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The case for an ancestral genetic system involving simple analogues of the nucleotides

(chemical evolution/origin of life/prebiotic chemistry/RNA catalysis)

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ABSTRACT **The idea that the first living systems on earth were based on self-replicating RNA molecules has recently become popular as a result of the discovery of ribozymes. However, there are several major problems associated with the prebiotic synthesis of ribonucleotides. In addition, there is the newly recognized problem of enantiomeric cross-inhibition, whereby template-directed polymerization involving one enantiomer of RNA is inhibited strongly by the presence of the other enantiomer. Here we propose that RNA was preceded in the evolution of life by a polymer constructed from flexible, acyclic, probably prochiral nucleotide analogues that were synthesized readily on the primitive earth. Several potentially prebiotic nucleotide analogues are considered in this context, and some of the consequences of this proposal are discussed.**

The possibility that certain RNA sequences might have catalytic activity and that very primitive living organisms may have contained catalytic RNA molecules rather than protein enzymes was first discussed extensively about 20 years ago (1–5). The unanticipated discovery of contemporary catalytically active RNA molecules derived from RNase P and from group I intervening sequences (6, 7) has made this idea increasingly popular (8–13). We wish to emphasize that there are several reasons for doubting that RNA itself was the first genetic material, even if it turns out that an RNA-only organism preceded organisms based on nucleic acids and proteins.

It has often been pointed out that the accumulation of substantial quantities of relatively pure mononucleotides on the primitive earth is highly implausible. First, the only prebiotic synthesis of ribose, the Butlerow reaction, yields a complex mixture of sugars; ribose never constitutes a major fraction of the products (14–16). Furthermore, ribose and the other sugars decompose rapidly on a geological time scale, with half-lives that are probably not more than a few hundred years at 0°C and pH 8 (17–19). Second, the known prebiotic syntheses of purine nucleosides by heating purines and ribose are inefficient, yielding at most a few percent of the β-ribosides, along with many isomeric nucleoside analogues (20, 21). The same reaction with pyrimidines and ribose does not give any detectable pyrimidine nucleosides (<0.1%).

It is possible that some efficient prebiotic synthesis of the β-ribosides, or some method of separating the β-ribosides from closely related isomers, will be discovered, but there is no basis in organic chemistry for optimism. It has become clear recently that the problem presented by the optical activity of the nucleosides is even more severe. There is no plausible mechanism for the enrichment of one of the optical isomers of a mononucleoside that could lead to an overall enantiomeric excess of even 1% (22–25). Since the template-directed polymerization of one enantiomer is likely to be inhibited strongly by the presence of the other (enantiomeric cross-inhibition), it is hard to see how replication could get started, even from a racemic solution of pure β-DL-nucleosides.

Enantiomeric Cross-Inhibition

Poly(D-C)-directed oligomerization of the D enantiomer of guanosine 5′-phospho-2-methylimidazole (2-MeImpG) is a very efficient reaction. The major products are the all 3′,5′-linked oligo(G)s ranging in length from the dimer to about the 30-mer (26). Poly(D-C)-directed oligomerization of racemic 2-MeImpG is far less efficient, resulting in a more complicated mixture of products up to about the 8-mer (27). Template-directed synthesis in this system is known to proceed in the 5′→3′ direction (28). L-2-MeImpG is incorporated much less readily than D-2-MeImpG, but once incorporated, the L isomer acts as a chain terminator. Presumably the L-guanosine residue distorts the helical structure at the 3′ end and terminates chain growth by preventing further D-guanosine incorporation (27).

The phenomenon of enantiomeric cross-inhibition has also been demonstrated in a system involving poly(D-C)-directed oligomerization of guanosine 5′-phosphorimidazole (ImpG) in the presence of Zn^{2+}. In this case the inhibitory effect of the L-guanosine residue is even more pronounced (27). Addition of activated D-adenosine, D-cytidine, or D-uridine mononucleotides to the reaction mixture does not inhibit oligo(D-G) synthesis in either system (28). This shows that the incorporation of L-guanosine derivatives depends on complementary pairing to the poly(D-C) template.

