Information transfer from peptide nucleic acids to RNA by template-directed syntheses

Jürgen G. Schmidt, Peter E. Nielsen and Leslie E. Orgel*

The Salk Institute for Biological Studies, PO Box 85800, San Diego, CA 92186, USA and 1The Center for Biomolecular Recognition, IMBG, Biochemistry B, The Panum Institute, Blegdamsvej 3c, DK-2100 Copenhagen N, Denmark

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ABSTRACT

Peptide nucleic acids (PNAs) are uncharged analogs of DNA and RNA in which the ribose–phosphate backbone is substituted by a backbone held together by amide bonds. PNAs are interesting as models of alternative genetic systems because they form potentially informational base paired helical structures. A PNA C10 oligomer has been shown to act as template for efficient formation of oligoguanylates from activated guanosine ribonucleotides. In a previous paper we used heterosequences of DNA as templates in sequence-dependent polymerization of PNA dimers. In this paper we show that information can be transferred from PNA to RNA. We describe the reactions of activated mononucleotides on heterosequences of PNA. Adenylic, cytidylic and guanylic acids were incorporated into the products opposite their complement on PNA, although less efficiently than on DNA templates.

INTRODUCTION

It has been suggested that the RNA world was preceded by a ‘biology’ based on some simpler genetic material (1–3). It is important, therefore, to investigate information-conserving transitions between different informational polymers (4). We have shown that heterosequences of DNA can direct the syntheses of complementary sequences of peptide nucleic acids (PNAs) (4–7). Here we report the use of heterosequences of PNA to facilitate the synthesis of complementary RNA sequences.

Template-directed polymerization of guanosine-5′-phospho-2-methylimidazolide (2-MeImpG) on a polyctidylic template is an efficient and regiospecific reaction that produces 3′-5′ linked oligoguanylates up to at least 40 residues in length (8–10). It has been shown that PNA C10 can substitute for corresponding RNA and DNA templates and efficiently facilitates oligomerization of 2-MeImpG (11). Nucleotides other than G are best incorporated into oligomers on heteropolymeric nucleic acid templates if their complements on the template are flanked by runs of C residues (12–16). In our studies of information transfer from PNA to RNA we designed a system as close as possible to those we previously used to study oligonucleotide synthesis on DNA and RNA templates. We carried out reactions on PNA templates of the type H-C5XC4-LysNH2 (X = A, C, G or T) and compared them with reactions on the corresponding DNA C5XC4 templates (Fig. 1). The reaction conditions were chosen to facilitate direct comparison with previously published results.

MATERIALS

Unless otherwise noted, all chemicals were reagent grade, purchased from commercial sources and were used without further purification. Nucleoside-5′-phosphoro-2-methylimidazolides (2-MeImpX, X = A, C, G or U) were synthesized by a published procedure (9). PNA templates H-C5XC4-LysNH2 (X = A, C, G or T), with a single lysine residue at the C-end, were obtained by solid phase synthesis using a published procedure and analyzed as previously described (17). PNA Boc-monomers were obtained from Perseptive Biosystems. Oligodeoxyribonucleotide templates were synthesized on a 391A DNA synthesizer (Applied Biosystems) to introduce the 3′-ribose residue. The primers were 5′-32P-labeled with ATP and T4 kinase (New England Biolabs) by the blunt end procedure (18) and purified by PAGE, followed by chromatographic purification on a Nensorb column (Nen DuPont). They were then end capped as uridine pyrophosphates using an obvious modification of a published procedure (19) and finally purified by PAGE. End capping prevents pyrophosphate formation in the primer extension reaction, which otherwise complicates the gel electrophoretic analysis.

All PAGE separations were run for ~2 h on a denaturing (8 M urea) 20% polyacrylamide gel at a constant current of 18 mA. Loading buffer was prepared by mixing 900 µl deionized formamide, 25 µl xylene cyanol (2%), 25 µl bromophenol blue (2%) and 50 µl 10× Tris–borate, EDTA buffer.

