioned at a greater distance from the CH_3 of thymine in the case of dT-I than in the case of dT-G.

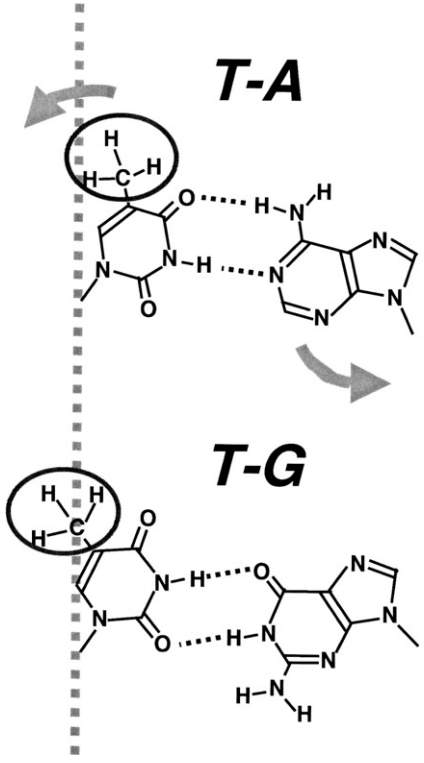

Fig. 4. The introduction of dT-G wobble base pair causes a shift in the position of CH_3.

5. Importance of the local conformation

The differences in the positioning of Ala could be due to the different sugar puckers of dT (Tamura and Schimmel, 2006). In a 3′-endo conformation, the C4′–C5′ bond of the ribose is leaned, compared to that in the case of 2′-endo conformation

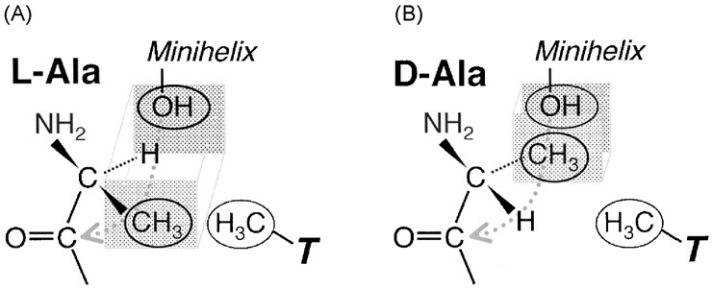

Fig. 5. Schematic representation of the positioning of (A) L-Ala and (B) D-Ala with respect to the 3′-OH of the minihelix and CH_3 of thymidine. The rectangular parallelepiped was used to show the depth of the space. The arrow indicates the nucleophilic attack of 3′-O of the minihelix.

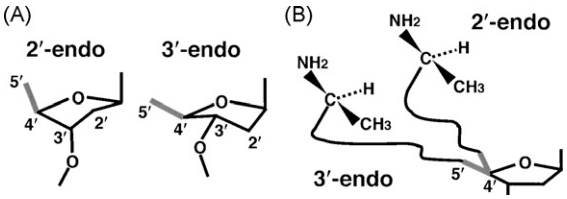

Fig. 6. (A) Schematic representation of the pucker of ribose ring (Saenger, 1984). (B) Possible differences of the spatial positioning of L-Ala based on the pucker differences of the ribose (modified from Tamura and Schimmel, 2006).

(more upright) (Fig. 6A). Due to the differences in the directions of the C4′–C5′ bond, the Ala connected to the 2′-endo sugar (through aminoacyl phosphate bond) could be positioned closer to the CH_3 of the thymidine than the Ala connected to the 3′-endo sugar (Fig. 6B). A 3′-endo pucker preference can be created by effecting a ribose 2′-*O*-CH_3 substitution (Venkateswarlu et al., 1999) (Fig. 7). Accordingly, a dT(2′-*O*-methyl)-G pair was introduced, and chiral-selective aminoacylation in the favor of the L-Ala product was restored (Fig. 7). This result suggests that the decrease in the aminoacylation of L-Ala in the case of dT-G is due to the steric hindrance between the methyl group of L-Ala and the methyl group of the thymidine caused by the combination of the wobble torsion and puckering of the the thymidine of dT-G.

