N-CARBAMOYL-α-AMINO ACIDS RATHER THAN FREE α-AMINO ACIDS FORMATION IN THE PRIMITIVE HYDROSPHERE: A NOVEL PROPOSAL FOR THE EMERGENCE OF PREBIOTIC PEPTIDES

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Abstract. Our previous kinetic and thermodynamic studies upon the reactional system HCHO/HCN/NH$_3$ in aqueous solutions are completed. In the assumed prebiotic conditions of the primitive earth ([HCHO] and [HCN] near 1 g L^{-1}, T = 25 $^\circ$C, pH = 8, [NH$_3$] very low), this system leads to 99.9% of α-hydroxyacetonitrile and 0.1% of α-aminoacetonitrile (precursor of the α-amino acid). The classical base-catalyzed hydration of nitriles, slow and not selective, can not modify significantly this proportion. On the contrary, we found two specific and efficient reactions of α-aminonitriles which shift the initial equilibrium in favor of the α-aminonitrile pathway. The first reaction catalyzed by formaldehyde generates α-aminoamides, precursors of α-aminoacids. The second reaction catalyzed by carbon dioxide affords hydantoins, precursors of N-carbamoyl-α-aminoacids. In the primitive hydrosphere, where the concentration in carbon dioxide was estimated to be higher than that of formaldehyde, the formation of hydantoins was consequently more efficient. The rates of hydrolysis of the α-aminoacetamide and of the hydantoin at pH 8 being very similar, the synthesis of the N-carbamoyl-α-amino acid seems then to be the fatal issue of the HCHO/HCN/NH$_3$ system that nature used to perform its evolution. These N-protected α-amino acids offer new perspectives in prebiotic chemistry, in particular for the emergence of peptides on the prebiotic earth.

1. Introduction

During the last decades, many important improvements have been made concerning the knowledge of the evolution of the Earth in the first billion years of its existence and particularly the knowledge of the first raw materials available before life arised. In fact, none of the primordial molecules have left any fossil-footprint. Nevertheless, refering to the recent informations based on the studies of atmospheres of other planets of our Solar system (Owen, 1985; Raulin, 1992; Lellouch, 1994; Toublanc, 1995), of meteorites (Wood, 1985) and interstellar clouds (Winnewiser, 1987), we start to get more pertinent ideas on what could have happened on our own planet in the distant past.

More than one hundred species of molecules were identified in the interstellar space. These molecules could have been synthesized in our atmosphere. Some of them, riches in energy (such as HCN (Abelson, 1966; Bar-Nun, 1975; Ferris, 1974 and 1975; Koberstein, 1973; Miller, 1957; Palm, 1962; Sanchez, 1966; Schavoand, 1959; Yuaso, 1975), CNO$^-$ (Yamagata, 1982), HCHO (Pinto, 1980), NO (Chamei-

des, 1981; Fegley, 1986; Yung, 1979)) were synthesized from the mixture of gases of different species.

According to recent estimations, a formaldehyde flux of 10^{11} mol/year in the original atmosphere could have produced the concentration of 2×10^{-2} mol L^{-1} in the hydrosphere. The hydrogen cyanide flux was estimated by another way: by comparison with the atmospheres of Neptune and Titan (Lellouch, 1994; Toublanc, 1995), the formation of an important bulk of HCN is highly probable.

The cyanates and nitrogen monoxide could also have been synthesized in the primitive hydrosphere. They could be considered as the raw materials for the production of ammonia (Summers, 1993).

The estimations of the partial pressure of carbon dioxide in the proto-atmosphere are quite large: they are included between 10 and 70 atm according to some authors (Owen, 1979; Kasting, 1986) and much fewer according to others (Pinto, 1980), approaching the today partial pressure of 3.3×10^{-4} atm.

All these molecules are considered, since a long time, as actors being able to play more or less important roles in the process of the emergence of life. The pioneering experiment of Miller (Miller, 1955) represents an important starting-point for the experimental research concerning the origin of α-aminoacids. On the basis of these first results, two main hypotheses were suggested to explain the formation of α-amino acids. One of them (Moser, 1968) proposes the base-catalyzed polymerisation of hydrogen cyanide, followed by hydrolysis. The other (Ponnamperuma, 1967) supposes the intermediate formation of α-aminonitriles 1, which can be hydrolyzed into α-amino acids via α-aminoamides 3 (according to Strecker (Strecker, 1850)) or via hydantoins (according to Bücherer-Berg (Bergs, 1929; Bücherer, 1934)). The last two reactions require the presence in the media of ammonia at high concentrations. The estimation of the total (t) concentration in ammonia, $[NH_3]_t$, in the primitive ocean is still imprecise (Summers, 1993; Miller, 1981; Bada, 1968). The last estimation of this concentration (3.6×10^{-6} mol $L^{-1} < [NH_3]_t < 70 \, 10^{-6}$ mol L^{-1}) led some authors (Summers, 1993) to hesitate about the efficiency of such a pathway in the process of the emergence of life.

In the present paper, on the basis of kinetic and thermodynamic studies, we propose a chemical pathway which does not lead to the emergence of free α-aminoacids on the prebiotic earth but rather to *N-carbamoyl-α-amino acids*. To obtain this result, we have analyzed the possible prebiotic signification of our previous work upon the Strecker (Taillades *et al.*, 1986) and the Bücherer-Berg (Rousset *et al.*, 1984) reactions. When necessary, we have completed our existing experimental data to make the discussion coherent.

2. Experimental Section

2.1. PRODUCTS

All the compounds are prepared and purified using the standard methods. The hydrochloride of α-aminoacetonitrile 1a (from Aldrich) is recristallized from ethyl alcohol before use and stored at low temperatures. The hydrochloride of α-amino-acetamide 3a (from Ega Chemie) is recristallized from ethyl alcohol. The α-amino-propionitrile 1b is prepared starting from a mixture of acetaldehyde, ammonium chloride and sodium cyanide in an ammonia-containing solution as previously described (Pascal *et al.*, 1980) and stored under the nitrate form. The α-amino-propionamide 3b is obtained by hydration of the corresponding nitrile in an aqueous basic solution of acetone (Commeyras *et al.*, 1977) and stored under the hydrochloride form. The hydantoin 5a (from Aldrich) is used in its commercial form. The 5-methylhydantoin 5b is obtained by the treatment of the α-aminopropionitrile 1b with a buffer aqueous solution of ammonium carbonate (Rousset *et al.*, 1980). The N-carbamoyl-glycine 6a and alanine 6b are obtained by treatment, at 50 °C, with an aqueous solution of potassium cyanate at pH = 4. After 18 hours, the reaction mixture is acidified (HCl 2 N) to make the hydantoic acid precipitate.