METHODS

Solutions of 5′-phosphoro-2-methylimidazolides (2-MeImpX) were prepared in Lutidine–HCl buffer (pH 7.8 at 4°C) and used

*To whom correspondence should be addressed. Tel: +1 619 453 4100; Fax: +1 619 558 7359; Email: orgel@salk.edu
immediately. Reactions were carried out in Eppendorf tubes in 10 µl volumes. The concentration of 2-MeImpG was always 50 mM. A second nucleotide derivative, 2-MeImpX (X = A, C or U), was present in most experiments at a concentration of either 50 or 100 mM. Reaction conditions for individual samples are noted in the figure legends. A mixture of 10 µl 1.0 M NaCl, 2 µl 1.0 M MgCl2, 10 µl primer stock solution (20 nM) and 10 µl template stock solution (20 µM), where appropriate, was prepared and evaporated to dryness. The residue was redissolved in 5 µl 0.2 M Lutidine–HCl buffer (pH 7.8 at 4°C), heated on a PCR heating block to 95°C for 10 min and cooled at 10°C/min to allow annealing of template and primer. The samples were then chilled on ice and appropriate aliquots of the solutions of 2-MeImpG and 2-MeImpX were added to reach the required concentrations. Samples were left for 5 days at 4°C. The reaction was terminated by addition of 1 µl 0.5 M EDTA solution (pH 8.0 at room temperature) and the reaction mixture evaporated to dryness. The residue was redissolved in 20 µl loading buffer and an aliquot containing ∼50 000 c.p.m. was taken and diluted to 10 µl with loading buffer. The resulting solution was heated to 95°C for 10 min, then chilled on ice and subjected to PAGE. The gels were visualized by autoradiography on film (Kodak). The radioactivity present in each band was determined using a phosphorimager (Molecular Dynamics) and independently by an optical transmission scan of the X-ray film (BioRad CCD 620). Yields are given relative to the total number of counts unless explicitly stated otherwise.

The nature of the phosphate linkages in the products was determined by RNase T1 and RNase A digestion. These enzymes are specific for 3′-5′ linkages. RNase A cleaves only after a pyrimidine residue and RNase T1 after a G residue. Both enzymes generate 3′-phosphate and 5′-hydroxyl groups at the cleavage site.

RNase A digestion

Reactions were carried out as described above. After incubation for 5 days the samples were shock frozen and kept at −80°C until analyzed. A 1 µl aliquot (50 000 c.p.m.) was transferred into a tube containing 18 µl 0.2 M sodium acetate buffer (pH 5 at room temperature). RNase A (10 U; Sigma) in 1 µl water was added and the samples were incubated at 37°C for 24 h. Samples were quenched with 1 µl 0.5 M EDTA solution (pH 8.0 at room temperature), evaporated to dryness and analyzed as previously described. A second sample was treated in the same way and then reincubated with a further 10 U enzyme for an additional 24 h to establish completeness of RNase digestion.

RNase T1 digestion

Reactions were carried out as described above. After incubation for 5 days the samples were shock frozen and kept at −80°C until analyzed. A 1 µl aliquot (50 000 c.p.m.) was transferred into a tube containing 18 µl 0.2 M Tris–HCl, 1 mM EDTA (pH 7.5 at room temperature). RNase T1 (10 U; Fluka) in 1 µl water was added and the samples were incubated at 37°C for 24 h. Sample work-up and analysis were performed in the same way as for the RNase A digests. A second sample was treated in the same way and then reincubated with a further 10 U enzyme for an additional 24 h to establish completeness of RNase digestion.

RESULTS

Extension of a UppG3′G primer on a C5TC4 template

The result of experiments in which the template was omitted are illustrated in Figure 2 A, lanes 1 and 2. When UppG3′G was incubated with 0.05 M 2-MeImpG alone a single product was obtained in ∼5% yield with mobility and resistance to RNase T1 appropriate for a 2′-5′ linked monoaduct (8,11). When 2-MeImpG was replaced by a mixture of 0.05 M 2-MeImpG and 0.05 M 2-MeImpA the amount of product formed did not change, suggesting that 2-MeImpG associates preferentially with the 3′-end of the primer.

The reaction of 2-MeImpG alone with primer (Fig. 1 c) in the presence of PNA C5TC4 template (Fig. 1 c) gave rise to four more or less equally abundant products in a combined yield of 37%
incorporation of A opposite T in the template is an
example of non-specific hydrolysis of RNA oligomers. 

In summary, incorporation of A opposite T in the template is an efficient reaction and displays high fidelity. The reaction on a PNA template is only slightly less efficient than that on DNA and has comparable fidelity.

Extension of a UppG3'T primer on a C5GC4 template

The reaction of 2-MeImpG with UppG3'T in the absence of template produced only one new product, whether or not 2-MeImpC was also present (Fig. 3A, lanes 1 and 2). We believe that this is the 2'-5' linked monoadduct (see above).

