Vol. 202, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1994 Pages 204-210

SUGAR-DNA MOLECULAR RECOGNITION: SPECIFIC INTERACTION OF α- 1,4- GLUCOPYRANOSE CHAINS WITH DNA IN THE MINOR GROOVE **

A. Gopala Krishna#, D. Balasubramanian$ and K. N. Ganesh#*

Division of Organic Chemistry, National Chemical Laboratory, Pune 411 008, INDIA

$ Centre for Cellular and Molecular Biology, Hyderabad 500 047, INDIA

Received May 24, 1994

Abstract: α(1,4) glucopyranose chains (dextrins) are shown to interact with DNA via hydrophobic interactions from the minor groove side, while α(1,6) dextrans do not bind DNA. The observed specific α(1,4) linked sugar-DNA interactions may have importance in recognition of DNA by ene-diyne class of antibiotics via their saccharide units which have mostly α(1,4) linkages.

A number of DNA binding natural products, in particular, the antibiotics chromomycin (1), nogalamycin (2), mithramycin (3), triostin (4) and the more recently discovered ene-diyne antibiotics (5) calcheamicin γ^{I} and esperamycin A posses oligosaccharide structures, which are implicated in DNA recognition (6). Based on molecular modelling (7), DNA cleavage experiments (8) and recent preliminary NMR studies (6d) with ene-diyne antibiotics, it has been speculated that the carbohydrate moiety in these antibiotics bind DNA in the minor groove. It has also been reported by one of us that polysaccharides with specific stereochemical constraints display reasonable amphiphilic property although devoid of obvious apolar moieties (9,10). This has been demonstrated experimentally with α-1,4- glucopyranose chains (linear dextrins, Figure 1) which has all hydroxyls disposed on one face of the molecule with the non-polar methines placed on the other side. Consequently, linear dextrins destabilize globular protein chains and bind to a fluorescent probe and enhance its emission (9).

These findings in literature prompted us to directly investigate sugar-DNA interactions by UV and fluorescence spectroscopic techniques using dansyl (DNS) as a sandwich probe. In this communication, it is demonstrated that α-1,4- linked dextrins preferentially

** NCL communication number 5936.

* Author to whom correspondence should be addressed. Fax. No. 091-0212-330233.

0006-291X/94 $5.00

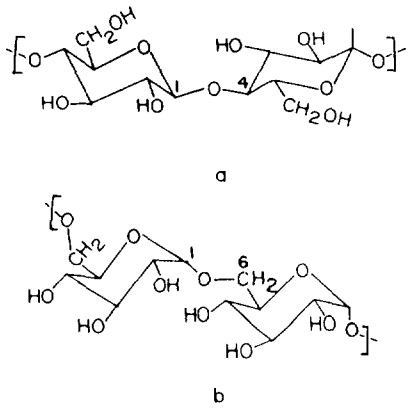

Figure 1. Chemical structures of (a) dextrin and (b) dextran.

interact with AT stretches in DNA via hydrophobic interactions in the minor groove with $K_a \sim 10\text{-}10^2\ M^{-1}$.

MATERIALS AND METHODS

All chemicals used were of the highest purity available. Dextrin-15 (Mr-1400) and dextran-4 (Mr~ 4000-6000), were purchased from Serva Chemical Co. Dansyl Chloride , maltose, maltotriose, CT-DNA were purchased from Sigma Chemical Co, ST Louis, USA. Netropsin was purchased from Boehringer Mannheim. All oligonucleotide sequences (1- CGCGAATTCGCG; 2- CGCGAAAAAATTTTTTCGCG; 3- ATATATATATAT: 4- CGCGCGCGCGCG; 5- CGCGAAT**U***CGCG where **U*** = 5-NH-dansyl-dU) were synthesized by β-cyanoethyl phosphoramidite chemistry on Pharmacia GA Plus DNA synthesizer and purified by FPLC and rechecked by HPLC.

DNA melting experiments were performed with or without oligosaccharide (30mM) in 10mM Tris buffer, pH 8.0, containing 100mM NaCl and 10mM $MgCl_2$ using Perkin Elmer Lambda 15 UV/VIS spectrophotometer. Fluorescence measurements were done on a Perkin Elmer model LS-50 B spectrometer. Samples dissolved in the above buffer were excited at 340nm and emission spectra recorded from 420 to 600nm using spectral bandwidths of 2.5nm. CD spectra were recorded on a Jobin Yvon instrument in the same pH 8.0 buffer. The crystal co-ordinates for netropsin were obtained from reference (11). Energy minimization calculations were done using the procedure of Rao (12,13), choosing the coordinates of Arnott and Scott (14) for the sugar rings. Association constants were calculated from the $1/\delta F$ Vs 1/L plots, where δF is the relative rise in fluorescence emission and L is the ligand concentration.

