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Alanyl-PNA Oligomers: A New System for Intercalation

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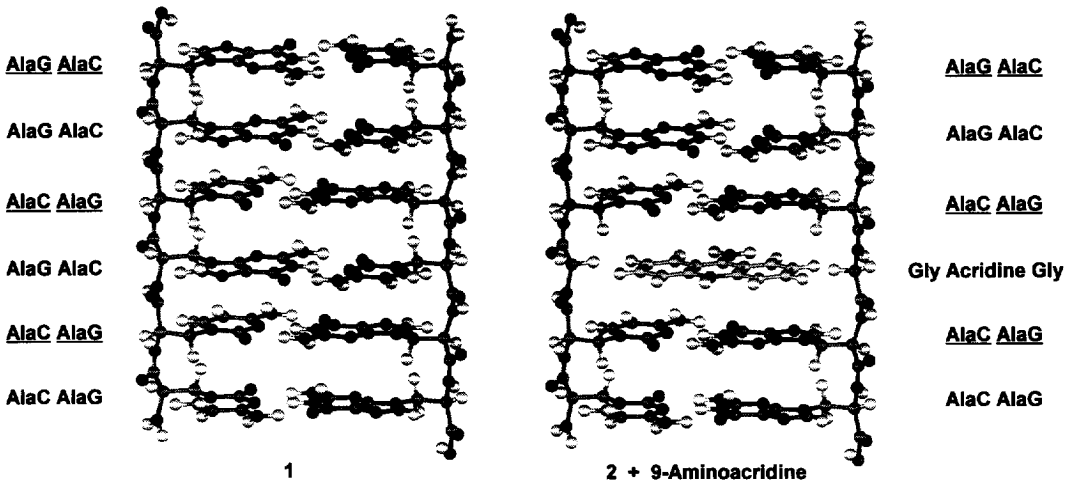
Abstract: An alanyl peptide nucleic acid double strand is described as an environment for examining intercalation. We observe that addition of 9-aminoacridine and ethidium bromide stabilizes an alanyl-PNA duplex containing an abasic site. © 1997 Elsevier Science Ltd.

The intercalation of planar aromatic molecules between base pairs of a DNA double strand is an area widely discussed because of the application as dyes, drugs, carcinogens and mutagens[1]. Two mechanistic features are always involved in the process of DNA intercalation[2]: First, unwinding of the DNA double helix necessary to create a base pair distance large enough for incorporation of a polycyclic aromatic molecule. This requires conformational reorientation which induces distortion of the sugar-phosphodiester backbone and extension of the helix. Second, intercalation is preceded by diffusion-controlled adhesion of the mostly positively charged drugs and dyes to the backbone of the double helix. Herein, we discuss intercalation into a alanyl peptide nucleic acid duplex which proceeds without lengthening of the double strand and adhesion to the backbone.

Figure 1 Model of an alanyl-PNA double strand based on Watson-Crick pairing of the enantiomeric oligomers H-(AlaG-AlaG-AlaC-AlaG-AlaC-AlaC)-Lys-NH_2 and H-(AlaG-AlaG-AlaC-AlaG-AlaC-AlaC)-Lys-NH_2 [3] (left) and an intercalation complex of the oligomers H-(AlaG-AlaG-AlaC-Gly-AlaC-AlaC)-Lys-NH_2 and H-(AlaG-AlaG-Gly-AlaG-AlaC-AlaC)-Lys-NH_2 with 9-aminoacridine (right). Lysine amides are omitted.



