J Mol Evol (1999) 49:165–168

JOURNAL OF MOLECULAR EVOLUTION

Production of Guanine from NH_4CN Polymerizations

Matthew Levy,* Stanley L. Miller, John Oró**

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0506, USA

Received: 18 September 1998 / Accepted: 31 March 1999

Abstract. The synthesis of adenine from the polymerization of concentrated ammonium cyanide solutions is well known. We show here that guanine is also produced by this reaction but at yields ranging from 10 to 40 times less than that of adenine. This synthesis is effective at both +80 and −20°C. Since high concentrations of NH_4CN are obtainable only by freezing, this prebiotic synthesis would be applicable to frozen regions of the primitive Earth, the Jovian satellite Europa and other icy satellites, and the parent body of the Murchison meteorite.

Key words: Guanine — Adenine — NH_4CN polymerization

Introduction

The prebiotic synthesis of adenine from concentrated ammonium cyanide solutions is a well-established reaction (Oró 1960; Oró and Kimball 1961, 1962). But in these investigations guanine could not be detected. A paper chromatography spot corresponding to guanine was found in an electric discharge reaction but the identification was only tentative (Yuasa et al. 1984). The only confirmed prebiotic synthesis of guanine is a three-step synthesis with frozen basic HCN solutions that react to form a HCN tetramer (diaminomaleonitrile) (Sanchez et al. 1966) followed by a two-photon photochemical rearrangement to amino imidazole carbonitrile (AICN) (Ferris and Orgel 1966). The reaction is completed by the use of the presumed prebiotic reagent cyanogen or cyanogen bromide (Sanchez et al. 1967, 1968) or from the reaction of aminoimidazole carboxamide (AICA) with guanidine or urea (Oró 1964). We show here that guanine is indeed produced in a "one-pot" synthesis from the polymerization of ammonium cyanide at yields between 10 and 40 times less than that of the adenine.

Materials and Methods

The reaction of 10 *M* NH_4CN was prepared from gaseous HCN and a solution of NH_4OH and heated in a round-bottom flask with condenser for 24 h at 80°C. The solution turned yellow almost immediately on mixing and quickly precipitated a black polymer on heating.

The solution of 0.1 *M* NH_4CN frozen at −20°C was prepared by one of us (S.L.M.) in 1972 from gaseous HCN and NH_3 and held continuously at −20°C for 25 years. The sample of 0.1 *M* NH_4CN frozen at −20°C for 2 months was prepared from NaCN and NH_4Cl. The −20°C samples turned brown after several months.

The polymer was centrifuged and the supernatant hydrolyzed in 6 *N* HCl for 24 h. For purposes of identification a sample was evaporated to dryness and chromatographed on Dowex 50 (H+) with 4 *N* HCl as eluant (Wall 1953). The adenine and guanine peaks were evaporated to dryness and chromatographed on a Beckman 110B HPLC using a 6.0 × 250-mm YMC ODS-AQ reversed-phase column and a Kratos UV spectrophotometer set at 260 nm. Peaks corresponding to A and G were collected using a 0.1 *M*, pH 4.8, phosphate buffer, evaporated to dryness, and rechromatographed using a 0.1 *M*, pH 2.5, phosphate buffer. In some cases a 0.1 *M*, pH 3.8, phosphate buffer was also used. The identification was confirmed by UV absorbance from 200 to 300 nm. The mass spectra of the isolated HPLC peaks were identical to the known adenine and guanine.

Routine quantitation was also achieved by chromatographing the hydrolyzed evaporated sample in 0.1 M, pH 4.8, phosphate buffer on the HPLC reversed-phase column. An example of this is shown in Fig. 1. The adenine and guanine peaks were then separately rechromato-

* *Present address:* Department of Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA

** Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX 77004, USA

Correspondence to: S.L. Miller; *e-mail:* smiller@ucsd.edu

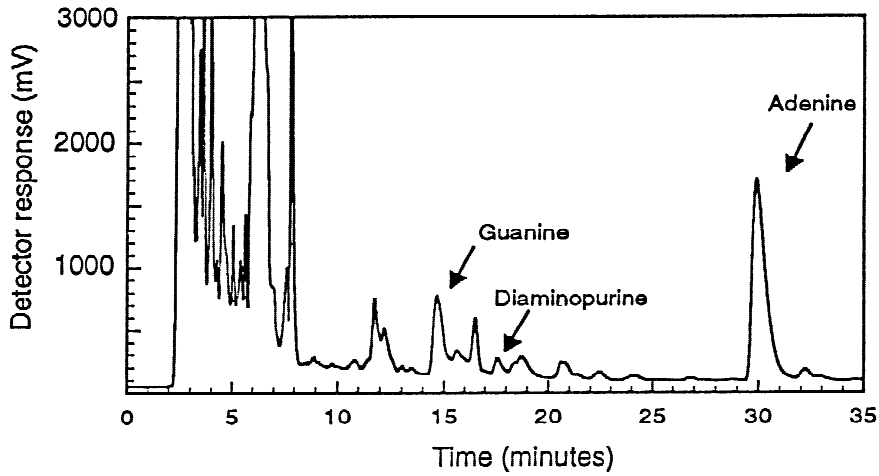

Fig. 1. HPLC chromatogram of crude [not separated on Dowex 50 (H^+)] acid-hydrolyzed 10 *M* NH_4CN heated at 80°C for 24 h. Guanine corresponds to ~9% of the area of the peak labeled. Adenine corresponds to ~95%. Diaminopurine could not be detected in the peak so labeled.

graphed at pH 2.5 and pH 3.8. The adenine peak from the first chromatographic run was found to be >95% adenine, but the guanine was found to be only ~9% guanine.

Results

Figure 1 shows the HPLC trace of the hydrolyzed supernatant from the reaction of 10 *M* NH_4CN at 80°C. The yields from this column were corrected for the percentages obtained upon rechromatographing the collected peaks. The yield of A was 0.027% and that of G was 0.0007% based on HCN (Table 1). We believe that the reason guanine was not previously detected in HCN polymerization reactions is the lack of sensitivity of paper chromatography compared to HPLC. Another factor may have been the loss of guanine by precipitation due to its very low solubility (4×10^{-5} *M*) at a neutral pH (DeVoe and Wasik 1984). In our experiments this problem was avoided by diluting all dried solutions in 0.01 *M* HCl, where the solubility is higher.

We also examined the fractions from the HCl elution from Dowex 50 (H^+) for diaminopurine and isoguanine. We could detect none, so the yield, if any, is less than 10% of the guanine yields.

The only way in nature to concentrate dilute NH_4CN solutions is by freezing since both HCN and NH_3 are more volatile than water. This would be an effective procedure in the frozen ocean model of prebiotic synthesis (Bada et al. 1994) or in the polar regions of an Earth of the present average temperature. This freezing procedure was used to concentrate the HCN in the tetramer synthesis experiments, but adenine was not looked for (Sanchez et al. 1966). Freezing at −2°C of 0.01 *M* HCN was effective in producing adenine, but only in the presence of NH_4OH (Schwartz et al. 1982).

In order to determine whether very low-temperature polymerizations will produce guanine as well as adenine, samples of 0.1 *M* NH_4CN were left frozen at −20°C for 25 years, thawed, and analyzed as the 80°C samples. The yields of adenine and guanine were 0.038 and 0.0035%, respectively, similar to the 80°C sample for adenine but considerably higher for guanine. These results also suggest that guanine synthesis relative to adenine is favored at lower temperatures.

Table 1. Percentage yield of adenine and guanine from the polymerization of NH_4CN, based on initial HCN concentration

	10 *M* NH_4CN (80°C, 24 h)	0.1 *M* NH_4CN (−20°C, 25 yr)	0.1 *M* NH_4CN (−20°C, 2 mo)
Adenine	0.028	0.038	5×10^{-4}
Guanine	0.0007	0.0035	1.4×10^{-4}

Because of the difficulty of repeating this long-term experiment, a fresh sample of 0.1 *M* NaCN and NH_4Cl was prepared and left at −30°C for 2 months. This yield of adenine and guanine was 5.0×10^{-4} and 1.4×10^{-5}%, or roughly a factor of 150 less than that of the 25-year sample. The time period, also less by a factor of ~150, suggests that synthesis was still going on in the −20°C samples.

Discussion

The mechanism for the synthesis of both adenine and guanine can be accounted for by the routes shown in Fig. 2. The adenine mechanism is based on the earliest proposal (Oró 1961) with later modifications (Shuman et al. 1979). The modifications for guanine are included. Formamidine is necessary for this route. The synthesis of this reagent under these very concentrated conditions is likely, whereas formamidine cannot play a role in more dilute NH_4CN reactions (Sanchez et al. 1967). It is to be noted that NH_3 is required for this mechanism. Cyanogen, or its equivalent, is also needed for guanine synthesis. This reagent has been recognized as a possible precursor to urea, guanidine, and oxalic acid in cyanide

