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Dissymmetric Recognition of the Helical Sense of Deoxyribonucleic Acid and Evidence for Binding of Reporter Molecules from the Minor Groove of DNA[1]

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Through the utilization of optically active DNP-derivatives of L- and D-proline, evidence is presented which suggests that nucleic acids exist as right-handed helices in solution. The results of ultraviolet absorption, circular dichroism, proton magnetic resonance (pmr), T_m of the helix-coil transition, viscometric, and binding studies are consistent with the above interpretation. It is shown that several types of DNA (i.e., salmon sperm, calf thymus, *Micrococcus luteus*, poly d(A-T)-poly d(A-T) and poly d(I-C)-poly d(I-C)) exist in a right-handed helical structure in solution. In addition, evidence is presented which strongly indicates that the 2,4-dinitroaniline ring of DNP-proline is intercalated between base-pairs of DNA and the prolyl side chain situated in the minor groove. Moreover, it is shown that the more sterically hindered DNP-derivatives exhibit a higher selectivity for A–T binding sites.

The structural investigation of nucleic acids is complicated by the fact that X-ray diffraction experiments have had to be carried out on fibers rather than single crystals. As a result, the resolution of the diffraction patterns is poor and the structures which are constructed are not clearly defined (*1*, *2*). For instance, the chirality of the B DNA structure has not been determined with certainty. It is assumed to be right-handed since the A DNA structure, as determined from X-ray diffraction, is right-handed and it is considered improbable that the chirality of the DNA molecule would change with increased hydration of the fiber (*3*). Yet, evidence has been presented in a number of cases which suggests that solution structures of DNA are quite different from their fiber structures. Most interesting are the structural studies on the synthetic polydeoxyribonucleotide poly d(I-C·d(I-C). X-ray diffraction patterns (*4*) of low humidity fibers of this DNA indicate that the polymer is left-handed. CD studies (*5*) confirm the contention that a left-handed helix exists, since the spectrum is inverted, exactly as predicted by Tinoco's theoretical treatment of optical activity of DNA. X-ray scattering studies by Bram also indicate that the solution structure of poly d(I-C)·d(I-C) is unique (*6*). However, refined X-ray diffraction studies of high-humidity fibers are consistent with a right-handed helix (*7*) which brings up the question: is poly d(I-C)·d(I-C) left- or right-handed?

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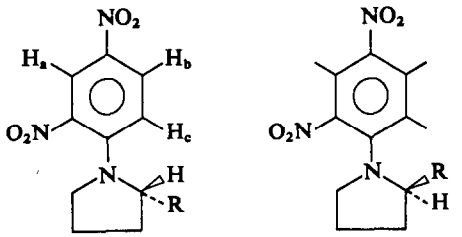
With the ambiguity in the poly d(I-C)·d(I-C) structure and the uncertainty in the chirality of the B DNA structure, an important contribution could be made if the chirality of double-helical nucleic acids in solution could be determined.

To this end the enantiomers L- and D-DNP-prolyl diammonium derivatives, *1* and *2*, respectively, were synthesized. The concept of employing an optically active agent to separate a racemic mixture (e.g., L brucine with 1 and d acids) is well known. The technique relies on the formation of two diasteromers which will naturally have different



L-*1* D-*2*

$$R = CONHCH_2CH_2\overset{+}{N}(\overset{d}{C}H_3)_2(CH_2)_3\overset{+}{N}(\overset{e}{C}H_3)_3 \cdot 2Br^-$$

physical properties and can be separated. Somewhat analogously, the optically active DNA molecule is used as the agent for the formation of diasteromeric complexes in solution with the L- and D-prolyl systems. These complexes are never isolated, but the physical properties and the binding strengths of these reporters to DNA are determined.

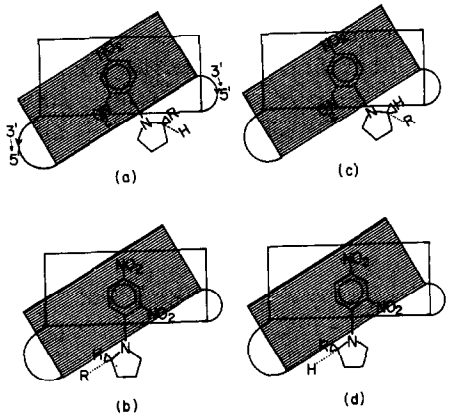


FIG. 1. Schematic illustration (top view) of the possible intercalated complexes of the D-prolyl derivative 2 (a and b) and L-prolyl derivative 1 (c and d) between base pairs of a right-handed DNA helix. The direction of the 3′ → 5′ sugar-phosphate chain with respect to the base pairs at the top (shaded) and bottom is indicated.

