THE PREBIOTIC ROLE OF ADENINE: A CRITICAL ANALYSIS

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Abstract. Adenine plays an essential role in replication in all known living systems today, and is prominent in many other aspects of biochemistry. It occurs among the products of oligomerization of HCN. These circumstances have stimulated the idea that adenine was a component in a replication system that was present at the start of life. Such replicators have included not only RNA, but also a number of simpler RNA-like alternatives which utilize a simpler backbone.

Despite these encouraging indicators, a consideration of the chemical properties of adenine reveals reasons that disfavor its participation in such a role. These properties include the following: (1) Adenine synthesis requires HCN concentrations of at least 0.01 M. Such concentrations would be expected only in unique circumstances on the early Earth. Adenine yields are low in prebiotic simulations, and if a subsequent high-temperature hydrolysis step is omitted, the reported yield does not represent adenine itself, but 8-substituted adenines and other derivatives. (2) Adenine is susceptibile to hydrolysis (the half life for deamination at 37 °C, pH 7, is about 80 years), and to reaction with a variety of simple electrophiles, forming a multiplicity of products. Its accumulation would not be expected over a geological time scale, and its regioselective incorporation into a replicator appears implausible. (3) The adenine-uracil interaction, which involves two hydrogen bonds (rather than three, as in guanine-cytosine pairing) is weak and nonspecific. Pairing of adenine with many other partners has been observed with monomers, synthetic oligonucleotides and in RNA. The hydrogen-bonding properties of adenine appear inadequate for it to function in any specific recognition scheme under the chaotic conditions of a prebiotic soup.

New and fundamental discoveries in the chemistry of adenine would be needed to reverse this perception. An alternative and attractive possibility is that some other replicator preceeded RNA (or RNA-like substances) in the origin of life.

1. Introduction

The speculation that life began with an 'RNA world' (Gilbert, 1986), in which RNA performed all catalytic and genetic functions has been cited frequently in origin of life literature. Although this idea has been disputed vigorously (see, for example, the summary by M.M. Waldrop (1989)), it has appeared in some textbooks of biochemistry as an accepted and established theory. In one case, for example, a section heading affirmed that 'the first "living molecule" was almost certainly a nucleic acid' (Watson *et al.*, 1987).

Before RNA could assume such a role, however, the spontaneous formation of the first RNA molecule would be needed. This could not take place however, unless the various RNA building blocks were produced in reasonable amounts in the presence of one another. Difficulties have arisen in the prebiotic synthesis and the stability of one key ingredient, D-ribose (Shapiro, 1984, 1988; Joyce *et al.*, 1987; Schwartz and De Graaf, 1993). The search for a reasonable prebiotic route to ribose continues (Muller *et al.*, 1990), but a number of authors have attempted to avoid this problem in another way. They have invoked RNA-like polymers, with a simpler backbone, at the start of life (Joyce *et al.*, 1987). The suggested substitute

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backbones have included glycerol phosphates (Spach, 1984; Schwartz and Orgel, 1985), other acyclic sugar analogs (Joyce *et al.*, 1987) crystalline lattices (Orgel, 1986a), amino and thiosugars (Schwartz and Orgel, 1985), pyrophosphate-linked oligomers (Schwartz *et al.*, 1987a), peptides (Cherny *et al.*, 1993) and others (Joyce and Orgel, 1993).

In another approach, alternative pentose and hexose backbones bearing the Watson-Crick bases have been synthesized, and their properties studied in detail (Hunziker *et al.*, 1993; Pitsch *et al.*, 1993). Structural complexity has not been reduced in these substances, but in the particular case of p-RNA (a 2′–4′ linked ribopyranosyl phosphate polymer), other advantages have been suggested which would qualify it as a candidate for the first genetic material (Pitsch *et al.*, 1993).

Both the RNA world and the RNA-like world theories, however, retain Watson-Crick pairing, A with U and G with C, as the basis of genetic template recognition. To explore the viability of this assumption, data concerning the likely prebiotic occurence and function of one base, adenine, has been assembled here. Evidence that favors the participation of adenine in a functioning replicating system at the start of life will be presented first, followed by a consideration of the factors that disfavor such a role for adenine.

2. Prebiotic Adenine: Favorable Indications

A number of circumstances have promoted optimism concerning a role for adenine in the very start of life:

(1) A noteworthy one-step synthesis of adenine from HCN was reported by Oró and his coworker in yields of up to 0.5% (Oró, 1960; Oró and Kimball, 1961). The following comments summarize the response of many workers to this achievement: 'The synthesis of the nucleoside bases is one of the success stories of prebiotic chemistry. Adenine is formed with remarkable ease from ammonia and hydrogen cyanide.' (Joyce and Orgel, 1993). 'The self-condensation of HCN to produce purines is such a remarkably simple and efficient reaction that it would be surprising if it did not have some relevance to the early history of life' (Joyce, 1989).