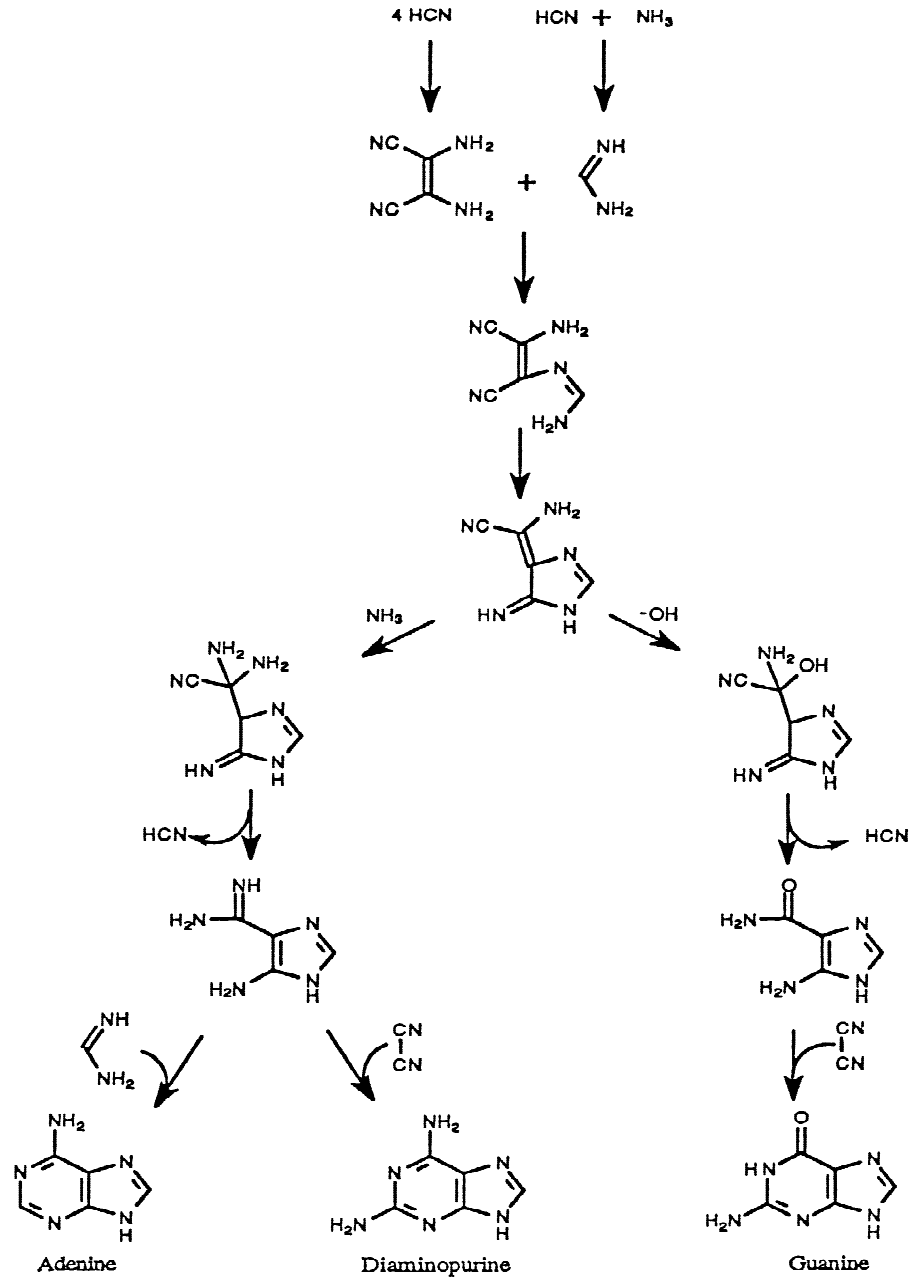

Fig. 2. Possible mechanisms for the synthesis of adenine, diaminopurine, and guanine from HCN and NH_3.

polymerizations. One route for the synthesis of cyanogen is from the HCN trimer (Ferris et al. 1973).

$$\mathrm{N{\equiv}C{-}CH(NH_2){-}C{\equiv}N} \xrightarrow{{}^{-}CN} \mathrm{N{\equiv}C{-}C(N^{-}){-}CH(NH_2){-}C{\equiv}N} \longrightarrow \mathrm{N{\equiv}C{-}C{\equiv}N + CH_2(NH_2){-}C{\equiv}N}$$

Other routes to cyanogen from HCN tetramer and oligomers can also be written.

The mechanism presented in Fig. 2 is an oversimplification since the polymerization of HCN is very complex and other precursors to adenine such as 2-cyano and 8-cyano adenine have been demonstrated (Voet and Schwartz 1983).

It is likely that diaminopurine is also produced in the HCN polymerization reaction as shown in Fig. 2. However, diaminopurine is readily hydrolyzed in 6 *M* HCl to guanine (87%) and isoguanine (7%). Thus most of the guanine may have arisen by the hydrolysis of diaminopurine.

