RESEARCH ARTICLE
PLANETARY SCIENCES

Earth and Planetary Physics
8: 868–877, 2024
doi: 10.26464/epp2024050

A plausible pathway to prebiotic peptides via amino acid amides on the primordial Earth

DingWei Gan[1,2], XiangMin Lei[3], RenWu Zhou[2*], SongSen Fu[1*], Jing Sun[2], RuSen Zhou[4], Kostya (Ken) Ostrikov[4], YuFen Zhao[1,5], and JianXi Ying[1*]

[1]Institute of Drug Discovery Technology, Qian Xuesen Collaborative Research Center of Astrochemistry and Space Life Sciences, Ningbo University, Ningbo Zhejiang 315211, China;

[2]School of Electrical Engineering, Xi'an Jiaotong University, Xi'an 710049, China;

[3]College of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin 300457, China;

[4]School of Chemistry and Physics, Queensland University of Technology, Brisbane, QLD 4000, Australia;

[5]Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

Key Points:

- A new prebiotic peptide origin path was found that meets the conditions of the primordial Earth.
- The unique energetics of the reactions lead to possible catalyst-free peptide formation.
- Amino acid amides could act as an enhancer to help amino acids participate in peptide formation.
- The amino acid amides were compatible with 20 amino acids and are capable of producing a diverse peptide library.

Citation: Gan, D. W., Lei, X. M., Zhou, R. W., Fu, S. S., Sun, J., Zhou, R. S., Ostrikov, K. K., Zhao, Y. F., and Ying, J. X. (2024). A plausible pathway to prebiotic peptides via amino acid amides on the primordial Earth. *Earth Planet. Phys.*, *8*(6), 868–877. http://doi.org/10.26464/epp2024050

Abstract: The prebiotic synthesis of peptides prior to ribosome-catalyzed processes remains an enigma. The synthesis of abiotic peptides from amino acids (AAs) is primarily constrained by high activation energies and unfavorable thermodynamics, necessitating the identification of plausible prebiotic alternatives for synthesizing prebiotic peptides. Here we present a plausible pathway to the formation of prebiotic peptides, wherein oligopeptides, oligopeptide amides, and cyclic oligopeptides can be directly synthesized from amino acid amides (AA-NH_2) under wet–dry cycle conditions without the need for any enhancers. The subsequent investigation revealed that AA-NH_2 demonstrated more favorable thermodynamic reaction effects than AAs in peptide formation. In contrast to the polymerization of AAs, the process of peptide formation through the polymerization of AA-NH_2 was significantly simplified. Additionally, AA-NH_2 was discovered to function as a "bridge" for the formation of peptides from AAs, thereby facilitating their participation in the synthesis of intricate peptide structures. On the basis of these findings, a plausible mechanism for the prebiotic origin network of peptides under primordial Earth conditions has been proposed. Overall, this research presents a plausible pathway for the generation of prebiotic peptides and peptide libraries within prebiotic environments.

Keywords: prebiotic chemistry; origin of life; chemical evolution; peptide

1. Introduction

The emergence of prebiotic peptides is a crucial event in the evolution of life. However, the mechanisms underlying their synthesis prior to ribosome origin remain enigmatic. It is widely postulated that prebiotic peptide synthesis occurs in two distinct stages: amino acid (AA) formation as the initial stage, followed by polymerization (Frenkel-Pinter et al., 2020). The process of polymerization requires the condensation of AAs accompanied by water loss, which poses a significant energy barrier. Consequently, the efficient formation of prebiotic peptides may require substrate activation (Martin, 1998; Frenkel-Pinter et al., 2020).

Numerous studies have been performed to find potential routes that can reduce the difficulties in chemical evolution (Chandru et al., 2020; Foden et al., 2020; Sanden et al., 2020). Trimetaphosphate has demonstrated its efficacy as an activator in prebiotic chemistry for the formation of prebiotic peptides and nucleotides (Ying JX et al., 2018a, 2021; Gan DW et al., 2022). Furthermore, metal ions have been reported as facilitating the formation of prebiotic peptides during evolutionary processes. For instance, Rode and colleagues documented Cu^{2+}-catalyzed peptide generation in the presence of a high concentration of NaCl (Schwendinger and

First author: D. W. Gan, 841230436@qq.com
Correspondence to: R. W. Zhou, renwu.zhou@xjtu.edu.cn
S. S. Fu, fusongsen@nbu.edu.cn
J. X. Ying, yingjianxi@nbu.edu.cn
Received 29 FEB 2024; Accepted 21 JUN 2024.
First Published online 13 AUG 2024.

Rode, 1989; Rode and Schwendinger, 1990; Jakschitz and Rode, 2012). Although various enhancers (e.g., activators and catalysts) are possibly present under primordial Earth conditions, finding a more facile approach to synthesize prebiotic peptides could be advantageous for the accumulation and evolution of biomolecules. Consequently, instead of initially synthesizing AAs followed by their dehydration for polymerization, we propose a simpler and more direct pathway for peptide formation under plausible prebiotic conditions.

The submarine hydrothermal system is considered the "cradle" of the origin of life. It consists of two types: the black smoker type, driven by the magma chamber beneath ocean-floor spreading zones, and the cooler (approximately 50–90 °C) Lost City type, not driven by magma. Both types are regarded as potential sites for the emergence of life (Martin et al., 2008). Alkaline lakes in the Archean era could have played a significant role in the origin and early evolution of life (Stüeken et al., 2015). Additionally, wet–dry cycling is commonly believed to facilitate condensation reactions under prebiotic conditions, leading to the formation of proto-biopolymers such as peptides, nucleic acids, and others (Da Silva et al., 2015; Forsythe et al., 2015; Becker et al., 2018). Forsythe et al. (2015) proposed that microenvironments might have existed on early Earth that resembled intertidal zones or land puddles, which promoted material flow during water influx and concentration reactions when dried (Forsythe et al., 2015).

Recently, non-AA substances, such as sulfhydryl acid and hydroxy acid, have been found to serve as raw materials in the reaction with AAs for prebiotic peptide synthesis (Forsythe et al., 2015; McKee et al., 2018; Frenkel-Pinter et al., 2022). Carbamoyl AAs are also considered potential peptide precursors (Gan DW et al., 2023). Amino acid amide (AA-NH_2), which existed in the primeval sea (Nishizawa et al., 1983; Mullen and Sutherland, 2007), is a kind of AA derivative that could potentially be utilized as a precursor for the synthesis of prebiotic peptides. Oró and Guidry (1960) and Kawashiro et al. (1980) investigated peptide synthesis from glycinamide (Gly-NH_2) in a concentrated ammonia solution (Oró and Guidry, 1960; Kawashiro et al., 1980). However, the concentrated ammonia solution would have been unsuitable under prebiotic conditions, where watery environments were likely more prevalent on early Earth (Martin et al., 2008).

In this work, we report a robust prebiotic system for peptide synthesis from AA-NH_2 (Figure 1a) and investigate the reactions between AA-NH_2 and 20 kinds of AAs to form prebiotic peptides (Figure 1b). The kinetic and thermodynamic obstacles for prebiotic peptide bond formation were resolved by wet–dry reactions. Our results indicated that AA-NH_2 could directly generate linear oligopeptides, oligopeptide amides, and cyclic oligopeptides without requiring activation by other chemical building blocks at mild temperatures and within a wide pH range. This is crucial for the accumulation of various prebiotic peptides prior to the emergence of ribosome. Furthermore, we propose a potential reaction mechanism suggesting that AA-NH_2 may serve as the key reactant in prebiotic peptide synthesis.

