Chapter 4
Prebiotic Synthesis of Bioorganic Compounds by Simulation Experiments

Kensei Kobayashi

Abstract A great number of prebiotic synthesis experiments under possible primitive conditions have been conducted since the 1950s. In most of the prebiotic synthesis experiments of the earlier era, strongly reducing gas mixtures were used as a primitive Earth atmosphere, and amino acids and other bioorganic compounds were successfully synthesized. Their formation mechanisms were explained by step-by-step modes, such as the Strecker synthesis. However, such a strongly reducing atmosphere is questioned and the contributions of extraterrestrial organics are under consideration. We learned that quite complex organic compounds could be formed under interstellar environments through the analysis of extraterrestrial samples and products of experiments simulating extraterrestrial conditions. Simulation experiments are also introduced to examine the possible origins of the homochirality of biomolecules. Experiments simulating submarine hydrothermal systems were also conducted. It is very difficult to verify the origin of life on the Earth, since relics of the prebiotic synthesis do not remain on the Earth. It would be possible, however, to examine possible origins of life in space through the synergy of planetary exploration and space experiments.

Keywords Prebiotic synthesis · Amino acids · Extraterrestrial organics · Homochirality · Submarine hydrothermal systems

4.1 Introduction

Chemical evolution is the concept used to explain that the first life was abiotically generated by the evolution of simple molecules into complicated and systemized organic compounds. Oparin and Haldane first presented this idea independently in the 1920s (Oparin 1953; Haldane 1929). It was assumed that it would be difficult to verify the chemical evolution processes experimentally in their era. In the early 1950s, however, pioneering experiments to examine chemical evolution pathways,

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K. Kobayashi (✉)
Yokohama National University, Yokohama, Japan
e-mail: kobayashi-kensei-wv@ynu.ac.jp

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including the historical spark discharge experiment by Miller (1953), were carried out. Following this experiment, a great number of experimental studies have been conducted to examine possible prebiotic chemical evolution pathways. Such works included experiments simulating the primitive Earth atmosphere, the primeval ocean, interstellar environments, and so on.

In this chapter, such experiments that were done under simulated primitive environments are introduced with a focus on our experiments, and a future perspective will be also provided.

4.2 Dawn of Experimental Prebiotic Chemistry

4.2.1 One-Pot Reactions

In the 1920s, Oparin and Haldane presented the theoretical idea of chemical evolution, but it was assumed that experimental verification of the idea would be quite difficult since such an “evolution” would require far greater time than could be provided in the laboratories of their period.

In the middle of the twentieth century, there were two hypotheses on the composition of the primitive Earth atmosphere, namely, a strongly reducing one and a nonreducing one. The former states that methane and ammonia were major constituents of the atmosphere, which was modeled based on the cosmic abundance and the atmospheres of the giant planets in our solar system (Urey 1952). In the latter theory, carbon dioxide and nitrogen were considered to be major constituents of the atmosphere, as seen on present terrestrial planets like Venus and Mars (Abelson 1966).

Garrison et al. (1951) performed simulation experiments based on the latter (nonreducing) atmospheric model. They irradiated aqueous solutions containing CO_2 and Fe^{2+} with high-energy helium ions from an accelerator to simulate the action of alpha rays resulting from radioactive nuclides in the primeval ocean. They detected formaldehyde (HCHO) and formic acid (HCOOH) in the products, but could not find evidence of important bioorganic chemicals such as amino acids since the system did not contain nitrogen.

Two years later, Miller (1953) followed the ideas of Urey (his supervisor) in that the primitive Earth atmosphere was strongly reducing and performed historical spark discharge experiments in a gas mixture of methane, ammonia, hydrogen, and water. In his first paper, five amino acids, namely, glycine, alanine, aspartic acid, β-alanine, and α-aminobutyric acid, were detected in the aqueous products by paper chromatography.

Since it was so astonishing that amino acids, most important bioorganics, were formed easily from simple molecules, many scientists started research in the field of experimental prebiotic chemistry. Most of these scientists used the strongly reducing gas mixtures containing methane and ammonia, which were exposed to various energy sources (Miller and Orgel 1974). They simulated the roles of solar ultraviolet light (Sagan and Khare 1971), thermal energy from volcanoes (Harada and Fox 1964), and shock waves associated with meteor impacts (Bar-Nun et al. 1970). All of these experiments resulted in the detection of amino acids in the products of these reactions. It was shown that formation of amino acids was not difficult to achieve if the primitive Earth had a strongly reducing atmosphere. On the other hand, nonreducing gas mixtures without methane and ammonia did not produce amino acids (Schlesinger and Miller 1983). Many people in the field therefore preferred the theory of a strongly reducing atmosphere to a nonreducing one. The composition of Earth's primitive atmosphere will be discussed in Chap. 10.

