

Chemical evolution toward the origin of life*

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Abstract: Numerous hypotheses about how life on earth could have started can be found in the literature. In this article, we give an overview about the most widespread ones and try to point out which of them might have occurred on the primordial earth with highest probability from a chemical point of view. The idea that a very early stage of life was the “RNA world” encounters crucial problems concerning the formation of its building blocks and their stability in a prebiotic environment. Instead, it seems much more likely that a “peptide world” originated first and that RNA and DNA took up their part at a much later stage. It is shown that amino acids and peptides can be easily formed in a realistic primordial scenario and that these biomolecules can start chemical evolution without the help of RNA. The origin of biohomochirality seems strongly related to the most probable formation of the first peptides via the salt-induced peptide formation (SIPF) reaction.

Keywords: origin of life; chemical evolution; amino acids; prebiotic chemistry; peptides.

INTRODUCTION

The question of the origin of life on earth is a topic that engages scientists of various scientific areas. Geologists have the difficult task of reconstructing the primordial scenario and environment that was present on earth around 4 billion years ago, a time for which most of the direct evidences have been destroyed or have at least undergone strong changes due to influences of plate tectonics, temperature, radioactivity, weather, water, etc., being the reason that the knowledge about the exact conditions at the time during the emergence of life on earth is still a little blurred. Chemists pick up the possible prebiotic conditions proposed by geologists and investigate possible pathways how organic molecules and biomolecules could be formed in such an environment, how they might have interacted, and how this might lead to more complex and finally “living” systems. Biologists usually see biomolecules in a different context, starting from the high complexity of a modern organism and looking for the very essential biological cycles and interactions and then trying to find how something similar but much more simple might have evolved. Astronomers try to achieve conclusions about the origin of our earth and about its physical development by examining other planets, and they also investigate whether our earth was severely affected by extraterrestrial objects like asteroids or meteorites that might have influenced the origin of life or even brought life to earth from another place in the universe.

Modern life is extremely complex, and it seems impossible that even the simplest contemporary cell could have evolved spontaneously. Most probably, evolution went a long way, starting from very simple organic molecules that formed larger biomolecules, which then started to interact with other classes of molecules in the environment. As soon as membrane-like structures able to form closed

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spheres were available, also less stable molecules and polymers that would otherwise hydrolyze or not even form at all in the harsh primordial environment had the opportunity to participate in chemical evolution inside these cell-like entities in a protected and more “comfortable” surrounding.

One severe problem in trying to understand such a possible pathway from simple molecules to the first cell is that in modern life one class of biomolecules is strongly dependent on the others and vice versa. DNA, for example, carries all the information that is needed to build up proteins, but by itself strongly depends on proteins that catalyze all the required reactions. This leads to a kind of hen and egg problem: what came first? An often-encountered answer is that there once was an “RNA world” before the proteins came into play [1,2]. RNA molecules are a little simpler than DNA, they are able to carry genetic information and can also have catalytic properties. From a chemical point of view, concerning possible formation pathways and stability under primordial conditions, however, the existence of unprotected poly-RNA has to be excluded—a salty ocean would have destroyed them within minutes. Peptides and proteins have major advantages concerning stability under such conditions, and they also possess all the properties needed to build up a kind of simple life form. This leads to the assumption that a primary peptide and protein world was much more likely than an “RNA world” at the starting point of life.

PREBIOTIC SCENARIO

When our earth was formed around 4.5 to 4.6 billion years ago, it was a very hot and hostile place due to heavy bombardment by meteors and asteroids during the first several hundred million years. Although scientists have diverging opinions about the environment where the first chemical steps toward life were done, there is a general agreement that liquid water is an essential prerequisite for any possible scenario. There is evidence that 4.3 [3] and even 4.4 [4] billion years (Gyr) ago, a liquid hydrosphere existed, although these first oceans might have been completely evaporated again by subsequent big impact events, and all (or at least the larger) biomolecules possibly formed would have been destroyed again. Around 4 Gyr ago, the bombardment of the earth declined and permanent, still rather hot oceans could form, being the most likely places where a chemical evolution could start to form a first form of life.

The atmosphere at that time is of crucial importance for this process, as the main source of the building blocks for biomolecules should have been atmospheric components. In a hot surrounding, the lightest gases like hydrogen and helium would have rapidly escaped the weak gravitational field of the earth. Other compounds like methane and ammonia are very labile under the UV radiation of the sun [5–7] and would, therefore, have been rapidly decomposed in the primordial atmosphere. Geologists also agree that there were only small amounts of free oxygen available before around 2.3 Gyr ago [8–11]. Some oxygen, however, could have been produced when CO₂ and water vapor were exposed to electric discharges and high-energy types of radiation as well as through thermal decomposition of oxidic minerals.

