

off set at ten seconds every hour under an atmosphere of "pre-purified" nitrogen. The monomer was collected in ampoules described elsewhere and sealed off under reduced pressure.

In contrast to our experience, the vinyl acetate obtained by Matheson and co-workers<sup>16</sup> from Eastman Kodak Company contained an inhibitor which could be removed by distillation from a prepolymerized mixture. The identity of these retarding and inhibiting impurities is open to speculation.

The rates of polymerization after the two methods of purification of vinyl acetate at 25° with 0.105 molar benzoyl peroxide as initiator are compared below

Method	Rate of polymeriz. $\times 10^6$ (mole l. <sup>-1</sup> sec. <sup>-1</sup> )
I	4.21
II	7.94
Matheson's Table II	
Second cut pre-irradiated	8.1
Best sample	8.5
Swain and Bartlett <sup>37</sup>	8.4

**Duroquinone.**—The duroquinone, a gift from Professor P. D. Bartlett, had been prepared by Dr. Harold Kwart according to the method of "Organic Synthesis"<sup>38</sup> and was used without further purification.

**Precipitation of Polymer.**—The concentration of polymer in solvent was adjusted with acetone so that when added dropwise to well stirred, filtered *n*-hexane with a 60–70° boiling range, a fibrous precipitate was formed. The polymers from the very dilute ampoule determinations were first concentrated by blowing air over the solution. The polymer thus obtained was first allowed to stand for several hours at room temperature in a vacuum desiccator charged with anhydrous phosphorus pentoxide, then dried to constant weight in a 60° oven. The ampoule determinations were further dried in a vacuum oven at 60°.

**Molecular Weight Determination.**—Solution viscosities in acetone at 25° were determined in an Ostwald-Fenske viscometer and kinetic energy corrections were applied. The inherent viscosities,  $[\eta] = c^{-1} \ln(\eta/\eta_0)$ , were converted to intrinsic viscosities by the equation (*c* in grams per deciliter)

$$[\eta] = \{\eta\} + 0.14 \{\eta\}^2 c$$

and then to number-average degrees of polymerization by the relationship

(37) C. G. Swain and P. D. Bartlett, *THIS JOURNAL*, **68**, 2381 (1946).

(38) L. I. Smith, "Organic Synthesis," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 254.

$$\log \bar{P}_n = 3.24 + 1.40 \log [\eta] \quad (23)$$

The latter equation was obtained in this Laboratory by Howard<sup>39</sup> as follows. From light scattering measurements on a series of low-conversion polyvinyl acetates, both fractionated and unfractionated, and with proper allowance for the minor effects of polydispersity, he obtained the equation

$$\log \bar{M}_v = 5.44 + 1.40 \log [\eta] \quad (24)$$

for polymers with *M* less than  $4 \times 10^6$ . Since low-conversion polyvinyl acetate has a molecular weight essentially controlled by transfer reactions, the "most probable" molecular weight distribution will be obtained in unfractionated samples, and with the viscosity exponent  $a = 1/1.40 = 0.71$ , this yields<sup>40</sup>

$$\bar{M}_v/\bar{M}_n = 1.85$$

which with (24) leads to (23). It should be remarked that equation 24 gives results somewhat different from the relation of Wagner,<sup>41</sup> which was based on osmotic molecular weights of fractionated high-conversion commercial polymer.

**Radioactivity Determination.**—The ampoule of polymer containing radiobenzene was opened, the polymer was precipitated and dried as described above. A portion of the polymer was set aside and labeled A. The residue was dissolved in 40 ml. of acetone, evaporated down to less than one ml., reprecipitated, dried and a portion set aside and labeled B. This procedure was repeated twice more, then the portion D was dissolved in 40 ml. of reagent benzene and evaporated to near dryness five times, then precipitated into *n*-hexane and dried to constant weight in a vacuum oven. The activities of CO<sub>2</sub> obtained from each of the samples A to D were determined in duplicate by Mrs. Clare M. Regan. The analyses of sample B were vitiated by a leak in the gas system, but those of samples A, B, D gave respectively 13.3, 13.2 and  $15.1 \times 10^{-3}$   $\mu$ curie/millimole. The average of these figures corresponds to the figure of 0.030 for (S/M)<sub>p</sub> quoted in the text.