Nucleic acid bases are another group of important bioorganics. Oro (1960) successfully synthesized adenine in an aqueous solution of hydrogen cyanide and ammonia. Ponnamperuma et al. (1963) found adenine when they performed an electron irradiation of a mixture of methane, ammonia, and water. It was suggested that adenine, among the nucleic acid bases, was abiotically formed most easily in reducing environments since adenine ($C_5H_5N_5$) is a more reducing base than guanine ($C_5H_5ON_5$), cytosine ($C_4H_5ON_3$), uracil ($C_4H_4O_2N_2$), and thymine ($C_5H_7O_2N_2$).

Sugars (carbohydrates) could be formed from a formaldehyde aqueous solution with a mild alkaline catalyst via a reaction known as the formose reaction (Butlerow 1861). There are, however, problems in synthesizing ribose, the sugar used in RNA, using the formose reaction under prebiotic conditions. These issues stem from the required high concentration of formaldehyde, and the yield of ribose was generally low, since so many other kinds of sugars and sugar-like compounds were formed in it.

4.2.2 Energetics and Formation Mechanisms

Table 4.1 summarizes the various energy sources available on the primitive Earth (Kobayashi and Saito 2000). Among them, the solar ultraviolet light flux is notably far larger than the others, followed by lightning (electric discharges). Amino acids could be formed by any of the energy sources listed here, and it was thus suggested that predominant energies such as solar UV and lightning were important in the abiotic synthesis of bioorganic compounds on the primitive Earth.

Miller and Urey suggested that amino acids were formed via the Strecker-type reactions in a spark discharge flask since they found hydrogen cyanide and aldehydes in their discharge products. The Strecker synthesis is a famous organic synthesis technique used to form amino acids, where hydrogen cyanide (HCN), aldehydes (RCHO), and ammonia (NH_3) are used as starting materials. These are mixed to form aminonitriles (NH_2-CHR-CN), and the subsequent hydrolysis of these aminonitriles produces amino acids (NH_2-CHR-COOH).

Table 4.1 Major energy sources for prebiotic synthesis on the primitive Earth

	Estimated flux	Reference	Amino acid formation	
Energy source	/eV m^{-2} $year^{-1}$		Strongly-reducing[a]	Mildly-reducing[b]
Solar radiation				
Total	6.8×10^{28}	1		
$\lambda < 200$ nm	2.2×10^{25}	1	+++	–
$\lambda < 150$ nm	9.1×10^{23}	1	+	–
$\lambda < 110$ nm	4.2×10^{22}	2	+	+
Thundering	1.8×10^{22}	3	++	+
	1.0×10^{24}	1		
Volcano heat	3.4×10^{22}	1	+	–
Radioactivity[c]	2×10^{23}	1	+	+
Cosmic rays	2.9×10^{21}	2	+	+++
Meteor impacts	1×10^{22}	1	+	++

Reference 1. Miller and Urey (1959), 2. Kobayashi et al. (1998), 3. Chyba and Sagan (1991)

[a] CH_4-NH_3-H_2O atmosphere

[b] CO-N_2-H_2O atmosphere

[c] 0–1.0 km deep of Earth crust

4.2.3 Step-by-Step Reaction Models

Simulation experiments using gas mixtures and various energy sources can be referred to one-pot reactions. Oro's adenine synthesis could be categorized as a one-pot reaction. Other types of experiments were also conducted on this subject. These used a selected number of starting compounds that were assumed to be present in prebiotic conditions as suggested by previous studies.

Not only was adenine detected upon the use of these experiments, but guanine was also detected by HCN polymerization (Levy et al. 1999). Pyrimidine bases (including uracil, cytosine, and thymine), however, could not be obtained from the HCN solution. Ferris et al. (1968) detected cytosine following a reaction between cyanoacetylene (CHCCN) with cyanate (HCNO). Uracil was obtained by the hydrolysis of cytosine (Miller and Orgel 1974). Thus it was noted that cyanoacetylene, not HCN, could be a candidate starting material for the production of pyrimidine bases.

After obtaining purines, pyrimidines, and sugars (ribose), the next targets for synthesis were nucleosides and nucleotides. Several studies on such syntheses were performed in the 1960s–1970s. Fuller et al. (1972) heated and dried a mixture containing a purine base (adenine, guanine, or hypoxanthine), ribose, magnesium ions, and trimetaphosphate and obtained purine nucleosides. Lohrmann and Orgel (1971) heated a mixture of uridine, inorganic phosphates, urea, and ammonium ions and obtained uridine phosphate. In both cases, these products were mixtures of isomers (e.g., α-nucleoside and β-nucleoside).

