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Interactions of DNA binding ligands with PNA–DNA hybrids

Pernilla Wittung, Seog K.Kim[1], Ole Buchardt[2], Peter Nielsen[3] and Bengt Nordèn*
Department of Physical Chemistry, Chalmers University of Technology, S-41296 Gothenburg Sweden, [1]Department of Chemistry, College of Science, Yeungnam University, Kyoungsan City, Kyoung-buk 712-749, Republic of Korea, [2]Department of Organic Chemistry, The H.C.Ørsted Institute, Universitetsparken 5, 2100 Ø Copenhagen and [3]Center for Biomolecular Recognition, The Panum Institute, Department of Biochemistry B, Blegdamsvej 3c, 2200 N Copenhagen, Denmark

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ABSTRACT

The interactions of two representative mixed-sequence (one with an AT-stretch) PNA–DNA duplexes (10 or 15 base-pairs) and a PNA_2/DNA triplex with the DNA binding reagents distamycin A, 4′,6-diamidino-2-phenylindole (DAPI), ethidium bromide, 8-methoxypsoralen and the Δ and Λ enantiomers of $Ru(phen)_2dppz^{2+}$ have been investigated using optical spectroscopic methods. The behaviour of these reagents versus two PNA–PNA duplexes has also been investigated. With triple helical poly(dA)/$(H\text{-}T_{10}\text{-}Lys\text{-}NH_2)_2$ no significant intercalative binding was detected for any of the DNA intercalators, whereas DAPI, a DNA minor groove binder, was found to exhibit a circular dichroism with a positive sign and amplitude consistent with minor groove binding. Similarly, a PNA–DNA duplex containing a central AATA motif, a typical minor groove binding site for the DNA minor groove binders distamycin A and DAPI, showed binding for both of these drugs, though with strongly reduced affinity. No important interactions were found for any of the ligands with a PNA–DNA duplex consisting of a ten base-pair mixed purine-pyrimidine sequence with only two AT base-pairs in the centre. Nor did any of the ligands show any detectable binding to the PNA–PNA duplexes (one containing an AATT motif). Various PNA derivatives with extentions of the backbone, believed to increase the flexibility of the duplex to opening of an intercalation slot, were tested for intercalation of ethidium bromide or 8-methoxypsoralen into the mixed sequence PNA–DNA duplex, however, without any observation of improved binding. The importance of the ionic contribution of the deoxyribose phosphate backbone, versus interactions with the nucleobases, for drug binding to DNA is discussed in the light of these findings.

INTRODUCTION

The interactions of double stranded DNA with small as well as with large molecules are often divided into contributions from the deoxyribose phosphate backbone and from the nucleobases themselves, although this segregation of binding/recognition components is not easily made. The main electrostatic component of the binding energy of cationic ligands is generally attributed to the negative phosphates of the DNA backbone, whereas the nucleobases contribute hydrophobic and dispersive bonding components but also considerable electrostatic components in terms of hydrogen and dipolar bonding (1,2). Specific interactions with the nucleobases naturally determine the sequence specificity exhibited by a ligand, for proteins they are also assisted by 'textured' properties such as helical pitch, indirectly determined by the base-sequence (3,4). However, the relative importance of electrostatic and hydrophobic contributions in the recognition/binding process is far from clear, even for rather simple ligands such as typical intercalators and minor groove binders.

We recently prepared a DNA analog termed PNA (peptide nucleic acid) containing an uncharged pseudo-peptide backbone composed of N-(2-aminoethyl)glycine units to which the nucleobases are attached (Figure 1a) (5–7). PNA has been found an excellent DNA mimic in terms of its ability to form Watson–Crick base-paired helical duplexes with complementary oligonucleotides (8). With a homopurine DNA strand two pyrimidine PNA strands form a triplex (7,9). Watson–Crick like PNA–PNA duplexes between complementary PNA strands have also been shown to form (10). In PNA only the nucleobases of DNA are retained and, furthermore, the backbone of PNA is uncharged. Therefore, the study of interactions of DNA recognizing ligands with these PNA-containing duplexes and triplexes could provide information specifically about the relative contributions of the nucleobases and the backbone to the ligand binding.