Due to the helical twist of DNA, successive base pairs can be visualized as in Fig. 1. The asymmetric center of the prolyl systems is such that the direction of the carboxyamido side chain (-CNH-R) is sterically unfavorable for intercalation of the L-enantiomer,

1, if the DNA helix is right-handed, whereas intercalation of the 2,4 dinitroaniline ring of the D-enantiomer, *2*, should occur. If the DNA helix is left-handed, then the argument is reversed (i.e., the L-enantiomer would intercalate between base pairs of the DNA helix). The proposed dissymmetric recognition of the helical twist of DNA by selective interaction with *1* and *2* is a unique application of chemical principles to macromolecular structure determination.

Recently, a report from our laboratory has shown that the helical sense of DNA in solution may be determined via the use of these L- and D-DNP-prolyl diammonium derivatives, *1* and *2* (*8*). This paper reports additional studies of these systems with several types of nucleic acids (i.e., salmon testes, *M. luteus* DNA, poly dG-poly dC, poly d(A-T)-poly d(A-T), and poly d(I-C)-poly d(I-C)). It is shown that the reporter molecules, *1* and *2*, have a high selectivity for DNA-type polymers and in particular for A-T binding sites. Moreover, the results are consistent with the interpretation that the deoxypolynucleotides are right-handed helices and form specific complexes with the nitroaniline reporter molecules, whereby the side chain of the latter is located in the minor groove.

RESULTS AND DUSCUSSION

The visible absorption spectroscopy results of the interaction of *1* and *2* with a number of DNA systems are given in Table 1. A number of interesting observations may be made from these data. (1) The D-enantiomer, *2*, shows a large hypochromic effect (34%) and a 3-nm red shift in the 4-nitroaniline transition on binding to salmon sperm DNA, (s.s. DNA), whereas the L-enantiomer, *1*, elicits a 12% hypochromism and no red shift. (2) Denatured s.s. DNA (obtained by heating native s.s. DNA at 100°C for 10 min followed by quenching in water/ice bath) shows no selectivity on the absorption spectra of the bound reporter molecules *1* and *2* (e.g., a 12- and 14% hypochromic effect and the absence of a red shift are noted, respectively). (3) All DNA samples studied, with the exception of poly dG-poly dC, elicit a greater hypochromicity with *2* than *1*. (4) Poly dG-poly dC shows no effect on the spectra of the reporter molecules. (*5*) The magnitude of the hypochromic effect of the reporters increases with increasing A-T/G-C ratio.

These results are consistent with the proposed model for dissymmetric recognition of right-handed, double-helical DNA in solution, since a larger hypochromicity is observed for *2* than for *1*. The hypochromism is the result of intensity interchange of electronic transition moments between chromophore and adjacent base pairs. The magnitude of the hypochromicity would be expected to depend upon the geometry of the complex as well as the proximity of chromophore to the bases of DNA (*9*). It is not possible at present to distinguish between either of these possibilities, but this problem does not detract from the fact that a dissymmetric recognition is indicated by the results. Indeed, when the s.s. DNA double helix is melted out the interaction differences between *1* and *2* disappear (i.e., the bound reporter molecules *1* and *2*, give a 12- and 14% hypochromic effect, respectively, and no red shift). Thus, it would appear that differential binding of the reporter to merely the sugar–phosphate backbone of DNA would not be a likely explanation of the absorption results. The apparent binding selectivity of *1* and *2* for A-T rich DNA will be discussed later in this section.

A further characterization of the effect of salmon sperm DNA on the absorption spectra of the L- and D-reporters is given in Fig. 2. As the DNA concentration is increased at a constant reporter molecule concentration (i.e., 1×10^{-4} M), the extinction coefficient of the reporters decreases until a base-pair/reporter ratio of approximately 18 is reached. Again, the extent of the hypochromicity is greater for the D-enantiomer than for the L-enantiomer at all B.P./R values. It would be expected that the decrease

