Plural Origins of the Molecular Homochirality in Our Biota

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We describe results of computer simulation calculations of mixed and homochiral oligoribotides showing that it is possible to bind L(D)-monomers at the terminal positions of chains built from D(L)-ribotides, but insertion at internal positions produces a large instability of the chains. Coupled with a proposed new mechanism for the growth of these linear polymers in prebiotic conditions, our results imply that enantiomeric cross-inhibition could have been bypassed to make all-D(L) oligoribotides dominant. We also show that racemic amino acids can form stable peptide β-strands, which rules out a similar origin for modern L-amino acids. Alternative schemes are proposed to account for the homochiralities of the amino acids and of other biomolecules.

Introduction

The bifurcation mechanism proposed for the origin and amplification of chiral polarity from racemic solutions (Frank, 1953; Fajszi and Czégé, 1981), experimentally confirmed in cases of growth of tridimensional structures (Kondepudi and Kaufman, 1990; Kondepudi and Sabanayagam, 1994; Epstein, 1995), has been challenged as inadequate in the case of self-replicating one-dimensional polymers in solution by the discovery of enantiomeric cross-inhibition (Joyce *et al.*, 1984; Joyce *et al.*, 1987). In this paper we describe results relevant to this problem, obtained by simulation techniques on the stability of *mixed* and *homochiral* oligoribotides and peptides. We have confirmed that ribotides of a given chirality can act as chain terminators to the growth of oligomers built from ribotides of the opposite chirality, but we have found besides that L(D)-ribotides cannot occupy internal positions of an all-D(L) oligoribotide strands without producing great instability in the chains. As a consequence, mixed oligoribotides could not grow if the template assisted mechanism, in response to environmental stresses, changed from the $(n - 1) + 1 \rightarrow n$ kinetics assumed by Joyce *et al.*, (1987) (n being the number of bases) to condensation reactions between diribotides or larger fragments. Homochiral oligomers on the other hand would not be affected by this constrain and would become the dominant molecular species. We also show that racemic amino acids can form stable peptide chains in the β-strand configuration, and, therefore, peptides could not act as chiral amplificators. We conclude that the homochiralities of ribotides, of amino acids, and of other biomolecules originated by differing mechanisms.

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Homochirality of the Ribotides

In their studies of poly (C_D)-assisted oligomerization of guanosine derivatives, Joyce *et al.* (1984) observed that the rate of growth of D-oligoribotide analogues is greatly .reduced by the presence of monomers of opposite chirality. They interpreted this phenomenon by assuming that the chains are built by the step-wise condensation of monomers at the 3' and/or 5' ends of the growing oligomers, in which case ribotides of opposite chirality act as chain terminators. This problem apparently does not arise in the case of three-dimensional structures such as crystals (Kondepudi and Kaufman, 1990), but it is important in the case of one-dimensional polymers.

Using the AMBER program (Weiner *et al.*, 1984, 1986), we have obtained pertinent results on the stability of homochiral and mixed oligoribotides (Lins *et al.*, 1994; Soares *et al.*, 1995). Confirming solid-model studies of Wald (1957) and

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Gol'danskii *et al.* (1984) on double-stranded DNA, we found that single-stranded homochiral oligoribotides are considerably more stable than mixed ones, although the excess energies of the latter decrease substantially if the D → L substitutions occur in the terminal 3' or 5' positions (Table I). We have also simulated the template-assisted growth of homochiral and mixed RNA and DNA duplexes in order to analyze the stability of the complementary interactions. As shown in Table I, although L-nucleotides can pair with all-D templates, their binding with adjacent D-nucleotides is weakened by 12 to 16 KJ·mol^{-1} of base pairs for middle positions in the chain, although terminal positions destabilize less the duplexes.

Table I. Excess energies of oligonucleotide chains[a].

Single chains with L-ribotides[b]	
L-ribotide position	$\Delta E_{average}$ (kJ mol^{-1})[c]
5'-end	6.3 ± 0.4
3'-end	18.9 ± 2.1
Internal	63.0 ± 8.4
Duplex chains[d]	
L-nucleotide in internal position	$\Delta E_{average}$ (kJ mol^{-1}) per base pair[c]
L-ribotide	13.4 ± 1.7
L-deoxyribotide	14.3 ± 2.9

[a] Numerical values obtained by molecular mechanics calculations using the AMBER force field including the solvent effects (water). The AMBER force field describes the potential energy function as (Weiner *et al.*, 1984, 1986):

$$E_{total} = \sum_{bonds} K_R(R-R_0)^2 + \sum_{angles} K_\theta(\theta-\theta_0)^2 +$$

$$\sum_{diedrals} \frac{V_n}{2}[1+\cos(n\varphi-\gamma)] +$$

$$\sum_{i>j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\varepsilon R_{ij}}\right] + \sum_{H\text{-}bonds}\left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right]$$

where the first term on the right-hand side measures the bonded atom interactions, the second term is a harmonic oscillator approximation for the bending of bond angles, the third term represents the torsion potential, the fourth is the non-bonded interactions and the last one describes the energy of possible hydrogen bonds. The Molecular Dynamics (MD) method has also been used in our calculations to allow for temperature variation.

