Conditions for Purine Synthesis: Did Prebiotic Synthesis Occur at Low Temperatures?

Abstract. *The rate of polymerization of hydrogen cyanide to aminomalononitrile and the tetramer, diaminomalonodinitrile, is quadratic in the total cyanide concentration. Since the reactions form part of a plausible prebiotic purine synthesis and since they compete with hydrolysis, concentration of cyanide may have been important. This may be achieved usefully by cooling to separate out ice.*

The synthesis of adenine (V) from hydrogen cyanide and ammonia, first demonstrated by Oro (*1*), is so remarkable that we believe it to have some relevance to the prebiotic accumulation of purines. We have, therefore, studied in detail the steps in this and related syntheses and have now confirmed our suggestion that aminomalononitrile (I) is an important intermediate (*2*). Aminomalononitrile is transformed into 4-aminoimidazole-5-carbonitrile (III) either by direct reaction with formamidine or by the photochemical rearrangement of the hydrogen cyanide tetramer (II) (*3*). Finally III and its hydrolysis product 4-aminoimidazole-5-carboxamide (IV) condense under quite mild conditions with aqueous cyanide, formamidine, cyanate, or cyanogen to give a variety of purines including adenine (V), hypoxanthine (IX), diaminopurine (VI), guanine (VIII), and xanthine (VII) (*4*). These reactions are summarized in Fig. 1.

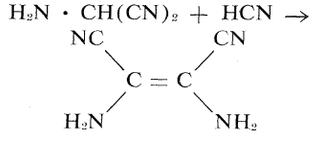
We now report on the kinetics of those early stages in hydrogen cyanide polymerization which give rise to aminomalononitrile and the hydrogen cyanide tetramer. The formation of tetramer either directly from hydrogen cyanide or from aminomalononitrile added to hydrogen cyanide can be followed by measuring the absorption of the solutions at 296 mμ, the position of the peak in the spectrum of the tetramer. Our conclusions are:

1) The rate-determining step in the polymerization under most conditions is the first step, namely the addition of CN^- to HCN. At 25°C, the reaction proceeds at maximum velocity when the *p*H is 9.2, the *pK* of hydrocyanic acid. The values of k in the rate law

$$\frac{d\,[\text{Tetramer}]}{dt} = k\,[CN^-]\,[HCN]$$

are 0.096 liter mole^{-1} day^{-1} (40°C); 0.0032 liter mole^{-1} day^{-1} (10°C).

2) The rate of appearance of tetramer in the earliest phase of the reaction is consistent with an intermediate step



We have studied this reaction in detail but have not completed the kinetic analysis. At constant *p*H, the rate is linear in the total cyanide concentration; at constant total cyanide concentration, the rate increases with *p*H in the range 8 to 10, suggesting that an anionic intermediate is involved. The half-life of aminomalononitrile is 3.2 minutes in 0.2M total cyanide at *p*H 9.2 and 25°C.

3) Although we have no experimental evidence for the proposed dimer of hydrogen cyanide we believe the scheme in Fig. 2 is soundly based, since the two proposed steps which we have not been able to follow are well known for closely similar reaction sequences.

4) The formation of dark-colored materials during the polymerization of hydrogen cyanide seems to be related to the presence of the tetramer. Addition of tetramer to freshly prepared hydrogen cyanide results in a much accelerated appearance of polymer.

The combination of the reaction sequence in Figs. 1 and 2 provides a route from cyanide, cyanate, and cyanogen to the purines including adenine, guanine, and hypoxanthine; all these steps may proceed in aqueous solution without the intervention of any other reagent. The presence of ammonia might make formamidine available, which would open up alternative pathways as indicated above.

We must next ask whether the concentration of cyanide required seems reasonable under prebiotic conditions. The main obstacle to the synthesis of imidazoles and purines from HCN is hydrolysis to formamide and formic acid. A second obstacle is the hydrolysis of aminomalononitrile which leads ultimately to the formation of glycine. (*4*). Our own studies (*4*) and those of Marsh and Martin (*5*) indicate that hydrolysis is predominant in 0.01M and polymerization in 1.0M solutions of hydrogen cyanide. As a rough guide we may say that the yield of purines formed from hydrogen cyanide would fall off very rapidly at cyanide concentrations less than 0.1M. We shall proceed on the hypothesis that proposed prebiotic processes which give a good yield are more probable than those which give a poor

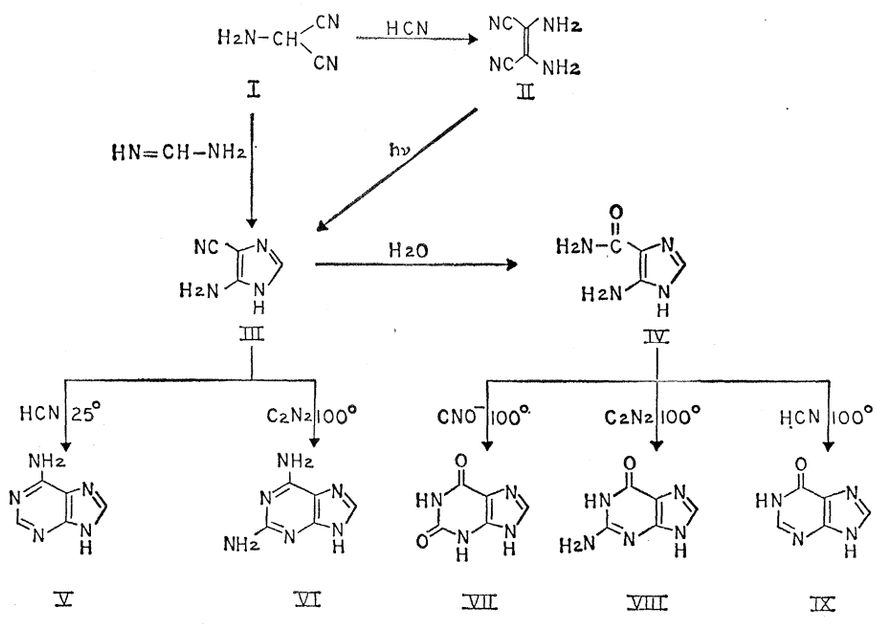


Fig. 1. Steps in the synthesis of purines from aminomalononitrile.