2.2. REACTION RATES AND EQUILIBRIUM CONSTANTS

The hydration of α-aminopropionitrile 1b was followed directly by ^{1}H NMR spectrometry (T60 and HA 100 from Varian) and or by UV spectrometry (DW2 from Aminco) (Pascal *et al.*, 1978; 1980).

The carbonatation of the α-aminopropionitrile 1b and the formation of the 5-methylhydantoin 5b were followed at various pH by ^{1}H NMR (HA 100 from Varian) (Rousset *et al.*, 1980).

The hydration at 25 °C of the α-aminoacetonitrile 1a (10^{-3} mol L^{-1} and 10^{-4} mol L^{-1}) in the presence of formaldehyde (5×10^{-3} mol L^{-1} and 8×10^{-2} mol L^{-1}) in a phosphate-buffer solution at pH = 8, 8.5 and 9. was followed by UV spectrometry (Uvikon 860 from Kontron) at $\lambda = 210$ nm, where only the amide absorbs ($\varepsilon_\lambda = 220$ cm^{-1} (mol L^{-1})$^{-1}$).

The equilibrium constant at 25 °C of the carbamate formation from α-amino-acetonitrile 1a was established from ^{1}HNMR spectra (AC 250 from Brucker), by integration of the NMR signals corresponding to the α-aminoacetonitrile 1a ($\delta = 3.68$ ppm (s, 2H, CH$_2$)) and to its carbamate ($\delta = 4.08$ ppm (s, 2H, CH$_2$)) in NaHCO$_3$ aqueous solutions (2×10^{-4} mol L^{-1} and 4×10^{-4} mol L^{-1}) at pH = 9. The spectra were monitored 4 hours after the preparation of the solutions of α-aminonitrile 1 (10^{-4} mol L^{-1}).

The kinetics of formation of hydantoin 5a from α-aminoacetonitrile 1a (0.05 mol L^{-1}) were followed by a sampling during two days at various temperatures (from 40 to 80 °C), in an aqueous solution of NaHCO$_3$ ((0.5 to 0.8) mol L^{-1}), adjusted to pH = 9. Samples taken from the reaction medium were diluted 100
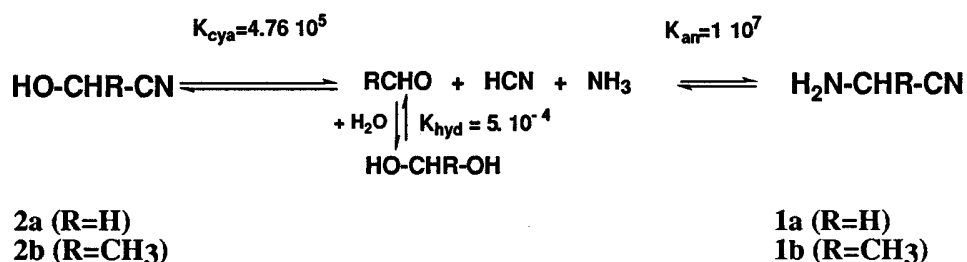
times in H_2O and then analyzed by UV spectrometry. The maximum of absorption at $\lambda = 220$ nm is due to the hydantoin ($\varepsilon_\lambda = 4500$ cm^{-1} $(mol\,L^{-1})^{-1}$).

The kinetics of hydrolysis of hydantoin 5a (10^{-3} $mol\,L^{-1}$) into hydantoic acid 6a are followed at various temperatures from 60 to 95 °C in a buffer solution of carbonate (pH = 9) or phosphate (pH = 7.5 and 8). The samples were analyzed by UV spectrometry during 24 hours (at pH = 9; T = 80 °C) or during 4 days (at pH = 7.5; T = 80 °C). The rate of hydantoin-concentration decay is evaluated from the absorbance measured at $\lambda = 230$ nm.

3. Results and Discussion

3.1. STABILITIES OF α-AMINOACETONITRILE AND α-HYDROXYACETONITRILE IN AQUEOUS SOLUTIONS

We recently studied (Moutou, 1995), in aqueous solutions, the equilibria (1) of formation of α-aminoacetonitrile 1a and of α-hydroxyacetonitrile 2a (Schlesinger, 1973) starting from the three-component system: HCHO/HCN/NH$_3$ (Scheme 1).

$$K_{cya}=4.76\ 10^5 \qquad\qquad K_{an}=1\ 10^7$$

$$\text{HO-CHR-CN} \xrightleftharpoons{\qquad} \text{RCHO} + \text{HCN} + \text{NH}_3 \xrightleftharpoons{\qquad} \text{H}_2\text{N-CHR-CN}$$

$$+ H_2O \;\bigg\|\; K_{hyd} = 5.\ 10^{-4}$$

$$\text{HO-CHR-OH}$$

2a (R=H)   **1a (R=H)**
2b (R=CH$_3$)   **1b (R=CH$_3$)**

Scheme 1. Initial equilibria (1) of the system (RCHO/HCN/NH$_3$) in diluted aqueous solutions at T = 25 °C and pH = 8. K_{cya}, K_{an} and K_{hyd} are given for $R = $ H.