(2) Adenine has also been prepared by subjecting a methane-ammonia-water mixture (hydrogen lowers the yields) to electron irradiation (Ponnamperuma *et al.*, 1963) or electrical discharges in a Miller-Urey type experiment (Yuasa *et al.*, 1984).

(3) The Fischer-Tropsch reaction (catalysis by iron oxide and other metallic oxides at 500 °C) produces some adenine from a mixture of carbon monoxide, hydrogen and ammonia (Hayatsu *et al.*, 1972).

The above reactions have encouraged conclusions that 'quite reasonable pathways' for prebiotic adenine synthesis existed (Schwartz *et al.*, 1987b) and that ade-

nine was 'readily generated' (Sharp and Eisenberg, 1987) and 'available' (Joyce, 1989) on the early Earth.

(4) Adenine has been identified in anhydrous formic acid extracts of the Murchison, Murray and Orgueil meteorites (Stoks and Schwartz, 1981).

(5) Many present-day biological cofactors (for example: NAD, NADP, FAD, Coenzyme A, Coenzyme B_{12}, S-adenosylmethionine, and ATP) contain an adenylate residue that does not participate directly in the catalysis of the relevant reaction (Benner, 1987; Joyce, 1989). They have been termed 'molecular fossils' (Eschenmoser and Lowenthal, 1992) and explained as 'vestiges of an ancient metabolism based on RNA catalysis' (Benner *et al.*, 1987, 1989). Earlier workers (Ponnamperuma *et al.*, 1963) noted that 'the apparent preference for adenine synthesis may be related to adenine's multiple roles in biological systems'. In these views, adenine is seen as a central participant in the start of life, and it has left its mark by its presence in key molecules since then.

3. Problems in the Prebiotic Synthesis of Adenine from HCN

The direct synthesis of adenine from HCN (Oró, 1960; Oró and Kimball, 1961) appears at first glance to meet the criteria for the ideal prebiotic synthesis: an important biochemical substance is prepared directly from a very simple precursor quite likely to have been present on the early Earth. On closer inspection of the conditions, however, a number of significant problems appear. The difficulties are separated into individual topics in the discussion below, which concentrates on the prebiotic likelihood of the conditions used to generate adenine, and the yields obtained. For more detailed reviews which include questions of mechanism, consult Miller and Orgel (1973), Ferris and Hagan (1984) and Ferris (1992).

(1) The requirement of a high HCN concentration ('HCN' will be used to represent the combined concentration of HCN and cyanide ion. The pH usually used for the reaction is 9.2, where the rate is maximal and both are present in approximately equal amounts):

In the initial studies by Oró and co-workers, HCN concentrations of 1-15 M were used, and yields of up to 0.5% of adenine were obtained. High concentrations of HCN favor the formation of adenine and other self-condensation products of HCN (the kinetics are higher than first-order) because they compete with hydrolysis to formamide and formate, which is first order in HCN (Sanchez *et al.*, 1967). Yields acan be improved even further by eliminating water entirely. Thus, adenine can be produced in 15% yield by heating a concentrated solution of HCN in liquid ammonia (Wakamatsu *et al.*, 1966). Yields fall sharply in aqueous solution, however, when less concentrated solutions of HCN are employed. For example, a synthesis which employed 1.0 M HCN afforded a 0.1% yield of adenine, while another run with 0.1 M HCN gave a yield of 0.03–0.04% (Ferris *et al.*, 1978). According to Sanchez *et al.* (1966): 'The yield of purines formed from HCN would fall off very rapidly at cyanide concentrations less than 0.01 M.'

What concentration of HCN was present in the prebiotic ocean? One estimate (Stribling and Miller, 1987) has placed it at 4×10^{-5} M under the most favorable circumstances of temperature and pH (0 °C and pH 7). Schwartz and Bakker have calculated that if all of the nitrogen now present on the Earth's surface were put into the oceans as HCN, the concentration of that substance would only come to 0.2 M. Of course, higher HCN concentrations could be achieved in limited environments, for example, a pond formed by a soft landing of a cometary nucleus (Clark, 1988) or a lake that froze in winter and melted in summer (Sanchez *et al.*, 1967). In the latter case, HCN would be concentrated in solution by the eutectic freezing of ice. In one synthesis conducted under freezing conditions, a yield of 0.004% of adenine was obtained, using an initial HCN concentration of 0.01 M (Schwartz *et al.*, 1982).

The limitation of adenine synthesis to only a few locations, under constrained conditions of pH and temperature would have an important drawback, however. The synthesis of an informational base is only the first of a number of reactions that must take place in succession to create a replicator. If a synthesis were discovered that was capable of producing adenine in abundance and distributing it globally, then the availability of adenine could be taken for granted. Subsequent steps could involve different reaction conditions and processes of low probability. If however, adenine were to be found only under unique circumstances, then the steps that incorporated it into a replicator would need to have higher probability and use chemicals also present in those locations.

