ON THE NATURE OF HYPERCHROMICITY IN POLYNUCLEOTIDE COMPLEXES[1]

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The formation of polynucleotide complexes causes changes in the UV absorption spectra. Many experimental and theoretical studies of the hypochromism observed in mixtures of poly (A+U)[4] at the absorption maximum (257 nm) have been published (Steiner 1961); less information is available about the hyperchromism at 280-295 nm. Although Rich and Kasha (1962) suggested that this hyperchromism is due to the interaction of n-π^* transitions, our spectroscopic studies on purines and pyrimidines (Kleinwächter, Drobnik and Augenstein, 1966) provided no evidence of n-π^* transitions in the 280-nm region of adenosine absorption spectra, and since uridine at neutral pH is in the keto form, it should have no non-bonding electrons.

Our present results suggest strongly that the hyperchromicity (280-290 nm) arises from interactions of relatively weak ("W") π-π^* transitions -- which are not present in pyrimidines and which are normally buried in the main absorption bands of adenosine and inosine -- and that the hypochromicity (255-258 nm) is the result of interactions of the most intense ("S")

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[4]Abbreviations: poly A, poly U and poly I refer to polyriboadenylic, -uridylic and -inosinic acids respectively; poly (AU), (A2U) and (A2I) designate the complexes of a given stoichiometry. Poly (nA+mU) refers to a mixture of n parts of poly A solution with m parts of an equimolar poly U solution, where n+m = 100.

π-π^* transitions present in both pyrimidines and purines.

The most critical observation leading to this conclusion is that whereas hyperchromism (288 nm) achieves a maximum value (of 75%) in a 1:1 complex of poly A and poly U, the addition of a second strand of poly U to give a 1:2 triple strand causes a continuing linear increase of hypochromism over that observed in the double strand. Apparently there is an approximately constant hyperchromicity (i.e., increase of absorbance) of 70-90% per purine residue present in the helical complexes since the hyperchromicity observed in the triple-stranded poly (A2I) is 280% and in double-stranded poly A is 140%.

Methods: The synthetic polynucleotides obtained from California Corporation for Biochemical Research were used without further treatment. Solutions were prepared in either 0.1 M NaCl buffered by 0.01 M phosphate at pH 7.0 (poly A and poly U mixtures) or 0.2 M sodium acetate (in the experiment with poly I): all concentrations were adjusted by measuring the optical density at the absorption maxima. The mixtures of constant total nucleotide concentration (but varying fractional contents) were prepared and after at least 3 hours the spectra were recorded on a Cary 15 spectrophometer over the interval 205-305 nm. A 1-cm light path was used except for mixtures of poly A + poly U where a 5-cm cell was needed in the region 283-305 nm to obtain better resolution of isosbestic points and hyperchromicity. In one set of measurements with poly A + poly U 0.001 M $MgCl_2$ was added and the spectra recorded at both room temperature and 58°C.

The positions of isosbestic points in a number of absorption spectra are given in Table I. Fig. 1 contains difference spectra for various mix-

Table 1. The positions (in nm) of isosbestic points in a set of absorption spectra obtained by the method of continuous variation of polynucleotide mixtures in 10^{-4}M solutions. The proportion (=n) of poly A is given in per cent. (*measurements in a 5-cm absorption cell).

Poly (nA+mU), room temp. with or without Mg^{++}				Poly (nA+mU), 58°C 0.001 M Mg^{++}				Poly (nA+mI)				
n	Points			n	Points			n	Points			
0- 30	215	232	283.2*	0- 30	-	-	283.6*	0- 30	-	229	262	
60-100	222	228	278.8*	40-100	-	-	282.0*	40-100	-	244	282	
35- 60	220	231	-									

tures of poly A with either poly U or poly I. The data in Fig. 1a confirm the previous report by Stevens and Felsenfeld (1964) that double-stranded poly (AU) forms only at room temperature, in the absence of Mg^{++} and with 1:1::A:U, whereas at high temperatures in the presence of Mg^{++} only triple-stranded poly (A2U) is formed. As can be seen in Fig. 1a, the most favorable region for the evaluating hyperchromism is 287-288 nm where the hypo-

