

PREBIOTIC ADENINE REVISITED: EUTECTICS AND PHOTOCHEMISTRY

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Abstract. Recent studies support an earlier suggestion that, if adenine was formed prebiotically on the primitive earth, eutectic freezing of hydrogen cyanide solutions is likely to have been important. Here we revisit the suggestion that the synthesis of adenine may have involved the photochemical conversion of the tetramer of hydrogen cyanide in eutectic solution to 4-amino-5-cyano-imidazole. This would make possible a reaction sequence that does not require the presence of free ammonia. It is further suggested that the reaction of cyanoacetylene with cyanate in eutectic solution to give cytosine might have proceeded in parallel with adenine synthesis.

Keywords: adenine, eutectic freezing, hydrogen cyanide tetramer, photo-isomerization, prebiotic synthesis, pyrimidines

1. Background

The general acceptance of the hypothesis that the modern biological world was preceded by an RNA world in which proteins, that is genetically coded polypeptides, played no part (Gesteland *et al.*, 1999; Joyce, 2002) makes the synthesis of RNA the central problem of prebiotic chemistry. It is possible that the RNA world was the very first organized 'biochemical world'. If this is the case, a supply of the components of RNA, nucleotides, must have accumulated *de novo* on the primitive earth. It is also possible that RNA is not a prebiotic molecule, because the RNA world was preceded by some other biological world, and the synthesis of nucleotides was totally dependent on the 'enzymes' of the earlier world. More complicated scenarios are also plausible, for example, the nucleoside bases may have formed prebiotically, and then have been converted to nucleotides by organisms of an earlier world that may or may not themselves have been dependent on prebiotic nucleoside bases.

The work of Juan Oro and his coworkers (Oro and Kimball, 1960, 1961, 1962; Oro, 1961a) provides the most compelling reason for believing that some at least of the chemical constituents of RNA may be prebiotic molecules. In a series of seminal papers, they showed that adenine could be synthesized by refluxing a solution of ammonium cyanide. The synthesis of such a complicated heterocyclic product from such simple precursors under such simple conditions is remarkable.



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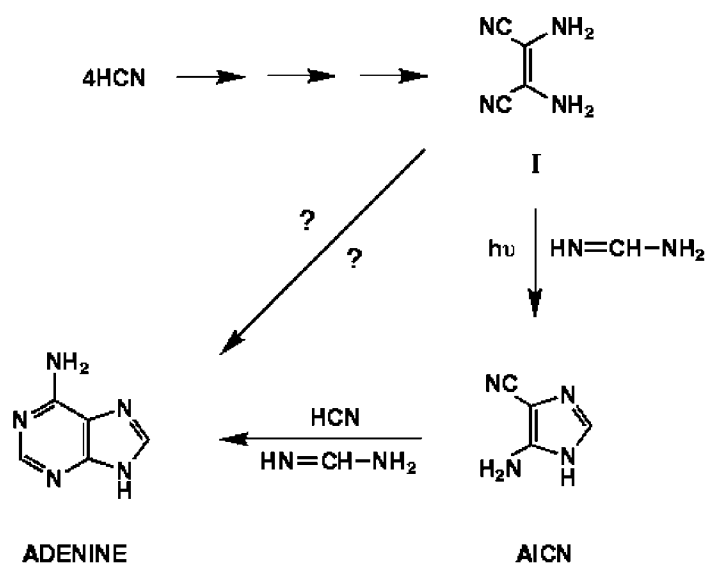


Figure 1. Proposed mechanisms for the synthesis of adenine from hydrogen cyanide.

Taken together with the central position of adenine in contemporary biochemistry and the ease with which hydrogen cyanide (HCN) is formed in reducing gas mixtures, (Miller, 1957) it is hard not to speculate that Oro's surprising synthesis has something to do with the origin of the RNA world. While admitting that Oro's work may provide a completely false clue (Shapiro, 1995), that purines may have formed in the atmosphere independently of HCN (Miyakawa *et al.*, 2000), or that adenine may have formed elsewhere in the solar system and been brought to the earth in meteorites or comets (Oro, 1961b; Chyba and Sagan, 1992), it seems reasonable to re-examine the possibility that adenine was indeed formed on the primitive earth by a mechanism related to the polymerization of HCN. Here we assume that HCN was synthesized in the earth's atmosphere. If HCN was formed in the deep-sea vents, different factors would have been important.

