

Role of Ferrocyanides in the Prebiotic Synthesis of α -Amino Acids

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Abstract We investigated the synthesis of α -amino acids under possible prebiotic terrestrial conditions in the presence of dissolved iron (II) in a simulated prebiotic ocean. An aerosol-liquid cycle with a prebiotic atmosphere is shown to produce amino acids via Strecker synthesis with relatively high yields. However, in the presence of iron, the HCN was captured in the form of a ferrocyanide, partially inhibiting the formation of amino acids. We showed how HCN captured as Prussian Blue (or another complex compound) may, in turn, have served as the HCN source when exposed to UV radiation, allowing for the sustained production of amino acids in conjunction with the production of oxyhydroxides that precipitate as by-products. We conclude that ferrocyanides and related compounds may have played a significant role as intermediate products in the prebiotic formation of amino acids and oxyhydroxides, such as those that are found in iron-containing soils and that the aerosol cycle of the primitive ocean may have enhanced the yield of the amino acid production.

Keywords Prebiotic synthesis · Ferrocyanides · Amino acids · Carboxylic acids · Aerosol chemistry · Strecker reaction

Introduction

Since Miller's famous experiment (Miller 1953) that probed the abiotic synthesis of amino acids under plausible prebiotic conditions, the investigation of the pathways that may have allowed prebiotic chemical evolution has become a fascinating field of research whose main questions are still unanswered. Amino acids are the monomers of proteins, which, along with nucleic acids, are the most important biopolymers in biochemistry. Amino acids are formed more readily from CH_4 -based atmospheres than any other biomonomer. Several routes have been proposed to account for

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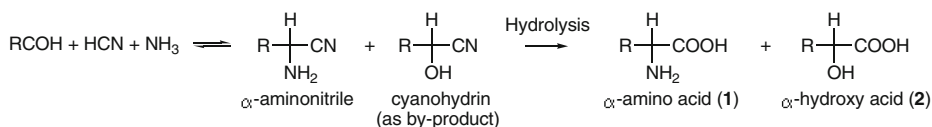
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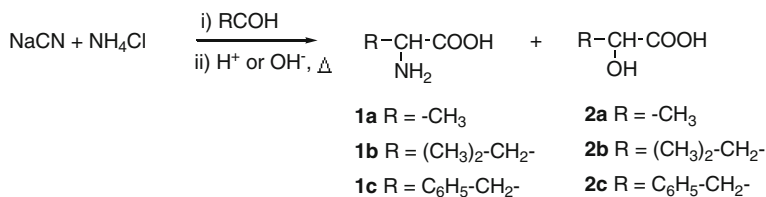
the appearance of amino acids in primitive Earth simulation experiments (see e.g., Lemmon 1970; Thompson and Sagan 1989; Miyakawa et al. 1999). One of these routes was proposed by Miller to explain the predominance of the α -amino acids in his experiment. The formation of amino acids in experiments with spark discharges and CH_4 is thought to follow a mechanism similar to that of the Strecker synthesis (Miller 1957a, Scheme 1). This hypothesis is supported by the fact that aldehydes and HCN are well-known products in prebiotic simulation experiments (see e.g., Miller 1957b; Stribling and Miller 1987). However, one must take into account that in an aqueous medium, the Strecker reaction is reversible, and unreacted starting material remains along with cyanohydrins as by-products (Scheme 1).

Miller's experiment and other prebiotic experiments used an environment with both liquid and gas phases: the gases are those of the prebiotic atmosphere, and the liquid phase is the primitive ocean. The incorporation of gases into the liquid phase and the natural mixing of products are required for these experimental protocols to be efficient. In this regard, marine aerosols have been shown to be very efficient containers for prebiotic chemistry, where reactants are concentrated and exposed to atmospheric sources of energy (see e.g. Shah 1970; Dobson et al. 2000; Tuck 2002; Donaldson et al. 2004; Tverdislov and Yakovenko 2008; Vaida and Tuck 2010; Lerman 2010; Ruiz-Bermejo et al. 2011; Soares et al. 2012; Griffith and Vaida 2012; Griffith et al. 2012). In this work, we used an aerosol production cycle as basic set-up to study the reaction yield of the Strecker reaction, on the basis of our previous results (Ruiz-Bermejo et al. 2007a) that showed the general efficiency of aerosols to enhance the yield of organic production. As a control, this setup was compared with standard stirred Strecker reactions for the production of three different amino acids. The syntheses of alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) were evaluated (Scheme 2). These amino acids were chosen because they are well-known reaction products in simulation experiments using CH_4 and relevant compounds in prebiotic chemistry (Friedman and Miller 1969; Johnson et al. 2008; Parker et al. 2011; Griffith and Vaida 2013).

One of the key unsolved facets of prebiotic chemistry on Earth is the environmental conditions that lead to biogenesis. The current geochemical models propose that the early Earth had a solid crust rich in iron, an atmosphere (without free oxygen), a huge pool of liquid water with iron in solution, a natural water-atmosphere cycle together with active volcanism that provided CH_4 and other gases to the atmosphere and strong incident solar UV irradiance. The existence of banded iron formations (Walker and Brimblecombe 1985; Holland 1989; Barley et al. 1997; Poulton et al. 2004) strongly suggests that dissolved iron (ferrous ion) was present in significant quantities in the ocean under the free O_2 atmosphere of the Archean epoch. It is also known that when solutions of Fe(II) and CN^- are mixed, $\text{Fe}_x(\text{CN})_y$ precipitates are formed (Sharpe 1976). In this context, ferrocyanides and ferricyanides have been suggested as compounds with prebiotic interest (Keefe and Miller 1996), and the ferrocyanide anion $[\text{Fe}(\text{CN})_6]^{4-}$ has been mentioned as a possible abundant component of the primitive ocean (Orgel 1974) and could have played a possible role in the prebiotic synthesis of adenine (Oró 1961). Indeed, on the modern Earth, the presence of ferrocyanides in volcanic hydrothermal environments in the Kuril Islands and on the Kamchatka Peninsula has been reported (Mukhin 1974; Mukhin et al. 1978). Arrhenius et al. (1994) proposed that cyanide forms ferrocyanides by reacting with Fe(II) , yielding (upon partial oxidation) an insoluble complex salt named