We have shown that the ratio of concentrations at the equilibrium of α-amino-acetonitrile 1a and α-hydroxyacetonitrile 2a at 25 °C and pH 8 is only controled by the concentration in free (f) ammonia independently of any kinetic considerations, as stated by Equation (1)

$$\frac{[H_2N - CH_2 - CN]}{[HO - CH_2 - CN]} = 21[NH_3]f \text{ at } 25\,^\circ C . \tag{1}$$

In the case of acetaldehyde (R = CH$_3$), another aldehyde probably present in the original hydrosphere, the ratio of concentrations [1b]/[2b] is approximately 10 times greater, according to an estimation, based on the constants of stability of 1b (7.6×10^6) and 2b (3.6×10^4) (Béjaud *et al.*, 1976).

With the recent estimations of the concentrations in free ammonia (2×10^{-5} $mol\,L^{-1}$), in formaldehyde and in hydrogen cyanide (both 2×10^{-2} $mol\,L^{-1}$), the

above equation shows that the concentration in α-aminoacetonitrile 1a would be smaller than 0.1% of the total concentration in both nitriles. We suppose here that the protonation is[*] the only free-ammonia consuming equilibrium. Formaldehyde and hydrogen cyanide seem thus to have been stored and stabilized under the α-hydroxyacetonitrile form 2a. The formation of this compound 2a, under kinetic control (Moutou, 1995), protects the starting reactants against degradation. The concentration of ammonia in the primitive atmosphere being very low, these equilibria could not have been shifted in favor of the α-aminoacetonitrile 1a (precursor of glycine) by the influence of the presence of NH_3 but rather by kinetic phenomena of selective reactions. That is what we will analyze below.

3.2. NON-SELECTIVE BASE-CATALYZED HYDRATION OF THE NITRILES HO-CH₂-CN, HCN AND H₂N-CH₂-CN INTO AMIDES

A first simple possibility to make this system evolve from its equilibrium position would be to consider, in the presumed initial conditions of the primitive earth (pH = 8, T = 25 °C) (Brack *et al*., 1991), the base-catalyzed hydrolysis of the three nitriles (HCN, 1a, 2a) into the corresponding amides according to the following kinetic law (Equation (2))

$$\frac{d[\text{RCONH}_2]}{dt} = k_0\,[\text{OH}^-][\text{RCN}] = k[\text{RCN}] \tag{2}$$

$$(R = CH_2OH, \; H \; or \; CH_2NH_2)$$

$$(k_0 \equiv k''_0 \; for \; R = CH_2OH; \; k_0 \equiv k'_0 \; for \; R = CH_2NH_2)$$

We have evaluated the apparent rate constants *k* for the three hydrations and these constants are given in the Scheme 2. The hydration of hydrogen cyanide in a very weak basic medium is extremely slow. The apparent rate constant *k*, obtained by extrapolation of the data established by Sanchez *et al.* (1967) at pH>8 and T>25 °C, is equal to 6.4×10^{-6} h^{-1}, leading to a half-life reaction time of 12.4 years.

The hydration of α-hydroxyacetonitrile 2a is also extremely slow in the same conditions. Starting from measures which were made at higher temperature (80 °C), we could estimate the α-hydroxyacetamide 4a formation rate constant: k''_o (80 °C) = 108 h^{-1} $(mol\ L^{-1})^{-1}$, giving k(80 °C) = 108×10^{-6} h^{-1} (Jammot, 1988). Taking the value of 16500 cal/mol for the activation energy of the hydration of α-hydroxyacetonitrile (Miller *et al*., 1975), we could calculate the rate constant of this hydration *k* (25 °C) = 1.4×10^{-6} h^{-1} (corresponding to k''_o (25 °C) = 1.4 h^{-1} $(mol\ L^{-1})^{-1}$) and $t_{1/2}$ = 56 years.

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[*] We admit, that the ratio $[NH_3]f/[NH_3]t$ is only pH-dependent: $[NH_3]f/[NH_3]t = K_a([H^+] + K_a)$; at 25 °C, K_a (NH_4^+) = 5.6×10^{-10} mol L^{-1}.
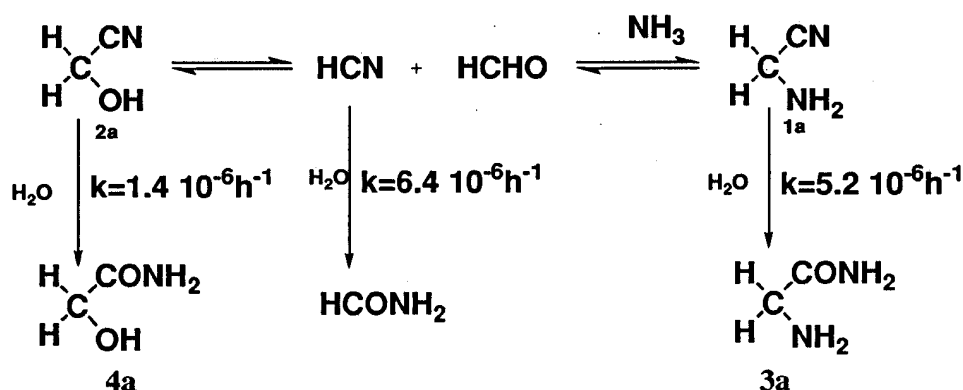
Scheme 2. The apparent rate constants k(h^{-1}) = k_o[OH$^-$] of base-catalyzed hydrations at pH = 8 and 25 °C of α-hydroxyacetonitrile 2a, hydrogen cyanide and α-aminoacetonitrile 1a.

The evaluation of the hydration rate constant $k_o^{'}$ for the α-aminoacetonitrile 1a is more difficult. In this case, we have to take into account a particular catalytic hydration process (see further in this text). Nevertheless, we can obtain an estimation using the Hammett-Taft expression (Equation (3)) with constants given by Miller and Van Trump (1975) for the hydrolysis of nitriles (pH = 8; T = 25 °C)

$$\log[k_0(h^{-1}(mol\ L^{-1})^{-1})] = -1.495 + 4.51\ \sigma*. \tag{3}$$

If $R = NH_2CH_2$, $\sigma* = 0.49$ and the above relation gives $k = 5.2 \times 10^{-6}$ h^{-1} and $t_{1/2} = 15.2$ years.