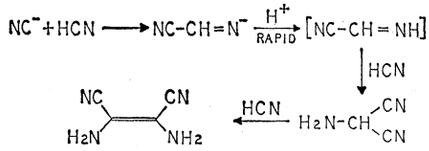


Fig. 2. Steps in the formation of aminomalononitrile and HCN-tetramer from hydogen cyanide.

yield provided they do not postulate unreasonable prebiotic conditions. We are well aware of the dangers implicit in this argument.

Under many conditions hydrogen cyanide is a major product formed in electric discharges through ammonia-methane mixtures. However, it is still difficult to see how 0.1*M* solutions of HCN could arise in large amounts. Such a high steady-state concentration in an extended water mass does not seem likely since the hydrolysis to formic acid requires at most a very few years at reasonable *p*H's and temperature. Perhaps, in a reducing atmosphere, raindrops in the neighborhood of an electrical storm might accumulate sufficient hydrogen cyanide, but we doubt whether this could account for the synthesis of large amounts of adenine. We were thus led to consider possible mechanisms for concentrating hydrogen cyanide from more dilute aqueous solutions.

The eutectic in the HCN–water system occurs at −23.4°C and contains 74.5 (moles) percent of HCN (*6*). Extrapolation of our data to these conditions suggested that tetramer formation should still proceed at an appreciable rate at the eutectic temperature in a solution having the composition of the eutectic mixture. Thus at about −22°C we might expect the proportion of cyanide transformed in a fixed time to be independent of the initial concentration of the HCN solution. We have now shown that tetramer formation in 0.01*M* HCN is accelerated by lowering the temperature from 25°C to −22°C and that at −22°C the rate of tetramer formation is roughly first-order in the initial cyanide concentration at least over the range 0.1*M* to 0.001*M*. The optimum rate of tetramer formation is attained at about −10°C.

In a typical experiment, solutions 0.01*M* in HCN and 0.005*M* in ammonia were made up at room temperature and left at 25°C, 0°C and −22°C for 3 days. Tetramer was clearly detectable only in the solution standing at −22°C; the yield was about 0.1 percent, five times the minimum amount which we could detect. In a series of control experiments sufficient methanol (40 percent by volume) was added to prevent the separation of ice; no tetramer was then detected. In an independent series of experiments with 1.0*M* HCN at 25°C we found that methanol accelerates tetramer formation slightly. These experiments prove that the increased rate of tetramer formation is indeed due to the effect of concentration during freezing. In certain of our experiments in which freezing occurred from below, the eutectic mixture separated as a droplet on the top of the ice, and it was then possible to follow the slow discoloration of the concentrated HCN solution at −22°C.

Recently, reports including some which may have relevance to prebiotic syntheses have dealt with reactions in eutectic (*7*) and solid ice phases (*7, 8*). We believe that these reports and our own experiments raise very directly the question: Did prebiotic synthesis occur at low temperatures? Many of the simple molecules, such as hydrogen cyanide, cyanic acid, cyanamide, formamide, hydrogen peroxide, and many ammonium salts, supposedly important in prebiotic synthesis, are extremely soluble in water and form low temperature eutectics. It seems quite likely that the Strecker synthesis of amino acids, the condensation of formaldehyde to sugars, and many similar reactions might proceed at a measurable rate in near-eutectic solutions at temperatures well below 0°C. At a later stage in biochemical evolution nucleotides and certain amino acids might have become concentrated in a similar way in a strong salt eutectic at low temperature, so favoring the operation of the template principle during polynucleotide formation and other condensations. If there is any basis to these speculations we may have to replace the usual picture of a warm, dilute, prebiotic medium with one more cold and much more concentrated, at least for some syntheses.

Thus concentration by freezing in raindrops and in, or above, lakes and other water masses must be added to evaporation and adsorption in the list of concentration mechanisms which may have been important on the primitive earth. Which played important roles in the evolution of life remains to be determined.

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References and Notes

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Circadian Rhythm in Pineal Tyrosine Hydroxylase

Abstract. *Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine synthesis. The rat pineal gland is richly innervated by sympathetic nerves from the superior cervical ganglia. The activity of tyrosine hydroxylase was measured in rat pineal gland at 4-hour intervals over a daily cycle of 12 hours of light (7 a.m. to 7 p.m.) and 12 hours of darkness. The results indicate a circadian rhythm with the maximum activity, at 11 p.m. to 3 a.m., about triple the low values observed at 3 p.m. The pattern is similar in phase to that previously reported for melatonin and hydroxyindole-O-methyl transferase activity.*

The level of hydroxyindole-*O*-methyl transferase (HIOMT) in rat pineal gland varies with environmental lighting, being high during hours of darkness and low during hours of light (*1*). Endogenous melatonin levels follow a circadian rhythm similar to that of HIOMT (*2*), while the endogenous serotonin content of the pineal shows a rhythm opposite in phase (*2, 3*). The serotonin rhythm also differs in persisting unchanged during continuous darkness (*3, 4*).

The HIOMT and serotonin rhythms are interrupted by removal of the superior cervical ganglia which inner-