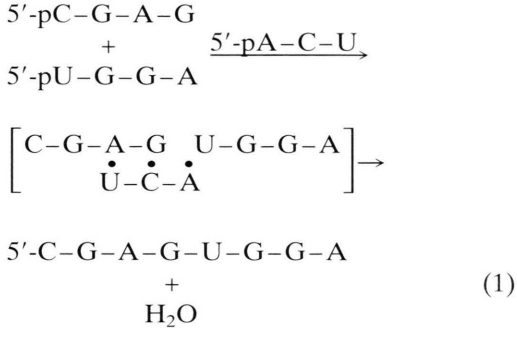
[b] Oligoribotide chains with L-ribotides substituting for the normal D-ribotides in the indicated position. The energy value shown for the internal position is the average of values calculated for the tetra-, penta-, and hexamers.

[c] Excess energy or relative energy compared to the normal oligoribotide (all-D-ribotides). $\Delta E_{average}$ is the average of the energy differences for the various lengths of the oligoribotide chains (tetra-, penta-, and hexamers).

[d] One of the chains is an all-D strand and the complementary chain contains one L-ribotide in the internal position, that is, other than the terminal (5' or 3' ends) position. The duplexes are made of three base-pairs.

These results are in agreement with Joyce *et al.*'s observation (1984) that in template directed reactions with a racemic mixture, monomers of the opposite handeness to the template are incorporated as terminators at the 3'(2') and 5' ends of the chains. However, our results depart from their interpretation (1987) that the binding of an L-ribotide (or L-deoxyribotide) is possible because L-*syn* isomers mimic D-*anti* isomers. We found instead that the torsional angle χ that minimizes the energies is situated between -104° and -150°, which corresponds to an *anti(clinal)* conformation.

Enantiomeric inhibition precludes the formation of long ribotide chains if the mechanism of chain growth is of the type $(n-1)+1 \rightarrow n$ (n = number of bases). It is possible, however that this mechanism is not the only one for the growth of unidimensional polymers, and that sometime during the prebiotic era, after small ribotides, both monochiral and mixed, were formed by that mechanism, further growth could occur through a different kinetic pathway, such as (Ferreira and Coutinho, 1993):



(1)

The change from a step-wise adddition of the one base to a fragment of size $(n+1)$, that is, a $(n-1)+1 \rightarrow n$ kinetics to our small *ribotide* fragments *[$m + q \rightarrow n$; $m > 1$, $q > 1$]* could have been produced by environmental factors, for example, by the inflow of ethyleneglycol or DMSO to produce a diluted-water reaction medium (De Meis, 1989), or of less common cations such as Mn^{2+} and

Zn^{2+}. It is reasonable to assume that in the presence of these factors both kinetic schemes could have coexisted and, gradually, as the concentration of oligomers in solution increased, pathway (1) came to be predominant in the case of all-D (or all-L) oligomers. However the process would be a very slow pathway for mixed fragments, even for those containing L-ribotides in the 3' and/or 5' positions, because the condensation products (such as 5'pCGAGUGGA) would contain L-enantiomers in unstable internal positions. Hence mixed oligoribotides of larger sizes would be kinetically inaccessible, and only chirally pure oligomers would grow.

Homochirality of Amino Acids

The occurrence of such processes in the prebiotic Earth must have been a very rare event. This difficulty becomes compounded if the same mechanism was also necessary for the amplification of the chiral polarity in other kinds of biopolymers. We have found, however, that it is unlikely that the homochirality of the amino acid residues has been achieved by such primary processes.

According to Wald (1957), protein α-chains would behave in an identical manner to polyribotide chains. Using the AMBER force field, we have done calculations involving segments of α-helices and β-strands (Soares *et al.*, 1995). As shown on Table II the instability produced by the insertion of D-residues on right-handed α-helices built from L-amino acids may be as high as 5 Kcal. mol^{-1}. However, for β-peptide strands there is no difference in the energies of the homochiral and mixed chains; hence they could not act as chiral amplificators. From the view-point of the "RNA World" (Gilbert, 1986; Westheimer, 1986; Zaug and Cech, 1986) simple peptides are more important than proteins at this early stage of evolution. Indeed, proteins must have been anticipated by translation, that is by tRNAs or some ancestors, and possibly by multiple rRNA structures, capable to catalyze peptide-bond formation (Noller *et al.*, 1992; Piccirilli *et al.* 1992). For these reasons the result shown for β-strands is more relevant than those for α-helices or other compact peptide structures present in proteins (Spach and Brack, 1979), and the homochirality of amino acids could not have been originated by a process similar to that responsible for the monochirality found in the nucleic acids.

Table II. Excess energies of mixed and homochiral peptide chains[a].