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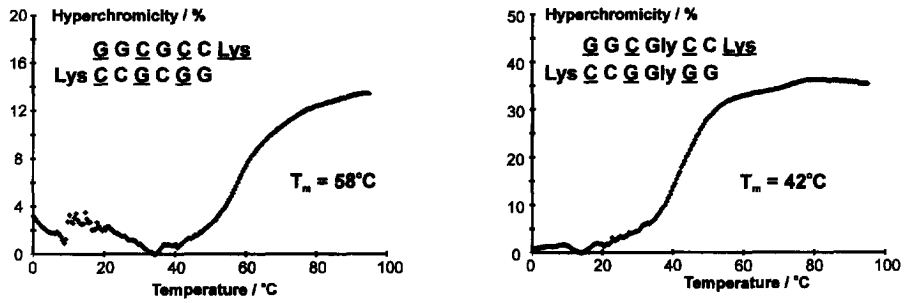
Recently, we introduced the double strand formation of alanyl peptide nucleic acids which probably are linear because the spacing between consecuting site chains is close to the base stacking distance of 3.5 Å (Figure 1). These alanyl-PNA complexes are based on the recognition of the nucleobases adenine, thymine[4], guanine and cytosine[5]. The backbone is formed by a regular peptide strand with alternating configuration of the nucleo amino acids. The nucleobases are attached at the β-position of the alanyl side chains. The stability of an equimolar mixture of the hexamers H-(AlaG-AlaG-AlaC-AlaG-AlaC-AlaC)-Lys-NH_2 and H-(AlaG-AlaG-AlaC-AlaG-AlaC-AlaC)-Lys-NH_2 (**1**)[3] is characterized by an UV melting temperature of T_m = 58°C (6 μM, 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7, 260 nm) based on six G-C Watson-Crick base pairs[5]. A double strand with one base pair missing was synthesized by replacing the nucleo amino acids by glycine in opposite position. The resulting double strand **2** formed by oligomers H-(AlaG-AlaG-AlaC-Gly-AlaC-AlaC)-Lys-NH_2 and H-(AlaG-AlaG-Gly-AlaG-AlaC-AlaC)-Lys-NH_2 is lacking three hydrogen bonds from the base pair and stacking interactions. Nevertheless, double strand **2** still has a T_m = 42°C (Figure 2)[6]. The potential of alanyl-PNA oligomers **2** to act as a host for DNA intercalators was examined.

Figure 2 UV melting curves of GC hexamer complex **1** (left) and the double strand **2** (right) which is suitable for intercalation because of the abasic position (6 μM, 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7, 260 nm).



The drugs and dyes tested for intercalation are shown in Figure 3. The intercalation was studied by examining the stabilization of the double strand while adding the intercalator[7]. Adenine (**3**) as well as proflavine (**4**) had no stabilizing effect. On the other hand, addition of 25 equivalents[8] of 9-aminoacridine (**5**) led to an increase of the melting temperature from T_m = 42°C to 52°C whereas the intercalator alone showed no sigmoidal transition (Figure 4, A). This strongly indicates intercalation of aminoacridine between GC base pairs. Incorporation of ethidium bromide (**6**) was also successful. A stabilization to T_m = 62°C was found (Figure 4, B) as well as a second melting point at T_m = 22°C, whereas the reference curve without PNA showed no temperature dependence. Further evidence for intercalation results from comparison of the CD-spectra of 9-aminoacridine and the intercalation complex **2** + **5**. A Cotton-effect was only observed for the achiral chromophor 9-aminoacridine when intercalated to alanyl-PNA (Figure 4, C). The drugs daunomycin (**7**) and actinomycin (**8**) failed to bind to alanyl-PNA complex **2** (not shown). The non binding of daunomycin is in agreement with daunomycin requiring a right-handed double helix geometry for intercalation[9]. For intercalation of the aromatic rings of actinomycin a minor groove binding of the peptide rings seems required[10].