(2) The involvement of ammonia: In most reported adenine syntheses, the concentration of ammonia present has been at least half that of HCN. One early review (Miller and Orgel, 1973) commented: 'The chemistry described above does not easily describe the synthesis of purines under prebiotic conditions because useful yields of adenine cannot be obtained except in the presence of 1.0 M or stronger ammonia. The highest reasonable concentration of ammonia or ammonium ion that can be postulated in oceans and lakes on the primitive earth is about 0.01 M.' More recent estimates of that concentration have run from 3.6 to 70 μM (Summers and Chang, 1993).

Mechanistic studies had indicated that the role of ammonia in the synthesis of adenine was limited, however. It reacted with HCN to produce formamidine that was needed to convert an intermediate HCN tetramer, diaminomaleonitrile, to an imidazole derivative. That same conversion could be carried out in good yield in a photochemical process. (Ferris and Kuder, 1970). Irradiation at 350 nm of a dilute, neutral, aqueous, tetramer solution for 9 hours converted it to the imidazole in yields of 70–82%. However, the complete synthesis of adenine from HCN in which all the intermediates in the synthesis have been exposed to UV irradiation has not been demonstrated.

The need for ammonia may also be circumvented by the inclusion of formaldehyde in the reaction mixture. In this case however, the principal purine product

is 8-hydroxymethyladenine rather than adenine. This is discussed under point (4) below.

(3) The requirement for a high-temperature hydrolysis step: Schwartz and Bakker (1989) have described this limitation of the HCN-ammonia oligomerization: 'None of the five bases that occur in nucleic acids has been identified among the initial oligomerization products formed in dilute solution. The detection of adenine requires an additional hydrolysis step; usually hydrolysis in 3M to 6M HCl at 100 to 110 °C for up to 18 hours. Such conditions cannot be considered to be prebiotic.' In one preparation, hydrolysis was conducted at 110 °C at pH 8.5 (Ferris *et al.*, 1978). Adenine was detected but not quantified. In the original studies of Oró and Kimball (1961) conducted with more concentrated HCN at 70 °C, small amounts of adenine were produced without the additional step. The amounts increased as the reaction time was extended, but the maximum reported was still less than one tenth of the yield after treatment with hot acid. In this case, hydrolysis was taking place under the reaction conditions.

Adenine 8-carboxamide has been identified as one product that is produced in the HCN oligomerization, and which then hydrolyzes at high temperature to produce adenine (Voet and Schwartz, 1983). It afforded 42% of the adenine formed upon hydrolysis. Presumably the remainder was linked to other HCN oligomerization products.

(4) The effect of other added substances: Schwartz and Bakker (1989) reasoned that formaldehyde was likely to be widely distributed on the early Earth and present in HCN oligomerizations. If the HCN is present in excess, then the formaldehyde is converted almost quantitatively to glyconitrile, which alters the course of the reaction. Some adenine is produced, but the major product is now 8-hydroxymethyladenine. The combined yield of both from a reaction run with 0.5 M HCN and 0.5 M glyconitrile was 0.03%. Of this product, 94% was 8-hydroxymethyladenine. These totals reflect yields after acid hydrolysis; if hydrolysis was omitted, the yields were about 20% less.In an earlier study (Schwartz *et al.*, 1982), a 0.02% yield was reported from 0.01 M HCN and 0.01 M glyconitrile. The product was considered to be adenine at that time It is not clear whether the yield reflects the presence of 8-hydroxymethyladenine as well.

(5) Summary: Orgel (1986b) has put forward the following prerequisite for the very first information system: 'its monomeric components must have been abundant components of a prebiotic mixture of organic compounds.' Adenine does not seem to meet this requirement. The oligomerization of HCN on the early Earth was restricted to those locations where sufficient concentrations of HCN (about 0.01 M) could be achieved. The product of oligomerization under those conditions was complex, but contained less than 0.1% of substituted adenines. No free adenine was present. 8-Hydroxymethyladenine may then be the most common purine product produced by this process. It should not be presumed that 8-hydroxymethyladenine would simply substitute for adenine in an early RNA, however. Substitution of adenine nucleosides at the 8-position favors their conversion from the anti con-

formation normally used for Watson-Crick pairing in DNA and RNA to a syn conformation (Saenger, 1984), which would alter its pairing mode. Suggestions have been made about very different prebiotic uses for 8-hydroxymethyladenine (Schwartz and Bakker, 1989), but they lie outside the scope of this review.

4. Alternative Sources of Adenine

Other preparations of adenine from simple chemicals have been reported which do not involve direct oligomerization of HCN. Adenine has been detected in the mixture produced from passing an electrical discharge through a methane-ammonia-water mixture under Miller-Urey conditions. However this procedure also utilized an acid hydrolysis and afforded low yields (0.001%) (Yuasa *et al.*, 1984). The detection of adenine among the products formed by electron irradiation of a similar mixture had been performed earlier (Ponnamperuma *et al.*, 1963). However, the identification had been based only upon paper chromatographic mobility, and no yield was reported. Such processes presumably involved HCN as an intermediate. The concentration of adenine (derived from HCN-related processes) in the prebiotic ocean is likely to have been very low, perhaps 3×10^{-5} M (Miller, 1987).

