CONCENTRATION BY EVAPORATION AND THE PREBIOTIC SYNTHESIS OF CYTOSINE

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Abstract. The efficient prebiotic synthesis of cytosine from urea and cyanoacetaldehyde (CA) has recently been claimed to be invalid on the basis of possible side reactions of the starting materials and the inapplicability of prebiotic syntheses using drying beach conditions. We therefore have investigated the synthesis of cytosine and uracil from urea and cyanoacetaldehyde at 100 °C under dry-down conditions, and in solution at 4 °C and –20 °C. We find that cytosine is produced from the low temperature experiments more efficiently than calculated from the Arrhenius extrapolation from higher temperatures, i.e., 60–120 °C. In addition, we find that CA dimer is as efficient as the monomer in cytosine synthesis. We also studied whether evaporating very dilute solutions of nonvolatile organic compounds will concentrate according to theory. Solutions as dilute as 10^{-4} M concentrate from pure water approximately according to theory. Similar solutions in 0.5 M NaCl have less than theoretical concentrations due to absorption, but concentrations near dryness were very high.

Keywords: cyanoacetaldehyde, cyanoacetaldehyde dimer, cytosine, dry-down conditions, evaporation, prebiotic synthesis, uracil

1. Introduction

In a recent article, Shapiro (1999) claims that the efficient prebiotic synthesis of cytosine and uracil from cyanoacetaldehyde and urea under concentrating conditions (Robertson and Miller, 1995) would not work because of dimerization and decarbonylation of the cyanoacetaldehyde, the decomposition of urea and other factors. These concentrating conditions would be found on drying beaches and drying lagoons; we will refer to these as dry-down conditions/experiments. No mention was made of the dry-down experiments with cyanoacetaldehyde and guanidine (Robertson *et al.*, 1996) which produce diaminopyrimidine, cytosine, isocytosine, and uracil in yields as high as 5.7% compared with 85% yields in solution. This claim that dry-down conditions are not prebiotic is curious since a large number of papers use dry-down experiments including the prebiotic synthesis of nucleosides (Fuller *et al.*, 1972a; Fuller *et al.*, 1972b), polyphosphates (Bishop *et al.*, 1972; Lohrmann and Orgel, 1971; Lohrmann and Orgel, 1973; Ostberg and Orgel,

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1972; Ostberg *et al*., 1973), nucleoside imidazolides (Burton *et al*., 1974), and peptides (Lorhmann *et al*., 1975; Sawai *et al*., 1975). Prebiotic dry-downs have also been used by Fox and Dose (1972), Oró, (Deamer and Oró, 1980; Eichberg *et al*., 1977; Oró and Stephen-Sherwood, 1976), Schwartz (Schwartz and Deuss, 1971; Schwartz, 1972; Schwartz and Chittenden, 1977), and many others (Flores and Leckie, 1973; Keefe and Miller, 1995; Usher and McHale, 1976; Usher, 1977; Usher and Yee, 1979; Yanagawa *et al*., 1984). The argument against the CA + urea process is based on the decomposition and dimerization rates of CA (Ferris *et al*., 1974), the decomposition of urea and the kinetic data for cytosine formation (Robertson and Miller, 1995). Rather than analyze the complicated kinetic argument we decided to run the reaction under dry-down conditions, as well as in aqueous conditions at 4 °C and -20°C.

We find that the cytosine yield in the dry-down experiment at 100 °C is in agreement with the calculated value by extrapolation from 60 °C to 120 °C, as obtained in most high temperature prebiotic syntheses, including the spark discharge and cyanide polymerization. In low temperature reactions, however the yields are higher than expected from high temperature extrapolation. At low temperature, we favor the mechanism involving CA dimer.

In addition, we show that evaporation of aqueous solutions of glycine, an amino acid readily formed under prebiotic conditions, follows approximately the theoretical equation derived from the mass conservation, which is defined as the molarity increase as the ratio of the initial and final volumes. These results suggest that the widely used evaporation process is a valid prebiotic process.

2. Experimental

Glycine was purchased from Sigma. Isoxazole and sodium methoxide were obtained from Aldrich. All other chemicals and solvents were purchased from Fisher. CA was synthesized from isoxazole and sodium methoxide and the concentration was determined by the absorbance at 248 nm (ε = 15,200 M^{-1} cm^{-1}) (Robertson and Miller, 1995). Cyanoacetaldehyde dimer was prepared from CA by reaction at pH 8 and room temperature for 10 days. CA dimer formation was monitored by an increase in absorbance at 310 nm.

The reaction of CA dimer (1 mM) with urea (2 M) was carried out in sealed glass ampoules heated at 100 °C. The pH was initially adjusted to 7 (1 mM sodium phosphate) but drifted to 9 over the course of reaction. The initial concentration of CA dimer was determined by the adsorbance at 310 nm (ε = 30,100 m^{-1} cm^{-1}).