The systematic inhibition by L-guanosine residues can be understood in terms of the stereochemistry of mononucleotides when bound to a complementary template. Guanosine mononucleotide binds to poly(C) by forming a Watson–Crick base pair (29). The orientation of the sugar-phosphate component of the monomer relative to the template is determined by the handedness of the ribose sugar (D vs. L) and the rotational conformation about the glycosidic bond (*syn* vs. *anti*). It has been postulated that enantiomeric cross-inhibition occurs because monomer addition to the 2′ (3′) terminus of a template-bound oligo(D-G) can involve either the *anti* D isomer or the *syn* L isomer (27). Model building suggests that, when bound to a poly(D-C) template, these two molecules adopt sufficiently similar orientations to allow the occasional misincorporation of an L-guanosine residue in a reaction designed to optimize incorporation of D-guanosine (Fig. 1).

It should be noted that the reactions described above are probably not historically relevant to the origins of life on the earth; they are intended to serve as general chemical models of an RNA-dependent RNA polymerase. However, these results suggest that enantiomeric cross-inhibition is a prob-

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Abbreviations: ImpG, guanosine 5′-phosphorimidazole; 2-MeImpG, guanosine 5′-phospho-2-methylimidazole.

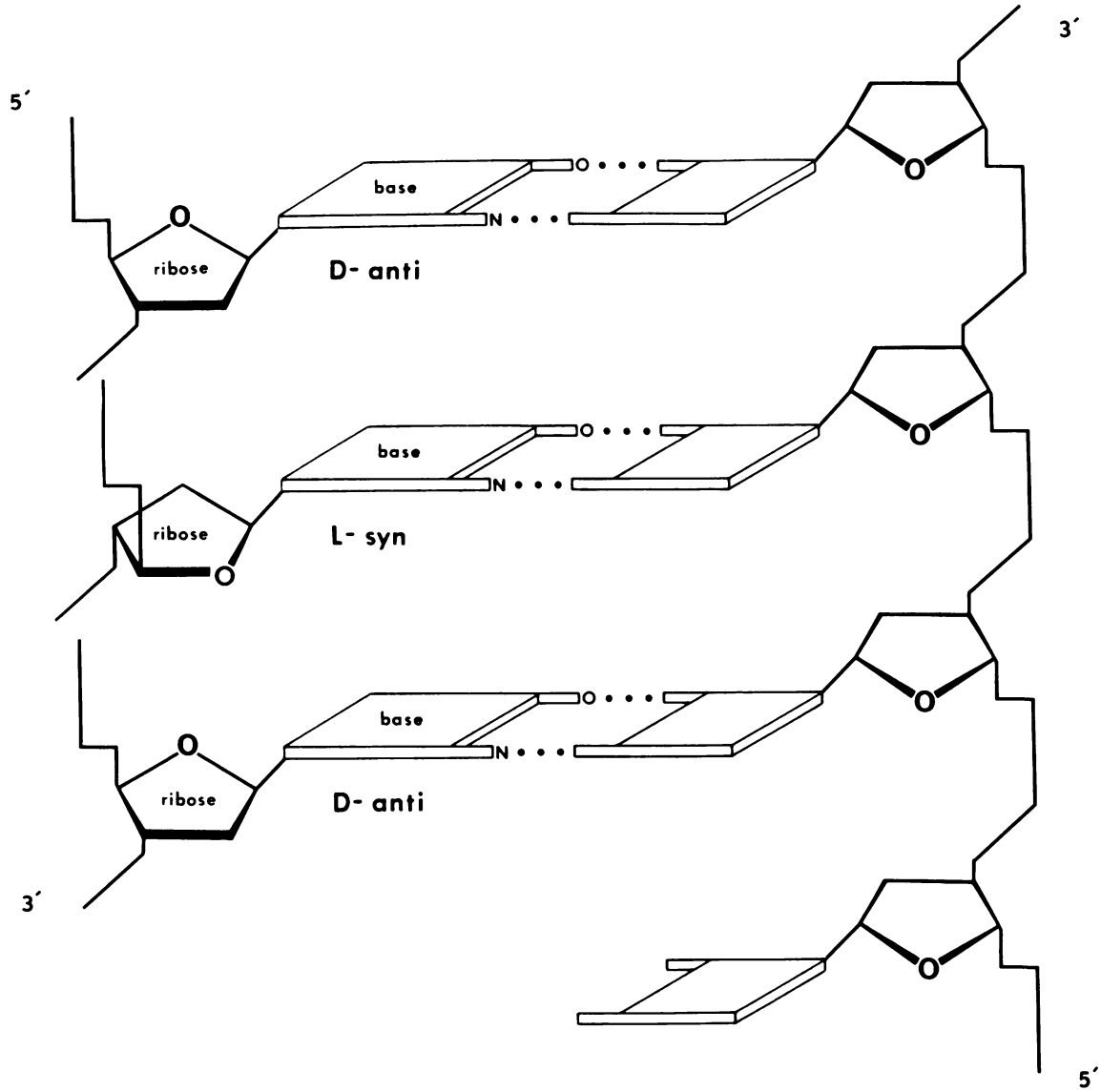

FIG. 1. Diagrammatic representation of *anti*-D- and *syn*-L-guanosine mononucleotide bound to a poly(D-C) template. The orientation of a template-bound monomer is fixed by Watson–Crick base pairing and by base-stacking interactions. The position of the sugar-phosphate component of the monomer relative to the template is determined by the handedness of the ribose sugar (D vs. L) and by the rotational conformation about the glycosidic bond (*syn* vs. *anti*). The D isomer in the *anti* conformation and the L isomer in the *syn* conformation are oriented antiparallel to the poly(D-C) template. The 5′ phosphate of *syn*-L-guanosine mononucleotide lies in close proximity to the 3′ hydroxyl of an adjacent *anti*-D-guanosine residue.