The main compounds of the primordial atmosphere, following the arguments of modern geology, were CO₂, N₂, and water vapor [11–13], which were constantly delivered by volcanic outgassing. This means that the early atmosphere was neutral or possibly due to some traces of oxygen or sulfur dioxide even slightly oxidizing. Some prebiotic chemists, however, still favor a reducing atmosphere, like the one assumed some decades ago, because a reducing atmosphere makes the formation of several organic compounds needed for life more easy. They try to collect arguments that reducing species such as methane, hydrogen, or carbon monoxide might also have been present in larger amounts [14,15]. Investigations of 3.8 billion-year-old rocks from West Greenland containing carbonate minerals show, however, that they could not have formed or existed in a reducing atmosphere [16,17].

The temperature after the formation of the first oceans can be assumed to have stayed at an elevated level (~70–100 °C) for a long time, although the activity of the sun was markedly lower 4 Gyr

ago. The high concentrations of CO₂ and water vapor in the atmosphere provided a strong greenhouse effect [18], maybe also with the intermediate help of some methane or ammonia [5,6].

The prebiotic ocean certainly contained large amounts of dissolved inorganic substances, mainly well-soluble salts of Na⁺, K⁺, Mg²⁺, Ca²⁺ (the salt concentration is assumed to have been as high as today [19]), and an increasing concentration of organic molecules according to Oparin's hypothesis [20]. Due to high concentrations of dissolved CO₂, the prebiotic sea must have been relatively acidic [21,22].

The earliest evidence for cell-like structures dates back 3.5–3.8 billion years, the later dating being based on findings of what seems to be fossilized cyanobacteria in the Apex Chert formation in the Warrawoona Group in the Eastern Pilbara near Marble Bar, Western Australia [23,24] and in Swaziland, South Africa. The earlier one considers ¹²C-enriched carbon deposits in the Isua supracrustal belt in West Greenland [25,26], a possible sign of biological carbon assimilation. This leaves a time window of a few hundred million years for the emergence of the first living systems.

AMINO ACID SYNTHESSES

For more than 50 years, various possibilities for the synthesis of amino acids on the prebiotic earth have been proposed and experimentally simulated. Diverse atmosphere compositions were exposed to energy sources, and in many cases the synthesis of amino acids resulted. Taking into account the plausibility of such reactions all over the earth, it seems reasonable to assume that atmospheric processes were the main source of amino acids and other small organic molecules, e.g., sugars.

In hydrothermal vent environments, the formation of amino acids is also possible, although under rather specific and most probably not very widespread conditions. Another possibility would be that amino acids came to the earth from space as ingredients of carbonaceous meteorites, but this assumption only moves the question of the process of amino acid synthesis to another location in our universe.

Gas-phase experiments

Most experiments related to prebiotic amino acid syntheses were performed in the gas phase. Stanley Miller's experiments [27–29], in which he showed that amino acids can easily form from a model atmosphere containing methane, hydrogen, ammonia, water, and some other simple compounds under the influence of electric discharges and heat, laid the foundation for this type of experiment and also for experimental prebiotic chemistry in general. After a reaction time of a few days, a multitude of organic molecules, such as formaldehyde, cyanide, organic acids, and some amino acids were detected. Miller's experiments were repeated under varying conditions (different composition of the "atmosphere", ultraviolet radiation, and other energy sources), leading also to other precursor molecules such as sugars and nucleic bases in some cases.

It was further shown that similar processes, e.g., Fischer–Tropsch-type syntheses (heterogeneous catalytic reactions on Fischer–Tropsch catalysts containing Fe, Co, or Ni), can also lead to intermediates and organic molecules under reducing atmospheric conditions [30].

One problem concerning the formation of organic molecules in such gas-phase reactions is that it works much better with strongly reducing atmospheric compositions comparable to the original Miller experiment. Most of the researchers had to add at least some reducing gases like hydrogen, methane, or carbon monoxide to obtain detectable amounts of organic molecules. Table 1 gives an overview of some of the gas-phase experiments to synthesize amino acids in assumed prebiotic atmospheres. Amino acid formation in a neutral atmosphere as assumed for the primordial earth by modern geochemistry, is much less efficient, however, but recently (2004) we could prove the formation of amino acids from an atmosphere consisting only of CO₂, N₂, and water vapor evaporating from the liquid phase. Electric discharges (60 kV, ~30 mA) between a submerged copper anode under water and a tungsten cathode in the

“atmosphere” were the energy source simulating lightning on the prebiotic earth. A photo of the apparatus is shown in Fig. 1.

Table 1 Summary of experiments related to gas-phase amino acid syntheses under simulated prebiotic earth conditions.

