

THE IMPROBABILITY OF PREBIOTIC NUCLEIC ACID SYNTHESIS

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ABSTRACT

Many accounts of the origin of life assume that the spontaneous synthesis of a self-replicating nucleic acid could take place readily. Serious chemical obstacles exist, however, which make such an event extremely improbable.

Prebiotic syntheses of adenine from HCN, of D,L-ribose from adenosine, and of adenosine from adenine and D-ribose have in fact been demonstrated. However these procedures use pure starting materials, afford poor yields, and are run under conditions which are not compatible with one another.

Any nucleic acid components which were formed on the primitive earth would tend to hydrolyze by a number of pathways. Their polymerization would be inhibited by the presence of vast numbers of related substances which would react preferentially with them.

It appears likely that nucleic acids were not formed by prebiotic routes, but are later products of evolution.

1. THE IMPORTANCE OF NUCLEOSIDE SYNTHESIS TO THEORIES CONCERNING THE ORIGIN OF LIFE

The development of the first molecular system capable of self replication represents a key step in the origin of life, one which is sometimes equated with the origin of life itself.

If such a system could be formed, together with a supply of subunits, and a suitable energy source, mutation and natural selection could take place, and evolution would begin.

This possibility was demonstrated by Sol Spiegelman and his coworkers in the 1960's in an elegant series of studies using RNA derived from the virus QB. (1) Manfred Eigen and his colleagues have elaborated these results, and used them as a basis for a complex theory of the origin of life. (2) Such schemes depend critically upon the spontaneous prebiotic assembly of the first nucleic acid. Very different attitudes appear in the literature, however about the likelihood of such an event. Some writers believe that is well documented:

"Indeed, numerous experiments in the field of primordial organic chemistry demonstrate quite clearly that biological macromolecules (amino acids, energy-rich nucleoside phosphates) could form and polymerize spontaneously to proteins and nucleic acids." (3)

"Here we simply start from the assumption that when self-organization began, all kinds of energy-rich material were ubiquitous, including in particular: amino acids in varying degrees of abundance, nucleotides involving the four bases A,U,G,C, polymers of both preceding classes" (4)

Other workers have expressed a much more pessimistic view, however:

"There have indeed been many interesting and detailed experiments in this area but the importance of this work lies, to my mind, not in demonstrating how nucleotides could have formed on the primitive Earth, but in precisely the opposite: these experiments allow us to see, in much greater detail than would otherwise have been possible, just why pre-vital nucleic acids are highly implausible." (5)

"The origin of nucleosides and nucleotides remains, in our opinion, one of the major problems in prebiotic synthesis." (6)

In attempting to decide between these opposing points of view, we must consider the ease of formation of the natural nucleosides and nucleotides, their stability, and the ability of the monomers to polymerize when surrounded by a host of competing substances.

2. SUMMARY OF PREBIOTIC NUCLEOSIDE SYNTHESIS

The following procedures are typical of the best ones

reported for adenosine synthesis. Prebiotic syntheses of the other natural nucleosides are less well worked out.

2.1 Adenine synthesis

Adenine can be prepared by oligomerization of HCN in concentrated solution (7). Lower concentrations (0.01 M NH_4CN) can be employed if the reaction is frozen at -2° for 2 months. A subsequent hydrolysis in 5 M HCl, at 100° for 18 hours is needed to complete the reaction. The yield is 0.04% (0.02% when catalytic glyconitrile is present) (8). Adenine is also formed by subsequent hydrolysis at pH 8.5 (14) and by a photochemical route which does not require a hydrolysis step (15).

2.2 Ribose synthesis

Formaldehyde is converted to a mixture of normal and branched-chain sugars by alkali. This is called the formose reaction. It can also be carried out by boiling 0.33 M formaldehyde with alumina at neutral pH for 5 hours (9). Sugars were also detected when 0.01 M formaldehyde was used, but no attempt was made to detect ribose (9,12). The highest yield of D,L-ribose reported in a formose procedure is 3.8%.

2.3 Adenosine synthesis

Pure adenine can be converted to adenosine in 2.3% yield by heating to dryness (100° , 2 hours) with a 15-fold excess of pure D-ribose, in the presence of magnesium salts (10).

3. CRITICISM OF PREBIOTIC NUCLEOSIDE SYNTHESIS

No report has appeared of the synthesis of a natural nucleoside from a simulated primitive atmosphere exposed to an energy source, nor has one been reported that started with a simple chemical mixture, such as one-carbon compounds and inorganic reagents. Nucleosides have generally been prepared from the appropriate base and sugar, and the synthesis of the base and sugar have been considered separately, as if they were independent problems. Even so, each of the procedures is seriously flawed.