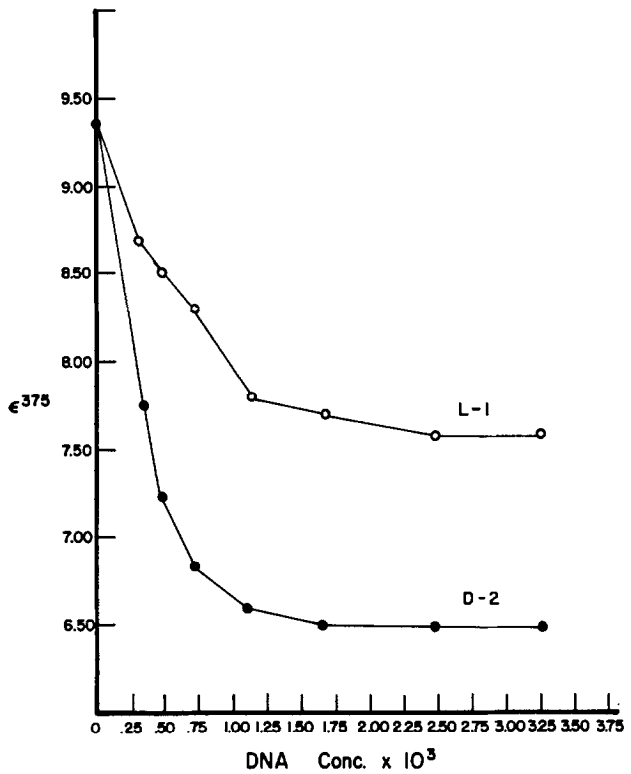


FIG. 2. The effect of increasing concentrations of salmon sperm DNA on the absorption of L- and D-prolyl reporter molecules. Spectra were recorded on a Cary 15 spectrophotometer at 22°C in 0.01 M 2-(*N*-morpholino) ethane-sulfonic acid buffer (0.05M Na^+) using a reporter concentration of 1.0×10^{-4}M.

in the extinction coefficient of the L- and D-reporters would reach a limiting value at a B.P./R ratio of 4 or 5. At this DNA to reporter ratio, there are enough base-pairs to bind all the reporter molecules via intercalation (*8*). The large B.P./R ratio required for a limiting value in the hypochromicity of *1* and *2* may be explained by assuming that these reporters have an affinity for specific intercalation sites and that these intercalation sites allow for a more intimate binding resulting in a greater hypochromicity (*10*). Therefore, as more base pairs are made available by the increase in DNA concentration, the L- and D-reporters are able to seek out the most favorable intercalation sites until all the reporters are bound to these favoured sites. From Table 1 it appears that these intercalation sites contain mostly A-T bases, since greater hypochromicity is observed for DNA of higher A-T content (see below).

TABLE 1

EFFECT OF VARIOUS NUCLEIC ACIDS ON THE ABSORPTION OF L- AND D-PROLYL REPORTER MOLECULES 1, AND 2, RESPECTIVELY[a]

Nucleic acid	Reporter	$\varepsilon^{max} \times 10^{-3}$	λ^{max}	%H[b]
S.s. DNA (n)[c]	L	8.4	375	12[d]
S.s. DNA (n)	D	7.1	378	34[d]
S.s. DNA (d)[c]	L	8.4	378	12
S.s. DNA (d)	D	8.2	378	14
M. luteus DNA (n)	L	8.4	375	12
M. luteus DNA (n)	D	7.8	375	21
Poly d(A-T)-poly d(A-T)	L	6.9	377	26
Poly d(A-T)-poly d(A-T)	D	5.9	380	48
Poly dG-poly dC	L	9.4	375	0
Poly dG-poly dC	D	9.4	375	0
Poly d(I-C)-poly d(I-C)	L	8.5	375	11
Poly d(I-C)-poly d(I-C)	D	8.5	376	17

[a] At ambient temperature in 10 mM NaCl, 0.1 mM EDTA, pH 7.3 ± 0.05. Absorption spectra were taken in 1-cm cells using a Cary 15 spectrometer at nucleic acid and reporter concentrations of 0.9 mM P/1 and 81 μM, respectively.

[b] Percent hypochromicity, %H, is defined as $(\varepsilon^{max}_{H_2O}/\varepsilon^{max}_{p} - 1)\,100$, where $\varepsilon^{max}_{H_2O}$ and ε^{max}_{p} are the extinction coefficients in the absence and presence of DNA.

[c] Native salmon sperm DNA (n) was heated at 100 °C for 10 min and quenched in ice-water bath to give the denatured DNA (d). The solution conditions are described under superscript [a].

[d] In 10 mM 2-(*N*-morpholino) ethane sulfonic acid buffer (MES) pH 6.2, 5 mM Na^+, and at a base-pair to reporter ratio of 19/1, the %H for native DNA-1 and 2 complexes is found to be 24 and 45%, respectively (see Table 2 and Fig. 2).