D-amino acid in peptide chains	$\Delta E_{average}$ (kJ mol^{-1})[b]
β-strand (0–50%)*	0.0
α-helix with 10%*	4.2 ± 3.4
α-helix with 20%*	8.0 ± 4.6
α-helix with 30%*	13.9 ± 4.6
α-helix with 40%*	17.6 ± 4.6
α-helix with 50%*	21.8 ± 3.4

[a] In all cases the amino acid was alanine. Numerical values obtained by molecular mechanics calculations using the AMBER force field. The parameters were the same used in the ribotide chain calculations (see Table I).
[b] Excess energy above the peptides built of only L-amino acids (all-L situation). $\Delta E_{average}$ is the average of the E (energy) differences (see Table I).
* The D-amino acid percentual correspond to percentual of D-amino acids in the polyalanine with 10 residues. For example, 10% of D-residues in peptide chain is the same as one D-residue in our polyalanine, 20%, 2 D-residues, 30%, 3 D-residues, etc. All possible positions was calculated to each percentile of the D-residue, for example: to 10% of the D-residue in the peptide chain (β-strand or α-helix), 1 L-alanine in the chain was replaced by one D-alanine in first, second, third, ..., and tenth position; for 20% two L-alanines was replaced by two D-alanines in positions 1 and 2, 2 and 3, ..., 9 and 10; 1 and 3, 2 and 4, ...,8 and 10; etc.

An alternative proposal (Ferreira, 1995) is that the homochirality of modern amino acids arose from stereochemical requirements of the peptide-oligoribotide interactions which must have followed the primitive RNA World. In our scheme the first increase in complexity of the RNA world was the result of interactions between the growing oligoribotides and the randomly synthesized peptides. These peptides, selected on the basis of specific interactions with homochiral ribotide sequences, were themselves homochiral. They were, in fact, the ancestors of ligase-like enzymes, that is, catalysts which increased the rates of condensation reactions of the type shown in reaction (1).

Homochirality of other Biomolecules

Milton *et al.* (1992) have recently prepared by total chemical synthesis the D and L forms of a protein, the HIV-1 protease. The enzyme enantiomers showed reciprocal chiral specificity on peptide substrates, and enantiomeric cross-inhibition was clearly shown. This discovery is a very defin-

itive proof of the stereochemical dependence of most biomolecules with respect to enzyme chirality.

Ribozymes, the simplest example known of molecules which are simultaneously genotype and phenotype were succeeded by assemblies of polyribotides and peptides. The further course of evolution involved compartimentalization (membranes made of phospholipids and proteins) and energy producing metabolites. These "new" biomolecules must have been selected by the stereochemical requirements of he primitive enzymes. In the same way, the homochirality of other biological compounds such as co-enzymes, hormones, neurotransmitters, etc., necessary to multicellular organisms, were also selected by the stereochemistry of the primitive enzyme systems.

The Case For a Terrestrial Origin the Homochirality in our Biota

Bifurcation theories of the origin of molecular homochirality require a beginning stage with a small chiral imbalance (Frank, 1953). This initial polarity, that is, a non-zero value for the difference between the numbers n of D and L isomers divided by its seem, $[(n_D-n_L)/(n_D+n_L) \neq 0]_{t=0}$ can be due to statistical fluctuations, or to the action of the electroweak force (Mason, 1982; Mason and Tranter, 1984; Tranter *et al.*, 1992). The violation of parity by the weak interaction ensures that enantiomeric pairs have different energies. Because the weak interaction supplies a constant chiral bias, its role in the origin of homochirality has gained acceptance in recent years. The energy bias is, however, very small compared with the average thermal energy of the biosphere. Therefore the idea has been proposed that chiral polarities originated either in very cold regions of space or in places, such as in the vicinity of neutron stars, with high intensity circularly-polarized ultraviolet radiation, and that the monochiral molecules were transported to the Earth (Rubenstein *et al.*, 1983; Gol'danskii and Kuz'min, 1989; Bonner, 1995). A non-terrestrial *scenario* for the origin of molecular homochirality encounters serious difficulties. Chiral amino acids racemize in solution with half-lifes of the order of 10^4-10^5 years (Bada *et al.*, 1973, 1974). In the primitive oceans these alien chiral molecules would undergo racemization in a time interval that it is too short compared with the 5×10^5 year old "window" which supposedly was the time necessary for the simplest self-replicating systems to arise about 3.7×10^9 years ago.

In the last ten years the great obstacle for the full acceptance of a terrestrial origin of homochirality has been the incapacity of experimentalists to avoid the phenomenon of enantiomeric cross-inhibition. We have now indicated that the accepted mechanism for the growth of oligoribotides, undoubtedly correct for the experimental conditions described by Joyce *et al.* (1984, 1987), could have changed during the prebiotic era to another mechanism which allows this difficulty to be bypassed, with the growth of monochiral polymers. Since our results are independent of the nature of the process responsible for the initial enantiomeric polarity, and are compatible with the view favoring statistical fluctuations in racemic solutions, we see no reason to abandon a terrestrial *scenario.*

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