Dry-down reactions with 1 mM CA and 10 mM urea were carried out in test tubes covered with Kimwipes to allow water vapor loss. Tubes were heated at 60, 80, and 100 °C and drying times were 16, 7, and 2 days, respectively. The pH was adjusted to the initial value of 7 (1 mM sodium phosphate) but drifted to 9 over the course of drying.

Low temperature reactions of 1 mM CA with 2 M urea were carried out at 4 and –20 °C in sealed tubes. The pH was initially adjusted with 1 molar NaOH to 7 and reactions were analyzed after 4 months for the 4 °C sample and 2 months for the –20 °C. Evaporation reactions were carried out in 1 L beakers that were dried under 14 mm Hg in a vacuum dessicator and shaken immediately prior to removing aliquots. Samples were taken periodically and analyzed for the concentration of glycine. Two separate experiments were run. One liter of 100 μM glycine was dried to 0.5 mL. In addition, 1 L of 100 μM glycine in the presence of 0.5 M NaCl was dried to 3 mL. The glycine concentration selected is consistent with those estimated for the primitive ocean (Stribling and Miller, 1987).

All HPLC analyses were performed on a Beckman Model 110B HPLC system using a YMC ODS-AQ reversed phase analytical column as previously described (Robertson and Miller, 1995). Identification was based on elution time, UV spectra, and coelution with a known compound. For glycine the mobile phase was 45% methanol in 0.1 M, pH 4.8 sodium phosphate. Prior to injection glycine was derivatized with *o*-phthaldialdehyde/*N*-acetyl cysteine (Zhao and Bada, 1995). The fluorescent derivatives were then analyzed by HPLC using a Gilman Spectro/glo filter fluorometer.

3. Results

3.1. Cytosine synthesis

A solution of 10^{-3} M CA and 10^{-2} urea was heated and dried down at 100 °C giving a yield of 0.23% cytosine and 0.08% uracil. Similar experiments at 80 and 60 °C gave comparable yields of cytosine of 0.2 and 0.05%, respectively.

We also tried the CA + 2 M urea synthesis at 4 °C, comparable to the temperature of the early ocean temperature, to see whether the Arrhenius extrapolation of the 80–120 °C data is valid. A solution of 10^{-3} M CA and 2 M urea buffered at pH 7 gave 0.010% compared with the calculated yield of 0.001% (Robertson and Miller, 1995) from

$$\log_{10} \mathrm{k}(\mathrm{M}^{-1}\mathrm{s}^{-1}) = 10.50 - \frac{6152}{\mathrm{T}},$$

where k rate constant, M is the molar concentration of urea and T is the absolute temperature. Surprisingly, uracil was also produced in a yield of 0.046%. The uracil is unlikely to come from hydrolysis of cytosine since the half-life for the reaction is 8500 yr at 4 °C (Levy and Miller, 1998). We also investigated the cytosine yield starting with 10^{-3} M CA + 2 M urea at –20 °C, the temperature of the freezer. The yield of cytosine after 2 months was 0.005% together with 0.02% uracil. The caluclated cytosine yield would be 1.6×10^5% for 2 M urea, but this calculated yield needs to be increased because of the concentration of urea on freezing.

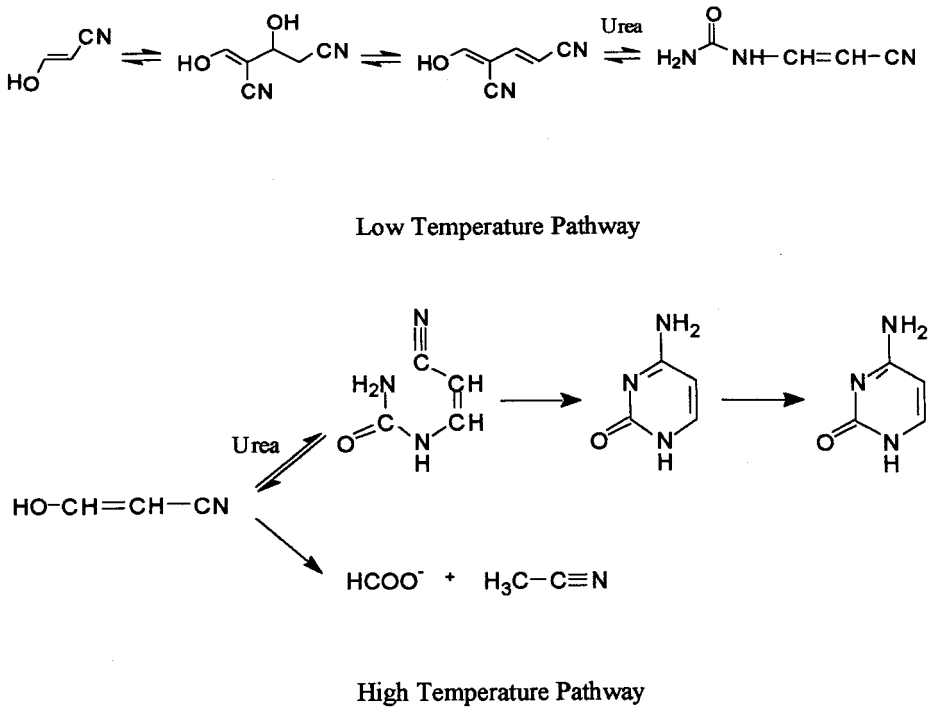
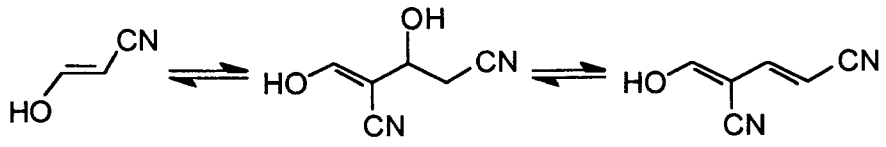