2. Materials and Methods

2.1 Wet–Dry Reactions

All wet and dry cycle reactions were placed in an oven at 80 °C, and samples for each reaction were placed in 1.5 mL Eppendorf tubes for a dry block, with the caps open. The volume of solution was then brought to 1 mL after each drying, unless stated otherwise.

For the alanine (Ala) wet–dry experiment, 0.1 M Ala was dissolved in 15 mL of aqueous solution and then divided into 1 mL quantities for two wet–dry cycles. The reaction temperature was set at 80 °C, and the pH was set at 3.0, 7.0, and 10.0. Each experiment was conducted three times in parallel.

For the alaninamide (Ala-NH_2) wet–dry experiment, 0.1 M Ala-NH_2 was dissolved in 15 mL of aqueous solution and then divided into 1 mL quantities for two wet–dry cycles. The reaction temperature was set at 80 °C, and the pH was set at 3.0, 7.0, and 10.0. Each experiment was conducted three times in parallel.

For the Ala-NH_2 and Ala wet–dry experiments, 0.05 M Ala-NH_2 and 0.05 M l-Ala were dissolved in 15 mL of aqueous solution and then divided into 1 mL for two wet–dry cycles. The reaction temperature was set at 80 °C, and the pH was set at 3.0, 7.0, and 10.0. Each experiment was conducted three times in parallel.

For the Ala-NH_2 and 20 respective AA wet–dry experiments, solution A was 0.1 M Ala-NH_2 dissolved in 50 mL of aqueous solution, and solution B was 0.05 M AA dissolved in 3 mL of aqueous solution. Because of the different solubilities of the AAs, isoleucine (Ile, 0.025 M), tyrosine (Tyr, 0.007 M), tryptophan (Trp, 0.025 M), glutamic acid (Glu, 0.017 M), and aspartic acid (Asp, 0.025 M) were mainly used. Two milliliters (2 mL) of solution A and 2 mL of solution

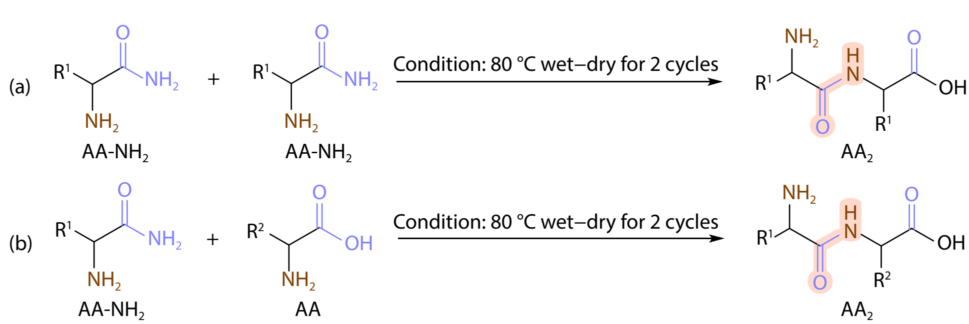

Figure 1. Prebiotic wet–dry reactions for peptide formation under primordial Earth conditions. (a) The wet–dry reaction of AA-NH_2 to form prebiotic peptides at 80 °C. (b) The wet–dry reaction of AAs with AA-NH_2 to form prebiotic peptides at 80 °C.

B were mixed 1:1 for two wet–dry cycle reactions. The reaction temperature was set at 80 °C, and the volume of the solution was increased to 1 mL after each drying step. The pH of the reaction solution was set at 7.0. Each experiment was performed three times in parallel. The final sample was analyzed by liquid chromatography–mass spectrometry (LC–MS), direct infusion electrospray high-resolution tandem mass spectrometry (MS/MS), and Fourier transform infrared spectroscopy (FTIR). A residual sample of 500 μL of solution B mixed 1:1 with ultrapure water was used to calculate the AA conversion after the reaction. For the variable pH wet–dry experiment, samples were dried under different pH conditions by the addition of a different number of equivalents of 1 M HCl or 1 M NaOH.

2.2 LC–MS

High-performance liquid chromatography (HPLC) was performed on a Thermo Scientific UltiMate 3000 standard HPLC/ultra HPLC (UHPLC) system. The HPLC flow rate was 1 mL·min^{-1}. The column was a 250 × 4.6 mm Phenomenex Luna 5 μm C18(2) 100A LC column, held at a temperature of 25 °C, with a 10 μL injection with needle wash. Solvent A was 0.1% formic acid in LC–MS grade water, and solvent B was LC–MS grade acetonitrile at a flow rate of 1 mL·min^{-1}. The gradient is listed in the Supporting Information. Mass spectrometry and MS/MS were performed on a Thermo Scientific Q-Exactive Plus system in positive mode. The MS instrument parameters were set as follows: spray voltage of 3800 V, sheath gas flow rate of 3 L·min^{-1}, and capillary temperature at 320 °C. Mass spectra were registered in the scan range from *m/z* 50 to 750.

2.3 Infrared Spectroscopy

Infrared data were obtained on a Thermo Nicolet 4700 FTIR spectrometer. Prior to analysis, samples were placed on Durapore hydrophobic polyvinylidene fluoride (PVDF) membranes with a pore size of 0.22 μm and allowed to dry. Samples were analyzed in an attenuated total reflectance (ATR) sample chamber. Spectra were background-subtracted from 500 to 2500 cm^{-1} and signal-averaged (16 scans per spectrum).

2.4 Computational Quantum Chemistry

Calculations were performed by using density functional theory with the B3LYP (Lee et al., 1988; Becke, 1993) functional and M062X (Zhao Y and Truhlar, 2008), as implemented in the Gaussian 16 program package (Frisch et al., 2016). The 6-31G* basis set (Krishnan et al., 1980) was used for geometry optimizations and frequency. Final energies for the fully optimized structures were obtained with the larger def2-TZVP basis set. Solvation free energies in water were calculated as single-point corrections on the optimized structures by using the integral equation formalism polarizable continuum model (IEFPCM) solvation model. Dispersion corrections were added by using the density functional theory-dispersion correction 3 (DFT-D3) method (Grimme et al., 2010, 2011). Analytical frequency calculations were carried out at the B3LYP/6-31G* level to obtain Gibbs free energy corrections at 298 K and to characterize the nature of stationary points. The zero-point energy (ZPE) correction factor at the B3LYP/6-31G* level was 0.9806, which we used and then performed Gibbs free energy calculations with Shermo (Lu T and Chen QX, 2021).

3. Results and Discussion

3.1 Prebiotic Peptide Formation from AA-NH_2 via Wet–Dry Reactions

To investigate the potential formation of peptides from AA-NH_2 in the wet–dry reactions, Ala-NH_2 was subjected to wet–dry cycles at 80 °C in an aqueous solution with pH 7.0 (Figure 2a). The products were characterized by using analytical techniques such as LC–MS and direct infusion electrospray high-resolution MS/MS.

The extracted ion chromatogram (EIC) of *m/z* 161.0921, combined with the MS/MS spectrum, was utilized for product analysis. The corresponding Ala_2 spectrum is depicted in Figures 2b and 2c. Notably, the reaction of Ala-NH_2 produced linear oligopeptides (AA_n) up to tetramers (Figures S1 and S2) and oligopeptide amides (AA_n-NH_2) up to hexamers (Figures S3–S6). Furthermore, a species with a mass of Ala_2 minus one water molecule and a retention time of 5.02 min was observed (Figure S7). This product was identified through MS/MS analysis as the cyclized form of Ala_2 (Figure S8), termed cyclo-Ala_2. Surprisingly, cyclo-Ala_3 and cyclo-Ala_4 were also detected by LC–MS (Figures S9 and S10), indicating that AA-NH_2 could generate not only linear peptides and their corresponding peptide amides but also cyclic peptides under prebiotic conditions. These findings highlight the substantial role played by AA-NH_2 in prebiotic peptide formation.

