Plural Origins of Molecular Homochirality in Our Biota
Part II. The Relative Stabilities of Homochiral and Mixed Oligoribotides and Peptides

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By computer simulations – molecular mechanics and molecular dynamics with the amber force field (Weiner *et al.*, (1986), J. Comp. Chem. **7**, 230–252) – we have determined the stabilities of oligoribotide strands built with D- and L-riboses, and of peptide chains with D- and L-amino acid residues. In particular, complementary double-chains of oligoribotides were studied, since they are an important feature of the growing mechanism of modern nucleic acids. Peptide chains on the other hand, grow without need of a template. We found that mixed oligoribotides are less stable than homochiral ones, and that this chiral effect is less noticeable in peptide chains. The results support the interpretation that L-riboses act as terminators to the template-assisted growth of oligo-r-G_D (enantiomeric cross-inhibition; Joyce *et al.*, (1987), Proc. Natl. Acad. Sci. USA **84**, 4398–4402). Based on this effect, a chemical pathway is proposed which could, under assumed prebiotic conditions, bypass the hindrance of homochiral growth.

Introduction

The amplification of molecular disymmetry by autocatalytic processes plays a central role in the theories of the origin of molecular homochirality in our biota, which are fundamental to our understanding of biogenesis itself (Frank, 1953; Wald, 1957; Bonner *et al.*, 1981; Fajszi and Czege, 1981; Kondepudi *et al.*, 1990). The emphasis on chiral **amplification** indicates that a complete solution of the problem does not depend solely on an initial chiral polarity, due either to statistical fluctuations or to a preferential disymmetric synthesis or decomposition of monomeric species caused by nuclear neutral currents (Mason, 1982; Bonner, 1988). Such processes produce at most a small chiral polarity, and one must still explain the perfect homochirality of the molecules of all living systems.

In a previous paper (Lins *et al.*, 1995) we discussed some results, obtained by computer simulation techniques, on the stability of homochiral and mixed oligoribotides and peptides. We also analyzed a chiral amplification mechanism similar to that of Wald (1957) and examined its significance with respect to the discovery of enantiomeric cross-inhibition (Joyce *et al.*, 1984; Joyce *et al.*, 1987).

In the present paper we have examined in greater detail the template-assisted growth of oligoribotide chains. The new results support our previous findings and add to our understanding of enantiomeric cross-inhibition. Surprisingly, we have found that cooperative binding is possible in mixed ribotide chains.

Wald's Model Revisited

Using very accurate solid models, Wald (1957) constructed segments of the DNA double helix and of protein α-helices and tried to quantify the effects of replacing the chirality of the deoxyribose (D→L) in DNA and of the naturally occurring amino acids in proteins (L→D).

He found that no helix is formed if C(3') and C(4') of the deoxyribose moieties have opposite handedness, and no base pairing is possible if, in addition, the C(1') are not all of the same chirality, the so called β-glycosidic bonds. Wald's findings are directly extensible to the ribose moiety of single stranded RNA, probably a more important ingredient of early life (Kruger *et al.,* 1982; Guerrier-Takada *et al.,* 1983; Cech, 1986a; Cech, 1986b; Zaug and Cech, 1986; Cech, 1987; Cech, 1990; Herschlag and Cech, 1990; Westheimer, 1986; Doudna and Szostak, 1989; Joyce, 1989; Noller *et al.,* 1992; Dai *et al.,* 1995). His main conclusion was essentially that mixed RNA or DNA strands are unstable. Furthermore, since the growth of such chains involves template-directed step(s), one can predict that the impossibility of Watson-Crick base-pairing would terminate the growth of mixed ribotide chains.

In the case of protein α-helices Wald found that, while the insertion of D residues at separate positions of an all-L right-handed helix is possible, the accumulation of D-amino acids residues would result in its destabilization.

On the basis of these results Wald proposed an equilibrium mechanism that could have enabled polyribotides and proteins to act as chiral **amplifiers**. Thus, for the growth of protein α-helices with an initial excess of, for instance, L residues, every D→L substitution would stabilize the chain, whereas the reverse would be true for L→D substitutions. Eventually one would have homochiral protein chains. The same mechanism, according to Wald, accounts for the existence of homochiral poly-D-ribotide strands.

