Enantiomeric Cross-Inhibition in the Synthesis of Oligonucleotides on a Nonchiral Template

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Prebiotic syntheses of chiral monomers always yield racemic mixtures. Living systems, however, utilize L-amino acids and D-nucleotides in their biopolymers. The generation of optical asymmetry by selection and amplification in an autocatalytic process is, therefore, an important element in many theories of the origin of life.[1−3] Replication of polynucleotides in template-directed syntheses is an obvious candidate for such an amplification step in a pre-"RNA world".[4,5] A serious objection to this suggestion is the observation that the efficiency of template-directed syntheses of RNA is limited by enantiomeric cross-inhibition.[6] Peptide nucleic acids (PNAs),[7−9] amide-linked, nonchiral analogues of RNA, have been "copied" into RNA[10] and constitute an alternative to chiral polynucleotides as an informational replicating system. Here, we use PNA as model for a hypothetical, nonchiral precursor of RNA in experiments re-examining enantiomeric cross-inhibition. We find that enantiomeric cross-inhibition is as serious in the polymerization of nucleotides on a PNA template as it is on a conventional RNA or DNA template.

L-Guanosine 5′-monophosphate (L-5′-GMP) was synthesized starting from L-ribopyranose (Sigma) following well-established procedures[11−15] and gave L-5′-GMP in 18% yield. All analytical data for L-5′-GMP were identical to those for the natural D-enantiomer. L-5′-GMP was converted to L-guanosine 5′-phosphoro-2-methylimidazolide (L-2-MeImpG) (Figure 1A) in 95% yield.[16] All reaction conditions in our template-directed reactions were chosen to facilitate comparison with previously published results; all peak assignments are in accord with those established in earlier publications.[10,17]

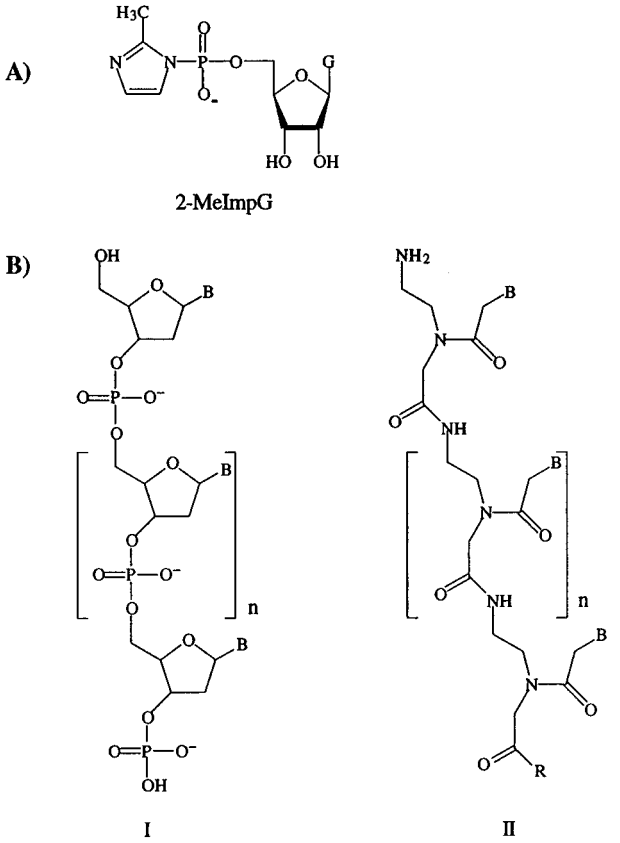

Figure 1. (A) Structure of D-2-MeImpG and (B) structure of DNA (I) and PNA (II). The PNA structure has been drawn to emphasize its relation to the standard DNA structure.

The reaction of 0.1 M D-2-MeImpG gives oligomers up to an octamer in good yields on a DNA C_{10} template after 24 h (Figure 2a), whereas the reaction with L-2-MeImpG gives only small amounts of a complex mixture of oligomers up to a maximum length of three (Figure 2b). Two sets of experiments were carried out with racemic mixtures of the activated nucleotides. In the first the concentration was reduced to 0.05 M for each enantiomer, thereby maintaining the overall concentration of activated nucleotides at 0.1 M. In the second set of experiments we used a 0.1 M concentration of each enantiomer to avoid dilution of the D-enantiomer. Oligomerizations using the lower concentration gave oligomers up to only a tetramer with yields diminished from those obtained in the reaction with the 0.1 M D-2-MeImpG alone (Figure 2d). In experiments with 0.1 M each of the nucleotides, higher yields of short oligomers were obtained, but the tetramer was still the longest detectable oligomer (Figure 2c). These results on a DNA template are similar to those reported in an earlier study of enantiomeric cross-inhibition on RNA.[6]