The oligomerization of amino acids and nucleosides has also been studied. Oligomerization is a condensation reaction and requires the removal of water

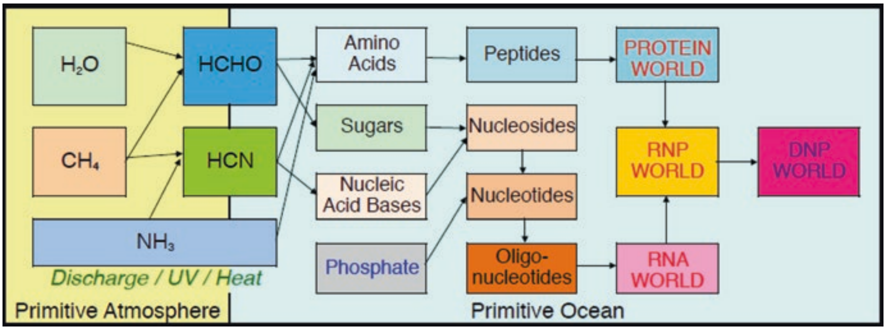

Fig. 4.1 Conventional stepwise scenario of chemical evolution

molecules from monomers. When amino acids were heated to dryness, peptides were often detected (e.g., Harada and Fox 1960). In the case of oligonucleotides, mononucleotides should have been activated. Phosphoimidazolide-activated nucleotides (ImpN) were often used in conjunction with metal ions and/or templates, and oligonucleotides were thus obtained (e.g., Sawai 1976).

After a great number of laboratory simulation experiments, a scenario outlining the processes from simple molecules to the existence of life was roughly expressed, as shown in Fig. 4.1. Here it may be referred to as a conventional stepwise scenario. Please note that each step was confirmed by the use of some in vitro simulation experiments, where high concentrations of starting materials were used without adding any inhibitors. Recent progresses in prebiotic syntheses of RNA will be introduced in Chap. 5.

4.3 Formation and Delivery of Extraterrestrial Organic Compounds

In the 1970s, it was recognized that ammonia in a terrestrial atmosphere was easily decomposed by solar UV radiation. A mixture of methane and nitrogen (N_2) was often used in experiments simulating abiotic synthesis in the primitive Earth atmosphere, and amino acids were still formed using such energies as spark discharges (Ring et al. 1972; Kobayashi and Ponnamperuma 1985b). However, the formation of amino acids by solar UV radiation was not achieved, since nitrogen cannot be dissociated by near UV light (Ferris and Chen 1975; Bar-Nun and Chang 1968). In the 1980s, planetary explorations of the solar system provided new insight into the formation of planets: they were formed by the collision of planetesimals, which lead to the formation of mildly reducing secondary planetary atmospheres (Abe and Matsui 1986a, b, Kasting 1990; Catling and Kasting 2017). Schlesinger and Miller (1983) performed spark discharge experiments using CH_4-N_2-H_2O, CO-N_2-H_2O, and CO_2-N_2-H_2O type gas mixtures, and found that mixtures containing CO or CO_2

produced a much smaller amount of amino acids than CH_4. Thus it is supposed that the formation of amino acids and other bioorganic compounds in the mildly reducing atmosphere is limited.

About the same period, a wide variety of organic compounds were found in extraterrestrial environments, such as in meteorites and comets. Kvenvolden et al. (1970) found amino acids in the Murchison meteorite, which was a CM2 carbonaceous chondrite that fell in Australia in 1969. The amino acids found in it were racemic mixtures, demonstrated their indigenousness. Complex organic compounds were also detected in cometary dusts by impact ionization mass spectrometry during the Vega 1 mission to Comet Halley (Kissel and Krueger 1987). Glycine was detected in a sample from Comet Wild 2 that was returned by the Stardust spacecraft (Elsila et al. 2009). These finding supported the formation of organic compounds in space and transfer to the Earth.

4.3.1 Abiotic Syntheses of Amino Acids in Simulated Space Environments

There are a number of scenarios that may be used to explain how organic compounds found in meteorites and comets formed, including formation in interstellar grains and in meteorite parent bodies. The former was first proposed by Greenberg and Li (1997) as follows (Fig. 4.2): interstellar molecules including H_2O, CO, CH_3OH, and NH_3 are frozen into silicate dusts that form ice mantles in molecular clouds since the temperature inside of the molecular clouds is quite low. The ice mantles are then irradiated by cosmic rays and cosmic ray-induced ultraviolet light, and complex organic molecules are thus formed. Such organic materials were

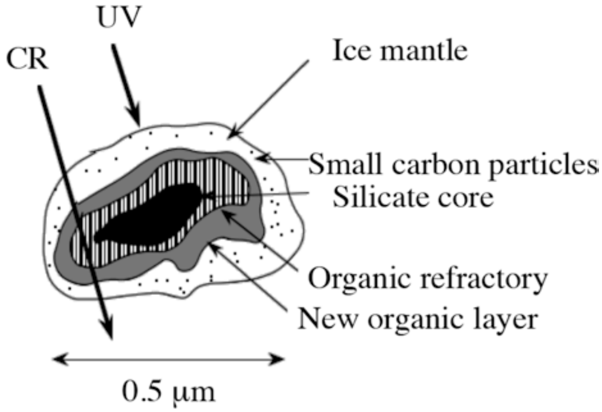

Fig. 4.2 Formation of organic compounds in interstellar dust environments. (Modified from Greenberg and Mendoza-Gomez 1993)

brought into asteroids and comets when they were formed from dusts in the early solar system.