DNA ligands are generally classified as major or minor groove binders or intercalators. In the present study the following DNA

*To whom correspondence should be addressed

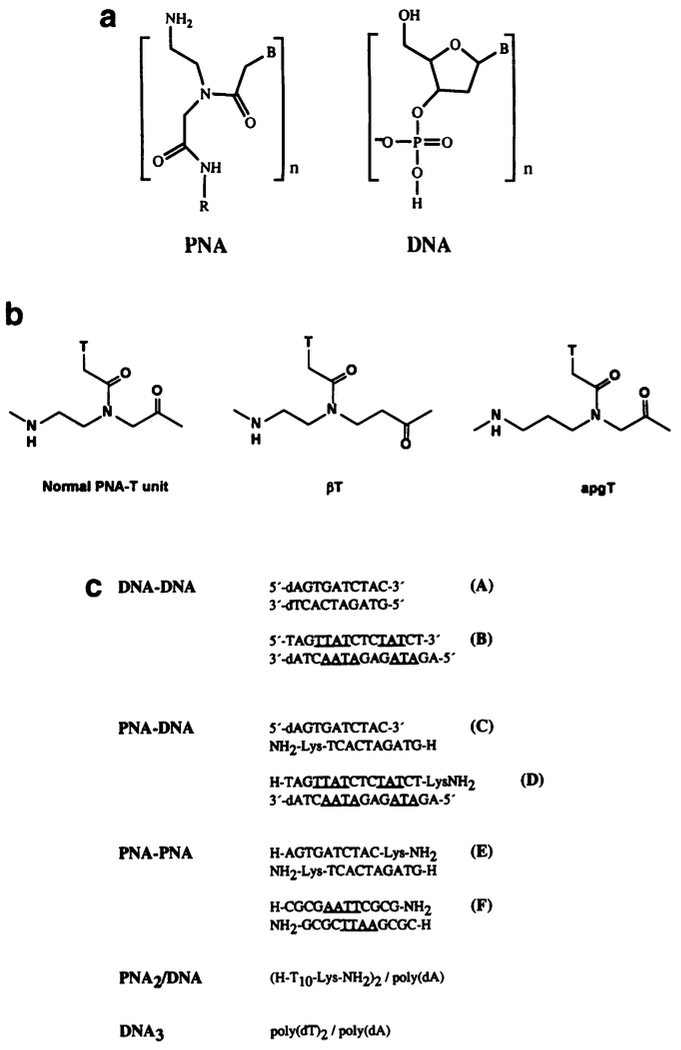

Figure 1. a. Structure of PNA compared to DNA. B = nucleobase and R = H or lysinyl amide for the PNAs discussed in this paper. The PNAs are written from the amino to the carboxy-terminal using normal peptide conventions: 'H' signifies a free amino group, while 'NH_2' signifies a terminal carboxamide. **b.** PNA derivatives with modified backbones. The structures 'βT' and 'apgT' provides an elongated backbone which should facilitate extension and unwinding upon intercalation. **c.** Oligonucleotide (DNA) and oligonucleopeptide (PNA) sequences used in the investigated complexes.

ligands have been investigated with respect to their interactions with PNA-containing duplexes and triplexes: distamycin A and 4',6-diamidino-2-phenylindole (DAPI), both known to bind preferentially to the minor groove of AT-rich sequences of B-DNA (11), ethidium bromide (12), 8-methoxypsoralen (8-MOP) (13), and the Δ and Λ enantiomers of $Ru(phen)_2dppz^{2+}$ all three intercalating (the latter only partially with its dppz wing between base-pairs in the DNA helix (14)) (Figure 2). Sequence complementary PNA and DNA oligomers of mixed purine-pyrimidine sequences were hybridized into PNA–DNA, PNA–PNA, or DNA–DNA duplexes. Potential triplex interactions were investigated using a homopyrimidine decamer, PNA-$(T)_{10}$, hybridized with a poly(dA) strand. In addition, a number of PNAs, with specific sequences, or in which the backbone had been modified, were studied with respect to drug interaction. Optical circular and linear dichroism, fluorescence and absorbance spectroscopies were used when appropriate for probing the interactions.

MATERIALS AND METHODS

Chemicals

For duplex studies the complementary sequences AGTGATCTAC (H-AGTGATCTAC-Lys-NH_2 for PNA and 5'-dAGTGATCTAC-3' for DNA) and GTAGATCACT (H-GTAGATCACT-Lys-NH_2 for PNA and 5'-dGTAGATCACT-3' for DNA) were used with either 2 antiparallel PNA strands (PNA–PNA), 2 antiparallel DNA strands (DNA–DNA), or one PNA strand and one DNA strand of each sequence in the antiparallel direction (PNA–DNA). Minor groove binding studies were also performed with the PNA–DNA duplex between the 15mer PNA H-TAGTTATCTCTATCT-Lys-NH_2 and the complementary DNA sequence 5'-dAGATAGAGATAACTA-3', the corresponding DNA–DNA duplex between 5'-dTAGTTATCTCTATCT-3'and 5'-dAGATAGAGATAACTA-3' oligonucleotides, as well as the PNA–PNA duplex with the self-complementary 12mer PNA H-CGCGAATTCGCG-NH_2. Intercalation studies with PNA–DNA duplexes containing lengthenings in the PNA backbone were performed with the PNA sequence H-GTAGAXCACT-Lys-NH_2 where X = βT or apgT (15) (See Figure 1b) hybridized to the corresponding DNA sequence 5'-dAGTGATCTAC-3'. A PNA_2/DNA triple helix was formed with the 10-mer PNA H-T_{10}-Lys-NH_2 and poly(dA) and a corresponding DNA triple helix was formed with poly(dT) and poly(dA). See Figure 1c for a summary of all sequences used.