Additional evidence for the dissymmetric recognition of a right-handed DNA in solution is provided by viscosity studies conducted with salmon sperm DNA. The results of the viscometric titration of s.s. DNA with the L- and D-reporters are shown in Fig. 3. It is noted that the specific viscosity of the DNA solution increases with increasing concentration of the D-enantiomer and levels off at a B.P./R ratio of 4.0. These results are consistent with an intercalation model (*11–13*). In contrast, the L-enantiomer causes a lowering of the specific viscosity of the DNA solution, which indicates a decrease in the effective length of the helix in the DNA-*1* complex. These results are consistent with a model in which partial intercalation of the nitroaniline ring of the L-enantiomer causes a distortion of the helical rod (i.e., bending at the point of contact). This bending of the DNA molecule would be expected from the steric interactions between the carboxyamido side chain of the L-enantiomer with the base pairs of DNA (see Fig. 1).

The pmr results shown in Table 2 are also consistent with a more intimate binding of *2* to DNA as compared to *1*. For example, the pmr signal of the Ha proton of *2* is upfield shifted by 64 Hz on binding to DNA at 39°C and also at 50°C. In contrast, the pmr signal of the Ha proton of *1* is upfield shifted by 52 Hz at 39°C and 50 Hz at 50°C on binding to DNA. Moreover, the entire pmr spectrum of the DNA-*2* complex shows

that the pmr signals of *2* are more broadened than the corresponding pmr signals observed for the DNA-*1* complex. The results are indicative that the dinitroaniline ring of *2* experiences greater shielding (i.e., via ring current anisotropy) and restricted tumbling than *1*. It is equally important to note that the upfield chemical shifts and line broadening of the pmr signals of the aromatic protons of *1* and *2* in the DNA complex can arise only from one type of binding, namely, intercalation (for details see Ref. (*14*)).

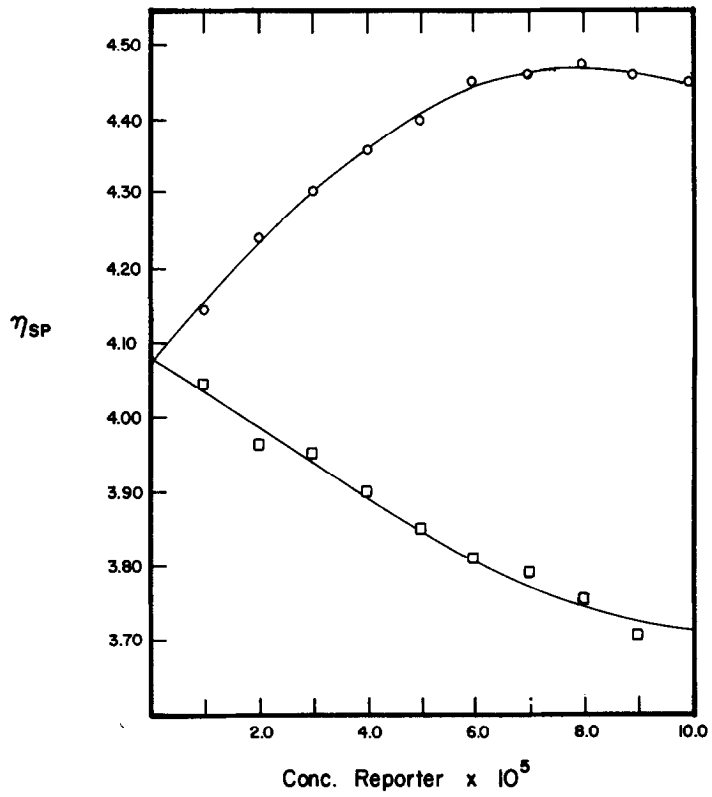


FIG. 3. The effect of L- and D-prolyl derivatives 1 and 2 on the specific viscosity of DNA (□——□, L-prolyl; ○——○, D-prolyl derivative). Viscosity measurements were carried out using 6.3×10^{-4} moles of phosphorus/1. in 0.01 M 2-(*N*-morpholino) ethanesulfonic acid buffer at 37.5°C using the low-shear Zimm viscometer.

The circular dichroism spectra of the free reporter molecules *1* and *2* (see Table 3) indicate that there are two optically active transitions (i.e., at 405 and 335 nm). These transitions have been shown by Gabbay to be due to the 2-nitroaniline (405 nm) and 4-nitro-aniline (335 nm) electronic transitions (*9*). It is noted that the D-enantiomer undergoes a larger change in the molar ellipticities,(θ), of the peaks at 405 and 335 nm as compared to the (θ) of the troughs of the L-enantiomer on binding to DNA. These induced CD changes in the DNA-*1* and DNA-*2* complexes are consistent with an intercalation mode of binding of the reporter molecules to DNA (*9*, *15*) and indicate a greater degree of interaction of *2* with DNA than *1*.

