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Influence of nucleotide sequence on dA·dT-specific binding of Netropsin to double stranded DNA

Ch.Zimmer[a], Ch.Marck[b], Ch.Schneider[b] and W.Guschlbauer[c]

[a]Zentralinst. Mikrobiol. und exp. Therapie, Forschungszent. Molekularbiol. und Med., Akad. Wiss. DDR, Abt. Biochem., Beuthenbergstr. 11, DDR-69-Jena, GDR and Services de [b]Biophys. et [c]Biochim., Dep. Biol., Cent. d'Etudes Nucléaires de Saclay, BP No. 2,F-91190, Gif-sur-Yvette, France

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ABSTRACT

Using CD measurements the complex formation of Netropsin (Nt) with poly(dA–dC)·poly(dT–dG) and its stability against high salt concentrations is compared with that of poly(dA)·poly(dT) and poly(dA–dT)·poly(dT–dA). It is experimentally shown that the insertion of a dG·dC pair in dA·dT sequences strongly reduces the specific interaction of Nt with DNA duplexes. The specificity of the interaction is strongly increased by two or more consecutive thymine residues as present in thymine isostichs of double stranded DNA's.

INTRODUCTION

Specific interaction of the oligopeptide netropsin (Nt) with double-stranded DNA occurs in regions rich in dA·dT base pairs, as demonstrated by a variety of spectroscopic and hydrodynamic measurements[1–5]. The strong binding affinity of Nt to duplex DNA strongly depends on dA·dT content[1,5], which is most efficient for the synthetic polymers poly(dA)·poly(dT) and poly(dA–dT)·poly(dT–dA) and has been related to clusters of dA·dT pairs in DNA[1,2,6]. The importance of dA·dT base pairs as strong Nt binding sites was further corraborated by CD studies with deoxy-oligomers[7,8] and synthetic polymers[1,4,9,10].

In this paper the interaction of Nt with double-stranded poly(dA–dC)·poly(dT–dG) is compared with that of the two pure dA·dT duplexes. The large differences in the salt stability of the Nt–polynucleotide complexes clearly demonstrate that the insertion of one dG·dC base pair into a dA·dT sequence is the crucial factor in the decrease of binding affinity of the oligopeptide.

MATERIAL AND METHODS

Nt hydrochloride used in these studies was isolated from Streptomyces netropsis[2] and kindly donated by H. Thrum (Jena). Extinction coefficients ε_{238}=19800 and ε_{296}=21500 were used[1]. Synthetic polynucleotides were purchased from Boehringer (Mannheim, G.F.R.); the following extinction coefficents

were used: poly(dA).poly(dT), ε_{260}= 6000; poly(dA-dT)·poly(dT-dA), ε_{260}=6800; poly(dA-dC)·poly(dT-dG), ε_{260}= 6500.

CD spectra were measured on a Roussel-Jouan dichrographe II-185, interfaced with a PDP-12 computer[11], in 1 cm cuvettes. CD data are given as $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$. Interaction spectra (iCD) are given as $\Delta\Delta\varepsilon = \Delta\varepsilon_{complex} - \Delta\varepsilon_{free\ DNA}$, reflecting the induced Cotton effect of bound Nt.

RESULTS

CD spectra of the Nt complex with DNA detect binding of this ligand to the double stranded structure by an induced Cotton effect at 315 nm[1,3,4,7]. Typical CD spectra of the Nt complex with poly(dA-dC)·poly(dT-dG) (fig. 1A)

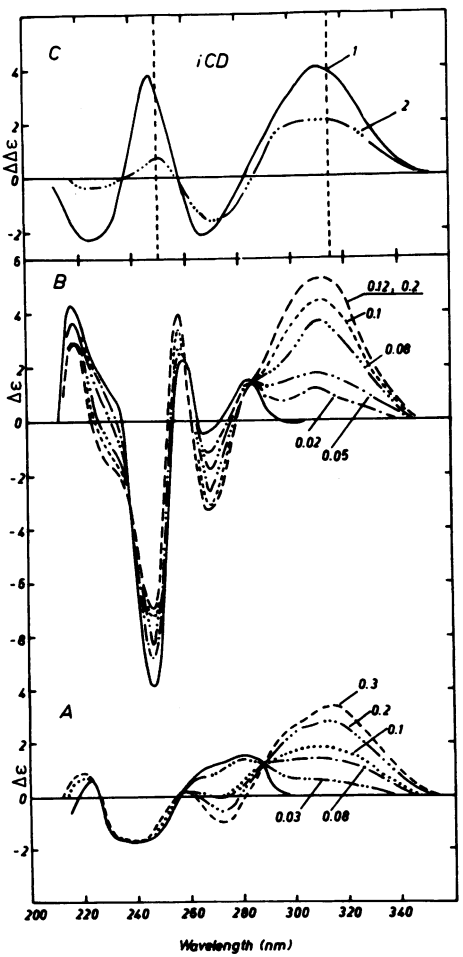

Figure 1: CD spectra of the Nt complexes poly(dA-dC)·poly(dT-dG) (A) and poly(dA)·poly(dT) (B) in 0.15 M NaCl. Numbers indicate r' (Nt/nucleotide). (C) CD interaction spectra (iCD) of the two duplexes in (A) and (B) at r'=0.1: 1: poly(dA)·poly(dT); 2: poly(dA-dC)·poly(dT-dG).