The PNA and DNA oligonucleotides were synthesised as described elsewhere (6,7,16). The synthetic polynucleotides poly(dA) and poly(dT) were purchased from Pharmacia. The ligands distamycin A, 4',6-diamidino-2-phenylindole (DAPI), ethidium bromide (EB), and 8-methoxypsoralen (8-MOP) were purchased from Sigma. The Δ and Λ enantiomers of $Ru(phen)_2dppz^{2+}$ (phen = 1,10-phenantroline; dppz = dipyrido[3,2-a:2',3'-c]phenazine) were synthesised as described elsewhere (14). The buffer used throughout this study was 5mM phosphate, pH 7.0. The duplexes between PNA–PNA, PNA–DNA, and DNA–DNA, as well as the triplex between $2PNA(T)_{10}$ and poly(dA), were formed by mixing equimolar amounts (2 to 1 for the triplex) of the strands and annealing at room temperature for one hour. The poly(dA)/$[poly(dT)]_2$ triplex was prepared by mixing poly(dT) and poly(dA), in a 2 to 1 concentration ratio, in the presence of 1mM $MgCl_2$ followed by heating to 90°C for 20 min and then annealing over night at room temperature. For spectrophotometric concentration determinations, the DNA molar extinction coefficients were also used for the PNA sequences. Concentrations of between 25μM to 50μM of base-pairs, or base-triplets, were normally used in the experiments. For binding constant estimations of the minor groove binders, titrations both at 25–50 μM and 250 μM base-pairs were performed.

Spectroscopic measurements

Circular dichroism (CD), defined as the differential absorption of left and right circularly polarised light was measured on a Jasco 720 Spectropolarimeter using either 10 mm or 1mm path length. Steady-state fluorescence measurements were carried out using an Aminco SPF-500 spectrofluorometer in the quanta correction