As mentioned in section 2, adenine has been produced under certain conditions in a high-temperature Fischer-Tropsch synthesis from carbon monoxide, hydrogen and ammonia (Hayatsu *et al.*, 1972). The yield from this process has been reported at 0.16% (Hayatsu and Anders, 1981). Most of the conditions that were tried yielded no adenine, however. It was suggested that such reactions may have taken place in the solar nebula and led to the production of organic materials in meteorites, but 'a detailed systematic study of this reaction would be very desirable, to see what conditions are required, and whether they are realistic for the solar nebula' (Hayatsu and Anders, 1981).

Meteorite analyses, however (reviewed in Stoks and Schwartz, 1981 and Cronin *et al.*, 1988) have fail to testify to any cosmic abundance of adenine. The total concentration of purines and pyrimidines identified was just above 1.3 parts per million, or about one-fiftieth of the amino acid total. The maximum concentration of adenine detected, from the Murchison meteorite, was 267 parts per billion (acidic hydrolysis at high temperature was performed during the analysis). Even this amount may be due to artefact : 'All of the purines and the pyrimidine found are common biological constituents and no biologically unknown or unusual analogues accompany them.... Thus, although blank runs testify to contamination-free procedures, the possibility that these compounds originated in terrestrial microorganisms in the sample should be kept in mind' (Cronin *et al.*, 1988).

In all, the above results offer little promise that adenine supplies on the prebiotic Earth were abundant. Hovever, we cannot exclude the possibility that other reactions, not yet identified, may have provided such a supply.

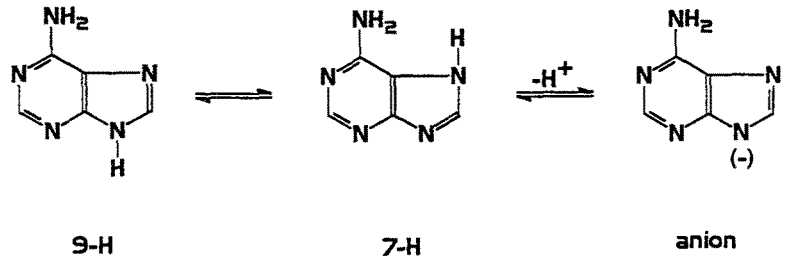

Fig. 1. Tautomerism and ionization of adenine.

5. Instability of Adenine: Hydrolytic Reactions

If adenine is produced only in trace amounts by prebiotic processes, then its use in a replicator requires either that it be concentrated by very selective processes, as yet unknown (Miller, 1987) or that it persist and accumulate over geological periods of time (Ferris *et al.*, 1978). The chemistry of adenine makes this latter alternative unlikely. Adenine is vulnerable to hydrolytic reactions, such as deamination and ring opening, and to reaction with a variety of other substances.

(1) Deamination of adenine: Adenine (Figure 1) and its derivatives are hydrolyzed in water to afford hypoxanthine (I, Figure 2) and its derivatives. The uncatalyzed rate of deamination for adenine at 85 °C has been measured (Frick *et al.*, 1987) at 1.3×10^{-8} s^{-1}, which would correspond to a half-life of 1.7 years. (The rate for adenosine was slightly slower: 8.6×10^{-9} s^{-1}, with a half life of 2.6 years). The authors assumed that the same energy of activation applied for the uncatalyzed reaction as had been determined for an alkali-catalyzed one (that relation had held for the case of cytidine), and extrapolated the rate of deamination of adenosine at 37 °C to be 1.8×10^{-10} s^{-1}. If the same assumption is made for adenine deamination, then a rate at 37 °C of 2.7×10^{-10} sec^{-1}, half-life = 81 years, can be estimated at 37 °C. By further extrapolation, we get for adenine at 25 °C, $k = 8.52 \times 10^{-11}$ sec^{-1}, half-life = 258 years and at 0 °C, $k = 5.47 \times 10^{-12}$ sec^{-1} and half-life = 4,000 years. At 25 °C, the rate of adenosine deamination is about one-sixth that of cytidine.

The uncatalyzed reaction predominates in neutral solution, but at low pH an acid catalyzed reaction ($k = 1.5 \times 10^{-6}$ M^{-1} s^{-1} at 85 °C for adenine) enhances the rate. A base-catalyzed demination of adenosine takes place in alkali, with a rate constant of 7.5×10^{-6} M^{-1} s^{-1} at 85 °C. Adenine dissociates to an anion (Figure 1) in base (pKa = 9.8; Dunn and Hall, 1975) and would probably be protected from alkali-catalyzed deamination at more alkaline pH. Apart from the above-mentioned catalysis, most nucleophiles had little effect on the rates (Frick *et al.*, 1987). However, a Cu^{2+}- montmorillonite clay combination (Strasak, 1991) appeared to have significant deamination activity for adenine.

