Azobenzene-Appended Oligonucleotides Form Unexpectedly Stable Triple-Helixes

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Modified oligo(T)s carrying an azobebzene at the 5'-ends form unexpectedly stable triple-helixes with oligo(A)/oligo(T) double-helix. The triplex-stabilizing activity of the azobenzene is comparable with (or greater than) that of thymine.

Recently, much attention has been focusing onto triple-helixes of DNA, because of their potential applications to biotechnology, therapy, and others.[1] However, they are rather unstable and only inefficiently formed under physiological conditions. Thus, stabilization of triple-helixes is one of the most urgent themes.[2] Furthermore, various functional residues should be attached to triple-helixes in order to extend their applications.

This paper reports a novel system, which fulfills both of these requirements. It is shown that oligo(T)s bearing an azobenzene at their 5'-ends form stable triple-helixes with oligo(A)/oligo(T) duplex.[3] Significantly, the activity of the azobenzene for the stabilization of triple-helix is comparable with (or even greater than) the activity of thymine, which forms Hoogsteen-type hydrogen-bonds with the Watson-Crick type A/T base-pair. The absence of hydrogen-bonding is satisfactorily compensated by other factors. Azobenzene has a number of important features as a modulator of the functions of triple-helixes: (1) easy chemical modification for the attachment of versatile functional moieties, (2) simple and well-defined structure, and (3) photo-induced structural change (*cis-trans* isomerization).

The modified oligonucleotides carry an azobenzene at the 5'-end of homothymidine, via different types of linkers (Figure 1). They were synthesized on an automated synthesizer by using the corresponding phosphoramidite monomer.[4] The

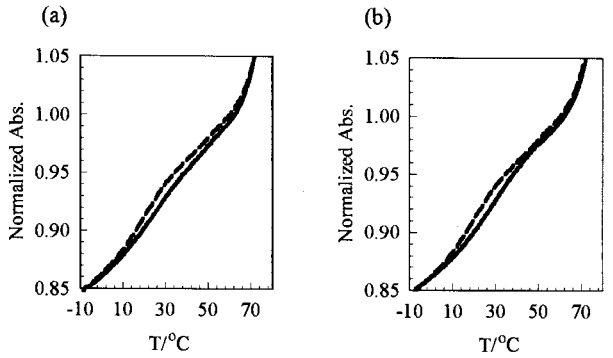
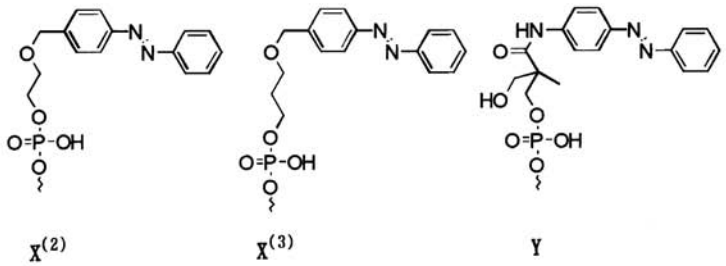


Figure 2. Melting curves of the triple-helixes of the modified oligonucleotides (the solid lines): (a) $\mathbf{X^{(2)}T_{11}/a/t}$, (b) $\mathbf{YT_{11}/a/t}$. The curves for the $\mathbf{T_{11}/a/t}$ are also shown (the broken lines).

A_{14}/T_{14} sequence for the triple-helix formation was placed in the middle of the duplex of two 32-mer DNA (**a** and **t**). The T_m values of the triple-helixes were measured by monitoring the absorbance at 280 nm on a JASCO model V-530 spectrophotometer, equipped with a programmed temperature-controller. The rate of temperature change was 1 °C/min. The concentrations of **a**, **t**, and the modified oligonucleotide were 2.0, 2.2, and 2.4 μmol dm^{-3} in pH 7.0 Hepes buffer (10 mmol dm^{-3}), respectively. Under these conditions, the **a/t** duplex (T_m = 73.0 °C) is completely formed.

The solid lines in Figure 2 depict the typical melting curves for the triple-helix formation between the **a/t** duplex and the modified oligonucleotide. For the purpose of comparison, the curves for the triple-helixes of the native oligonucleotide $\mathbf{T_{11}}$ (with the **a/t** duplex) are also presented (the broken lines). It is noteworthy that the T_m value of the $\mathbf{X^{(2)}T_{11}/a/t}$



$X^{(2)}T_{11}$: 5'- $X^{(2)}$TTTTTTTTTT-3'
$X^{(3)}T_{11}$: 5'- $X^{(3)}$TTTTTTTTTT-3'
YT_{11} : 5'- YTTTTTTTTTT-3'

$X^{(2)}T_{14}$: 5'- $X^{(2)}$TTTTTTTTTTTTT-3'
$X^{(3)}T_{14}$: 5'- $X^{(3)}$TTTTTTTTTTTTT-3'
YT_{13} : 5'- YTTTTTTTTTTTT-3'

t : 5'-GCCACGAAATTTTTTTTTTTTTTAAACCGACG-3'
a : 3'-CGGTGCTTTAAAAAAAAAAAAAATTTGGCTGC-5'

Figure 1. The modified oligonucleotides bearing an azobenzene at the 5'-end. The DNA duplex **a/t** involving the A_{14}/T_{14} sequence for triple-helix formation is also presented.