The model of alanyl-PNA duplex **2** suggests that intercalation is possible only at abasic sites because alanyl-PNA is already linear and fully extended with a base pair distance of about 3.5 Å. This is different from DNA where intercalation usually is not restricted to one position. The regioselectivity of intercalation to alanyl-PNA was proven by attempted intercalation of 9-aminoacridine (**5**) to double strand **1** (Figure 4, D). Addition of the intercalator resulted in a destabilization of 23°C. This unexpected destabilization of **1** in presence of acridine requires further investigations. Nevertheless, the difference of alanyl-PNA double strands **1** and **2** in binding acridine points to a specificity of intercalation only at abasic sites which makes alanyl-PNA a perfect tool to control the regioselectivity and the amount of incorporated intercalator.

Figure 3 Compounds tested for intercalation

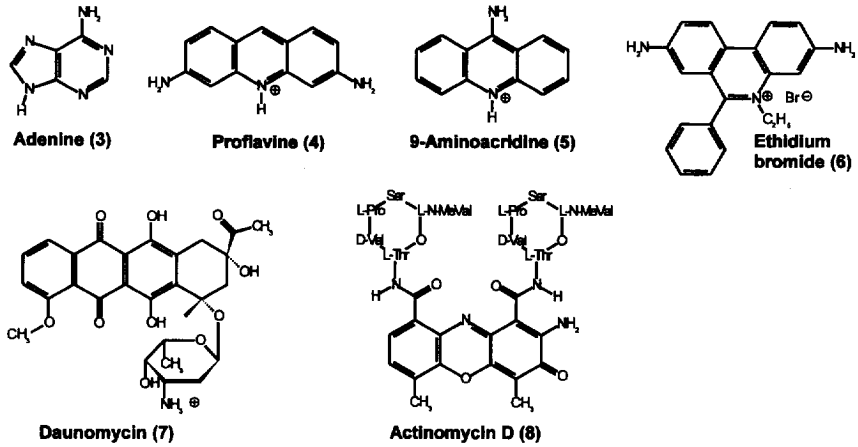
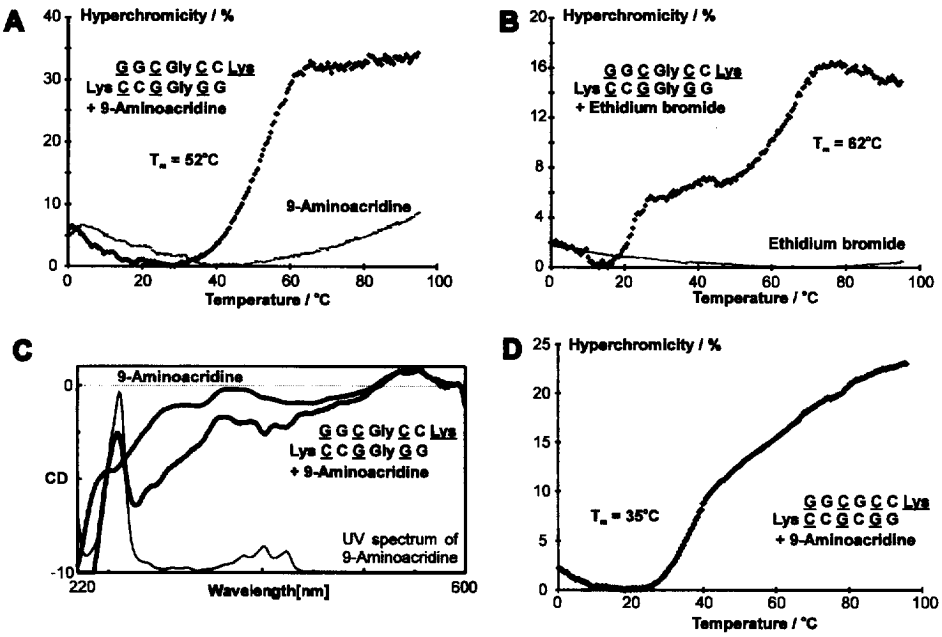


Figure 4 Alanyl-PNA double strand **2** with 9-aminoacridine compared to 9-aminoacridine without PNA (270 nm) (**A**), **2** with ethidium bromide compared to ethidium bromide without PNA (250 nm) (**B**), CD-spectra of **2** with 9- aminoacridine compared to 9-aminoacridine without PNA and UV-spectrum of 9-aminoacridine (**C**); alanyl-PNA double strand **1** with 9-aminoacridine (280 nm) (**D**). Conditions: 6 μM PNA, 150 μM intercalator, 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7.