3.2 Formation of Prebiotic Peptides from AA-NH_2 in a Wide pH Range

To investigate the feasibility of achieving the formation of prebiotic peptides via AA-NH_2 across a wide pH range, Ala-NH_2 was subjected to two wet–dry cycles at 80 °C under various pH conditions (pH 3.0, 7.0, and 10.0). The formation of AA_n, AA_n-NH_2, and cyclo-Ala_n was confirmed under all three pH conditions (Figures S1–S22). The results demonstrated that the chain length and peptide yield were influenced by the solution pH (Figure 3). Ala_2 and Ala_3 were observed in all three pH gradient reactions for Ala-NH_2, with the highest production of both Ala_2 and Ala_3 occurring at pH 10.0. However, Ala_4 was detected at pH 7.0. When the outcomes between reactions conducted at pH 3.0 and 10.0 were compared, it was evident that longer peptides were formed under neutral conditions (pH 7.0), indicating a preference for peptide bond formation in this system.

3.3 Prebiotic Peptide Formation via Wet–Dry Reactions of AA-NH_2 with AAs

It has been reported that AAs can serve as reactants for the production of peptides (Ying JX et al., 2018b; Holden et al., 2022). In addition, the coexistence of AAs and AA-NH_2 under primordial Earth conditions could possibly aid the evolution of prebiotic peptides. Thus, we investigated the mixture of AA-NH_2 (0.05 M) with AAs (0.05 M) to form peptides, and the reaction without Ala-NH_2 to form peptides was considered a comparison. The pH values for the wet–dry reactions were set at 3.0, 7.0, and 10.0, with a temperature of 80 °C. Finally, LC–MS and FTIR were used to

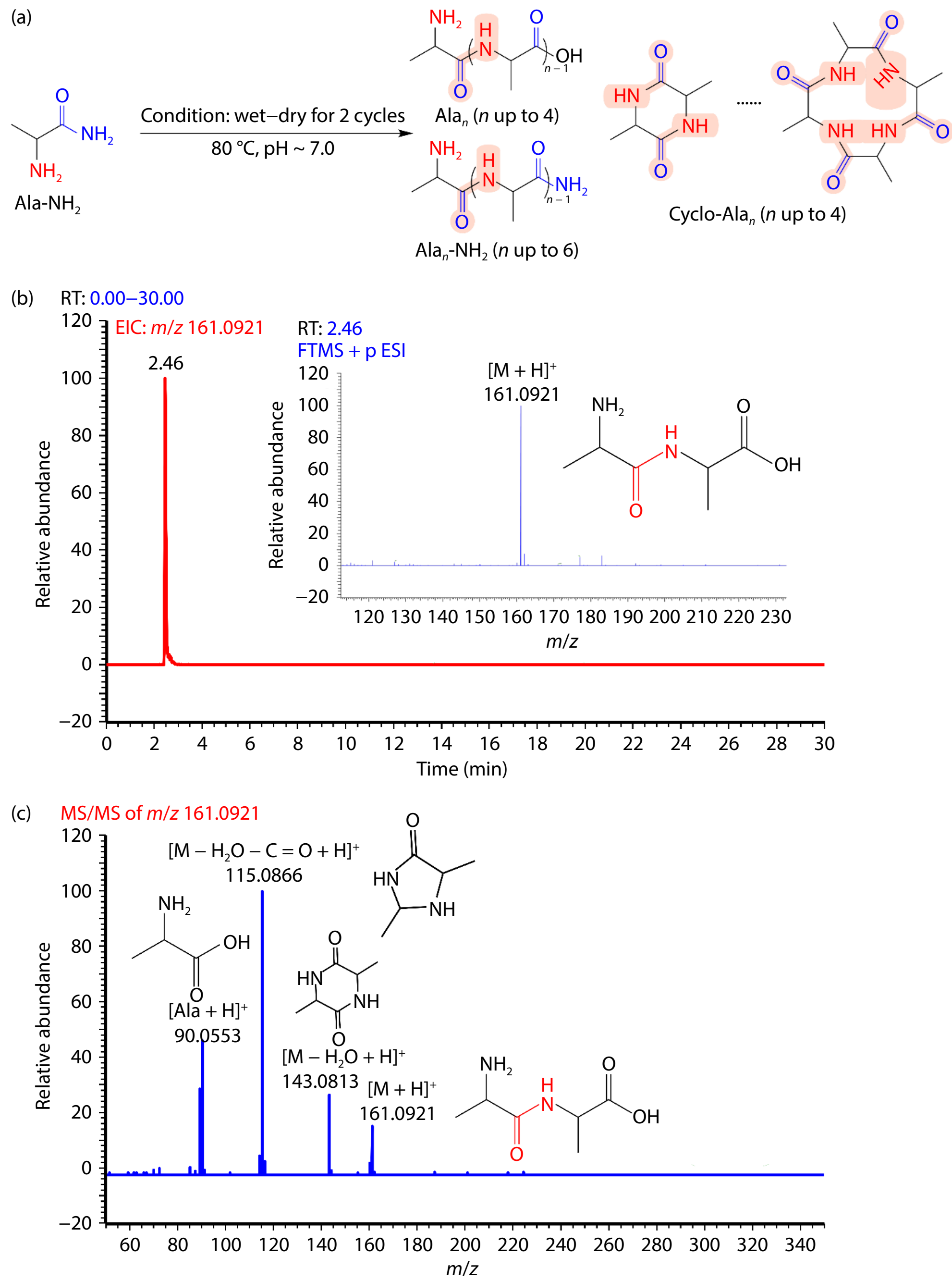

Figure 2. Analytical data confirmed the formation of prebiotic peptides in the wet–dry reaction. (a) The reaction scheme for peptide, peptide amide, and cyclic peptide formation from Ala-NH_2. The formation of Ala$_n$ (n = 2, 3, 4) is verified here and in Figures S1 and S2. The formation of Ala$_n$-NH_2 (n = 2, 3, 4, 5, 6) is verified in Figures S3–S6. The formation of cyclo-Ala$_n$ (n = 2, 3, 4) is verified in Figures S7–S10. (b) Extracted ion chromatogram (EIC)-MS profile of the product. The EIC of m/z 161.0921 was analyzed, which corresponded to Ala$_2$. The retention time (RT) of Ala$_2$ was 2.46 min. p: positive ion mode. (c) The MS/MS profile of m/z 161.0921, which confirms the formation of Ala$_2$.

analyze the resulting samples.

The presence of amide bonds in the product oligopeptides was confirmed by FTIR (Figure 4a). The amide I band (1600–1700 cm^{-1}) comprised mainly peptide backbone C=O stretching vibrations. The wet–dry reaction of Ala and Ala-NH_2 resulted in the appearance of amide absorption bands (amide I and amide II), direct reporters of amide bond formation (Rozenberg and Shoham, 2007). One of the products was confirmed to be Ala$_2$ by comparison with a pure Ala$_2$ authentic standard.