The RNA bridging oligo and the aminoacyl phosphate deoxyribooligonucleotide exist as an A-form double helix (Arnott et al., 1986), and the puckering of the sugars assumes a 3′-endo conformation (Saenger, 1984). Although the dT of dT-I necessarily have a 3′-endo conformation similar to the normal A-form double helix, the dT of dT-G might assume a 2′-endo conformation (Fig. 8). A 2′-endo preference is associated with a dT-G wobble; this could in part be because of the water bridge between the 2-amino of G and the 3′-O of the ribose of dT (Tamura and Schimmel, 2006) (Fig. 8). The water bridge could fix the conformation of the thymidine into 2′-endo; this resulted in the position the CH_3 of L-Ala being much closer to the CH_3 of the thymine as compared to the case of dT-I, which has a 3′-endo conformation (Fig. 8). The NMR structure of the

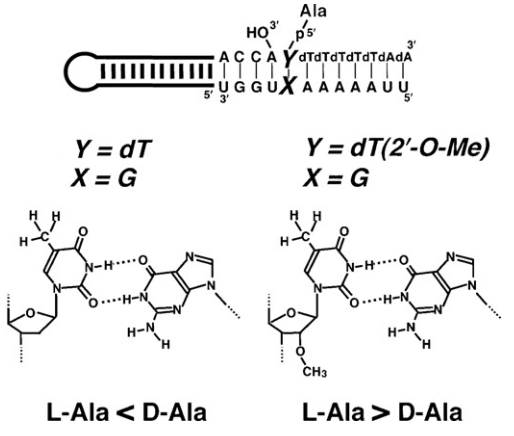

Fig. 7. The comparison of the aminoacylation using wobble base pairing (dT-G, dT(2′-*O*-Me)-G) at the position closest to the amino acid-attachment site. The inequalities at the bottom show the chiral-preferences of the amino acids in each reaction.

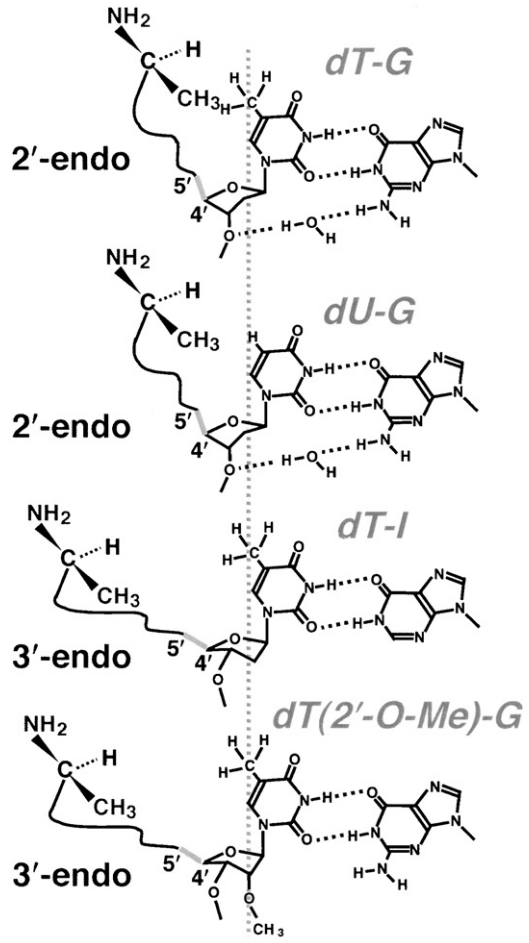

Fig. 8. Possible conformations of the position closest to the amino acid attachment site; dT-G, dU-G, dT-I, dT(2′-*O*-Me)-G. A sugar-pucker is sensitive to steric clash between the pendant group of a base and the amino acid side chain.

oligonucleotiedes containing dT-dG has been analyzed, and the dT has been observed to exhibit the 2′-endo conformation (Isaacs et al., 2002). Surprisingly, the distance between the 3′-O of T and the H of the amino group of G is 7.2 Å, which is perfect to accommodate a water molecule to form the hydrogen bonds (Isaacs et al., 2002).

6. Real conformation in the chiral-selective reaction

The above results show that the CH_3 of L-Ala is distal to the 3′-OH of A, while the CH_3 of D-Ala crowds this 3′-OH (Tamura and Schimmel, 2006) (Fig. 5). In order to confirm the relative positioning of Ala shown in Fig. 5, *N*-acetyl-Ala was also used. This selectivity was similar to that of non-acetylated Ala (Tamura and Schimmel, 2006). The most stable isomer of Gly-Na^+ is known to contain the metal ion complexed between the carbonyl and amine groups in a bidentate mode (Cerda et al., 1998). In addition, the bidentate coordination of Na^+ between the 2 carbonyl *O*-atoms of *N*-acetyl-Gly has been indicated (Cerda et al., 1998), suggesting that Ala and *N*-acetyl-Ala attached to the oligonucleotides via acyl phosphate bonds have a similar conformation in the non-enzymatic aminoacylation of an RNA minihelix. This is because the reaction system contains Na^+ (Tamura and Schimmel, 2004, 2006). These conformations in

both Ala and *N*-acetyl-Ala are consistent with their identical chiral selectivity. In these situations, the amino group of L-Ala could be located on the outside of the helix (Fig. 5). The positioning of the 3′-O immediately prior to a nucleophilic attack could be crucial in determining the efficiency of the transition intermediate formation.

