Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1249–1253

BIOORGANIC & MEDICINAL CHEMISTRY

A Possible Prebiotic Synthesis of Purine, Adenine, Cytosine, and 4(3H)-Pyrimidinone from Formamide: Implications for the Origin of Life

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Received 3 November 2000; accepted 15 December 2000

Abstract—The synthesis of prebiotic molecules is a major problem in chemical evolution as well as in any origin-of-life theory. We report here a plausible new prebiotic synthesis of naturally occurring purine and pyrimidine derivatives from formamide under catalytic conditions. In the presence of $CaCO_3$ and different inorganic oxides, namely silica, alumine, kaolin, and zeolite (Y type), neat formamide undergoes the formation of purine, adenine, cytosine, and 4(3H)-pyrimidinone, from acceptable to good yields. The role of catalysts showed to be not limited to the improvement of the yield but it is also relevant in providing a high selectivity in the products distribution.

Introduction

Any origin-of-life theory based on the evolution of a self-replicating genetic system, that is accurate enough to undergo Darwinian evolution, involves the use of chemically stable compounds, and stabilizing micro-environments. Stability is required to allow both low-error template processes, and the accumulation of precursors in chemical conditions permitting polymerization to oligomers. Several examples of synthetic reactions producing prebiotic pregenetically-relevant compounds have been described starting from CO, H_2 and NH_3 in the presence of Fe or Ni at 200–400 °C,[1] from ammonium cyanide,[2] via cyanoacetylene by sparkling mixtures of $CH_4 + N_2$[3] and cyanoacetaldehyde.[4] Hydrogen cyanate (HCN) chemistry provides a preferential route for the prebiotic synthesis of purines and pyrimidines.[5] A set of conditions that would favour the synthesis versus degradation has been described,[6] essentially consisting in a high concentration of HCN and in relatively low temperature. It was pointed out,[6] that a major problem for the accumulation of the nucleobases adenine (A), uracil (U), guanine (G), cytosine (C), and thymine (T) on early Earth is in their rapid rates of degradation at high temperatures, irrespective of whether DNA or RNA were the original informational molecules. However, several considerations, mainly based on the evidence indicating warm conditions in the early evolution of the Earth, have led to suggest that life originated under high-temperature conditions (80–110 °C or higher, up to 350 °C).[7–14] These considerations led to the conclusion that an origin of life based on a four-letter code and on the present-day compounds would only be possible if it occurred very rapidly (< 100 yrs) at relatively low temperatures, and in the presence of stabilizing micro-environments. In addition to the condensation into nucleobases, HCN hydrolyzes to formamide and then to formic acid.[15] Therefore, in a HCN-chemistry based scenario, formamide could be a possible additional down-the-line candidate for the synthesis of nucleobases.[5,16] Pioneering studies have shown that purine can be synthesized from neat formamide when treated at high temperature.[17–19] Noteworthy, the introduction of

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0968-0896/01/$ - see front matter
PII: S0968-0896(00)00340-0

HCN in the reaction vessel gave adenine as major product.[19] ^{13}C NMR and ^{13}C, ^{15}N coupling NMR studies of adenine prepared from doubly enriched potassium cyanide, clearly indicated the presence of both molecules of HCN and formamide in the adenine ring.[20] A new synthesis of purine and pyrimidine derivatives from formamide under catalytic conditions would provide a more efficient prebiotic route. Silica, alumina, and more complicated metallic oxides such as perovskites, spinels, clays and zeolites, were present in the early Earth. These inorganic oxides have already shown to be of value in a large range of abiotic catalytic transformations due to acidic or basic properties, to cation-exchange capacity, and to the possibility to accommodate copious quantities of water or other polar molecules.[21] In the latter case, a network of cages and channels might provide a local microenvironment to stabilize and template the new formed prebiotic molecules into oligomers.[22] As an example, oligomers of glycine have been formed in the presence of clays under experimental conditions simulating natural prebiotic processes.[23] Moreover, certain purines and pyrimidines (such as thymine), which are not normally intercalated into clays from aqueous solution, are taken up in the presence of adenine, with which they may pair in the interlamellar region.[21] In an effort to investigate a possible role of metallic oxides on the prebiotic synthesis of nucleic bases from formamide we treated neat formamide in the presence of $CaCO_3$, silica, alumina, kaolin, zeolite (Y type) as representative prebiotic models of heterogeneous catalysts.