The subsequent results showed that pH 7.0 exhibited greater advantages for peptide extension in comparison with pH values of 3.0 and 10.0 (Figure 4b and Figures S23 and S24), which was similar to the result of the wet–dry reaction from Ala-NH_2. Notably, only Ala$_2$ production was detected when a single reactant, Ala, was used (Figure 4b and Figures S23–S27). This observation suggests that the combined reaction of Ala-NH_2 with Ala contributed more substantially to peptide formation than did the reaction involving Ala alone.

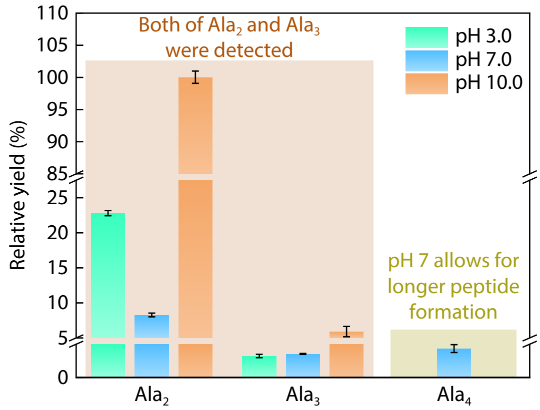

Figure 3. The pH effect on peptide formation via the wet–dry reaction of Ala-NH_2. The LC–MS data confirmed the formation of Ala_n. The average EIC peak area of Ala_2 generated at pH 10.0 was set to 100%. The error bars for triplicate experiments (n = 3) are plotted as the standard deviations.

Next, computational quantum chemistry was used to investigate whether Ala-NH_2 contributed more to peptide formation than did Ala. As Figure 4 shows, compared with the reaction of Ala, the reaction of Ala-NH_2 with Ala needed less energy to reach the corresponding transition state product (TS, Figures 4c and 4d). As a result, the reaction of AA-NH_2 with AA might be more likely. This result further indicated that Ala-NH_2 exhibited more favorable peptide-forming activity than did Ala. The idea that mixtures of AA-NH_2 and AAs produced oligopeptides was particularly attractive from an origin-of-life perspective because the presence of simple AA-NH_2 and AAs has been verified under prebiotic conditions (Miller, 1953; Oró and Guidry, 1960; Wolman et al., 1972; Miller and Bada, 1988). Peptide formation became even more favorable upon addition of Ala-NH_2 to the reaction mixture containing Ala (Figure 4b and Figures S23 and S24), potentially because of its role as an activator agent that promotes peptide synthesis by facilitating the involvement of Ala.

3.4 AA-NH_2 Effectively Reacts with 20 Respective AAs

To further investigate whether AA-NH_2 acts as an activator to aid AAs as a part of the peptide, a reaction of Ala-NH_2 (0.1 M) with 20 AAs (0.05 M) for two wet–dry cycles at 80 °C was performed. The pH was adjusted to 7.0 because of its enhanced effect in promoting the synthesis of longer peptides. Subsequently, LC–MS analysis confirmed the formation of peptides resulting from the reaction

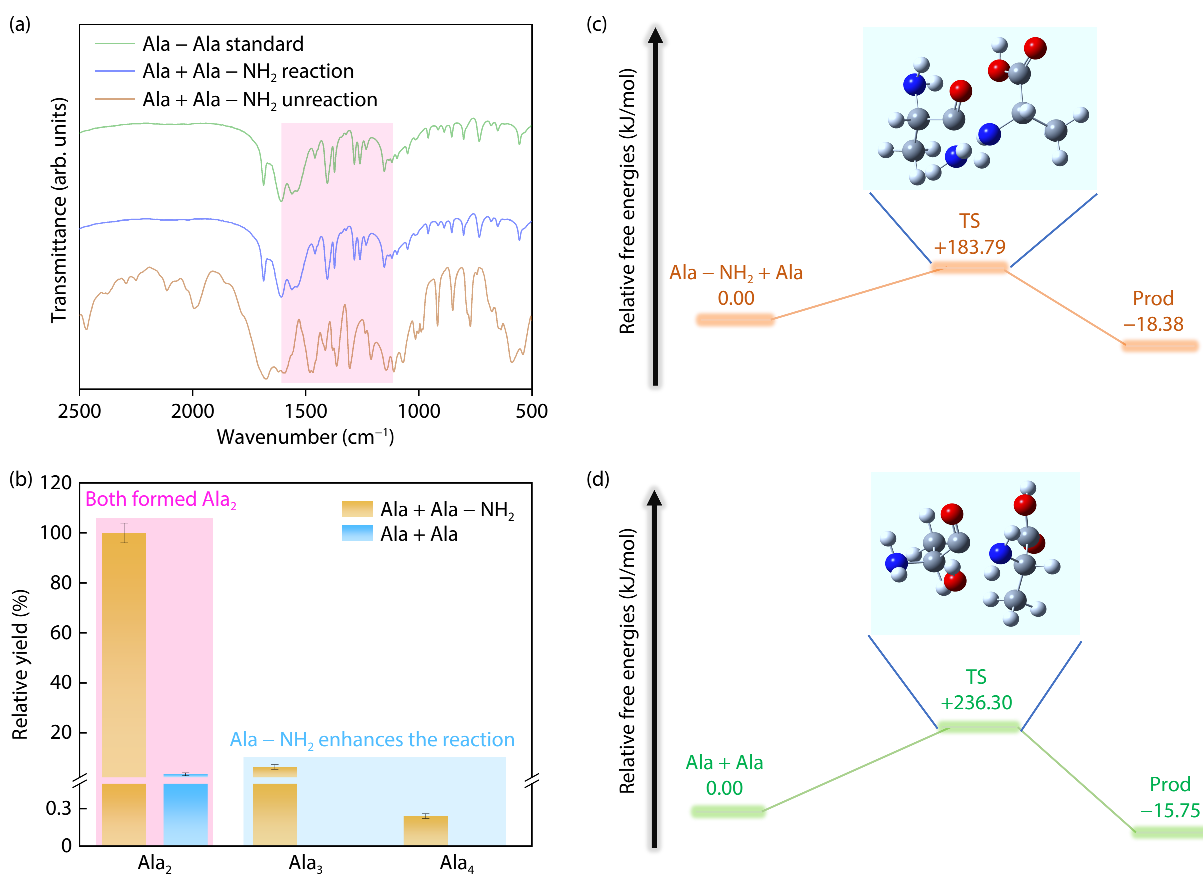

Figure 4. Peptide formation via wet–dry reaction of Ala-NH_2 with Ala. (a) Fourier transform infrared spectroscopy verification of the product mixtures, showing shifts in the C=O band and in the amide regions upon wet–dry reaction of Ala and Ala-NH_2, supporting peptide formation. (b) Effect of Ala-NH_2 on Ala-forming peptides at pH 7.0. The reaction of Ala-NH_2 with Ala and the reaction of Ala were compared. The average EIC peak area of Ala_2 formed by the reaction of Ala-NH_2 with Ala was taken as 100%. The error bars for triplicate experiments (n = 3) are plotted as the standard deviations. (c) Free energy profile for the formation of Ala_2 via reaction of Ala-NH_2 with Ala. (d) Free energy profile for the formation of Ala_2 via reaction of Ala.

between Ala-NH_2 and 20 respective AAs (Figures S28–S47).