are compared with those of poly(dA)·poly(dT) (fig. 1B). Both polymer duplexes exhibit similar induced Cotton effect in the region around 315 nm upon Nt binding (fig. 1C), but with higher efficiency for poly(dA)·poly(dT) as indicated by the greater amplitude. The iCD spectra of these Nt complexes of synthetic deoxypolynucleotides are similar in shape to those observed with natural DNAs (ref. 1,3,4). They are composed of two positive and two negative dichroic bands. The CD amplitudes depend on the dA·dT content of the polymer duplex at a given Nt binding ratio. The CD maxima of the poly(dA)·poly(dT) complex are located about 5 nm lower than those of the other complexes. Thus the iCD spectra (fig. 1C) and in particular the induced CD band around 315 nm which directly reflects the induced CD of bound Nt, suggest that the Nt complex formation with double stranded DNA is not only affected by the base composition, but also by the nucleotide sequence

The CD titration curves of poly(dA)·poly(dT) and poly(dA–dC)·poly(dT–dG) (fig. 2) likewise indicate that a much higher Nt ratio is required to reach the saturation level, i.e. binding is less effective.

In figures 3 and 4 results on the stability of Nt–duplex complexes against high salt concentration are presented. The amplitude of the 315 nm CD band of the Nt complex of poly(dA–dC)·poly(dT–dG) at r'=0.1 is strongly decreased, if the ionic strength is increased from 0.15 M to 0.5 M NaCl, indicating removal of Nt from the complex (fig. 3A). From the iCD spectra in fig. 3 it follows that the Nt complex of poly(dA–dC)·poly(dT–dG) is most sensitive and completely dissociated at 0.8 M NaCl. In contrast, poly(dA)·poly(dT) is highly stable even at 4 M NaCl (fig. 3D), while poly(dA–dT)·poly(dT–dA) shows intermediate stability (fig. 3C), in accordance with previous results[1,4]. The iCD spectrum of poly(dA–dC)·poly(dT–dG) (fig. 3B) is

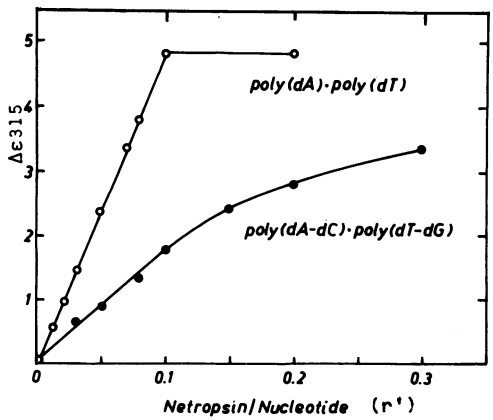

Figure 2: Comparison of CD titration at 315 nm of duplex polymers in 0.15 M NaCl.

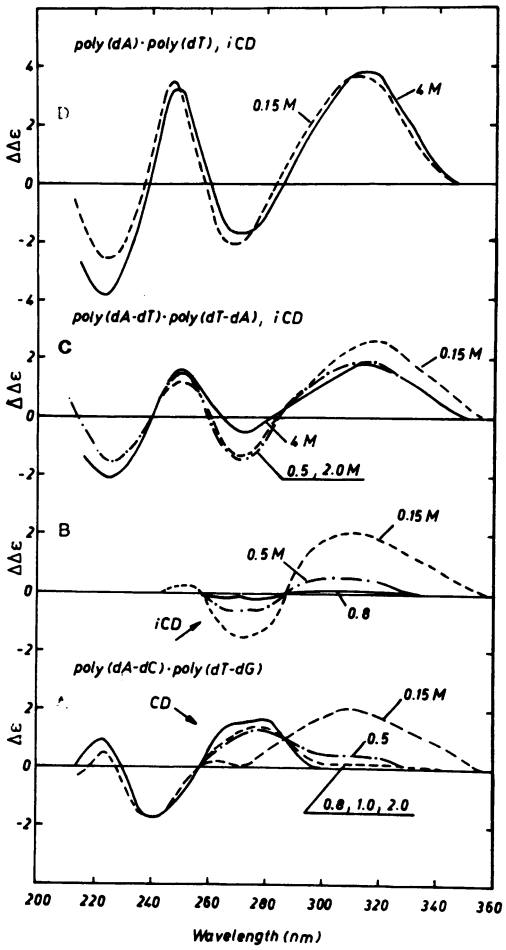

Figure 3: Influence of salt concentration on the CD spectrum of the Nt-complex with poly(dA-dC)·poly(dT-dG) (A); of the iCD spectra of poly(dA-dC)·poly(dT-dG) (B), poly(dA-dT)·poly(dT-dA) (C) and poly(dA)·poly(dT) (D). The Nt concentration was r'=0.1. Numbers on the spectra are NaCl concentrations.

very small at 0.5 M NaCl and disappears at 0.8 M NaCl , while it is practically unchanged in the case of the two other Nt-duplex complexes. The dependence of Nt binding on salt concentration shown in fig. 4 compares the relative stability of the Nt complexes of the three deoxynucleotide duplexes used in this investigation. It is immediately striking that poly(dA-dC)·poly(dT-dG) shows a sharp cooperative decrease which approaches zero above 0.5 M NaCl (fig. 4, curve 3), poly(dA)·poly(dT) (curve 1) and poly(dA-dT)·poly(dT-dA) (curve 2) show small changes only, even at 4 M NaCl. This clearly shows a pronounced lowering of the Nt interaction as a consequence of the insertion of a dG·dC base pair into the dA·dT sequence.

