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Antiparallel Thymine-Thymine-Duplexes in Oligonucleotides Containing α- and β-Thymidine

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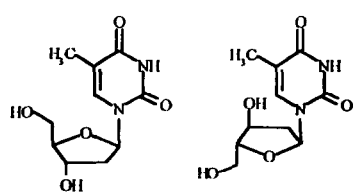
Abstract: *Oligonucleotide-sequences (14 to 21 nucleotides in length) consisting only of α-thymidine, and alternating ($^{\alpha}$T-$^{\beta}$T)-sequences were shown to form antiparallel duplex-structures as determined by UV melting curves and CD spectroscopy.*

The base-recognition mode in natural nucleic acids is crucial to their biological role and prompts on one hand the question on the influence of the nature of the bases[1,2], and on the other hand the question about the role of the underlying sugar-phosphate-backbone in the mode and selectivity of base-pairing[3]. It has recently become clear that besides the Watson-Crick type of complementary base-pairing, also Hoogsteen type of purine-pyrimidine-pairing[4] (as in triple-helices of the (U-A-U), (T-A-T) and (C^+-G-C) type)[5-7] and purine-purine-pairing (as in triple-helices of the (A-A-T) and (G-G-C)-type[8,9] and in G-tetrads[10,11]) occur in natural DNA. In oligonucleotides consisting of 2',3'-dideoxy-β-D-glucopyranosyl nucleosides, purine-purine base-pairs of the (A-A)- and (G-G)-type were observed besides the natural Watson-Crick (A-T)- and (G-C)-base-pairs[12]. Pyrimidine-pyrimidine base-pairing so far was only observed in the case of poly(S^2U) and polyC (at low pH)[13], as well as in a phosphate-methylated hexamer of β-thymidine[14]. In our investigations of the pairing properties of oligonucleotides containing α- and β-thymidine, we observed self-pairing in pure α-thymidine- and in alternating (α,β)-thymidine sequences. While for an α-thymidine-octamer, a duplex structure was already proposed based on CD spectroscopy[15,16], the second pairing-motif is new. Since no indication on the structure of the α-thymidine duplex was given, we decided to explore the pairing behavior of both types of oligonucleotides.

Figure 1

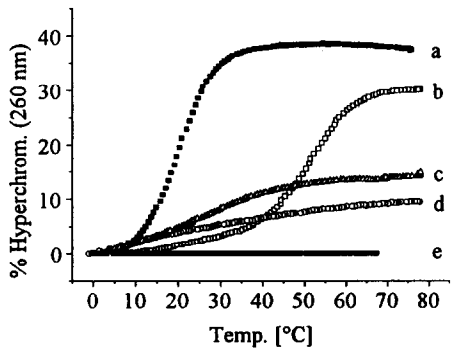


β-Thymidine ($^{\beta}$T) α-Thymidine ($^{\alpha}$T)

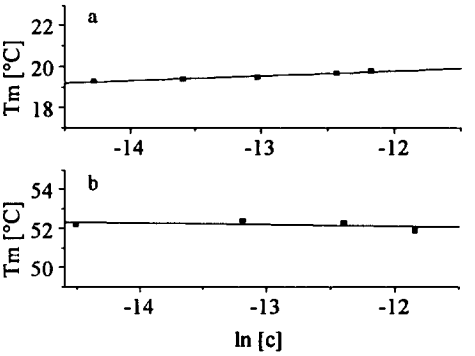
1 d[($^{\alpha}T_{20}$)-C]
2 d($^{\alpha}$T-$^{\beta}$T)$_{10}$
3 d($^{\alpha}$T-$^{\beta}$T)$_7$
4 d($^{\alpha}T_{10}$-$^{\beta}T_{10}$)
5 d($^{\beta}$T)$_{20}$

The sequences **1-5** shown in (Fig. 1) were prepared according to standard cyanoethyl phosphoramidite chemistry on a DNA synthesizer. The compatibility of both anomeric nucleoside-forms in the synthesis was previously established[17]. Crude oligonucleotides were purified by ion exchange- and/or reversed phase-hplc to homogeneity in both chromatographic systems.