The Ala attached via an acyl phosphate bond is believed to possess a very flexible conformation because most of the bonds are single bonds. However, out of the very flexible conformations, the aminoacylation reaction occurs only in a suitable and specific conformation (reactive conformation) since there are certain restrictions in the regulation of the reaction (Bürgi-Dunitz angle (Bürgi et al., 1974)). At least in "reactive conformation", the positioning of Ala could be predicted to be not far from the above indications. In contrast, the reaction does not occur in other conformations (non-reactive conformation). These results support the notion that subtle, chemical and/or structural parameters determine the chiral-selectivity that occurs most probably during the aminoacyl transfer step.

7. Origin of L-amino acids

Thus, the aminoacylation of an RNA minihelix could have determined the homochirality of amino acids in biological systems (Tamura and Schimmel, 2004). The preference for the L-amino acids is quite apparent (4 times more favorable than that for the D-amino acids) as compared to those observed in other cases (parity violation effect, etc. (Bonner, 1996; Chyba and Sagan, 1992; Chyba et al., 1990; Hegstrom, 1987; Oró, 1961)). Once the L-amino acids were selected in the primitive aminoacylation system, it is straightforward that proteins, which are generated from the L-amino acids bearing tRNA, are composed of L-amino acids. The peptide bond-forming machinery could have evolved from the prebiotic peptide synthesis in a simple manner and then developed into the modern ribosome (Tamura and Alexander, 2004; Tamura and Schimmel, 2001, 2003).

In this context, it is noteworthy that the contemporary RNA is composed of D-ribose and not L-ribose because the chirality of the ribose of RNA would be the determinant of the homochirality of amino acids. This raises another issue: what is the origin of D-ribose in the RNA world?

In order to answer this question, the process of prebiological RNA synthesis should be considered. Functional RNA molecules could have been synthesized by repeated oligomerization of short nucleotides. Therefore, the next issue to be addressed is how D-ribose-based RNA was selected over L-ribose-based RNA.

Using activated guanosine 5′-mononucleotides, Joyce and Orgel and their co-workers demonstrated chiral selection in the template-directed oligomerization of nucleotides (Joyce et al., 1984). The D- and L-isomers of guanosine 5′-phosphorimidazole or guanosine 5′-phosphor-2-methylimidazole were added to the poly C system, which was composed of D-ribose. They demonstrated that only the D-isomers could be oligomerized (Joyce et al., 1984). Thus, poly C-directed oligomerization of activated guanosine mononucleotides proceeds readily if the monomers are of the same optical handedness as the template (Joyce et al., 1984). Eschenmoser and his co-workers attempted to perform template-directed auto-oligomerization using the short pyranosyl-RNA oligonucleotide 2′,3′-cyclophosphates (Bolli et al., 1997). All possible combinations of homochiral and heterochiral diastereomers were used; finally, oligomerization proceeded chiroselectively, that is, the final products became homochiral oligomers (Bolli et al., 1997). This experiment gives us an insight into the origin of D-isomer RNA.

Let us consider a mixture consisting of racemic enantiomer pairs and that the short oligonucleotides are to co-oligomerize stochastically by chirospecific ligation to D- and L-libraries each consisting of higher oligomers. In such a situation, these 2 libraries would consist of equal amounts of homochiral all-D and all-L oligomers. However, symmetry breaking would occur inevitably because in the formation of both the D- and L-libraries, the number of possible sequences with growing oligomer length would be far beyond the number of sequences actually formed (Fig. 9). If the co-oligomerization proceeded stochastically, any given sequence in both libraries would actually occur only once. Thus, the sequence composition of both libraries would not be identical, suggesting that in principle, these 2 libraries have different chemical properties (Fig. 9). If the system were to evolve eventually against internal and external selection pressures, the emerging winner sequences would be homochiral and would belong to either the D- or L-library.