Scheme 1 Strecker synthesis for the production of α -amino acids



Scheme 2 Formation of alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) *via* Strecker shynthesis

Prussian Blue, $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 15\text{H}_2\text{O}$ [(ferric hexacyanoferrate(II))], as a precipitate. In a previous publication, we reported the easy formation of Prussian Blue using aqueous aerosols of ferrous salts, a CH_4 -based atmosphere and spark discharges. The formation of this insoluble salt led to a clear decrease in amino acid production (Ruiz-Bermejo et al. 2007b). In the Strecker reaction, HCN incorporation is necessary for the production of amino acids. Any sink that captures HCN, such as Prussian Blue, should partially inhibit the formation of amino acids. To investigate this possibility, the reversibility of this pathway was explored using ferrocyanides as sources of cyanide; the corresponding aldehydes for the formation of **1a**, **1b** and **1c**; and UV radiation within an aerosol-cycle set-up. Prussian Blue and ammonium ferrocyanide $[(\text{NH}_4)_4\text{Fe}(\text{CN})_6 \cdot 1.5\text{H}_2\text{O}]$, tetraammonium hexacyanoferrate(II) were used as plausible prebiotic iron-cyanide complexes. Ammonium ferrocyanide is formed under plausible prebiotic conditions from heating of ammoniacal solutions of Prussian Blue (Ruiz-Bermejo et al. 2009).

Thus, in this study, we explored the interaction of a plausible prebiotic hydrosphere, the Earth's crust, solar irradiance and the atmosphere, and we investigated the processes that led to the incorporation of HCN into organic molecules, producing precipitates, as by-products, that are consistent with those observed on Earth.

Materials and Methods

Strecker Reaction Using Free Cyanide

To a solution of 73.5 mg of NaCN and 80.2 mg of NH_4Cl in 5 mL of water (0.3 M) was added the corresponding aldehyde: 83.5 μL of acetaldehyde for the formation of alanine (**1a**), 137 μL of isobutyraldehyde for valine (**1b**) and 175 μL of phenylacetaldehyde for phenylalanine (**1c**). Then, the bubble-aerosol-droplet cycle was established using an ultrasonic aerosol generator (BONECO model 7035). The system was maintained at a constant temperature (38 °C) with active aerosol generation for 24 h. Then, the crude reaction products were treated with 200 mg of NaOH and heated at 150 °C for 1 h or freeze dried. The dried residues were heated at 110 °C in 500 μL of 6 N HCl for 24 h (see Scheme 2). The alkaline solutions of amino acids were neutralised with HCl and freeze dried, and the acidic solutions were directly freeze dried (experiment A, Table 1). Control experiments were carried out at room temperature and 38 °C (experiment B and C, respectively, Table 1) under the indicated conditions without aerosols.

Strecker Reaction Using Ferrocyanides

Prussian Blue was prepared from aqueous solutions of $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3 \text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ (Ludi 1981). Ammonium hexacyanoferrate(II), $(\text{NH}_4)_4\text{Fe}(\text{CN})_6 \cdot 1.5 \text{H}_2\text{O}$, was prepared according to the method of Brauer (1963).

Table 1 Yields of the Strecker reactions from free cyanide for alanine (**1a**), valine (**1b**) and phenylalanine (**1c**). Experiment A: experiment using aqueous aerosols Experiment B: control experiment with stirring at room temperature. Experiment B: control experiment with stirring at 38 °C. The reaction time was 24 h in all experiments. Yield **1** (%) corresponds to the production of amino acids. The ratio **1:2** corresponds to the relationships between the yield in amino acids and in hydroxy acids

R	Experiment A		Experiment B		Experiment C	
	Ratio 1:2	Yield 1 (%)	Ratio 1:2	Yield 1 (%)	Ratio 1:2	Yield 1 (%)
-CH ₃ (a)	9:1	93	3:2	33	3:1	75
(CH ₃) ₂ -CH ₃ (b)	6:1	68	6:1	62	7:1	63
C ₆ H ₅ -CH ₂ - (c)	7:1	81	1:1	35	3:2	58

To an aqueous solution of (NH₄)₄Fe(CN)₆·1.5 H₂O (1.6 g in 5 mL of water, experiment 1, Table 3) or to an ammonium solution of Prussian Blue (600 mg in 5 mL of 5 N NH₄OH, experiment 2, Table 3) under an inert atmosphere was added the desired aldehyde (1 M). Then, aerosols were generated, and each system was allowed to react for 48 h. The final mixture of each system was extracted with chloroform. The organic layer was dried using a N₂ stream, and the residue was hydrolysed as described above. The same experiments were performed at 38 °C as a control (experiments 3 and 4, Table 3). A concentration of 1 M of aldehyde is actually improbable in a prebiotic scenario but it was chosen in order to permit comparison with previous results about the prebiotic synthesis of glycine in the presence of FeCl₂ (Summers and Lerner 1998).

In the case of phenylalanine (**1c**), after acid hydrolysis with HCl, two phases were separated. One was the supernatant, which was freeze-dried, yielding a white solid, and the other was an oily residue, which was dissolved with CHCl₃ and dried using a N₂ stream. Both fractions were analysed by GC-MS.