These results can explain the presence of both adenine and guanine in the Murchison meteorite on the basis of a frozen aqueous solution of NH_4CN in the interstitial aqueous environment of an asteroid. Adenine and guanine are in about-equal abundance in Murchison instead of the factor of 10 to 40 less guanine in our NH_4CN polymerization. This difference may be due to the isolation procedure from Murchison, which will be discussed in another paper. The three-step photochemical route

would not be applicable on an asteroid into which ultraviolet light could not penetrate. The elegant photochemical route would also be less efficient on a frozen Earth than the simple freezing of dilute NH_4CN, where UV light would be scattered and absorbed by surface ice. This synthesis of both adenine and guanine may be an important process on Europa and other icy satellites (Levy et al. 1999).

Because both the rates of synthesis and the hydrolytic stability (Levy and Miller 1998) of A and G are similar, our results suggest that guanine would be as readily available as adenine on the primitive Earth. It is therefore reasonable that guanine and adenine were incorporated into the first genetic material. The preferential synthesis of these two purines also suggests that they may be components of the genetic molecules of other independent origins of life.

Acknowledgment. We thank Kevin Nelson for the mass spectra and the NASA Specialized Center of Research and Training for an NSCORT fellowship (M.L.) and grant support.

References

Bada JL, Bigham C, Miller SL (1994) Impact melting of frozen oceans on the early Earth: implications for the origin of life. Proc Natl Acad Sci USA 91:1248–1250

DeVoe H, Wasik SP (1984) Aqueous solubilities and enthalpies of solution of adenine and guanine. J Sol Chem 13:51–60

Ferris JP, Orgel LE (1966) An unusual photochemical rearrangement in the synthesis of adenine from hydrogen cyanide. J Am Chem Soc 88:1074

Ferris JP, Donner DB, Lobo AP (1973) Possible role of hydrogen cyanide in chemical evolution: the oligomerization and condensation of hydrogen cyanide. J Mol Biol 74:511–518

Levy M, Miller SL (1998) The stability of the RNA bases: Implications for the origin of life. Proc Natl Acad Sci USA 95:7933–7938

Levy M, Miller SL, Brinton K, Bada JL (1999) Prebiotic synthesis of adenine and amino acids under Europa like conditions (submitted for publication)

Oró J (1960) Synthesis of adenine from ammonium cyanide. Biochem Biophys Res Commun 2:407–412

Oró J (1961) Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions. Nature 191:1193–1194

Oró J (1964) Prebiological synthesis of nucleic acid constituents. In: Kretovich VL, Bach AN (eds) Problems of evolutionary and industrial biochemistry. Institute of Biochemistry Academy of Sciences USSR, Moscow, pp 63–69

Oró J, Kimball AP (1961) Synthesis of purines under possible primitive Earth conditions. I. Adenine from hydrogen cyanide. Arch Biochem Biophys 94:217–227

Oró J, Kimball AP (1962) Synthesis of purines under possible primitive Earth conditions. II. Purine intermediates from hydrogen cyanide. Arch Biochem Biophys 96:293–313

Sanchez RA, Ferris J, Orgel LE (1966) Conditions for purine synthesis: Did prebiotic synthesis occur at low temperatures? Science 153: 72–73

Sanchez RA, Ferris JP, Orgel LE (1967) Studies in prebiotic synthesis. II. Synthesis of purine precursors and amino acids from aqueous hydrogen cyanide. J Mol Biol 30:223–253

Sanchez RA, Ferris JP, Orgel LE (1968) Studies in prebiotic synthesis. IV. Conversion of 4-aminoimidazole-5-carbonitrile derivatives to purines. J Mol Biol 38:121–128

Schwartz AW, Joosten H, Voet AB (1982) Prebiotic adenine synthesis via HCN oligomerization in ice. BioSystems 15:191–193

Shuman RF, Shearin WE, Tull RJ (1979) Chemistry of HCN. 1. Formation and reactions of N-(aminomethylidene)diaminomaleonitrile, an HCN pentamer and precursor to adenine. J Org Chem 44:4532–4536

Voet AB, Schwartz AW (1983) Prebiotic adenine synthesis from HCN—Evidence for a newly discovered major pathway. Bioorg Chem 12:8–17

Wall JS (1953) Simultaneous separation of purines, pyrimidines, amino acids, and other nitrogenous compounds by ion exchange chromatography. Anal Chem 25:950–953

Yuasa S, Flory D, Basile B, Oró J (1984) Abiotic synthesis of purines and other heterocyclic compounds by the action of electrical discharges. J Mol Evol 21:76–80