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Self-Pairing PNA with Alternating Alanyl/Homoalanyl Backbone

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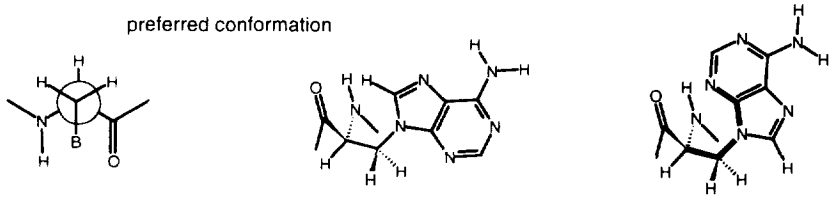
Abstract: A PNA hexamer with alternating D-homoalanyl adenine/L-alanyl thymine building units forms a self-pairing double strand as indicated by temperature dependent UV and CD spectroscopy. Simple model studies suggest a linear, antiparallel A-T pairing complex.

Recently, we investigated a modified peptide nucleic acid (PNA)[1] containing alanine amino acids with alternating configuration and the nucleobases adenine and thymine substituted in β-position of the side chain[2]. For an adenine thymine alternating octamer H-(AlaA-AlaT)$_4$-Lys-NH_2 as well as an adenine hexamer H-(AlaA-AlaA)$_3$-Lys-NH_2 [3] self-pairing was observed. Based on model studies we expect these complexes to be linear. Here we would like to present a self-pairing PNA oligomer with a modified backbone. We will discuss the influence of an additional methylene group within the side chain on the binding specificity and the geometry of the double strand.

Examining the conformation of a β-nucleobase substituted alanyl unit, there are three staggered conformations for the rotation of the Cα-Cβ bond, of which only one avoids interactions similar to a 1,5 repulsion between base and backbone (Figure 1). Preferentially, the nucleobase should be orientated antiperiplanar to Hα in a way, that proton H8 (purine) or H6 (pyrimidine) respectively, is directed towards the backbone. The other conformation having the ring system synclinal to the β-hydrogens is unfavorable because of 1,5 repulsion and steric interaction between ring and backbone.

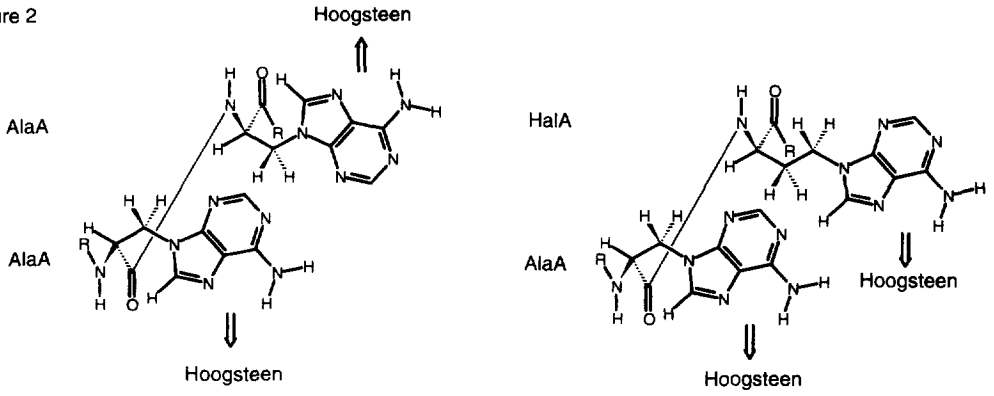
Figure 1



The extension of the alanyl side chain by adding a methylene group leads to higher flexibility and provides a longer distance between base and backbone. Comparing the base-substituted alanyl to the - extended - homoalanyl amino acid (Figure 2) the order of proton donor and acceptor positions of the base are converted. The Hoogsteen binding sites in an alanyl/homoalanyl alternating oligomer are oriented alike. Another consequence of the additional methylene group is shown in Figure 3: In an alanyl PNA model A-T pairing is possible either for antiparallel strand orientation and homochiral base pair connectivity or *vice versa* (parallel, heterochiral)[2]. By using an ethylene spacer in the side chain of every second amino acid this specificity between strand orientation and configuration of the amino acids of a base pair is altered.