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(39) R. O. Howard, Ph.D. thesis, M.I.T., 1952.

(40) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 313.

(41) R. H. Wagner, *J. Polymer Sci.*, **2**, 21 (1947).

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## Hypochromism in Polynucleotides<sup>1</sup>

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The change in light absorption which occurs when a polymer changes from an ordered (native) to a disordered (denatured) structure is attributed to the interaction between the dipoles induced in the chromophores by the light. Calculated values for the relative absorption coefficients of native deoxyribonucleic acid (DNA) and its mononucleotides are consistent with the experimental fact that the light absorption of native DNA is about 60% of the absorption of its mononucleotides.

### Introduction

The ultraviolet light absorption of a polynucleotide is considerably less than the sum of the absorptions of its constituent nucleotides. Furthermore, the absorption of the polymer is very sensitive to the environment. High temperature, high concentration of urea, temporary extremes of pH and low ionic strength all cause an increase in the molar ab-

sorption coefficient. However, the shape of the absorption curve and the wave length maximum are not affected. Hydrolysis of the polymer causes similar changes. These results have been presented in detail previously.<sup>2–5</sup> Recent experiments have used this change in light absorption to study the

(1) This work was supported in part by research grant A-2220 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service and by an unrestricted grant from Research Corporation.

(2) G. H. Beaven, E. R. Holiday and E. A. Johnson, "The Nucleic Acids," Vol. I, E. Chargaff and J. N. Davidson, Ed., Academic Press, Inc., New York, N. Y., 1955, p. 493.

(3) P. D. Lawley, *Biochim. Biophys. Acta*, **21**, 481 (1956).

(4) R. Thomas, *ibid.*, **14**, 1231 (1954).

(5) K. K. Reddi, *ibid.*, **27**, 1 (1958).

secondary structure of polynucleotides.<sup>6-10</sup> The stability of the native deoxyribonucleic acid (DNA) helix<sup>11</sup> and other naturally occurring and synthetic polynucleotide structures can be conveniently determined by measurements of optical density. To gain the most complete information from these measurements it is necessary to understand the reasons for the change in light absorption. Although earlier explanations have been qualitative,<sup>1-10</sup> a quantitative approach to the absorption of a polynucleotide can be made by considering the interaction between the dipoles induced in the individual nucleotides by the incident light. Analogous methods have been used to interpret the spectra of molecular crystals,<sup>12</sup> the shift in the absorption maximum in dye polymers<sup>13</sup> and the optical activity of molecules.<sup>14,15</sup>

### Theory

We shall discuss the theory qualitatively in this section and present the final equations. The exact derivation will be outlined in the Appendix.

The interaction of light with matter can be related formally to the dipole induced in the material by the oscillating electric field of the light. The absorption of light is directly proportional to the square of the electric dipole transition moment. The transition moment  $\mu_{oa}$  is the dipole moment associated with the transition from ground state  $o$  to state  $a$  in the molecule. Its magnitude is easily determined from the area under an absorption curve, but its direction must be obtained from a study of oriented systems with polarized light. The transition moment is characteristic of the electronic structure of the molecule or group, therefore any change in bonding will in general change the spectrum. We would expect then that a polymer would have a different spectrum from the monomer. The changes would be associated with the quantum mechanical interactions of electron exchange, overlap, etc., but coulombic forces would also contribute. The quantum mechanical overlap effects are very short range and primarily influence the groups directly bonded; therefore in certain polymers such as polynucleotides, the main interactions affecting the spectrum may be coulombic. This hypothesis is strengthened by the observation of spectral changes with no concurrent changes in primary chemical bonding.

We shall ascribe the hypochromism in polynucleotides solely to the coulombic interaction between electrons in different bases. The leading term in the interaction is dipole-dipole and is the

only one we will consider. On this basis we can immediately make some qualitative conclusions about the change in absorption on forming a polymer.

