Right-Handed Triplex Formed between Peptide Nucleic Acid PNA-T8 and Poly(dA) Shown by Linear and Circular Dichroism Spectroscopy.

Nielsen, P. E. et al. *J. Am. Chem. Soc.* **1993**, *115*, 6477-6481.

UV-Topic: CD and LD Spectroscopy

Chem Topic: Biopolymers

PNA (peptide nucleic acid) are oligonucleotide analogues in which the entire desoxyphosphate backbone is replaced by a chemically completely different but structurally homomorphous polyamide backbone composed of (2-aminoethyl)glycine units. PNA retains hybridization properties of natural DNA.

Linear and circular dichroism spectroscopy are used to characterize binding stoichiometry, conformation, and chain flexibility of the DNA-PTA complex formed between a single strand poly(dA) chain and a complementary eightmer of PNA.

CD: differential absorption of left and right circularly polarized light.

LD: differential absorption of linearly polarized light (para. and perp.) to the flow direction.

LD^r: reduced linear dichroism is LD divided by the absorbance measured without flow.
$LD^r = LD/A_{iso}$

LD^r is proportional to an orientation factor S that describes the degree of orientation of the DNA helix axis relative to the flow direction. It is thus possible to gain conformational information from the measurement of LD^r.

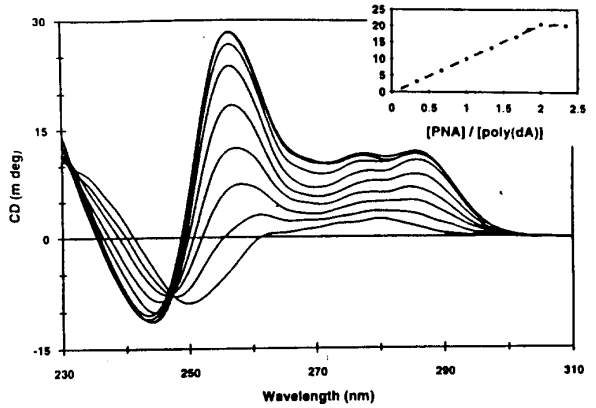

Figure 1. Circular dichroism titration of poly(dA) (constant concentration) with PNA H-T_8-LysNH_2 (PNA-T_8). The mixing ratios, [PNA-T_8]/[poly(dA)], are the following from bottom to top at 260 nm: 0.00, 0.33, 0.67, 1.00, 1.33, 1.67, 1.83, 2.00, and 2.33. The CD spectra for the ratios 2.00 and 2.33 were found to be almost identical. The concentration of the poly(dA) was 25 mM, the pathlength was 1 mm, and each spectrum was averaged 10 times.

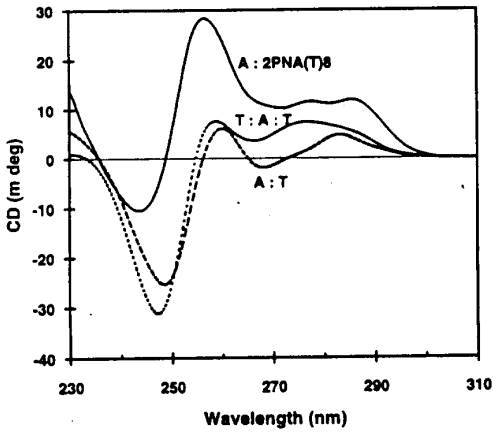

Figure 2. CD spectrum of poly(dA)[PNA-T_8]$_2$ compared with CD spectra of duplex poly(dA)poly(dT) and triplex poly(dA)[poly(dT)]$_2$. Conditions as in Figure 1.

Stoichiometry: CD titration: 1 mol poly(dA) saturated by 2 mol PNA-T8. Triplex Isosbestic points, thus only two compounds present (poly(dA) and triplex).

Chirality: Fig 2 shows the same sign pattern as the poly(dA)-poly(dT) duplex which is known to form a right-handed helical structure.

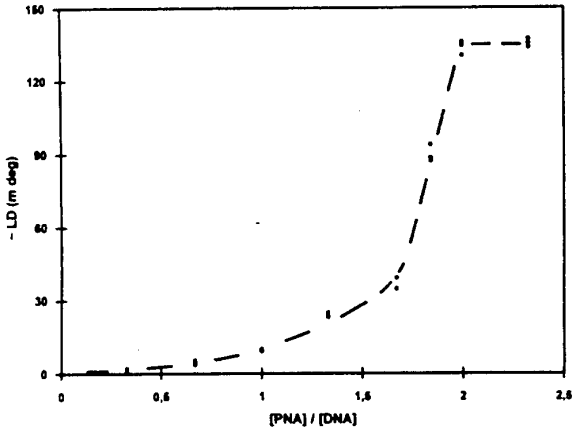

Figure 4. Flow linear dichroism spectra of PNA-T_8 + poly(dA) mixtures. The mixing ratios are the same as those in Figure 1. (A, top) Increasing the PNA/DNA ratio gives an increasingly stronger negative LD band with a maximum at 258 nm. The LD spectra for the ratios 2.00 and 2.33 are almost identical. Shear gradient was 3600 s^{-1} and pathlength was 1 mm. The conversion factor between ellipticity units of the Jasco instrument and dichroism in absorbance units, θ (deg), is 33.0 × ΔA (absorbance units). (B, bottom) Negative LD signal at 258 nm plotted versus the PNA/DNA ratio (three experiments). LD increases rapidly when approaching the stoichiometry of two PNA thymines per DNA adenine. The LD is saturated at this stoichiometry.

Stiffness. Poly-(dA) is flexible and shows no LD, but the triplex shows LD and it must be rigid (Fig 4). The triplex of poly-(dA) with PNA is much like the triplex with poly-(dT).

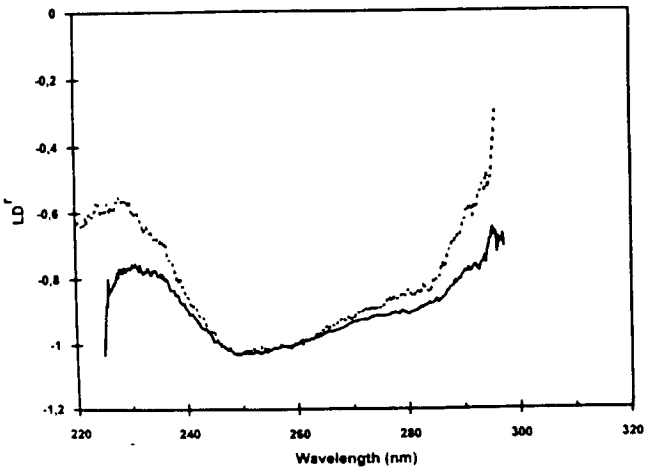

Figure 5. Reduced linear dichroism spectra, $LD^r(\lambda)$, of complex poly-(dA)[PNA-T_8]$_2$ (—) and of triplex poly(dA)[poly(dT)]$_2$ (- - -). LD^r spectra of both systems are scaled to –1 at the LD maxima at 258 nm.