Back in 1957 Wald had good reasons to chose for his examples α-helices and double-helical DNA, whose general structures had been discovered a few years before. Nowadays, it is possible to study the issue of the stability of chiral and mixed structures from a broader viewpoint with the aid of computer simulation techniques. It is convenient to study oligoribotides and peptides separately, especially if we want to obtain from structural data information on the growth of these different heteropolymers, since complementarity by base-pairing is a feature of oligoribotide growth, but entirely absent in the case of peptide chains.

Mixed and Homochiral Oligoribotides

Oligomerization of activated mononucleotides in the laboratory invariably requires the presence of specific molecular templates. Perhaps the most efficient template-directed reaction systems are those involving poly-rC_D and guanosine nucleotides, described by Joyce *et al.,* (1984, 1987). Oligomerization of a solution of the D enantiomer of guanosine 5'-phospho-2-methylimidazole in the presence of a poly-rC_D produces 3',5'-linked oligo(G)s ranging in length up to 30mers (in the absence of the template, only dimers and traces of trimers are formed). If one uses a racemic mixture of the monomeric guanosine derivative in the presence of the same template, only small amounts of dimers and trimers are obtained: there is thus a reduction in the efficiency of the oligomerization. Clearly the L isomer inhibits the polymerization of the mirrored enantiomer. By analogy, the same effect is expected in the case of a poly-rC_L template: efficient oligomerization should occur from a solution of the L-guanosine derivative, but not from a racemic solution. This phenomenon was called **enantiomeric cross-inhibition** by Joyce *et al.* (1984, 1987), and considered to be a serious problem for all theories of the origin of molecular homochirality in living systems and, consequently, to the theories of the origin of life.

To compare our computational results with the experimental data of Joyce *et al.,* (1984, 1987) we have chosen a large fragment of poly-rC_D as a template in association with penta-rG, both monochiral and mixed. We have constructed monochiral ribotide sequences starting from crystallographic data of the A form RNA double helix ($\phi = -67.2°$, $\psi = -74.8°$, $\theta_1 = -179.1°$, $\theta_2 = +58.9°$, $\theta_3 = -150.0°$). Form A was the chosen secondary structure because of the high incidence of such structure in RNA fragments, such as in "hairpins" and RNA-DNA hybrids (Dickerson *et al.,* 1982) and because ribose sugars do not fit into a helix of the B and Z types. The monochiral structures had their energies minimized; the ribose chirality of the penta-rG strands in all positions was systematically inverted and the mixed structures in complementary association with poly-rC_D strands also had their energies minimized by the same process. The difference in stabilities are evaluated by the relative potential energy differences, that is,

$$\Delta E_{pot} = E_{pot} \text{ (mixed)} - E_{pot} \text{ (monochiral)}, \quad (1)$$

where a positive ΔE_{pot} means that the mixed structure is *less stable* than the corresponding homochiral one. The results for several D-ribose → L-ribose substitutions in pentaribotides are shown in Table I.

In a previous paper (Lins *et al.*, 1996) using the same procedure for the single strands of tetra-, penta- and hexaribotide chains, we had concluded that the D-ribose → L-ribose substitution is more favorable at the 5'-position than at the 3'-position of the oligoribotide chains. In addition, a much larger destabilization occurred when the D-ribose → L-ribose substitutions are at the internal positions (2, 3, and 4). We now find for the template-bonded chains, that the simultaneous substitution at 5'- and 3'-positions leads to a significant stabilization of the mixed penta-rG strand compared to the monochiral one. Hence, L-riboses act as terminators to the poly-rC$_D$ template-assisted growth of poly-rG$_D$. These results are consistent with the experiments of Joyce *et al.*, (1984, 1987), in which only oligoribotides containing L-ribose in the external positions (5' and 3') were observed to any significant degree. We also found that of the two ends, L-ribose binds more easily to the 5'- than to the 3'-position, thus corroborating again the results obtained by Joyce *et al.*, (1984, 1987) at low concentrations.