Strecker Reaction Using Ferrocyanides and UV Radiation

In another group of experiments, the reaction mixtures containing the ferrocyanides were irradiated with UV light (254 nm) using a Hg lamp (Spectroline[®] 11SC-1, Spectronics Corporation) for 48 h. The crude products were processed as described above (Schemes 4 and 5).

In all cases, the reaction yields were calculated based on the initial amount of aldehyde.

Instrumental Analyses

Gas Chromatography–Mass Spectrometry (GC-MS) GC-MS analyses in full-scan mode were performed on a 6850 network GC system coupled to a 5975 VL MSD with a triple-axis detector operating in electronic impact (EI) mode at 70 eV (Agilent). The GC system included an HP-5MS column (Crossbond 5 % diphenyl-95 % dimethyl polysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness), and He was used as the carrier gas.

Infrared Spectroscopy IR spectra were obtained using a Nexus Nicolet FTIR spectrometer. The spectra were obtained in CsI pellets using the reflectance mode of operation from 4,000 to 450 cm⁻¹.

X-Ray Powder Diffraction The X-ray analysis was performed using a Seifert X-Ray Diffraction System, model XRD 3003 TT (from 5° to 70°, step 0.1°, step time 3 s).

Analytical Procedure

The amino acid:carboxylic acid ratios were calculated from the peak areas in the GC-MS chromatograms. The following protocol was used for the preparation of the samples: i) Approximately 2 mg of each sample in 50 μL of anhydrous dimethylformamide plus 50 μL of MSTFA containing 1 % TMCS [N-methyl-N-trimethylsilyl-trifluoroacetamide containing 1 % trimethylchlorosilane from Thermo Scientific] was heated at 85 $^{\circ}\text{C}$ for 1 h to obtain the respective TMS derivative. ii) The derivatised samples were analysed by GC-MS using the following GC oven program: 60 $^{\circ}\text{C}$ (initial temperature) with a hold of 1.5 min, heated to 130 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$ with a hold time of 11 min and heated to 180 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$ with a hold time of 8 min. Two microlitres of each sample was injected. The temperature of the injector was 220 $^{\circ}\text{C}$, and the injections were performed in split mode. The detector temperature was 280 $^{\circ}\text{C}$. The flow rate was 1.1 mL/min. As a rule, the assignments of the GC-MS peaks attributed to amino acids and carboxylic acids were verified by comparison with the retention times and mass spectra of external standards (purchased from Sigma-Aldrich and Fluka).

Results and Discussion

Strecker Reaction Using NaCN and NH_4Cl

The Strecker reaction, which was first reported in 1850, is the oldest known synthesis of α -amino acids (Strecker 1850), and as previously reported, the use of aqueous aerosols significantly improves the rough yield for the production of α -amino acids (19.33 μmol against 12.54 μmol) from CH_4 and spark discharges in prebiotic simulation experiments (Ruiz-Bermejo et al. 2007a, Table 2). To quantify this increase in yield and to test the real effect of the aerosols on the production of amino acids, we performed the Strecker synthesis of alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) using NaCN, NH_4Cl and the appropriate aldehyde (Scheme 2) using aqueous aerosols (Table 1, experiment A). A set of control experiments, without aerosols, was run in parallel at room temperature (experiment B) and 38 $^{\circ}\text{C}$ (temperature of the ultrasonic bath, experiment C). The yields of all experiments are reported in Table 1.

As it shown in Table 1, in all experiments, the corresponding α -hydroxy acids (the final products of the hydrolysis of cyanohydrins) were identified as by-products (**2a**, **2b** and **2c**). The data in Table 1 also show that an increasing temperature increased the total yield of amino acids and that the use of aqueous aerosols notably improved the overall formation of amino acids, likely due to the partial inhibition of cyanohydrin production.

The effect of the aqueous aerosols was highly significant in the cases of alanine (**1a**) and phenylalanine (**1c**). Kendall and McKenzie (1929) reported a total yield of alanine (**1a**) of approximately 55 % from a Strecker reaction using NaCN, NH_4Cl and acetaldehyde as reactants. This result is comparable to the one reported in Table 1 for experiment B. However, the presence of aqueous aerosols increased the yield to up to 93 %. Figure 1 clearly summaries the results for the Strecker synthesis of alanine (**1a**) under the conditions used in this study.

To date, the standard Strecker synthesis has not been effective for the synthesis of phenylalanine (**1c**). Because of the presence of a methylene group activated by the adjacent phenyl and formyl groups, phenylacetaldehyde readily polymerises in the presence of acids or bases or at elevated temperature. When an attempt was made to produce the corresponding aminonitrile under the conventional conditions for the Strecker reaction, only a small amount of phenylalanine (**1c**) was recovered from the reaction mixture, and most of the phenylacetaldehyde was

Table 2 Amount of amino acids formed by CH₄ based atmosphere and spark discharges