Author	Reactants	Energy source	Results reported	*
Miller [27]	CH ₄ , NH ₃ , H ₂ O, H ₂	Electric discharges	Simple amino acids, organic compounds	–
Garrison et al. [33]	CO ₂ , H ₂ O	40 MeV helium ions	Formic acid, formaldehyde	(+)
Abelson [34]	CO, CO ₂ , N ₂ , NH ₃ , H ₂ , H ₂ O	Electric discharges	Simple amino acids, HCN	–
Bar-Nun et al. [35]	CH ₄ , NH ₃ , H ₂ O	Shock wave	Simple amino acids	–
Harada, Fox [36]	CH ₄ , NH ₃ , H ₂ O	Thermal energy (900–1200 °C)	14 proteinogenic amino acids	(+)
Lawless, Boynton [37]	CH ₄ , NH ₃ , H ₂ O	Thermal energy	Glycine, alanine, aspartic acid, β-alanine, <i>N</i> -methyl-β-alanine, β-amino- <i>n</i> -butyric acid	–
Groth, Weysenhoff [38]	CH ₄ , NH ₃ , H ₂ O	Ultraviolet light (1470 and 1294 Å)	Simple amino acids (low yields)	–
Sagan, Khare [39]	CH ₄ , C ₂ H ₆ , NH ₃ , H ₂ O, H ₂ S	Ultraviolet light (>2000 Å)	Simple amino acids (low yields)	–
Yoshino, Haratsu, Anders [40]	H ₂ , CO, NH ₃ , montmorillonite	Temperature of 700 °C	Glycine, alanine, glutamic acid, aspartic acid, histidine, lysine, arginine	–
Kobayashi et al. [41]	CO, N ₂ , H ₂ O	Proton irradiation	Various amino acids	+
Palm, Calvin [42]	H ₂ , CH ₄ , NH ₃ , H ₂ O	Electron irradiation	Glycine, alanine, aspartic acid	–
Miyakawa, Kobayashi, Sawaoka [43]	CO, N ₂ , H ₂ , H ₂ O	High-temperature plasma	Glycine, alanine, aspartic acid	(+)
Kobayashi et al. [44]	CO, CO ₂ , N ₂ , H ₂ O	Proton irradiation	Glycine, alanine, aspartic acid, serine, threonine, glutamic acid	+
Plankensteiner, Reiner, Schranz, Rode [31,32]	CO ₂ , N ₂ , H ₂ O	Electric discharges	Glycine, alanine, valine, serine, proline, lysine, histidine	+

*The probability to occur on prebiotic earth according to recent geochemical data: –, little or no probability; (+), possible under special circumstances; +, possible.

Under various conditions (room temperature, 80 °C; water, sodium chloride solution), several amino acids including glycine, alanine, valine, serine, proline, lysine, and histidine [31,32] were formed, and although the yields are relatively small in a neutral atmosphere, such reactions should have produced substantial amounts of amino acids on the primordial earth, continuously occurring almost everywhere on the planet over a long time span.

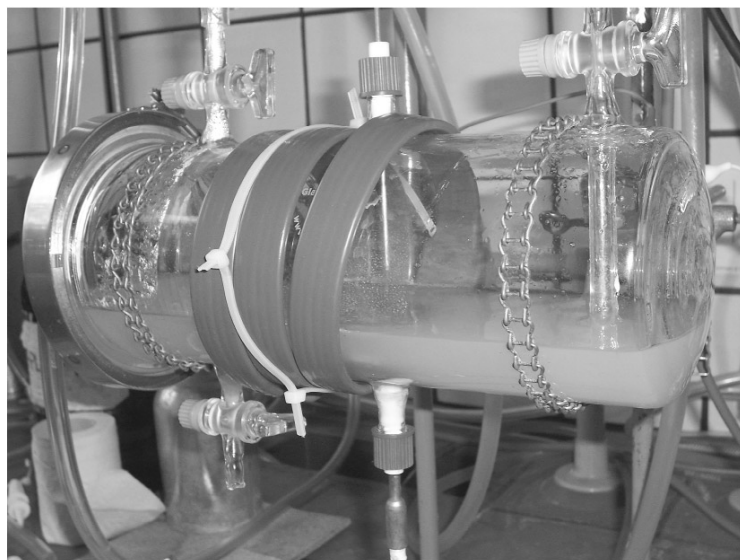


Fig. 1 Apparatus for the experimental formation of amino acids in a simulated prebiotic $N_2/CO_2/H_2O$ atmosphere by electric discharge. The liquid phase is in this case a suspension of montmorillonite in water.

Hydrothermal vents

Hydrothermal vents have increasingly become a focus of interest in geological studies, and some of these investigations also refer to their possible role in the synthesis of biologically relevant molecules [45].

Wächtershäuser and coworkers, and some other groups have postulated that life might have originated in hydrothermal vent environments on the surface of pyrite (FeS_2) [46–49]. Under quite specific conditions, in particular, high partial pressure of carbon monoxide and high temperature, a pendant to the reductive citric acid cycle as a kind of first metabolism is assumed to form pyruvate, which then, but under different and much milder conditions, can further react to amino acids. The occurrence of environments providing such specific conditions may have been more widespread on the primordial earth than today, but compared to the huge volume of the atmosphere as production site for amino acids, the quantities obtained in the hydrothermal vents would have been rather marginal, even if the atmosphere was of a neutral character, thus providing relatively moderate yields.

Other ways of amino acid synthesis in hydrothermal solutions have been proposed, starting from NH_4HCO_3 , C_2H_2 , H_2 , and O_2 [50], however, it is difficult to estimate to what extent these specific conditions could be realized locally on the primitive earth.

