TOPOGRAPHY OF NUCLEIC ACID HELICES IN SOLUTIONS. X. THE SYNTHESIS AND SELECTIVE INTERACTIONS OF REPORTER MOLECULES WITH POLYNUCLEOTIDES*

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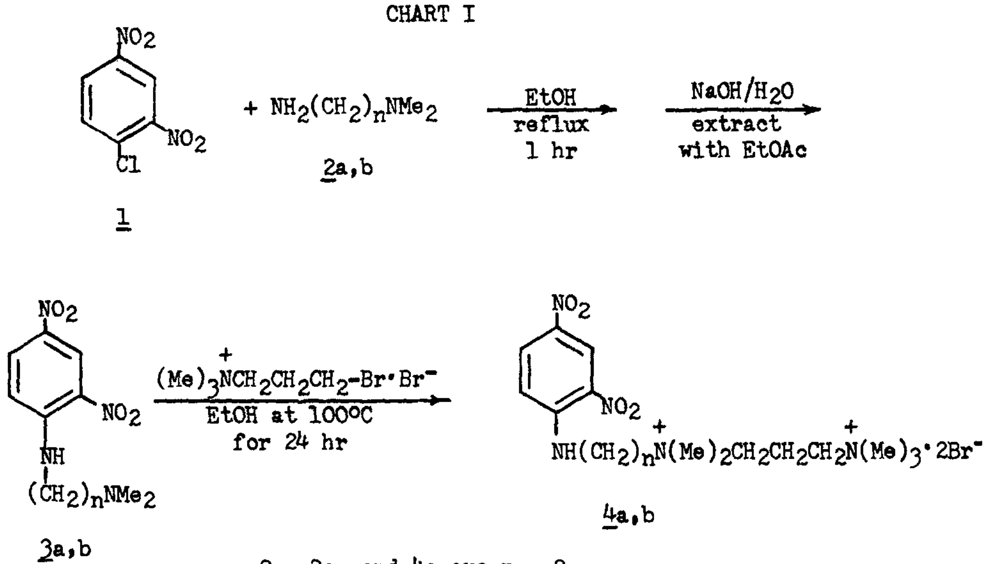
This paper reports the synthesis and interactions of reporter molecules with nucleic acids. (Reporter molecule is used in the same sense as described by Hille and Koshland, 1964, 1967). The synthetic methods used are outlined in Chart I. The visible and near u.v. absorption spectra of compound 4a is

CHART I



2a, 3a, and 4a are n = 2
2b, 3b, and 4b are n = 3

shown in Figure 1. The effect of polynucleotides on the absorption spectra of the bound reporter molecules is summarized in Table 1.

Several interesting points may be made. (1) The transition in the 350 mμ region is probably a $\pi^* \leftarrow \pi$ type since it has an extinction coefficient, ε_{max},

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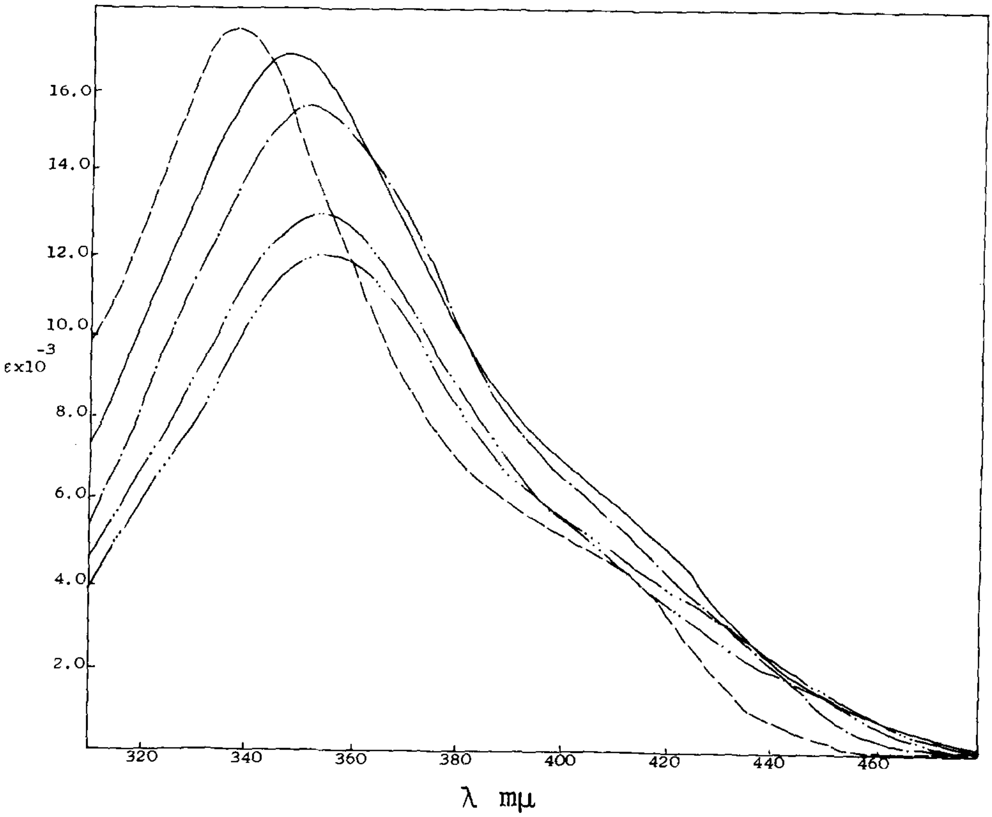