Figure 2

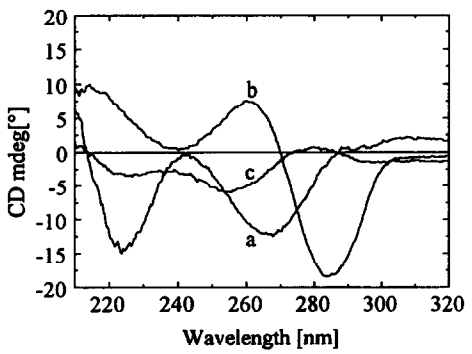


UV-melting-curves of a) 2 (4.5μM), b) 1 (1.9μM), c) 4 (2.5μM) in 10 mM Tris·HCl, 50 mM $MgCl_2$, pH 7.0, and d) 1, e) 2 in 10mM Tris·HCl, 8M urea, pH 7.0.

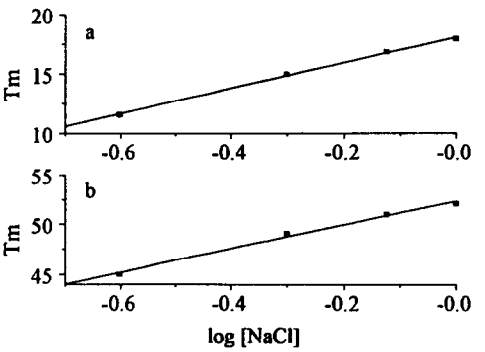


Melting-temperatures vs oligonucleotide-concentrations: a) 2 and b) 1 in 10mM Tris·HCl, 50 mM $MgCl_2$, pH 7.0.

Figure 3



CD-spectra of a) 1 (2μM, 4°C) in 10 mM sodium-cacodylate, 0.1M NaCl, pH 7.0, and b) 2 (4.3μM, 9°C), c) 4 (4.1μM, 9°C) in Tris·HCl, 50 mM $MgCl_2$, pH 7.0.



Melting-temperature vs. log([NaCl]) in Tris·HCl, pH 7.0: a) 2 (4.2μM), b) 1 (2.6μM).

In neutral buffer containing 50 mM of $MgCl_2$ both, the pure (αT)-sequence **1** and the alternating (αT-βT)-sequence **2** showed cooperative and reversible UV melting curves (Fig. 2, left) with T_m-values of 52.2° and 19.2° resp. and hyperchromicities of 30% and 37% resp. The melting profile still remains in both, if no electrolyte is added to the buffer. Addition of urea (8M) results in complete loss of any cooperative transition and reduces the hyperchromicity to 9% for **1** and to 0% for **2**. Also the shorter tetradecamer **3**

shows a sharp melting-transition in neutral buffer containing NaCl and spermine (Tab.1). Omission of salt and spermine thus abolishes this transition completely. Under the conditions mentioned, sequence **5** shows, as expected, no melting at all. These results indicate that **1**, **2** and **3** do form base-paired duplex structures. In order to assess the molecularity of the transitions, we measured melting curves of **1** and **2** at different oligonucleotide concentrations. From the fact, that essentially no change in T_m-values over a 10-15 fold concentration range was observed (Fig. 2, right), we conclude that in both cases *intramolecular* base pairing occurs. This is only conceivable if one infers *hairpin*-structures in which the base-paired region is necessarily aligned in an antiparallel orientation. A further indication for hairpin formation comes from the higher electrophoretic mobility of **1** with respect to **5** in a 20%-nondenaturing acrylamide gel (data not shown). From differentiated UV melting curves we calculated *van't Hoff* duplexation enthalpies and -entropies for **1** and **2** (50mM $MgCl_2$, Tab.1) according to the method of *Marky & Breslauer*[18]. Inspection of these data show that the pairing enthalpy in **2** is stronger by approximately 20 kcal/mol with resp. to **1**. The fact that **1**, however, is thermodynamically more stable than **2**, is a consequence of the unfavorable pairing entropy in the alternating sequence **2**.