Reaction of 2-MeImpG in the absence of 2-MeImpC on the PNA C5GC4 template yielded a monoadduct in 19% yield and hardly any detectable longer products (Fig. 3A, lane 3). The corresponding reaction of 2-MeImpG on the DNA C5GC4 template (Fig. 3A, lane 6) produced a higher total yield of products (29%) and significant amounts of longer oligomers up to the decamer. Production of the decamer in 1–2% yield indicates that reaction on the DNA template proceeds by G-B base pairing on the DNA template.

The reaction of an equimolar mixture of 2-MeImpG and of 2-MeImpC on the PNA C5GC4 template (Fig. 3A, lane 4) produced 49% of oligomers up to the nonamer, which is formed by base pairing on the DNA template.
in 6% yield. The corresponding reaction of a mixture of 2-MeImpG and 2-MeImpC on DNA C5GC4 (Fig. 3A, lane 7) gave a higher yield of products (73%) than was obtained on the PNA template. The occurrence of larger fractions of longer oligonucleotides on the DNA template also suggests that the elongation reactions are more efficient on a DNA than on a PNA template. Reactions of mixtures of 2-MeImpG and 2-MeImpC at equimolar concentration reduced the fraction of products originating from guanosine read-through to <3% for the PNA and <1% for the DNA template. Addition of 2 equiv 2-MeImpC to the reaction of 2-MeImpG on the PNA C5GC4 template reduced misincorporation of guanosines further and improved the overall reaction yield to 53%. Neither the yield nor the extent of misincorporation was influenced by addition of a second equivalent of 2-MeImpC in the reaction with 2-MeImpG on the DNA C5GC4 template.

The RNase T1 digestions revealed that the majority of read-through product from reactions with 2-MeImpG alone on the PNA template (Fig. 3B, ii, lane 3) is 2′-5′ linked. The corresponding reaction on a DNA template (Fig. 3B, ii, lane 6) yielded a majority of 2′-5′ linked monoadduct, which is not extended further, and a minority of 3′-5′ linked product which is extended to longer oligomers. RNase T1 digestion (Fig. 3B, ii, lanes 4, 5, 7 and 8) of the products from reactions of mixtures of 2-MeImpG and 2-MeImpC on either template proved that the majority of the first new phosphodiester is 3′-5′ linked. Small amounts of 2′-5′ linked pentamers that are formed are not further elongated. The relative amount of read-through products accumulated in the pentameric band was higher for reactions on the PNA template than on the DNA template.

RNase A digestion (Fig. 3B, i, lanes 2–10), which selectively cleaves oligomers after pyrimidine residues, confirmed that only cytosine was incorporated opposite guanosine on both PNA and DNA templates. The mobility of the product of RNase A digestion corresponded to that expected for the primer elongated by one C residue and terminated by a 3′-phosphate group.

In summary, incorporation of C opposite G on a PNA template is fairly efficient and has reasonably high fidelity. It is somewhat less efficient than the corresponding reaction on a DNA template. Incorporation of C opposite G is somewhat less efficient than incorporation of A opposite T, whether a PNA or a DNA template is used.

Extension of a UppG3rG primer on a C5AC4 template

Reactions of 2-MeImpG in the presence or absence of 2-MeImpU and in the absence of template (Fig. 4A, lanes 1 and 2) gave results analogous to those previously described for non-template reactions involving A or C. The yield of the single monoadduct obtained with 2-MeImpG was not affected by addition of 2-MeImpU to the reaction mixture. Failure of RNase T1 to digest this monoadduct shows that it contains a 2′-5′ phosphodiester bond.

Mismatches read-through reactions containing only 2-MeImpG as substrate and PNA C5AC4 as template (Fig. 4A, lane 3) gave 20% guanosine oligomers up to the nonamer. This result for misincorporation of guanosines opposite adenosine of PNA C5AC4 is similar to that described previously for the G-G read-through on PNA C5GC4. Read-through on the corresponding DNA C5AC4 template yielded a total of 35% (Fig. 4A, lane 6) of products. The longest detected product for reaction of 2-MeImpG on the DNA template was the decamer.

Products of reaction of 2-MeImpG in the presence of 2-MeImpU on the PNA C5AC4 template are illustrated in Figure 4A, lanes 3–6. It is immediately clear that incorporation of U opposite A in the template was the least efficient of the three reactions we have studied. Additionally, uracil-containing products were not clearly separated from homoguanosine oligonucleotides of equal length. This makes interpretation of the gel pattern difficult. It is only possible to deduce the nature of the products indirectly with the aid of results obtained from RNase digestion (Fig. 4B).

