TOPOGRAPHY OF NUCLEIC ACID HELICES IN SOLUTIONS. XXVIII. EVIDENCE FOR A DYNAMIC STRUCTURE OF DNA IN SOLUTION.

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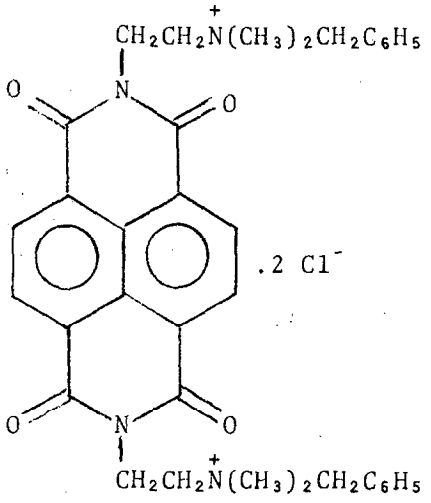
Summary

Proton magnetic resonance (pmr), ultraviolet absorption, induced circular dichroism (CD), and viscometric evidence is presented which show that reporter molecules 1 and 2 bind to DNA via an intercalation process. Preliminary kinetic studies show that the DNA·1 complex forms rapidly (i.e., <1 msec), whereas the DNA·2 complex forms at a considerably slower rate ($t_{\frac{1}{2}}$>100 msec). The kinetic results, and the steric requirements for intercalation of 2 can be explained on the basis of a dynamic structure of DNA.

Through the use of hydrogen-exchange techniques (1-5) as well as the rates of reactions of formaldehyde with native and denatured deoxyribonucleic acid (6), it has been shown that the conformation of DNA is "subject to continuous thermally-induced local fluctuations and distortions" (4). One type of transient conformation involves unstacking of adjacent base-pairs with a concomitant H-bond breakage to form an open DNA structure. This communication presents further evidence for the dynamic structure of DNA in solution as well as additional data on the interaction specificity of novel reporter molecules with DNA.

To accomplish the above objective, two types of reporter molecules were synthesized (1 and 2), and their interaction specificities with native salmon sperm DNA (s.s. DNA), polydAT-polydAT, and polydG-polydC were examined. It is noted that the 1,8-naphthylimide ring of 1 may intercalate readily between base-pairs of DNA without the necessity of breaking H-bonds between base-pairs. However, in order to intercalate the 1,8,4,5-naphthyldiimide ring of 2 between base-pairs of DNA, unstacking of adjacent base-pairs and local melting (H-bond breakage)



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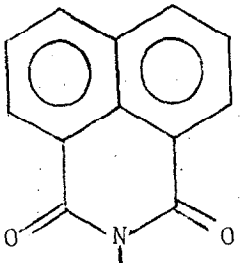

$CH_2CH_2\overset{+}{N}(CH_3)_2(CH_2)_3\overset{+}{N}(CH_3)_3 \cdot 2Br^-$

1

of the helix must occur. (If the 1,8,4,5-naphthyldiimide ring of 2 intercalates, the N-benzyl, N,N-dimethyl side chains must occupy opposite grooves in DNA (i.e., one side chain in the minor, the other in the major groove) since the distance between side chains of 2 is approximately 11.2 A^{o}. Moreover, it has been shown in this laboratory that a t-butyl group on an aromatic ring blocks the intercalation process (7). Since the N-benzyl, N,N-dimethyl group of 2 is larger than a t-butyl group, it is therefore concluded that intercalation of the aromatic ring of 2 must be preceeded by local opening, i.e., melting, of the helix. This conclusion is supported by the kinetic study (see below)).