The CD spectra of **1** and **2** (Fig. 3, left) reflect the considerable differences between the two structures. We analyzed the pairing behavior of the block sequence **4** by means of CD-spectroscopy and found the spectrum of **4** to be of the same type as that of **1**, indicating ($^{\alpha}$T-$^{\alpha}$T)-pairing with the ($^{\beta}$T)-end of **4** unpaired. The oligonucleotides **1** and **2** also show a linear dependence of the melting temperatures from the log([NaCl]) in the concentration range 0.25-1M (Fig. 3, left). The slopes of plots of T_m vs. log([NaCl]) for **1** and **2** (Tab. 2) are in the same range as values obtained for natural DNA-hairpins[19].

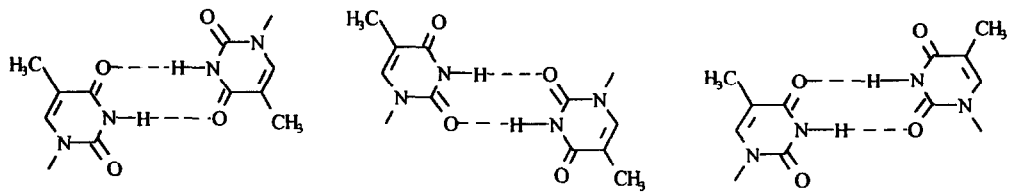
Table 1

	Tm [°C] 50 mM $MgCl_2$	Tm [°C] No salt	ΔH_{vH} [kcal/mol]	ΔS_{vH} [cal/K·mol]	$\Delta G^{25°}$ [kcal/mol]	δTm/δlog[NaCl]
1 (1.9μM)	52.2	29.6	-31.9	-98.1	-2.6	11.7
2 (4.5μM)	19.2	~0	-52.4	-179.3	1.2	10.6
3 (2.4μM)	9.6*	---	---	---	---	---

*10mM Tris.HCl, pH 7.0; *) +1mM spermine, 0.1M NaCl, no $MgCl_2$;*

It is clear that the data presented here are not sufficient to describe a detailed structure of the two types of duplexes. While only ($^{\alpha}$T-$^{\alpha}$T)-base-pairing can occur in the case of **1**, base-pairs of the type ($^{\alpha}$T-$^{\beta}$T), or alternating stretches of ($^{\alpha}$T-$^{\alpha}$T)- and ($^{\beta}$T-$^{\beta}$T)- base-pairs are possible in **2** and **3**. Three constitutional different base-pairs, held together by two hydrogen bonds each, have to be taken into consideration (Fig. 4). Simple model building, starting from antiparallel duplex structures and assuming that the nucleobases are anti-oriented in both, α- and β-thymidine (as generally observed in duplex-structures of α-[20] and β-DNA, and as observed in the free nucleosides themselves[21]), suggests a right-handed helix with base-pairs of the (WC-RWC)-type in **1** and with ($^{\alpha}$T-$^{\beta}$T)-base-pairs of the (WC-WC)- or (RWC-RWC)-type in **2** and **3** (Fig. 4). A detailed investigation of the mode of base-pairing still has to be done.

Figure 4



WC - WC RWC - RWC WC - RWC

It is interesting to note with respect to nature's choice of β-nucleosides as constituents of DNA and RNA, that the strength of self-association in thymine-containing oligo-deoxyribonucleotides is strongest in the case of their α-, weakest in their β-, and in between in their alternating α,β-anomeric form. Further exploration of the pairing behavior of oligonucleotides containing α- and β-nucleosides might lead to DNA-analogues with base-pairing selectivities different from that of natural DNA.

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