If the groups, *i.e.*, their transition moments, are randomly oriented with respect to each other there is no net effect on the spectrum. However, if the aggregate contains colinear transition moments there will be an increase in absorption (hyperchromism), while parallel stacking of the moments will cause a decrease in absorption (hypochromism). This is illustrated in Fig. 1. The strength of the effect will depend on the cube of the distance between groups.

The equation (derived in the Appendix) relating the absorption of a polymer to the monomer, with only dipole-dipole interaction between chromophores, is

$$F_{oa}/f_{oa} = 1 - \frac{K\lambda_{oa}^2 f_{oa}}{N} \sum_{i=1}^N \sum_{j \neq i}^N G_{ij} \mathbf{e}_i \cdot \mathbf{e}_j - \frac{4K\lambda_{oa}^2}{N} \sum_{a' \neq a} \sum_{i=1}^N \sum_{j \neq i}^N \frac{G_{ij} \mathbf{e}_i \cdot \mathbf{e}_j f_{oa'} \lambda_{oa'}^2}{\lambda_{oa}^2 - \lambda_{oa'}^2} \quad (1)$$

$F_{oa}$  = oscillator strength per chromophore for the polymer in absorption band  $oa$

$f_{oa}$  = oscillator strength for the monomeric chromophore in band  $oa$

$K = 3e^2/8\pi^2 mc^2 = 1.07 \times 10^{-6} \text{ \AA}$ . The charge and mass of an electron are respectively,  $e$  and  $m$ . The speed of light in vacuum is  $c$

$\lambda_{oa}$  = wave length ( $\text{\AA}$ ) of maximum absorption for the monomer in band  $oa$

$N$  = the number of chromophores in the polymer

$G_{ij} = \left[ \mathbf{e}_i \cdot \mathbf{e}_j - \frac{3(\mathbf{e}_i \cdot \mathbf{r}_{ij})(\mathbf{e}_j \cdot \mathbf{r}_{ij})}{r_{ij}^2} \right] \frac{1}{r_{ij}^3}$

$\mathbf{e}_i, \mathbf{e}_j$  = unit vectors in the direction of the respective transition moments in chromophores  $i$  and  $j$

$r_{ij}$  = distance between centers of group  $i$  and  $j$

The oscillator strengths  $f_{oa}$  or  $F_{oa}$  are obtained from the experimental absorption curves from the equation<sup>16</sup>

$$f_{oa} = \frac{2303 mc^2}{\pi e^2 N_0} \int \epsilon_{oa} d\omega = 4.32 \times 10^{-9} \int \epsilon d\omega \quad (2)$$

$N_0$  = Avogadro's number

$\epsilon_{oa}$  = molar (mole of chromophore) extinction coefficient in l./mole cm.

$\omega$  =  $\lambda^{-1}$  in  $\text{cm}^{-1}$

The  $f$ 's are unitless and of order of magnitude one. If the absorption curves for monomer and polymer are similar in shape, for example, if their half-widths are equal, then the ratio  $F_{oa}/f_{oa}$  in eq. 1 can be replaced by the ratio of maximum absorption coefficients.

The second term in eq. 1 represents the interaction between the same transition in different residues. The last term represents the interactions of the transition moment under consideration  $oa$  with all other transitions  $oa'$  in the system. Only groups oriented with respect to the  $oa$  moment and fairly close to it will contribute, however, as the geometric term  $G_{ij}$  is zero for random relative orientations or large distances. The hypo- or hyperchromism is seen to depend on the intensity of the absorption band considered (second term) and also to depend on the intensity of other neighboring absorption bands (third term).

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(7) R. C. Warner, *J. Biol. Chem.*, **229**, 711 (1957).

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(9) P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, *ibid.*, **45**, 482 (1959).

(10) A. Pour-El, Ph.D. Thesis, Univ. of California, Berkeley, 1959.

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(14) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

(15) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci. U. S. A.*, **43**, 723 (1957).