RESULTS AND DISCUSSION

Table I shows 't_m' values of three different DNA duplexes in the presence and absence of α-1,4- dextrin and α-1,6- dextran. A lowering of 't_m' was observed specifically with α-1,4-dextrins which caused duplex destabilization, not seen with dextran- DNA complexes. The CD spectra of DNA (not shown) were unchanged in presence of any sugars, ruling out any gross perturbation in the base stacking or polymorphic transitions in DNA.

Table I
Tm of DNA duplexes

DNA	Alone	+ Dextrin-15 #	+ Dextran-4
CT.DNA	86.1	85.2 (-0.9)	86.1
$d(CG)_2(AT)_2(CG)_2$	61.5	55.7 (-5.8)	61.5
$d(CG)_2(AT)_6(CG)_2$	67.5	58.3 (-9.2)	67.5

Values in brackets denote the change in T_m, negative sign indicates destabilization.

N,N-Dimethylamino napthalene sulfonyl chloride (DNS) binds to DNA[1] and this association leads to a saturation enhancement of its fluorescence (Figure 2). This is accompanied by a slight blue shift in the emission wavelength. DNS binding to DNA is strong ($K_a \sim 10^5$-$10^6 M^{-1}$) and was found to be fairly independent of DNA sequence. Addition of sugars to DNA-DNS complex led to a further enhancement of fluorescence, reaching a second limit (Figure 2). Among the various saccharides, α-1,4- dextrin elicited a fluorescence enhancement whose magnitude expressed as a percentage of initial rise (DNA-DNS complex) was significantly highest. The percent enhancement also increased with the number of sugar units in saccharide. In contrast, the α-1,6- dextrans were very poor in this assay. The association constants (Table II) computed from the fluorescence data (15) were in the range of 30-80 M^{-1}, with a maximum for DNS-dextrin-$(AT)_6$ complex (entry-e). Among the DNA sequences studied dextrin exhibited higher binding to AT sequences than CG sequences (entry e and f). In comparison to DNA-DNS-dextrin ternary complex, the fluorescence increase was negligible upon DNS binding to dextrin, in the absence of DNA. The results suggest that α-1,4- dextrins bind to DNA while α-1,6- dextrans do not, thus supporting the data from UV-melting experiments.

DNS is a non-intercalating fluorophore and the observed changes in its fluorescent behaviour upon association with DNA is indicative of its binding in the relatively hydrophobic, low dielectric minor groove of DNA just as other non-polar dyes (16). Netropsin, a classical minor groove binding drug when added incrementally to DNA-DNS-dextrin complex, led to a gradual reduction in DNS fluorescence and a slight red shift in fluorescence emission (17) (Figure 3). This effect originates from displacement of DNA bound dextrin in the minor groove by netropsin, whose binding affinity is much higher than that of sugars. Further, titration of dextrin into fluorescent DNA **5** in which DNS is covalently linked at C-5 of dU (18), did not show any change in fluorescence of DNS. In this DNA, the conjugated DNS is necessarily projected into the major groove of DNA, and the results imply that dextrin has no interactions in the major groove of DNA and selectively interacts from the minor groove side.

[1]Under the experimental conditions, in aqueous solutions, DNS does not form covalent adduct with DNA and hence the binding is non-covalent.

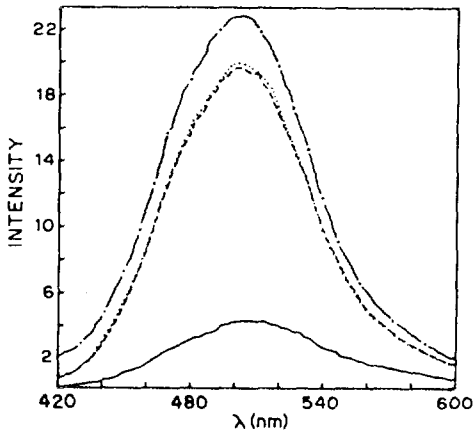

Figure 2. Fluorescence spectra of DNS (1.86×10^{-6} M) in 10mM Tris buffer, pH 8.0 [———]; DNS + **1** (260nM)[- - -]; DNS + **1** + Dextran (30mM) [....]; DNS + **1** + Dextrin (30mM) [-.-.-].