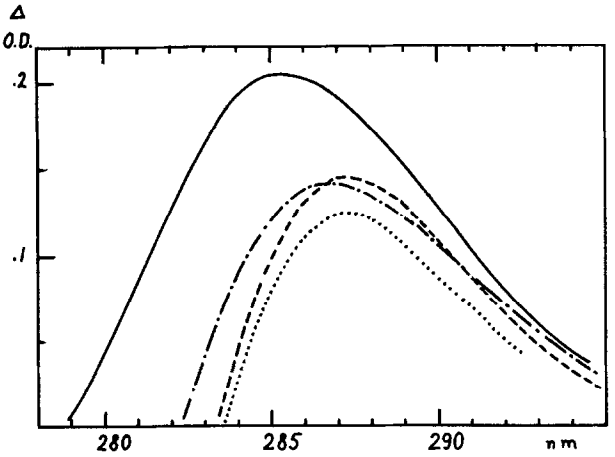

Fig. 1a

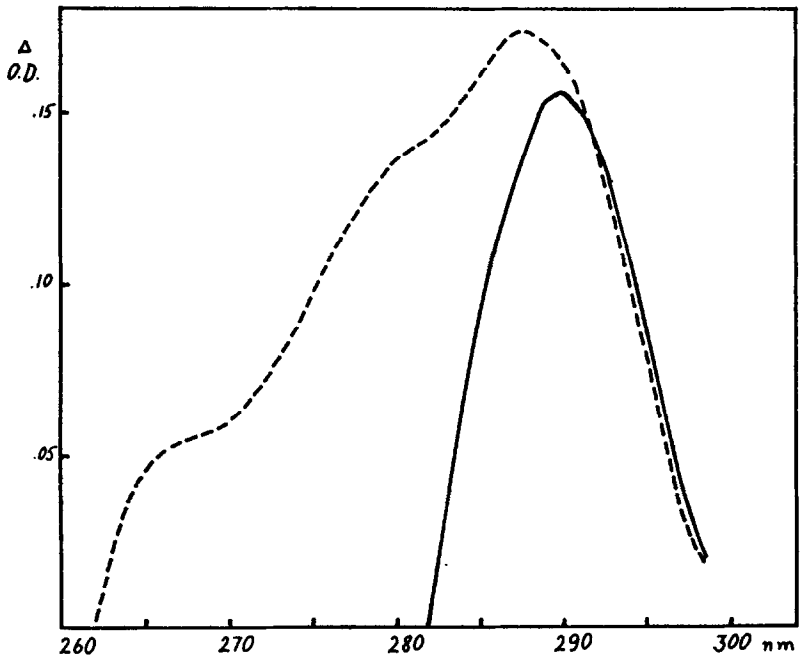

Fig. 1b

Figure 1. Difference spectra of polynucleotide mixtures
(a) poly A + poly U, 10^{-4}M measured in a 5-cm cell: (——) poly (60A+40U) minus poly A, no Mg^{++}, room temp; (----) poly (25A+75U) minus poly U, no Mg^{++}, room temp; (-.-.-) poly (60A+40U) minus poly A, 0.001M Mg^{++}, 58°C; (····) poly (25A+75U), 0.001M Mg^{++}, 58°C
(b) poly A + poly I, 1.1 x 10^{-4}M, 1 cm cell: (——) poly (40A+60I) minus poly A; (----) poly (30A+70I) minus poly I.

chromism observed at shorter wavelengths is minimal.

The mixing curves obtained at room temperature without added Mg^{++} are in Fig. 2a. The deviation of the experimental points from the two lines near their intersection, indicates that the equilibrium constants are not completely shifted toward complex formation although in all cases the equilibrium ratios are either 1:1 or 1:2. Nevertheless, the hyperchromicity curves for poly A-poly I mixtures never had a break at the ratio of 1:2 where the minima in the hypochromicity curves are observed.

Thus, the hyperchromicity of the triple-stranded poly (A2U) must be the same as that of the corresponding mixture of poly U and poly (AU). This conclusion is further supported by measurements with Mg^{++} present (Fig.2b). At room temperature, the absorbances measured at 288 nm for 50-100% poly A are slightly below the values expected if only poly A and poly (AU) were present; thus, even under these conditions some poly (A2U) must be formed. Heating greatly enhances its formation since the measured OD values are very close to those expected for mixtures of poly A and poly (A2U) when n = 34-100.