Extraterrestrial origin of amino acids

Additional organic material including amino acids was imported to earth via asteroids, meteorites, and organic dust particles. Especially, the Murchison meteorite that came down in Australia in 1969 has been very well investigated in this aspect. It contains a wide variety of organic substances, such as amino acids (proteinogenic and nonproteinogenic ones), hydroxy acids, sulfonic acids, phosphonic acids, etc. [51–53]. How such organic molecules can be formed in space was shown, when ice films containing H_2O , CH_3OH , NH_3 , and HCN were irradiated with UV light [54]. Amino acids like glycine, alanine, and serine are formed in this scenario that seems highly plausible for interstellar ice grains.

It is difficult to estimate how much organic material delivered from space has contributed to the total pool of biologically important small molecules on the early earth. Optimistic estimations say that the endogenous production and the exogenous delivery might have been somehow comparable [55,56], but this strongly depends on the early atmosphere and on the survival rate of organic molecules in the impact events.

All in all, the availability of amino acids on the primordial earth does not seem to be a major difficulty on the way to the origin of life, and, therefore, the most crucial question concerning early chemical evolution is the further involvement of these molecules in it, in particular their oligomerization.

SYNTHESIS OF RIBOSE, NUCLEOBASES, AND PHOSPHATES AND THEIR ASSEMBLY

In the 19th century, Alexander Michailovich Butlerov discovered the formose reaction, a polymerization reaction of formaldehyde forming a mixture of carbohydrates in aqueous solution catalyzed by calcium hydroxide [57–59]. This condensation reaction provides glycolaldehyde as first product, which is later converted to glyceraldehyde and a variety of tetrose, pentose, and hexose sugars. However, it had not been possible so far to achieve a Butlerov reaction forming ribose, providing a pathway to the sugar component of the nucleotides. This indicates that the ribose concentration in the “primordial soup” was very low and that this sugar was only a minor component of the heterogenous mixture of formed sugars [60,61].

Furthermore, a high formaldehyde concentration would have been needed in the primordial scenario, and this is highly questionable, as the reactions of other compounds present in the “prebiotic soup” with formaldehyde would rapidly convert it into numerous other products. Besides that, the pH of the primordial ocean was rather acidic than basic due to the high carbon dioxide concentration in the atmosphere [62] and thus unfavorable for the Butlerov condensation. Further, under such reaction conditions, any produced sugars are rapidly decomposed again [61], if they are not protected by complexation with borates [63] or silicates [64].

Polyphosphates and inorganic phosphates are the most plausible source of phosphate in a prebiotic scenario, but the main problem for the association of phosphate with nucleic bases and ribose—besides the lack of a plausible reaction mechanism under primitive earth conditions—is the availability of dissolved phosphate in the slightly acidic prebiotic sea: polyphosphates would quickly hydrolyze and phosphate ions precipitate as insoluble compounds of metal ions like Ca^{2+} as indicated by many rocks and minerals consisting of metal phosphates.

In the 1950s, Juan Oro showed for the first time a possible way to the formation of relevant nucleobases as building blocks for RNA. Adenine was produced in appreciable yields by refluxing a solution of hydrocyanic acid or ammonium cyanide for six hours [65–69]. Further experiments investigated related reactions by varying the reaction conditions and obtained further nucleobases [70–76]. The major problem with all these reactions is the high starting concentration of HCN or NH_4CN needed, which seems more than questionable on the primitive earth. Enrichment of nucleobases can be achieved via freezing of aqueous solutions [77], but that is again in contradiction to a hot primordial earth with vigorous volcanism.

If all three essential parts—ribose, phosphates, and nucleobases—were formed in adequate amounts under the rough earth conditions, they would have to be assembled to nucleotides and further polymerize to RNA molecules. Related to the specific composition and complex structure of nucleic acids, the probability that they could have been formed in such a hostile environment is almost zero.

The formation of purine ribonucleosides upon heating of their constituents in an otherwise pure solvent has been shown experimentally, but even in this clean environment only a small percentage of the products has biologically relevant linkages [78,79]. Analogous reactions with pyrimidines have failed completely so far [78]. Furthermore, a chemical process with the correct regioselectivity for the assembly of all the building blocks is almost impossible to imagine in the chemical environment of the prebiotic scenario [80]. Reactions like oligomerization of phosphorimidazolides of nucleobases on

montmorillonite [81] work well under laboratory conditions but are very hard to imagine in a realistic primordial soup. All these aspects and arguments almost exclude the nucleic acids as a candidate for the first step of chemical evolution toward life.

STABILITY/CARRIERS OF INFORMATION/REPLICATORS

Nucleic acids are the present carriers of information and responsible for replication, and they do their job in a very efficient but complicated way with the help of a major biochemical (protein-based) machinery. However, at the beginning of life, peptides and proteins could have preceded them, as peptides can also work as information-carrying molecules and can self-replicate, as shown recently [82–84], albeit not as efficiently as nucleic acids. Under the rough conditions of the primitive earth, they would have had many advantages, however.

In addition to the previously discussed problems in the formation of nucleic acids under primordial earth conditions, another strong argument against a “RNA world” is the physical and chemical instability of nucleic acids and, in particular, their polymers, which rapidly decompose in hot salty solution. The decomposition of ribose occurs very rapidly at temperatures around 80 °C and at pH values of 7 and higher [85,86]. Experiments regarding the stability of the four nucleobases in aqueous solutions at elevated temperature and over a wide pH range showed that at 100 °C their half-life time ranges from days to years, the shorter values being associated with the prebiotically more significant lower pH values [87]. Besides these aspects, the presence of salts and metal ions would further increase the decomposition rates [86–88].