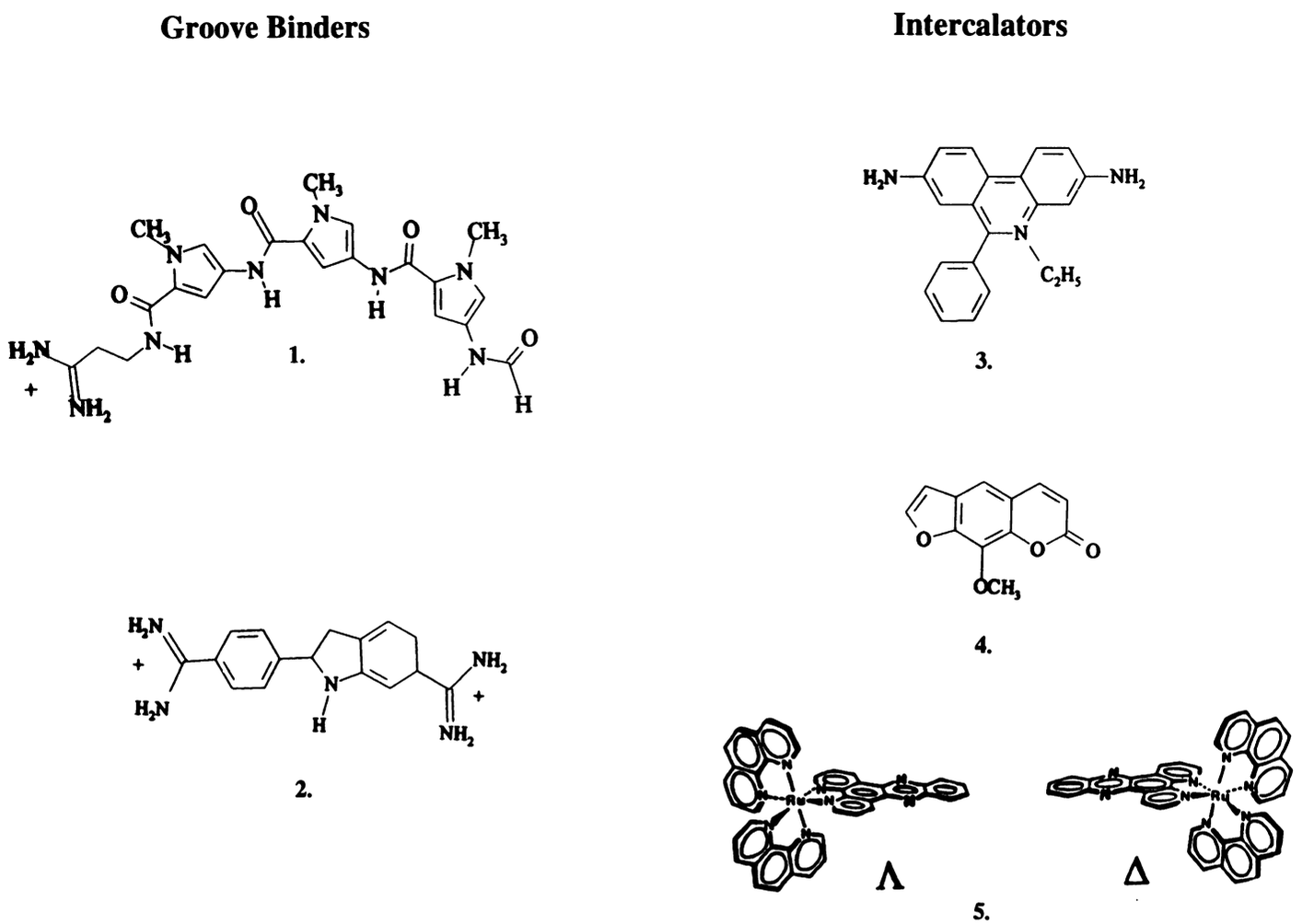

Figure 2. Chemical structures of the ligands studied: Distamycin A (**1**); 4′,6-diamidino-2-phenylindole, DAPI (**2**); ethidium bromide, EB (**3**); 8-methoxypsoralen, 8-MOP (**4**); and the Δ and <SYMBOL 76 Λ enantiomers of $[Ru(phen)_2dppz]^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine) (**5**).

Table 1. Summary of interactions of DNA binding ligands with double and triple helical PNA−DNA complexes. See text and Materials and Methods for explanation

Ligand (charge)	DNA duplex	DNA:PNA duplex	PNA:PNA duplex	DNA triplex (T:A:T)	triplex dA/$(pnaT_{10})_2$
Distamycin A (+1)	Minor groove binding	AT-binding[1]	No binding	Minor groove binding	No significant binding
DAPI (2+)	Minor groove binding	AT-binding[1]	No binding[4]	Minor groove binding	Minor groove binding
8-MOP (non-ionic)	Intercalates	No significant binding[2]	No binding	–	–
EB (2+)	Intercalates	None or very poor binding[2,3]	No binding	Intercalates[5]	No binding
Δ(Ru)dppz (2+)	Intercalates	None or very poor binding[3]	No binding	Intercalates	No binding
Λ(Ru)dppz (2+)	Intercalates	None or very poor binding[3]	No binding	Intercalates	No binding

[1]A PNA−DNA duplex with a central AATA motif (D) showed binding (Figure 3 and 4), however, the mixed sequence (C) with only AT did not.
[2]PNAs with an extended backbone (Figure 1b) to facilitate intercalative opening did not show any binding either (see text). Photocrosslinking indicated very weak binding of 8-MOP.
[3]Less binding than to the single-stranded oligonucleotide was observed.
[4]A self-complementary PNA forming a PNA−PNA duplex containing a central AATT motif (F) did not show any binding either.
[5]According to Scaria, P.V. and Shafer, R.H. (22).