Mixtures of poly A and poly I for n = 48-100 show only one set of

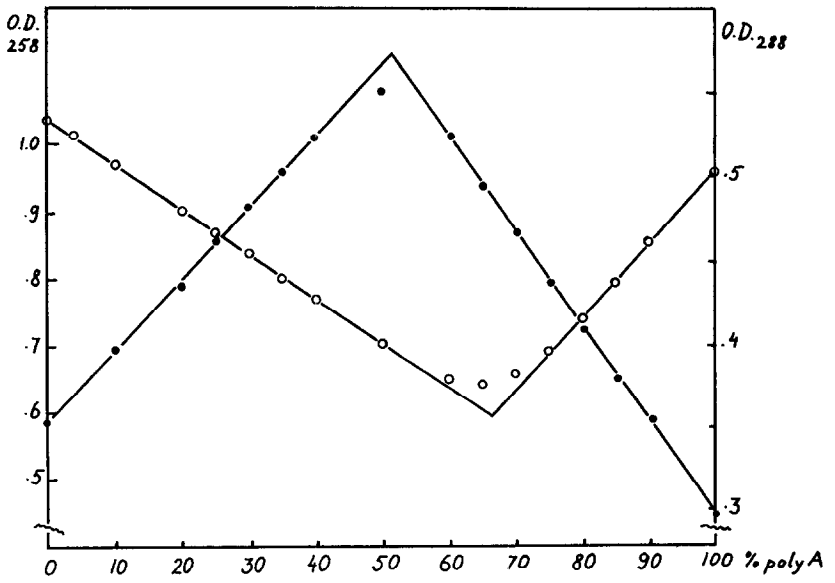

Fig. 2a

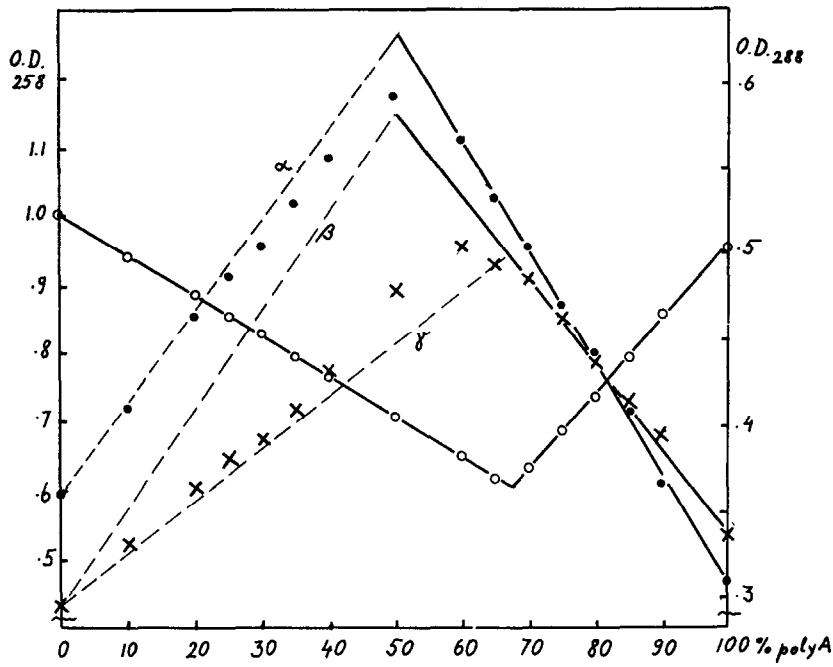

Fig. 2b

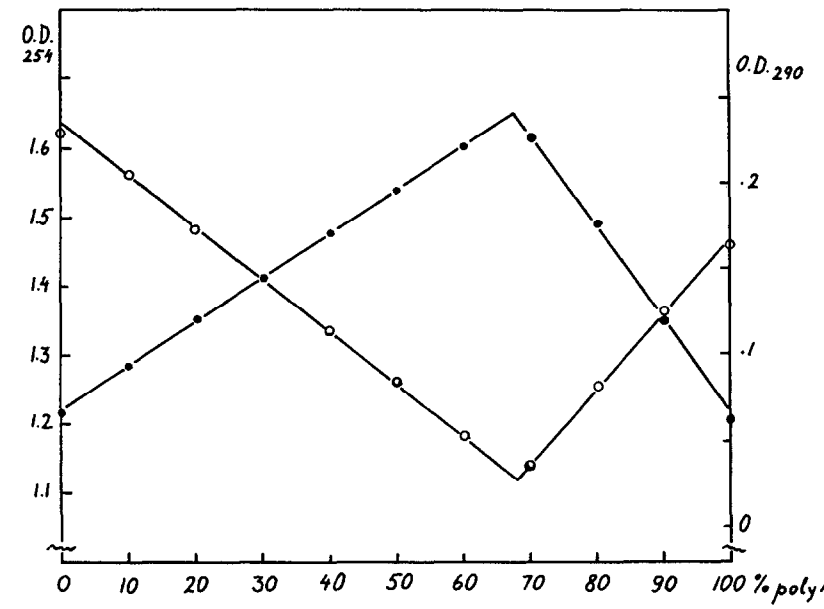

Fig. 2c

Figure 2. Mixing curves of polynucleotides.
(a) Poly A + poly U, no Mg^{++}, room temperature. Open circles are optical density at 258 nm in a 1-cm cell. Filled circles show OD at 288 nm in a 5-cm cell.
(b) Poly A + poly U, 0.001M Mg^{++} present. Open circles are OD at 258 nm in a 1-cm cell at room temperature after 6 hours standing. Filled circles are OD in a 5-cm cell at 288 nm under similar conditions. Crosses are OD at 288 nm in a 5-cm cell at 58°C. Dashed lines are theoretical values: α - assuming only poly A and doublestrand poly (AU) present at room temperature; β- for a similar mixture at 58°C; and γ-when only poly A and triple-stranded poly (A2U) are present presumably.
(c) Poly A + poly I. Open circles refer to the OD at 254 nm, the filled circles to the OD at 290 nm. All measurements in a 1-cm cell.

isosbestic points (Table I); thus no 1:1 complex must exist under our experimental conditions. The difference spectrum between poly A (100A) and the mixture poly (40A + 60I) is peaked at 290 nm. The other curve in Fig. 1b, which shows the difference in OD between poly I and the mixture poly (30A + 70I) is more complicated because of complexity in the poly I absorption spectrum: we used the OD's at 254 and 290 nm to construct the mixing curves given in Fig. 2c inasmuch as this curve has a rather flat maximum at 288 nm. Since no 1:1 complex is present both curves intersect at n = 34 where there is about 28% hypochromicity and 280% hyperchromicity.