TABLE 2

CHEMICAL SHIFTS (ppm) FROM THE INTERNAL STANDARD SODIUM 2,2-DIMETHYL-2-SILAPENTANE-SULFONATE (DSS) AND LINE WIDTH IN Hz AT HALF-HEIGHT ($\Delta \nu 1/2$) OF FREE AND DNA-BOUND REPORTER MOLECULES, 1 AND 2, AT VARIOUS TEMPERATURES[a]

Reporter	Temp, °C	Chemical Shift (δ)[b]			
		H_a	H_b	H_c	$H_d + H_e$
1	39	873 d_1	829 q	707 d_2	318 (1.5)
1	90	870 d_1	829 q	708 d_2	318 (1.5)
DNA-1	39	821 (7)	[c]	[c]	319 (3.0)
DNA-1	50	823 (4)	789 (10)	682 (20)	318 (2.5)
2	39	873 d_1	829 q	707 d_2	318 (1.5)
2	90	870 d_1	829 q	708 d_2	318 (1.5)
DNA-2	39	809 (8)	[c]	[c]	319 (5)
DNA-2	50	809 (5)	766 (15)	[c]	318 (3)

[a] Sonicated low molecular-weight ss DNA was used at 0.16 mole of phosphorus/l. in D_2O. The concentration of reporter molecule was 0.02 M. Spectra were taken on a Varian XL-100. It should be noted that the pmr spectrum of DNA in the temperature range of 20–70°C is completely broadened and indistinguishable from base-line noise.[2]

[b] The chemical shift, δ (Hz), and the multiplicity of the pmr signal are given as follows: d_1, doublet with $^1J_{H_a-H_b} = 2.5$ Hz; d_2, doublet with $^2J_{H_b-H_c} = 9.3$ Hz; q, doublet of doublets with $^1J = 2.5$ and $^2J = 9.3$ Hz.

[c] Proton signal is indistinguishable from base-line noise.

Melting temperature studies on various double-stranded nucleic acids with L- and D-reporters show a preferential stabilization of the double helix relative to the random coil by the D-enantiomer (see Table 4). Two additional observations may be made. (1) All the nucleic acid systems show a greater stabilization of the T_m with *2* than with *1* at several different reporter concentrations. (2) The degree of helix stabilization is a function of the A-T/G-C ratio of the DNA. For instance, $\Delta T_m (\Delta T_m = T_m^D - T_m^L)$ at 7.5×10^{-5} M of *1* and *2* is 1.5, 2.2, and 8.3° for *M. luteus*, salmon sperm DNA, and poly d(A-T) · poly d(A-T), respectively.

Again, these results suggest a greater binding of the D-enantiomer to the double-stranded nucleic acids than the L-enantiomer. Since the binding of the reporters to helical DNA will be dependent on the free energy contributions of electrostatic, hydrophobic, and electronic forces, the greater stabilization of the T_m by the D-enantiomer is not surprising since this enantiomer intercalates to a greater extent than the L-enantiomer.

It has been mentioned previously that reporter molecules *1* and *2* appear to bind preferentially to A-T-rich regions of DNA (e.g., see Tables 1 and 4). In order to obtain additional evidence for this effect and to explain the mechanism of the binding selectivity, binding studies using equilibrium dialysis were carried out. In addition, evidence is presented which suggests that intercalation of the 2,4-dinitroaniline ring of reporter molecules *1*, *2*, and *3* occurs in the minor groove of DNA. For example, the chromophore of reporter molecule *3*, may intercalate to DNA to form complexes whereby the diammonium side chain may lie in the major and/or minor groove of the helix. Since

TABLE 3

EFFECT OF SALMON SPERM DEOXYRIBONUCLEIC ACID (SS DNA) ON THE ABSORPTION AND CIRCULAR DICHROISM SPECTRA OF THE L AND D ENANTIOMERS 1 AND 2, RESPECTIVELY[a]

	Absorption spectra					Circular dichroism							
	H_2O-buffer		SS DNA			H_2O-buffer				SS DNA			
Reporter	λ_{nm}^{max}	ε^{max}	λ_{nm}^{max}	ε^{max}	%H[b]	λ_1	$[\theta]_1 X 10^{-4}$	λ_2	$[\theta]_2 X 10^{-4}$	λ_1	$[\theta]_1 X 10^{-4}$	λ_2	$[\theta]_1 X 10^{-4}$
L-1	374	9,400	376	7600	24	405	−1.22	335	−1.18	405	−1.14	333	−0.94
D-2	374	9,400	379	6500	45	405	1.14	335	1.04	390	0.88	330	0.48