Numerous research groups have extended Oro's original observations in different directions. Possible mechanisms of the condensation reaction have been considered in some detail (Sanchez *et al.*, 1966a; Ferris and Orgel, 1965, 1966a, b; Sanchez *et al.*, 1967, 1968). The possibility of using ammonium formate to replace HCN advantageously in the later steps of adenine synthesis has been demonstrated (Zubay and Mui, 2001), and many modifications of the conditions of the reaction to make it more plausibly prebiotic have been considered (reviewed in Zubay and Mui, 2001; Miyakawa *et al.*, 2002a, b). The present discussion is mainly concerned with the obstacles facing the realization of the reaction under plausibly prebiotic conditions, although this will necessitate some initial discussion of possible mechanisms for adenine synthesis.

It seems clear that the first reasonably stable product of the polymerization of HCN or ammonium cyanide in aqueous solution is the HCN tetramer (I), diamino-maleodinitrile (Figure 1). The subsequent steps remain obscure. The tetramer, once formed, initiates a further polymerization reaction which leads to the precipitation of an intractable, dark-colored solid from which adenine can be released by hydrolysis with acids or bases. Under some conditions a further amount of adenine is present in free solution (Miyakawa *et al.*, 2002b). In model experiments it has been shown that HCN tetramer reacts with formamidine to give adenine (Figure 1), and it has been proposed that this reaction is involved in Oro's synthesis of adenine (Sanchez *et al.*, 1967, 1968). More recent work suggests that much of the adenine may be formed in a different way, via adenine 8-carboxamide and related compounds (Voet and Schwartz, 1983). For the present discussion, it is only necessary to note that that formation of adenine and complex polymerization products from HCN depends on the formation of the HCN tetramer as an intermediate and/or polymerization initiator.

The key to adenine synthesis, therefore, is the formation of HCN tetramer, and therein lies the major difficulty. Kinetic studies show that under the most plausible prebiotic conditions, hydrolysis of HCN to formamide and ultimately ammonium formate competes very effectively with tetramer synthesis if the HCN concentration falls below 10^{-1} to 10^{-2} M (Sanchez *et al.*, 1967). It seems impossible to achieve such a high concentration in the bulk of the oceans, and very difficult to see how a very volatile substance could concentrate substantially in lakes or tide-pools. Evaporation of a lake for example, would lead to the volatilization of much of the HCN that it contained.

In 1966, Sanchez *et al.* suggested a solution of this problem in a paper entitled 'Conditions for Purine Synthesis: Did prebiotic synthesis occur at low temperatures?'. They showed that HCN solutions can be concentrated by cooling below 0 °C; ice crystallizes out of the solution until a eutectic is formed at -23.4 °C containing 74.5 (moles) percent of HCN. Eutectic solutions obtained in this way gradually deposit a typical intractable dark polymer (Sanchez *et al.*, 1966a). Sanchez *et al.* did not isolate adenine in their experiments, but in 1982 Schwartz and his coworkers reinvestigated this reaction and were able to obtain adenine by HCN polymerization in 'eutectic' solution and to identify it unambiguously. They estimated that the yield of adenine was 0.004% after 98 days at -2 °C (or 0.02% in the presence of glycolonitrile) (Schwartz *et al.*, 1982). Miller and his co-workers have re-investigated the hydrolysis of hydrogen cyanide over a much wider range of pH and temperature. In their recent paper entitled 'Cold Origin of Life: Implications Based on the Hydrolytic Stabilities of Hydrogen Cyanide and Formamide' they too conclude that eutectic cooling is a plausible mechanism for concentrating dilute hydrogen cyanide solutions sufficiently to permit the prebiotic formation of the HCN tetramer and subsequently more complex molecules including adenine (Miyakawa *et al.*, 2002a). In an accompanying paper they describe the products obtained from a solution of ammonium cyanide that had been maintained at -78 °C

for 27 years (Miyakawa *et al.*, 2002b). Their results confirm and extend the earlier conclusions of Schwarz and his coworkers (1982).