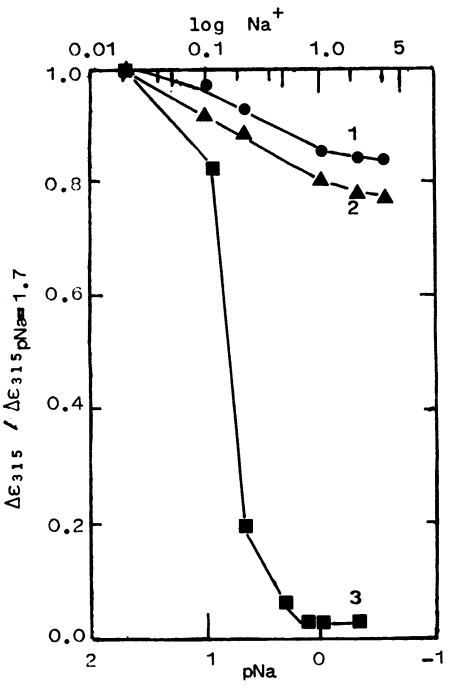

Figure 4: Dependence of the stability of the Nt complexes of synthetic duplex polymers on salt concentration at r'=0.1 Nt/pucleotide.
1: poly(dA)·poly(dT),
2: poly(dA-dT)·poly(dT-dA),
3: poly(dA-dC)·poly(dT-dG).

The induced CD band at 315 nm was normalized, taking its intensity at pNa=1.7 (0.02 M Na^+) as 1.0.

DISCUSSION

Previous conclusions for a possible importance of the base pair sequence of DNA in the Nt binding as predicted from CD and hydrodynamic studies[1,2,4-6] are now experimentally verified by the comparative results with poly(dA-dC)·poly(dT-dG). A decreased affinity of Nt for the double stranded DNA structure containing alternating sequences of dA·dT and dG·dC pairs is directly indicated by the titration curves in fig. 2. As shown in figs. 3 and 4 the increased sensitivity against salt dissociation is a consequence of the insertion of a dG·dC base pair between the dA·dT pairs. Nt probably covers three or four base pairs[3,4,7,10,12,13]. The specific interaction of Nt with neighbouring dA·dT pairs is achieved by hydrogen bonding with the keto groups of thymine in the minor groove[1,3-5,10,13]. It appears to be disturbed as a result of the elimination of a specific hydrogen bond acceptor site in the minor groove. Steric hindrance effects in the minor groove are introduced by the replacement of adenine by guanine bearing the amino group[5].

The results of figs. 3 and 4 experimentally show the decreasing binding affinity of Nt for the deoxynucleotide sequences:

d(A–A–A–A–A–A) / d(T–T–T–T–T–T) > d(A–T–A–T–A–T) / d(T–A–T–A–T–A) ≫ d(A–C–A–C–A–C) / d(T–G–T–G–T–G)

In previous studies binding constants of Nt complexes have been reported for poly(dA–dT)·poly(dT–dA) ($K_a \sim 10^8 M^{-1}$) at low ionic strength[12] and for DNA's[4,5] ($K_a \sim 10^5 M^{-1}$ to $10^7 M^{-1}$). These results reflect mainly overall binding constants. These binding data and the respective association constants of DNA complexes with distamycin A[4] for strong ($K_a \sim 10^9 M^{-1}$) and weak ($K_a \sim 10^6 M^{-1}$) interaction sites indicate that differences in binding constants of three orders of magnitude should account for the relatively drastic lowering of affinity of Nt to dG·dC containing sequences.

The sequence specificity of Nt for consecutive thymine sequences will explain several applications of this oligopeptide. The lowering of the buoyant density of DNA upon complex formation with Nt[14–16] in concentrated CsCl solutions is apparently due to binding to certain dA·dT rich sequences. The action of restriction endonucleases[17] as well as of DNase I is inhibited at runs of dA·dT pairs, while cleavage proceed in mixed sequences[18]. Blocking of certain other enzymes, like DNA- and RNA-polymerases by Nt is dependent on the dA·dT content of the DNA used[1]. This appears consistent with recent results[19] which indicate that the minor groove is the main site of attack of RNA polymerase. The strong binding of Nt to dA·dT rich sequences appears to prevent the access by these enzymes to their specific interaction sites. Since interspersion of these sequences with dG·dC pairs reduces the affinity of Nt to these sites, the oligopeptide can thus be displaced by the competing enzyme.

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