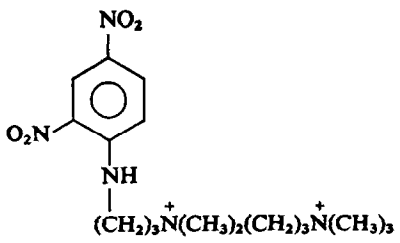
[a] At ambient temperature in 0.01 M 2-(*N*-morpholino) ethanesulfonic acid (Mes) buffer, pH 6.2 (0.005 M in Na^+). Absorption spectra were taken in 10-mm cells using a Cary-15 spectrometer at DNA and reporter concentrations of 2.5×10^{-3} moles of phosphorus/liter and 1×10^{-4} M, respectively. Under these conditions, the reporter molecules are fully bound. Circular dichroism spectra were taken in 50-mm cells using a Jasco J-20 spectrometer at DNA and reporter concentrations of 2.7×10^{-3} moles of phosphorus/liter and 9.1×10^{-5} M, respectively. [b]Per cent hypochromicity ($\%H = [\varepsilon_{H_2O}^{max}/\varepsilon_P^{max} - 1)]100$), where $\varepsilon_{H_2O}^{max}$ and ε_P^{max} are the extinction coefficients in the absence and presence of DNA.

TABLE 4

THE EFFECT OF REPORTER MOLECULES 1 AND 2 ON THE T_m OF THE HELIX-COIL TRANSITION OF VARIOUS NUCLEIC ACID SYSTEMS[a]

	T_m(°C)									
		Concn 1 (L) × 10^5					Concn 2 (D) × 10^5			
Nucleic acid	—	3.75	7.5	11.25	15.5	—	3.75	7.5	11.25	15.50
SS DNA	65.4	66.9	69.7	70.0	72.3	65.4	69.8	71.9	73.4	75.7
M.L. DNA	77.1	77.9	78.9	—	—	77.1	78.5	80.4	—	—
Poly d(A-T)-poly d(A-T)	42.3	48.3	51.2	51.6	56.8	42.3	55.7	59.5	61.7	63.0
Poly d(I-C)-poly d(I-C)	36.0	37.7	38.7	40.3	—	36.0	4.07	41.4	43.9	—

[a]In sodium phosphate buffer (0.01 M Na^+) and 1.0×10^{-4} M EDTA at pH 6.7 ± .02. In all cases 1.25×10^{-4}M P/liter of nucleic acid was used.



the electrostatic repulsion between phosphate anions is greater across the minor than in the major groove it would be expected that the positively charged side chain of the reporter molecule would lie in the minor groove. However plausible this argument may be, it does not constitute proof, and further experimental verification is necessary. To resolve this problem the binding of reporter molecules *1-3* to various deoxypolynucleotides with different A-T to G-C base content was examined. The data are presented in Table 5. Several interesting observations may be made. (1) In all cases the D-enantiomer

TABLE 5

THE APPARENT BINDING CONSTANT, K_a, OF REPORTER MOLECULES 1–3 TO VARIOUS NUCLEIC ACIDS[a]

Nucleic acid	AT/GC	$K_a \times 10^{-3}$		
		1 (L-Polyl)	2 (D-Prolyl)	3
Poly dG-poly dC	0	0.13 (±0.08)	0.20 (±0.01)	4.14 (±0.18)
M. luteus DNA	0.39	1.63 (±0.12)	2.97 (±0.08)	18.7 (±0.3)
S.S. DNA	1.38	1.80 (±0.10)	3.40 (±0.10)	15.9 (±0.2)
Poly d(A-T)-poly d(A-T)	∞	2.25 (±0.28)	7.90 (±0.15)	10.5 (±0.2)
Poly d(I-C)-poly d(I-C)[b]	—	0.59 (±0.01)	2.15 (±0.11)	4.55 (±0.09)

[a] Equilibrium dialysis studies were carried out in 0.01 M sodium phosphate buffer pH 7.3 (0.01 M Na^+) at 25°C. In all cases, reporter concentrations of 50 μM and a base-pair to reporter ratio of 10 ± 1 was used. [b]Base-pair to reporter ratio was 4.5.

binds more strongly than the L-enantiomer, which is consistent with the previous data. (2) Reporter molecule *3* binds more strongly to all the DNA systems than the prolyl reporters, *1* and *2*, and exhibits a preference for A-T-rich DNA (i.e., $K^{\text{poly dA-T}}/K^{\text{poly dG-polydC}} = 2.53$); the binding constants listed in Table 5 were carried out at a b.p./R = 10 so that even for *M. luteus* DNA (28% AT) there are sufficient A-T base pairs to bind all the reporter molecule). (3) Reporters *1* and *2* show a marked increase over *3* in selectivity for A-T-rich DNA. The selectivity ratios (i.e., K^{AT}/K^{GC}) for reporter molecules *1–3* are found to be 18, 39.5, and 2.53, respectively.