The AA conversion rates of the 20 reactions were compared by calculating the average EIC peak area ratio of the consumed AAs to the AA mother liquor. Multiple parallel experiments were performed to reduce the error. The results showed that the reactivities of the 20 AAs were different (Figure 5). Hydrophilic AAs, such as glutamic acid (Glu), glutamine (Gln), arginine (Arg), serine (Ser), and asparagine (Asn), exhibited greater efficiency in this system, whereas aromatic AAs, such as phenylalanine (Phe), showed resistance. Notably, Glu displayed the highest conversion rate, at 53.9%, whereas Ile had the lowest, at only 1.2%. Previous experimental data indicated that AAs were also detected in samples reacted with AA-NH_2 (Figure S48), suggesting hydrolysis of AA-NH_2 leading to corresponding AA formation. Thus, it is likely that true conversion rates were slightly higher than our calculated values.

It is worth noting that we observed the presence of both dipeptides and tripeptides in certain reactions. Taking the reaction of Ala-NH_2 with Ser as an example, we analyzed the MS/MS spectra of these peptides to determine their sequences. The analysis revealed that the dipeptides consisted of Ala-Ser and Ser-Ser, whereas the tripeptides comprised Ala-Ala-Ser and Ala-Ser-Ser (Figures 6a and 6b and Figure S49). It is interesting that the LC–MS results showed that Ala-Ser was greatly synthesized but that Ser_2 was rarely formed (Figure 6c). Further results revealed that the major tripeptide was Ala-Ala-Ser (Figure 6d). The results showed that the amino group at the carboxyl end of AA amide attacked the amino group of the other AAs, which was further verified in our recent work (Zhang L et al., 2024). These findings suggest that peptide formation involving Ser may have been challenging under primordial Earth conditions. However, with the assistance of Ala-NH_2 as an activator, Ser could have participated more effectively in peptide formation for enhanced evolutionary processes.

3.5 The Mechanism of Prebiotic Peptide Formation Based on AA-NH_2

On the basis of the results above, we have proposed a plausible mechanistic route network for abiotic peptide synthesis (Figure 7). In an aqueous solution, AA-NH_2 can undergo hydrolysis with H_2O to yield AA, which can then be condensed through dehydration to form peptides (Frenkel-Pinter et al., 2020). Additionally, AA-NH_2 can engage in nucleophilic attack with the amino group of the AA, resulting in the formation of AA_2 and the release of NH_3. By following a similar approach, when AA-NH_2 encounters AA_2, it gives rise to the generation of AA_3. Continuously, AA_n can gradually be formed. It is important to note that without any additional additives present, AA-NH_2 has the inherent capability of undergoing self-rearrangement into AA_2-NH_2 and even AA_n-NH_2. Subsequent reaction with H_2O via a hydrolysis process leads to the formation of AA_n. Remarkably, cyclo-AA_n can be produced from AA_n-NH_2. The mechanism has been reported in our recent work (Zhang L et al., 2024), where we show its universality and superiority in prebiotic chemistry.

4. Conclusions

Collectively, the results presented above demonstrate that AA-NH_2 could have played a key role in prebiotic peptide formation. The plausibility of our argument rests on the following points: (i) the suitability for abiotic synthesis of the building blocks of life; (ii) the presence of AA-NH_2 under primordial Earth conditions; (iii) the unique energetics of the reaction, leading to possible catalyst-free peptide formation; and (iv) the compatibility of AA-NH_2 with the 20 AAs to produce a diverse library of abiotic peptides.

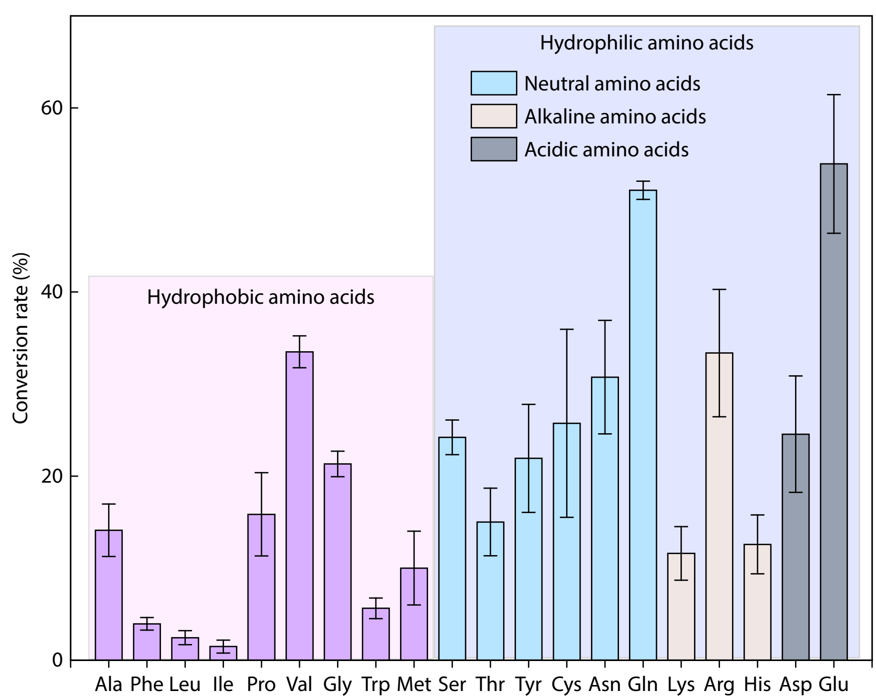

Figure 5. The AA conversion rates of the 20 reactions further showed the importance of AA-NH_2 in the synthesis of prebiotic peptides. The error bars for triplicate experiments (n = 3) are plotted as the standard deviations.