The importance of simultaneous substitutions at the extreme ends is evident when comparing the individual relative energies, that is, the oligo-rG-

Table I. Difference of total energy between monochiral and mixed pentaribotides (oligo-G), in association with the template (poly-C).

Pentaribotides (oligo-G)		
Position of L-ribose	ΔE_T (kJ/mol)	χ Angle conformation
All D-ribose	0.00	*anti*
5'	−15.1	*anti*
3'	+43.1	*anti*
5' and 3'	−11.7	*anti*
2 (internal)[a]	+51.0	*anti*
3 (internal)[a]	+39.7	*anti*
4 (internal)[a]	+38.5	*anti*

[a] The positions 2, 3 and 4 correspond to the internal position of the oligoribotides.

ribotides containing L-ribose in position 5' is stabilized by -15.1 kJ/mol relative to the monochiral, whereas the substitution at the 3'-position leads to a destabilization of +43.9 kJ/mol, which would yield a destabilization of +14.6 kJ/mol for the 5', 3'-disubstituted case, if only additive effects were operative. However, when minimization of the oligo-rG-ribotides containing L-ribose in positions 3' and 5' is performed a stabilization of -11.7 kJ/mol is obtained. It is thus clear that there are some cooperative effects acting when simultaneous substitutions are performed at the two ends. In order to investigate the origin of this cooperative effect a detailed analysis of each component of the total energy is presented in Table II and Figure 1. The bonded term in Figure 1 refers to the valence force field, that is, stretch (bond), bend (theta), torsion (phi) and improper torsion (out of plane) listed in
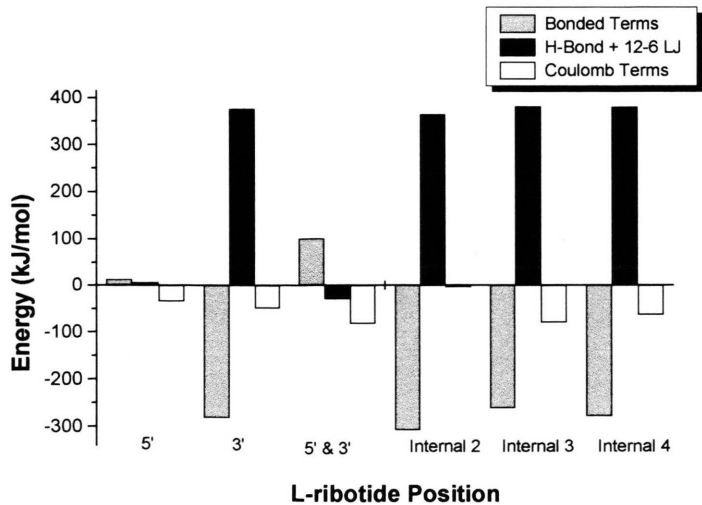

Fig. 1. Graphic representation of the individual energy differences term between monochiral and mixed pentaribotides. *Bonded terms* refer to column 1–4, *H-Bond* and *12–6 Lennard-Jones non-bonded terms* of columns 5+6, and *Coulomb term* to column 7 of Table II.

Table II. The non-bonded terms are splitted into two parts, namely, the hydrogen-bond plus the 6–12 Lennard-Jones (H-bond + 6–12 LJ) and the coulombic interaction.

It seems clear from Table II and Figure 1 that the stabilization due to the substitution at the 5'-positions is related to the very small variation of all terms when compared with all the other substitutions, except the simultaneous substitution at the 5'- and 3'-positions. In particular, the balance between the large stabilization due to the torsional term (φ in Table II) and the large destabilization due to the 6–12 Lennard-Jones non-bonded term ("non-bonded" in Table II) appears to be the cause for the destabilization of the substitutions, except for the 5' and simultaneous 5' and 3' substitutions. Thus, the cooperative effect of the simultaneous substitution seems to be related to the compensation of the large distortion of the dihedrals as well as the non-bonded terms. More specifically, the simultaneous substitution leads to a inversion of the relative contribution of those important terms, that is, the torsional term becomes positive (destabilization) and the 6–12 Lennard-Jones non-bonded term becomes negative (stabilization).