		Control experiment ^a	Aqueous aerosol experiment ^b	Aqueous aerosol experiment+FeCl ₂ ^c
		Amount (μmol)	Amount (μmol)	Amount (μmol)
α-amino acids				
Glycine	C ₂ H ₅ NO ₂	2.89	3.93	3.434
Aminomalonic acid	C ₃ H ₅ NO ₄	0.001	1.06	0.565
Alanine	C ₃ H ₇ NO ₂	8.68	12.98	4.630
Serine	C ₃ H ₇ NO ₃	0.07	0.14	0.074
Aspartic acid	C ₄ H ₇ NO ₄	0.10	0.34	0.220
2-Aminobutyric acid	C ₄ H ₉ NO ₂	0.40	0.18	d
Glutamic acid	C ₅ H ₉ NO ₄	0.16	0.08	0.154
Ornithine*	C ₅ H ₁₂ N ₂ O ₂	0.24	0.43	–
Histidine*	C ₆ H ₉ N ₃ O ₂	–	d	–
t-Leucine	C ₆ H ₁₃ NO ₂	–	0.19	0.150
Total(μmol)		12.54	19.33	9.23
β-amino acids				
β-alanine	C ₃ H ₇ NO ₂	0.21	1.16	0.126
Isoserine	C ₃ H ₇ NO ₃	0.12	0.34	0.105
3-Aminobutyric acid	C ₄ H ₉ NO ₂	1.02	0.68	0.245
3-Aminoisobutyric acid	C ₄ H ₉ NO ₂	1.34	1.65	0.387
Total(μmol)		2.69	3.83	0.863
α-amino acids N-sustitued				
Sarcosine	C ₃ H ₇ NO ₂	1.01	0.45	0.240
N-Methylalanine	C ₄ H ₉ NO ₂	0.09	d	0.194
Total(μmol)		1.1	0.45	0.434
Other amino acids				
Iminodiacetic acid	C ₄ H ₇ NO ₄	d	0.03	–

* Tentatively assigned

^a Experiment using a pool of liquid water at 38 °C (Ruiz-Bermejo et al. 2007a)

^b Experiment using aqueous aerosols (Ruiz-Bermejo et al. 2007a)

^c Experiment using saline aqueous aerosols of FeCl₂ (Ruiz-Bermejo et al. 2007b)

converted into an oily material. Masao-Nakamura et al. (1975) solved this problem by using a solvent mixture (water and ethanol or methanol), HCN, concentrated ammonium hydroxide and ammonium carbonate and by heating the reaction mixture at 120 °C, achieving phenylalanine yields of up to 93 % (**1c**). In the present study, we demonstrated that the use of aqueous aerosols produces an extremely high yield of phenylalanine (**1c**) (81 %, Table 1) when using under milder conditions and without organic solvents.

The comparison of the different experiments, A, B and C (Table 1), for valine (**1b**) production revealed that our yields are comparable to the one reported by Gaudry (1946), who obtained this amino acid with a total yield of 65 % from isobutyraldehyde.

In summary, the yields of α-amino acids from the Strecker synthesis are maintained or significantly improved when using an aerosol cycle.

In general, the results of the new experiments presented in this work (Table 1) are in good agreement with our previous data (Table 2) for the production of α-amino acids from a CH₄

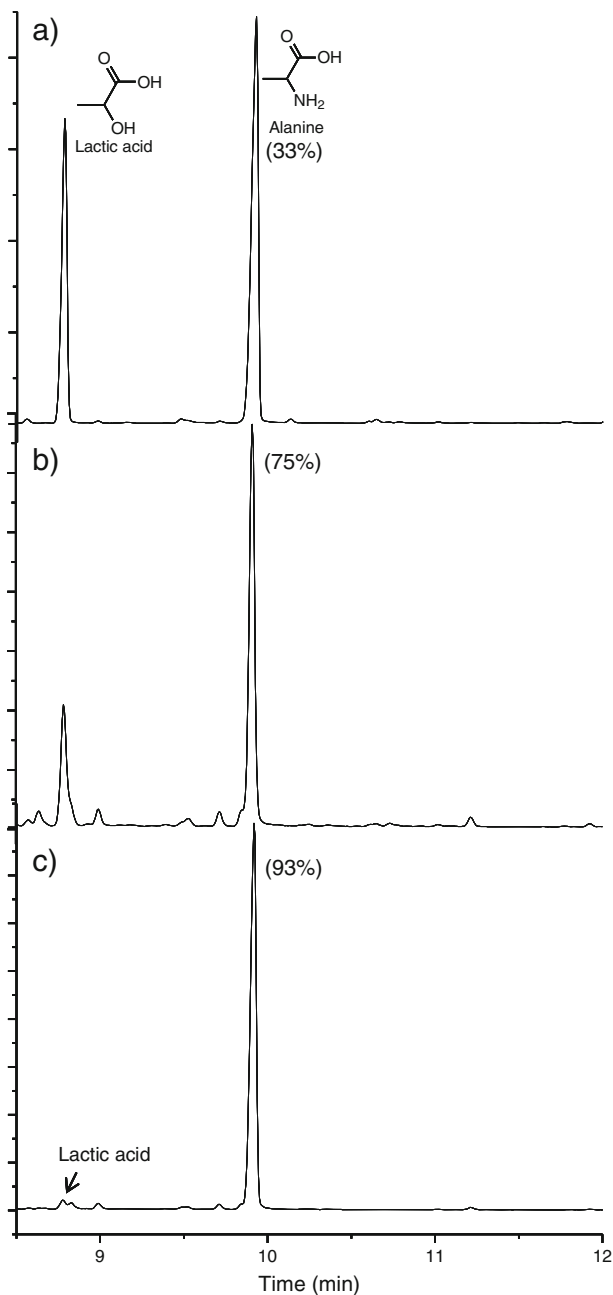


Fig. 1 Alanine Strecker reaction. **a** Experiment a room temperature (experiment C). **b** Experiment at 38 °C (experiment B). **c** Experiment with aqueous aerosols (experiment A). The presence of aerosols significantly increased the yield of alanine

atmosphere and spark discharges (Ruiz-Bermejo et al. 2007a). In particular, under those prebiotic reductive conditions the yield of alanine (**1a**) increased by 49 % when using the

aqueous aerosols with respect to the control experiment using liquid water (Ruiz-Bermejo et al. 2007a, Table 2). Here, we demonstrated that in the absence of other cross reactions, such as is the case of a discharge experiment, the presence of aqueous aerosols increased the absolute yield for alanine up 93 %.