Figure 1. Near u.v. spectra of reporter molecules 4a at 25.0°C; 4a in 95% EtOH (----), in H_2O/buffer (——), in presence of rU (—·—), in rA (—··—), and in rA-rU (—····—). See Table 1.

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of 16,000 characteristic of an allowed transition, and a red shift in the maximum is observed in going from 95% ethanol to 0.01M sodium phosphate buffer (338 → 350 mμ, and 343 → 356 mμ for n = 2 and 3 respectively). (2) Interaction of the reporter molecule with polynucleotides leads to a complex in which the environment of the chromophore is more polar than that of H_2O since a further shift to the red is observed (350 → 355 mμ and 356 → 362 mμ for n = 2 and 3 respectively (excluding polypyrimidine nucleotide)). This result is anticipated since the electrostatic binding of the reporter molecule to adjacent phosphate anions of the polynucleotide would place the 2,4-dinitroaniline ring in close proximity to the negatively charged phosphodiester group. (3) Substantial hypochromism is observed when the reporter molecule is bound to the polyribo-

Table 1. The Effect of Polynucleotides on the Absorption Spectra of Reporter Molecules 4a and 4b at 25.0 ± 0.2°C[a]

Conditions	Reporter Molecule 4a (n = 2)				Reporter Molecule 4b (n = 3)			
	$\lambda^{max}_{m\mu}$	ϵ^{max}	% H[b]	P/R[c]	$\lambda^{max}_{m\mu}$	ϵ^{max}	% H[b]	P/R[c]
95% EtOH	338	16,600	--	--	343	16,050	--	--
H_2O/buffer[d]	350	16,600	--	--	356	16,160	--	--
rU[d]	351	15,300	8.4	30	357	14,550	11.1	29
rC[d]	352	15,200	9.1	30	357	15,020	7.5	29
rI[d]	355	13,600	22.0	28	360	13,510	19.5	29
rA[d]	355	12,800	29.6	28	361	12,450	29.8	29
rA/rU[d]	355	11,600	43.1	29	362	11,020	46.5	29
rI-rC[d]	355	11,800	40.7	29	362	11,510	40.5	29

[a]Values of λ^{max} and ϵ^{max} in the presence of polynucleotides reported in this table are limiting values.

[b]% hypochromicity (% H) = $\left[\epsilon^{max}_{H_2O}/\epsilon^{max}_{p} - 1.0\right]100$.

[c]P/R indicates the ratio of phosphate/reporter molecule.

[d]In 0.01M sodium phosphate buffer (0.01M in Na^+), pH 6.50.

nucleotides. The extent of hypochromism appears to depend on the nature of the nucleic acid system and not on the number of methylene groups, n, between the ring nitrogen and the quaternary nitrogen of the reporter molecule. The percentage hypochromicity increases for the following series, rU ≈ rC < rI < rA < rA-rU ≈ rI-rC, which is also the order of increased stacking of the bases (Felsenfeld and Miles, 1967). The results suggest that the hypochromism of the reporter molecule depends on the secondary structure of the polynucleotide as well as the nature of the bases.