The presented data give credence to formation of a ternary complex (DNA-DNS-dextrin) in which the fluorophore DNS is sandwiched between the hydrophobic face of α-1,4- dextrin and DNA in the minor groove. The complexation originates due to the non-polar surface of polysaccharides since dextrans, which are relatively less amphiphilic show much reduced binding. The interaction though predominantly hydrophobic, may also partly involve some of the sugar hydroxy groups in association with DNA phosphate (19), since aqueous salt solutions (50mM-3M) dissociated dextrin from DNA, leading to a fall in DNS fluorescence. It may be pointed out that DNA binding saccharide units in ene-diyne antibiotics are mostly α-1,4- linked and comprise 6-deoxy sugars and rhamnose units that are suggested to bind DNA by hydrophobic interactions (20).

Table II
Carbohydrate- DNA binding

Sugar	DNA*	$K_a(M^{-1})$#
a) Maltose	**1**	5.0
b) Maltotriose	**1**	31.0
c) Dextrin-15	**1**	51.9
d) Dextrin-15	**2**	6.3
e) Dextrin-15	**3**	76.0
f) Dextrin-15	**4**	--
g) Dextrin-15	**CT.DNA**	31.6
h) Dextran-4	**1/2/3/4/ CT.DNA**	--

Caculations of the binding constants from the fluorescence data were done according to (15). * For DNA sequences see Materials and Methods.

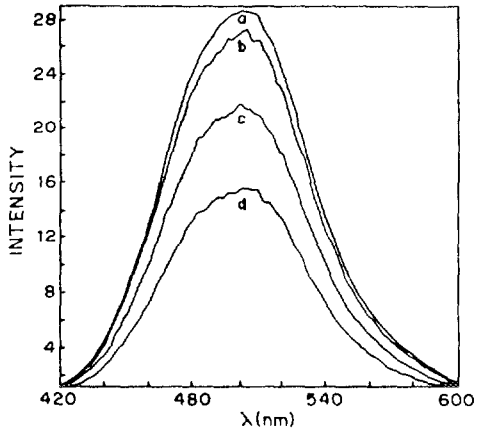

Figure 3. Fluorescence quenching of DNS-DNA-Dextrin complex in presence of (a) 0μM, (b) 5μM, (c) 37μM and (d) 105μM of Nertropsin. All other conditions are same as in Fig. 2.

In addition to presence of suitable hydrogen bonding donor/acceptor groups, feature necessary for association is the complementarity between the curvature of the groove surface and that of the binding agent, which must be helicaly concave (21). The minor groove binding drugs such as netropsin and distamycin have molecular structures which are of crescent shape (11), with an inherent bend that geometrically complements the minor groove of DNA. In contrast to dextran which is not curved (Figure 4), the energy minimized molecular stereostructure of α-1,4- dextrin has shape similarity to netropsin. The origin of strong binding of netropsin related compounds to DNA in the minor groove is attributed to enthalpic effects, resulting from the replacement of spine of hydration with

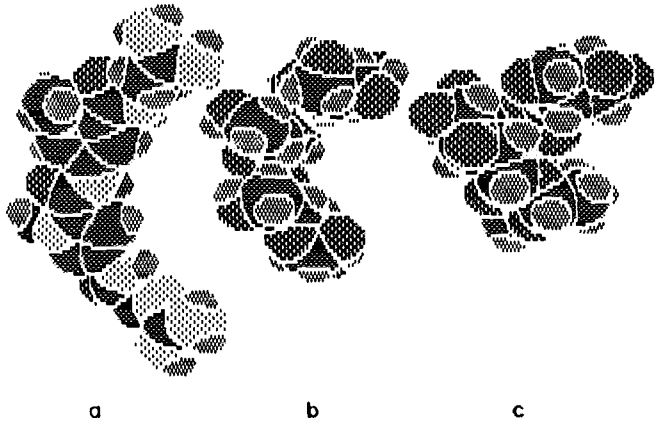

Figure 4. Energy minimized conformations of: (a) netropsin, (b) dextrin trimer and (c) dextran trimer shown in space- filled mode.

formation of specific DNA-netropsin hydrogen bonds (22). On the other hand, α-1,4-dextrins, interact with DNA possibly via pair hydrophobic interactions (23), without any specific hydrogen bonds, thus leading to a weak binding.

Though the binding of dextrins to DNA *per se* is not very strong, the important point is the significant difference in the binding constants of dextrins and dextrans. While dextrins bind to DNA, inspite of lack of any "DNA reading moities" dextrans do not show any affinity at all. Although the examples shown here are from a limited number of saccharides, the concept of hydrophobic interaction in minor groove as demonstrated may generally be applicable to all sugar-DNA interactions.