As the primitive earth had not yet formed an ozone layer, high-energetic UV irradiation would efficiently decompose unprotected nucleobases and RNA molecules within a short time [89]. Dissolved ions and several organic compounds in the sea water absorb a large portion of the damaging radiation of wavelengths below 220 nm, thereby protecting most amino acids that absorb in this region. Nucleobases, on the other side, are most sensitive to UV light around 260 nm and, therefore, are much less protected against this additional damaging influence.

Information theoretical approaches comparing the error-resistance of an RNA vs. a peptide system also show strong preferences for the second case. Nowadays, replication of RNA or DNA is accompanied by frequent errors that are corrected by a complex enzymatic repair machinery in modern organisms. In a pure RNA world, however, such mistakes would quickly lead to catastrophic events such as “selfish RNA”, “short circuit”, and “population collapse”, all of which end in a loss of catalytic activity of the system, as was established by computer simulations of Manfred Eigen’s hypercycles [90] performed by his coworkers [91]. Freeman Dyson showed with his “toy model” [92] that a larger set of building blocks (instead of 4 different nucleobases, one could assume 8–10 amino acids) is much more tolerant against errors and would thus allow the stepwise production of more complex systems. If an error occurs, the “wrong” amino acid might have similar properties as the original one and the newly formed peptide would still be autocatalytically active, not interrupt the evolutionary pathway and possibly even lead to a favorable “mutant”.

PEPTIDE SYNTHESSES

As all arguments presented in the previous chapters have indicated that peptides and proteins appear the presumably only choice to form polymers in chemical evolution, the consideration of oligomerization of amino acids toward a possible “peptide world” becomes the most essential topic for the evolutionary scenario. While large amounts of liquid water are an important prerequisite for any chemical evolution scenario, the formation of peptides in aqueous solution is a rather unfavorable reaction, both thermodynamically and kinetically, as the condensation of amino acids to peptides requires the removal of water from the reaction partners. In the case of diglycine formation in water at a temperature of 85 °C,

the equilibrium constant is 8.41×10^{-3} , and thus less than 0.01 % of glycine would be converted into diglycine in aqueous solution. Considering the principle of LeChatelier, this condensation reaction can only be carried out in substantial amounts under special conditions. Moreover, the zwitterionic form of the amino acids leads to deactivation of the amino group for a nucleophilic attack at the carboxyl-C of other amino acids or peptides. To overcome these thermodynamic and kinetic barriers, some possible pathways for peptide formation have been proposed and experimentally investigated.

Melting processes

By heating mixtures of amino acids containing large amounts of acidic or basic amino acids to temperatures of ~ 180 °C under anhydrous conditions to get a melt without decomposition of the amino acids, polymerization occurs [93–95] and so-called “proteinoids” with high molecular weights were formed. However, these proteinoids contain only marginal amounts of peptide bonds, they are mostly based on ester-like connections [96]. Furthermore, the requirement of an excess of acidic or basic amino acids and the absence of disturbing other molecules or salts is not compatible with any accepted prebiotic scenario nor the typical distribution of amino acid yields in Miller-type experiments. For this reason, melting processes do not seem to be a feasible way to peptides and proteins.

Hydrothermal vent systems

Wächtershäuser and others achieved peptide formation under rather specific conditions that might have occurred in hydrothermal vent environments. In an aqueous slurry of (Ni,Fe)S and in the presence of CO and H₂S or CH₃SH as a catalyst and condensation agent, di- and tripeptides were formed at elevated temperatures at pH values between 7 and 10 [97,98]. These conditions and problems inevitably connected to them such as high hydrolysis rates and racemization of the amino acids do not lend this scenario much credibility as a main production site for biologically relevant peptides.

Oligomerization of the most simple amino acid glycine can be quite easily achieved, for example, in a simulated submarine hydrothermal system where an aqueous solution of glycine was circulated through a flow reactor in which the fluid was alternately heated to over 200 °C in a high-pressure chamber and then cooled down again [99]. When some CuCl₂ was added, oligomers up to hexaglycine were found.

Condensation reagents

A large number of attempts to form peptides under “possible prebiotic conditions” in solution implied substances that could act as condensation reagents when present in sufficient amounts. Under optimal conditions reagents such as cyanates [100], trimetaphosphates [101], imidazole [102–104], triazole, cyanamides [105,106], linear and cyclic inorganic polyphosphates [107,108], ATP [109] and UTP [102] have been shown to yield considerable amounts of peptides from simple amino acids. The existence of these condensation reagents in the required concentrations in the aqueous environment of the primordial earth has never been plausibly argued, however.