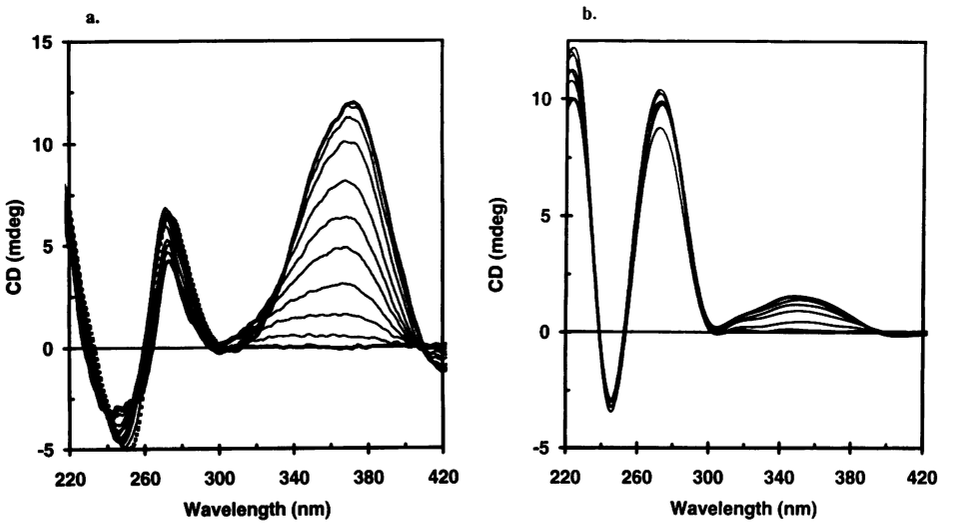

Figure 3. Circular dichroism of a 15 base-pair PNA–DNA duplex (D), which incorporates an ATA and an AATA-motif, upon titration with minor groove binder DAPI (**b**) and the corresponding DNA–DNA duplex (B) titration (**a**). The basepair concentration was 25 μM in (**a**) and 50 μM in (b). The DAPI concentration ranges from 0–20 times the duplex concentration in (a), and 0–5 times the duplex concentration in (b). The CD in the absorption band above 300 nm is induced as a result of interaction with the helical nucleic acid structure. The CD amplitude reflects the amount of bound drug.

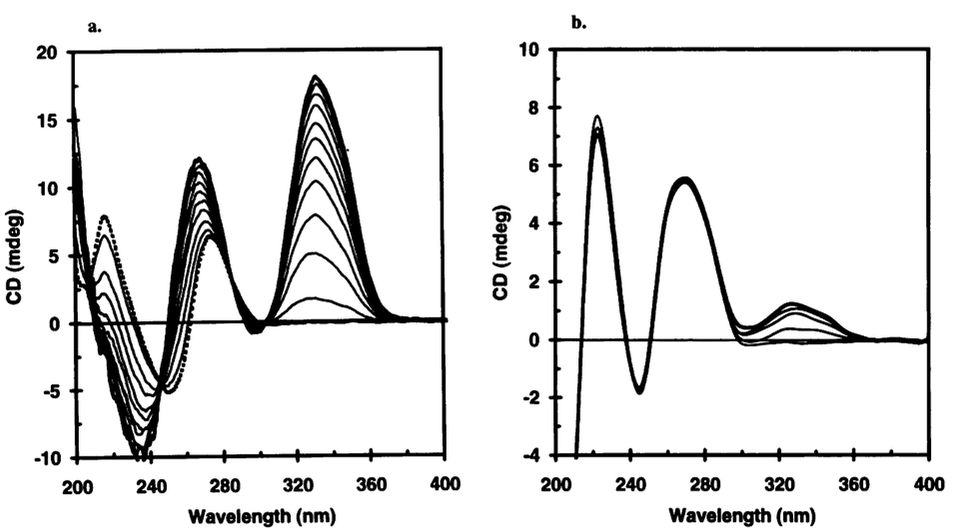

Figure 4. Circular dichroism of a 15 base-pair PNA–DNA duplex (D), which incorporates an AATA-motif, upon titration with minor groove binder Distamycin A (**b**) and the corresponding DNA–DNA duplex (B) titration (**a**). The basepair concentration was 25 μM and the Distamycin A concentration ranging from 0–25 times the duplex concentration in (a), and 0–7 times the duplex concentration in (b).

mode. The emission spectra were measured upon excitation at appropriate wavelengths specific for the respective drug analysed. Isotropic absorbance spectra were recorded on a Varian Cary 2300 spectrophotometer.

Binding affinities

Owing to the rather small amounts available of the PNA reagents, complete stability constant determinations were not possible to perform. Sizes of binding constants were estimated from titration curves (from deviation from a linear dependence at the point of half saturation). As a control also the increased dissociation of complex upon dilution (10×) was checked. It should be emphasized that these estimates are very approximate since they are based on the assumption of linear dependence between oserved spectral change and amount of bound drug.

RESULTS

The interactions of six typical minor groove binders and intercalators, with DNA–DNA, PNA–DNA and PNA–PNA duplexes have been screened using, depending of sensitivity, absorbance, circular dichroism or fluorescence techniques. For this purpose decamer strands of a mixed purine-pyrimidine sequence of DNA and PNA, and the corresponding

complementary strands were used. For minor groove binding also a 15-mer sequence with an AATA motif was used (Figure 1c). The interactions of the drugs with a $(PNA(T)_{10})_2$/poly(dA) triplex were compared with their binding to a normal $(poly(dT))_2$/poly(dA) triplex. The results are summarized in Table 1 and refer to significant binding observable for nucleotides in the sub-millimolar range.

Minor groove binders

DAPI, a synthetic trypanosid drug, and distamycin A, a naturally occurring agent, are both known to bind preferentially to the minor groove of AT-rich DNA sequences (11). Both drugs showed binding to the PNA–DNA duplex (D), containing a central AATA motif, but neither of them bound to a PNA–DNA duplex (C) lacking such an AT stretch. The binding was evidenced from the appearance of induced circular dichroism in the drug absorption bands in the 300–350 nm region (Figure 3b (DAPI) and 4b (distamycin A)). Both drugs are achiral and, hence, exhibit no CD in absence of interaction with the chiral nucleotide duplexes. Also in the absorption spectra of DAPI (not shown) a hypochromic effect (some 18% at 340 nm) and a small red-shift (5 nm) accompanied the binding, comparable with that observed for minor groove bound DAPI to DNA (17).