Mixed and Homochiral Peptides

We have seen that Wald used α-helices in his discussion on chiral amplification. Indeed, α-helices are a very common secondary structure in many proteins and it is known that during protein folding α-helices appear within 10^{-3} s of the complete folding process which takes approximately 10^2 s (Englander, 1993; Jennings and Wright, 1993; Miranker *et al.*, 1993). We studied changes in the stability of polyalanine α-helices obtained by the insertion of D-residues in fragments of right-handed α-helices (Lins *et al.*, 1996). One should note that, since proteins depend on the existence of a translational machinery for their synthesis, simple peptides are more important than segments of protein secondary structure as far as early evolution is concerned, and for this reason we have also studied the L→D alanine substitution in the less compact β-strands.

The methodology used was the same as that described for the oligoribotide calculations. Potential energy minimization of segments of peptide chains both in the α-helix and β-strand conformations were performed in which the amber force field was used (Weiner *et al.*, 1984; Weiner *et al.*, 1986). The change in the chirality of a residue is accomplished by exchanging the hydrogen atom and the side chain R on the α-carbons.

Our α-helices contained 10 residues of alanine with torsion angles φ = -63°, ψ = -42°, and ω = 180° (Blundell *et al.*, 1983). The potential energy of the all-L polyalanine right-handed α-helix was the zero of the stability scale. An all-D polyalanine right-handed α-helix has an energy of +30.5 kJ/mol. This difference must be attributed solely to the interactions between L or D residues and the remainder of the peptide chain, as a consequence of its helicity. Earlier results (Scott and Scheraga, 1966; Ramachandran, 1968) yielded lower values for this energy difference. We have calculated the potential energies of decameric polyalanine α-helices with L→D substitutions in many positions. The main result is that for polyalanine α-helices L→D substitutions in contiguous regions decreases somewhat the stability of the whole structure.

Table II. Values of the individual energy differences between monochiral and mixed pentaribotides (oligo-G), in association with the template (poly-C), All energies in kJ/mol.

Ribotides	Bond	*Theta*	*Phi*	Out of Plane	Hydrogen Bond	Non-bonded	Coulomb
All D-ribose	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L-ribose 5'	0.0	−12.1	27.2	−2.1	−4.6	11.3	−34.7
L-ribose 3'	−0.5	−50.7	−221.3	−10.0	16.7	358.1	−49.0
L-ribose 5' and 3'	3.3	26.4	65.3	5.4	−11.3	−18.4	−82.4
L-ribose 2[a]	−2.9	−64.0	−231.4	−9.6	13.4	350.2	−5.0
L-ribose 3[a]	0.0	−27.6	−224.3	−9.2	16.7	364.4	−80.3
L-ribose 4[a]	1.7	−37.2	−233.5	9.2	7.9	372.4	−63.6

[a]The positions 2, 3 and 4 correspond to the internal position of the oligoribotides.

However, we observed in almost all cases the φ and ψ torsional angles within the range of values allowed for the usual all-L right-handed α-helices. In the same way, the longitudinal hydrogen bonds connecting a residue *i* with another one at (*i* + 4) are maintained (Lins *et al.*, 1996).

We have performed less extensive calculations involving residues different from alanine. The disruption brought about by L→D substitutions can in some cases be much more significant. This is particularly noticeable in the case of L→D substitution of valine, isoleucine and threonine, which have a bifurcation at their β-carbons. It should be mentioned that these residues, even in the case of all-L situations, are known to be uncommon in α-helices (McGregor *et al.*, 1987; Horovitz *et al.*, 1992).

We have also examined by the same techniques the stability of substituted polyalanine β-strands (φ= -139°, ψ = +135° and ω = 180°). In the minimization process there is a significant change in these angles, indicating that small peptide segments (5 residues in our case) do not have a unique conformation. The distortion of the chain leads almost to a fully extended conformation in which the helical chirality is practically zero. This occurs both with the mixed and the homochiral strands. According to our calculations even homochiral polyalanine β-sheets as independent units, either parallel or anti-parallel are unstable (Lins *et al.*, 1996). This can be explained by the fact that although alanine fits well in α-helices the same does not occur in the β-sheet structure (Creighton, 1983). Brack and Spach (1979a, 1979b) synthesized a series of poly (leucyl-lysyl) samples with D and L residues along the chains. Infrared spectroscopy and CD measurements indicated the presence of β-sheets of varying width. They also proposed that either system acted as a chiral amplifier, but after the discovery of ribozymes the evolutionary meaning of such findings is difficult to assess.