lem that must be addressed by any theory of the origins of life based on the replication of oligonucleotides.

Since only two systems were investigated, we cannot exclude the possibility that there may be some other system of activated nucleotides in which the *anti* D isomer and the *syn* L isomer adopt sufficiently different conformations that incorporation of the "wrong" enantiomer is minimized. On the basis of the similarity in stereochemistry between the *anti* D and *syn* L conformations of the nucleosides, this does not seem very likely, although it may be possible. Even then, incorporation of the correct enantiomer would enrich the incorrect enantiomer in a small pool of nucleotides making discrimination progressively more difficult. Another possible solution to the problem of enantiomeric cross-inhibition is to postulate a system in which the two enantiomers of the riboside are coincorporated, but the "wrong" enantiomer produces only minimal distortion of the helical structure so that chain termination does not occur. This calls for a very close structural homology between the two conformations of each mononucleoside involved in the reaction system and again seems improbable given the constraint of the rigid furanose ring. However, as we shall see, it is not improbable if the ring is replaced by an acyclic analogue.

Advantages of Flexible Nucleotide Analogues

Here we propose a different solution that minimizes all of the problems discussed above. We suggest that RNA was preceded in the evolution of life by a polymer of simple, flexible, possibly prochiral nucleotide analogues that were synthesized readily on the primitive earth and that polymerized rapidly. Replication of the polymer occurred by complementary synthesis based on Watson–Crick hydrogen-bonding. Several potentially prebiotic nucleotide analogues that might be considered in this context are shown in Fig. 2.