The evidence for intercalation of reporters 1 and 2 between base-pairs of DNA is briefly summarized. (1) The proton magnetic resonance signals of the aromatic and side chain protons of 1 and 2 are totally broadened and are indistinguishable from base-line noise in the

presence of salmon sperm DNA. (For a detailed discussion of the pmr signals of intercalated molecules see references (8) and (9)). (2) It is noted from the viscometric titration studies shown in figure 1 that

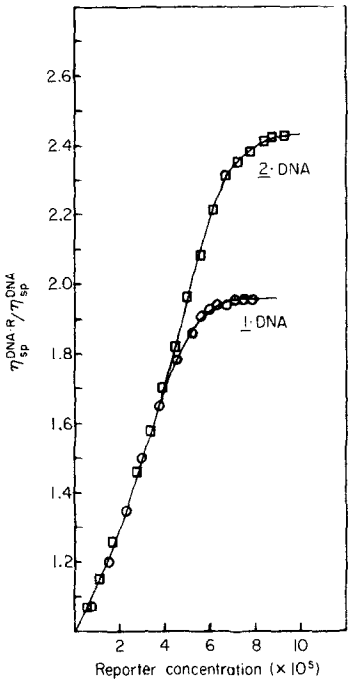

Figure 1. The effect of increasing concentration of 1 (o-o), and 2 (□-□) on the specific viscosity of ss DNA solution (2.65×10^{-4} M P/1) in 0.01 M MES buffer pH 6.2.

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the relative specific viscosity, $\eta_{sp}^{complex}/\eta_{sp}^{DNA}$, of the DNA solution increases with increasing concentrations of reporters 1 and 2 and finally levels off at a base-pair to reporter ratio of 2.30 and 1.74 for reporters 1 and 2, respectively. It should be noted that intercalation of planar molecules between base-pairs of DNA leads to an increase in the length of the helix and is usually accompanied by an increase in the viscosity of the solution (10-12). (3) A large hypochromic effect and an induced circular dichroism in the absorption band

Table I. Effect of Salmon Sperm DNA (ss DNA), PolydAT-PolydAT and PolydG-PolydC on the Absorption and Induced Circular Dichroism Spectra of Reporter Molecules 1 and 2.

Reporter	Absorption Spectra[a]											Circular dichroism[c]			
	H_2O buffer		PolydAT-PolydAT			PolydG-PolydC			ss DNA			ss DNA			
	λ^{max}_{nm}	ε^{max}	λ^{max}_{nm}	ε^{max}	%H[b]	λ^{max}_{nm}	ε^{max}	%H	λ^{max}_{nm}	ε^{max}	%H	λ^1	$[\theta]^1$	λ^2	$[\theta]^2$
1	344	12,480	347	6,360	114	347	7,610	78	348	5,670	120	357	1800	316	-1500
2	381	29,700	381	11,700	149	384	12,000	144	384	10,950	171.	408	- 900	355	-5500

[a] Absorption spectra were taken in 10 mm cells using a Cary 15 spectrometer at a nucleic acid concentration and reporter concentration of 1.0×10^{-4} M P/l and 5×10^{-6} M, respectively, in 0.01 M 2-(N-Morpholino)ethanesulfonate buffer (MES) pH 6.30 and 0.005 M Na^+ at ambient temperature.

[b] Percentage hypochromicity (%H) = $(\varepsilon^{max}_{H_2O}/\varepsilon^{max}_{p} - 1.0)\ 100$, where $\varepsilon^{max}_{H_2O}$ and ε^{max}_{p} are the extinction coefficients in the presence and absence of the polynucleotides.

[c] Circular dichroism spectra were taken in 50 mm cells using a Jasco J-20 spectrometer and the same concentration conditions as above.

Table II. Kinetics of the Binding of 1 and 2 to Nucleic Acids.

		k_{obs}, sec^{-1} [a]						
		DNA (C x 10^{-6})	PolydAT-PolydAT (C x 10^{-6}) [d]					PolydG-PolydC (C x 10^{-6}) [d]
R (C x 10^{-6})	T°C	97.0	129.0	69.3	34.7	17.3	8.7	58.1
1 (2.50)	20	b	-----	b	----	----	----	b
2 (2.50)	20	c	11.95	9.15	7.30	5.40	4.40	3.48
2 (1.25)	20	c	12.40	8.85	6.80	----	----	3.57
2 (2.50)	10	c	-----	3.86	----	----	----	1.36
2 (2.50)	30	c	-----	23.3	----	----	----	9.30

[a] Stop-flow kinetics were carried out on a Durrum stop-flow apparatus thermostatted with a Lauda K-2/R circulating bath. The reaction was followed at 348 and 383 nm for reporters 1 and 2, respectively, in BPES buffer (0.08 M Na_2HPO_4, 0.02 NaH_2PO_4, 0.18 M NaCl, and 0.01 M di NaEDTA) pH 6.90.

