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Synthesis and oligomerization of N^{δ}-Boc-N^{α}-(thymin-1-ylacetyl)ornithine

Kenneth H. Petersen*[§], Ole Buchardt[⊗] and Peter E. Nielsen*[♣]

Center for Biomolecular Recognition the H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, ♣ IMBG Department of Biochemistry B, The Panum Institute, Blegdamsvej 3c, DK-2200 Copenhagen N, Denmark

Abstract: The synthesis of a new DNA analogue based on ornithine is described. Only the thymine analogue was synthesised. A 10 mer entirely made up of the thymine monomer forms a triple helix with poly RNA A. The T_m of the triplex was 21°C. Copyright © 1996 Elsevier Science Ltd

The development of peptide nucleic acid (PNA) has demonstrated that pseudopeptide backbones can effectively mimic the phosphodiester(deoxy)ribose backbone of nucleic acids[1-3]. This is of great interest for anti-sense and anti-gene therapeutics[4-6] as well as for DNA based diagnostics and molecular biology technics[7-9] , but may also have implications for our understanding of the origin of the primordial genetic material of life.[10-13] In particular, it would be of interest to study DNA mimics composed of recognized biomolecules or molecules of likely prebiotic origin. Thus PNA analogues containing the naturally occurring amino acid ornithine have been suggested in this connection.[10] We now report the synthesis and preliminary DNA mimicking properties of an ornithine derived PNA.

The synthesis of the monomer is shown in Figure 1. Only the L-form of the monomer was synthesised. The starting material was N^{α}-tertbutyloxycarbonyl-N^{δ}-benzyloxycarbonylornithine. First the

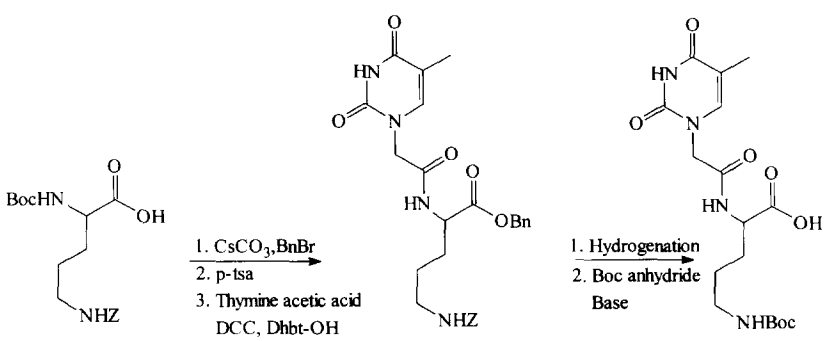

Figure 1. The synthesis of the ornithine based monomer.

[§] Present address: DAKO A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark
[⊗] The late

acid group was converted to the benzylester using benzylbromide and cesium carbonate. This was done to ease handling and increase the solubility of the intermediates. The Boc group was removed by treatment with p-toluenesulfonic acid. Thymine acetic acid was then coupled to the free amino group using DCC and Dhbt-OH. Removal of the Z group and the benzyl group by hydrogenation yields the unprotected monomer. The monomer was then protected at the δ-amino group by reaction with Boc anhydride using Schotten-Baumann conditions.[14,15]

To test the optical purity of the ornithine monomer (T_O), it was reacted with (L)-leucinmethylester hydrochloride in the presence of HBTU and DIEA, and the two diastereomers were separated by reverse phase HPLC chromatography. The result showed that the monomer contained 2,4 % of the D-form. A 10-mer and a 15-mer was synthesised using the solid phase synthesis protocol for PNA synthesis.[16] The oligomers were purified by reverse phase HPLC and characterised by FAB^+ or MALDI-TOF-MS.[17,18] Binding of a T_o-decamer $(T_o)_{10}$ to poly A was indicated by a ~ 20% hypochromicity upon mixing, and from a Job plot (Figure 2), we conclude that a PNA_2/RNA triplex is formed. Thermal denaturation experiments gave the results shown in Figure 3. While Poly A alone exhibits a smooth thermal transition without distinct "T_m's", a new transition with T_m = 25 °C can be assigned to the $(T_o)_{10}$ - poly A triplex.