The above kinetic studies show that the hydration of the three nitriles (HCN, 1a, 2a) occur at similar rates at pH 8. This classical base-catalyzed hydration could thus not have disturbed the initial equilibria (1). Even after a base-catalyzed hydration of nitriles, the proportion α-hydroxyacetonitrile 2a/α-aminoacetonitrile 1a remains under thermodynamic control. In the absence of a specific catalytic hydration, the α-hydroxyacetonitrile 2a is still the major product of equilibria (1). The hydrolysis of nitriles (and aminonitriles in particular) being very slow even in mild conditions, the intervention of such species in the prebiotic synthesis of α-amino acids has been considered to be very unlikely.

We show below that two specific and efficient reactions could have shifted equilibria (1) in favor of the α-aminoacetamide 3a, precursor of the α-aminoacid (glycine).

3.3. SPECIFIC FORMALDEHYDE-CATALYZED HYDRATION OF α-AMINOACETONITRILE INTO α-AMINOACETAMIDE

The hydration of an α-aminonitrile can be catalyzed in a basic medium by a carbonyl compound (Pascal *et al.*, 1978 and 1980) which can be formaldehyde as shown in Scheme 3
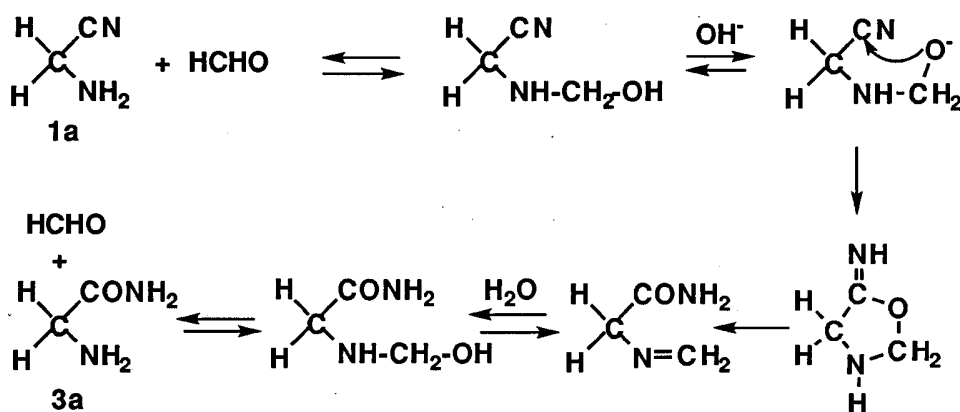
Scheme 3. Mechanism of formaldehyde-catalyzed hydration of α-aminoacetonitrile 1a, leading to α-aminoacetamide 3a.

In diluted medium, the condensation of formaldehyde with an α-aminonitrile leads, through a rapid pre-equilibrium, to the formation of a gem-aminoalcohol. This latter is in equilibrium in basic medium with the aminoalcoholate. The intramolecular cyclisation of the aminoalcoholate, strongly favored, leads to the corresponding imino-5-oxazolidine. This intermediate converts then very quickly into an imine of the α-aminoamide. The hydrolysis of this last product easily gives the α-aminoacetamide 3a and regenerates the catalyst. The reaction rate of this catalytic process is given by the Equation (4)

$$v_1 = k_1 [OH^-][H_2CO]_t[H_2N - CH_2 - CN] . \tag{4}$$

When α-aminopropionitrile 1b was used instead of 1a, we found a reaction rate constant k_1 equal to 1×10^6 h^{-1} (mol L^{-1})$^{-2}$ (Pascal *et al.*, 1980). In the present work, in the case of the α-aminoacetonitrile 1a, we obtained $k_1 = 0.3 \times 10^6$ h^{-1} (mol L^{-1})$^{-2}$. Such rate constants are 10^5 times greater than those of the classical base-catalyzed hydration (Scheme 2). The Equation (5a) presents the ratio v_1/v_o' of the two base-catalyzed hydration rates of α-aminoacetonitrile 1a (v_1 for the formaldehyde-catalyzed and v_0' for the simple base-catalyzed)

$$\frac{v_1}{v_o'} = \frac{k_1 [OH^-][H_2CO]_t[H_2N - CH_2 - CN]}{k_0' [OH^-][H_2N - CH_2 - CN]} = \frac{k_1[H_2CO]_t}{k_0'} . \tag{5a}$$

Such a ratio is only controled (Equation (5b)) by the total concentration in formaldehyde, $[HCHO]_t$. With the previous calculated value $k_o' = 5.2$ h^{-1} (mol L^{-1})$^{-1}$ and with $k_1 = 0.3 \times 10^6$ h^{-1} (mol L^{-1})$^{-2}$, we obtain

$$\frac{v_1}{v_o'} = \frac{0.3 \times 10^6[H_2CO]_t}{5.2} = 0.58 \times 10^5 [H_2CO]_t . \tag{5b}$$

The rate v_1 of the formaldehyde catalyzed hydration will be greater than $v_{o'}$ only if the formaldehyde concentration is greater than 1.7×10^{-5} mol L^{-1}. With the bottom limit estimation of 2×10^{-2} mol L^{-1} for [HCHO]$_t$ in the original hydrosphere, the rate v_1 becomes 1.2×10^3 times greater than v_o'. We can see that in such conditions, the hydration of the α-aminoacetonitrile 1a will be carried out for 99.9% by the formaldehyde-catalyzed hydration and only for less than 0.1% by the simple base-catalysis.