Though it is possible to observe simple molecules such as CO and CH_3OH in the ice mantles of molecular clouds, it is not easy to detect complex molecules. Instead, a number of laboratory simulation experiments have been conducted to see what kinds of organic molecules are formed in the ice mantles. The first report of amino acid formation in simulated ice mantles was reported by Briggs et al. (1992). They irradiated a frozen mixture of H_2O, CO, and NH_3 on a metal substrate using UV light from a hydrogen lamp at 12 K. After irradiation, they warmed up the substrate to room temperature and analyzed the product residue using GC/MS. They detected glycine as well as a number of other organic molecules.

Kobayashi et al. examined the possible roles of cosmic ray particles in place of ultraviolet photons in the formation of complex organic molecules in the ice mantles. Ice mixtures of H_2O, CO, and NH_3 on metal substrates were irradiated with 3 MeV protons from a van de Graaff accelerator (Tokyo Institute of Technology) (Fig. 4.3), and it was found that several amino acids existed in the hydrolyzed products (Kobayashi et al. 1995; Kasamatsu et al. 1997). The energy of the cosmic rays is considered too high and passes through the ice mantles with little interaction with other molecules. Kobayashi et al. (2010) used 290 MeV/u carbon beams, whose energy is close to typical cosmic rays that exist in space, which were generated by HIMAC accelerator (National Institute of Radiological Sciences). A frozen mixture of H_2O, CH_3OH, and NH_3 at 77 K was irradiated with carbon beams, and the resulting product was acid hydrolyzed and was subjected to amino acid analysis. A number of amino acids were detected by HPLC and/or GC/MS. It was shown that cosmic ray particles, which deposit only a small part of their energy, can form complex organic molecules including amino acid precursors.

Since UV sources are more easily available than accelerators, more UV irradiation experiments have been performed than particle irradiation experiments, and

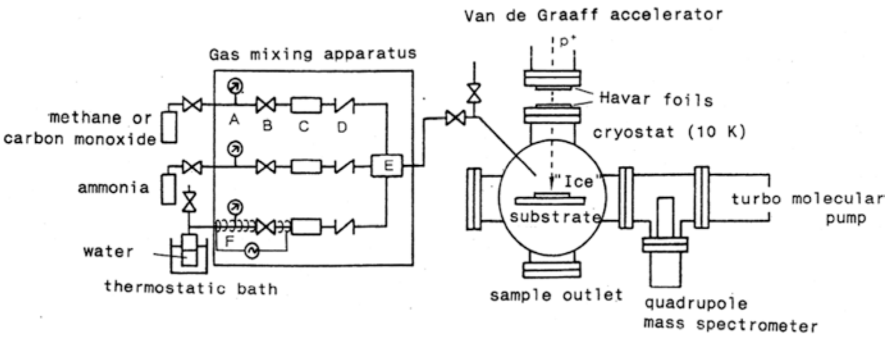

Fig. 4.3 Proton irradiation of a simulated ice mantle of interstellar dust particles (ISDs). (**a**) Pressure gauge, (**b**) needle valve, (**c**) thermal mass-flow meter, (**d**) control valve, (**e**) mixer. A gas mixture of CO (or CH_4), NH_3, and H_2O was frozen on a metal substrate located in a cryostat and was irradiated with high-energy protons from a van de Graaff accelerator via Havar foils. (Modified from Kobayashi et al. 1995)

many kinds of racemic amino acids were identified in the irradiation products after hydrolysis (Munos Caro et al. 2002; Bernstein et al. 2002). These experiments support the formation of amino acids in space environment.

4.3.2 Abiotic Syntheses of Amino Acids and Insoluble Organic Matter in Simulated Meteorite Parent-Body Environments

Most of the organic carbon found in carbonaceous chondrites is often referred to as IOM (insoluble organic matter), which has quite complex macromolecular structure (Pizzarello 2006). One of the possible scenarios of the formation of IOM is via aqueous alteration or a hydrothermal reaction in the parent bodies of the meteorites. Cody et al. (2011) proposed aqueous solution of formaldehyde could yield insoluble organic matter since water ice melted via heat provided by the decay of ^{26}Al. The IOM obtained by their experiments had a structure similar to the IOM found in carbonaceous chondrites. Further studies showed that the incorporation of ammonia in this reaction could produce organic solids containing imidazole, pyridine, and pyrrole structures (Kebukawa et al. 2013).