The binding most likely occurs in the minor groove, as indicated by the induced positive circular dichroism signal in the 350 nm absorption band (Figure 3b for DAPI and 4b for distamycin A) resembling the spectrum of DAPI when bound in the minor groove of DNA (18). The specific CD of bound DAPI was estimated to $\Delta\epsilon = +7\ M^{-1}cm^{-1}$ which compares reasonably well with the values observed for the minor groove DAPI complexes with poly[(dA-dT)]$_2$ ($\Delta\epsilon = +22\ M^{-1}cm^{-1}$, (19)) AT-containing oligonucleotide duplexes ($\Delta\epsilon = +9-+12\ M^{-1}cm^{-1}$, (19)) and the present corresponding DNA–DNA double helix B ($\Delta\epsilon = +13\ M^{-1}cm^{-1}$).

The binding affinities of DAPI as well as distamycin A, for the AT-rich PNA–DNA duplex (D), were both estimated to $5\times10^5M^{-1}$ per site (with two AT sites for DAPI and one for distamycin A). With the DNA–DNA duplex (B) saturation is reached at a considerably higher drug/duplex ratio than for PNA–DNA suggesting more (unspecific) binding sites available (Figure 3a and 4a); the avarage binding constant per site was here estimated to be $\sim10^7\ M^{-1}$ in agreement with studies of DAPI binding to oligonucleotides of varied sequence (20). The PNA–DNA duplex (C), with a mixed sequence, does not show any binding of either DAPI or distamycin A. Binding thus occurs only to the PNA–DNA duplex (D), most likely to the AATA site for distamycin A and to both the AATA site and the ATA site for DAPI.

In accordance with the observed decreased binding affinity, when replacing one strand in a DNA–DNA duplex with PNA, the PNA–PNA duplexes, including one with a central AATT stretch (F), were found completely unable to bind any of these minor-groove drug molecules.

The triplex formed between poly(dA) and PNA-$(T)_{10}$ has previously been characterized using linear and circular dichroism and been shown to form a base stacking structure similar to that of the normal DNA T-A-T triplex (9). From the appearance of an induced circular dichroism (Figure 5), similar to that observed for DAPI complex with a DNA T-A-T triplex (Kim *et al.*, to be published), DAPI is also found to bind to the PNA_2/DNA-triplex. The flow linear dichroism spectrum was found to be very

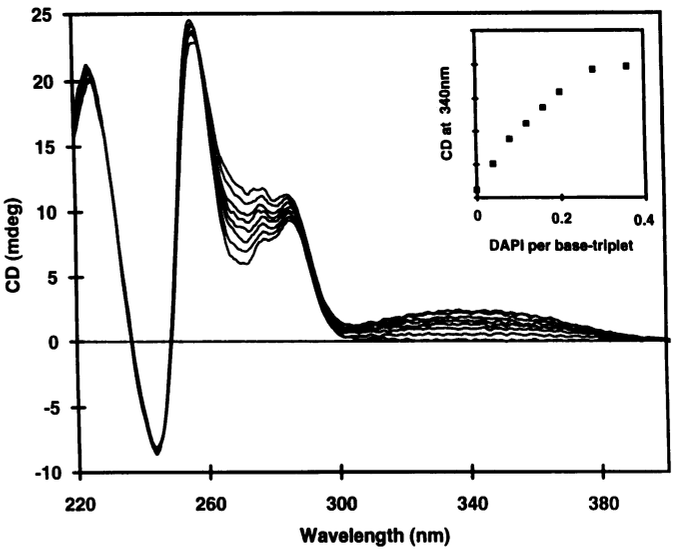

Figure 5. Circular dichroism of poly(dA)/$(PNA(T)_{10})_2$ triplex titrated with increasing amount of DAPI. The binding is observed as described in Figure 3. The base-triplet concentration is 50 μM. CD at 340 nm as a function of added DAPI per base-triplet is seen in the insert.

similar to that obtained for DAPI bound to the normal DNA triplex (results not shown) suggesting similar binding geometries in the minor groove at an angle roughly 45° relative to the fiber axis (9). A binding constant of approximately $3\times10^4M^{-1}$ (per base-pair) was estimated. This is a 20 fold reduction compared to the affinity of DAPI for [poly(dA-dT)]$_2$ (20). With the cationic distamycin A the difference is even larger and to this PNA_2/DNA triplex no significant binding of distamycin A was detected.