2. Photo-isomerization of HCN Tetramer

In the light of the general acceptance of the RNA world hypothesis and a revised view of the likely composition of the primitive atmosphere, it is interesting to revisit, after almost thirty years, alternative scenarios for the prebiotic synthesis of adenine that involve the photochemical isomerization of the HCN tetramer to 4-amino-5-cyano-imidazole (AICN). In dilute aqueous solution HCN tetramer is photo-isomerized rapidly and efficiently, for example in Californian sunlight, to AICN (Figure 1) (Ferris and Orgel, 1966a; Sanchez *et al.*, 1967), a molecule that is readily converted under laboratory conditions to the biologically important purines (Sanchez *et al.*, 1968) (Figure 1). Scenarios involving this photo-isomerization reaction should be considered as alternatives to reaction sequences that do not involve a photochemical step. Electric discharges in an atmosphere containing nitrogen, methane and carbon monoxide would have provided short-lived, highly localized sources of HCN. Solution in raindrops, followed by freezing and exposure to sunlight in the atmosphere, would then have led directly to the synthesis of AICN in low yield (Sanchez *et al.*, 1967). The amount of AICN that formed in this way would have been small because tetramer formation at sub-zero temperatures is slow. Nonetheless, this mechanism should not be ignored, since it provides a way of converting HCN tetramer to AICN prior to the appearance of large amounts of polymer.

A more promising scenario postulates that the photochemical step occurred after the deposition of frozen solutions of hydrogen cyanide on the surface of the earth. There are advantages to such a scenario, for it explains the appearance of a surface layer of ice containing far more total HCN than would have been present if a solution in equilibrium with the bulk oceans had been frozen. Furthermore, it makes it plausible that an enriched surface layer would have been directly exposed to sunlight. No doubt the proportion of HCN converted to AICN in this way would have been small, but perhaps not as small as that obtained in other prebiotic syntheses, for example a yield of 3×10^{-4} based on HCN was obtained from frozen solutions in the dark after 27 yr at -78°C (Miyakawa *et al.*, 2002b). It is hard to estimate photochemical yields under plausibly prebiotic conditions, since the reaction is sensitive to many factors, for example the pH of the solution and the time, intensity and wavelength of irradiation. Under laboratory conditions, yields of AICN up to 0.2% have been obtained by irradiation of polymerizing HCN solutions (Sanchez *et al.*, 1967). Yields under prebiotic conditions could have been considerably larger or smaller than this.

The post-irradiation history of solutions containing AICN is crucial for the formation of adenine. The synthesis of adenine in 7.7% yield from AICN and

HCN at 30 °C after 22 months has been described (Sanchez *et al.*, 1968). More recent laboratory work is also relevant. Zubay has shown that AICN reacts efficiently with ammonium formate at 94 °C to give adenine (Zubay and Mui, 2001). Since ammonium formate is the product of the hydrolysis of HCN, there are many prebiotic scenarios for the evaporation of aqueous solutions containing AICN and a large excess of ammonium formate.

Oro's adenine synthesis and its more prebiotic variants begin with ammonium cyanide. This presents a difficulty because the presence of substantial amounts of free ammonia in the atmosphere or dissolved in surface waters on the primitive earth may not be realistic (Levine *et al.*, 1982). The scenarios involving a photochemical step are advantageous because they are not dependent on the presence of free ammonia. The addition of HCN to AICN yields adenine directly (Sanchez *et al.*, 1968).

Almost all prebiotic syntheses, including those of adenine, have been challenged on the grounds that they deal with simple idealized models and ignore the complexity of realistic prebiotic environments (Shapiro, 1995, 1999, 2002). No doubt similar objections can be made to the scenarios that are recommended here. One can only respond to some of the most obvious criticisms.

1. There never was a reducing atmosphere. It seems clear that there was never a methane/ammonia/water atmosphere similar to that employed in early experiments on prebiotic synthesis. However an atmosphere containing large quantities of nitrogen and some methane and carbon monoxide seems possible (Kasting and Brown, 1998; Kasting, personal communication) and such an atmosphere might provide an adequate source of HCN (Stribling and Miller, 1987).
2. Other reactions would consume any HCN formed, thus making HCN tetramerization unlikely. The reaction of HCN that would be most likely to compete with tetramer formation is that with formaldehyde, since formaldehyde would form along with HCN in the atmosphere. This reaction would lead ultimately to the formation of glycolic acid, or glycine if ammonia were present in sufficient quantities. However it is not clear that the amount of formaldehyde formed would exceed the amount of HCN under all potentially prebiotic conditions (Stribling and Miller, 1987).
3. Only a fraction of a percent of HCN would be converted to adenine. Other products formed from HCN would interfere with the reactions needed to make use of adenine in subsequent prebiotic reactions. However, selective adsorption on minerals, for example montmorillonite (Winter and Zubay, 1995; Cohn *et al.*, 2001), might lead to substantial enrichment of adenine. No doubt there are other plausible enrichment and concentration mechanisms.