Heterogeneous systems

Clay minerals such as kaolinite and montmorillonite can catalyze the formation of peptides on their active surface and edges [110–113]. As their reactivity is rather low and the condensation limited to a few simple amino acids, their main role can be seen in subsequent processes, in concentrating peptides and stabilizing them against hydrolysis through adsorption, and in promoting a chain elongation of formed

peptides [113]. Pure silica and alumina have also been investigated [114–118] in this context, showing even more favorable properties than composite natural minerals.

The salt-induced peptide formation (SIPF) reaction

Introduction

The most simple and universal explanation for peptide formation under realistic prebiotic conditions known to date is the salt-induced peptide formation (SIPF) reaction [119,120] that forms peptides from all amino acids investigated so far in aqueous solution in the presence of sodium chloride and Cu(II) ions at temperatures of 60–90 °C within a few days.

Monte Carlo simulations of NaCl solutions had shown that at concentrations higher than 3 M the first hydration shell of the sodium ions becomes unsaturated [121,122], resulting in a dehydrating effect that can shift the thermodynamical equilibrium of peptide formation. Divalent metal ions are able to form complexes with amino acids, bringing the reaction partners into close vicinity and activating them. Investigations including numerous metal ions showed that Cu(II) is by far the most suitable one for the purpose of peptide formation [123]. The ubiquitous presence of sodium chloride in the ocean and in lagunas on the primordial earth can be taken for granted, and the so-called “green zones” in pre-cambrian rocks, mainly consisting of the copper-containing minerals malachite and azurite, are good evidence for the presence of Cu(II) ions in sufficient amounts at this early stage of chemical evolution. Subsequent investigations have shown that the SIPF reaction performs well within a wide range of environmental conditions concerning temperature, concentrations, and atmospheric composition [124]. The best results are achieved between 80 and 90 °C, the range most probable for a primitive ocean that had just condensed and thus had a temperature not much below the boiling point of water.

Reaction mechanism of the SIPF reaction

Investigations about the mechanism of the SIPF reaction by potentiometric and spectrophotometric methods showed that among several complex species present in this reaction depending on the pH value [125], $[\text{CuCl}]^+$, $[\text{Cu}(\text{AA})\text{Cl}]^+$, and $[\text{Cu}(\text{AAH})\text{Cl}]^{2+}$ are dominant. On the other hand, Monte Carlo simulations and neutron diffraction studies of a solution containing 0.5 M CuCl_2 and 5 M NaCl determined $[\text{CuCl}(\text{H}_2\text{O})_5]^+$ as the predominant Cu(II) species [126], pointing at the presence of one chloride ligand in all copper complexes.

In the active SIPF complex connecting two amino acids (see Fig. 3), one of them chelates the monochlorocuprate species, and a second (protonated) amino acid (or peptide) only binds end-on via the carboxyl group (because of the chloride ligand). Two water molecules stay bound above and below the Cu(II) ion at Jahn–Teller elongated positions. Peptide yields are drastically reduced when chloride is replaced by other anions, indicating that the chloride ligand provides the necessary steric and electronic preconditions for the formation of the peptide linkage [124]. The partial charges in this complex favor a nucleophilic attack of the amino group of the chelating amino acid at the other (protonated) amino acid's carboxyl carbon. The complexation of the copper ion by the newly formed peptide is weaker than that by the amino acids; it is, therefore, released to the solution and the reaction can start again with copper complexation by another amino acid.

Whenever one reaction partner is a peptide and the second one an amino acid, the amino acid will form the chelate ligand and the peptide will be coordinated via a carboxyl oxygen only.

The dehydrating effect of the sodium ions is directly related to the hydration enthalpy of this cation and is reflected in the primary peptide formation. Other cations, e.g., Mg(II), Ca(II), or Ba(II) could remove water more readily, but their disadvantage is a shift of the pH to lower values, which increases hydrolysis rates and also moves the pH-dependent Cu(II) complex species distribution away from its optimum. K(I) could act in a similar way to Na(I) if present at equal concentrations [127]. However, Na(I) seems to be the optimal compromise between dehydrating effect and complex species

formation, apart from its highest probability among all cations to have been present in large amounts under primordial conditions.

Peptide synthesis in the SIPF reaction

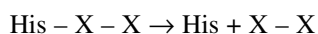
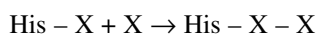
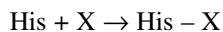
The first SIPF experiments were done with constant volume, and in this case high Cu(II) and NaCl concentrations are necessary to achieve peptide synthesis. This type of reaction mimics the situation presumably present in hot salt lakes and big lagunas on the prebiotic earth. The subsequently employed evaporation cycle experiments simulate reaction conditions expected in tidal zones, small lagunas, and puddles, providing constant cycles of evaporation and subsequent redissolution through tidal activities or rainfall [128]. The initial NaCl concentration of 0.5 M corresponds to the concentration present in today's oceans, and during an evaporation cycle performed by keeping the solution at a temperature of 85 °C, which leads to total evaporation of the water within one day, concentrations increase to the required values. After a redissolution of the residue with water, another evaporation cycle starts.

The SIPF reaction is also compatible with reactions on the surface of clay minerals. In the same reaction system, shorter peptides synthesized by the SIPF reaction can be simultaneously converted to higher oligopeptides on the surface of clay minerals and, at the same time, protected against hydrolysis [129,130].