In a similar way, we can compare (Equation (6)) the rate v_1 of the formaldehyde-catalyzed hydration of the α-aminoacetonitrile 1a with the rate v_o'' of the simple base-catalyzed hydration of α-hydroxyacetonitrile 2a*.

$$\frac{v_1}{v_0''} = \frac{k_1 \, [\text{OH}^-][\text{H}_2\text{CO}][\text{CH}_2 - (\text{CN})\text{NH}_2]}{k_0'' \, [\text{OH}^-][\text{CH}_2 - (\text{CN})\text{OH}]} =$$

$$\frac{k_1 [\text{H}_2\text{CO}] \, [\text{CH}_2 - (\text{CN})\text{NH}_2]}{k_0'' \, [\text{CH}_2 - (\text{CN})\text{OH}]} \, . \tag{6}$$

Taking again $k_1 = 0.3 \times 10^6$ h^{-1} (mol L^{-1})$^{-2}$ and $k_o'' = 1.41$ h^{-1} (mol L^{-1})$^{-1}$, we finally obtain

$$\frac{v_1}{v_o''} = 0.2 \times 10^6 \, [\text{H}_2\text{CO}]_t \, \frac{[\text{CH}_2 - (\text{CN})\text{NH}_2]}{[\text{CH}_2 - (\text{CN})\text{OH}]} \, . \tag{7}$$

We have shown above that the equilibria (1) between the three reactants HCHO, HCN, NH$_3$ in water lead to an excess of α-hydroxyacetonitrile 2a, the α-amino-acetonitrile 1a concentration being very weak (less than 0.1%). With a medium concentration in ammonia in the primitive ocean equal to 2×10^{-5} mol L^{-1}, the above relation gives

$$\frac{v_1}{v_0''} = 4.2 \times 10^6 \, [\text{H}_2\text{CO}]_t [\text{NH}_3]_f = 84 \, [\text{H}_2\text{CO}]_t \, . \tag{8}$$

If we take [HCHO]$_t = 2 \times 10^{-2}$ mol L^{-1}, we can see that in the original hydrosphere conditions the rate v_1 of formation of the α-aminoacetamide 3a, precursor of glycine, will be 1.7 times faster than the rate of formation of the α-hydroxyacetamide 4a. In these conditions (pH = 8, [HCHO] = 2×10^{-2} mol L^{-1}), the rate of formation of the α-aminoacetamide 3a will be (Equation (9))

$$v_1(\text{mole L}^{-1})\text{h}^{-1} = 0.3 \times 10^6 [\text{CH}_2 - (\text{CN})\text{NH}_2][\text{H}_2\text{CO}]_t [\text{OH}^-]$$

$$v_1(\text{mole L}^{-1})\text{h}^{-1} = 6 \times 10^{-3} [\text{CH}_2 - (\text{CN})\text{NH}_2] \, . \tag{9}$$

* We don't take into account the low catalytic effect of the borates ions already described for the hydration of cyanohydrines (Jammot *et al.*, 1989)

The reaction rate v_1 is furthermore 15 times greater than the reaction rate v_d (Equation (10)) of decomposition of the α-aminoacetonitrile 1a** (Pascal *et al.*, 1978)

$$v_d = 4 \times 10^{-4} [CH_2 - (CN)NH_2] . \tag{10}$$

We can thus conclude (Scheme 4) that in such conditions the initial equilibrium between HO-CH$_2$-CN and H$_2$N-CH$_2$-CN was not maintained and was strongly shifted in favor of the α-aminoamide. With $[NH_3]_t = 2 \times 10^{-5}$ mol L^{-1} and $[HCHO]_t = 2 \times 10^{-2}$ mol L^{-1} (at pH = 8 and T = 25 °C), the rate v_1 of formation of the α-aminoacetamide 3a will be not only 1.7 times that of α-hydroxyacetamide 4a (as it is at the equilibrium), but will become much greater.
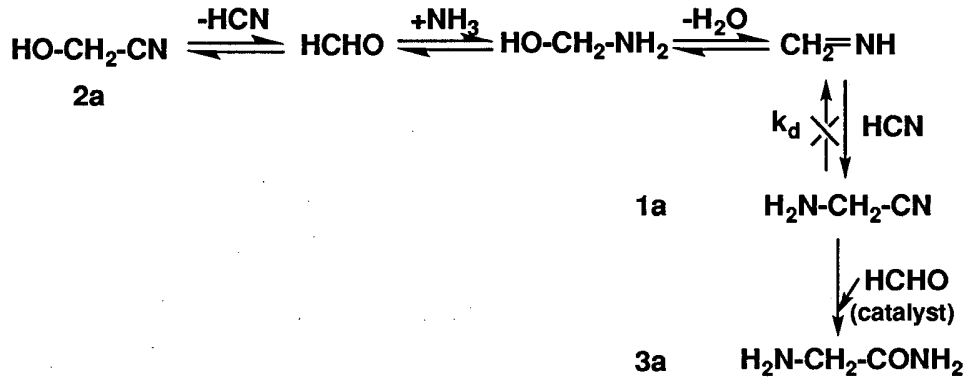
Scheme 4. Reversible mechanism of formation of the α-aminoacetonitrile 1a. When $[HCHO]_t > [HCN]_t$, the equilibrium $[HOCH_2CN]/[H_2NCH_2CN]$ is shifted in favor of the α-amino-acetamide 3a.

In fact, the main problem in such studies is to evaluate the concentration in formaldehyde in the primitive ocean (the maximal value is evaluated to 2×10^{-2} mol L^{-1}). In the presence of hydrogen cyanide, which plays a main role in prebiotic chemistry, the concentration in formaldehyde is controled by the apparent constant K'_{cya} of formation of the cyanohydrin at pH 8 (Schlesinger, 1973) which is quite great

$$K'_{cya} = \frac{[CH_2(OH)(CN)]}{[CH_2O]_t [HCN]_t} = 4.44 \times 10^5 \ (\text{mole L}^{-1})^{-1} . \tag{11}$$

With such a high stability of the cyanohydrin 2a, whatever the formaldehyde concentration is, the only condition to make the formaldehyde-catalyzed hydration of the α-aminonitrile possible is: $[HCHO]_t > [HCN]_t$. Such a situation is compatible with the processes of prebiotic syntheses already described (Calvin, 1969). In a first prebiotic period, when $[HCN]_t$ was high ($> [HCHO]_t$), the synthesis of bases
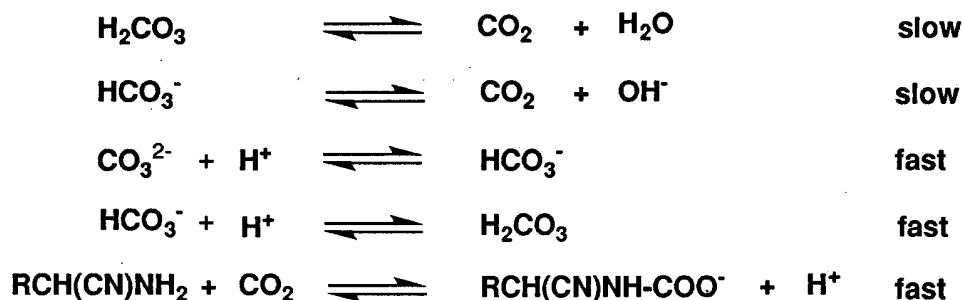

** In a neutral or weakly basic medium, the reaction rate v_d is almost independent of pH: the slow step of the decomposition is the cutting of the C-CN single bond.