In order to interpret these results, several pertinent features of the Watson–Crick–Wilkins-type double helix should be cited. (a) The major and minor grooves of the W–C–W helix arise as a result of the position of substitution of the sugars, i.e., the sugar–phosphate backbone connected to the individual bases is closer on one side of the plane of the base-pairs than the other, (b) substitution at the 5 position of pyrimidines will

place the group in the major groove (e.g., the thymine methyl group) and (c) the additional H-bond in G-C base-pair lies in the minor groove. Hence, it is clear that there is greater crowding (i.e., steric hindrance in the major groove next to A-T than to G-C sites and lesser steric hindrance in the minor groove next to A-T than to G-C sites). The results of the equilibrium dialysis experiments (Table 5), which indicate a higher affinity of the reporter molecules *1–3* to A-T sites, strongly suggest that the reporter molecules are binding to DNA from the minor groove. However, it may be argued that electronic factors between the 4-nitroaniline ring and the bases in the intercalated complex play the major role in accounting for the binding specificity exhibited by these reporter molecules. The results shown in Table 5, however, suggest that this is not the likely case. For example, reporter molecules *1–3* have an identical chromophore (i.e., 2,4-dinitroaniline ring, and, therefore, the electronic factors involved in the intercalated complex should also be identical). Hence, the binding specificity (i.e., the ratio of K_a for poly d(A-T)-poly d(A-T) to K_a for poly dG-poly dC) would be expected to be similar for reporter molecules *1–3*. The selectivity ratio, K_a^{A-T}/K_a^{G-C}, for reporter molecules *1–3* is found to be 18, 39.5, and 2.53, respectively (Table 5). The high selectivity of *1* and *2* for A-T sites would, therefore, appear to be best interpreted in terms of steric hindrance to formation of the intercalated complex next to G-C sites. This effect would be expected to be greater for the DNA-*1* and *2* than DNA-*3* complexes due to the presence of a larger steric group adjacent to the intercalating chromophore in the former (i.e., the prolyl ring). On this basis it is reasonable to suggest that the 2,4-dinitroaniline containing reporter molecules *1–3* bind to nucleic acids from the minor groove. (It should be noted that additional arguments (*9*) based on comparison of the binding of reporter *3* to DNA and RNA helices support the above conclusion).

It is well-known that small molecules may exhibit more than one type of binding to DNA. For example, aromatic molecules such as the dyes, acridine orange and proflavine, have been shown to exhibit two types of binding, namely, (i) intercalation between base-pairs (strong binding) and (ii) weak binding to the periphery of the helix (*16*). The second mode of binding will take place after saturation of the strong binding sites has occurred (i.e., at low base-pair to dye ratio). Therefore, the possibility exists that the differences in the binding affinities of reporters *1–3* to DNA which are reported in Table 5 may be due to differences in the mode of binding (i.e., intercalation type vs peripheral-type binding). The proton magnetic resonance (pmr) experiments, discussed earlier, were conducted at a base-pair to reporter ratio (B.P./R) of 4 and definitively show that the 2,4-dinitroaniline ring of *1* and *2* is intercalated between base-pairs of DNA. In addition, Scatchard-type plots obtained by a spectral titration technique (*8*) show that reporters, *1*, *2*, and *3* exhibit one strong binding site per $5.4 \pm .3$, $4.5 \pm .3$, and $4.0 \pm .1$ base-pairs of DNA, respectively. Thus, under the conditions of the equilibrium dialysis experiments (Table 5) where the b.p./R is 10, only one type of binding to DNA is possible for the reporters, namely, intercalation between base-pairs rather than weak binding to the periphery of the helix. Nonetheless, equilibrium dialysis studies were also conducted at a higher b.p./R ratio than that shown in Table 5. For example, at a b.p./R of 20 (e.g., 2×10^{-3} M P/1 of s.s. DNA and 5×10^{-5} M of *1–3*) the values of the binding constants are found (in all cases) to be approximately 20% higher than those obtained at a b.p./R of 10. Thus, it is concluded that the reporters, *1–3*, have the same binding mode to DNA (i.e., intercalation between base-pairs), under the conditions of

the equilibrium dialysis experiment (Table 5). This conclusion is also supported by the uv, induced CD, and viscometric studies previously mentioned.

