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Direction control in DNA binding of chiral D-lysine-based peptide nucleic acid (PNA) probed by electrospray mass spectrometry

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The DNA binding abilities of peptide nucleic acids (PNAs), both achiral and bearing three adjacent D-lysine-based monomers in the middle of the strand ("chiral box" PNA), were studied by means of electrospray mass spectrometry (ESI-MS). In contrast with achiral PNA, "Chiral box" PNA was confirmed to exert high direction control (antiparallel *vs*. parallel DNA target) in DNA binding.

Peptide nucleic acids (PNAs) are DNA mimic pseudopeptides, first described by Nielsen and co-workers in 1991,[1] which have attracted a great interest because of their highly specific and very stable DNA binding.[2] Due to their outstanding behavior, PNAs are currently used in a plethora of biological applications which require specific DNA recognition, such as detection of point mutations, genetic probing by *in situ* hybridisation, PCR clamping, inhibition of transcription and translation and many others.[3] Since their discovery, many modifications of the original PNA monomeric units have been reported, in order to improve the stability of the binding to the complementary nucleic acids and/or the specificity of the complexation.[4] Among all the proposed modifications, one of the most interesting is the introduction of a stereogenic centre on the α carbon of the aminoethylglycine unit (Figure 1).[5]

In a previous paper we reported that a chiral D-lysine-based PNA bearing three adjacent chiral monomers in the middle of the strand ("chiral box" PNA), was able to exert direction control (*i.e*. antiparallel *vs*. parallel[6]) in DNA binding, as deduced by the absence of a melting temperature in the parallel DNA-PNA mixture.[7]

Such a behavior is far from typical for standard achiral PNAs, which usually show poor direction control, binding both antiparallel and parallel targets, although with a marked preference for antiparallel DNAs.[8] In order to have independent confirmation of this outstanding ability with a technique different from standard melting temperature determination, we addressed the use of electrospray mass spectrometry (ESI-MS). Actually, ESI-MS has been extensively exploited in the last years to study DNA binding molecules[9] and spectra of $(PNA)_2$-DNA triplexes have also been reported.[10] Anyway, no systematic studies have yet been performed on the binding specificity of PNA-DNA duplexes by this technique. Therefore, in order to confirm the outstanding direction control exerted by the "chiral box" PNA, an extensive ESI-MS study was performed.

Mass spectrometer conditions were first carefully optimized in order to detect the PNA-DNA duplex signal in the negative ion mode even at low concentrations (5 μM for each strand); in particular buffer concentration, cone voltage and desolvation temperature were found to be important parameters affecting the signal of the duplex. The optimal buffer concentration (ammonium formate, pH = 7) was found to range from 5 to 10 mM. The signal intensity strongly decreased below 5 mM (due to the increasing intensity of sodiated and polysodiated signals) and above 10 mM (due to salt-induced ionization suppression). The cone voltage was optimal when kept to the minimum allowed from the instrument to give rise to viable signals, in our case 15 V. Even at 25 V no signals due to single strand DNA (ssDNA) or to the duplex were any more detectable, due to the in-source fragmentation of labile oligonucleotide molecules. Finally, the optimal desolvation temperature for duplex detection was found to be around 40–60 °C. Higher temperatures resulted in the gradual decrease of the duplex signal and in the corresponding increase of the ssDNA signal. Other instrumental parameters (gas flows, capillary voltage, source temperature) were found to influence the duplex signal in a very limited way†. Using optimized conditions, first ESI-MS spectra of an achiral PNA (sequence H-GTAGATCACT-NH_2, Figure 1) mixed with short synthetic oligonucleotides corresponding either to the antiparallel or to the parallel target (DNA sequences: antipar. 5′-AGTGATCTAC-3′, par. 5′-CATC-TAGTGA-3′) were recorded‡. Original and reconstructed spectra are reported in Figs 2 and 3.

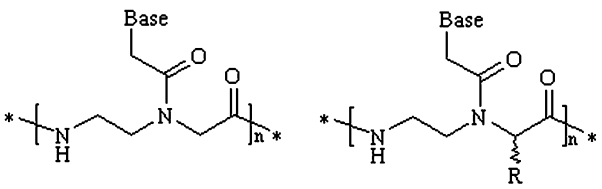

Fig. 1 Achiral (left) and chiral (right) PNA residues.

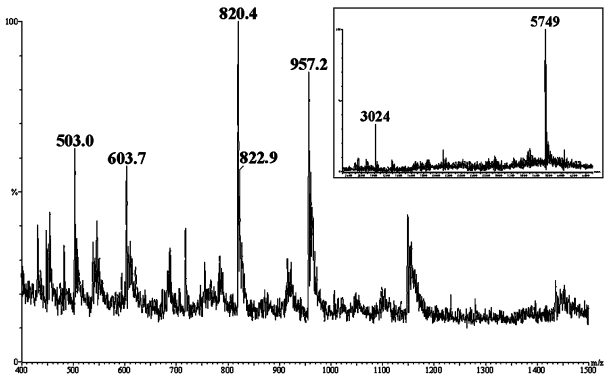

Fig. 2 ESI-MS spectrum of the achiral PNA–antiparallel DNA duplex with the corresponding reconstructed spectrum (insert).

