Strand Displacement Binding of a Duplex-Forming Homopurine PNA to a Homopyrimidine Duplex DNA Target

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Molecules that bind sequence specifically to double-stranded DNA in a predictable way are of high interest in chemistry, biology, and medicine, in particular if the molecules can be designed to bind desired DNA targets. In this context, triple-helix-forming oligonucleotides[1] and, more recently, strand-invading peptide nucleic acids (PNA)[2] have attracted considerable attention.

Sequence-specific binding of homopyrimidine PNAs to homopurine targets in double-stranded DNA takes place via strand displacement upon the formation of an internal Watson−Crick−Hoogsteen base-paired PNA_2−DNA triplex.[3] Such binding is thermodynamically favored because of the extraordinary stability of PNA_2−DNA triplexes employing the homopyrimidine triplex motif involving T·A-T and C^+·G-C Hoogsteen−Watson−Crick triplets, in which the two PNA pyrimidine strands are preferably antiparallel.[4] However, both the binding efficiency and sequence discrimination are kinetically controlled.[5]

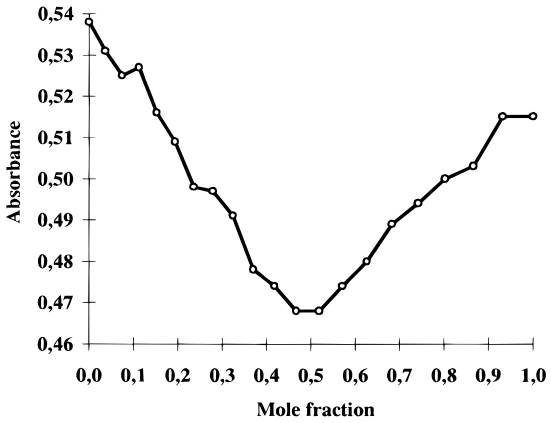

Figure 1. Titration of the binding of PNA H-GAGAGGAAAA-$LysNH_2$ to oligonucleotide 5′-d(TTTTCCTCTC) (Job plot). The measurements were performed at 20 °C in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0.

It is well established that DNA can also form triplexes in which the third strand is homopurine. This triplex motif involves A·A-T and G·G-T triplets in which the two purine strands are antiparallel.[1c] In order to explore the possibilities of this purine triplex motif in a PNA context and thereby potentially expand the recognition repertoire of PNA, we synthesized a homopurine decamer PNA: AAAAGGAGAG in either of the two orientations.[6]