Results

The temperature upper limit for the synthesis of nucleobases from formamide is set by its boiling point (211 °C). Azeotropic effects for a 5–95% formamide solution are limited.[24] The lowest temperature at which formamide undergoes appreciable thermal decomposition is 180–190 °C.[24,25] Evaluation of the steady state concentration of a formamide water solution at various temperatures in prebiotic conditions is a procedure full of uncertainty, because it would depend on which reaction or on which combination of the many reactions yielding formamide is considered, on the rate of hydrolysis (strictly dependent on pH), etc. In the ''drying-lagoon'' model,[4] a solution of formamide would easily become highly concentrated upon heating, given its solubility and non-volatility. In increasingly anhydrous condition, hydrolysis of formamide becomes less relevant. Thus, the most favorable set of conditions for the synthesis would be high formamide concentration, the presence of a catalytic system, a local stable microenvironment, and a temperature higher than 100 °C and lower than 180 °C. On the basis of these suggestions, all experiments were performed with neat formamide **1** (5.7 g., 5 mL, 0.12 mol) at 160 °C for 48 h in the presence of selected inorganic catalysts (1.0% w/w). Besides low amounts of unidentified by-products (high temperature reactions performed on a one carbon fragment yield always very complex reaction mixtures) we focused our attention on the identification of the more abundant purine and pyrimidine derivatives. The main reaction products, purine **2**, adenine **3**, cytosine **4**, and 4(3H)-pyrimidinone **5**, are shown in Figure 1. Their analysis and identification were performed by gas chromatography–mass spectrometry in comparison with samples of authentic products (Table 1).[26]

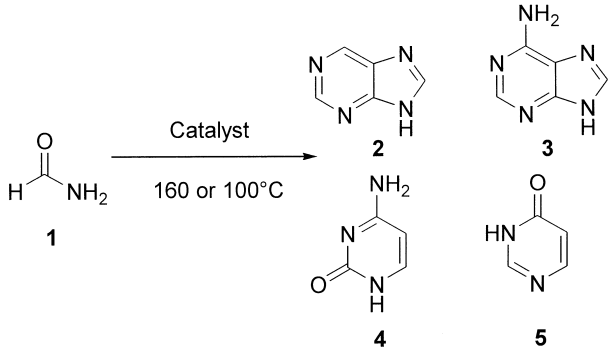

Figure 1.

Table 2. Condensation of formamide

Entry	Catalyst[a]	Temperature (°C)	Product (s)[b]	Yield (mg/g)[c]
1	No catalyst	160	**2**	34.1
2	$CaCO_3$	160	**2**	214.9
3	Kaolin	160	**2**, **3**, **4**	51.3, 0.6, 2.0
4	Zeolite	160	**2**, **3**, **4**	82.5, 0.6, 4.4
5	Alumina	160	**2**, **3**, **4**, **5**	39.7, 0.7, 1.4, 2.0
6	Silica	160	**2**, **3**, **4**, **5**	4.0, 0.9, 4.2, 1.5
7	Kaolin	100	**2**	3.0

[a]Reactions were performed in the presence of 1.0% w/w of catalyst.
[b]Products were identified by comparison of their retention times and mass spectra with those of authentic samples.
[c]quantitative evaluation was performed by capillary gas-chromatographic analysis using a HP 5890 III gas chromatograph with FID detector equipped with a column SP-2380, using a temperature programme of 100–250 °C per min with He as carrier gas. 6-Methoxypurine was used as internal standard. Because of the uncertainty of the number of formamide molecules involved in the synthesis of recovered products the yield were calculated as mg of product formed for gram of formamide.

Table 1. Selected mass spectrometric data[a]

Product	MS (m/z) data (%)
2	120 (M, 100), 93 (M-HCN, 37), 66 (M-2×HCN, 19)
3	135 (M, 100), 108 (M-HCN, 38), 81 (M-2×HCN, 26), 66 (M-69, 35), 54 (M-3×HCN)
4	111 (M, 100), 95 (M-NH_2, 20), 83 (M-CO, 32), 69 (M-NCO, 45), 68 (M-HNCO, 35), 41 (M-HNCO-HCN, 58)
5	96 (M, 100), 69 (M-HCN, 25), 68 (M-CO, 35), 54 (M-NCO, 47), 53 (M-HNCO, 34)

[a]Mass spectroscopy was performed with Hewlett-Packard 5971 mass-selective detector on a Hewlett-Packard 5890 III gas chromatograph with FID detector.

