A NEW TYPE OF AT-SPECIFIC LIGAND CONSTRUCTED OF TWO NETROPSIN-LIKE MOLECULES


Institute of Molecular Biology, USSR Academy of Sciences, Vavilov str., 32, 117984 Moscow V-334, USSR

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1. Introduction

Solving the problem of recognition of specific nucleotide sequences on double-stranded DNA by regulatory proteins is one of the main goals of molecular biology. In systems modelling protein–nucleic acid recognition one can make use of low molecular weight compounds possessing affinity for definite short base sequences of DNA [1,2]. Among these compounds, antibiotics distamycin (DM) and its analog netropsin (Nt) are the most thoroughly studied [3–7]. Stereochemical models for DM–DNA and Nt–DNA complexes have been suggested and corroborated [3–7]. According to these models upon binding of DM (or Nt) to DNA the oligopyrrolcarboxamide backbone of the antibiotic molecule is localized in the minor DNA groove forming a helix isogometric to the DNA β-helix. The AT specificity of binding is a consequence of formation of hydrogen bonds which connect the antibiotic amide groups with thymine O2 and adenine N3 atoms lying in the same polynucleotide chain and exposed in the minor DNA groove. The binding is stereospecific in a sense that CO+NH direction in the antibiotic carboxamide groups coincides with C5′→C3′ direction in the corresponding polynucleotide chain.

Here we describe a new approach to designing sequence specific ligands. Each ligand contains two netropsin-like molecules linked by a flexible chain in such a way that the CO+NH directions in the two moieties are antiparallel. This approach allows an increase in the number of AT-specific recognition centers in a ligand molecule, thereby increasing its binding specificity. We have obtained a series of ligands each capable of interacting by its two Nt-like fragments with two stretches of adenine and thymine bases lying in the opposite DNA strands. Fig.1 shows chemical formulae of the compounds synthesized which are distinguished by the number of methylene groups in a flexible chain linking the two Nt-like fragments.

2. Materials and methods

Compounds 1a–d were synthesized by interaction of compound II with di-4-nitrophenylsuccinate [8], di-N-hydroxysuccinimide esters of adipic, suberic and decane dicarboxilic acids, respectively. The condensations were carried out in N,N-dimethylformamide solution, the ratio of an activated ester and compound II being 1:2:1. The reaction products la–d were isolated by 2-fold chromatography on a Sephadex LH-20 column (150 × 2.5 cm) with 62–75% yield. Their homogeneity was proved by electrophoresis on FN-16 paper (Filtrak, GDR) in 1 M AcOH (pH 2.4) at 20 V/cm. Electrophoretic mobilities (against glycine) were 0.8 ± 0.02 for compounds la–d and 1.4 ± 0.02 for compound II.

The UV spectra were measured with a Cary 118 spectrometer (USA) using 1 cm pathlength cells. The CD spectra were obtained with a Jobin-Yvon Mark III dichrograph (France) applying 0.1 cm, 1 cm and 2 cm pathlength cells. Fluorescence spectra were measured with an Aminco SPF-1000 CS instrument (USA). Poly(dA) · poly(dT) was from P.L. Biochemicals (USA); calf thymus DNA was from Sigma (USA). All polynucleotides were used as supplied without further purification. All polynucleotide solutions were dialysed for 6 h against 0.06 M phosphate buffer.
3. Results and discussion

The absorption spectra of compound 1c and the Nt-like compound II are given in fig.2. The shapes of the two absorption spectra are similar but the molar extinction of 1c is, however, twice as large as that of II. The additive contribution of two Nt-like fragments demonstrates that these two moieties do not interact with each other in solution. Addition of DNA markedly affects the spectrum of 1c (fig.2). The observed spectral changes exhibit a similarity to those detected on binding compound II to DNA. Similar results were obtained from binding compounds 1a, 1b and 1d to DNA.

