A Highly Enantio-Selective Hexitol Nucleic Acid Template for Nonenzymatic Oligoguanylate Synthesis

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The effect of L-guanosine 5′-phosphoro-2-methylimidazole (L-2-MeImpG) on the nonenzymatic oligomerization of the D-enantiomer (Figure 1a) on a poly(D-C) template has been studied in some detail because of its relevance to prebiotic chemistry.[1] It was shown that the L-enantiomer is a potent inhibitor of the oligomerization and is incorporated as a chain terminator in short oligo(D-G) products. A similar result was obtained when the poly-(D-C) template was replaced by an achiral peptide nucleic acid (PNA) C_{10} template.[2] This enantiomeric cross-inhibition was attributed to the ability of the L-guanosine to base-pair with cytosine residue on a template with normal Watson−Crick geometry and then to adopt a *syn*-conformation. This would bring the 5′-phosphate group of the activated L-monomer close to the 3′-hydroxyl group of the growing oligo(D-G) strand.

Hexitol nucleic acids (HNAs) are DNA analogues built up from standard nucleobases and a phosphorylated 1,5-anhydrohexitol backbone (Figure 1b). The six-membered hexitol ring can be considered as a mimic of a furanose ring, frozen in its 2′-exo, 3′-endo conformation.[3,4] HNA oligomers are able to hybridize with complementary DNA or RNA oligomers and with their own complements.[5] In preliminary studies we have found that the D-HNA oligomer hC_{10} is a very efficient template for the oligomerization of D-2-MeImpG. It seemed possible that a D-HNA template, because it is frozen in a single conformation, would prove more enantioselective than a standard nucleic acid template. Here we compare enantiomeric cross-inhibition in the oligomerization of a mixture of D- and L-2-MeImpG on C_{10} (RNA), dC_{10} (DNA) and hC_{10} (HNA) templates under the conditions used in earlier studies.[1]

Oligomerization of the D- or L-enantiomer of 2-MeImpG yields identical patterns of products in the absence of a template; only dimers and smaller amounts of trimers are formed. Furthermore, the D-templates do not direct oligomerization of L-2-MeImpG (data not shown). The products that we obtained using a dC_{10} or a C_{10} template (Figure 2a−f) are analogous to those previously reported for a poly(C) template.[1] A dC_{10} or a C_{10} template catalyzes efficient oligomerization of D-2-MeImpG (Figure 2a,d). In both cases strong enantiomeric cross-inhibition by the L-enantiomer is observed (Figure 2b,c,e,f). Decreasing the reaction temperature from 0 °C to −10 °C has little effect on the degree of enantiomeric cross-inhibition or the regiospecificity of the reaction (Figure 2c−f).

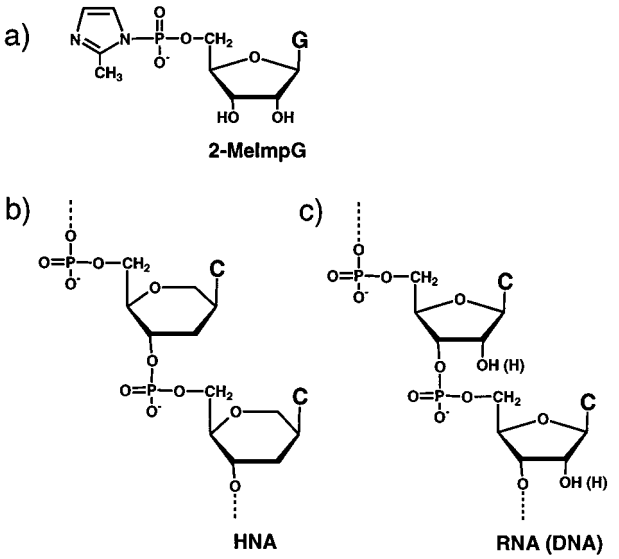

Figure 1. Structures of (a) guanosine 5′-phosphoro-2-methylimidazole (2-MeImpG), (b) hexitol nucleic acid (HNA), and (c) RNA and DNA.

An hC_{10} template facilitates efficient oligomerization of D-2-MeImpG (Figure 2g). The HPLC profiles in Figure 2h,i show the product distributions obtained in the template-directed polymerization of an equimolar mixture of D- and L-2-MeImpG on an hC_{10} template at 0 °C (Figure 2h) and at −10 °C (Figure 2i). The main peaks (Figure 2h,i) were shown to correspond to 3′-5′-linked oligo(D-G)$_n$ products by co-chromatography with the corresponding oligo(G)$_n$'s synthesized in a reaction of D-2-MeImpG on a C_{10} template. This assignment was confirmed by showing that intensity of the peaks which represent 3′-5′-linked oligo(D-G)$_n$ increased steadily as the proportion of L-2-MeImpG in a reaction mixture of D- and L-2-MeImpG decreased. When we reduced the reaction temperature from 0 °C (Figure 2h) to −10 °C (Figure 2i), we observed a substantially more selective formation of 3′-5′-linked oligo(D-G)$_n$ products. This contrasts sharply with our finding for C_{10} or dC_{10} templates.