The "nonchiral" PNA C_{10} strand cannot, in principle, distinguish between the nucleotide enantiomers since it is capable of adopting equivalent left- and right-handed helical conformations. A PNA C_{10} template, as anticipated, supported almost equally effective oligomerization of D- and L-2-MeImpG (Figure 3, parts a and b). Very minor differences in the efficiency of the reaction and in the product distribution can be attributed to the chiral L-lysine that is attached to the template in order to increase its solubility.[8] The reaction of a solution

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(1) Wald, F. *Ann. N. Y. Acad. Sci.* **1957**, *69*, 352−368.
(2) Bonner, W. A.; Blair, N. E.; Dirbas, F. M. *Origin Life Evol. Biosphere* **1981**, *11*, 119−134.
(3) Ponnamperuma, C.; Chela-Flores, J. *Chemical evolution-origin of life: proceedings of the Trieste Conference on Chemical Evolution and the Origin of Life*; Ponnamperuma, C., Chela-Flores, J., Eds.; A. Deepak Publishing: Hampton, VA, 1992.
(4) Gesteland, R.; Atkins, J. F. *The RNA WORLD. The Nature of Modern RNA Suggests a Prebiotic RNA World*; Gesteland, R., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1993; Vol. Monograph 24.
(5) Joyce, G. F.; Orgel, L. E. *The RNA World*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1993.
(6) Joyce, G. F.; Visser, G. M.; van Boeckel, C. A. A.; van Boom, J. H.; Orgel, L. E.; van Westrenen, J. *Nature* **1984**, *310*, 602−604.
(7) Wittung, P.; Nielsen, P. E.; Buchardt, O.; Egholm, M.; Nordén, B. *Nature* **1994**, *368*, 561−563.
(8) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497−1500.
(9) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Nordén, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566−568.
(10) Böhler, C.; Nielsen, P. E.; Orgel, L. E. *Nature* **1995**, *376*, 578−581.
(11) Recondo, E. F.; Rinderknecht, H. *Helv. Chim. Acta* **1959**, *17*, 1171−1173.
(12) Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505.
(13) Hóly, A.; Sôrm, F. *Collect. Czech. Chem. Commun.* **1971**, *36*, 3282−3299.
(14) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234−1255.
(15) Jenny, T. F.; Benner, S. A. *Tetrahedron Lett.* **1992**, *33*, 6619−6620.
(16) Inoue, T.; Joyce, G. F.; Grzeskowiak, K.; Orgel, L. E.; Brown, J. M.; Reese, C. B. *J. Mol. Biol.* **1984**, *178*, 669−676.
(17) Joyce, G. F. *Nonenzymatic template-directed synthesis of informational macromolecules*; Cold Spring Harbor Press: Cold Spring Harbor, NY, 1987; Vol. LII, pp 41−51.

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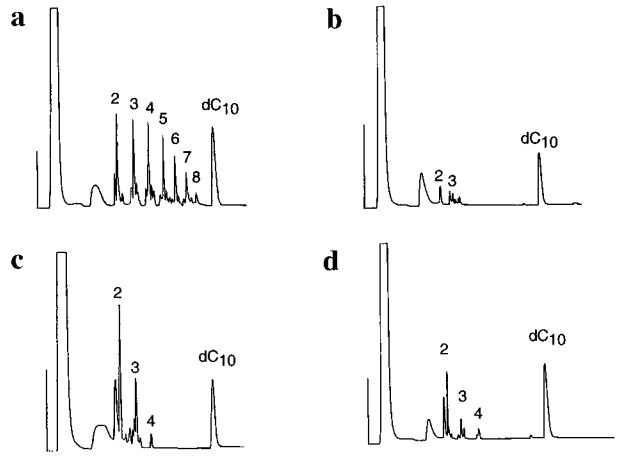