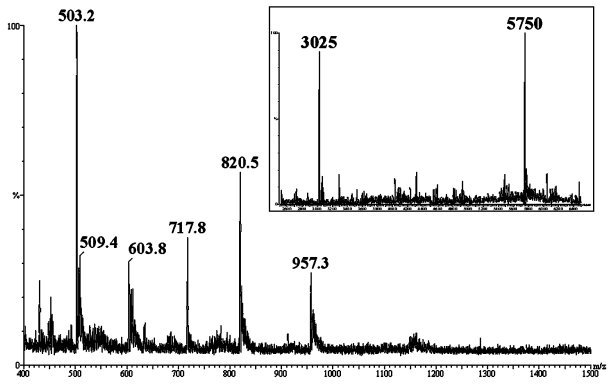

Fig. 3 ESI-MS spectrum of the achiral PNA–parallel DNA duplex with the corresponding reconstructed spectrum (insert).

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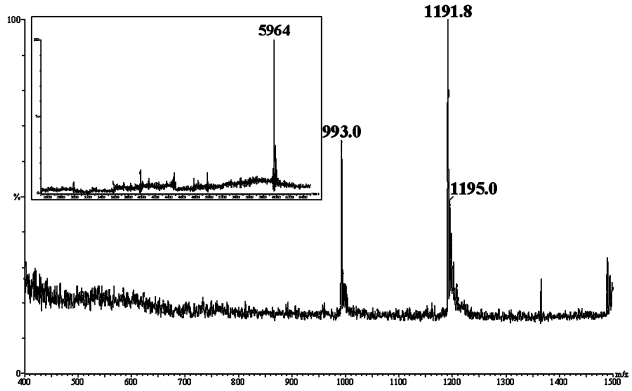

Fig. 4 ESI-MS spectrum of the "chiral box" PNA–antiparallel DNA duplex with the corresponding reconstructed spectrum (insert).

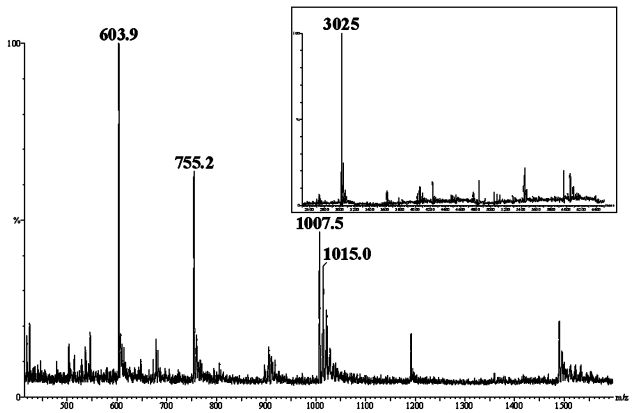

Fig. 5 ESI-MS spectrum of the "chiral box" PNA–parallel DNA duplex with the corresponding reconstructed spectrum (insert).

From the reconstructed spectra it is quite clear that in both cases a stable duplex is formed (calculated monoisotopic MW for PNA-DNA duplexes 5750 Da, found MWs 5749 ± 1 Da and 5750 ± 1 Da,), thus confirming the lack of direction specificity in DNA binding by a standard achiral PNA. However, the more stable antiparallel duplex gives a higher duplex/ssDNA intensity ratio, as compared to the less stable parallel duplex (calculated monoisotopic MW for single strand DNA 3025 Da, found MWs 3024 ± 1 Da and 3025 ± 1 Da). The sequence specificity was confirmed by an ESI-MS experiment performed on the same PNA mixed with a scrambled DNA, used as a control (sequence 5′-AGCGACGACTTGA-3′): in the reconstructed spectrum only the signal of the ssDNA was detectable (data not shown).

In contrast, a homologous "chiral box" PNA (sequence H-GTAG*ATC*ACT-NH_2, where monomers in italics are based on D-lysine, Figure 1)‡ gave rise to a strong duplex signal in the reconstructed ESI spectrum (calculated monoisotopic MW for PNA-DNA duplex 5963 Da, found MW 5964 ± 1 Da,) when mixed with the antiparallel DNA sequence, with no detectable ssDNA (Figure 4).

On the other hand, in contrast with what was observed with achiral PNA, the "chiral box" PNA did not form a duplex with the parallel DNA target, the corresponding signal in the reconstructed spectrum not being significantly different from background noise, while the MW of the free ssDNA was absolutely dominant (Figure 5).