[b] The kinetics of binding of 1 to nucleic acids were found to be faster than the capability limit of the instrument (i.e., <1 msec).

[c] The reaction of DNA with 2 did not follow simple first order kinetics; however, it can be fitted to a series of simultaneous first order processes (E. Gabbay, and R. DeStefano, work in progress).

[d] First order kinetics are observed for polydAT-polydAT and PolydG-PolydC for at least 3 half-lives. The rate constants given above are reproducible to ±3%.

of the reporter molecules 1 and 2 are obtained upon binding to salmon sperm DNA, polydAT-polydAT, and polydG-polydC (Table I). These observations also suggest an intercalation mode of binding of reporters 1 and 2 to DNA (8).

Preliminary kinetic studies of the intercalation process of reporter molecules 1 and 2 to nucleic acids were studied by stop-flow techniques (Table II). The results support the "breathing model" of DNA as postulated by von Hippel et. al. (1,2,4,6). For example, the following points should be noted. (1) Intercalation of the 1,8-naphthyl imide ring of 1 between base-pairs of nucleic acids occurs rapidly (<1 msec), whereas the corresponding process of intercalation of 2 with nucleic acids is significantly slower. (2) The binding of 2 to polydAT-polydAT and to polydG-polydC exhibit first order kinetics for at least 3 half-lives; however, complex kinetics are obtained with salmon sperm DNA. (Complex kinetics of binding of reporter 2 to DNA may be due to the presence of 10 different possible intercalation sites in the latter (13)). (3) The reaction of 2 with polydAT-polydAT and polydG-polydC is first order with respect to reporter concentration. For example, first order kinetics are observed for the binding 2 to polydAT-polydAT at DNA phosphate to reporter ratios which varied from 103/1 to 3.5/1 (Table II). (4) A sequence-dependent dynamic structure of nucleic acid may also be indicated by the data shown in Table II, i.e., the observed rate constant, k_{obs}, for intercalation of 2 to polydAT-polydAT is approximately 2.5 times as great as that noted for intercalation to polydG-polydC.

The above kinetic data is consistent with a "dynamic" structure of DNA helix in solution. The slower rates of binding of reporter 2 to nucleic acid helices, as compared with 1, together with the steric requirements for intercalation of 2 strongly suggests that the helix exist in rapid equilibrium between a closed and an opened helical

structure. This conclusion is completely in line with the "breathing model" proposed by von Hippel, et al. (1,2,4,6).

Current work is in progress to ascertain (i) the kinetic order in polynucleotide concentration, (ii) the dissociation rate constants, and (iii) to fit the kinetics of binding of 2 to s.s. DNA to a set of parallel first order processes.

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References

1. Printz, M.P., and von Hippel, P.H., Proc. Nat. Acad. Sci., Wash., 53, 363 (1965).
2. idem, Biochemistry, 7, 3194 (1968).
3. Marshall, T.H., and Grunwald, E., J. Am. Chem. Soc., 91, 4541 (1969).
4. McConnel, B., and von Hippel, P.H., J. Mol. Biol., 50, 297, 317 (1971).
5. Hanson, C.V., J. Mol. Biol., 58, 847 (1971).
6. von Hippel, P.H., and Wong, K.Y., J. Mol. Biol., 61, 587 (1971).
7. Gabbay, E., and DeStefano, R., unpublished results (1972).
8. Gabbay, E.J., Gaffney, B.L., and Glaser, R., Symp. N.Y. Acad. Sci., 171, 810 (1970).
9. Gabbay, E.J., Sanford, K., and Baxter, C.S., Biochemistry, 11, 3429 (1972).
10. Lerman, L.S., J. Mol. Biol., 3, 18 (1961).
11. Cohen, G., and Eisenberg, Biopolymers, 8, 45 (1969).
12. Müller, W., and Crothers, D.M., J. Mol. Biol., 35, 251 (1968).
13. Gabbay, E.J., Destefano, R., and Sanford, K., Biochem. Biophys. Res. Commun., 46, 155 (1972).