Amino acids were also found from similar experiments. When a mixture of formaldehyde, glycolaldehyde (CH_2OHCHO), ammonia, and water with an alkaline catalyst ($Ca(OH)_2$) was heated at 90–250 °C for 72 h, both water-soluble and insoluble organic compounds were formed. The soluble fraction produced amino acids after acid hydrolysis (Kebukawa et al. 2017). It was shown that both IOM and amino acid precursors could be formed in the interior of meteorite parent bodies in the early stages of the solar system.

4.3.3 Formation of Enantiomeric Excesses of Amino Acids in Extraterrestrial Environments

Terrestrial organisms generally use L-amino acids (except achiral glycine) to synthesize proteins, while abiotically formed amino acids were fundamentally racemic mixtures, i.e., a 1:1 mixture of D- and L-amino acids (Fig. 4.4). Why then were only L-amino acids selected when life was born on the Earth? The riddle of the origin of the homochirality of these amino acids (and other biomolecules) has been one of the largest subjects in the study of chemical evolution toward the generation of life. Though a number of hypotheses for this have been presented (Bonner 1991), most of them have not been supported by cosmogeochemical evidence.

Amino acids found in meteorites were thought to be fundamentally racemic mixtures since they are formed abiotically. Cronin and Pizzarello (1997) reported that some of the amino acids found in the Murchison meteorite had L-enantiomer excesses. Though L-excesses of protein amino acids have been judged to stem from

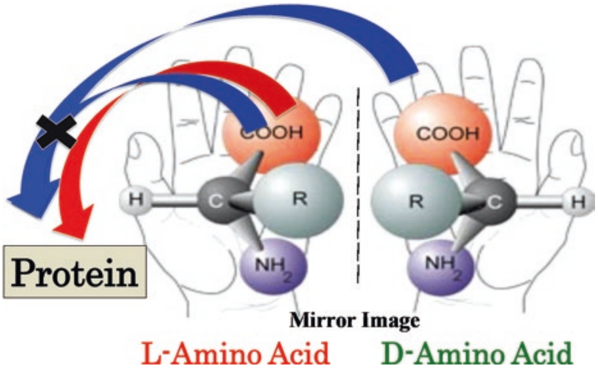

Fig. 4.4 Amino acid enantiomers. (Modified from Kobayashi et al. 2010)

contamination from the terrestrial biosphere, this group showed that α-methyl amino acids such as isovaline also have some L-excesses. This tendency has been confirmed by several investigators (Elsila et al. 2016). It was suggested that these enantiomeric excesses (ee's) of amino acids were generated by physical asymmetry in space. Among several candidates, circularly polarized ultraviolet light (CPL-UV) has been considered most promising as the cause of this physically asymmetric formation. CPL-UV from neutron stars was first noted as a potential cause, but there is little chance to be exposed to CPL-UV, since it is highly directional. On the other hand, widely distributed CPL regions were recently found (Fukue et al. 2010), which are more plausible triggers for asymmetric formation of amino acids in space.

The generation of ee's of amino acids by CPL-UV has been often explained by asymmetric decomposition. One of the amino acid enantiomers decomposes more than the other after irradiation of the left- or right-handed CPL-UV, which was shown theoretically and experimentally (Inoue 1992). A problem in this scenario is that UV photons may have decomposed most of the amino acids to obtain significant ee's of the amino acids resulting in a few remaining quantity.

Takano et al. (2007) irradiated not free amino acids, but amino acid precursors using CPL from a synchrotron. Amino acid precursors were synthesized from CO, NH_3, and H_2O by proton irradiation, which were quite complex organic molecules (Takano et al. 2004). After right-CPL was irradiated on the complex amino acid precursors, a small amount (0.44%) of ee's of D-alanine was detected, while the left-CPL resulted in 0.65% of ee's of L-alanine. The detected ee's were small but statistically significant. It is notable that the yield of amino acids after hydrolysis following CPL-UV irradiation was not greatly changed from unirradiated precursors. It can be shown that the amino acids were not asymmetrically decomposed but rather asymmetrically altered (Fig. 4.5).