(guanine, adenine) took place. Later on, in a second period, when [HCN] decreased, the formaldehyde concentration became more important ($>[HCN]_t$) and this could have allowed the prebiotic synthesis of sugars.

The above studies thus show that the formaldehyde-catalyzed hydration of aminonitriles could have shifted the equilibria (1) to the right and favored the formation of the α-aminoamide, precursor of the α-aminoacid.

3.4. SPECIFIC CONVERSION OF α-AMINOACETONITRILE INTO HYDANTOIN IN THE PRESENCE OF CARBON DIOXIDE

We propose now another chemical pathway than hydration which could have occured in the prebiotic conditions and shifted the initial equilibria (1) to the same direction than the above-mentioned. The process implies the Bücherer-Berg (Bergs, 1929; Bücherer, 1934) reaction we have studied for a long time (Rousset *et al.*, 1980 and 1984). This reaction, specific to α-aminonitriles, uses carbon dioxide and leads to hydantoins 5 (Scheme 6) and N-carbamoylaminoacids 6 (Scheme 7), precursors of α-aminoacids. Contrary to the previous formaldehyde-catalyzed-formation studied above, this way does not need any hypothesis upon the chronological formation of formaldehyde and hydrogen cyanide in the primitive hydrosphere. In the presence of appreciable concentrations in carbonates and hydrogeno-carbonates ions, the carbon dioxide slowly formed can quickly react on the α-aminonitrile 1 to afford the corresponding carbamate (Scheme 5)

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O \qquad \text{slow}$$

$$HCO_3^- \rightleftharpoons CO_2 + OH^- \qquad \text{slow}$$

$$CO_3^{2-} + H^+ \rightleftharpoons HCO_3^- \qquad \text{fast}$$

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \qquad \text{fast}$$

$$RCH(CN)NH_2 + CO_2 \rightleftharpoons RCH(CN)NH\text{-}COO^- + H^+ \qquad \text{fast}$$

Scheme 5. Formation of carbamates by reaction of CO_2 on α-aminonitriles.

If C_t represents the total concentration in carbonates (Equation (12)),

$$C_t = [CO_2] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \qquad (12)$$

the apparent equilibrium constant K_{carb} of carbamates formation is given by the following equation (Equation (13))

$$\frac{[RCH(CN)NH - COO^-]}{[RCH(CN)NH_2]\ C_t} = k_{Carb} \qquad (13)$$

We have earlier shown that in the case of α-aminopropionitrile 1b (R = CH$_3$) we took as a model, the K_{carb} constant is almost pH independent around pH 8. At this pH, this constant is at its maximum (at 25 °C, K_{carb} (1b) = 3.6 (mol L^{-1})$^{-1}$ (Rousset *et al.*, 1980)) and corresponds furthermore to the best compromise between a sufficient proportion of dissolved CO$_2$ and an acceptable stability of the α-aminonitrile 1. In the present work, the K_{carb} constant was also determined for the α-aminoacetonitrile 1a (R = H): K_{carb} (1a) = 9.0 (mol L^{-1})$^{-1}$.

Near pH 8, the [carbamate]/[aminonitrile] ratio (Equation (13)) thus only depends on C_t.

We have also studied the mechanism of transformation of such carbamates (Rousset *et al.*, 1984) into hydantoins and we have shown that the initial intramolecular cyclisation (Scheme 6) is the slow (k_2) step. The mechanism of this process is quite similar to that (Scheme 3) proposed for the aldehyde-catalyzed hydration of α-aminonitriles 1. That is to say that the imino-5-oxazolidinone so obtained rearranges quickly (k_3) into an α-isocyanatoamide.

Scheme 6. Mechanism of the Bücherer-Berg conversion of α-aminonitriles 1 into hydantoins 5 in presence of CO$_2$.

This compound, by a rapid intramolecular cyclisation, leads exclusively to the hydantoin 5 according to the kinetic law $v_2 = k_2\ K_{carb}[\text{CH}_2\ (\text{CN})\text{NH}_2].C_t$. We have determined in the present work the rate constant of the first slow cyclisation at 25 °C (for R = H): $k_2 = 0.045\ \text{h}^{-1}$. In the same conditions, the reverse ring-opening reaction rate constant, k_{-2}, is negligible at pH = 8, so that in presence of carbonic anhydride the equilibria (1) must be only shifted in favor of the hydantoin.

The prebiotic signification of this reaction depends on the concentration of carbonic anhydride in the early hydrosphere. In the today oceans, the distribution

of CO_2 between the atmosphere, the sea-water and the solid sediments is one of the most difficult problem of the marine chemistry (Riley, 1970). The origin of this difficulty lies in the slowness with which the reactions concerned take place and attain the equilibria in a complex heterogeneous multiphase system.

Without taking into account the variations of pH with temperature and depth, the total concentration in carbonates C_t in the today oceans can be estimated between 2 and 3×10^{-3} mol L^{-1}. It is generally admitted that, since the early Precambrian period, the partial pressure of CO_2 in the air (3.3×10^{-4} atm) and its concentration in water ($C_t = 1.12 \times 10^{-5}$ mol L^{-1}) did not significantly changed.