In principle, the glycerol-derived acyclonucleosides (compound **II**) can be derived from glycerol, formaldehyde, and a base. Condensation of glycerol with formaldehyde yields a mixture of hemiacetals and cyclic acetals. Heating such a mixture with one of the four bases should yield the

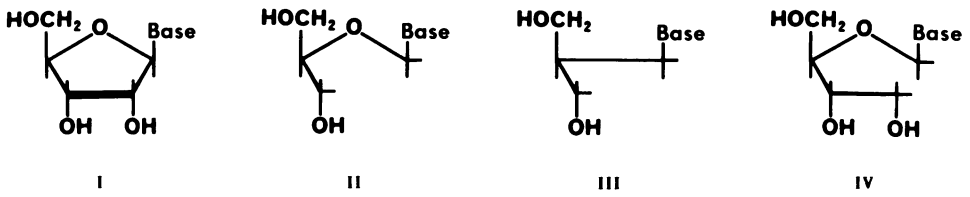

FIG. 2. Comparative structure of a nucleoside and of three acyclic nucleoside analogues. Compounds: **I**, nucleoside; **II**, glycerol-derived acyclonucleoside; **III**, acrolein-derived nucleoside analogue; **IV**, erythritol-derived nucleoside.

acyclonucleoside together with the isomeric 1-substituted glycerol compounds. The acrolein-derived nucleoside analogues (compound **III**) should be obtainable from acrolein, formaldehyde, and the bases or from acetaldehyde, formaldehyde, and the bases. The bases are claimed to react readily with acrolein to give 3-substituted proprionaldehydes (30). In the presence of formaldehyde and a reducing agent, compound **III** should be formed. Standard methods of prebiotic phosphorylation would convert any of these analogues to a mixture of mono- and diphosphates.

The schemes described above seem more plausible than the proposed prebiotic syntheses of ribonucleosides. Glycerol is much more stable than ribose and could have accumulated in relatively large amounts. It could have condensed with the bases without the coproduction of large amounts of competing isomers. Acrolein is a major product in electric-discharge experiments (31) and can be synthesized from acetaldehyde and formaldehyde (32). It remains to be determined whether acrolein would react preferentially with purines and pyrimidines rather than with competing nucleophiles. There are many compounds related to compounds **II** and **III** that have optically active carbons but are effectively prochiral in the present context. For example, the erythritol nucleoside (compound **IV** in Fig. 2) is very flexible, and the extra CH_2OH group that it contains might not interfere with duplex stability or with the polymerization reactions.

It is probably an oversimplification to suppose that the first genetic material had a completely uniform backbone. We leave open the possibility that families of related nucleotide analogues copolymerized. It is known, for example, that nucleoside 5′-phosphates copolymerize readily with deoxynucleoside 3′,5′-diphosphates (33).

The efficiency with which monomer units containing an activated phosphomonoester group oligomerize in an aqueous environment depends on two factors: the intrinsic reactivity of the nucleophile and the detailed orientation of the nucleophile relative to the activated phosphate. The latter topic will be taken up in detail in the next section, in the context of template-directed synthesis. Here we emphasize that, in general, activated phosphate groups react much more efficiently with phosphomonoesters to give pyrophosphates than with alcohols to give phosphodiesters. For example, activated nucleoside 3′,5′-diphosphates condense efficiently in aqueous solution (33), while activated nucleoside 5′-phosphates do not. It should also be noted that activated monophosphates of the acyclonucleosides (compound **II**) cyclize rapidly, thus preventing their oligomerization. The same would probably be true for any acyclic monophosphate that could undergo ring closure to form a five- or six-membered ring. For these reasons, the diphosphate derivatives seem to be more plausible prebiotic monomers than the monophosphates.

In addition to the difficulties associated with enantiomeric cross-inhibition in racemic systems, template-directed synthesis even in an optically homogeneous system faces two major problems. First, the reaction between the 3′ OH group of a growing oligonucleotide chain and the activated 5′ phosphate of the incoming nucleotide is extremely sensitive to the orientation of the activated phosphate (26). Therefore, an activated phosphate that leads to the efficient condensation of a particular mononucleotide with a particular terminal nucleotide is unlikely to act as efficiently for some other combination of incoming monomer and terminal acceptor. It may prove difficult or impossible to find a "consensus" activating group for phosphate that permits replication of a wide variety of oligonucleotide sequences. Second, inter- and intramolecular template self-structure is a serious problem for those templates that have a high degree of self-complementarity. In the next section we will discuss the ways in which use of an acyclic derivative might help to overcome these problems.