In the absence of any catalyst, purine **2** was obtained in low yield as the only recovered product (Table 2, entry 1). When the reaction was performed in the presence of $CaCO_3$, we obtained a high increase in the yield of **2** (Table 2, entry 2). In this case other purine and pyrimidine derivatives were not obtained in appreciable yield. A different behavior was found in the presence of kaolin, zeolite, alumina, and silica. In the presence of kaolin and zeolite, an increase in the yield of **2** was again observed, in addition to the presence of adenine **3** and cytosine **4** (Table 2, entries 3 and 4). In particular, with zeolite, that is characterized by a micropourus structure as a favourable microenvironment and dehydrating system, the yield of purine **2** was found second only to $CaCO_3$. The presence of adenine in the reaction mixtures suggests that the dehydration of formamide to HCN, an essential step in the synthesis of **3**, is an operative process in the latter two cases. Moreover, it is reasonable that HCN might be involved also in the formation of **4**. In the presence of alumina there is not an appreciable increase in the yield of **2**. In this case, besides the previously observed products **3** and **4**, 4(3H)-pyrimidinone **5** was also obtained in the reaction mixture (Table 2, entry 5). To the best of our knowledge there are no reports in the literature dealing with the direct synthesis of cytosine **4** and 4(3H)-pyrimidinone **5** from formamide. Formamide is widely used in the primary synthesis of pyrimidine derivatives involving formation of three bonds with others reagents, for example the consensation of two molecules of formamide with ketone derivatives yielding 4-substituted pyrimidines via *N*-formylformamidine as reactive intermediates.[27,28]

Prebiotic syntheses of pyrimidines include ultraviolet light induced dehydrogenation of dihydrouracil on silica,[29] the formation of orotic acid from cyanide polymerization,[30] and the synthesis of cytosine and uracil by condensation of cyanoacetaldehyde and urea.[4,31] We have not studied in detail the mechanism of formation of compounds **4** and **5** under our experimental conditions.

It is interesting to note that 2-cyanoacetamide, a plausible three carbon atom precursor of pyrimidine derivatives, was identified by mass-spectroscopy [$m/z = 84$ (M^+, 30%)] and by comparison with an authentic sample (data not shown). A different reaction pathway was observed in the presence of silica under analogous experimental conditions. In this case, the yield of purine **2** was found to be lower with respect to the reaction performed in the absence of catalyst, while cytosine **4** and 4(3H)-pyrimidinone **5** were recovered as the main reaction products (Table 2, entry 6). Clearly, in the presence of silica the formation of pyrimidine derivatives is favoured. In part, a similar effect was obtained also in the presence of alumina. In a selected representative case (kaolin) the reaction was performed also at 100 °C to study the effect of the temperature on the reaction pattern. Under these experimental conditions, purine **2** was obtained in low yield as the only recovered product (Table 2, entry 7). In this case the reaction temperature appears to be a crucial factor for the synthesis of adenine and pyrimidine derivatives, probably because of a less efficient generation of HCN. Independently from the specific reaction conditions, these results show that formamide is a plausible prebiotic substrate for the synthesis of purine and pyrmidine derivatives and that a major role could have been played by inorganic catalysts in improving the yield and changing the distribution of reaction products.