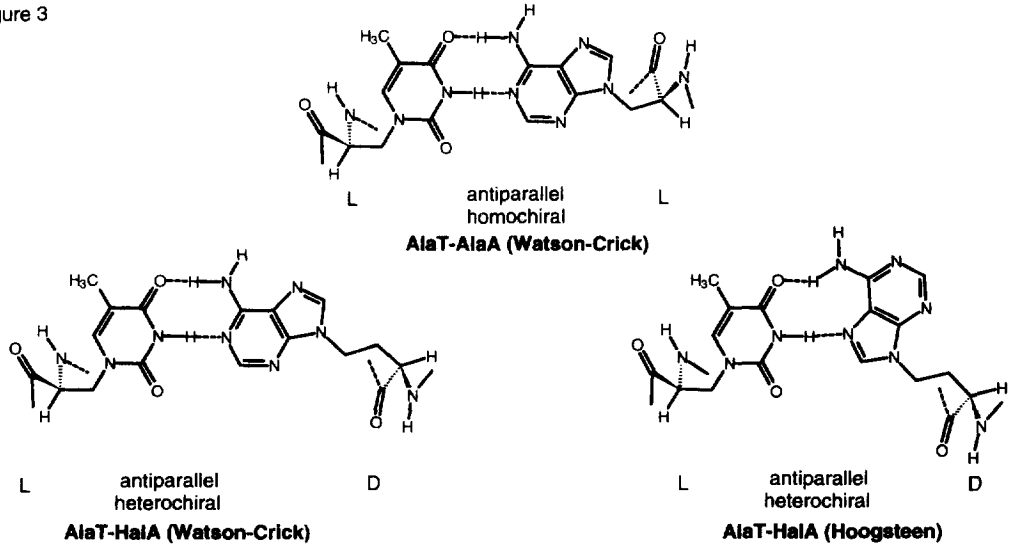
Irrespective the pairing mode, double strand formation with alternating homoalanyl/alanyl amino acids should only be possible in an antiparallel, heterochiral or in a parallel, homochiral (not shown) manner. Whereas for the H-(AlaA-AlaT)$_n$-Lys-NH$_2$ alanyl oligomer antiparallel self pairing is not expected for geometric reasons, an homoalanyl/alanyl alternating oligomer should form an antiparallel double strand. Furthermore, since in this case the steric interaction between base and backbone is not important, Hoogsteen pairing of homoalanyl-bound adenine is more likely to occur as compared to alanyl adenine.

Figure 2



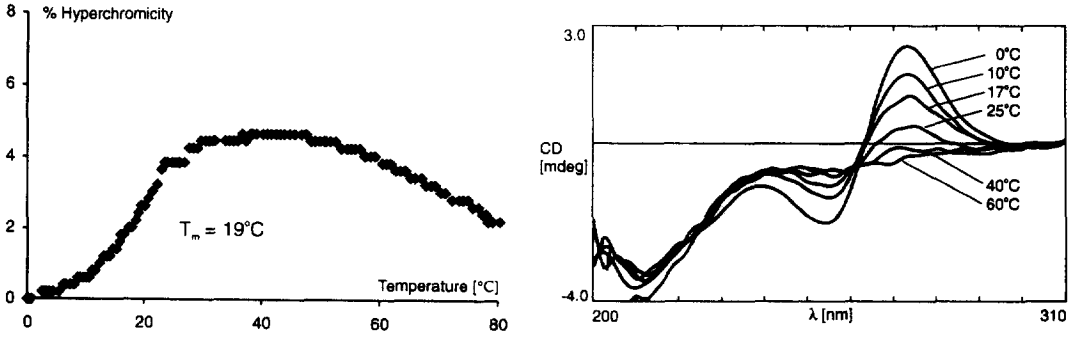
To study the pairing specificity discussed above, we synthesized the hexameric PNA H-(HalA-AlaT)$_3$-Lys-NH$_2$ by oligomerization of the *N-tert.*-butoxycarbonyl protected amino acids on the solid support using HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)[4] as a coupling reagent. The adenine substituted homoalanine monomer was synthesized as described by Taddei[5] and the thymine substituted alanine derivative was prepared following the procedure of Lohse[6]. It was not necessary to protect the *N*6 amino function of the adenine for oligomerization. The C-terminal lysine amid was introduced in analogy to Buchardt and Nielsen[7] to increase the water solubility. The oligomer obtained after acidic cleavage from the solid support was purified by HPLC on a RP-C18 column and characterized by ^{1}H-NMR spectroscopy[8] and MALDI-TOF MS (1386.6 Da, $C_{57}H_{72}N_{30}O_{13}$, MH^+ calc. 1385.6 Da).

Figure 3



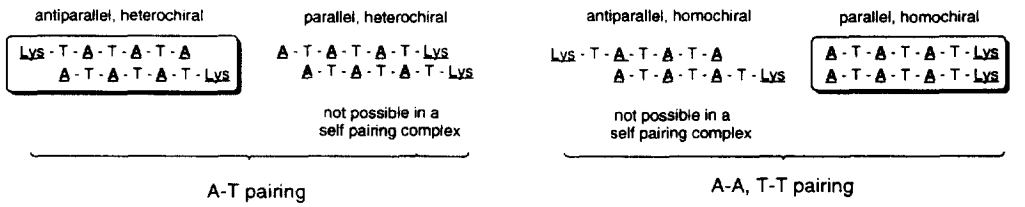
A sigmaoid melting curve indicates the formation of a double strand as known from the temperature dependent UV spectra of DNA-DNA complexes. The melting temperature is dependent on the stability of the double strand. As illustrated in Figure 4, oligomer H-(HalA-AlaT)$_3$-Lys-NH$_2$ shows a cooperative depairing process with a melting temperature of T_m = 19°C (6 µM, 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7, 260 nm, 0.5°C/min). The process is reversible in a way that the absorption at 0°C was reached again by cooling, the pairing however, started at temperatures lower 5°C and was completed after about 30 minutes. The CD spectra were measured at various temperatures under the conditions described above. The UV melting profile correlates with decrease of the CD intensity when increasing the temperature. The 0°C curve has a strong Cotton effect with a maximum at 274 nm and a minimum at 255 nm, whereas the very similar CD curves at 40 and 60°C only show low intensity.