An induced circular dichroism (CD) in the absorption band of the bound reporter molecule is also observed. Figure 2 shows the CD results for 4a bound to the single-stranded rA and rI, as well as to the double-stranded rA-rU and rI-rC helices. Table 2 contains a summary of the results including the molar

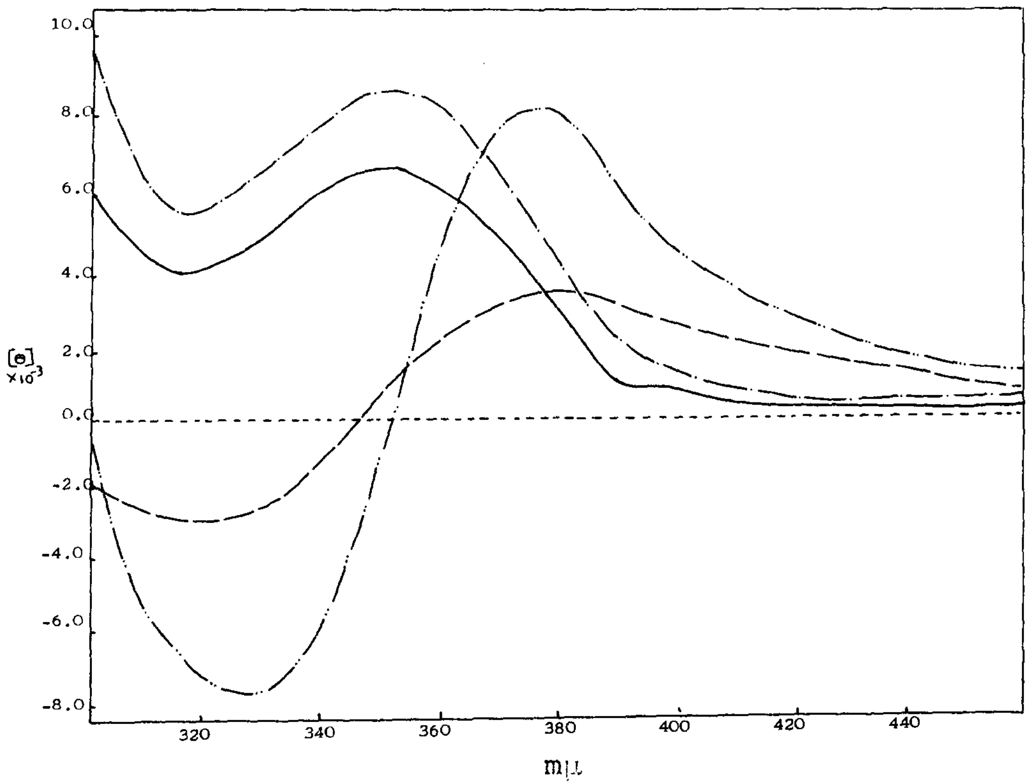

Figure 2. CD of reporter molecule $\underline{4}$a in the presence of rA (—···—), rI (— —), rA-rU (———), and rI-rC (—·—). See Table 2 for experimental conditions.

Table 2. The Effect of Various Polynucleotides on the Induced CD of Reporter Molecules $\underline{4}$a and $\underline{4}$b[a,b]

Polynucleotide	Molar Ellipticity							
	$\underline{4}$a (n = 2)				$\underline{4}$b (n = 3)			
	$[\theta]^P \times 10^{-3}$	$\lambda^P m\mu$	$[\theta]^t \times 10^{-3}$	$\lambda^t m\mu$	$[\theta]^P \times 10^{-3}$	$\lambda^P m\mu$	$[\theta]^t \times 10^{-3}$	$\lambda^t m\mu$
rA	8.14	374	-7.18	323	5.11	375	-4.38	325
rI	3.35	370	-2.40	320	2.43	360	-0.97	310
rA-rU	6.70	347	--	--	6.15	345	--	--
rI-rC	8.62	350	--	--	13.4	350	--	--
rC	No detectable CD				No detectable CD			
rU	No detectable CD				No detectable CD			

[a]CD curves were measured in a Cary 60 Recording Spectropolarimeter equipped with a Model 6001 CD accessory at ambient temperature in 10 mm cells. The solution contained 1.67×10^{-4}M of $\underline{4}$a or $\underline{4}$b in 0.01M sodium phosphate buffer (0.01M in sodium), pH 6.40-6.50.