Intercalators

Typical DNA intercalators such as ethidium bromide, 8-methoxypsoralen and the Δ and Λ enantiomers of $Ru(phen)_2dppz^{2+}$ were tested with respect to their interaction with the PNA–DNA duplex, the PNA–PNA duplex and the $(PNA(T)_{10})_2$/poly(dA) triplex using, when appropriate, absorbance, circular dichroism and/or fluorescence.

8-MOP is known to bind rather weakly to DNA ($K = 10^3M^{-1}$ (13)) but being a non-ionic molecule it was considered a potentially suitable ligand for the PNA–DNA hybrids as a result of the non-charged character of the PNA part. However, no significant binding could be detected spectroscopically neither to the PNA–DNA duplex nor to the PNA–PNA duplex under conditions at which 8-MOP did bind to the corresponding DNA–DNA duplex. These results were corroborated by photocrosslinking experiments showing that under conditions that resulted in 80 % interstrand crosslinking of a DNA duplex (5′-AGTGATCTAC-3′ + complement, (A)), less than 2% of a product attributed to a PNA–DNA interstand crosslink was obtained using the corresponding PNA–DNA duplex (C).

In the micromolar concentration range no significant binding occurs of ethidium bromide to PNA–DNA and PNA–PNA duplexes. At higher concentrations (50 μM base-pairs) a certain fluorescence increase (not shown) indicates some binding,

however, less than to a corresponding single strand of DNA. Also, the absence of the characteristic induced circular dichroism for the DNA–DNA complex suggests that this is not a function of intercalated ethidium bromide, but rather an effect of external binding ($K \sim 10^2 M^{-1}$ per base-pair).

The PNAs with modified backbones shown in Figure 1b were also studied with respect to interaction with intercalators. Extentions of the backbone were introduced below and above a central thymine residue in duplex C in order to reduce the rigidity of the amide-backbone that might restrict opening between base-pairs (breathing) and hence intercalation. These modified PNAs have been shown to form stable complexes with DNA (15). However, no improved binding of ethidium bromide, or 8-MOP, was detected by flourescence and circular dichroism respectively.

Neither the Δ nor the Λenantiomer of $Ru(phen)_2dppz^{2+}$, which are known to bind very strongly to double-stranded DNA ($K \approx 10^8 M^{-1}$), with the dipyrido[3,2-a:2′,3′-c]phenazine ring intercalated (14), did show any significant intercalative binding to the PNA-containing duplexes or triplexes as judged from fluorescence and absorbance measurements. As deduced from a certain fluorescence emission increase (data not shown) there is, just as for ethidium bromide, a weak, virtually non-specific binding of both enantiomeric forms of the metal-complexes to single-stranded DNA, and an even weaker binding to the PNA–DNA duplex, probably to its DNA strand.

DISCUSSION

It is generally accepted that the electrostatic attraction by the deep electronegative potential well of the minor groove in AT-rich regions of duplex DNA is a determinant component for binding of cationic DNA ligands such as DAPI or distamycin A. It is therefore not surprising that these minor groove binders bind less efficiently to an AT stretch-containing PNA–DNA duplex. The reduced binding affinity can be explained by the reduction of negative charge of the duplex due to the non-ionic PNA strand. In agreement with this interpretation, no binding at all is found with the charge neutral PNA–PNA duplexes. As to the poly(dA)/$(PNA\text{-}T_{10})_2$ triplex, anticipated to display a minor groove with negative charge from the single poly(dA) strand, DAPI, but not distamycin A, is found to bind. Distamycin A carries only a single positive charge (on its terminal amidino group) whereas DAPI has one positive charge at each end, a difference that may suffice to make the binding negligible to the nucleic triple helix structure with its negative charge being reduced from −3 per base-triplet to −1.

For the DNA intercalator ethidium bromide, just as for the minor-groove drugs, a major component of the binding energy is electrostatic and, therefore, a reduced binding affinity to a PNA–DNA duplex and a PNA–PNA duplex would be expected. The lack of significant binding could be due to this effect but other possibilities exist. We speculated that at least part of the explanation could be an unability of the PNA backbone to accomodate the helix extension (by 3.4 Å) and unwinding (presumably 10–30°, (21)) required for intercalative binding. We therefore studied two PNA derivates in which the backbone had been extended, at either side of one base, to facilitate helix extension and unwinding at this position. The lack of binding even to such modified PNA–DNA duplexes is taken as evidence that steric constraints by the backbone towards extension and unwinding is not the main obstacle to intercalative binding. However, it is reasonable that the breathing rate of the PNA-containing hybrid duplexes, which would influence intercalation, is decreased as a result of the strongly reduced repulsion between the backbone units. Further studies are in progress in order to clarify the conditions required for intercalation.