Properties of the SIPF reaction

The SIPF reaction has some important properties, strongly pointing to its relevance for prebiotic peptide formation and evolution, besides its preference for the biologically relevant α -amino acids over β - and γ -amino acids due to their better complex formation ability with the copper ion [131] and its good conservation of optical purity when working with only one enantiomeric form of the amino acids, especially at low concentrations: Although the SIPF reaction works with all amino acids investigated so far, the dipeptide yields strongly depend on the amino acids used. Extensive investigations with pure amino acids and their binary mixtures have clearly demonstrated that specific peptide linkages are preferentially formed. This can be explained by the different side chains that on the one hand have different steric properties and, on the other hand, provide different electronic charge distributions, thus leading to different complex formation abilities. A comparison of the preferred SIPF products with the occurrence of peptide linkages in membrane proteins of the oldest still existing organisms, namely archaea and procaryonta, shows an astonishing similarity that can hardly be explained by pure chance [119,132], but rather indicates a kind of "fingerprint" of the SIPF reaction in early proteins.

In the course of these extended investigations on the formation of peptides from binary amino acid mixtures, it was found that some amino acids catalyze the formation of homo-dipeptides of other amino acids. This effect, called "mutual amino acid catalysis", can be achieved most effectively with glycine, diglycine, and its anhydride DKP (diketopiperazine) and histidine [133–136]. The reaction mechanism for this catalytic effect, taking histidine as example, can be explained as follows, X being another amino acid:



This means that higher oligopeptides containing the catalyst are formed first. A subsequent hydrolysis breaks the catalyst-X bond and produces the homo-dipeptide X-X and sets free the catalyzing molecule, which can react again with other X molecules. Even amino acids that do not form substantial peptide yields when present alone can readily produce peptides via this pathway. Figure 2 shows the L-divalinaline yields without an additional catalyst compared to the yields obtained with the catalytic help of glycine and L-histidine.

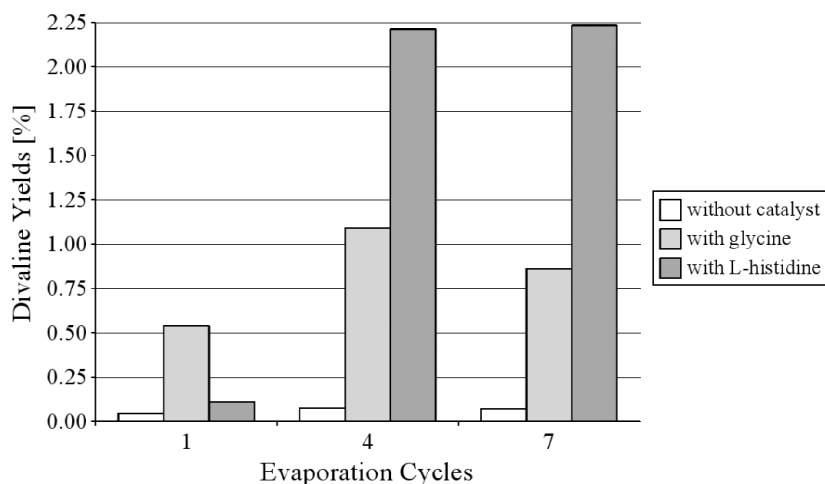


Fig. 2 Dipeptide yields of L-valine with a starting concentration of 40 mM in the SIPF reaction without a catalyst and with the catalytic effect of glycine (5 mM) [137] and L-histidine (5 mM) [136].

HOMOCHIRALITY OF BIOMOLECULES

One principal characteristic of life on earth that should be kept in mind in any attempt to reconstruct the emergence of life is that the essential chiral molecules, such as amino acids, sugars, and other molecules essential for the metabolic pathways, occur in only one enantiomeric form. In the case of amino acids, only the L-forms are incorporated into proteins, and in RNA and DNA, only D-sugars are contained in the backbone. This biohomochirality is essential for various biochemical reactions like the synthesis of specific proteins directed by DNA/RNA, the replication of DNA and the large number of specific reactions catalyzed by proteins, where a precise fitting of the reaction partners is required. For steric reasons, this is only possible with homochiral polymers but not with polymers that contain both enantiomers of the building blocks, as such an enantiomeric impurity would impede the formation of the stabilizing and shape-defining secondary structures like α -helices or β -sheets.

The observations that the SIPF reaction prefers the formation of homochiral peptides and that, furthermore, L-amino acids are favored over their D-counterparts in some cases have become further strong arguments, therefore, that this reaction could have played a decisive role in the formation of the first peptides leading to the basis for living systems. L-valine has a much better performance in the SIPF reaction than its mirror image D-valine [137] and for alanine a persistent preference for the L-form was observed as well [138] (see Table 2). Even if such a preference exists only for a few of the essential amino acids, this would direct the further evolution into a L-protein world. Thus, the SIPF reaction would not only be responsible for the formation of the first peptides on earth but also figure as most plausible among several hypotheses about the origin of biohomochirality [139].