Conclusions

The mixtures of the organic components of nucleic acids that are formed from single molecules such as ammonia, formaldehyde and HCN are usually very complex,[5,32,33] so that the selection of particular derivatives is hard to understand. Moreover, the rapid rates of degradation of purine and pyrimidine derivatives at high temperature is a relevant problem. Accordingly, it has been assumed that the presence of inorganic components with layer or porous cavity structure could provide the occurrence of a favourable local microenvironment. This could introduce a selectivity in the product distribution and possibly increase the thermal stability of nucleobases through molecular recognition processes or simply by their isolation from the external environmental conditions. We have hypothesized that formamide condensation performed in the presence of inorganic oxides can provide a framework for one plausible pathway of prebiotic synthesis of purine and pyrimidine derivatives. This hypothesis satisfies some of the accepted prerequisites for prebiotic chemical evolution. Among the examples studied, the reaction performed in the presence of $CaCO_3$ gave purine as the only recovered product in a yield that is very high for a prebiotic synthesis. Alumina, kaolin, and zeolite also catalyzed the formation of purine, even if adenine, cytosine, and the previously not reported 4(3H)-pyrimidinone were obtained in acceptable yields. A different reaction pattern was observed with silica, in which case pyrimidine derivatives became the main reaction products. Thus, a wide range of selectivity and yield could be obtained depending on the catalyst used. No matter how plausible and efficient the described syntheses are (especially so for their qualitative aspects), the problem remains of the measured low stability of the nucleobases at temperatures of 100 °C or higher.[6] Recently, we have reported that above 100 °C in a formamide solution, both purines and pyrimidines can be degraded back to low molecular weight fragments among which formamide. Adenine, for example, undergoes nucleophilic opening of the imidazole ring producing pyrimidine derivatives such as, 6-amino-5-formamido-4-[*N*-(2′-deoxy-β-D-ribofuranosyl)]-pyrimidine, 4,6-diamino-5-formamido pyrimidine, and 2,6-diamino-4-oxo-5-formamido pyrimidine.[34,35] Consequently, formamide, a product of degradation of nucleobases, represents a substrate for their synthesis, suggesting a prebiotic synthesis with possible elements of cyclicity. The problem can also be solved invoking the possibility that guanine might be produced under different reaction conditions, that the GC base-pair was not used in the first genetic material, or that life arose quickly, or that a different letter code was initially used.

An additional solution could be provided by an as-yet-undescribed, possibly catalyzed, competitively rapid condensation of the nucleobases into more stable, higher order molecular structures. In the absence of experimentally verified solutions for this bias, the formamide-based catalyzed synthesis of nucleobases described here should be considered only as a chemically attractive possibility for prebiotic processes.

Experimental

Mass Spectroscopy (MS) was performed with Hewlett-Packard 5971 mass-selective detector on a Hewlett-Packard 5890 III gas chromatograph with FID detector. NMR spectra were recorded on a Bruker (200 MHz) spectrometer and are reported in δ values. Infrared spectra were recorded on a Perkin Elmer 298 spectrophotometer using NaCl plates. All solvents were ACS reagent grade and were redistilled and dried according to standard procedures. Formamide (>99%, Fluka), Kaolin, calcium carbonate, zeolite (Y type), silica, alumina, and 6-methoxypurine (Aldrich) were used without further purification.

Formamide condensation. General procedure

Formamide (5.7 g., 5 mL, 0.12 mol) in the presence of the catalyst (1.0% w/w; calcium carbonate, kaolin, zeolite Y type, and silica) was heated at 160 °C for 48 h. The reaction in the presence of kaolin was also performed at 100 °C. The reaction mixture was filtered to remove the catalyst and evaporated under high vacuum. Gas-chromatography and gas chromatography–mass spectrometry of the reaction products were performed using a SP-2380 column (30 m×0.25 mm and 0.25 mm film thickness), and an isothermal temperature profile of 100 °C for the first 2 min, followed by a 10 °C/min temperature gradient to 250 °C and finally an isothermal period at 250 °C for 30 min. The injector temperature was 280 °C. Chromatography grade helium was used as the carrier gas. The fragmentation patterns were compared to those of authentic samples. Selected mass spectrometry data are reported in Table 1. 6-Methoxypurine (from Aldrich) was used as internal standard. Because of the uncertainty of the number of formamide molecules involved in the synthesis of recovered products the yields were calculated as mg of product formed for gram of formamide and are reported in Table 2. In a selected case (kaolin) the reaction crude was dissolved in chloroform:methanol (9.0:1.0) and purified by flash-chromatography. The structures of products were determined by usual spectroscopic techniques (^{1}H NMR, ^{13}C NMR, FT-IR) and mass-spectroscopy (EI) and confirmed by comparison with authentic commercial samples. Data of 4(3H)-pyrimidinone **5** are reported as a selected case: mp 166–169 °C; ^{1}H NMR (DMSO-d_6) δ 6.35 (d, 1H, J=5.0 Hz, CH), 7.95 (d, 1H, J=5.0 Hz, CH), 8.20 (s, 1H, CH), 12.8 (br. s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 118.45 (CH), 145.10(CH), 147.02 (CH), 163.33 (C);%]; ν_{max} (Nujol) 3460, 3300, 2890, 1680, 1600, 1475 cm^{-1}. Mass spectrometry data are reported in Table 2.

Acknowledgements

This work was supported by a grant from the Italian MURST.

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