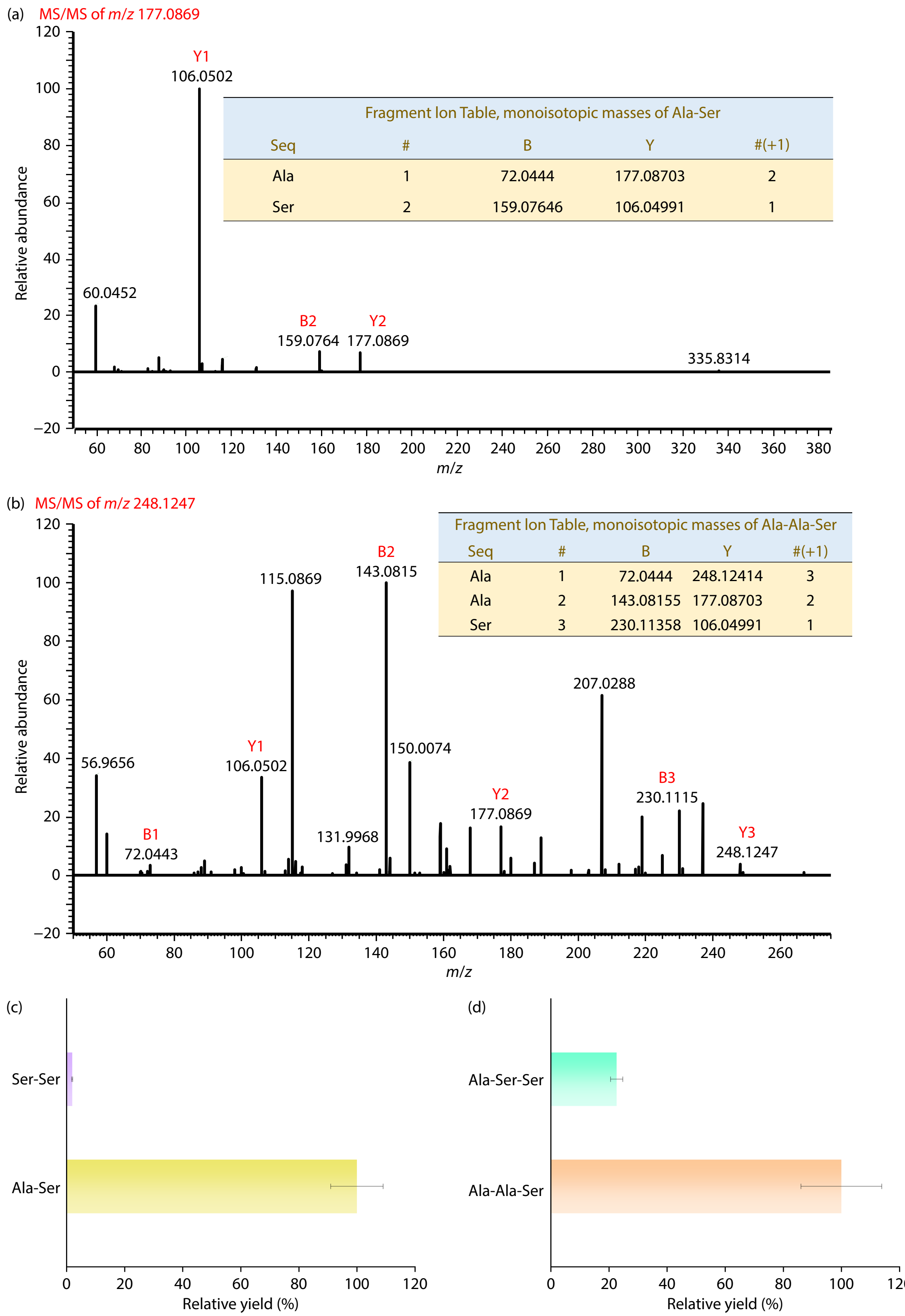

Figure 6. The reaction of Ala-NH_2 with Ser indicated that Ala-NH_2 could help Ser participate in peptide synthesis. (a) MS/MS of *m/z* 177.0869. As the fragment ion table shows, *m/z* 177.0869 corresponded to Ala-Ser. (b) MS/MS of *m/z* 248.1247. As the fragment ion table shows, *m/z* 248.1247 corresponded to Ala-Ala-Ser. (c) The relative yields of the dipeptides Ser-Ser and Ala-Ser. The average EIC peak area of Ala-Ser was taken as 100%. The error bars for triplicate experiments (n = 3) are plotted as the standard deviations. (d) The relative yields of the dipeptides Ala-Ala-Ser and Ala-Ser-Ser. The average EIC peak area of Ala-Ala-Ser was taken as 100%. The error bars for triplicate experiments (n = 3) are plotted as the standard deviations.

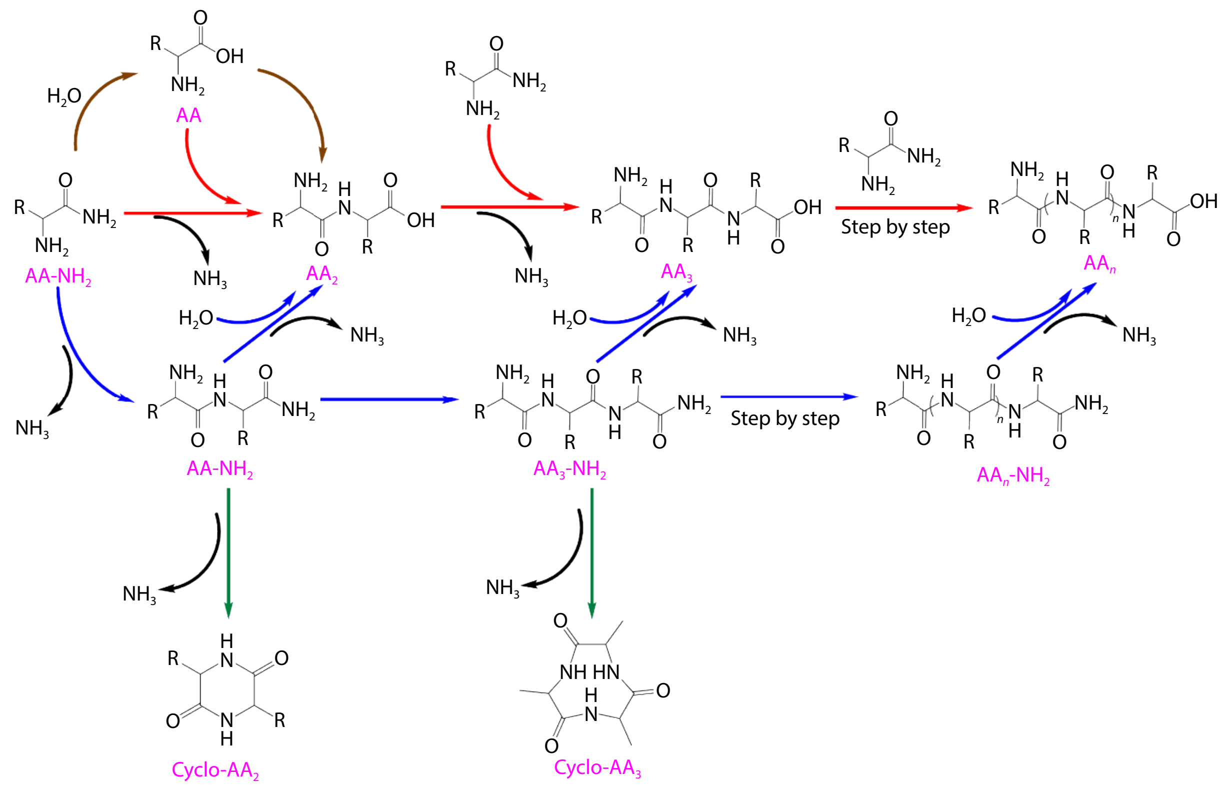

Figure 7. A plausible mechanistic route network for the abiotic synthesis of peptide libraries. Route 1 is represented by a brown line: AA-NH$_2$ first generated AA, and then AA further formed peptides. Route 2 is represented by a red line: AA-NH$_2$ reacted with AA, releasing NH$_3$ to form AA$_2$, then step by step to form AA$_n$. Route 3 is represented by a blue line: AA-NH$_2$ first generated AA$_2$-NH$_2$ and then formed AA$_n$-NH$_2$ step by step; AA$_n$-NH$_2$ then dehydrates and cyclizes itself to form cyclo-AA$_n$.

We emphasize that this study revealed the formation of prebiotic peptides containing both amino- and carboxy-terminal ends. These peptides are likely to have been favorable for the abiotic synthesis of the building blocks of life, which differs from polymers generated by hydroxy acids and thioesters (Forsythe et al., 2015; Frenkel-Pinter et al., 2022). Furthermore, the reaction presented in this article can generate peptide amides (AA$_n$-NH$_2$). C-Terminal amination of a peptide can decrease its overall charge and enhance peptide stability, whereas terminal closure would bring it closer to the parent protein. This modification has the potential to enhance the biological activity of the peptide (Wollack et al., 2009), which is meaningful for modern peptide products.

The presence of AA-NH$_2$ on primordial Earth as a prebiotic compound is highly plausible. It has been experimentally confirmed that AA-NH$_2$ can be synthesized through the prebiotic reaction of isocyanides, as reported by Mullen and Sutherland (2007). In addition, Bolm et al. (2018) verified that the AA-NH$_2$ can be formed by mechanochemical activation of iron cyano complexes with α-aminonitriles under primordial Earth conditions. Parker et al. (2014) also demonstrated the prebiotic source existence of AA-NH$_2$ by simulated spark discharge experiments.

