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DNA Binding Properties of Oligodeoxynucleotides Containing Pyrrolidino *C*-Nucleosides

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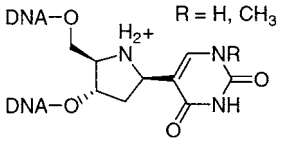
ABSTRACT



We have incorporated pyrrolidino-*C*-nucleosides (pyrrolidino-pseudonucleosides) containing the base uracil and *N*-1-methyl uracil into oligodeoxynucleotides and compared their thermal duplex and triplex stabilities with unmodified or pseudouridine-containing oligodeoxynucleotides. We find relative destabilizations of triplex formation by ca. −13 to −1 °C per modification (relative to thymidine) in a strongly sequence dependent mode. Duplex formation is less destabilizing and more homogeneous with −4 to −2 °C per modification.

In our ongoing search for oligonucleotide analogues with enhanced binding properties to double stranded DNA, we became interested in triplex-forming oligonucleotides (TFOs) containing pyrrolidino-*C*-nucleosides. The design strategy for enhanced duplex target recognition was based on earlier findings on 2′-aminoethoxy-modified TFOs. Here, dual recognition of the target purine strand via base−base interaction and via salt bridge formation between a phosphate residue and the charged aminoethyl side-chain was proposed to be responsible for the observed, strong increase in affinity to DNA targets.[1] This opened the way to interesting biological applications.[2]

We reasoned that a similar dual recognition could occur with the pyrrolidino-*C*-nucleosides dpψU and dpψT (Figure 1) by recognition of an adenine base by the uracil or the *N*-1-methyl-uracil (structural thymidine equivalent) base, and a nonbridging *pro-R*-phosphate oxygen at position $n + 1$ that is recognized by the charged pyrrolidino nitrogen (Figure 1). Distances between the nitrogen and the *pro-R*-oxygen were expected to be in the range of 3.2 to 4.0 Å, based on a recent X-ray structure of a partial DNA-triplex.[3] The C-glycosidic bond in the pyrrolidino nucleoside analogues is necessary to confer chemical stability and basicity to nitrogen. It has been shown previously that the aminal function in pyrrolidino nucleosides is unstable unless the ring nitrogen is acylated.[4]

We have already reported on the synthesis of the corresponding nucleosides dpψU and dpψT, and the corresponding phosphoramidite building blocks, in which the pyrrolidino ring nitrogen is Fmoc-protected.[5] We now incorporated these building blocks into oligodeoxynucleotides using standard solid-phase phosphoramidite chemistry on a DNA-synthesizer. Coupling efficiences were typically >97%. Standard ammonia deprotection led to the cleavage of all base and phosphate protecting groups, including the base-labile Fmoc groups on the pyrrolidino ring nitrogen. All modified

(1) (a) Cuenoud, B.; Casset, F.; Hüsken, D.; Natt, F.; Wolf, R. M.; Altmann, K.-H.; Martin, P.; Moser, H. E. *Angew. Chem., Int. Ed.* **1998**, *37*, 1288−1291. (b) Blommers, M. J.; Natt, F.; Jahnke, W.; Cuenoud, B. *Biochemistry* **1998**, *37*, 17714−25. (c) Sollogoub, M.; Darby, R. A.; Cuenoud, B.; Brown, T.; Fox, K. R. *Biochemistry* **2002**, *41*, 7224−31.

(2) (a) Puri, N.; Majumdar, A.; Cuenoud, B.; Natt, F.; Martin, P.; Boyd, A.; Miller, P. S.; Seidman, M. M. *J. Biol. Chem.* **2001**, *276*, 28991−98. (b) Puri, N.; Majumdar, A.; Cuenoud, B.; Natt, F.; Martin, P.; Boyd, A.; Miller, P. S.; Seidman, M. M. *Biochemistry* **2002**, *41*, 7716−24.

(3) Rhee, S.; Han, Z.-J.; Liu, K.; Miles, H. T.; Davies, D. R. *Biochemistry* **1999**, *38*, 16810−15.