On the aerosol experiments the efficiency of nucleation can be observed on sight, by the appearance of an optically thick cloud of aerosols in suspension above the liquid bulk (see Fig. 2). To characterize the size of the aerosols an experiment was implemented in a previous work with a sodium chlorate solution at 38 °C and an aerosol cycle (Osuna-Esteban et al. 2008). For this solution the minimum theoretical aerosol diameter is 1 μm but aerosol droplets grew due to droplet coalescence. We can conclude that under this set-up the typical sizes of the droplet seen by scanning electron microscopy (SEM) imaging were about 30 μm and up to 100 μm , as observed by their salt relics.

Let us mention that aerosols may have also played some role in the original Miller-Urey experiments since aerosols are easily produced above a solution while heated. In fact during his research Miller tested and compared several setups. One of them explored the synthesis induced by lightning in a steam-rich volcanic eruption or geyser. The cloud generated by this steam as it cools down and nucleates in aerosol droplets can be observed by inspection (see Fig. 1 in Bada 2013). In this alternative experimental set-up he identified five different amino acids with product yields somewhat higher than in the classical configuration. This supports our understanding of the positive influence of aerosols on the amino acid production yield.

In addition, the other experimental conditions used in this study, temperature and initial concentration of cyanide, are discussed below due to their relevance in a prebiotic scenario. Our experiments using aqueous aerosols reach a temperature of 38 °C (ultrasonic bath temperature). This temperature can be considered relative high. However, regarding the temperature environment, it is unclear what were the thermal conditions at the sea level at the prebiotic era. However, due to the greenhouse effect of the volcanic gases (such as CO_2



Fig. 2 Aqueous aerosol formed using an ultrasonic generator

and CH₄) and serpentinization emissions of CH₄ a warmer environment during the early Archean epoch is more than plausible (Emmanuel and Ague 2007). Two crucial observations provide constraints for the environmental conditions during the Archean: (1) sediments, such as the Banded Iron Formations, were formed in liquid water, so mean surface temperatures must have been above freezing; (2) isotopic data from sediments formed between 3.3 Ga and 3.5 Ga indicates that temperatures may have been as high as 70 ± 15 °C (Knauth and Lowe 2003). At the same time, the concentration of HCN in a prebiotic aqueous scenario is important under a synthetic point of view. Taking into account the production rates of HCN in the primitive atmosphere and the experimental hydrolysis rates, the steady state concentration of HCN in the primitive ocean could be in the range 4 × 10⁻⁶ – 2 × 10⁻⁸ M at pH 8 between 0° and 25 °C (Stribling and Miller 1987). Miyakawa et al. (2002) estimated this concentration to be approximately 2 × 10⁻⁶ M at pH=8 and 0 °C. Thus, a concentration of 0.3 M in cyanide is unlikely in the prebiotic scenario of the early Earth. However, this concentration was chosen taking in consideration that: (1) it is enough high to prevent the hydrolysis of cyanide [the hydrolysis processes of HCN are dominant in dilute solutions (Sanchez et al. 1967)]; (2) it is comparable to the concentrations of cyanide used by Summers and Lerner (1998) for the prebiotic Strecker synthesis of glycine.

On the other hand, if the route for the production of α-amino acids in experiments with spark discharges and CH₄ is actually the Strecker reaction, then any reduction in the HCN concentration will lead to a reduction in the yield of the α-amino acids, independent of the presence of aqueous aerosols. This result was indeed clearly observed in a previous work for the case of alanine (**1a**) (Ruiz-Bermejo et al. 2007b, Table 2). In the experiment using saline aqueous aerosols of FeCl₂, the production of alanine decreased by up 64 % due to the formation of Prussian Blue, which acts as sink for cyanide. And, in general the rough yield in α-amino acids decreased from 19.33 μmol to 9.23 μmol (Ruiz-Bermejo et al. 2007b, Table 2).

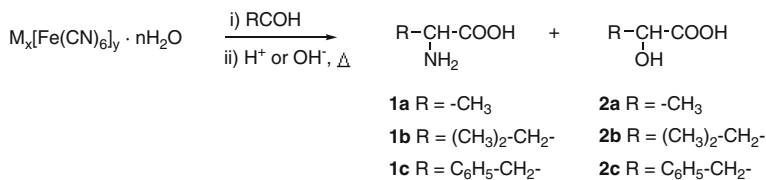
Strecker Reaction Using Ferrocyanides

Next, taking into account the results reported above, the Strecker reactions for alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) were performed using ferrocyanides, that is, ammonium ferrocyanide and Prussian Blue, in the presence of aqueous aerosols (Scheme 3, Table 3) to study the reversibility of this process. Specifically, we tested the ability of ferrocyanide to serve as a source of cyanide in the formation of α-amino acids.

Phenylalanine (**1c**) was detected in only trace amounts in the experiment using aqueous aerosols and ammonium ferrocyanide when the hydrolysis was performed under acidic conditions (experiments 1, Table 3). Similarly, β-phenyllactic acid (**2c**) could be quantified or identified only when aqueous aerosols and acidic hydrolysis were used (27 % and traces from ammonium ferrocyanide and Prussian Blue, respectively; experiments 1 and 2, Table 3, Scheme 3). Therefore, in the case of the formation of phenylalanine (**1c**) by the Strecker reaction, the form of cyanide used, free or metal-bound, is a key factor. For alanine (**1a**) and valine (**1b**), the use of Fe-coordinated cyanide led to a significantly lower production of these amino acids than the use of free cyanide did. However, the aqueous aerosols again enhanced the amino acid production.