Through a direct measurement of heat in an aqueous medium, thermochemistry has shown that an AA amide bond stores more energy than a peptide bond (Dobry and Sturtevant, 1952; Benzinger et al., 1959). Thus, it is reasonable to assume that prebiotically formed AA-NH$_2$ has already overcome thermodynamically unfavorable conditions for providing peptide bonds, based on thermochemical studies. Furthermore, computational quantum chemistry results have indicated that AA-NH$_2$ exhibits a greater and more beneficial contribution to peptide formation than does AA.

It is important to note that AA-NH$_2$ facilitates the participation of 20 AAs in peptide evolution. In the presence of AA-NH$_2$, AAs were observed to form longer peptide chains and generate a greater number of peptides (Figure 4b). Furthermore, we have discovered that AA-NH$_2$ can assist dipeptides in forming tripeptides. Taking the reaction system involving Ala-NH$_2$ and Ser as an example, LC–MS results showed that the tripeptide formed was not only Ala-Ala-Ser but also small amounts of Ala-Ser-Ser (Figure 6d and Figure S49). According to our proposed mechanistic speculation (Figure 7), for the generation of Ala-Ser-Ser, Ser first forms Ser$_2$ (Gorlero et al., 2009), and then the further formation of Ala-Ser-Ser begins with the activation of AA-NH$_2$. These results indicate that AA-NH$_2$ promotes the involvement of AAs in the formation of dipeptides and their subsequent elongation into oligopeptides. Therefore, the coexistence between AA-NH$_2$ and AAs may provide a more favorable condition for generating a diverse peptide library, which is beneficial for the emergence of life with complexity and diversity.

In conclusion, this work has successfully resolved a long-standing controversy regarding the possibility of catalyst-free reactions leading to peptide formation by utilizing the characteristic of AA-

XX (AA analogs). This unique feature allows for the simultaneous fulfillment of both the presence of a reaction precursor and the energetic requirements under the primordial Earth environment. The oversight of this potentiality over several decades can be attributed to most researchers focusing on AA-forming peptides as the primary route for investigation, often using catalysts, whereas only a few researchers have explored non-AA-forming peptides for synthesizing prebiotic-derived peptides. On the basis of these distinctive attributes, this research has detailed a novel pathway for prebiotic peptide synthesis without any activators, indicating that the prebiotic peptide could be directly formed from plausible prebiotic AA analogs. The findings of this work not only indicate the potential formation of prebiotic peptides from AA-NH_2, but also provide support for a scenario in which the coexistence of AA-NH_2 and AAs promotes peptide evolution, potentially contributing to the origin of life under primordial Earth conditions.

Conflict of Interest Statement

The authors declare no competing financial interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 42388101, 92256203, and 42003062), Fundamental Research Funds for the Provincial Universities of Zhejiang (No. SJLY2023007), the Technology and Engineering Center for Space Utilization, Chinese Academy of Sciences (No. YYWT-0901-EXP-16), and the Ningbo Top Talent Project (No. 215-432094250). The Analysis Center of the Institute of Drug Discovery Technology is acknowledged for collecting the LC-MS data.

References

Becke, A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.*, *98*(7), 5648–5652. https://doi.org/10.1063/1.464913

Becker, S., Schneider, C., Okamura, H., Crisp, A., Amatov, T., Dejmek, M., and Carell, T. (2018). Wet–dry cycles enable the parallel origin of canonical and non-canonical nucleosides by continuous synthesis. *Nat. Commun.*, *9*(1), 163. https://doi.org/10.1038/s41467-017-02639-1

Benzinger, T., Kitzinger, C., Hems, R., and Burton, K. (1959). Free-energy changes of the glutaminase reaction and the hydrolysis of the terminal pyrophosphate bond of adenosine triphosphate. *Biochem. J.*, *71*(2), 400–407. https://doi.org/10.1042/bj0710400

Bolm, C., Mocci, R., Schumacher, C., Turberg, M., Puccetti, F., and Hernández, J. G. (2018). Mechanochemical activation of iron cyano complexes: A prebiotic impact scenario for the synthesis of α-amino acid derivatives. *Angew. Chem. Int. Ed.*, *57*(9), 2423–2426. https://doi.org/10.1002/anie.201713109

Chandru, K., Jia, T. Z., Mamajanov, I., Bapat, N., and Cleaves, H. J., II. (2020). Prebiotic oligomerization and self-assembly of structurally diverse xenobiological monomers. *Sci. Rep.*, *10*(1), 17560. https://doi.org/10.1038/s41598-020-74223-5

Da Silva, L., Maurel, M. C., and Deamer, D. (2015). Salt-promoted synthesis of RNA-like molecules in simulated hydrothermal conditions. *J. Mol. Evol.*, *80*(2), 86–97. https://doi.org/10.1007/s00239-014-9661-9

Dobry, A., and Sturtevant, J. M. (1952). Heats of hydrolysis of amide and peptide bonds. *J. Biol. Chem.*, *195*(1), 141–147. https://doi.org/10.1016/S0021-9258(19)50882-1

Foden, C. S., Islam, S., Fernández-García, C., Maugeri, L., Sheppard, T. D., and Powner, M. W. (2020). Prebiotic synthesis of cysteine peptides that catalyze peptide ligation in neutral water. *Science*, *370*(6518), 865–869. https://doi.org/10.1126/science.abd5680

Forsythe, J. G., Yu, S. S., Mamajanov, I., Grover, M. A., Krishnamurthy, R., Fernández, F. M., and Hud, N. V. (2015). Ester-mediated amide bond formation driven by wet–dry cycles: A possible path to polypeptides on the prebiotic earth. *Angew. Chem. Int. Ed.*, *54*(34), 9871–9875. https://doi.org/10.1002/anie.201503792

Frenkel-Pinter, M., Samanta, M., Ashkenasy, G., and Leman, L. J. (2020). Prebiotic peptides: molecular hubs in the origin of life. *Chem. Rev.*, *120*(11), 4707–4765. https://doi.org/10.1021/acs.chemrev.9b00664

Frenkel-Pinter, M., Bouza, M., Fernández, F. M., Leman, L. J., Williams, L. D., Hud, N. V., and Guzman-Martinez, A. (2022). Thioesters provide a plausible prebiotic path to proto-peptides. *Nat. Commun.*, *13*(1), 2569. https://doi.org/10.1038/s41467-022-30191-0

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Petersson, G. A.,…Fox, D. J. (2016). Gaussian, Inc., Wallingford CT.

Gan, D. W., Ying, J. X., and Zhao, Y. F. (2022). Prebiotic chemistry: The role of trimetaphosphate in prebiotic chemical evolution. *Front. Chem.*, *10*, 941228. https://doi.org/10.3389/fchem.2022.941228

Gan, D. W., Guo, Y. T., Lei, X. M., Zhang, M., Fu, S. S., Ying, J. X., and Zhao, Y. F. (2023). Urea-mediated warm ponds: prebiotic formation of carbamoyl amino acids on the primordial Earth. *Earth Planet. Sci. Lett.*, *607*, 118072. https://doi.org/10.1016/j.epsl.2023.118072

Gorlero, M., Wieczorek, R., Adamala, K., Giorgi, A., Schininà, M. E., Stano, P., and Luisi, P. L. (2009). Ser-His catalyses the formation of peptides and PNAs. *FEBS Lett.*, *583*(1), 153–156. https://doi.org/10.1016/j.febslet.2008.11.052

Grimme, S., Antony, J., Ehrlich, S., and Krieg, H. (2010). A consistent and accurate *ab initio* parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.*, *132*(15), 154104. https://doi.org/10.1063/1.3382344

Grimme, S., Ehrlich, S., and Goerigk, L. (2011). Effect of the damping function in dispersion corrected density functional theory. *J. Comput. Chem.*, *32*(7), 1456–1465. https://doi.org/10.1002/jcc.21759

Holden, D. T., Morato, N. M., and Cooks, R. G. (2022). Aqueous microdroplets enable abiotic synthesis and chain extension of unique peptide isomers from free amino acids. *Proc. Natl. Acad. Sci. USA*, *119*(42), e2212642119. https://doi.org/10.1073/pnas.2212642119

Jakschitz, T. A. E., and Rode, B. M. (2012). Chemical evolution from simple inorganic compounds to chiral peptides. *Chem. Soc. Rev.*, *41*(16), 5484–5489. https://doi.org/10.1039/c2cs35073d

Kawashiro, K., Morimoto, S., and Yoshida, H. (1980). On the reaction of aminoacetonitrile and glycine amide in aqueous ammoniacal solutions. *Viva Origino*, *8*, 41–47.