The CD spectra of compounds 1c and II complexed with poly(dA)·poly(dT) are shown in fig.3, from which it can be seen that they are similar. However, the molar ellipticity of the complex 1c·poly(dA)·poly(dT) is twice as large as that of the complex II·poly(dA)·poly(dT). This observation can be explained if both Nt-like fragments of 1c bind to DNA independently in virtually the same manner as does II. This conclusion is also supported by our measurements of sizes of DNA sites occupied by molecules of 1c and II. Fig.4 represents the dependences of CD amplitude at 315 nm obtained on titration of poly(dA)·poly(dT) and calf thymus DNA with 1c.
Fig. 3. CD spectra of compounds 1c (1) and II (2) complexed with poly(dA)·poly(dT). Ligands were $2.6 \times 10^{-5}$ M, DNA was $4.1 \times 10^{-5}$ M (basepairs). The experimental conditions were identical to those in fig. 1.

and II. From measured saturation levels of binding of these compounds we conclude that II and 1c occupy 5 and 10 basepairs, respectively, on poly(dA)·poly(dT). Similar experiments show that compounds 1a, 1b and 1d occupy 7, 10 and 11 basepairs, respectively. The saturation level of binding of 1c to calf thymus DNA (58% AT) is lower than that of II, reflecting a higher A1-specificity of 1c in comparison with II.

Fig. 4. The CD titration of poly(dA)·poly(dT) (●) and calf thymus DNA (○) with compounds 1c (1) and II (2). $\Delta \varepsilon_{315}$ is the CD amplitude of the complex measured at 315 nm, $r = C/[P/2]$, where $C$ is the total ligand molar concentration, $[P/2] = 3 \times 10^{-5}$ M is the concentration of DNA (in basepairs).

Fig. 5. Displacement of fluorescent analog of Nt (Ila) from poly(dA)·poly(dT) by compound 1c, $I_{510}$ is the intensity of fluorescence of Ila measured at 510 nm and excited at 340 nm; $r$ is as in fig. 4. (○) Compound Ila complexed with poly(dA)·poly(dT); (●) compound Ila in the free state.

To estimate the binding constant of 1c to DNA we studied the competition for binding sites on poly(dA)·poly(dT) between 1c and the fluorescent Nt analog Ila whose binding constant to poly(dA)·poly(dT) is known to be $2 \times 10^6$ [7]. Poly(dA)·poly(dT) was first saturated with Ila, whose fluorescence, being low in the free state, increases dramatically upon binding to DNA. DNA saturated with Ila was then titrated with 1c. Displacement of Ila from DNA was accompanied by a decrease in the fluorescence intensity measured at 510 nm. From the results presented in fig. 5 one can conclude that 1c displaces completely the fluorescent ligand Ila from DNA, when one 1c molecule is bound/10 basepairs ($r = 0.1$). This

Fig. 6. Schematic presentation of the geometry of complexes formed between Nt and DNS (a) as well as between compound 1c and DNA (b).
demonstrates that 1c occupies the same binding sites on DNA as does Nt, i.e., 1c molecules are attached to AT pairs in the minor groove of DNA. From these data it can also be estimated that the binding constant of 1c to poly(dA) \cdot poly(dT) is \( >100 \text{ times} \) larger than that determined for binding of IIa. If two Nt-like fragments in 1c act independently upon binding then we can estimate the binding constant of 1c to be of the order of \( 10^{-12} \text{ M}^{-1} \), that is about the square of the binding constant of IIa. Experimental data on competition between the fluorescent analog IIa and 1b and 1d demonstrates that the latter bind to poly(dA) \cdot poly(dT) more strongly than does IIa. Evidently, each of the two Nt-like fragments in these molecules is bound to DNA. Compound 1a forms a less-stable complex with DNA, presumably because the linking chain is too short and independent binding of the two Nt-like fragments is not possible. These experiments and the data in [5–7] on binding of various analogs of DM to DNA point toward the model for the binding of compounds 1b–d to DNA illustrated in fig.6. In the complex, the two Nt-like fragments of the ligand molecule are bound to the opposite DNA strands in such a way that the complex structure possesses a \( \sim 2\)-fold rotational symmetry. The binding involves hydrogen bond formation between amide groups of each Nt-like fragment and 3 successive adenine (or thymine) residues lying in the same polynucleotide chain. Since thymine O2 and adenine N3 atoms in AT pairs are related by 2-fold rotational symmetry, one can conclude that upon binding of these ligands to DNA the adenine is approximately equivalent to a thymine. The proposed model agrees with our experiments showing that compounds 1b, 1c and 1d possess an extremely high affinity for AT-rich DNA sequences and presumably utilize the symmetry of the DNA sequence. These ligands may interact strongly with AT-rich sequences included in the so-called ‘Pribnow box’ [9,10] in procaryotic promoters and may serve as efficient inhibitors of transcription.

References