Marcelus et al. (2011) also found such ee's after a frozen mixture of CH_3OH, NH_3, and H_2O (80 K) was irradiated with CPL-UV. It was found that the amino acid

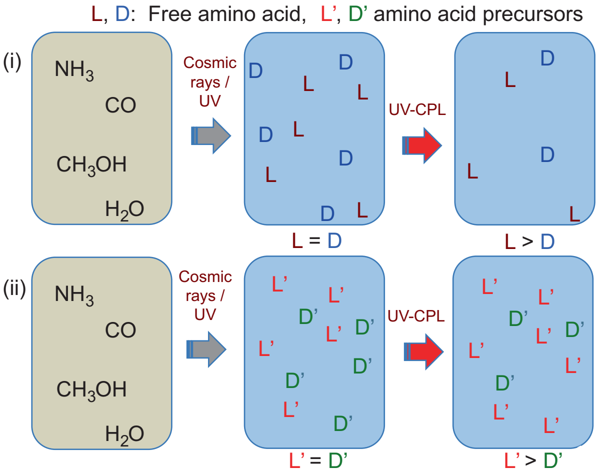

Fig. 4.5 Generation of enantiomeric excesses of amino acids in space. (*i*) Conventional asymmetric decomposition of free amino acids. More free D-amino acids are decomposed by irradiation with circularly-polarized light (CPL) than free L-amino acids. (*ii*) Newly proposed asymmetric alteration of amino acid precursors. Complex organics were formed in interstellar space having both L- and D-amino acid moieties equally. A part of the D-amino acid moieties were converted to the L-amino acid moieties by irradiation with CPL

precursors were formed in the ice by UV irradiation, and further CPL-UV radiation at room temperature formed 0.71–1.34% ee's.

In the CPL-UV-triggered chirogenesis hypothesis, the chance to generate an L-amino acid favoring world is 50%, since there are both left-CPL regions and right-CPL regions in space (Fukue et al. 2010). Another group of hypotheses are those based on the parity violation. One example of the parity violation is that electrons generated during β-decays are always left-spin polarized. It is expected that spin-polarized electrons can induce a similar effect as CPL on amino acid decomposition/alteration. Compared to the CPL experiments, spin-polarized electron experiments are difficult. One possible way to examine the effects of these electrons is to use natural β-ray sources, which produce left spin-polarized electrons. When racemic mixtures of amino acids were irradiated with β-rays from a strong ^{90}Sr-^{90}Y source, optical activity was observed in the irradiated samples, but not in the samples taken before irradiation (Burkov et al. 2008; Gusev et al. 2008). Further experiments are needed in this field, particularly those with right spin-polarized electrons.

4.3.4 Delivery of Extraterrestrial Organic Compounds

We are now sure that there are a wide variety and a large amount of organic compounds including amino acid precursors and nucleic acid bases in space. The next question is how to deliver these compounds to the primitive Earth. Though we know that some carbonaceous chondrites have delivered organic compounds safely to the Earth, organics in larger meteorites would have decomposed during bolide impacts. It was suggested that cosmic dusts could have delivered more organics than meteorites and comets (Chyba and Sagan 1992; Barbier et al. 1998). Cosmic dusts are, however, so tiny that they are exposed to strong solar UV radiation during their stay in space. Cosmic dusts (micrometeorites) have been collected in the terrestrial biosphere such as in ice from Antarctica, and they have been found to contain a high percentage of organics, but most of these are complex insoluble organic matter (IOM). It is difficult to conclude that they could carry such bioorganics as amino acids since they have been in contact with terrestrial materials.

Yamagishi et al. (2009) have been conducting a space experiment named Tanpopo by utilizing the International Space Station since 2015. In this mission, super low-density silica aerogel is exposed on the Exposed Facility of the Japanese Experiment Module (JEM-EF) and is collecting dusts flying near the ISS. Amino acids from the captured dusts will be analyzed to examine the scenario that extraterrestrial amino acids and other bioorganics were delivered by cosmic dusts.

4.4 Abiotic Synthesis and Alteration of Organic Compounds in Simulated Primitive Earth Environments

Though organic compounds including amino acid precursors could have been formed either in interstellar space or in planetary atmospheres, life cannot be generated there. Since water is essential for life and a major molecule consisting terrestrial organisms is water, it is natural that terrestrial life was first generated in a hydrosphere – either in the ocean or in land water. Cold or warm (up to 100 °C) water media having plenty of sunlight were considered as sites of the generation of life on the Earth up until the 1970s.

4.4.1 Prebiotic Synthesis in Simulated Submarine Hydrothermal Conditions

Corliss et al. (1979) first discovered submarine hydrothermal vents at the Galapagos spreading center. Superheated seawater over 300 °C was erupting from chimneys on the deep seafloor. Such submarine hydrothermal systems have a number of merits for their use as sites for chemical evolution toward the origin of life: (1) the hydrothermal fluid contains such reducing species as hydrogen, methane, and ammonia, maintaining reducing environments even at present on Earth; (2) seawater is superheated by magma and then quenched when it erupts into cold seawater; (3) the hydrothermal fluid contains high concentrations of essential metal ions such as iron, manganese, and zinc, which can work as biotic and prebiotic catalysts (Kobayashi and Ponnamperuma 1985a, b); (4) the deep sea is a dark world, where organic compounds were free from decomposition from strong solar UV radiation before the formation of the ozone layer. It was also suggested that the last universal common ancestor (LUCA) or commonote of terrestrial organisms were hyperthermophiles, as suggested by the 16S rRNA universal phylogenetic tree (Pace 1991), which agrees with the idea that the first terrestrial life was generated near superheated media (see also Chap. 7).