In the primitive hydrosphere, the partial pressure of CO_2 is still unknown. The estimations vary between 70 atm (Owen, 1979; Kasting, 1986) and 2×10^{-4} atm (Pinto *et al.*, 1980). It seems nevertheless clear that during a certain period the partial pressure of CO_2 in the hydrosphere was high. The concentration of the dissolved CO_2 in the protooceans was consequently great. The acidity thus increased was responsible for the erosion of shores. Later on, the progressive condensation of water and the decrease of athmospheric CO_2 partial pressure transformed the protooceans into the present oceans (pH $\approx$ 8).

Taking into account all these considerations, if we choose for the total concentration in carbonates in the primitive oceans the today concentration $C_t = 2.5 \times 10^{-3}$ mol L^{-1}, which is obviously a minimised value, the ratio [carbamate]/[aminonitrile] at the equilibrium will be

$$\frac{[\text{RCH(CN)NH} - \text{COO}^-]}{[\text{RCH(CN)NH}_2]} \approx 1\% \, (R \,=\, \text{CH}_3) \text{ and } 3\% \, (R \,=\, \text{H}) \,. \tag{14}$$

In the above conditions, the ratio (Equation (15)) of reaction rates of the hydantoin 5a formation (v_2) and of the formaldehyde-catalyzed α-aminoacetonitrile 1a hydration (v_1) can be written

$$\frac{v_2}{v_1} \;=\; \frac{\text{k}_2 \, \text{K}_{\text{carb}}[\text{CH}_2(\text{CN})\text{NH}_2] \, C_t}{\text{k}_1 \, [\text{CH}_2(\text{CN})\text{NH}_2] \, [\text{H}_2\text{CO}]_t [\text{OH}^-]} \,. \tag{15}$$

Taking $k_2 = 0.045$ h^{-1}, $K_{carb} = 9$ (mol L^{-1})$^{-1}$, $k_1 = 0.3 \times 10^6$ h^{-1} (mol L^{-1})$^{-2}$ and [OH$^-$] = 10^{-6} mol L^{-1}, we obtain (Equation (16))

$$\frac{v_2}{v_1} \;=\; 1.35 \, \frac{C_t}{[\text{H}_2\text{CO}]_t} \,. \tag{16}$$

At 25 °C and pH = 8, with $C_t = 2.5 \times 10^{-3}$ mol L^{-1} (minimised value) and $[\text{HCHO}]_t = 2 \times 10^{-2}$ mol L^{-1}, the reaction rates v_1 and v_2 are comparable: with such concentrations, hydantoins and α-aminoamides should thus have been formed at similar rates. In spite of the existing doubts, the partial pressure in CO_2 in the primitive hydrosphere was very likely much higher (> 1 atm). In this case, v_2 becomes much greater than v_1 that is to say that the formation of hydantoins is

much favorised compared to the formation of α-aminoamides. We can conclude at this stage that the initial equilibrium between the three species HCHO, HCN, NH_3 selectively leads to hydantoins.

In the course of the study of the prebiotic signification of this chemical evolution, we wondered if this selectivity was conserved in the following step of the pathway. For this purpose, we determined and compared the hydration rate constants at pH 8 and 25 °C of both the hydantoin (leading to the N-carbamoylglycine) and the α-aminoamide (leading to glycine).

3.5. BASE-CATALYZED HYDROLYSIS OF α-AMINOACETAMIDE INTO GLYCINE AND OF HYDANTOIN INTO N-CARBAMOYL-α-AMINO ACID

The kinetic of hydrolysis of α-aminoacetamide 3a was studied in detail by Van Trump and Miller (1975). At pH>10, the reaction is of first order towards the concentration in hydroxyl ion. It corresponds to the classical mechanism of the attack of the hydroxyl ion on the amide. On the contrary, at pH<10 the attack of the hydroxyl ion on the protonated form of the α-aminoacetamide 3a ($pK_a = 8$) is significant. Such behaviour has for consequence a relatively small variation of the apparent rate constant of hydrolysis in the interval: 7.5<pH<8.5. At pH = 8 and 25 °C, the rate constant we have calculated on the basis of previous data (Van Trump and Miller, 1975) is equal to 3.6×10^{-5} h^{-1} ($t_{1/2} = 2.2$ years).

The hydrolysis of hydantoin 5a takes place in two consecutive and slow steps. The first step occurs in a basic medium and leads to the N-carbamoyl-glycine 6a. The second step is the base-catalyzed hydrolysis of 6a into glycine. At pH 8 and at 25 °C, this hydrolysis is quite difficult and will therefore not be taken into account here.

On the basis of kinetic data obtained in a basic medium (pH>11; Figure 1), Blagoeva *et al.* (1978) proposed a mechanism of hydrolysis (Scheme 7) involving the slow attack of OH^- ions on hydantoin under the 5a form (whereas the majority of the molecules stay under the 5a$'$ form):

This attack leads to the tetrahedral adduct 5a$''$ which transforms itself following two kinetically-significant ways: one of them ($k_3[OH^-]$) leading to N-carbamoyl-glycine 6a and the other one (k_{-1}) returning back to the hydantoin 5a. In the present work, we studied the hydrolysis of the hydantoin at pH between 7 and 9 at high temperatures (80 °C, 100 °C, 120 °C) and we made an extrapolation of the values obtained at 25 °C.

To understand completely our results (Figure 1), it is necessary to take into account both the spontaneous decomposition (k_2) of the tetrahedral intermediate and the fraction of the hydantoin under its non dissociated form ($pK_a = 9.04$).