Tacticity of Acyclopolynucleotides

The use of an achiral monomer eliminates the problem of enantiomeric cross-inhibition, but it raises a completely equivalent problem related to the tacticity of the newly synthesized oligomer chain. When the prochiral acyclonucleoside derivative is incorporated into a nucleic acid-like oligomer, the central carbon atom of each monomeric unit becomes chiral. Therefore, each monomeric unit can adopt either a D-like or L-like conformation in the chain. The formation of a syntactic oligomer (one in which all subunits have the same handedness) is equivalent to the formation of an optically homogeneous oligonucleotide. Regions of atacticity in an acyclonucleoside oligomer (where both D-like and L-like subunits are present) are equivalent to regions of optical heterogeneity in an oligonucleotide.

Oligomers of acyclic derivatives are likely to be able to replicate even if they contain a few "atactic" residues, not because the monomers are achiral, but because they are sufficiently flexible (34). We have suggested (Fig. 1) that D-nucleotides in the *anti* conformation and L-nucleotides in the *syn* conformation have similar three-dimensional structures. This similarity is even more striking when we compare the conformations of the two enantiomers of a glycerol-derived acyclonucleotide or acyclonucleoside 1,3-diphosphate incorporated in an oligomer chain. Model building using Corey–Pauling–Kolton models suggests that the two conformations are so similar that they can be interchanged without producing any significant distortion of the chain. It also may be possible to interchange conformers of the acyclic compounds that differ with respect to the rotation about the C_1^1 carbon in a way that is analogous to the difference between the α and β conformations of a ribonucleotide (35).

Energy minimization calculations by M. Hirshberg and M. Levitt (personal communication) strongly suggest that oligomers of the glycerol-derived acyclonucleoside monophosphates form a double helix very similar to the RNA double helix and that the energy of interaction between pairs of complementary oligomers is only slightly decreased if a single *syn* L-like residue is incorporated into a syntactic *anti* D-like chain. It must be emphasized that this may be equally true for a flexible, chiral molecule such as the erythritol nucleoside (compound **IV**). It is the flexibility of acyclic monomers that permits *syn* L-like and *anti* D-like residues to adopt virtually identical conformations and so permits elongation to contin-

ue irrespective of which conformer occupies the terminal position in the growing chain. It is quite unexpected that *syn* L residues in an otherwise syntactic *anti* D chain do not greatly decrease the stability of the double helix. We would be surprised if the low energy cost of substitution of one enantiomer for the other in polymers of this kind did not have some significance for prebiotic evolution.

The glycerol-derived acyclonucleoside diphosphates and the deoxynucleoside 3′,5′-diphosphates are the only compounds for which relevant experimental evidence is available (33). Template-directed reactions of these compounds, as anticipated, seem to be much less sensitive to orientation than do the corresponding reactions of the nucleoside 5′-phosphates. The three compounds investigated, the diimidazolides of adenosine 3′,5′-diphosphate and guanosine 3′,5′-diphosphate and the glycerol-derived acycloguanosine 1′,3′-diphosphate, all undergo efficient condensation on complementary homopolynucleotide templates. Detailed HPLC analysis suggests that the polymers formed from deoxyguanosine 3′,5′-diphosphates contain 3′,3′ and 5′,5′ linkages in addition to 3′,5′ linkages. This implies that partially atactic polymers of the more flexible acycloguanosine derivative could also form readily. While the synthesis of pyrophosphate-linked oligomers on pyrophosphate-linked templates has not been attempted, analogy with known reactions suggests that it would proceed efficiently and that partially atactic polymers would be able to support template-directed synthesis.