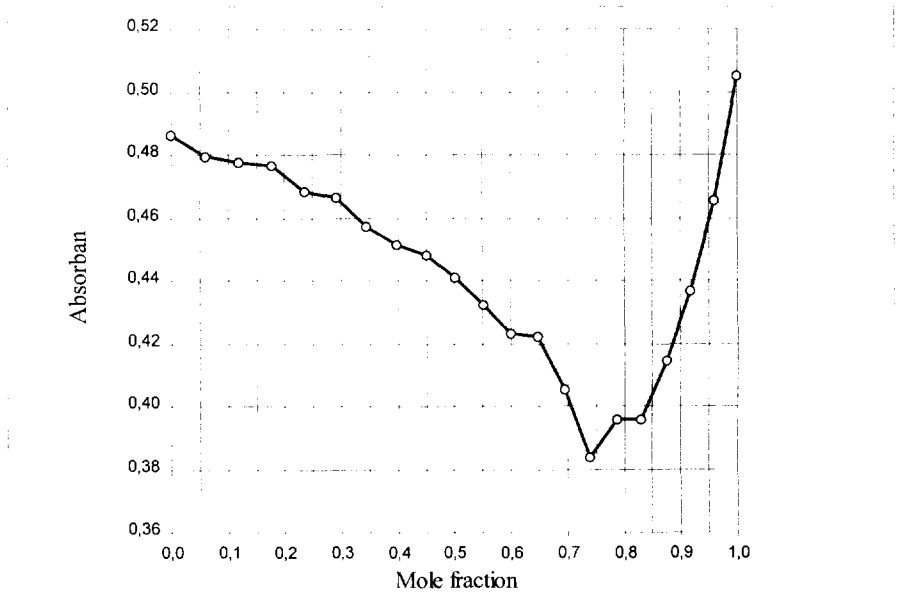

Figure 2. Titration of poly A with $(T_O)_{10}$.

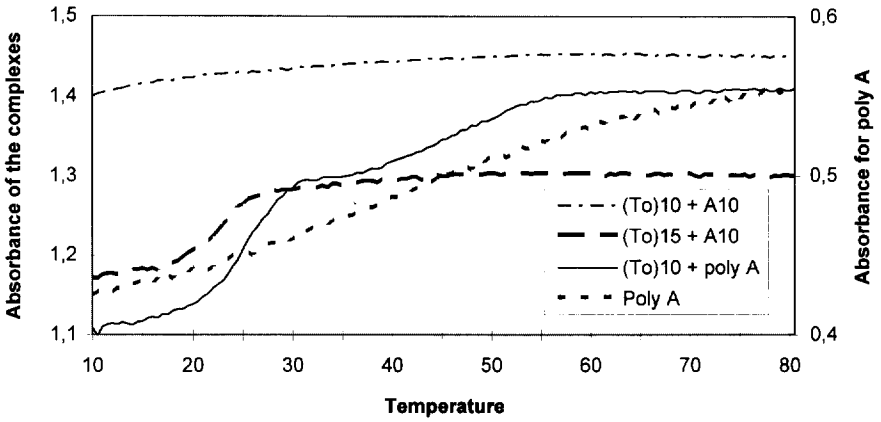

Figure 3. The UV melting curves for Poly A, $(T_o)_{10}$/ Poly A, $(T_o)_{10}/A_{10}$ and $(T_o)_{15}/A_{15}$.

The second transition in the melting curve is assigned to poly A alone. The T_o -decamer was also mixed with a dA_{10} oligomer, but in this case no stable complex could be detected (above 10 °C). Likewise no complex was detected with poly(dA). Thus the ornithine PNA appears to recognize RNA significantly better than it does DNA. In accordance with the above results, the T_o 15-mer showed a distinct transition with a T_m of 23 °C when hybridised to A_{10} and T_m= 31 °C when hybridised to poly A.

A PNA decamer with one T_o (H-AGT GAT$_o$ CAT C-NH_2) showed a T_m of 46 °C when hybridised to a complementary RNA. The T_m of a corresponding complex with the all glycine PNA was 54 °C and thus a single substitution with one T_o resulted in a decrease in T_m of 8 °C. It should be emphasised, however, that an ornithine unit in an aminoethylglycine PNA context will result in "out of register" nucleobases.

The present results clearly demonstrate that an ornithine PNA analogue is a structural DNA mimic for recognizing RNA albeit not as effective as the original aminoethyl glycine PNA. The ornithine PNA structure is isomeric with the propionyl PNA structure,[19] and therefore is characterized by having retained the nucleobase distance of PNA, but has a one atom extension between the backbone and the nucleobase. Thus, it is noteworthy that thymine decamers of these two PNA analogues form complexes with poly A of similar thermal stability, and it may therefore be argued that the less efficient DNA hybridization by the ornithine PNA is "primarily" due to the extended backbone nucleobase distance and less to the structural isomerization of the backbone.