We conclude that the recycling of cyanide captured in the form of ferrocyanides can participate in the formation of α-amino acids, although, as expected, the yields were notably lower than the yields obtained from free cyanide. In addition, the role of aqueous aerosols was very significant in these Strecker-like syntheses.

Thus, the results presented here indicate that metal-bound cyanide is able to participate in the Strecker synthesis, though the yield is poor. This result apparently contradicts the previous



Scheme 3 Strecker synthesis of alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) using ferrocyanides

results of Summers and Lerner (1998). These authors used the glycine Strecker reaction as model to test the effect of metal-bound cyanide, achieving a glycine yield of 0.88 % using free cyanide in an iron-free medium and yields of approximately 0.5–2.5 % in the presence of $FeCl_2$. These data suggest that the Strecker synthesis seems to have little sensitivity to whether the cyanide is free or bound to $Fe(II)$. However, the amount of glycine obtained by Summer and Lerner under their conditions was far lower than our yields of amino acids synthesised from free and Fe -coordinated cyanide. Our previous results (Ruiz-Bermejo et al. 2007b), summarized in Table 2, indicated that the glycine yield increased by 36 % in the presence of aqueous aerosols, but this yield decreases by 12 % in the presence of $FeCl_2$.

Strecker Reaction Using Ferrocyanides and UV Radiation

We also performed the Strecker reaction using ammonium ferrocyanide and Prussian Blue in the presence of UV radiation and aqueous aerosols because UV radiation was ubiquitous on the early Earth and because it is known that the ferrocyanide moiety can be photochemically oxidised in the near-UV range by water, liberating HCN and producing α - and γ - $FeOOH$ as oxidation products (Arrhenius 1990). Tiwari (1983) and Tiwari and Sharma (1985) demonstrated that amino acids can be produced from soluble $K_4Fe(CN)_6$ and formaldehyde in aqueous solution using UV light radiation on the basis that potassium ferrocyanide degrades, releasing HCN. However, in the reports of these pioneering studies, no yields were given, and the identification of amino acids was performed only by paper and thin layer chromatography.

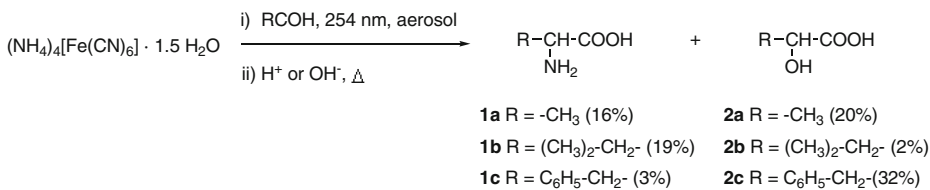
The yields for all amino acids synthesised in this work are shown in Schemes 4 and 5.

The yields in amino acids in all experiments with UV radiation were higher than the yields obtained in the experiments using ferrocyanides without radiation (Table 3). Therefore, the UV-induced photodegradation of ferrocyanides, which releases HCN, improved the production of α -amino acids in this Strecker-like reaction. This experimental

Table 3 Experiment 1: $M=(NH_4)_4$, $x=4$, $y=1$, $n=1.5 H_2O$, i.e. $(NH_4)_4[Fe(CN)_6] \cdot 1.5 H_2O$; experiment using aqueous aerosols. Experiment 2: $M=Fe$, $x=4$, $y=3$, $n=15 H_2O$, i.e. $Fe_4[Fe(CN)_6]_3 \cdot 15 H_2O$; experiment using aqueous aerosols+ NH_4OH (5N). Experiment 3: $M=(NH_4)_4$, $x=4$, $y=1$, $n=1.5 H_2O$, i.e. $(NH_4)_4[Fe(CN)_6] \cdot 1.5 H_2O$; experiment with stirring at 38 °C. Experiment 4: $M=Fe$, $x=4$, $y=3$, $n=15 H_2O$, i.e. $Fe_4[Fe(CN)_6]_3 \cdot 15 H_2O$; experiment with stirring at 38 °C+ NH_4OH (5N)

R	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Ratio 1:2	Yield 1 (%)	Ratio 1:2	Yield 1 (%)	Ratio 1:2	Yield 1 (%)	Ratio 1:2	Yield 1 (%)
-CH ₃ (a)	5 : 1	10	6:1	6	11:2	6	3:1	4
(CH ₃) ₂ -CH ₃ (b)	5:4	4	4:1	2	5:9	4	–	–
C ₆ H ₅ -CH ₂ - (c)	–	d	–	–	–	–	–	–

d detected but the ratio signal:noise <10



Scheme 4 Yields of the Strecker synthesis of alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) using ammonium ferrocyanide, as source of cyanide, in the presence of aqueous aerosols and UV radiation

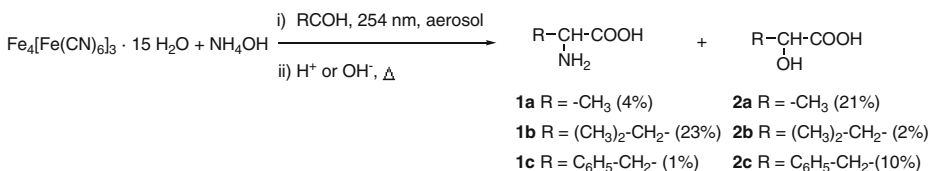
protocol shows that the ratios of the products in the Strecker reaction are effectively altered and that the amino acid production yield increases, indicating that a recycling mechanism that liberates HCN for the formation of amino acids is possible.

Figure 3 shows all the products obtained from the photodegradation of Prussian Blue and ammonium ferrocyanide in the presence of isobutyraldehyde and aqueous aerosols. In this last case (Fig. 3b), the UV radiation seemed to rearrange and/or to decompose the isobutyraldehyde, leading to the production of other organics of prebiotic and biological interest, such as glycine, alanine and several α -hydroxy acids.