3.1 Adenine synthesis

The direct condensation of HCN to adenine requires two steps for optimal yield: one in cold base, the other in hot acid. The final yield is very small.

3.2. Ribose synthesis

The formose reaction would be spoiled if amines or cyanide were present, as the formaldehyde would be consumed. The reaction requires concentrated formaldehyde solutions, for appreciable yield of products. The sugars that are formed can decompose quickly. The reaction mixture is complex: "The formose product can be regarded as a carbohydrate analog of petroleum, in that it contains so many carbohydrates of varying molecular weight and isomeric structure" (11).

It has been concluded: "We do not believe that the formose reaction as we and others have carried it out is a plausible model for the prebiotic accumulation of sugars" (12).

3.3 Nucleoside synthesis

The conditions used are very different than those used for adenine and ribose synthesis. Pure adenine and pure D-ribose were used. The yield was small. What yield would be expected if the crude adenine reaction mixture and the formose product were employed? Further, the procedure fails entirely for pyrimidine nucleosides.

4. HYDROLYSIS OF BASES AND NUCLEOSIDES

Bases, nucleosides and nucleotides are unstable in aqueous solution and hydrolyze slowly by a number of pathways. These reactions have been summarized in a review by this author (13). Only a brief overview will be given here.

Glycosyl cleavage is the most important hydrolysis path for deoxyribonucleosides (except those of cytosine). This process is slowest at 7.4, and is catalyzed by acids and bases. Half-lives for purine deoxyribosides are about 25 years, pH 7.4, 37°. The half-life for thymidine is about 150 years, under these conditions. Ribonucleosides are considerably more stable. Adenosine and guanosine have half-lives for glycosyl cleavage of 2500 years, under the above conditions.

Cytosine, and its nucleosides, deaminate to uracil derivatives in aqueous solution, with a half-life of about 50 years (pH 7.4, 37°). This rate is accelerated in the presence of acids, bases, or nucleophilic catalysts. Adenosine undergoes several reactions in alkaline solution, including N-glycosyl cleavage, deamination to inosine, and imidazole ring opening, which ultimately affords 4,5,6-triaminopyridine. The last two reactions have not been studied in neutral solution but probably occur at a slow rate. Deamination of adenine in DNA

takes place in neutral solution, with a rate perhaps 1% that of cytosine.

Adenine resists attack by alkali because of its anionic character ($pK = 9.8$). In neutral or weakly basic solution it is uncharged, and should exhibit the same reactions as adenosine. Guanine and its nucleosides also carry a negative charge in alkaline solution, and resist degradation. They may undergo deamination and ring opening reactions in weakly basic solution, but this has not been explored. The most stable nucleoside is uridine. It decomposes, with a half-life of perhaps 3,500 years, by glycosyl and ring cleavage reactions.

The slow instability of bases and nucleosides puts additional constraints upon any plausible prebiotic synthesis of nucleic acids, as no slow build-up in concentration of monomers over geological periods of time would be expected to occur.

5. COULD THE NATURAL NUCLEOTIDES FIND ONE ANOTHER?

The rate of synthesis of an oligonucleotide of length n would increase with the n^{th} power of the monomer concentration, and fall off to a comparable extent as the number of other reactive hydroxyl-bearing components of the prebiotic mixture increased. Natural ribonucleosides, for example, would be swamped by many closely related, incorrect ones. Consider adenosine analogs, as an illustration:

Adenine can react at positions other than N-9. There are two other monoaminopurines, three diaminopurines, and one triaminopurine. Eight straight chain aldopentoses exist, and sixteen aldohexoses; each of the 24 has 4 different ring forms. By selecting one sugar and one base from the above, we can generate 2,640 nucleosides. Much larger numbers would be obtained by including branched chain sugars, amino sugars, ketoses, reduced sugars, as well as ketopurines, methylpurines, and derivatives of hundreds of other heterocyclic systems.

Many other substances, capable of polymerization, would be present in the prebiotic soup: alcohols, hydroxyethers, aminoalcohols, hydroxyacids, cyanohydrins, etc. (Chemical Abstracts listed 6 million organic compounds in the period, 1965-1983.) Nucleosides would react with them in preference to combining with one another, precluding the formation of an RNA chain.

6. CONCLUSION

Consideration of the existing chemistry of nucleic acid components leads to the conclusion that the spontaneous formation of even a short nucleic acid chain under prebiotic conditions was a highly improbable event. It is likely that nucleic acids were first assembled with the aid of enzymes, and are later products of biochemical evolution. This conclusion would be reversed only if much more effective routes for the prebiotic synthesis and polymerization of nucleotides were discovered.

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