These results confirm the high direction control of "chiral box" PNA, independently from the classical melting temperature determination. Moreover, it has been shown that ESI-MS is a suitable technique to analyze PNA-DNA duplexes and that it may be a useful tool in hybridization specificity studies on PNA.

Notes and references

† Optimal conditions used for recording ESI-MS spectra of PNA-DNA mixed solutions are the following: PNA and DNA are dissolved in double distilled water (5 μM each strand) with 10 mM ammonium formate buffer (pH = 7). The solutions were directly perfused into the ESI-MS spectrometer (Micromass ZMD 4000 equipped with a single quadrupole analyzer) at 10 μl min^{-1} rate. ESI conditions: negative ion mode, capillary voltage 3,0 kV, cone voltage 15 V, desolvation gas (N_2) 400 l h^{-1}, cone gas (N_2) 100 l h^{-1}, source temperature 120 °C, desolvation temperature 60 °C. Reconstructed spectra showing the actual MW corresponding to a multicharged pattern were obtained from the original spectra by using the program MaxEnt 1.0 (Micromass). The MaxEnt algorithm uses the method of maximum entropy to produce true molecular mass spectra from multiply-charged electrospray spectra, by finding the simplest molecular mass spectrum (spectrum of maximum entropy) that could account for the observed *m*/*z* data.

‡ Achiral PNA was synthesized by solid phase synthesis as previously reported.[7,8] Highly optically pure chiral box PNA was synthesized by the solid phase submonomeric strategy recently developed in our group.[11] Oligonucleotides were purchased from MWG Biotech AG (Ebersberg, DE)

1 P. E. Nielsen, M. Egholm, R. M. Berg and O. Buchardt, *Science*, 1991, **254**, 1497; M. Egholm, O. Buchardt, P. E. Nielsen and R. H. Berg, *J. Am. Chem. Soc.*, 1992, **114**, 1895.
2 M. Egholm, C. Behrens, L. Christensen, R. H. Berg, P. E. Nielsen and O. Buchardt, *J. Chem. Soc., Chem. Commun.*, 1993, **9**, 800; M. Egholm, O. Buchardt, L. Christensen L, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden and P. E. Nielsen, *Nature*, 1993, **365**, 566.
3 E. Uhlmann, A. Peyman, G. Breipohl and D. W. Will, *Angew. Chem. Int. Ed.*, 1998, **37**, 2796; P. E. Nielsen, *Curr. Opin. Biotech*, 2001, **12**, 16; H. Stender, M. Fiandaca, J. J. Hyldig-Nielsen and J. Coull, *J. Microb. Meth.*, 2002, **48**, 1.
4 P. E. Nielsen and G. Haaima, *Chem. Soc. Rev.*, 1997, **26**, 73; B. Falkiewicz, *Acta Biochim. Pol.*, 1999, **46**, 509; K. N. Ganesh and P. E. Nielsen, *Curr. Org. Chem.*, 2000, **4**, 931.
5 K. L. Dueholm, K. H. Petersen, D. K. Jensen, M. Egholm, P. E. Nielsen and O. Buchardt, *Bioorg. Med Chem. Lett.*, 1994, **4**, 1077; G. Haaima, A. Lohse, O. Buchardt and P. E. Nielsen, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1939; A. Puschl, S. Sforza, G. Haaima, O. Dahl and P. E. Nielsen, *Tetrahedron Lett.*, 1998, **39**, 4707.
6 In the accepted convention for PNA oligomers the PNA C-terminus corresponds to oligonucleotide 3′-end, whereas the PNA N-terminus corresponds to oligonucleotide 5′-end. Accordingly, in a parallel PNA-DNA duplex the PNA N-terminus is facing the DNA 5′-end, whereas in an antiparallel PNA-DNA duplex the PNA N-terminus is facing the DNA 3′-end.
7 S. Sforza, R. Corradini, S. Ghirardi, A. Dossena and R. Marchelli, *Eur. J. Org. Chem.*, 2000, 2905.
8 E. Uhlmann, D. W. Will, G. Breipohl, D. Langner and A. Ryte, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2632; S. Sforza, G. Haaima, R. Marchelli and P. E. Nielsen, *Eur. J. Org. Chem.*, 1999, 197.
9 S. A. Hofstadter and R. H. Griffey, *Chem Rev.*, 2001, **101**, 377.
10 M. C. Griffith, L. M. Risen, M. J. Greig, E. A. Lesnik, K. G. Sprankle, R. H. Griffey, J. S. Kiely and S. M. Freier, *J. Am. Chem. Soc.*, 1995, **117**, 831.
11 S. Sforza, T. Tedeschi, D. Ciavardelli, R. Corradini, A. Dossena and R. Marchelli, *Eur. J. Org. Chem.*, 2003, 1056.