(4) (a) Altmann, K.-H. *Tetrahedron Lett.* **1993**, *34*, 7721−24. (b) Altmann, K.-H.; Freier, S. M.; Pieles, U.; Winkler, T. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1654−57.

(5) Häberli, A.; Leumann, C. J. *Org. Lett. 2001*, *3*, 489−92.

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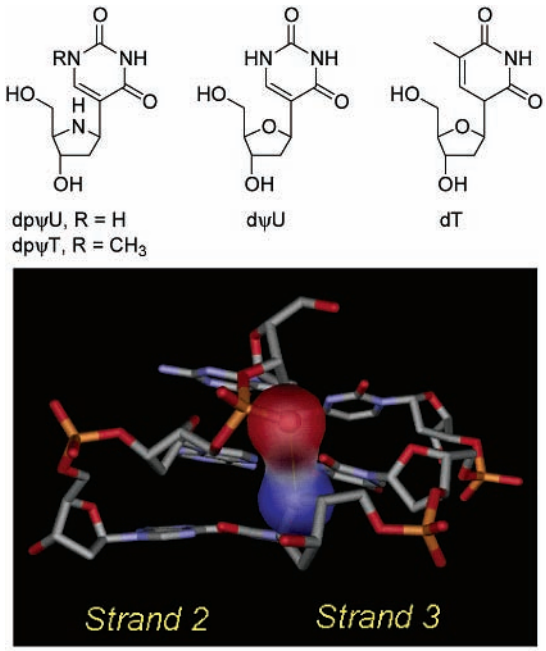

Figure 1. Top: Chemical structures of the nucleoside analogues under investigation. Bottom: Anticipated phosphate recogniton by the protonated pyrrolidine ring nitrogen as modeled into a segment of a DNA parallel triplex. Strand 2 denotes the purine strand and strand 3 the TFO strand. The Watson−Crick pyrimidine strand was omitted for clarity.

oligonucleotides were routinely analyzed by ESI-MS (see Supporting Information).

To investigate the triple-helix formation in the parallel binding motif, we prepared the TFO 15-mers as well as their double stranded DNA target 21-mer described in Table 1. Thermal stabilities (T_m data) of triplexes were obtained from UV-melting curve analysis at pH 6.0. At this pH the pyrrolidino ring nitrogen is expected to be fully protonated. NMR titration of the free nucleoside analogue dpψU revealed a pK_a of 7.9 (Supporting Information). As expected, de- and renaturation curves at the applied temperature gradient of 0.5 °C/min were not superimposable due to slow binding kinetics. Table 1 summarizes T_m data determined from the melting curves.

From inspection of the data in Table 1, two major differences between the target affinity of the pyrrolidino-containing TFOs relative to the unmodifed TFO become apparent. First, the TFOs containing dpψT bind less efficiently to their target than TFOs containing the nonmethylated dpψU. Second, there is a large sequence effect in target binding, depending on the nearest neighbors of the pyrrolidino nucleosides (TFOs **1**−**3**). Two dT as nearest neighbors destabilize least (ΔT_m/mod ca. −1 to −5 °C), a dC and a dT unit intermediate (ΔT_m/mod ca. −4 to −6 °C), and two dC residues most (ΔT_m/mod ca. −10 to −12 °C). The ΔT_m/mod data seem to be additive, as the T_m values of TFOs with two or more pyrrolidino residues (TFOs **4**−**6**) do not dramatically deviate from the expected values, irrespective of whether the pyrrolidino nucleosides are next to each other