It is clear that L-2-MeImpG inhibits oligomerization of D-2-MeImpG on an hC_{10} template (Figure 2h,i), but the inhibition is much less severe than is observed on a C_{10} or dC_{10} templates (Figure 2b,c,e,f). The most striking differences between the products formed on hC_{10} and C_{10} or dC_{10} templates are seen for G_6 and longer oligomers. These are formed in greater yield and with much increased regiospecificity when an hC_{10} replaces a standard nucleic acid template (Figure 1c). The regiospecific formation of an *n*-mer requires $n - 1$ successive regiospecific reaction steps; therefore, it is surprising that at −10 °C all D-oligomers as long as the 6- and 7-mer are formed in substantial yield with only very small amount of side-products (Figure 2i).

Enantiomeric cross-inhibition is due to the ability of L-2-MeImpG to compete with the D-enantiomer for the binding site on the C residue adjacent to the 3′-terminus of the growing oligo-(G) chain. If L-2-MeImpG, after binding, is unable to form a covalent bond to the growing oligo(G) chain, it will behave as a competitive inhibitor; however, if it does form a covalent bond,

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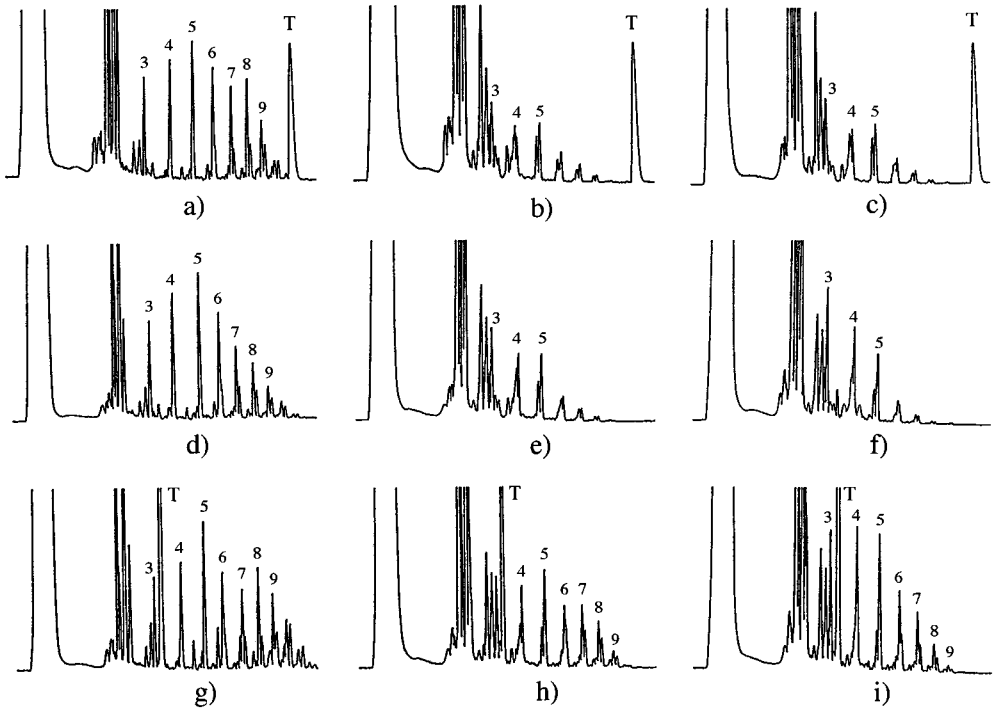

Figure 2. Oligomerization of D-2-MeImpG and L-2-MeImpG on different templates after 5 days: dC_{10} (DNA) template (a−c); C_{10} (RNA) template (d−f); hC_{10} (HNA) template (g−i); D-2-MeImpG, 100 mM at 0 °C (a, d, g); a mixture of D-2-MeImpG (100 mM) and L-2-MeImpG (100 mM) at 0 °C (b, e, h) and at −10 °C (c, f, i). The numbers above the peaks indicate the length of the 3′-5′ linked oligo(D-G)$_n$ products, T indicates the template. Reaction conditions for the polymerization were chosen to permit comparison with earlier work.[1] The C_{10} template was cleaved with RNase A before analysis. HPLC analyses of the reaction mixtures were performed on an RPC5 column as previously described.[7]

it will terminate the chain irreversibly. Our results are most plausibly explained if we suppose that chain termination is important on RNA and DNA templates but less so on an HNA template, particularly at −10 °C. The conformation of the L-nucleotide in the transition state for a covalent bond formation is unknown. The previous suggestion that a *syn*-conformation is present[1] may need to be modified, since it has been shown that a (D-C):(L-G) base pair within an otherwise D-helical structure forms a stable Watson-Crick-type base-paired structure in which L-G has an S-type sugar geometry and a low anti-glycosyl conformation.[6]

The increased enantioselectivity of the oligomerization at −10 °C could indicate that L-2-MeImpG is excluded from the "active site" by competition with D-2-MeImpG at low temperature. Alternatively, L-2-MeImpG may occupy the "active site" but, at low temperature, may be confined to a conformation that prevents covalent bond formation. The strong, observed temperature dependence supports the latter interpretation.

The finding that a hexitol nucleic acid template can select the D-isomer from an enantiomeric mixture is not directly relevant to prebiotic chemistry since we cannot suggest a plausible prebiotic synthesis of the hexitol nucleotides. Nevertheless, our observations show that efficient selection of one optical isomer from a racemic mixture of nucleotides during nonenzymatic oligonucleotide synthesis on an analogue of a nucleic acid template is possible. They suggest that there may be chiral potentially prebiotic nucleic acid-like polymers that can replicate without significant enantiomeric cross-inhibition.

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