The non-ionic 8-methoxypsoralen, whose binding to normal DNA is solely due to dipole-dipole, dispersive and hydrophobic interactions upon stacking close to the nucleobases, did not show any significant (or only very weak) binding to the PNA–DNA duplex. In this case, a decreased breathing, unfavourable helix extension unwinding geometry, appears the only plausable explanation for the highly reduced affinity compared to DNA.

CONCLUSIONS

Replacement of one DNA strand in duplex DNA (or two strands in triplex DNA) by the DNA mimic PNA, deviod of the negatively charged phosphate backbone, leads to a drastic reduction in binding capability of both minor groove binders and intercalators. The origin of the decreased binding is considered to be a reduced electrostatic potential, as a direct effect on the Coulombic interaction between the drug and the nucleic acid, and, an effect (for intercalators) of decreased breathing frequency either due to lower internucleotide repulsion or to a decreased flexibility of the pseudopeptide backbone.

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REFERENCES

1. Saenger, W. *'Principles of Nucleic Acid Structure'* Springer-Verlag, Berlin, (1984).
2. Pullman, B. in *'Molecular Mechanisms of Carcinogenic and Antitumor Activity'* (Eds. Chagas, C. and Pullman, B.) ISBN 88–7761–023–9 Adenine Press, N.Y. pp. 3–31 (1987).
3. Travers, A. *'DNA–Protein Interactions'* Chapman & Hall, London (1993).
4. Steitz, T.A. *'Structural Studies of Protein-Nucleic Acid Interaction. The Sources of Sequence-Specific Bindings.'* Cambridge University Press, Cambridge (1993).
5. Nielsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. *Science* **254**, 1497–1500 (1991).
6. Egholm, M., Buchardt, O., Nielsen, P.E. and Berg, R.H. *J. Am. Chem. Soc.* **114**, 1895–1897 (1992).
7. Egholm, M., Buchardt, O., Nielsen, P.E. and Berg, R.H. *J. Am. Chem. Soc.* **114**, 9677–9678 (1992).
8. Egholm, M. Buchardt, O., Christensen, L., Behrens, C., Freire, S.M., Driver, D. Berg, R.H., Kim, S.K. Nordén, B. and Nielsen, P.E. *Nature* **365**, 566–568 (1993).
9. Kim, S.K., Nielsen, P. E., Egholm, M., Buchardt, O., Berg, R.H. and Nordén, B. *J. Am. Chem. Soc.* **115**, 6477–6481 (1993).
10. Wittung, P., Nielsen, P.E. Buchardt, O., Egholm, M. and Norden, B. *Nature* **368**, 561–563 (1994).
11. a. Zimmer, C. *Prog. Nucleic Acid Res. Molec. Biol.* **15**, 285–318 (1985) **b.** Zimmer, C. and Währent, U. *Prog. Biophys. Molec. Biol.* **47**, 31–112 (1986).
12. Houssier, C., Hardy, B. and Fredericq, E. *Biopolymers* **13**, 1141–1160 (1974).
13. Hansen, B., Bjerring, P., Buchardt, O., Ebbesen, A., Kanstrup, G., Kamp, P., Knudsen, P.H., Nielsen, P., Norden, B. and Ygge, B. *J. Med. Chem.* **28**, 1001–1010 (1985).

14. Hiort, C., Lincon, P. and Nordén, B. *J. Am. Chem. Soc.* **115**, 3448–3454 (1993).
15. Hyrup, B., Egholm, M., Nielsen, P., Wittung, P., Nordén, B. and Buchardt, O. *J. Am. Chem. Soc.* **116**, 7964–7970 (1994).
16. Egholm, M., Behrens, C., Christensen, L. Hansen, H.F., Vulpius, T., Flensburg, C. Dueholm, K., Petersen, K.H., Berg, R.H., Nielsen, P.E. and Buchardt, O. *J. Org. Chem.* (in press).
17. Kubista, M. Åkerman, B. and Nordén. B. *Biochemistry* **26**, 4545–4553 (1987).
18. Nordén B., Kubista, M. and Kurusev, T. *Quart.Rev. Biophys.*, **25**, 103–164 (1992).**19.** Eriksson, S. Kim, S.K. Kubista, M. and Nordén B. *Biochemistry* **32**, 2987–2998 (1993).
20. Jansen, K.Nordén, B. and Kubista, M. *J. Am. Chem. Soc.* **115**, 10527–10530 (1993).
21. Waring, M.J. in *'Molecular Basis of Antibiotic Action'* (Eds. Gale, E.F., Lundliffe, F., Reynolds, P.E. and Waring, M.J.) Wiley, London, pp. 258–401 (1981).
22. Scaria,P.V. and Shafer, R.H. *J. Biol. Chem.* **266**, 5417–5423.