3. Compatibility of Purine and Pyrimidine Synthesis

While the main purpose of this review is to discuss adenine synthesis, something must be said about the compatibility of proposed prebiotic syntheses of the purine and pyrimidine bases. The plausibility of a scheme for the synthesis of the nucleoside bases would be greatly enhanced if the pyrimidines and the purines could be formed under similar conditions. This is not always true of published scenarios. Miller and his coworkers, for example, have recently suggested that the purines were formed in eutectic solution (Miyakawa, 2002b), but that the pyrimidines were obtained in drying lagoons in which urea had become concentrated to saturation (Robertson and Miller, 1995; Nelson *et al.*, 2001). Given the great difficulty of understanding the geochemistry of the latter process (Shapiro, 1999, 2002), there must have been few if any places where products from one of these two very different environments could have encountered those from the other. Scenarios involving synthesis in eutectic solution are not subject to the same kind of criticism.

Cyanoacetylene is obtained along with hydrogen cyanide when a methane-nitrogen atmosphere is subjected to an electric discharge or upon heating a methane nitrogen mixture. Cyanoacetylene reacts with cyanate (1.0 M) to give cytosine in good yield (Sanchez *et al.*, 1966b; Ferris *et al.*, 1968). Cyanate is unlikely to be formed directly in an electric discharge, but it is formed quantitatively when cyanogen undergoes hydrolysis. It has been speculated that the reaction of cyanoacetylene with either cyanate or cyanogen in a eutectic solution would slowly yield cytosine (Ferris *et al.*, 1968). The quantitative basis for this speculation is the observation that cyanovinyl urea is formed from cyanoacetylene and 1.0 M cyanate or cyanogen after one day at 30 °C in 4–13% yield, depending on the pH, etc. Cyanovinyl urea cyclizes to cytosine almost quantitatively with a half time of 20 hr at pH 10 and 30 °C. The formation of cyanovinyl urea is likely to proceed at least as efficiently in a eutectic at –10 °C as in solution at 30 °C, but the half time might increase from days to years or tens of years. The cyclization reaction would have a half-life of about 100 days at pH 8 and 30 °C and hence of years to hundreds of years at –10 °C.

Unfortunately, no direct experimental evidence is available on the reaction of cyanoacetylene with cyanate in eutectic solution, but Miller and his coworkers have provided data on a closely related reaction, that of cyanoacetaldehyde, the hydrolysis product of cyanoacetylene, with urea (Nelson *et al.*, 2001). This is intrinsically a slower reaction than that of cyanate with cyanoacetylene but still yields 0.005% of cytosine and 0.02% uracil after only two months at –20 °C. Miller and his coworkers do not comment on the relevance of this low temperature reaction to the origin of life, but it could be argued that it is far more plausible as a prebiotic synthesis than the higher temperature reaction suggested in the drying lagoon model.

The reaction of cyanoacetylene with cyanate (cyanogen) in frozen raindrops or in the layers of ice formed when the raindrops deposit on the surface of the earth

seems the more plausible route to cytosine, but in the absence of experimental evidence the reaction of cyanoacetaldehyde, formed by the hydrolysis of cyanoacetylene, with urea in a eutectic cannot be excluded. The main conclusion is that eutectic concentration provides a mechanism for the synthesis of pyrimidine bases that parallels the synthesis of adenine from HCN.

HCN is formed in substantially larger amounts than cyanoacetylene when an electric discharge is passed through a methane/nitrogen atmosphere. Consequently, the formation of maleonitrile and fumaronitrile, and later glycolic acid and possibly aspartic acid (Sanchez *et al.*, 1966b; Ferris *et al.*, 1968), will always compete with the formation of pyrimidines. The possibility that the prebiotic synthesis of purines and pyrimidines could have occurred in parallel would depend on the formation of some frozen droplets in which cyanogen was a major component, comparable in amount to HCN. There is no experimental evidence available to show whether or not this is possible.

4. Conclusion

It is difficult to decide whether or not adenine is a prebiotic molecule and, if it is, whether it was formed on the primitive earth or introduced by comets or meteorites. On the one hand, it is possible to raise numerous serious but not necessarily fatal objections to any prebiotic synthesis of adenine that has been proposed. On the other hand, adenine is a complex molecule available from a nitrogen-methane atmosphere through a remarkably simple procedure, making it hard to believe that it is a coincidence that adenine is also a central molecule of modern biochemistry. It therefore seems likely that adenine is a prebiotic molecule. If it was formed from HCN on the primitive earth, eutectic concentration and a photochemical step probably were involved. It is also possible that pyrimidines were formed from cyanoacetylene and cyanate in eutectic solutions, but without the need for photochemistry.

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