With respect to a possible role of an ornithine like PNA in a prebiotic scenario, it would be of importance to prepare purine monomers in order to study mixed purine/pyrimidine sequences and explore the

possibility of forming ornithine PNA duplexes. One may argue that due to the presumed absence of "helicase activity" in a prebiotic world a genetic material that formed not too stable duplexes would be advantageous in order to facilitate strand separation in a replication process.

Finally we find it noteworthy that nucleobase-amino acids have been identified in plants e.g. in the form of a 3-(1-uracylalanine).[20] Although this probably does not fit into a DNA mimic, it demonstrates that this type of compounds are naturally occurring, and that PNA-like molecular fossils may be found.

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References and Notes

1. Nielsen P. E.; Egholm M.; Berg R. H.; Buchardt O. *Science* **1991**, 254, 1497-1500.
2. Egholm M.; Buchardt O.; Christensen L.; Behrens C.; Freier S. M.; Driver D. A.; Berg R. H.; Kim S. K.; Norden B.; Nielsen P. E. *Nature* **1993**, *365*, 566-568.
3. Nielsen P. E. *Annu. Rev. Biophys. Biomol. Struc.* **1995**, 24, 167-183.
4. Nielsen P. E.; Egholm M.; Buchardt O. *Gene* **1994**, 149, 139-145.
5. Hanvey J. C. et. al. *Science* **1992**, 258, 1481-1485.
6. Bonham M. A. et. al. *Nucleic Acids Research* **1995**, 23, 1197-1203.
7. Demidov V.; Frank-Kamenetski M. D.; Egholm M.; Buchardt O.; Nielsen P. E. *Nucleic Acids Research* **1993**, 21, 2103-2107.
8. Ørum H.; Nielsen P. E.; Egholm M.; Berg R. H.; Buchardt O.; Stanley C. *Nucleic Acids Research* **1993**, 21, 5332-5336.
9. Ørum H.; Jørgensen M.; Koch T.; Nielsen P. E.; Larsson C.; Stanley C. *Biotechniques* **1995**, 19, 472-480.
10. Nielsen P. E. *Origins of life and the evolution of the biosphere* **1993**, *23*, 323-327.
11. Wittung P.; Nielsen P. E.; Buchardt O.; Egholm M.; Norden B. *Nature* **1994**, 368, 561-563.
12. Schwartz A. W. *Current Biology* **1994**, 4, 758-760
13. Böhler C.; Nielsen P. E.; Orgel L. E.; *Nature* **1995**, 376, 578-581.
14. Keller O. ; Keller W. E. ; Look G. v. ; Wersin G. *Org. Synth.* **1985**, *63*, 160-170.
15. ^{1}H-NMR(DMSO-d^6): 11.32 (s, 1H,CONHCO); 8.52 (d, 1H, H^6); 7.49 (s, 1H, CONH); 6.86 (t, 1H, CONH); 4.42 (s, 2H, NCH_2CO); 4.27 (t, 1H, $NCHCO_2$); 2.99 (t, 2H, CH_2NH); 1.9-1.2 (m, 4H, CH_2CH_2); 1.83 (s, 3H, CH_3 in T); 1.45 (s, 9H, CH_3C)
^{13}C-NMR (DMSO-d^6): 173.21; 166.95; 164.43; 155.62; 150.97; 142.40; 107.88; 77.46; 51.91; 49.01; 28.72; 28.33; 26.08; 11.93
MS-FAB$^+$ (Calc.) Found: (399.2) 399.3
Elemental analysis (Calc.) Found: C (51.23) 51.66; H (6.58) 6.62; N (14.07) 13.48
16. Christensen L.; Fitzpatrick R.; Gildea B.; Petersen K. H.; Hansen H. F.; Koch T.; Egholm M.; Buchardt O.; Nielsen P. E.; Coull J.; Berg R. H. *J. Pep. Sci.* **1995**, 3, 175-183.
17. FAB$^+$-MS (Calc.) found: T_{10} ; 2947,3; 2949,5; MALDI-TOF (Calc.) found: T_{15} ; (4347) 4352
H-AGT GAT_o CAT C-NH_2; (2741) 2741.
18. The synthesis of $(T_o)_{10}$ using Fmoc chemistry was described during the revision of this manuscript: Lioy E.; Kessler H.;*Liebigs ann.* **1996**, 201-204.
19. Hyrup B.; Egholm M.; Nielsen P. E.; Wittung P.; Norden B.; Buchardt O. *J. Am. Chem. Soc.* **1994**, 116, 7964-7970.
20. Gmelin R. Z.; *Physiol. Chem. Hoppe-Segler's* **1959**, 316, 164.

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