Methodology

For the simulations treated here, the macroscopic variation of the volume is negligible ($\Delta V \approx$ 0), since the processes should occur in condensed media. In addition, the degrees of freedom for a chirality change in different monomeric species are the same. Thus, it is expected that the entropy

variation of a D↔L should also be negligible ($\Delta S \approx 0$). As a result, the free energy change can be properly approximated by the change in the internal energy ($\Delta G \approx \Delta E$).

All numerical values were obtained by molecular mechanics calculations using the amber force field (Weiner *et al.*, 1984, 1986), in the Biosym software (Biosym Program, 1993), with the potential energy function given by

$$E_{total} = \sum_{bonds} K_R(R-R_0)^2 + \sum_{angles} K_\theta(\theta-\theta_0)^2 +$$
$$\sum_{dihedrals} \frac{V_n}{2}[1+\cos(n\phi-\gamma)]$$

$$+ \frac{1}{VDW}\sum_{i>j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^6}\right] + \frac{1}{EEL}\sum_{i>j}\left[\frac{q_iq_j}{\varepsilon(R)R_{ij}}\right]$$

$$+ \sum_{\text{H-bonds}}\left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right] + \sum_{constraints} K^*_R(R-R_0)^2, \quad (2)$$

where all the variables, except for the distances (*R*), angles (θ) and dihedrals (φ), are constants of the force field, obtained via parametrization. In Eqn. (2), R_0 and θ_0 are the equilibrium bond distance and bond angle, respectively, with K_R and K_θ being the force constant related to these motions. The torsional motion is described by a Fourier series of symmetry, *n*, a corresponding potential V_n and a phase angle given by γ. The 6−12 Lennard-Jones and Coulomb interactions between atoms *i* and *j* at the R_{ij} distance are described by the constants A_{ij} and B_{ij} and the atomic charges q_i and q_j. In addition, the dielectric function $\varepsilon(R)$ is taken to be linear with the interatomic distance R_{ij}. The amber force field treats the hydrogen-bond interaction as a special case represented by a 10−12 potential function described by the C_{ij} and D_{ij} constants. This force field allows to keep part of the system rigid via a penalty function characterized by a very large force constant K^*_R. A partial physical interpretation of this force field can be described as follows: *i*) the first three terms in Eqn. (2) are referred to as the bonded interactions; *ii*) the fourth and fifth terms are the so called non-bonded ones; *iii*) and the last term is included in order to keep part of the molecular system restrained. The bonded terms are described by a harmonic valence force field for stretch (1st term), bending (2nd term) and torsion or improper dihedral (3rd term). The non-bonded terms are represented by the 4th, 5th and 6th terms of Eqn. (2),

where the 6–12 Lennard-Jones potential simulates the van der Waals interactions (attractive London dispersion interactions and the repulsive interactions due to the Pauli exclusion), except when the pairs of atoms are involved in hydrogen bonds, the van der Waals function is replaced by a 10–12 function (6th term). The electrostatic interactions are described by the Coulomb potential due to point charges (5th term). It should be noted that the 1–4 van der Waals and the 1–4 electrostatic interactions are scaled, typically, by a factor of 0.5, *i.e.*, VDW = EEL = 2. This scaling is important to compensate for the steepness of the 6–12 function and charge redistribution during a pairwise interaction. In addition, the distance dependent dielectric function, $\varepsilon(R)$, is implemented in order to reduce the magnitude of the long range electrostatic interactions as compared to a constant dielectric. This distance dependent dielectric function is important when the parametrization of the force field is performed for solvated biomolecules. Finally, the special treatment for the hydrogen-bonding interaction via an 10–12 function is important to fine tune the H-bond distances and energies. It should be emphasized that for all calculations we have not employed any cutoff for the potential function.