At pH = 8 and at 25 °C, the apparent rate constant of hydrolysis of the hydantoin, leading to N-carbamoylglycine 6a, is $(2.2 \pm 0.8) \times 10^{-5}$ h^{-1}. This value is quite small but very similar to the rate constant (3.6×10^{-5} h^{-1}) of hydrolysis of the α-aminoacetamide 3a, leading to glycine.
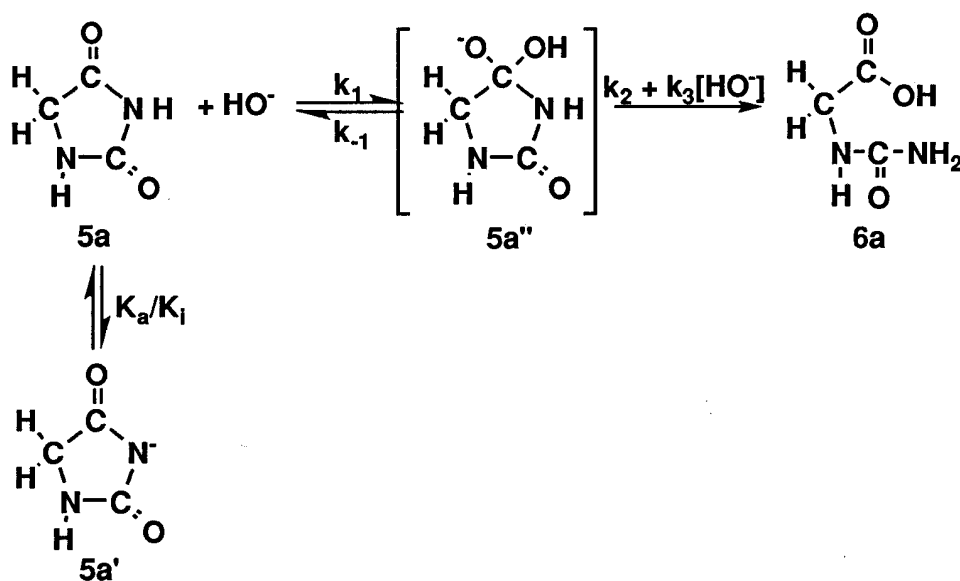
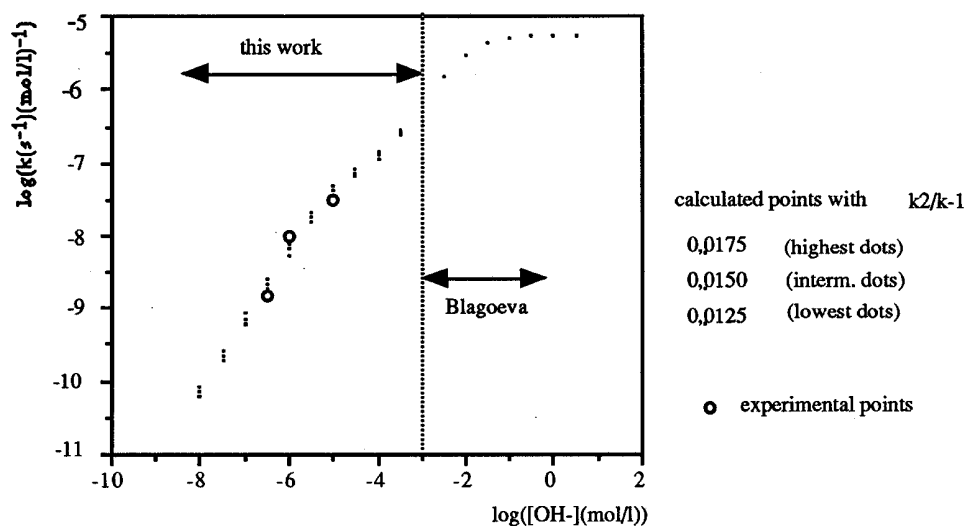*Scheme 7.* Mechanism of hydrolysis of hydantoin 5a into N-carbamoyl-α-amino acid 6a.

Figure 1. Logarithm of the reaction rate constant k (s^{-1} (mol $L^{-1})^{-1}$) of hydantoin 5a-hydrolysis as a function of log([OH⁻](mol L^{-1})). Experimental points and calculated ones on the basis of Blagoeva's relationship: $k = k_1[OH^-]$ A/(1+[OH⁻]K_a/K_i)/(1+A) with A = k_2/k_{-1}+[OH⁻]k_3/k_{-1} and for three k_2/k_{-1}-values (0.0125, 0.0150, 0.0175) and with: $k_1 = 0.495s^{-1}$ (mol $L^{-1})^{-2}$; k_3/k_{-1} = 120 (mol $L^{-1})^{-1}$; $K_a = 0.3981 \times 10^{-9}$ (mol $L^{-1})^{-2}$; $K_i = 1 \times 10^{-14}$ (mol $L^{-1})^{-1}$. The best fit between the three experimental points (o) and the curve is obtained for $k_2/k_{-1} = 0.0137$.

This result thus involves that the selectivity of the precedent step is conserved. We can therefore conclude that the formation of the N-carbamoylglycine (and by

extension of N-carbamoyl-α-amino acids) seems to be the fatal prebiotic issue of a non destructive chemical evolution of the system HCHO/NH$_3$/HCN.

4. Conclusion

In the assumed prebiotic conditions (T = 25 $^\circ$C, pH = 8), the three-component system HCHO/NH$_3$/HCN leads to an equilibrium between the α-hydroxyaceto-nitrile ($>$99.9%) and the α-aminoacetonitrile ($<$0.1%). Our studies presented in this paper show that this equilibrium could have been kinetically shifted in favor of the precursor of the α-amino acid by two catalytic reactions, using respectively formaldehyde and carbon dioxide (both prebiotic reactants) as catalyst. These two ways produce in a first time α-aminoamides or hydantoins and are much more efficient than the classical base-catalyzed hydration of nitriles. The concentration in the primitive hydrosphere in carbon dioxide being higher than that of formaldehyde, the way which leads to the formation of hydantoins was certainly much favorised. The rates of hydrolysis of both hydantoins and α-aminoamides are comparable, so that N-carbamoyl-α-amino acids appear to be the preferential issue that nature chose to preserve the initial system from degradation and to make it further evolve. N-carbamoyl-α-amino acids, which are N-protected α-amino acids, have never been mentioned as intermediates in prebiotic chemistry. In spite of their small reactivity compared to that of α-amino acids, these compounds seem to offer great potentialities. We have indeed recently shown (Collet *et al*., 1996) that they are activated by a mixture of NO and O$_2$ to afford N-carboxyanhydrides, well known precursors of peptides. The formation of N-carbamoyl-α-amino acids in the primitive hydrosphere is thus not a 'cul de sac'. We can even wonder if these NCA could not have been a source of energy for other coupling reactions. We are currently studying such developments.

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