Figure 4 shows a comparison of the yields obtained for phenylalanine (**1c**) under some of the different conditions assayed. The chromatograms presented in Fig. 4c and d correspond to the same experiment. The supernatant after acid hydrolysis was richer in phenylalanine (**1c**) (Fig. 4c), whereas the main product in the oily residue was β -phenyllactic acid (**2c**) (Fig. 4d). Other products of prebiotic interest, such as phenylpyruvic acid, were detected at a yield of 2 % in these experiments (Fig. 4d).

In addition, when alanine (**1a**) and phenylalanine (**1c**) were synthesised using aqueous aerosols from free cyanide, the production of aminonitriles was increased relative to the production of cyanohydrins. By contrast, for the same syntheses using Fe-bound cyanide and UV radiation, the opposite effect was observed. In the case of the valine (**1b**), the opposite effects were observed. The nature of the cyanide compound and the level of UV radiation are important factors that seem to influence the Strecker reaction. It is known that in the production of amino acids *via* HCN polymerisation, UV radiation enhances the polymerisation of HCN. This process must cause internal rearrangements, including disproportionation. The irradiation times (i.e., the total dose) strongly affects the reaction yields (Abelson 1966). Indeed, the yields were lower than when free cyanide was used because the ferrocyanides, under the conditions assayed, are only partially decomposed and others compounds are produced. For example, in the experiments using Prussian Blue and UV radiation, the formation of $\text{Fe}(\text{NH}_4)\text{[Fe(CN)}_6\text{]}$ and ammonium ferrocyanide was observed.

Another interesting result observed in our experiments using UV radiation was that the photodegradation of Prussian Blue and ammonium ferrocyanide led to the formation of oxyhydroxides, in agreement with the previous results of Arrhenius (1990). α -FeOOH



Scheme 5 Yields of the Strecker synthesis of alanine (**1a**), valine (**1b**) and phenylalanine (**1c**) using Prussian Blue in the presence of aqueous aerosols and UV radiation

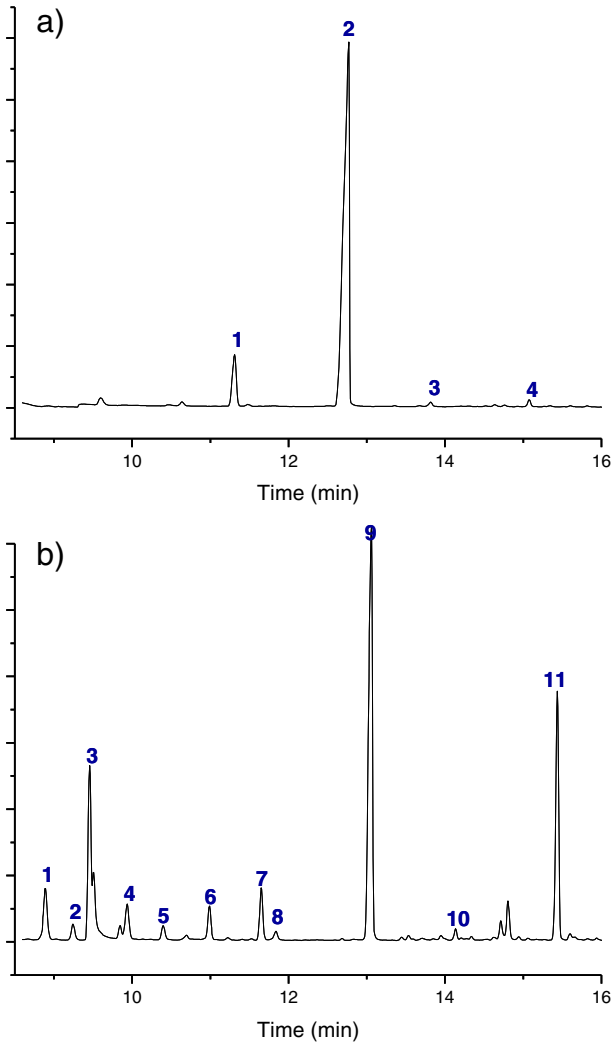


Fig. 3 Valine Strecker reaction using aqueous aerosols, UV radiation and **a** $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot 15 \text{H}_2\text{O}$ (*Prussian Blue*): 1. α -hydroxyvaleric acid, 2. valine (II), 3. α -ketoisovaleric acid and 4. glycine (III); and **b** $(\text{NH}_4)_4[\text{Fe}(\text{CN})_6] \cdot 1.5 \text{H}_2\text{O}$: 1. α -hydroxybutyric acid, 2. hydroxy-acetic acid, 3. valine (I), 4. alanine, 5. glycine (II), 6. α -aminoisobutyric acid, 7. α -hydroxyvaleric acid, 8. α -aminobutyric acid, 9. valine (II), 10. α -ketoisovaleric acid and 11. glycine (III). In this last case, the UV radiation led to rearrangement reactions that produced other organics of prebiotic interest

(goethite) and γ -FeOOH (lepidocrocite) are the most common forms of iron(III) oxide-hydroxide and the most important mineral carriers of iron soils. Their presence is very suggestive within the context of prebiotic chemistry because, as has been recently shown, oxyhydroxides may act as catalysts in amino acid oligomerisation reactions and in the production of purine and pyrimidines under possible prebiotic conditions (Shanker et al. 2011, 2012).