Figure 1. Synthesis pathway under dry-down conditions in the low and high temperature experiments.

It is clear that the observed yield of cytosine at low temperatures is far higher than that calculated from the Arrhenius extrapolation from the 60–120 °C data. The observed yield of cytosine at low temperatures is higher than expected because the reaction mechanism may involve the CA dimer at lower temperatures (Figure 1). We favor this low temperature mechanism which would have a different activation energy from the high temperature mechanism and would explain the higher than expected yields of cytosine at low temperatures. These reactions are being investigated further.

3.2. CA DIMER + UREA

Shapiro states dimerization of CA will ruin the CA + urea reaction (Shapiro, 1999). CA does indeed dimerize with a very complex pH rate and equilibrium profile (Raulin and Toupance, 1975):



Cyanoacetaldehyde

Indeed, we find most of the CA converted to dimer in the dry-down and the 4 °C experiments. Rather than sort out all these factors, we heated 10^{-3} M dimer and 2 M urea. The yield of cytosine after 3 days at 100 °C was 12.8% compared to 12.0% starting from CA. These results show that CA dimer is as good a starting reagent as CA. A more extensive investigation of the dimer reaction is underway.

3.3. CA VS. CYANOACETYLENE AS A PREBIOTIC REAGENT

Something needs to be said about CA as a prebiotic reagent. CA was first proposed as prebiotic (Miller, 1957) because it is a Strecker precursor to aspartic acid. It is presumably involved in the HCN polymerization synthesis of aspartic acid, although other mechanisms are possible. The source of CA in the spark discharge experiments may have been cyanoacetylene which was shown to be efficiently synthesized by a spark from $CH_4 + N_2$ but not from $CH_4 + NH_3$ (Sanchez *et al.*, 1966). However, some of the CA may have been formed as a direct result of the spark as does acetaldehyde, propanol, etc. In the presence of water, cyanoacetylene is rapidly converted to CA

$$HC{\equiv}C{-}C{\equiv}N + H_2O \longrightarrow HO{-}HC{=}CH{-}C{\equiv}N$$

with phosphate being an excellent catalyst for this reaction, adding to make cyanovinyl phosphate (Ferris, 1968; Ferris *et al.*, 1970). Ammonia would also be a catalyst producing

$$H_2N{-}HC{=}CH{-}C{\equiv}N$$

followed by hydrolysis to CA. This may be responsible for the low cyanoacetylene yield from spark discharges with $CH_4 + NH_3$. The most potent prebiotic catalyst would probably be HS^- which is particularly efficient in nucleophilic additions to triple bonds. Thus, HS^- is about 10^4 times more reactive than OH^- in the nucleophilic addition to the triple bond of phenyl acetylene (Friedmann *et al.*, 1971). Although there are no kinetic data available, it is likely that in an ocean containing HS^- as mercaptide ions cyanoacetylene would react very quickly followed by hydrolysis to CA. Thus providing further evidence that cyanoacetylene is an unlikely prebiotic compound whereas its hydrolysis product CA could have been the souurce of pyrimidines and aspartic acid.