Krishnan, R., Binkley, J. S., Seeger, R., and Pople, J. A. (1980). Self-consistent molecular orbital methods. XX. A basis set for correlated wave functions. *J. Chem. Phys.*, *72*(1), 650–654. https://doi.org/10.1063/1.438955

Lee, C., Yang, W. T., and Parr, R. G. (1988). Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B*, *37*(2), 785–789. https://doi.org/10.1103/PhysRevB.37.785

Lu, T., and Chen, Q. X. (2021). Shermo: A general code for calculating molecular thermochemistry properties. *Comput. Theor. Chem.*, *1200*, 113249. https://doi.org/10.1016/j.comptc.2021.113249

Martin, R. B. (1998). Free energies and equilibria of peptide bond hydrolysis and formation. *Biopolymers*, *45*(5), 351–353. https://doi.org/10.1002/(SICI)1097-0282(19980415)45:5<351::AID-BIP3>3.0.CO;2-K

Martin, W., Baross, J., Kelley, D., and Russell, M. J. (2008). Hydrothermal vents and the origin of life. *Nat. Rev. Microbiol.*, *6*(11), 805–814. https://doi.org/10.1038/nrmicro1991

McKee, A. D., Solano, M., Saydjari, A., Bennett, C. J., Hud, N. V., and Orlando, T. M. (2018). A possible path to prebiotic peptides involving silica and hydroxy acid-mediated amide bond formation. *ChemBioChem*, *19*(18), 1913–1917. https://doi.org/10.1002/cbic.201800217

Miller, S. L. (1953). A production of amino acids under possible primitive earth conditions. *Science*, *117*(3046), 528–529. https://doi.org/10.1126/science.

117.3046.528

Miller, S. L., and Bada, J. L. (1988). Submarine hot springs and the origin of life. *Nature*, *334*(6183), 609–611. https://doi.org/10.1038/334609a0

Mullen, L. B., and Sutherland, J. D. (2007). Simultaneous nucleotide activation and synthesis of amino acid amides by a potentially prebiotic multi-component reaction. *Angew. Chem. Int. Ed.*, *46*(42), 8063–8066. https://doi.org/10.1002/anie.200702870

Nishizawa, M., Makino, Y., and Egami, F. (1983). A probable prebiotic peptide formation from glycineamide and related compounds in a neutral aqueous medium participation of nucleoside and 5′-mononucleotide. *J. Mol. Evol.*, *19*(2), 179–183. https://doi.org/10.1007/BF02300756

Oró, J., and Guidry, C. L. (1960). A novel synthesis of polypeptides. *Nature*, *186*(4719), 156–157. https://doi.org/10.1038/186156a0

Parker, E. T., Zhou, M. S., Burton, A. S., Glavin, D. P., Dworkin, J. P., Krishnamurthy, R., Fernández, F. M., and Bada, J. L. (2014). A plausible simultaneous synthesis of amino acids and simple peptides on the primordial Earth. *Angew. Chem. Int. Ed.*, *53*(31), 8132–8136. https://doi.org/10.1002/anie.201403683

Rode, B. M., and Schwendinger, M. G. (1990). Copper-catalyzed amino acid condensation in water—A simple possible way of prebiotic peptide formation. *Origins Life Evol. Biosphere*, *20*(5), 401–410. https://doi.org/10.1007/BF01808134

Rozenberg, M., and Shoham, G. (2007). FTIR spectra of solid poly-l-lysine in the stretching NH mode range. *Biophys. Chem.*, *125*(1), 166–171. https://doi.org/10.1016/j.bpc.2006.07.008

Sanden, S. A., Yi, R. Q., Hara, M., and McGlynn, S. E. (2020). Simultaneous synthesis of thioesters and iron–sulfur clusters in water: two universal components of energy metabolism. *Chem. Commun. (Camb.)*, *56*(80), 11989–11992. https://doi.org/10.1039/D0CC04078A

Schwendinger, M. G., and Rode, B. M. (1989). Possible role of copper and sodium chloride in prebiotic evolution of peptides. *Anal. Sci.*, *5*(4), 411–414. https://doi.org/10.2116/analsci.5.411

Stüeken, E. E., Buick, R., and Schauer, A. J. (2015). Nitrogen isotope evidence for alkaline lakes on late Archean continents. *Earth Planet. Sci. Lett.*, *411*, 1–10. https://doi.org/10.1016/j.epsl.2014.11.037

Wollack, J. W., Zeliadt, N. A., Mullen, D. G., Amundson, G., Geier, S., Falkum, S., Wattenberg, E. V., Barany, G., and Distefano, M. D. (2009). Multifunctional prenylated peptides for live cell analysis. *J. Am. Chem. Soc.*, *131*(21), 7293–7303. https://doi.org/10.1021/ja805174z

Wolman, Y., Haverland, W. J., and Miller, S. L. (1972). Nonprotein amino acids from spark discharges and their comparison with the Murchison meteorite amino acids. *Proc. Natl. Acad. Sci. USA*, *69*(4), 809–811. https://doi.org/10.1073/pnas.69.4.809

Ying, J. X., Fu, S. S., Li, X., Feng, L. B., Xu, P. X., Liu, Y., Gao, X., and Zhao, Y. F. (2018a). A plausible model correlates prebiotic peptide synthesis with the primordial genetic code. *Chem. Commun. (Camb.)*, *54*(62), 8598–8601. https://doi.org/10.1039/C8CC04767G

Ying, J. X., Lin, R. C., Xu, P. X., Wu, Y. L., Liu, Y., and Zhao, Y. F. (2018b). Prebiotic formation of cyclic dipeptides under potentially early Earth conditions. *Sci. Rep.*, *8*(1), 936. https://doi.org/10.1038/s41598-018-19335-9

Ying, J. X., Ding, R. W., Liu, Y., and Zhao, Y. F. (2021). Prebiotic chemistry in aqueous environment: A review of peptide synthesis and its relationship with genetic code. *Chin. J. Chem.*, *39*(8), 2264–2272. https://doi.org/10.1002/cjoc.202100120

Zhang, L., Zhang, M., Guo, X. F., Gan, D. W., Ye, Y., Zhao, Y. F., and Ying, J. X. (2024). A model for N-to-C direction in prebiotic peptide synthesis. *Chem. Commun.*, *60*(20), 2748–2751. https://doi.org/10.1039/D3CC06101A

Zhao, Y., and Truhlar, D. G. (2008). The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.*, *120*(1-3), 215–241. https://doi.org/10.1007/s00214-007-0310-x