In summary, we have demonstrated a direct evidence for interaction of α-1,4-dextrins with DNA in the minor groove by hydrophobic interactions. The results have implications in understanding the structural basis of sugar-DNA complexations and may assist rational design of DNA binders for antitumour activity.

Acknowledgment: AGK acknowledges financial support from CSIR, India.

REFERENCES

1. Gao, X., And Patel, D. J. (1989) Q. Rev. Biophys. 22, 93-138.
2. Liaw, Y. -C., Gao, Y. -G., Robinson, H., van der Marel, G. A., van Boom, J. H. Wang, A. H. -J. (1989) Biochemistry. 28, 9913-9918.
3. Fox, K. R., and Howarth, N. R. (1985) Nucleic Acid Res. 13, 8695-8714.
4. Wang, A. H. -J., Ughetto, G., Quigley, G. J., Hakoshima, T., van Der Marel, G., van Boom, J. H., Rich, A. (1984) Science. 225, 1115-1120.
5. Review: Nicolaou, K. C., and Dai, W. -M. (1991) Angew. Chem. Int. Ed. Engl. 193, 1387-1416.
6. (a) Walker, S., Valentine, K. G., and Kahne, D. E., (1990) J. Am. Chem. Soc. 112, 6428-6429; (b) Walker, S., Yang, D., and Kahne, D. E., (1991) ibid. 113, 4716-4717; (c) Silva, D. J., and Kahne, D. E., (1993) ibid, 115, 7962-7970; (d) Walker, S., Murnick, J., and Kahne, D. E., (1993) ibid, 115, 7954-7961.
7. Hawley, R. C., Kiessling, L. L., and Schreiber, S. L. (1989) Proc. Natl. Acad. Sci. USA 86, 1105-1109.
8. (a) Aiyar, J., Danishefsky, S. J., and Crothers, D. M. (1992) J. Am. Chem. Soc. 114, 7552-7554; (b) Nicolaou, K. C., Tsay, S. -C., Suzuki, T., and Joyce, G. F. (1992) ibid. 114, 7555-7557.
9. Balasubramanian, D., Raman, B., Sundari, C. S. (1993) J. Am. Chem. Soc. 115, 74-77.
10. (a) Raman, B., Sundari, C. S., Balasubramanian, D. (1992) Ind. J. Biophys. Biochem. 29, 143-147; (b) Sundari, C. S., Raman, B., Balasubramanian, D. (1991) Biochim. Bophys. Acta. 1065, 35-41.
11. Berman, H. M., Neidle, S., Zimmer, C., and Thrum, H. (1979) Biochim. Biophys. Acta. 561, 124-131.
12. Rao, V. S. R., Yathindra. N., and Sundarajan, P. R. (1969) Biopolymers. 8, 325-333.
13. Yathindra, N., and Rao. V. S. R. (1970) Biopolymers. 9, 783-790.
14. Arnott, S., and Scott, W. E. J. Chem. Soc., Perkin Trans. II. (1972) 324-335.
15. In Fluorescence Spectroscopy: An Introduction to Biology and Medicine (Eds.: A. J. Pesce, C. -G. Rosen, T. L. Pasby), pp 237. Marcel Dekker Inc., (1971), New York.
16. Jin, R., and Breslauer, K. J. (1988) Proc. Natl. Acad. Sci. 85, 8939-8942.

17. From the fluorescence data of DNA-DNS-Netropsin copmplex, K_a for netropsin-DNA binding was calculated to be 4×10^4, well in agreement with literature reported data. See Luck, G., Triebel, H,. Waring, M. J,. and Zimmer, C. (1974) Nucl. Acids. Res. 1, 503-530.
18. Barawkar, D. A., and Ganesh, K. N. (1993) BioMed. Chem. Lett. 3, 347-352.
19. Pelmore, H., Eaton, G., and Symons, M. C. R.(1992) J. Chem. Soc. Perkin. Trans. 2. 149-150.
20. Ding, W. -D., and Ellestad, G. A. (1991) J. Am. Chem. Soc. 113, 6617-6620.
21. Neidle, S., Pears, L. H., and Skelly, J. V. (1987) Biochem. J. 243, 1-13.
22. (a) Kopka, M. L., Yoon, C., Goodsell, D., Pjura, P., and Dickerson, R. E. (1985) J. Mol. Biol. 183, 553-563: (b) Marky, L. A., and Breslauer, K. J. (1987) Proc. Natl. Acad. Sci. USA. 84, 4359-4363.
23. Wood, R. H., and Thompson, P. T. (1990) Proc. Natl. Acad. Sci. USA. 87, 946-949.