Table 1. T_m Data (260 nm) of TFO-Melting from the Duplex Target

duplex	5'-d-CGTAAAAAGAGAGAGAGATCG		
target	3'-d-CGATTTTTCTCTCTCTCTAGC		
TFOs	**1**	5'-d-TTTTTCXCTCTCTCT	
	2	5'-d-TTTTXCTCTCTCTCT	
	3	5'-d-TTTXTCTCTCTCTCT	
	4	5'-d-TTTXXCTCTCTCTCT	
	5	5'-d-TTTXTCTCTCXCTCT	
	6	5'-d-XXXXXCTCTCTCTCT	
TFO	modification	Tm[a)]	ΔTm/mod[b)]
1	X=dpψU	33.0	-10.1
	X=dpψT	30.3	-12.8
	X=dψU	38.0	-5.1
2	X=dpψU	38.4	-4.7
	X=dpψT	37.0	-6.1
	X=dψU	41.5	-1.6
3	X=dpψU	40.7	-2.4
	X=dpψT	38.1	-5.0
	X=dψU	41.8	-1.3
4	X=dpψU	35.8	-3.7
	X=dpψT	34.3	-4.4
	X=dψU	37.9	-2.6
5	X=dpψU	29.6	-6.8
	X=dpψT	23.9	-9.6
	X=dψU	36.1	-3.5
6	X=dpψU	37.5	-1.1

[a] Single strand concentration = 1.2 μM. Buffer: 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$, pH 6.0. T_m of target duplex = 57.0 ± 1.0 °C. [b] T_m of reference triplex with 5′-d-TTTTTCTCTCTCTCT = 43.1°C.

or spaced by 6 nt. Interestingly one exception is TFO **6**, in which the five consecutive dpψU units are less destabilizing than expected from the ΔT_m/mod data. The TFOs containing deoxypseudouridine, dψU, behave similarly although the corresponding triplexes are more stable compared with that of the pyrrolidino TFOs, but in all cases less stable than that of the unmodified reference TFO.

From the available data on dψU and dpψU it can be concluded that both the sugar and the attachment of the base contribute to the destabilizing effect observed for the pyrrolidino TFOs, relative to a dT unit. An interesting and unexpected finding is the discrepancy in triplex stability between dpψT and dpψU containing TFOs. While it is generally accepted that a methyl group in position 5 of the pyrimidine nucleosides promotes triplex formation, due to advantageous hydrophobic interactions,[6] the opposite is encountered here. A possible reason for this behavior might be a preference for the syn conformation of the base in the pyrrolidino nucleosides due to intramolecular H-bond formation (Figure 2a). Accordingly, dpψT would have to adopt the less preferred anti-conformation, as in Figure 2b, to recognize the target base adenine by two H-bonds, which would lead to a negative effect on triplex stability.

(6) Xodo, L. E.; Manzini, G.; Quadrifoglio, F.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1991**, *19*, 5625−31.

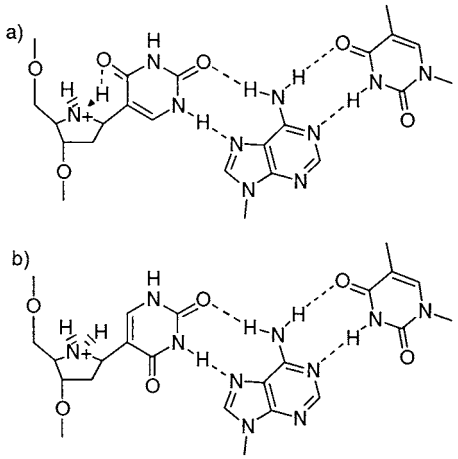

Figure 2. Two possible modes of Hoogsteen recognition of adenine by dpψU. The syn conformation, as in part a, could be preferred due to H-bond formation between O(4) of the base and the protonated pyrrolidino ring nitrogen.

The fact that dC-residues as nearest neighbors destabilize triple-helix formation in the pyrrolidino-TFOs might have to do with a disadvantageous intrastrand accumulation of positive charges, as the base cytosine in a third strand needs to be protonated as well in order to form a Hoogsteen base-pair with its target guanine. Alternatively differences in the distance between the backbones of the purine strand and the TFO as a function of the base sequence might modulate the electrostatic contribution to binding of the presumed salt bridge. The question on why no stabilization at all is observed due to charge complementation, as in the case of the 2′-O-aminoethyl TFOs, remains open so far and calls for high-resolution structural data. It is not excluded that the distances of ca. 3−4 Å, as inferred from the X-ray structure based model, are too long for efficient salt bridge formation, and that there is no space to contract the two backbones of the directly interacting strands without energetic penalty.