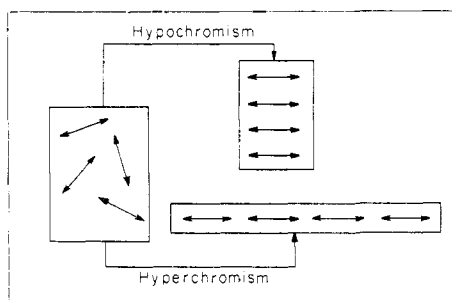


Fig. 1.—The arrows represent the transition moments in the chromophores. A change from a random array to an ordered array will cause either an increase (hyperchromism) or a decrease (hypochromism) in light absorption.

**Deoxyribonucleic Acid (DNA).**—We shall apply eq. 1 to the naturally occurring polynucleotide DNA and show that its use is consistent with the limited experimental information available. Many simplifying assumptions are made; however, as more knowledge about the spectrum of the bases becomes known a more detailed analysis will be possible.

The Watson-Crick model<sup>17</sup> of DNA consists of two right handed helices of the same pitch and radius with a common center. There are 10 residues per turn with a pitch of 33.6 Å. The base nitrogen atoms attached to the backbone chain are on a radius of 4.6 Å. The planar bases are perpendicular to the helix axis. Each purine on one helix is hydrogen bonded to a coplanar pyrimidine on the other helix. The structures of the bases and the hydrogen bonding (as slightly modified by Pauling and Corey<sup>18</sup>) are shown in Fig. 2.

The oscillator strengths for the 2600 Å. band of the four chromophores in DNA were obtained by use of eq. 2 from the large scale drawings of spectra given in "The Nucleic Acids."<sup>2</sup> The values obtained for adenosine, guanosine, cytidine, and thymidine are  $f_A = 0.30$ ,  $f_G = 0.40$ ,  $f_C = 0.17$  and  $f_T = 0.21$ . Changes in spectra on forming the phosphate esters of these compounds or on substituting deoxyribose for ribose are negligible. For our calculation we assume that one DNA chain consists of a random sequence of purines and pyrimidines and we use a mean oscillator strength for each base. Calf thymus DNA which has been most intensively studied has the base ratio of  $(A + T)/(G + C) = 1.33$ <sup>19</sup> and  $A = T$ ,  $G = C$ . With this weighting factor  $f = 0.27$ .

To calculate the geometrical factors in eq. 1 we use a cylindrical coordinate system with the position of a point specified by a vector  $r$ .

$$r = a \cos \gamma i + a \sin \gamma j + zk$$

$i, j, k$  = right handed set of orthogonal unit vectors with  $k$  along the helix axis.  
 $a$  = radius of helix  
 $\gamma$  = angle of polar coordinates in a plane  
 $z$  = distance along helix axis

We choose the centers of the bases on a radius of

(17) (a) J. D. Watson and F. H. C. Crick, *Proc. Roy. Soc. (London)*, **223A**, 80 (1954); (b) R. Langridge, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins and L. D. Hamilton, *J. Biophys. Biochem. Cytology*, **3**, 767 (1957).

(18) L. Pauling and R. B. Corey, *Arch. Biochem. Biophys.*, **65**, 164 (1956).

(19) E. Chargaff, ref. 2, p. 354.

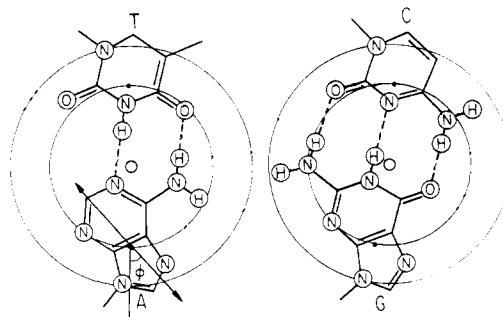


Fig. 2.—The structure and orientation of bases in a Watson-Crick helix. The bases are adenine (A), thymine (T), guanine (G) and cytosine (C). The transition moments in the quartz ultraviolet are in the plane of the ring of each base, but their orientations, characterized by the angle  $\phi$ , are not yet known.