In summary, we describe first qualitative results of the intercalation to a new class of PNA-oligomers which requires a binding mechanism different from DNA intercalation. 9-Aminoacridine and ethidium bromide were shown to bind to alanyl-PNA **2**. The intercalation to PNA-PNA complexes based on a *N*-(2-

aminoethyl)glycine backbone failed completely as described by Nordèn *et al.*[11]. With regards to these experiments our results indicate that the required conformational changes in the unwinding process might hamper intercalation rather than the uncharged backbone. Work studying the thermodynamics of binding, the selectivities and geometric requirements is under way. The results also indicate that alanyl-PNA should be a valuable system to investigate the electron transfer through nucleobase stacks[12]. Alternative transport mechanisms using phosphodiesters could be excluded, the stacking is well defined but different from DNA and the regiochemistry and stoichiometry of intercalation easily controlled.

Acknowledgments

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References and Notes

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2. Saenger, W.; 'Principles of Nucleic Acid Structure' Springer Verlag New York **1984**, 350.
3. Abbreviations used: AlaG (β-*N*9-guaninyl-L-alanine), AlaC (β-*N*1-cytosinyl-L-alanine), Lys-NH_2 (lysine amide introduced for solubility). The amino acids with D-configuration are underlined. The synthesis of the PNA oligomers will be described in 5 and Diederichsen, U.; (manuscript in preparation).
Oligomers were purified by HPLC (RP C-18) and characterized by ESI-MS (H-AlaG-AlaG-AlaC-AlaG-AlaC-AlaC-LysNH$_2$ *m/z* = 1368.4 $(MH)^+$, 674.2 $(MH_2)^{2+}$; H-AlaG-AlaG-AlaC-AlaG-AlaC-AlaC-LysNH$_2$ *m/z* = 674.0 $(MH_2)^{2+}$; H-AlaG-AlaG-AlaC-Gly-AlaC-AlaC-LysNH$_2$ *m/z* = 1183.2 $(MH)^+$, 592.6 $(MH_2)^{2+}$; H-AlaG-AlaG-Gly-AlaG-AlaC-AlaC-LysNH$_2$ *m/z* = 1223.2 $(MH)^+$, 612.5 $(MH_2)^{2+}$).
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5. Diederichsen, U.; *Angew. Chem. Int. Ed. Engl.* in press. For comparison: DNA oligomer $d(GC)_3$ has a melting point T_m = 47°C (De Prisco Albergo, D.; Marky, L. A.; Breslauer; K. J., Turner, D. H.; *Biochemistry* **1981**, *20*, 1409) and a PNA-PNA decamer containing all four nucleobases has a T_m = 67°C (Hyrup, B.; Nielsen, P. E.; *Bioorg. Med. Chem.* **1996**, *4*, 5).
6. It is quite likely that the double strand regains stacking and compensates for the hole e.g. by helicalization around the glycine units.
7. Sample preparation: 150 μM intercalator (purchased from Sigma-Aldrich) were added to a 6 μM solution of a 1:1 mixture of oligomers H-(AlaG-AlaG-AlaC-Gly-AlaC-AlaC)-Lys-NH_2 and H-(AlaG-AlaG-Gly-AlaG-AlaC-AlaC)-Lys-NH_2 in 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7. After heating to 60°C the sample was kept at 0°C for 3 h. The melting curve was measured in 3h at a wavelength not interfering with the main absorption band of the intercalator (Figure 4).
8. The concentrations were estimated by UV absorption at 260 nm (PNA), 400 nm (9-aminoacridine) and 481 nm (ethidium bromide).
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