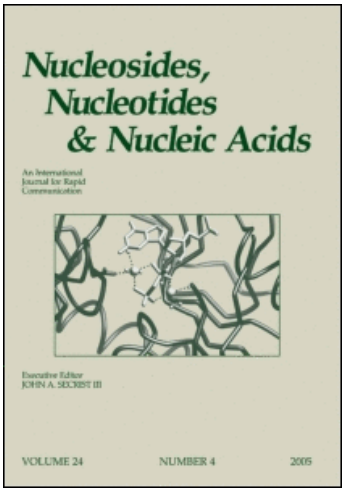

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TRIPLEX FORMATION BETWEEN DNA AND MIXED PURINE-PYRIMIDINE PNA ANALOG WITH *LYSINES* IN BACKBONE

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ABSTRACT. Melting UV experiments and mixing curves indicated slow triplex formation between *lysine* comprising PNA and DNA complement in 100mM Na^+ solution.

Peptide-nucleic acid (PNA), a nuclease resistant DNA analog, was synthesized and described some years ago [1]. Neutral backbone of PNAs enables them to bind DNA and RNA targets at low and moderate ionic strength with affinity higher than that for corresponding oligonucleotides (ODN) [2]. However reduced solubility in physiological solutions and limited cellular uptake is a side effect of backbone neutrality of PNAs. To overcome these problems, we have synthesized mixed purine-pyrimidine PNA analogs with four positively charged *lysines* instead of neutral *glycines* in the backbone and studied the effect of positive charges on a complex formation between PNA and DNA target.

Materials and methods

PNA and DNA synthesis. The PNAs were synthesized as previously described[3] and characterized by electrospray mass spectrometry. DNA base sequence was designed to bind PNAs in antiparallel orientation (N-terminus of PNA binds to 3'-end of DNA target) (FIG. 1).

Melting experiments. PNAs and DNA target were combined in a 1:1 ratio at total strand concentration of 8 μM in 100 mM Na^+, 10 mM phosphate buffer, 0.1 mM EDTA,

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H-CGCTTGGCAGTCTC (PNA)

H- CGCT_kT_kGGCAGT_kCT_kC (*lysine* PNA analog)

5'-GAGACTGCCAAGCG (DNA complement)

FIG. 1. DNA complement, PNA base sequence, and position of *lysyl* units (T_k) in PNA analog. By analogy to peptides, PNA sequences are written from amino (N) to carboxy (C) terminus [2].

pH 7.0. Mixtures of PNA and DNA were heated to 95° for 10 minutes and cooled slowly at the bench for indicated times. Absorbance vs. temperature curves were measured at a heating rate of 0.7°/min. T_m values were determined as the temperatures of the maximum in the first derivative of dissociation curves.

UV Mixing curves: PNAs and their complementary DNA target were mixed at different molar fractions ranging from 0 to 1.0 in the buffer used for melting experiments with total strand concentration 8 μM and annealed as described above. We ran spectra for each mixture between 210-330 nm at 20°. Maximum hypochromicity was observed at 280 and 284 nm (see results).

Results and Discussion

Mixed purine-pyrimidine PNAs with one *lysine* at C terminus bind single stranded DNA and RNA targets in duplex fashion obeying the Watson-Crick hydrogen bonding rules [2]. Replacement of some neutral *glycyl* units for positively charged *lysyl* ones in the PNA sequence was expected to increase PNA affinity to DNA target due to additional electrostatic attraction between PNA and DNA backbones. However UV melting experiments demonstrated that T_m remained the same (72.7±0.5°) and melting transition became slightly broader (FIG. 2). One of the reason for transition broadening can be disproportionation leading to mixture of structures in solution. To test our hypothesis, we melted PNA:DNA complexes and measured UV mixing curves after 1 and 6 hours incubation at 20°. Melting and mixing curves demonstrated that PNA with neutral

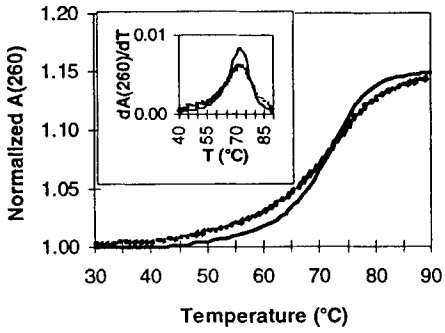

FIG.2. Absorbance vs. temperature profiles for complex of DNA complement with PNA (—) and lysine PNA analog (---) in phosphate buffer, pH 7.0 with 100mM Na^+. In the insert: first derivatives of melting curves.