Our model offers a plausible and consistent abiotic scenario in which oxyhydroxide deposits are the final precipitated by-products of a set of cycled reactions that capture and

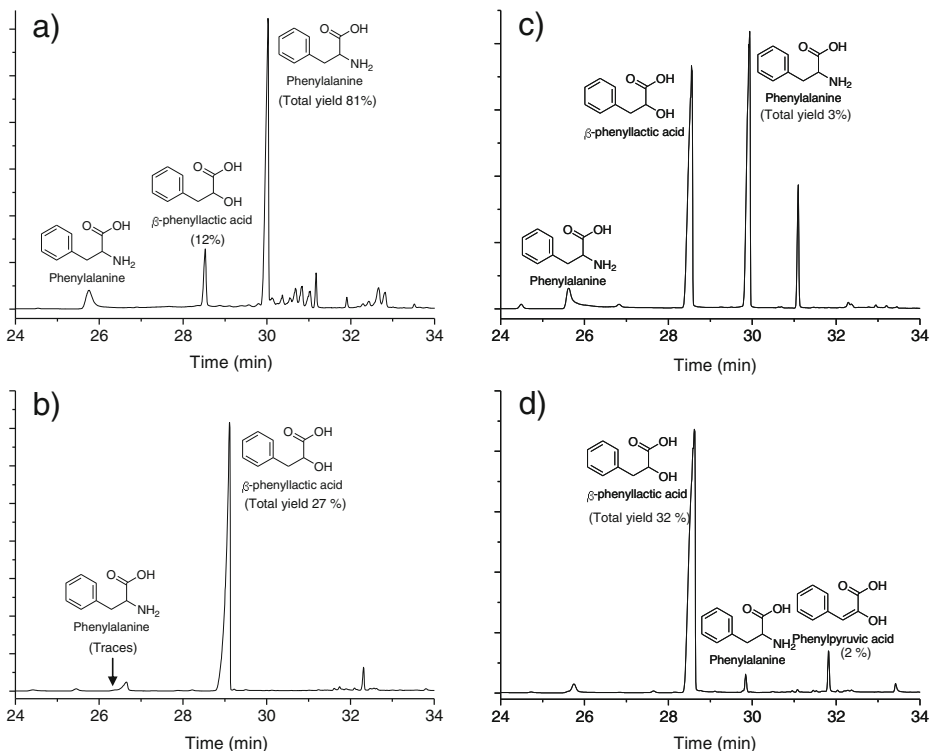
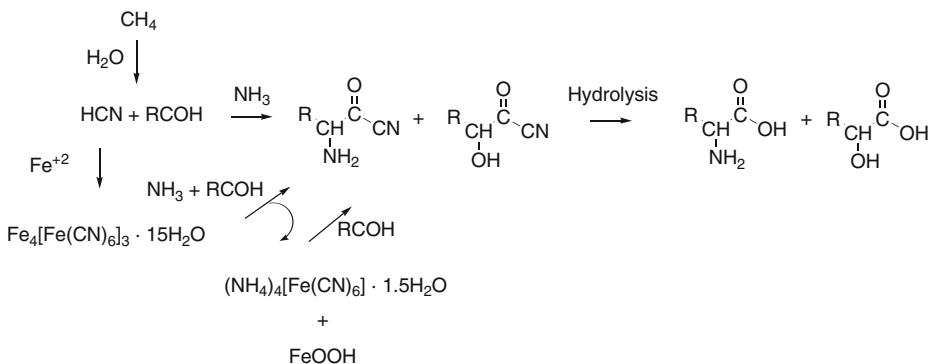


Fig. 4 Phenylalanine Strecker reaction in the presence of aqueous aerosols. **a** Phenylalanine from free cyanide. In the chromatogram, the first peak assigned to phenylalanine corresponds to the derivative with one trimethylsilyl group, and the second peak is the disubstituted derivative. **b** Phenylalanine Strecker reaction from ammonium ferrocyanide. In this case, only trace amounts of phenylalanine was detected, but the secondary product of the reaction, β -phenyllactic acid, was obtained in high yield (27 %). **c** Phenylalanine Strecker reaction from ammonium ferrocyanide and UV radiation. Acidic fraction (supernatant after acid hydrolysis). Phenylalanine was obtained with a total yield of a 3 %. **d** Phenylalanine Strecker reaction from ammonium ferrocyanide and UV radiation. Organic fraction (oily fraction after acid hydrolysis). β -phenyllactic acid was obtained with a total yield of a 32 %. The yield of phenylpyruvic acid was 2 %

UV radiation



Scheme 6 Model of a set of cyclic reactions that capture and liberate HCN in the presence of iron and UV radiation, allowing for the formation of amino acids under possible prebiotic conditions

liberate HCN in the presence of iron and UV radiation, allowing for the sustained formation of amino acids under prebiotic conditions (Scheme 6).

In this work, we have investigated a prebiotic synthesis pathway where iron has a critical role in the chemistry of the hydrosphere and in the production of organics. Even to date, and due to the fact that iron is a micronutrient of most life forms, the iron supply via aerosols to the ocean has a critical impact on nitrogen fixation and the CO₂ cycle, since it affects photosynthesis (Mahowald et al. 2005).

Conclusions

The yields of α -amino acids from the Strecker synthesis are notably improved when using an aerosol cycle, independently of the nature of cyanide, free or metal bounded.

The formation of Prussian Blue or similar precipitates may have partially inhibited the formation of amino acids in early stages of prebiotic chemistry. In this work, we showed that, in the later stages, this or other ferrocyanides may have served as HCN sources when exposed to UV radiation. We observed that this reaction liberates HCN, sustaining the continuous formation of amino acids and oxyhydroxides. Reaction mechanisms such as the one presented herein must have occurred in this prebiotic era to reverse the HCN-consuming reactions and favour the formation of amino acids. If this was a natural process in the prebiotic era, the precipitated products should be found ubiquitously on Earth.

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