The deamination of adenine, and of cytosine, proceed at sufficient rates for the reaction to be of genetic significance. In present-day organisms, separate, specific

I II

Fig. 2. Deamination (I) and ring-opening (II) products of adenine.

glycosylases are available to remove the reaction products, hypoxanthine and uracil, from DNA (Lindahl, 1993).

(2) Ring opening of adenine. In the study of Frick *et al.* (1987), other side reactions of adenosine contributed to its decomposition in alkali, including opening of the imidazole ring to afford 4,5,6-triaminopyrimidine, (II, R = H, Figure 2) (Gordon *et al.*, 1957; Jones *et al.*, 1966; Mian and Walker, 1968; Garrett and Mehta, 1972). Adenine again appeared to be protected from this reaction in strong alkali, due to the formation of its anion, Figure 1). At pH values less than about 9.5, however, this protection would not apply. A half-life at 37 °C of 200 years at neutral pH has been estimated for this reaction on the basis of the data in alkali (Shapiro, 1981).

Adenine derivatives also undergo imidazole ring opening when exposed to gamma rays or X-rays,to produce, initially, 4,6-diamino-5-formamidopyrimidine (II, R = CHO, Figure 2) (Bonicel *et al.*, 1980; Breimer, 1984; Chetsanga and Grigorian, 1985). This reactivity would not be of relevance for most prebiotic simulations, but could apply to those which invoke an extraterrestrial origin for adenine.

6. Reactivity of Adenine

The instability of adenine on a geological time scale makes its wide spread prebiotic accumulation unlikely. Under special circumstances, however (for example, in areas with local high concentrations of HCN), some adenine or substituted adenine might form. Further transformations would be needed in order to incorporate it into a replicator. Presumably this would take place within the environment that generated it, as the limited half-life of adenine makes extensive redistribution implausible.

In considering such transformations, we can generalize and include not only adenine, but any prebiotic subunit that is to function as part of a macromolecular template. Two separate areas of the subunit must be distinguished: the *attachment site* (N-9 for adenine in a RNA-like molecule), which should react to incorporate the subunit into the macromolecule, and the *recognition site* (N-1 and amino

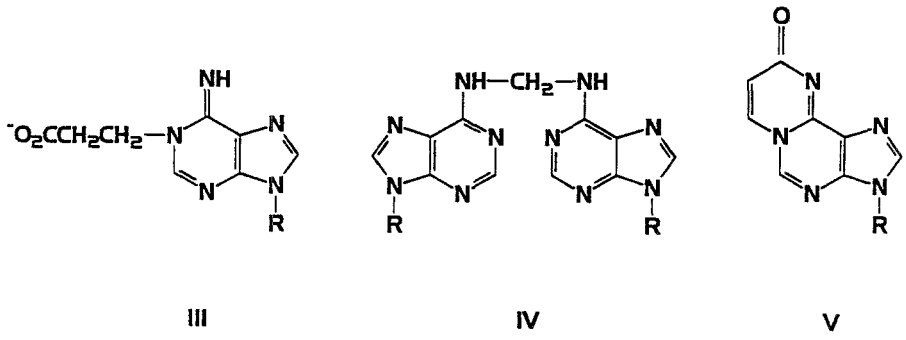

Fig. 3. Some reaction products of adenines with simple reactants.

hydrogen for adenine), which must avoid such reactions, and function in replication after the macromolecule has been assembled. Ideally, chemical reactions should take place specifically at a unique attachment site, as reactions elsewhere would deplete supplies of the subunit, and also possibly result in the incorporation of misaligned subunit into the polymer. In the discussion below, we will focus on the regiospecificity of adenine reactions. A more detailed consideration of the chemistry of adenine and its derivatives can be found in Lister (1971), Kochetkov and Budovskii (1971) and other works.

Adenine has four ring nitrogen atoms and an amino group, and all are potentially reactive. Removal of the proton would be required for the ring N bearing a proton to react: this can achieved by tautomerism (adenine exists 80% as the 9-H tautomer and 20% as the 7-H tautomer; Gonnella *et al.*, 1983) or ionization (Figure 1). In practice, no regioselectivity is displayed; all five of the nitrogens in adenine can be substituted, though position 7 appears to be the slowest in most reactions.

A number of plausible prebiotic substances, aldehydes and alpha-beta unsaturated carbonyl compounds in particular, react readily with aqueous solutions of adenine derivatives under mild, potentially prebiotic conditions (25-45 °C, neutral or mildly acidic or alkaline pH, hours to days of reaction time). Typical reactants include acrylonitrile (Solomon *et al.*, 1984), acrylonitrile oxide (Solomon *et al.*, 1993), acrylic acid (Segal *et al.*, 1987), acrolein (Sodum and Shapiro, 1988), diethyl pyrocarbonate (Leonard *et al.*, 1971), ethyl propiolate (Roques *et al.*, 1992), and formaldehyde (Chaw *et al.*, 1980). Reaction most commonly involves the adenine 1-position (III, Figure 3, acrylic acid product) the amino group (IV, Figure 3, formaldehyde product) or both, with cyclization (V, Figure 3, ethyl propiolate product).