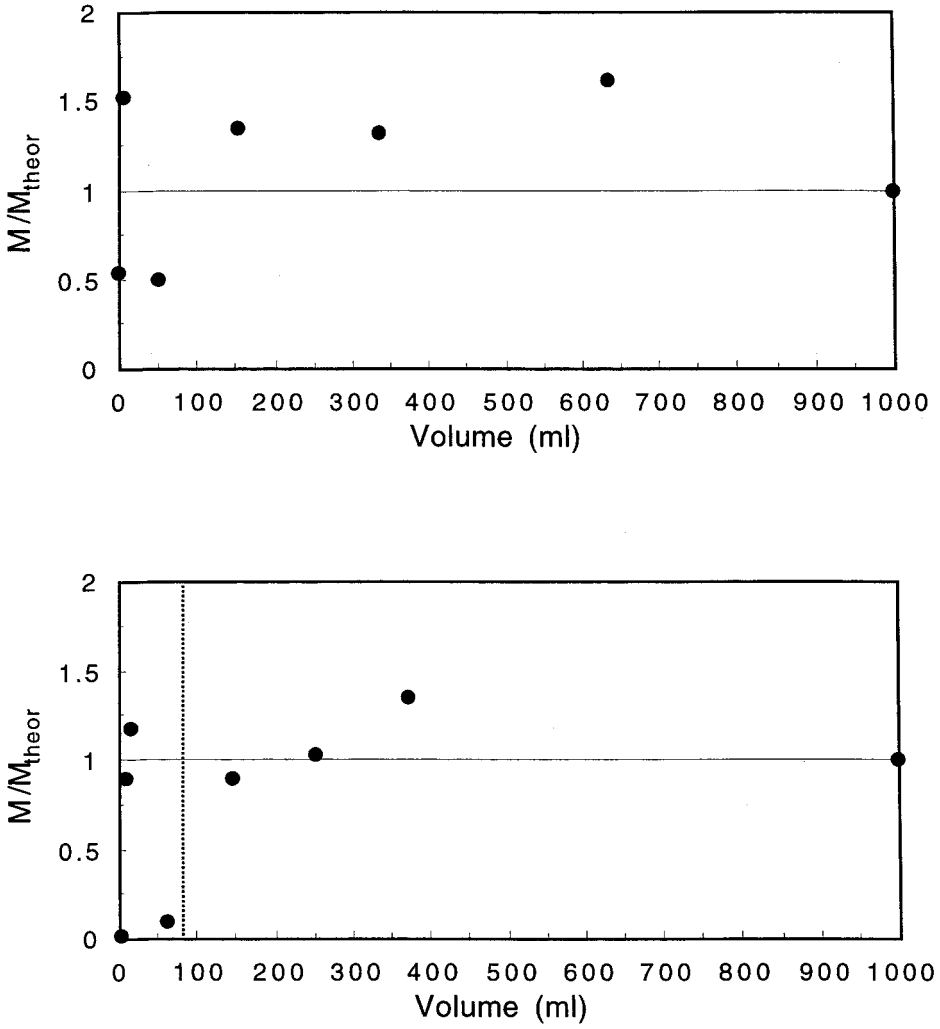


Figure 2. The measured glycine concentrations (●) as a function of the initial concentration and volume for the evaporation of dilute solutions in pure water (top) and in the presence of 0.5 M NaCl (bottom). The initial concentrations were glycine = 100 μM and the initial volume was 1000 mL. The dashed line in the bottom figure indicates the volume at which the NaCl becomes saturated.

3.4. CONCENTRATION BY EVAPORATION

This is a widely used prebiotic process and is considered the most efficient concentration method available. Dry-down experiments demonstrate the application of concentration by evaporation to prebiotic syntheses. However, the process of evaporation has never been tested for losses and other problems. We therefore

evaporated 10^{-4} M glycine from 1 L to 0.5 mL. The theoretical concentration given as a function of remaining volume is

$$M = \frac{M_0 V_0}{V},$$

where M is the concentration in volume V and M_0 and V_0 are the initial concentrations and volumes. Figure 2a shows the molarity of glycine as a function of remaining volume. Figure 2b shows the same experiment with glycine but using 0.5 M NaCl in place of pure water. Subsituting other amino acids such as 2-aminoethyl glycine and aminoisobutyric acid in these experiments gave similar results. These results show that the evaporation process follows the theoretical curve approaximately.

4. Discussion

We will not here analyze where the kinetic calculations of Shapiro went wrong except to say that using preliminary kinetic data without a pH rate profile and mechanism can lead to substantial errors in extrapolations. A similar example was the Shapiro calculation (Shapiro, 1994; Shapiro, 1995) of the stability of adenine based on acid hydrolysis data (Frick *et al*., 1987) rather than the neutral hydrolysis rate from the pH rate profile (Levy and Miller, 1998) which leads to an error of a factor of 458.

The effectiveness of evaporation demonstrated here needs to taken with caution. The reproducibility of these experiments is only approximate because of differences in the shape of the vessels, differences in absorption on the surfaces of salt crystals, and fluid inclusions in the crystals that alter the theoretical assumptions. However, the lack of accurate agreement with theory does not affect the prebiotic usefulness of evaporation as long as the solution becomes concentrated, even though the amount of concentrated solution is less than predicted by theory.

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