Reaction of an equimolar mixture of 2-MeImpG and 2-MeImpC on a PNA C5AC4 template is illustrated in Figure 4A, lane 4. It gave a 23% total yield of elongation products. A 3′-5′ linked pentamer was the predominant product (9.2%); longer oligomers were produced in ∼7.3% total yield. The hexamer and...
longer oligomers were obtained in roughly equal amounts, indicating that the rate limiting step in formation of longer oligomers was addition of 2-MeImpG to primer that had already been elongated by one residue. Reaction of an equimolar mixture of 2-MeImpG and 2-MeImpU on the corresponding DNA C₅AC₄ template (Fig. 4A, lane 7) gave a higher yield of oligomers (38%). In general, longer oligomers formed a higher proportion of products in reactions on a DNA template than on the corresponding PNA template, suggesting that elongation beyond the first added residue is more efficient on DNA. Doubling the concentration of 2-MeImpU in the reaction mixture had little effect on the products of these reactions (Fig. 4A, lanes 5 and 8).

The results of RNase digestion are of particular interest for this case, since PAGE separation of untreated reaction samples was insufficient to discriminate uridine-containing products from products of misincorporation of G. RNase T₁ digestion (Fig. 4B, ii, lanes 2–10) confirmed that the majority of the longer products were formed in reaction sequences in which the first addition led to a 3′-5′ phosphodiester linkage. All products with retention times longer than the primer were cleaved to give a product, C, with the expected mobility of the 3′-phosphate of the primer. RNase T₁ digestion, however, did not distinguish between U with the expected mobility of the 3′-phosphate of the primer were cleaved to give a product, C, of the reactions that we have studied, whether a PNA or a DNA template is used. The efficiency of incorporation of U is greater on a DNA template than on a PNA template.

**Extension of a UppG₄G primer on C₅XC₄ templates**

If G-T wobble base pairing and G-G or G-A mispairing are important, replacement of UppG₄G by UppG₄rG as primer should inhibit incorporation of the correct bases on our templates (Fig. 5A) and facilitate formation of homopolymeric oligoGs. This is clearly confirmed by the electrophoretograms. In general, the primers elongated efficiently when only 2-MeImpG was present and either equally or less efficiently when a second base was added. Furthermore, the mobilities of the major bands were the same and consistent with homoG oligomers, whether a second base was present or not. This contrasts with our results obtained with the shorter primer and confirms that even in the presence of the correct base, mispairing with the template is the most productive mode of association for UppG₄rG.

**Control reactions of primers on a PNA C₁₀ template**

We have included two control experiments using a PNA C₁₀ in all our experiments (lanes 9 and 10 in all figures). Reaction of 2-MeImpG alone on this template was described previously (11). Addition of any other activated ribonucleotide 2-MeImpX (X = A, C or U) to reactions of 2-MeImpG on PNA C₁₀ had no influence on the yield or the product distribution. We found no evidence for incorporation of bases other than G on PNA C₁₀.

**DISCUSSION**

The most important conclusion that can be drawn from the results of our experiments is that information can be transferred from PNA to RNA in a template-directed primer extension reaction. Efficiency of the reactions was somewhat lower on a PNA than on a DNA template, but this is not surprising and probably not significant. The 2-methylimidazolides of the nucleosides were
selected to optimize efficiency of oligomerizations on RNA and DNA, but no corresponding search for the optimum substrate has been made in the case of reactions on the PNA template. It is entirely possible that the relative efficiency would be reversed if we used substrates that were optimized for template-directed reactions on PNA.

Misincorporation of G opposite T, G or A in the template was always significant when there was no second base present in the reaction mixtures. However, the presence of the base complement to the heterobase in the template suppressed misincorporation of G almost completely.

The regioselectivity of the template-directed reactions was similar on DNA and PNA templates. On both templates the heterobase was incorporated via a 3'-5' phosphodiester bond. Furthermore, extension of the 3'-5' linked monoaduct occurred almost entirely via addition of further 3'-5' linked G residues. These results were not unexpected, since we already had evidence that a trimer bound to PNA was long enough to guarantee a preference for formation of 3'-5' linkages (11,20). Perhaps surprisingly, misincorporation of G opposite heterobases in the template also seemed to be regiospecific, with a preference for 3'-5' linked G residues.

Incorporation of nucleotides opposite internal residues on a template was assisted by neighboring template-bound nucleotides. Consequently, addition of the last nucleotide on a ribonucleic acid template was assisted by neighboring template-bound nucleotides. Therefore, loss of the terminal base during template-directed replication was an even more severe problem for PNA than RNA templates.

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