Simple alkylating agents (halides, sulfates, epoxides) also react readily with adenine: The reactivity of adenine in this case has been summarized: 'In adenine, all ring nitrogen atoms are available for alkylation.... In neutral media, which may be an aqueous solution or a dipolar aprotic solvent..., the major alkylation product is the 3-alkyladenine, but some of the 9- and 1- alkyl isomers will also be

present.' (Lister 1971, pages 342–3). Greater selectivity can be obtained in alkali: the 9-isomer predominates, but the 3-isomer is also formed.

A number inorganic reactants could also deplete adenine supplies. For example, nitrous acid affords hypoxanthine (Shapiro and Pohl, 1968), hydroxylamine gives N^6-hydroxylaminopurine (Budowsky *et al.*, 1971) and hydrogen peroxide (or acyl peroxides) yield adenine 1-N-oxide (Stevens and Brown, 1958; Stevens *et al.*, 1958; Subbaraman *et al.*, 1969). The above reactions would apparently preclude any prebiotic union of adenine with ribose, as part of a path to RNA. The prebiotic synthesis of adenosine would require the regioselective substitution of adenine at N-9 by ribose to form a β-ribofuranosyl derivative. Ribose synthesis appears at any event to be incompatible with the amine and cyanide-rich solutions used to generate adenine (Schlesinger and Miller, 1973; Shapiro, 1988). If they should be formed together by some other prebiotic circumstance, however, it is unlikely that ribose could compete with the other types of reactant listed above for the available adenine. Adenine and ribose do not react with one another in aqueous solution, over a pH of 2-11, temperature 30-100 °C (Fuller *et al.*, 1972a,b). When they are heated in the dry state at 100 °C (Fuller *et al.*, 1972a,b), a mixture of N^6-ribosyladenines is produced (Maurel and Convert, 1990). Existing evidence thus strongly disfavors the idea that adenine could be incorporated into RNA under prebiotic circumstances.

Adenine thus reacts readily with water and numerous substances likely to be present in a prebiotic soup. Reactivity is not limited to the desired attachment site, but occurs at the recognition site and elsewhere: In addition to the 1- and 6-positions, reaction can also occur at the 3, 7 and 9-positions. This suggests that any adenine formed in prebiotic processes will be dispersed rapidly among a multitude of products, excluding adenosine. This may in fact have happened during the syntheses of adenine in HCN solutions. Substituted adenines were formed initially, and adenine was released only after a high-temperature hydrolytic step. A portion of the bound adenine was present as the 8-carboxamide, but the majority remains unidentified (Voet and Schwartz, 1983). It has been noted that the HCN oligomerization mixture exhibits intense mutagenic activity (Giner-Sorolla and Oró, 1993), so it seems likely that chemical transformations of the type described in this section altered any adenine that was produced directly.

7. Infidelity and Weakness of Adenine Hydrogen Bonding

We have seen in previous sections that a number of difficult obstacles hinder the prebiotic regioselective incorporation of adenine into any potential replicator. If its presence in a replicator offered profound and unique advantages, however, then we could argue that these benefits justified the acceptance of very implausible prebiotic sequences. In this section we will consider whether adenine offers such advantages, with particular emphasis on the strength of the hydrogen bonding of adenine to uracil and the fidelity of its recognition of a unique partner. In a prebiotic

environment, with a host of competing substances present at low concentration, such strength and accuracy of recognition would be essential for template function. The discussion will be limited to 9-substituted adenines, because information is lacking on the template abilities of polymers containing adenine residues which are substituted at other positions.

(1) Strength of the adenine-uracil pair: Adenine, because of its geometry, can form only two hydrogen bonds to another base. This limitation makes makes adenine to uracil (or thymine) hydrogen bonding considerably weaker than that of guanine with cytosine or 2,6-diaminopurine with uracil, which can pair with three hydrogen bonds. For this reason, the melting temperature of DNAs from many species rises linearly in proportion to the fraction of GC base pairs, and AT rich regions of DNA are the first to melt upon heating (Stryer, 1988). No interaction can be detected between A and U monomers at biological concentrations in aqueous solution, due to competition by water (Voet and Rich, 1970). Adenine and uracil do not form hydrogen bonds in dimethylsulfoxide, under conditions where guanine-cytosine recognition can be observed (Dyllick-Brenziger, 1980). In chloroform, however, the interaction of soluble adenine and uracil derivatives can be observed (Voet and Rich, 1970). In this solvent, the interaction constant between 1-cyclohexyluracil and 9-ethyladenine is about 100 M^{-1}. Guanine-cytosine interactions under these circumstances are about 1000 times stronger (Pitha *et al.*, 1966).