Table 1. The T_m values (in °C) of the triple-helixes between the **a/t** duplex and the modified (or native) oligo(T)s.[a]

Modified oligo(T)		Native oligo(T)		$[MgCl_2]$/mol dm^{-3}
$\mathbf{X^{(2)}T_{11}}$	25.3	$\mathbf{T_{11}}$	20.6	0.4
$\mathbf{X^{(3)}T_{11}}$	24.6	$\mathbf{T_{12}}$	26.8	
$\mathbf{YT_{11}}$	29.1			
$\mathbf{X^{(2)}T_{14}}$	23.1	$\mathbf{T_{13}}$	18.0	0.1
$\mathbf{X^{(3)}T_{14}}$	25.8	$\mathbf{T_{14}}$	22.5	
$\mathbf{YT_{13}}$	26.0			

[a]On duplicated runs, the T_m values were identical with the values presented here, within ±1.0 °C.

triple-helix (25.3 °C) is higher than the value (20.6 °C) for the $\mathbf{T_{11}}$**/a/t** triple-helix. Moreover, its T_m is close to the value for the $\mathbf{T_{12}}$**/a/t** triple-helix (see Table 1). The $\mathbf{X^{(3)}T_{11}}$**/a/t** triple-helix is almost as stable as the $\mathbf{X^{(2)}T_{11}}$**/a/t.** Still more stable triple-helix is obtained when an azobenzene is attached to the 5'-end of $\mathbf{T_{11}}$ via an amide linker ($\mathbf{YT_{11}}$, see Figure 2(b)). The T_m of the $\mathbf{YT_{11}}$**/a/t** triple-helix (29.1 °C) is by 8.5 °C higher than the corresponding value for the native triple-helix $\mathbf{T_{11}}$**/a/t**, and exceeds even that (26.8 °C) of the $\mathbf{T_{12}}$**/a/t**. The terminal azobenzene stabilizes the triple-helix in a similar (in some case greater) magnitude as does the thymine (at the 5'-end), although it does not form hydrogen-bonds with the Watson-Crick A/T base-pair. Apparently, the hydrogen-bonding is replaced by other interactions. The same conclusion was obtained for the triple-helix formation of $\mathbf{X^{(2)}T_{14}}$, $\mathbf{X^{(3)}T_{14}}$, and $\mathbf{YT_{13}}$ (see Table 1).

The enormous stabilization of the triple-helixes by the azobenzene is associated with its intercalation into the base pairs in the **a/t** duplex.[2] The azobenzene residues in the present oligonucleotides mostly take their *trans*-forms (with respect to the stereochemistry of the N=N bond),[5] which are planar [6] and sufficiently apolar. Furthermore, they are placed near the duplex on the triplex formation. Thus, the intercalation preferentially occurs, although azobenzene is not a typical intercalating agent. Consistently, negative circular-dichroism (CD) was weakly induced around 360 nm on the triple-helix formation: the values of $\Delta\varepsilon$ (mol^{-1} dm^3 cm^{-1}) were –3.5 and –5.4 for $\mathbf{X^{(2)}T_{11}}$**/a/t** and $\mathbf{X^{(3)}T_{11}}$**/a/t** at -5 °C. This indicates that the long axis of the azobenzene is almost parallel to the A-T base-pairs in the **a/t** duplex.[7] The intercalation stabilizes the triple-helixes, in place of the Hoogsteen-type hydrogen-bonds in the native $\mathbf{T_{12}}$**/a/t** triple-helix. The argument is supported by the fact that the triple-helix was substantially destabilized when the *trans*-azobenzene was isomerized to the *cis*-form by irradiating UV-light.[8] After the UV irradiation, the T_m values of the $\mathbf{X^{(2)}T_{11}}$**/a/t** and the $\mathbf{X^{(3)}T_{11}}$**/a/t** triple-helixes were 6.1 and 10.9 °C, respectively. The *cis*-azobenzene is non-planar [6] and more polar than is the *trans*-isomer. Thus, it cannot be accommodated between two A/T base-pairs, and does not stabilize the triple-helix.

In conclusion, an azobenzene, tethered to the 5'-end of oligo(T), notably promotes the triple-helix formation with oligo(A)/oligo(T) duplex. By attaching appropriate functional residues to the azobenzene, the systems can be made still more sophisticated and used for versatile applications. Photo-regulation of their functions is also promising.

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4 In the synthesis of the phosphoramidite monomers for the $\mathbf{X^{(2)}}$ and $\mathbf{X^{(3)}}$ residues, ethylene glycol or propylene glycol was coupled with 4-bromomethylazobenzene in the presence of NaH. The structures of the products were completely characterized by ^{1}H-NMR. The synthesis of the phosphoramidite monomer for the Y residue was described in Ref. 3a.

5 The azobenzene in the modified oligonucleotides takes mostly (>80%) the *trans*-form, as confirmed by HPLC (a Merck LiChrospher 100 RP-18(e) column; linear gradient from 5/95 (acetonitrile/H_2O) to 50/50 at 25 min).

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7 This CD corresponds to the π–π* transition of the azobenzene. According to the literature (R. Lyng, A. Rodger, and B. Nordén, *Biopolymers*, **32**, 1201 (1992)), negative CD is induced only in this conformation.

8 The photo-isomerization of the azobenzene in the modified oligonucleotides was accomplished by irradiating the light from a 150 W Xenon lamp for 15 min through an appropriate filter. Infrared light was cut off by water-filter. By this treatment, the fraction of the *cis*-isomer was kept almost constant at 70 % throughout the measurement.