Figure 2. Oligomerization of D-guanosine 5′-phosphoro-2-methylimidazolide (2-MeImpG) on a $(dC)_{10}$ template: (a) 0.1 M D-2-MeImpG; (b) 0.1 M L-2-MeImpG; (c) a mixture of 0.1 M D- and 0.1 M L-2-MeImpG; (d) a mixture of 0.05 M D- and 0.05 M L-2-MeImpG. Reactions were analyzed after 1 d. Reaction conditions: 4 °C; 1.2 M NaCl; 0.2 M $MgCl_2$; 0.005 M template; and 0.2 M 2,6-lutidine buffer, pH 7.8 (at room temperature). Reaction solutions were prepared in 0.7 mL Eppendorf tubes in 5 μL volumes. First stock solutions of NaCl and $MgCl_2$ with the template were coevaporated to dryness. To start the reaction, the residue was redissolved in a freshly prepared solution of 2-MeImpG in 2,6-lutidine buffer. At appropriate times 1 μL of a reaction solution was added to 100 μL of an aqueous solution containing 0.01 M HCl and 0.002 M EDTA. The resulting solution (pH 2.8−3.0) was kept at 37 °C for 24 h to hydrolyze surviving phosphoroimidazoles and then neutralized with aqueous NaOH. A 10 μL sample of this solution was mixed with 1 mL of starting buffer (pH 12) and analyzed by HPLC on RPC-5 as previously described.[20,21] The reaction products were eluted with a linear gradient of $NaClO_4$ (pH 12, 0−0.08 M, 60 min). UV absorption monitored at 254 nm.

containing equal 0.05 M concentrations of D- and L-2-MeImpG on a PNA C_{10} template gives only short oligomers, including a trace of the tetramer (Figure 3d). When the concentration of each enantiomer is increased to 0.1 M, the yield of short oligomers increases, but the tetramer is still the largest detectable oligomer (Figure 3c). This clearly shows that the absence of longer products on a PNA template is due to enantiomeric cross-inhibition.

A somewhat more detailed analysis of the HPLC elution profiles is possible. Comparison of Figures 2a and 3a shows that the trimer peak is more complex when PNA is used as a template in place of DNA. The individual subpeaks must correspond to 2′−5′-linked and pyrophosphate-linked products in addition to the 3′−5′-linked products.[18,19] However, longer oligomers are represented by a single predominant peak in both cases. This suggests that cooperative association with monomers is sufficient to stabilize a helical structure with a DNA template, but a homochiral trimer is required in the case of a PNA template.

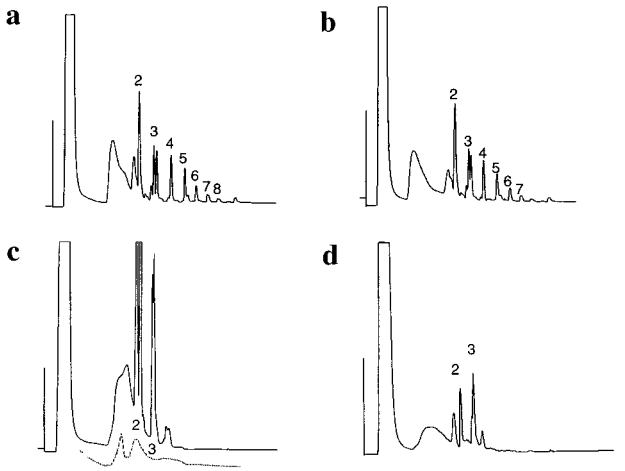

Figure 3. Oligomerization of L- and D-guanosine-5′-phosphoro-2-methylimidazolide (2-MeImpG) on a PNA C_{10} template: (a) 0.1 M D-2-MeImpG; (b) 0.1 M L-2-MeImpG; (c) a mixture of 0.1 M D- and L-2-MeImpG (dashed line gives reaction without template); (d) a mixture of 0.05 M D- and L-2-MeImpG. Reactions were analyzed after 1 d. Reaction conditions and analysis are as described in the caption to Figure 2.

We have not studied the PNA-directed reactions of bases other than G. Perhaps enantiomeric cross-inhibition would be less severe for other bases, particularly for the pyrimidines which do not readily adopt the syn-configuration. Nevertheless, it now seems unlikely that the choice of a new template, whether chiral or achiral, will overcome enantiomeric cross-inhibition so generally as to permit the template-directed replication of oligomers long enough to seed the direct emergence of the RNA world from a solution of racemic activated ribomononucleotides. The origin of the RNA world from ribomononucleotides presumably depended on the availability of substrates that were chirally-enriched, selectively adsorbed on chiral mineral surfaces, or synthesized by a chiral catalyst formed in a pre-RNA informational system.

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(18) Bridson, P. K.; Orgel, L. E. *J. Mol. Evol.* **1980**, *144*, 567−577.
(19) Inoue, T.; Orgel, L. E. *J. Am. Chem. Soc.* **1981**, *103*, 7666−7667.
(20) Lohrmann, R.; Orgel, L. E. *J. Mol. Biol.* **1980**, *142*, 555−567.
(21) Joyce, G. F.; Inoue, T.; Orgel, L. E. *J. Mol. Biol.* **1984**, *176*, 279−306.