These experiments show that hyperchromicity at the appropriate wavelength of 288 nm is a valuable tool for detecting double or triple stranded structures in mixtures of poly (nA+mU). Further, any explanation of hyperchromism resulting from the formation of multistranded structures of poly A and poly U must take into account that adding a second poly U strand to poly (AU) to form triple-stranded poly (A2U) makes no contribution to the hyperchromism in the long wavelength region of the spectra. A simple interpretation is that the hyperchromism observed depends only on the helical arrangement of adenosine residues in poly A + poly U complexes, but upon the helical arrangement of both adenosine and inosine residues in the poly A + poly I complexes. If so, we suggest the following regarding the nature of the observed hyperchromism: we concluded from emission and absorption studies (Drobnik, Kleinwächter and Augenstein, 1965) that two types of π-π^* bands exist in the 230-300 region of purine spectra and designated them arbitrarily as S (=strong, solvent insensitive) and W (= weak, solvent sensitive) bands. We also presented evidence that in both adenosine and inosine the W band lies on the low energy side of the S band and is more or less buried in it. Therefore, the hypochromicity may be correlated with decrease in the intensity of the S band, whereas the hyperchromicity may be caused by an increase in intensity of the W band. There is no W band in the uridine spectra, so that consistent with Fig. 2 only interactions among

these transitions in the purine constituents must contribute to the hyperchromism at 287-288 nm.

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