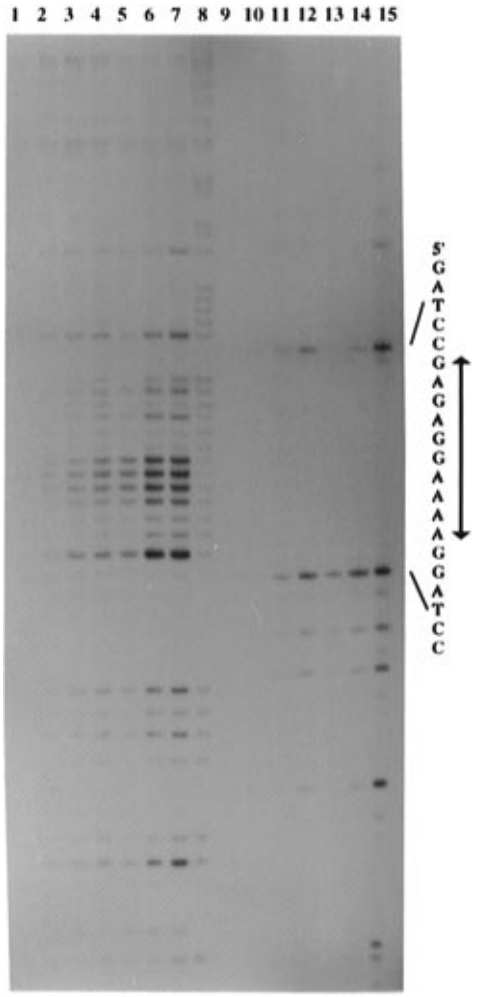

Figure 2. Binding of PNA H-AAAAGGAGAG-$LysNH_2$ (parallel, lanes 2−4 and 10−12) or H-GAGAGGAAAA-$LysNH_2$ (antiparallel, lanes 5−7 and 13−15) to a double-stranded 5′-d(TTTTCCTCCT) DNA target cloned into the *Bam*H1 site of pUC19. The plasmid was cleaved with *Pvu*II and 3′-^{32}P-labeled at the *Hin*dIII site. The 156 bp *Hin*dIII−*Pvu*II fragment was purified by gel electrophoresis and incubated with 0 (lanes 1 and 9), 1 (lanes 2, 5, 10, and 13), 3 (lanes 3, 6, 11, and 14), or 10 μM (lanes 4, 7, 12, and 15) PNA for 1 h in 10 mM Tris-HCl, pH 7.2 at room temperature. Probing with diethyl pyrocarbonate (DEPC) (5%) was performed for 5 min at room temperature, and probing with $KMnO_4$ (1 mM) was performed for 15 s at room temperature. DEPC or $KMnO_4$ reaction was detected as DNA strand breaks following piperidine treatment. An A + G sequence reaction was run in lane 8. The DNA samples were analyzed by high-resolution polyacrylamide gel electrophoresis and autoradiography. The autoradiogram is presented in the figure (for further details, cf.: Jeppesen, C.; Nielsen, P. E. *FEBS Lett.* **1988**, *231*, 172−176). The sequence around the PNA target (arrow) is indicated to the right.

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Table 1. Thermal Stability of PNA−DNA Complexes[a]

	T_m, °C	
oligonucleotide	H-AAAAGGAGAG-$LysNH_2$	H-GAGAGGAAAA-$LysNH_2$
5′-d(CTCTCCTTTT)	69	57
5′-d(TTTTCCTCTC)	56	67
5′-d(TTTTCCACTC)	50	
5′-d(CTCACCTTTT)	52	
H-CTCTCCTTTT-$LysNH_2$	70	50
H-TTTTCCTCTC-$LysNH_2$	50	69

[a] Buffer: 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0. Heating rate: 0.5 deg/min at 5−90 °C.

The binding of these PNAs to complementary deoxyoligonucleotides was analyzed by thermal denaturation (Table 1). The results revealed a surprisingly high T_m (67−69 °C)[7] for the antiparallel complex (which is approximately 10 °C more stable than the parallel complex). Previously we have found that decamer mixed-sequence PNA−DNA duplexes melt around 55 °C,[8] whereas T_m's around 70 °C are characteristic of decamer PNA_2−DNA triplexes.[4] Since, in analogy to DNA triplexes, homopurine PNAs might form PNA−PNA−DNA triplexes, we determined the stoichiometry of the complex by Job plot analysis (Figure 1), which clearly indicated the complex to be a PNA−DNA duplex. Furthermore, the thermal transition did not show hysteresis which is otherwise characteristic of triplex formation with monomeric PNA.[9]

Thus this homopurine PNA−DNA duplex has a thermal stability comparable to that of a PNA_2−DNA triplex and could therefore be expected to bind to a double-stranded DNA target by strand displacement. Indeed, as shown by the experiment presented in Figure 2, upon incubation with the PNA, adenines of a double-stranded DNA target becomes reactive toward diethyl pyrocarbonate, an acylation reagent that reacts preferentially with adenines and to a lesser extent with guanines (at the N7 (or N3) position) of a perturbed DNA helix or a single-stranded DNA region.[10] We therefore take this reactivity as evidence for strand invasion.[11] This conclusion is further substantiated by $KMnO_4$ probing experiments which showed that thymines proximal to the putatively displaced PNA target reacted with permanganate in the presence of PNA (Figure 2, lanes 9−15). Thus, as previously observed for homopyrimidine PNA−dsDNA strand displacement complexes,[3a,c] the DNA duplex flanking the PNA target is sufficiently perturbed to expose thymines to attack by permanganate. Furthermore, the PNA antiparallel to the target bound more efficiently than the parallel PNA.[12] We were not able to detect binding by gel shift, and consequently, the complex must be of lower stability than the homopyrimidine triplex strand displacement complexes, despite the comparable thermal stabilities of the PNA−DNA complexes involved. Therefore, strand displacement by PNA appears not to be solely a consequence of a very stable complex with the target DNA strand. Other factors which could be of dynamic nature must also play an important role.

In conclusion, we have shown that a duplex-forming PNA can bind to a double-stranded DNA target by strand invasion, albeit with lesser efficiency. Thus general PNA strand invasion by mixed purine−pyrimidine sequence PNA should be possible using PNAs that bind their single-strand targets more strongly. However, the results also show that other properties of the complex are of importance (most probably related to dynamics) and need to be analyzed and controlled.

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(7) An analysis of seven PNA−DNA decamer complexes indicates the thermal stability of these (to a first approximation) to be linearly correlated to the purine content of the PNA strand, and not, as seen for DNA−DNA duplexes, directly correlated to the total G-C content.

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(11) Chemical probing with dimethyl sulfate of guanine N7 of the DNA target did not show any protection, and we therefore see no indication of conventional PNA−DNA_2 triplex binding to the target.

(12) At higher PNA concentrations both the diethyl pyrocarbonate and the permanganate hypersensitivity extended partly through the short (30 base pairs) and thus easily denaturable end of the DNA fragment (Figure 2, lanes 7 and 15). We ascribe this to unspecific PNA binding induced by the target-specific PNA binding.