Having the oligonucleotides **1**−**6** at hand we also approached the question on how pyrrolidino nucleosides influence DNA double helix formation. We therefore prepared the antiparallel DNA complement to **1**−**6** and collected the corresponding T_m data (Table 2).

As expected, all de- and renaturation curves in the duplex series were superimposable at the applied temperature gradient of 0.5 °C/min, reflecting equilibrium binding conditions. With regard to affinity, the same order as for the triplex stability applies, that is dT > dψU > dpψU > dpψT. The relative destabilization in general is less extreme (−1.8 to −5.9 °C/mod) as in the case of the corresponding triplexes. Interestingly, the observed sequence effects are much less pronounced and even inverted in the duplex context. Thus, dC residues as nearest neighbors are less destabilizing than dT residues next to the pyrrolidino nucleosides. In the case of the pseudouridine (dψU)-containing oligonucleotides, roughly the same sequence specificity order is observed. Again, the ΔT_m/mod values

Table 2. T_m Data (260 nm) for Melting of the Duplexes between **1**−**6** and Their Antiparallel DNA Complement

DNA target		3'-d-AAAAAGAGAGAGAGA	
	1	5'-d-TTTTTCXCTCTCTCT	
	2	5'-d-TTTTXCTCTCTCTCT	
	3	5'-d-TTTXTCTCTCTCTCT	
	4	5'-d-TTTXXCTCTCTCTCT	
	5	5'-d-TTTXTCTCTCXCTCT	
	6	5'-d-XXXXXCTCTCTCTCT	
TFO	modification	T_m[a]	ΔT_m/mod[b]
1	X=dpψU	42.2	-3.0
	X=dpψT	40.4	-4.8
	X=dψU	43.8	-1.4
2	X=dpψU	41.1	-4.1
	X=dpψT	39.6	-5.6
	X=dψU	43.4	-1.8
3	X=dpψU	40.7	-4.5
	X=dpψT	39.6	-5.6
	X=dψU	42.9	-2.3
4	X=dpψU	36.7	-4.3
	X=dpψT	36.1	-4.6
	X=dψU	39.8	-2.7
5	X=dpψU	37.9	-3.7
	X=dpψT	33.5	-5.9
	X=dψU	39.7	-2.8
6	X=dpψU	36.2	-1.8

[a] Single strand concentration = 1.2 μM. Buffer: 140 mM KCl, 7 mM NaH_2PO_4, 0.5 mM $MgCl_2$, pH 7.0. [b] T_m of reference duplex with 5′-d-TTTTTCTCTCTCTCT = 45.2 °C.

seem to be additive within this simple nearest neighbor context, irrespective of the number and position of the pyrrolidino modifications.

Oligonucleotides containing pyrrolidine substitutes for deoxyribose with unnatural bases or as abasic units have been prepared in the past and investigated as inhibitors for DNA glycosylases[7] or as inhibitors for the cytotoxic heterodimeric protein ricin.[8]

The first preliminary results on the base-pairing properties of pyrrolidino pseudonucleosides, as reported here, leave a number of questions open with regard to molecular recognition of DNA duplex targets that need to be addressed in the future. Efforts toward the synthesis of pyrrolidino pseudoisocytidine and of fully modified, zwitterionic pyrrolidino-DNA are currently underway and will be reported in due time.

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(7) (a) Schärer, O. D.; Nash, H. M.; Jiricny, J.; Laval, J.; Verdine, G. L. *J. Biol. Chem.* **1998**, *273*, 8592−97. (b) Deng, L.; Schärer, O. D.; Verdine, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 7865−66.

(8) Chen, X.-Y.; Link, T. M.; Schramm, V. L. *J. Am. Chem. Soc.* **1996**, *118*, 3067−68.

Supporting Information Available: Experimental procedures for oligonucleotide synthesis, melting curve analysis, MS-data for TFOs, and pK_a measurement of dpψU. This material is available free of charge via the Internet at http://pubs.acs.org.

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