In the earlier stages of experiments simulating submarine hydrothermal systems, autoclaves were often used, where possible starting materials were dissolved in water and heated at a high pressure following pressurization. For example, Yanagawa and Kobayashi (1992) heated an aqueous solution containing various metal ions (Fe^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Cu^{2+}, and Ba^{2+}) and NH_4^+ at 325 °C for 1.5–12 h in an autoclave under pressurization by an 80 kg cm^{-2} gas mixture of CH_4 and N_2. The resulting products contained amino acids following hydrolysis.

Imai et al. (1999) developed a flow reactor simulating submarine hydrothermal systems. An aqueous solution of glycine was injected into the reactor and heated at 250 °C and then quenched at 0 °C, which yielded a hexamer of glycine in the solution. Kurihara et al. (2012) examined the possible formation of organic aggregates in simulated submarine hydrothermal systems. First a mixture of CO, N_2, and H_2O was irradiated with high-energy protons to simulate possible organic formation in a primitive atmosphere. Water-soluble complex organic compounds containing amino acid precursors were formed. The products were heated at 300 °C under a pressure of 25 MPa and then quenched to 0 °C in a flow reactor. The resulting products contained organic aggregates with amino acid precursors. These results suggested that it was possible that in submarine hydrothermal systems, the dehydration condensation of biomonomers and the formation of organic aggregates in an aqueous solution had taken place.

4.4.2 Reconsideration of the Stepwise Scenario of Chemical Evolution

It has been controversial as to whether proteins and RNA were formed earlier than other organic materials. After the discovery of ribozymes (Krueger et al. 1982), Gilbert (1984) proposed the hypothesis that the first terrestrial life was based on RNA only, which is known as *the RNA World hypothesis*. Since it was proven that RNA cannot only store and replicate genetic information but also catalyze chemical reactions, the RNA World hypothesis is quite fascinating (see Chap. 6). It has been claimed, however, that abiotic synthesis of RNA is much more difficult than that of proteins. The synthesis of proteins requires two steps: (1) synthesis of amino acids and (2) polymerization of amino acids. On the other hand, the synthesis of RNAs requires five steps: (1) synthesis of nucleic acid bases, (2) synthesis of ribose, (3) synthesis of nucleosides from bases and ribose, (4) synthesis of nucleotides from nucleoside and phosphate, and (5) polymerization of nucleotides. Among these steps, the abiotic synthesis of nucleoside is the most difficult.

A great number of abiotic synthesis experiments have been conducted, most of which are based on the stepwise scenario of chemical evolution, as shown in Fig. 4.2. It could be said that each step is possible in vitro, but usually high concentrations of the starting materials are required to obtain the products. Powner et al. (2009) proposed a novel pathway of nucleotide synthesis in "prebiotically plausible" conditions (Fig. 4.6). Here they did not use nucleic acid bases and ribose as starting materials, but instead used high concentrations of starting molecules without any possible inhibitors and changed the reaction conditions of each reaction step (see Chap. 5 in detail).

Generally speaking, the prebiotic formation of oligopeptides is easier than that of oligonucleotides, since amino acids could be abiotically synthesized more easily than nucleotides. A great number of experiments have been conducted to condense amino acids via heating (Fox and Harada 1960), the use of clays as catalysts (Bujdák

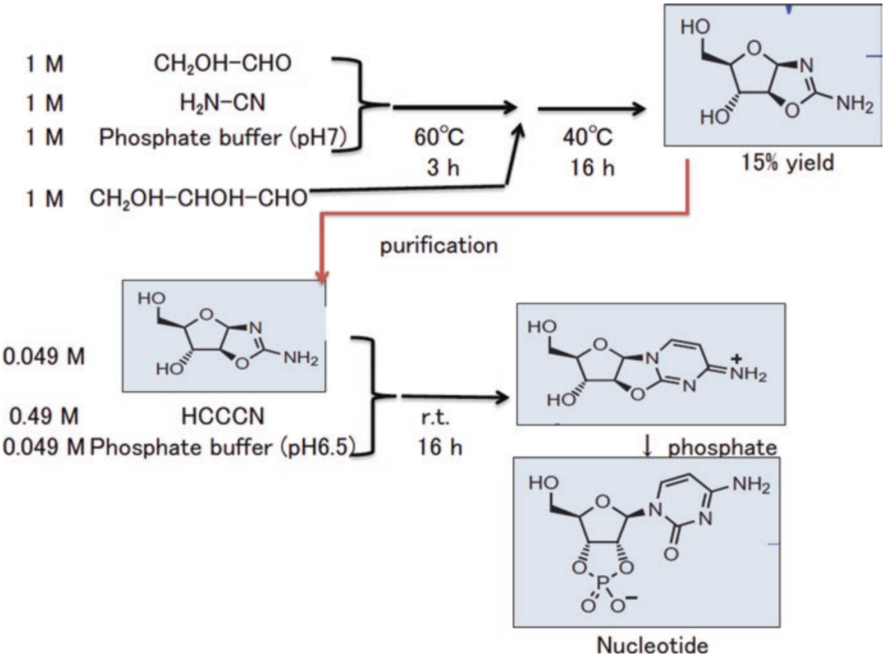

Fig. 4.6 Abiotic synthesis of nucleotides. (Based on the supplementary information from Powner et al. 2009)