3 Å. Adjacent bases in the same chain are separated by  $\gamma_{ij} = \gamma_j - \gamma_i = 36^\circ$ . The angle between hydrogen bonded ring centers on different helices is  $175^\circ$ . The value of  $z_{ij}$  for adjacent bases on the same helix is always 3.36 Å.

The direction of the transition moment  $e$  in each base is specified by angles relative to a coordinate system ( $e_r, e_t, e_a$ ) fixed to the center of each base.

$$e = \sin \theta \cos \phi e_r + \sin \theta \sin \phi e_t + \cos \theta e_a$$

(axial)  $e_a = k$   
(radial)  $e_r = \cos \gamma i + \sin \gamma j$   
(tangential)  $e_t = -\sin \gamma i + \cos \gamma j$

In these planar bases the transition moments in the quartz ultraviolet are in the plane of the ring, therefore  $\theta = 90^\circ$ .

$$e = \cos \phi e_r + \sin \phi e_t$$

As we do not know the directions of these transition moments, we assume that they are the same in the purines and pyrimidines and obtain our results in terms of  $\phi$ .

We also need, in principle, information about the neighboring transitions in the bases, sugars and phosphates. However the 2000 Å. absorption of the bases is probably the only significant contribution to the third term of eq. 1. For this absorption we take the oscillator strengths equal to the 2600 Å. absorption (this is probably correct within a factor of two), and we assume that the transition direction is perpendicular to the previous one. This latter assumption is usually valid for adjacent bands in  $\pi$  electron systems.

We now have all the information needed to use eq. 1. Ignoring end effects and summing over 9 bases on either side of an average base  $i$ , we obtain the curve in Fig. 3. The horizontal lines in the figure are experimental results for (a) DNA at  $99^\circ$ , (b) DNA heated for 15 minutes at  $99^\circ$  then cooled to room temperature, (c) native DNA at room temperature. The experiments were done with calf thymus DNA in a pH 7, 0.2 ionic strength phosphate buffer. As the ratio of oscillator strengths is approximately equal to the ratio of extinction coefficients, these results are very similar to previous data obtained by many investigators.<sup>1-10</sup> It is seen that the calculated hypochromism is consistent with the results for the native DNA.

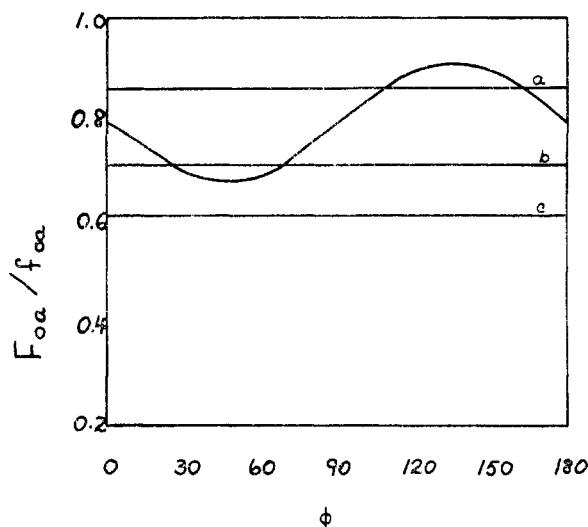


Fig. 3.—The calculated relative light absorption at 2600 Å. for native DNA and its mononucleotides is shown as a function of  $\phi$  (defined in Fig. 2). The horizontal lines are for (a) DNA at 99°, (b) DNA heated for 15 minutes at 99° then cooled to room temperature, (c) native DNA at room temperature.

### Discussion

The use of induced dipole interactions to treat optical properties is well established.<sup>12-15</sup> We therefore have no reservations about the general relation between structure and absorption discussed in the preceding section. Whatever other phenomena affect the absorption of a polynucleotide,<sup>20</sup> the electrostatic forces considered here will be a factor. The exact quantitative effect of the dipolar interaction, however, must await further information about the absorption spectra of the mononucleotides. Lacking this information we will postpone further calculations on the hypochromism of denatured DNA and of other polynucleotides. Instead we will conclude with some generalizations which only depend on the nature of dipole-dipole interactions.