Energy-minimization calculations suggest that the use of a glycerol-derived acyclonucleoside in place of a ribonucleoside results in a slightly more favorable enthalpy (about 1 kcal) of double-helix formation. However, the entropy cost for incorporation of an acyclonucleoside into a double helix must be larger than the corresponding cost for a ribonucleoside, since 2 additional rotational degrees of freedom are lost in the former case. The maximum cost, corresponding to 2 degrees of rotational freedom for each residue, is therefore about $2RT$ (1.2 kcal) for each base pair. The net result is likely to be a slight destabilization of double-helical RNA or DNA, which would not be large enough to lead to the dissociation of the double-chain structures under appropriate conditions of temperature, pH, and ionic strength.

We note that destabilization of the double-helical structure might be an advantage rather than a disadvantage from the point of view of replication, provided it is achieved by modifying the backbone, and not by decreasing the strength of hydrogen-bonding or the extent of base stacking. The incoming monomer would then be held as tightly on the template as in a standard double helix, so that chain extension need not be adversely affected. On the other hand, internal self-structure due to short, complementary sequences in the template would be greatly weakened. Thus, one might expect that chain growth would be efficient and yet would not be strongly inhibited by self-structure.

Helical Properties of Acyclopolynucleotides

Helical structures based on achiral or prochiral monomers have special properties with respect to chirality. Consider a linear syntactic homopolymer joined by phosphodiester linkages and having hydroxyls at both ends. The 2′ carbon of each glycerol subunit is a chiral center (although in syntactic chains containing an odd number of subunits, the 2′ carbon of the middle subunit is achiral). Yet the polymer as a whole is a *meso* structure and, therefore, is not optically active. Syntactic polymers containing more than one base are optically active, unless their base sequence is a palindrome (of the form abcd...dcba). When a homopolymer is phosphorylated at one end, the plane of symmetry is lost, and the molecule becomes optically active.

To add a twist to the problem, consider a symmetrical syntactic homopolymer with either a phosphate or a hydroxyl group at each end. Such a polymer can adopt completely equivalent left- and right-handed helical conformations because it contains a mirror plane perpendicular to the long axis of the molecule. A symmetrical double helix, say of syntactic poly(acyclo-C) with syntactic poly(acyclo-G), would adopt left- or right-handed helical conformations with equal probability. However, once the symmetry of one (or both) strands is destroyed (for example, by the addition of a single phosphate group or by substituting a heterobase in the sequence), the energies of chains adopting the two different helical twists must be slightly different so that one helical direction is preferred. The energy effect is likely to be small in most cases so that domains of both right-handed and left-handed helical structure could occur within the same molecule.

Transition to an RNA Genome

In summary, there are several reasons to believe that life on earth did not originate with self-replicating RNA molecules. In addition to the problems associated with the prebiotic synthesis of β-ribosides, there is the newly recognized problem of enantiomeric cross-inhibition. We believe that polymers constructed from flexible, acyclic, prochiral analogues such as the acyclonucleosides, or even polymers of flexible chiral analogues offer a more plausible alternative. We envision at least three phases in the evolution of the genetic material, beginning with an acyclic system, then making a transition to RNA, and finally settling on DNA. It is possible that even simpler systems preceded that, which involved prochiral nucleoside analogues (36).

If RNA is such an unlikely candidate for use as the first genetic material, then why should it be any more suitable at a later stage in evolution? Presumably, many of the problems associated with the replication of RNA were solved by development of appropriate catalytic function in an ancestral genetic polymer. For example, a chiral polymer constructed from flexible monomers may have catalyzed the preferential synthesis of D-ribosides, thereby eliminating the problem of enantiomeric cross-inhibition. In order for the transition from a prochiral system to an RNA system to have been a favorable event, the new system must have provided all the essential functions of the old one and offered an overall selective advantage as well. The relative inflexibility of the furanose ring, the presence of the 2′ OH groups along the chain, and the enhanced nucleophilicity of the 2′(3′)-*cis*-glycol endow polyribonucleotides with characteristic chemical properties that were not possessed by their ancestors. Apparently the physical and chemical properties of RNA were advantageous for its primitive role as information carrier and catalyst and eventually allowed it to usurp the function of some precursor genetic material during the early history of life on earth.

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