Figure 4 UV- and CD-spectra of H-(HalA-AlaT)$_3$-Lys-NH$_2$ (6 µM, 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7)



In principle, double strand formation of hexamer H-(HalA-AlaT)$_3$-Lys-NH$_2$ can be realized by formation of either 6 A-T basepairs or 3 A-A and T-T interactions each. The latter arrangement seems to be less likely for this oligomer since only the adenine bearing side chains are extended and therefore the smaller thymine bases should not be able to form H-bonds. Furthermore, our model studies suggest that A-T self-pairing of oligomer H-(HalA-AlaT)$_3$-Lys-NH$_2$ should occur only with antiparallel strand orientation. As an A-T basepair in this self-pairing complex has to be heterochiral, the strand orientation is defined to be antiparallel. From the experiments presented here we can not exclude A-A pairing with parallel strand orientation, and we can not decide whether the Watson-Crick or Hoogsteen pairing mode applies. Currently, we examine this problem by incorporation of *N*7-carbaadenine as well as by NMR conformational analysis.

Figure 5 Self pairing possibilities of D-homoalanyl adenine [A]/L-alanyl thymine [T] alternating oligomers



In summary, we prepared a PNA oligomer with an homoalanyl/alanyl backbone and alternating configuration of the amino acids. This hexamer H-(HalA-AlaT)$_3$-Lys-NH_2 is self-pairing in a stable double strand (T_m = 19°C) as shown by UV- and CD-spectroscopy. Especially for the PNA complex described herein, there might be an excellent potential for specific binding of a third strand. Either the Hoogsteen binding site (in a Watson-Crick pairing double strand) or the Watson-Crick site (in a Hoogsteen pairing double strand) of all purine bases should be oriented on the same side of the pairing complex.

Acknowledgments

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References and Notes

1. The name **P**eptide **N**ucleic **A**cid (PNA) was introduced by Buchard and Nielsen for an oligomer containing a polyamide with nucleobase substituted side chains. (For a discussion of the 'not strictly chemically correct name' see Nielsen, P. E.; Egholm, M.; Buchardt, O.; *Bioconjugate Chem.* **1994**, *5*, 3.). The abbreviation PNA is common today at least for the description of the Nielsen-type of oligomer. We decided to use the abbreviation PNA for our oligomers as well, since our backbone is a real peptide and the functionality for recognition and storage of information is based on the DNA/RNA nucleobases. In this way PNA is a chimera between peptides and nucleic acids. Peptide nucleic acid (PNA) was used for nucleo homoalanyl oligomers before. (Lenzi, A.; Reginato, G.; Taddei, M.; *Tetrahedron Lett.* **1995**, *36*, 1713.) Garner suggests 'Peptide based Nucleic Acid surrogates (PNA). (Garner, P.; Yoo, J. U.; *Tetrahedron Lett.* **1993**, *34*, 1275.)
2. Diederichsen, U.; *Angew. Chem.*, submitted.
3. Abbreviations used: AlaA (β-*N*9-adeninyl-L-alanine), AlaT (β-*N*1-thyminyl-L-alanine), HalA (γ-*N*9-adeninyl-L-homoalanine), Lys-NH_2 (lysineamide), The amino acids with D-configuration are underlined.
4. Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F.; *J. Chem. Soc., Chem. Commun.* **1994**, 201.
5. a) Lenzi, A.; Reginato, G.; Taddei, M.; *Tetrahedron Lett.* **1995**, *36*, 1713. b) Lenzi, A.; Reginato, G.; Taddei, M.; Trifilieff, E.; *Tetrahedron Lett.* **1995**, *36*, 1717.
6. a) Lohse, P.; Synthese und Eigenschaften von Nukleodipeptamidinium-Salzen; thesis Nr. 9937 in the laboratory of A. Eschenmoser; ETH Zürich **1992**. b) Lohse, P; Oberhauser, B.; Oberhauser-Hofbauer, B.; Eschenmoser, A.; *Croatica Chim. Acta*, in press.
7. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O.; *Science* **1991**, *254*, 1497.
8. ^{1}H-NMR (250 MHz, D_2O): δ (ppm) 8.29-8.15 (m, 6H, H2, H8 adenine), 7.36-7.31 (m, 2H, H6 thymine), 7.25 (s, 1H, H6 thymine), 4.58-3.75 (m, 19H, Hα, Hβ alanine, Hγ homoalanine), 2.92 (t, 2H, J=8.1 Hz, Hε Lys), 2.50-2.05 (m, 6H, Hβ homoalanine), 1.85-1.55 (m, 4H Lys), 1.65 (s, 6H, thymine), 1.57 (s, 3H, thymine), 1.34 (m, 2H, Lys).

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