and Rode 1999), and many other techniques. It was shown that oligopeptides could be formed from free amino acids. Amino acids are bifunctional molecules, such that it would be possible to synthesize long oligomers. It should be noted, however, that not only amino acids but also carboxylic acids, which are monofunctional molecules, were formed together with amino acids in many prebiotic synthesis experiments (Miller 1955). If such monofunctional molecules and amino acids coexisted, the elongation of oligopeptides would be terminated.

One of the largest differences between the abiotic world and the biotic world is that the former contains many more kinds of molecules than the latter. Schmitt-Kopplin et al. (2010) reported that extracts from the Murchison meteorite contained a large variety of molecules having more than 14,000 elemental compositions, which was estimated by comprehensive analysis using electrospray ionization-Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). It is of importance to examine whether stepwise reactions toward the formation of biotic molecules could occur in systems containing a wide variety of abiotically available molecules.

4.5 Future Prospects

4.5.1 Nobel Insights from Planetary Exploration

Explorations to the moon and other planets started in 1959, and probes have visited all planets of the solar system, a number of their satellites, dwarf planets (Ceres and Pluto), asteroids, and comets. Among them, the Voyager and Cassini missions to the Saturnian moons (Titan and Enceladus) have given us a better understanding of prebiotic chemistry.

Titan is the largest satellite of Saturn, having a dense atmosphere predominantly composed of nitrogen and methane (Coustenis and Raulin 2015, see also Chap. 26). Astrobiologists have been highly interested in the chemistry of Titan's atmosphere due to the implications it may have in relation to the prebiotic chemistry that could have occurred in a slightly reducing primitive Earth atmosphere. The Voyager mission found the presence of various hydrocarbons and nitriles together with an orange haze in Titan's upper atmosphere; the haze was made of complex organic aerosols.

Sagan and Khare (1979) synthesized complex organic aerosols by plasma discharges in a simulated Titan atmosphere and named them *tholins*. They also found that amino acids were formed after the hydrolysis of *tholins* (Khare et al. 1986). A large number of experiments have been conducted to simulate possible organic reactions in Titan's atmosphere (Coll et al. 1998; Kobayashi et al. 2017). Most of them simulated possible reactions in Titan's upper atmosphere using energetic electrons and ultraviolet light, but it was shown that tholins containing amino acid precursors could be formed in dense gas mixtures in Titan's troposphere by way of such energy supplies as cosmic rays (Taniuchi et al. 2013).

The formation of complex amino acid precursors (*tholins*) by plasma discharge and particle irradiation in a simulated Titan atmosphere did not seem to result from a step-by-step process like the Strecker synthesis. Here instead, large molecular weight organics were directly formed by flash and quench type mechanisms. Such kinds of pathways should be considered for prebiotic reactions in the primitive Earth atmosphere and interstellar media.

It is not easy to presume the actual primitive Earth conditions, because they are lost on the present Earth. We are now getting more and more information on the organic reactions occurring in extraterrestrial bodies by way of planetary exploration. We will therefore be able to use these bodies as natural chemical evolution laboratories.

4.5.2 Space Experiments

In laboratory experiments simulating prebiotic conditions, a single energy has been usually used to examine the possible formation and alteration of organic compounds. Currently, however, we can perform space experiments by utilizing satellites and space stations, where actual space environments are available to examine prebiotic chemistry. For example, EXPOSE-E, EXPOSE-R, and EXPOSE-R2 were conducted from 2008 to 2016 by using European and Russian exposure facilities on the International Space Station (ISS), where a number of chemical and biological samples were exposed to space environments (Cottin 2015). The Tanpopo mission started in 2015 on the Exposure Facility of the Japanese Experiment Module (Kibo), which includes the exposure of microbial and chemical samples and a collection of dusts flying at high speed by using ultralow density silica aerogel (Kawaguchi et al. 2016).

In space, it would be possible to utilize the full solar spectrum and cosmic radiation at the same time, which would make possible to examine possible organic reactions in space that may have occurred on a primitive planet before the formation of the ozone layer. Such experiments might be done to examine the possible synergy of plural energies in prebiotic chemistry.

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