The strength of interaction between adenine and uracil in chloroform increases with the acidity of the proton donated by the adenine partner. The interaction constant is 30 M^{-1} with a dihydrouracil derivative (pKa near 11; uracil has a pKa of 9.2), and 240 M^{-1} for a 5-bromouracil derivative (pK = 7.8). Electronic factors are also involved, as the complex of 9-ethyladenine and phenobarbitol (5-ethyl-5-phenyl-barbituric acid, pK 7.3) in chloroform has an association constant of 1200 M^{-1} (Voet and Rich, 1970). The strongest reported complexation of a substituted adenine, 120,000 M^{-1} (Zimmerman *et al.*, 1991), was observed in the complex of a 'molecular tweezer' cavity designed to fit the adenine, with a carboxylic acid functioning as the hydrogen-bonding partner (IX, Figure 4).

For effective interaction of adenine and uracil in aqueous solution, cooperative multiple-unit pairing is needed within a suitable polymer framework. Effective interaction of oligo (A) and oligo (U) can be observed with tetramers, but it requires 0.12 M total nucleotide concentration at 0 °C, with 0.04 M Mg^{2+} (Gennis and Cantor, 1970). Monomeric adenines can interact with poly(U), but complexes of poly(A) with uracil monomers are unstable (Orgel, 1987). For this reason, efforts at template-directed oligomerization of activated uracil nucleotides on a poly(A) template have failed (Orgel, 1987; Stribling and Miller, 1991).

Reduction of the stereochemical homogeniety of the backbone diminishes the strength of A-T interaction: The substitution of a flexible glycerol-based residue for a more rigid ribofuranosyl ring in an A-T pair greatly destabilized the double helix that contained this feature (Schneider and Benner 1990). Further, A-U and

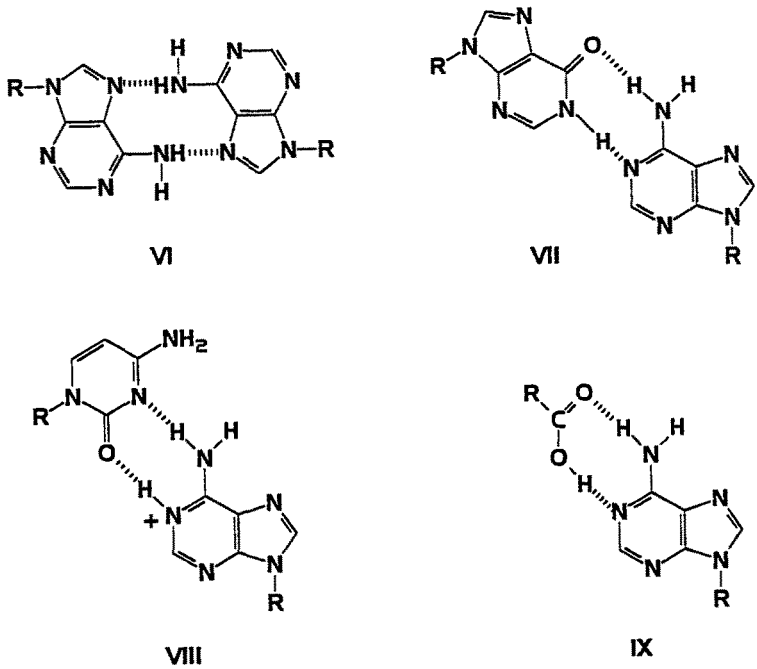

Fig. 4. Some alternative hydrogen bonding partners of adenine.

A-T interactions are weakened by acid or alkali, which cause protonation of A (pKa about 4) and ionization of T and U (pKas between 9 and 10) (Sowers *et al.*, 1987). The interaction of A and U appears to be too weak for their mutual recognition to act effectively as an organizing force in a prebiotic mixture.

(2) Selection of other partners by adenine: Infidelity in partner selection by adenine creates another problem. For bonding at its amino and N-1 sites (other positions may also be used), adenine requires a combination of a H-bond acceptor and H-bond donor in an appropriate geometry; many chemical substances can meet this requirement. As the above discussion illustrates, the greater the acidity of the donor proton, the stronger the interaction. In appropriate environments, a number of amides, imides and carboxylic acids have been used as hydrogen-bonding partners for adenine derivatives (Rebek, 1990; Shea *et al.*, 1993).

The specific Watson-Crick pairing of adenine with thymine in DNA has of course been important in maintaining the integrity of genetic information during replication. But factors other than the innate specificity of A for T have been crucial in maintaining great fidelity during replication: the steric and electronic requirements of the replicative enzymes, the presence of only four activated nucleotide triphosphates, and the stereochemical requirements of the 2-deoxy-D-ribofuranosyl backbone. This last point was illustrated recently when it was found that adenine prefers to pair with itself (VI, Figure 4) within a synthetic