In order to measure the stability, it is important to explore the conformational space of the oligomers in the presence of a fixed template, and try to determine the global minimum of the potential energy surface. The molecular dynamics (MD) method (Allen and Tildesley, 1989) has been chosen to explore the phase space of the system. The approach consists of a temporal propagation of the Cartesian coordinates of atoms which are allowed to move (the template is kept fixed). It is expected that if this propagation is performed for a long enough time all the possible configurations of the phase space will be visited. Keeping a record of all these configurations, it is possible then to take the most stable ones and perform an energy minimization procedure. In our MD simulations, the temperature was varied from 900 K down to 300 K with 20 ps for thermalization and 200 ps acquisition time. The energy minimization was performed by initially running 100 steps of the steepest descent method after which the conjugate gradient method was switched on to complete the minimization. The final RMS of the gradient of all structures was less than 0.04 kJ/nm. As mentioned before, cutoffs are not used in our simulations.

Since tetraribotides and larger homochiral oligoribotides are found to be more stable than the corresponding mixed ones we predict that the latter cannot grow at a rate comparable to that of homochiral ones. Although it is generally assumed that thermodynamic arguments do not justify kinetic predictions, there are strong reasons to believe that they are correct in this case. First, except for the small entropy of mixing, the free-energies of racemic monomers are the same as those of chiral enantiomers. Secondly, the energy change for the conversion of D (or L) ribotides into an all-D (all-L) oligoribotide is much smaller than that for the conversion of racemic monomers into a mixed oligoribotide of the same size. Furthermore the reaction profiles must be alike, since the processes consist of condensation reactions involving similar groups. Hence, one can expect that homochiral oligomers will grow at a faster rate than mixed ones.

Discussion

One of the main results of the calculations is that the stability of oligoribotides is much more dependent on the homochirality of their constituent monomers than is the case for peptide chains.

Joyce *et al.* (1987), have proposed that in enantiomeric cross-inhibition L-ribotides can fit the next site of the growing chains in the *syn* conformation. Our modeling has confirmed this supposition but, as shown in Table I, we also found that when phosphodiester bonds are formed in the growing chain the L-ribotides assume the *anti* conformation.

The enantiomeric cross-inhibition is considered such a serious problem to the theories of the origins of life that it has been proposed that the "RNA World" had evolved from ancestral systems based on simpler acyclic analogues of the nucleotides (Joyce *et al.*, 1987) containing prochiral centers, or even from other kinds of polymers such as peptide nucleic acid, or PNA (Eghohom *et al.*, 1992; Mesmaker *et al.*, 1994). It is not explained however, how the known chiral RNA strands could have been formed from these precursors. It is possible, of course, that biogenesis on Earth did occur along these lines. Nevertheless, we have re-

cently (Lins *et al.*, 1996) retaken the suggestion that RNA-like polymers were the first self-replicating-systems. We proposed a mechanism through which the enantiomeric cross-inhibition could have been bypassed. In a first phase dimers were synthesized from activated monomers independently of the template fragments. Concurrently the autocatalytic formation of homochiral tetramers occurred in the way described by von Kiedrowski (1986) and by Zielinski and Orgel (1987). Further growth of oligoribotides probably occurred not exclusively by the well known step-wise addition of monomers, in which enantiomeric cross-inhibition could act, but also by a different pathway involving the condensation of large fragments (Ferreira and Coutinho, 1993). The bonds formed by the latter process are at internal positions of the resulting oligoribotides.

Because mixed oligoribotides do not pair easily with complementary fragments (see Table I), and D(L) oligoribotides containing L(D) monomers at internal positions are very unstable (Tables I and II), only homochiral species (all-D and all-L) could take part in this step, characterized by some of the growth fragments acting as catalytic templates.

In this model, both all-D and all-L fragments would be formed, but not mixed ones. These catalytic templates would guarantee that only homochiral oligoribotides could grow. One should persist in searching for adequate physico-chemical parameters (hopefully present in the prebiotic conditions), which allow these types of processes to occur. For example, perhaps they could happen in water diluted by ethylene glycol, DMSO, formamide or the presence of specific divalent cations.

Finally, the results support the idea (Lins *et al.*, 1996) that primitive RNA acted as a chiral amplifier, whereas the homochirality of α-amino acids originated from stereochemical requirements of subsequent RNA-peptide interactions.

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