[b]The ratio of moles of polynucleotide phosphorus to moles of reporter molecules in solution in all cases reported ranged from 28.2-29.5.

ellipticities of the (positive) peaks and (negative) troughs together with the associated wavelengths. Similar studies were made with rC and rU and no detectable induced CD in the absorption band of 4a and 4b was noted. A dependence of the observed molar ellipticity on the P/R (moles polynucleotide phosphorus/ moles of reporter molecules) ratio was found; as $P/R \rightarrow 0$ the molar ellipticity $[\theta] \rightarrow 0$; as $P/R \rightarrow \infty$, $[\theta]$ appears to approach a limiting value characteristic of dye and polynucleotide. In addition, aqueous solutions of 4a and 4b in the absence of polynucleotides obey Beer's Law over the concentration range used in these studies (10^{-5}-10^{-3}M), which indicates no aggregation of the reporter molecules under these conditions. The single-stranded polymers--i.e., rA and rI--induce a double Cotton effect in 4a and 4b with a crossing point near the u.v. absorption maximum of the chromophores. Such a result is usually taken to be indicative of chromophore-chromophore stacking, since double Cotton effects are characteristic of exciton-type interactions, which are favored by such geometries (Kasha, 1963; McRae and Kasha, 1964; Bush and Tinoco, 1967). In the case of the double-stranded rA-rU and rI-rC helices, however, single positive Cotton effects are observed for 4a and 4b. From these results we can deduce the following: (1) CD is induced upon binding of the reporter molecule to the polynucleotide by interactions which are specific to both dye and polymer. For example, as n varies from 2 to 3, the molar ellipticity, θ, decreases for the single chains (rI and rA); remains essentially constant for the double-stranded rA-rU helix; and increases by one-half for the rI-rC helix (Table 2). (2) The interactions are apparently dependent upon the secondary structures of the polynucleotides, since induced CD is not observed in the presence of the polypyrimidines, rU and rC. This result may be explained in terms of the lower propensity of pyrimidine bases to stack as compared with the purine bases (Guttman and Higuchi, 1957; Ts'O, Melvin, and Olson, 1963; Ts'O and Chan, 1964; Bush and Tinoco, 1967, and references therein). In solution, several free bond rotations are allowed per monomer of a polynucleotide; i.e., two oxygen-phosphorus bond rotations, two carbon-oxygen bond rotations, one carbon-carbon bond, and one carbon-nitrogen bond rotation. In polypyrimidines these rotations are expected to occur more freely than in polypurines. Therefore, it is not surprising that rU and rC do not induce CD in the absorption band of the bound reporter molecule, since the complex formed is not as rigid as that obtained with stacked single- or double-stranded helices. In the case of polypurines a double Cotton effect is observed in the induced CD of the bound reporter molecule which is suggestive of cooperative binding. Cooperative binding of diammonium salts to single-chain polynucleotides is anticipated, since the complex formed would freeze out a number of rotations in the polynucleotide and lead to local stacking of bases. Such interactions would favor binding of a second reporter molecule to an adjacent site and lead to chromophore-chromophore stacking.

At this point we must consider the origin of the hypochromism and CD found for the transitions of the bound reporter molecule in the 310-460 mμ region. There are several possible explanations: (1) Upon binding to the polymer, a conformational change in the chromophore might reduce the strength of the transition; e.g., twisting of the nitro or the amino group out of plane of the 2,4-dinitroaniline ring system. The induced CD would arise from the local asymmetric environment. (2) An ordered array of chromophores, in particular an array which brings about the stacking of chromophores' transition moments, is predicted to cause hypochromism and circular dichroism (Kasha, 1963; Bush and Tinoco, 1967). (3) The hypochromism of the bound reporter molecule may arise from the interactions of the transition moment of the $\pi^* \leftarrow \pi$ of the reporter with the transition moment of the higher energy $\pi^* \leftarrow \pi$ of the purine or pyrimidine rings. It has been shown by Tinoco that a card-stacking arrangement of two transition moments of two chromophores would lead to hypochromism for the lower energy transition (Tinoco, 1960, 1961). The induced CD would arise from local asymmetric environment. Although our evidence is not unambiguous, it appears that explanations 2 and 3 are operating. Since the reporter molecules 4a and 4b appear not to aggregate in solutions, and non-zero limiting values of the molar ellipticities, $[\theta]$, in the presence of polynucleotides are observed at high P/R ratios (in the range of 30-110; Gabbay, unpublished results), site induced CD is strongly implicated for the double-stranded helical structure. This is in contrast to the behavior of the induced CD of acridine orange bound to DNA which is attributed to exciton interactions. For this system the induced CD displays a dependence on the P/R ratio; i.e., increasing to a maximum from a zero value at low P/R ratio and then decreasing on furt'er increase of P/R ratio greater than 10 (Gardner and Mason, 1967).

Further studies of these and other systems are in progress in an attempt to establish more conclusively the nature of the reporter-polymer interactions involved in the phenomena we have discussed.

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