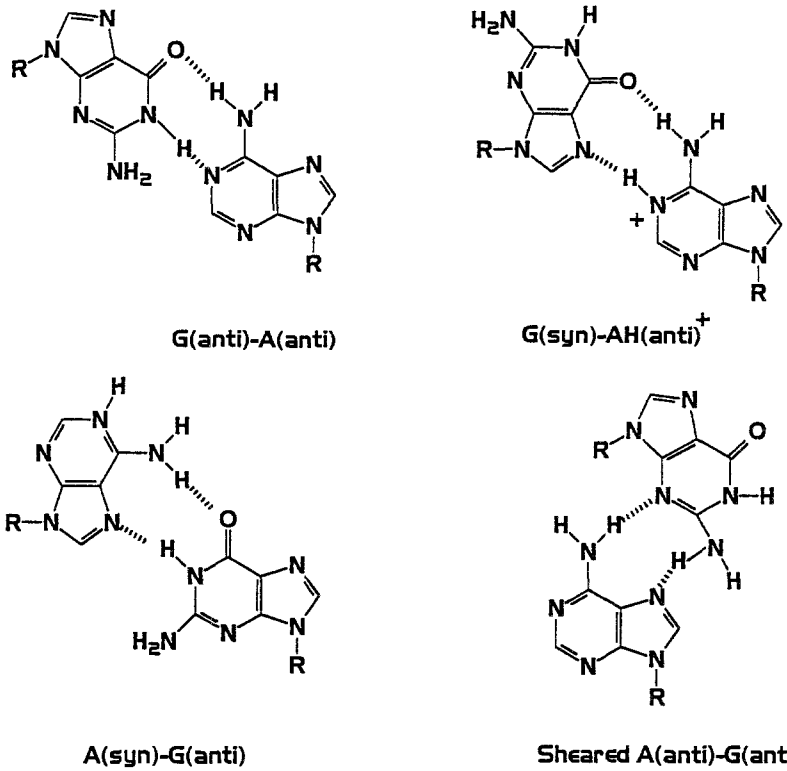

Fig. 5. Four modes af adenine-guanine hydrogen bonding.

'homo-DNA' (2',3'-dideoxy-β-D-glucopyranosyl) oligomer helix (Eschenmoser and Loewenthal, 1992; Hunzicker *et al.*, 1993).

In an RNA environment, adenine shows considerable infidelity in partner selection. For example, four distinct A-G mismatches are known (Figure 5); such mismatches are a common structural element in RNA folding (Chou *et al.*, 1992). A-G and A-A pairs have been observed in synthetic DNA oligomers as well (Maskos *et al.*, 1993). A-I wobble pairing (VII, Figure 4) occurs in tRNA. Adenine to protonated cytosine mismatches (VIII, Figure 4) have been observed in synthetic oligonucleotides. At pH 5.5 or less, the stability of such pairs can exceed that of A-T pairs (Brown *et al.*, 1990). A recent investigation concerning the absence (with only two exceptions) of adenosine from the wobble position of tRNA anticodons concluded: 'Our results would seem to support the notion that A is avoided in this position because it cannot sustain the discrimination that is needed for translational fidelity in the reading of such codons... Adenosine would then be a highly promiscuous wobbler, to be avoided at all costs except in cases where it is necessary for a single tRNA to read all four codons in a family' (Boren *et al.*, 1993).

Some of the features that support adenine infidelity are innate to its chemical structure: its ability to make only two hydrogen bonds has already been mentioned.

Another lies in the existence of an alternative hydrogen bond acceptor site at N-7. This position, in combination with the amino group, can accept the same set of partners as the Watson-Crick face, but with different geometrical requirements. It can thus support Hoogsteen pairing, triple helix formation and mispair formation (for example A-A and A_{syn}-G pairs).

We can conclude that the specific recognition of A and U (or T) appears to work effectively only under tightly constrained circumstances: within the context of a uniform D-ribo(or deoxyribo)furanosyl $3' \rightarrow 5'$ phosphodiester (or closely related) backbone, and under a limited range of pH and ionic strength values. In a prebiotic soup, a great variety of substances might be expected to occur that would serve as competitors of uracil for binding to adenine. It appears unlikely that adenine could function effectively under such chaotic conditions.

8. Conclusions

Adenine plays an essential role in replication in all known living systems today, and is prominent in many other aspects of biochemistry. Despite this, a consideration of its intrinsic chemical properties suggests that it did not play these roles at the very start of life. These properties include the low yields in known syntheses of adenine under authentic prebiotic conditions, its susceptibility to hydrolysis and to reaction with a variety of simple electrophiles, and its lack of specificity and strength in hydrogen bonding at the monomer and mixed oligomer level.

New and fundamental discoveries in the chemistry of adenine would be needed to reverse this perception. An alternative and attractive possibility is that some other replicator preceeded RNA (or RNA-like substances) in the origin of life (Cairns-Smith, 1982, Shapiro, 1986, Stribling and Miller, 1987, Orgel and Crick, 1993). This possibility has been described aptly by Orgel (1986b): 'The nature of the original "bases" is a subject for speculation. Perhaps as we learn more about earlier and earlier phases in the evolution of life, the familiar features of double-helical DNA will fade away, one-by-one. Like the Cheshire Cat, it will leave behind a "grin", the principle of structural complementarity between monomeric subunits.'

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