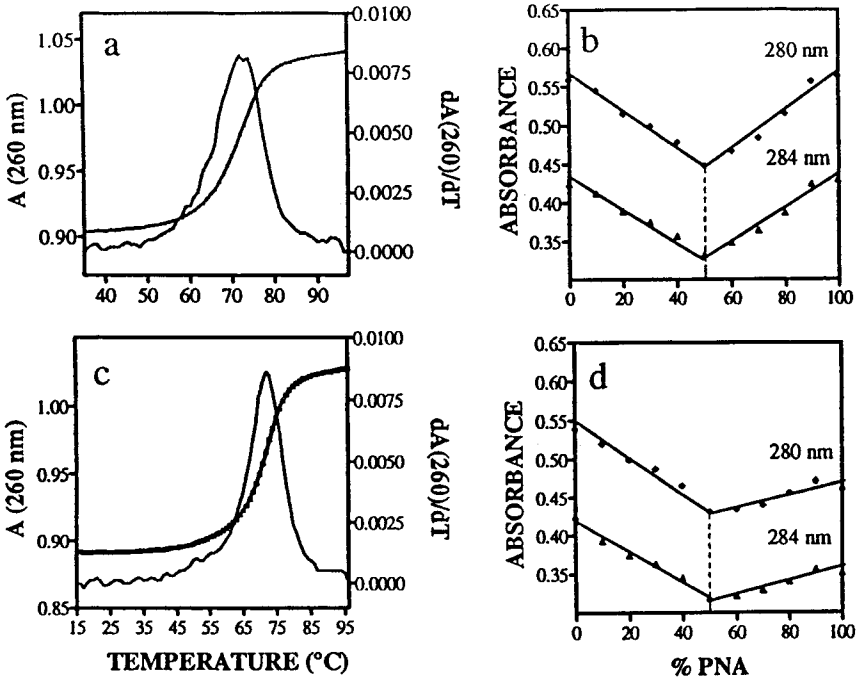

FIG.3. Absorbance at 260 nm and first derivative curves vs. temperature and mixing curves at 280 and 284 nm measured after 1 h (a, b) and 6 hs (c, d) incubation at 20° for complexes of DNA with PNA.

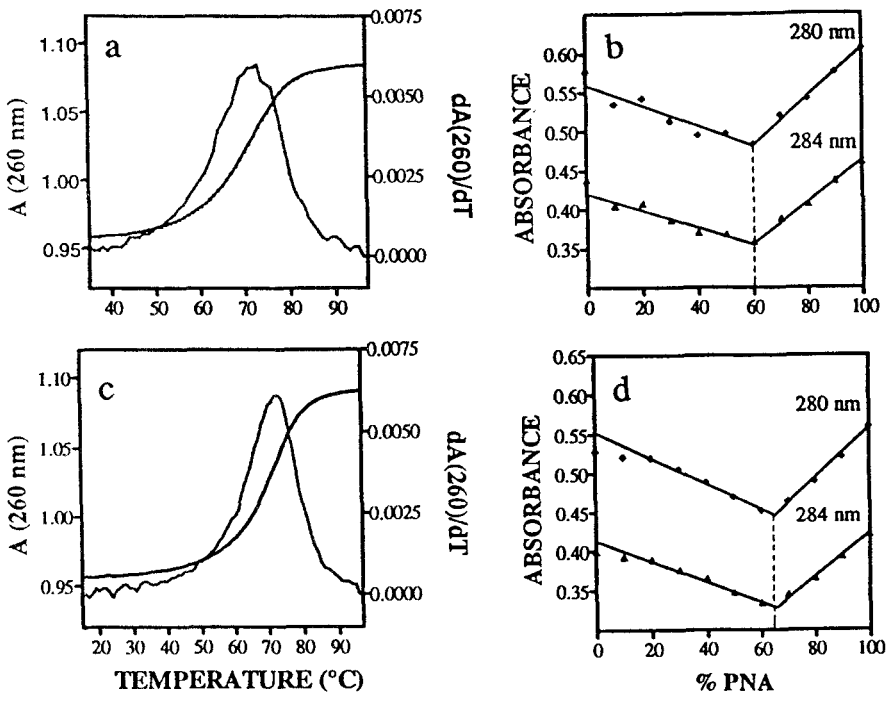

FIG.4. Absorbance at 260 nm and first derivative curves vs. temperature and mixing curves at 280 and 284 nm measured after 1 h (a, b) and 6 hs (c, d) incubation at 20° for complexes of DNA with *lysine*-PNA analog.

backbone bound complementary *ss*DNA in duplex fashion and no changes in binding were observed with time (FIG. 3).

Complex of lysine PNA analog with DNA target also demonstrated one cooperative transition after 1 and 6 hours incubation at 20° (FIG. 4-a, c). However the minimum in mixing curves measured after one hour incubation was at 60% PNA suggesting there was a mixture of duplex and triplex structures (FIG. 4-b). After 6 hours, we observed shift of mixing curve minimum to 64-65% PNA (FIG. 4-d) suggesting equilibrium slowly shifted toward triplex formation.

Thus the data indicated that replacement of four *glycines* for positively charged *lysines* in PNA backbone resulted in slow disproportionation leading to triplex formation driven apparently by additional electrostatic interaction between DNA and PNA backbones since high salt concentration (1 M NaCl) provided only duplex formation in 1:1 mixture judging by mixing curves experiments. We have no data on triplex structure however triple-strand complex formation reported for PNAs and ODNs with neutral backbones under similar conditions [1, 4] suggests formation both Py/Pu/Py and Pu/Py/Pu triplets in the complex.

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