An extensive amount of data have been obtained by a number of different techniques supporting the proposed model for the dissymmetric recognition of DNA in solution. Under the experimental conditions used in the various experiments reported in this paper, all DNA samples assayed with the reporter molecules *1* and *2* are apparently right-handed in solution, even the controversial poly d(I-C)·d(I-C). Although the evidence for this conclusion is substantial, conclusive proof requires the use of the reporters *1* and *2* with a known left-handed DNA sample. Since Fric *et al.* (*17*) have reported the synthesis of left-handed polyribonucleotides containing L sugars, it might appear that it would be possible to test these systems with reporters *1* and *2*. Unfortunately, the L- and D-enantiomers do not show selective binding to polyribonucleotides (*18*). This finding reinforces our argument since the 2′-OH group on the ribose sugar ring is known to lie in the minor groove of the RNA double helices (e.g., polyA-polyU, polyI-polyC, etc.). Consequently, the steric environment for intercalation of the 2,4-dinitroaniline ring of *2* would be unfavourable. Thus, the results with RNA systems support the hypothesis that reporter type molecules (i.e., *1–3*) bind in the minor groove of double-stranded nucleic acid helices.

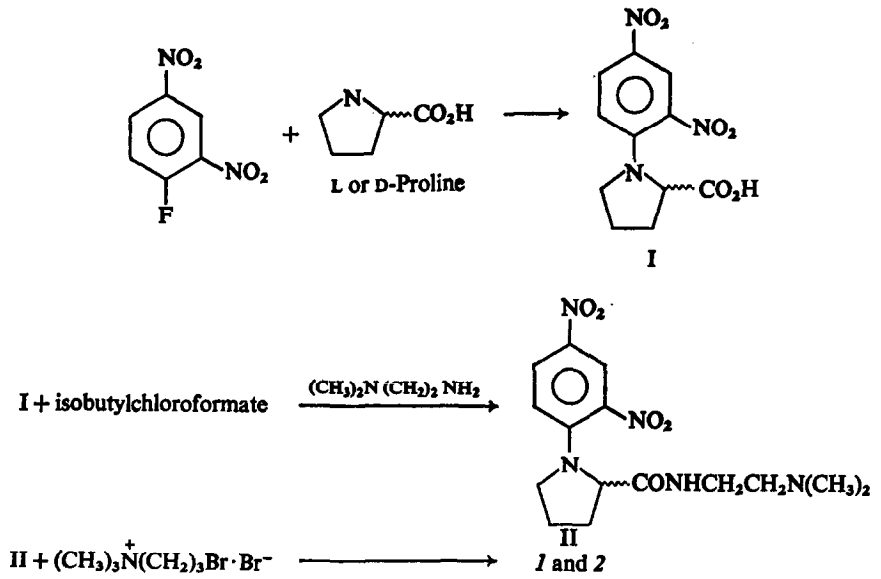
EXPERIMENTAL SECTION

Materials and Methods

Poly d(A-T)poly d(A-T) (lot no. 30), poly dG-poly dC (lot no. 32), and *M. luteus* DNA were purchased from Miles Laboratories. The *M. luteus* DNA was further purified to remove the 2–3% residual protein by the phenol-extraction procedure (*19*). Salmon sperm DNA was obtained from Worthington Biochem. Corp. The alternating Poly d(I-C)-Poly d(I-C) was a kind gift from Dr. R. D. Wells. Stock solutions of the polymers were made in 10 mM 2-(N-morpholino) ethane sulfonic acid buffer (MES) pH 6.2, 5 mM Na^+ and stored at 0°C. The stock solutions were removed and diluted in the various buffer as indicated in the tables and figures.

Equilibrium dialysis experiments were run in Plexiglas blocks at ambient temperature according to the method of Wells and Larson (*20*). Time studies showed that equilibration was complete in 16 hr. *Tm* of the helix-coil transition, ultraviolet studies, circular dichroism, proton magnetic resonance, and viscometric studies were carried out according to previous published procedures (*8*, *21*).

Reporter molecule *3*, was synthesized according to Gabbay (*9*), and the L- and D-enantiomers, *1* and *2* were synthesized according to the following chart. The DNP-proline derivative, I, was converted to the amide, II, by the mixed-anhydride procedure (*22*) and the latter was treated with *N,N,N*-trimethyl-*N*-3-bromopropylammonium bromide (*10*) to yield reporter molecules *1* and *2* in 70% yield. The reporter molecules were found to be exceedingly hygroscopic and light sensitive. They were kept in frozen solutions and stored in the dark. Pmr, IR, uv, CD, and elemental analysis (theorit. C, 41.18, H, 5.88; found for *1*, C, 40.95, H, 6.07; for *2* ,C, 41.06, H, 5.95) results are consistent with the assigned structure.



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