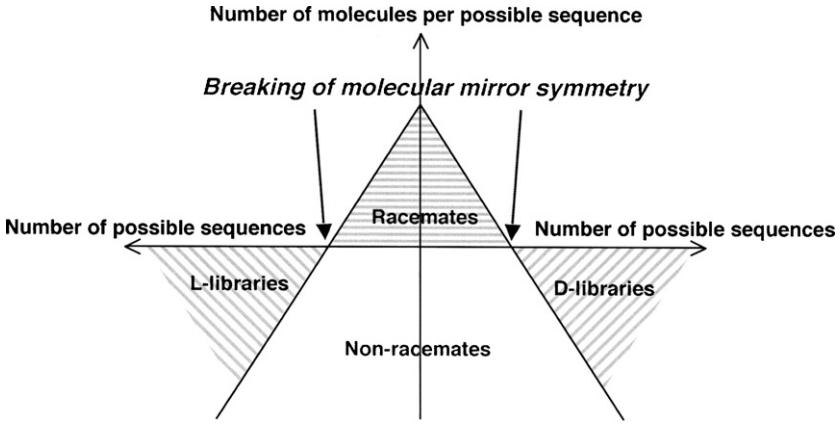

Fig. 9. Symmetry breaking by stochastic chiroselective co-oligomerization in oligonucleotides (modified from Bolli et al., 1997).

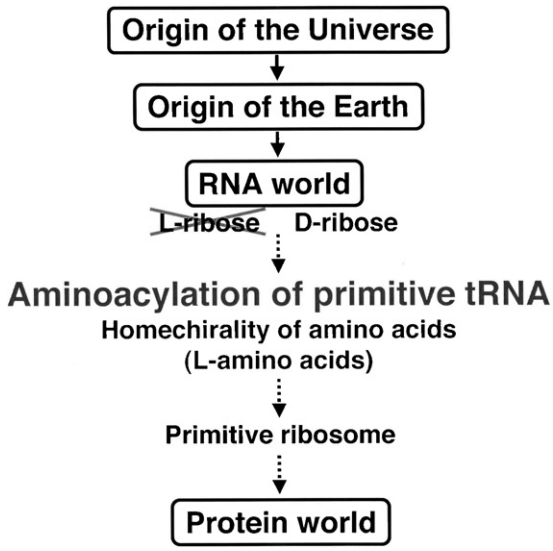

Fig. 10. Possible evolutionary process of the origin of amino acid homochirality.

Therefore, during these processes, RNA in the D-libraries that exhibited an important chemical ability (for the subsequent evolution of the biological system thereafter) would have been selected (Fig. 10). For example, in a constructed homochiral RNA world, RNA molecules with different sequences (belonging to either the D- or L-library) could have catalyzed different reactions. A ribozyme may have accidentally evolved in the D-libraries for the specific catalysis of the formation of D-sugars before such a ribozyme evolved in the L-libraries for the catalysis of L-sugars. Thus, the D-libraries would have gained a kinetic advantage over the L-libraries, thereby tipping the balance toward the preferential use of L-amino acids. If the RNA world hypothesis holds true, D-ribose-based RNA could have been used to select L-amino acids (Fig. 10). Therefore, the specificity of the RNA sequences may have been a critical factor in the selection of D-nucleotides and L-amino acids, and this would also have been strongly related to the appearance of the genetic code (Root-Bernstein, 2007).

8. Conclusion

The origin of the homochirality of amino acids is still an unsolved issue. As physicists insist, the intrinsic property of elementary particles might help in providing an answer to the theme. However, as described, we should focus on the peculiarity of the evolution of the aminoacylation of tRNA in the biological system. tRNA aminoacylation is definitely the first step where the amino acids encounter the RNA, and the non-enzymatic aminoacylation of an RNA minihelix described here clearly shows chiral-selective results (preference for L-amino acids). Chiral selectivity occurs as a result of the spatial constraints of the molecules participating in the reaction. Minihelix corresponds to one-half of the modern tRNA and is believed to be an ancient form of tRNA. The formation of the L-aminoacylated-minihelix might have been used to synthesize primitive proteins that were eventually composed of L-amino acids, possessing the same homochirality as the present-day proteins (Fig. 10).

Several lines of evidence suggest that the RNA world really existed in the primitive stage of biological evolution. Thus, once the D-ribose-based RNA world was established, the L-amino acids could have been selected during the aminoacylation using the mechanism shown here (Fig. 10). Why then did D-ribose exist in the RNA world? It occurred by "chance and necessity": symmetry breaking in homochiral RNA formation and the appearance of a specific sequence in the D-nucleotide libraries. There must have been a definite process to ensure that the sequence-based mechanism functioned in the RNA world. Future experiments will provide insights regarding the basis using which this mystery can be solved. Since the Earth rotates around its own axis, some special effects of this rotation (for example, the Coriolis force) might have influenced the origin of life on the Earth. If this is true, we might be able to specify at least the hemisphere of the Earth in which life originated.

Scientists often avoid addressing the issue of the origin of life and homochirality as research topics, partly because owing to certain intrinsic characteristics, this issue is considered to fall under the purview of "history". However, now is the time to experimentally address this issue. The chiral-selective aminoacylation of an RNA minihelix is a good and an important example from this standpoint. This kind of experimentation would lead to profound science.

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