The basic foundations of the L-preference of the SIPF reaction can be seen in a connection of physical and chemical phenomena: As a consequence of parity violation in weak nuclear forces, two enantiomers of a chiral molecule do not have exactly the same ground-state energy [140,141] and, therefore, slightly different rates of formation and stabilities. In the case of amino acids, this effect is mostly in favor of the L-forms [142–145], but it is much too small to be responsible for a selective homochirality of biomolecules. However, a pseudo-tetrahedral geometry of the main ligands of the SIPF complex apparently provides an efficient amplification mechanism for these parity-violating energy differences (PVED) between the two chiral forms of amino acids. As the SIPF complex is distorted toward a tetrahedral conformation, a central chirality at the copper ion is produced which can be further increased by chiral induction through chiral amino acid ligands. Due to its high atomic number, the

copper center provides principally much larger PVEDs (proportional to Z^5 , Z being the atomic number) than carbon atoms. Ab initio calculations have shown that the active SIPF complexes of valine (Fig. 3) and alanine are significantly more distorted toward a tetrahedral arrangement than that of proline [139], for which no preference of either L- or D-form is observed, thus confirming the assumption that the distorted SIPF complex is an essential step on the way to preferably use L-amino acids on the way to biologically relevant peptides and proteins.

Table 2 Dipeptide yields of the L- and D-forms of some amino acids obtained in the SIPF reaction after 7 evaporation cycles and the corresponding L/D stereoselectivity factors. Starting concentration of the amino acids was 40 mM (20 mM tryptophan), and, except in the alanine case, no additional catalyst was used (data taken from [137,138]).

Amino acid	Dipeptide yields (%)	L/D factor
L-valine	0.069	7.67
D-valine	0.009	
L-alanine*	2.93	1.13
D-alanine*	2.60	
L-proline	6.19	0.98
D-proline	6.30	
L-tryptophan	0.0016	1.00
D-tryptophan	0.0016	
L-lysine	6.96	0.94
D-lysine	7.41	
L-serine	0.19	1.06
D-serine	0.18	

*With 5 mM glycine as a catalyst.

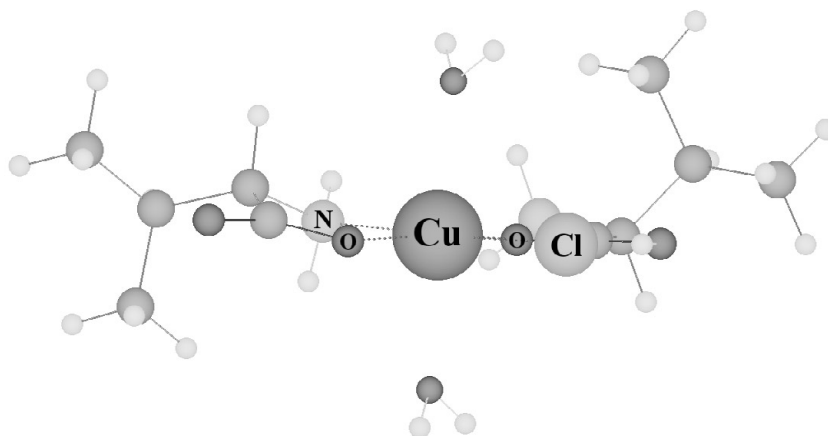


Fig. 3 Ab initio optimized geometry of a SIPF complex with two L-valine ligands showing the tetrahedral-like distortion of the “plane” formed by the central Cu(II) ion, the chloride, and the two amino acid ligands.

CONCLUSION AND FURTHER CHEMICAL EVOLUTION

As has been pointed out, an initial “RNA world” would have encountered such severe problems concerning formation and stability of RNA and even its components in a scenario compatible with modern geology for the time when life arose on earth that it can almost be ruled out. On the other hand, the formation of amino acids in atmospheric processes is feasible, even in a neutral atmosphere consisting of N_2 , CO_2 , and H_2O and their assembly to even enantiomerically enriched peptides with the help of NaCl and Cu^{2+} ions constitutes no major difficulty in such an environment.

In this context, it is important to outline that a peptide-based evolution is by no means a dead-end street: Numerous recent investigations have revealed hitherto unknown features of peptides like the ability of self-replication [82–84] and spontaneous self-assembly to membrane-like structures and nanovesicles [146–148]. Inside such cell-like compartments, a protected aqueous environment could have provided more suitable conditions for other biomolecules like RNA that could accumulate within and start to interact with themselves and with peptides and other compounds as well. It has already been demonstrated that tripeptides are able to bind nucleotides with high affinity and specificity [149], which might have provided a starting point for interactions between proteins and RNA/DNA that later revolutionized the replication mechanism and finally, after a long evolutionary path, led to the perfect interplay and cooperation between these classes of biomolecules in today’s organisms, as illustrated in ref. [146].

The hypothesis of a primary “peptide world”, claiming that peptides and proteins were the first class of biomolecules formed on the primordial earth, thus representing the first stage of chemical evolution, now seems more acceptable and realistic than ever before and should, therefore, be widely investigated to provide answers to numerous still open questions and to arrive at a comprehensive picture of the origin of life in a realistic prebiotic scenario.

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