It is seen from eq. 1 that the hypochromism in a given polynucleotide is mainly a measure of the relative orientation of the bases. If one base has little preferred orientation relative to another in the polymer, then the absorption is nearly equal to that of the monomer; however, if the bases are stacked parallel, there is a significant decrease in absorption. The increase in optical density on denaturation (high temperature, pH extremes, etc.) is caused by disordering of the bases. However, this does not mean that the absorption of DNA is directly a measure of the fraction of bases coiled into a Watson-Crick helix. This helix, although sufficient to cause hypochromism, is not a necessary condition for hypochromism. The hypochromism of single chain polynucleotides whether of high molecular weight<sup>8,9</sup> or of low molecular weight<sup>22</sup> can be understood without need for postulating a helix.

If we wish to consider a partially denatured poly-

nucleotide as containing a certain fraction of ordered residues and the remainder disordered, we may ask if optical density is a linear function of the fraction ordered and therefore a quantitative measure of denaturation. The answer for practical purposes is yes, if the ordered regions are at least 7 or 8 residues long. That is, as the potential for dipolar forces varies as  $r^{-3}$ , a base with a few ordered neighbors considers itself to be in an infinite ordered chain.

Hypo- or hyperchromism in other ordered arrays such as the polypeptide  $\alpha$ -helix are also predicted by this theory. Quantitative calculations, however, have not yet been made.

### Appendix

To calculate the light absorption of the polymer we must relate the electronic wave function of the chromophore in the polymer to that of the monomer. The desired wave functions have been obtained previously by Moffitt, Fitts and Kirkwood<sup>15</sup> in their treatment of the optical activity of a polymer. We shall follow their derivation and apply the results to the absorption of the polymer.

**Intensity of Absorption.**—As the notation tends to be confusing we shall give a summary of it first. The electronic wave functions of the residue are  $\phi$  while those of the polymer are  $\psi$ . Superscripts refer to the order of the approximation, *i.e.*, 0, 1, 2. Subscripts *o* or *a* or *a'* refer to the ground or excited states; subscripts *i, j, k* label the residue. The monomer wave functions are real and orthonormal.

For a polymer with *N* identical units the zero order ground state is

$$\psi_0^0 = \prod_{i=1}^N \phi_{oi} \quad (A1)$$

The excited state with one residue excited to the *a*-th level is

$$\psi_{aj} = \frac{\phi_{aj}}{\phi_{oj}} \prod_{i=1}^N \phi_{oi} \quad j = 1, 2, \dots, N \quad (A2)$$

For two residues excited

$$\psi_{ajk} = \frac{\phi_{aj}\phi_{ak}}{\phi_{oj}\phi_{ok}} \prod_{i=1}^N \phi_{oi} \quad j, k = 1, \dots, N \quad (A3)$$

The  $N \psi_{aj}$  are orthonormal, real wave functions for an energy level which is *N* fold degenerate in zero order. In first order the energy level is split into *N* energy levels with correct zero order orthonormal wave functions  $\psi_{aK}^0$  which are linear combinations of the  $\psi_{aj}$

$$\psi_{aK}^0 = \sum_{i=1}^N C_{aKi} \psi_{ai} \quad (K = 1, \dots, N) \quad (A4)$$

The zero order ground state is perturbed by all excited states (in which two residues are excited at the same time) to give the first order ground state.

$$\psi_0^1 = \psi_0^0 - \sum_{a'} \left[ \sum_{i=1}^N \sum_{j \neq i} \frac{(V_{ij})_{aia'j;0} \psi_{aia'j}}{h(\nu_{0ai} + \nu_{0a'j})} \right] \quad (A5)$$

The sum over *j* includes all other molecules and groups in the solution including solvent.

$\nu_{0ai}$  = positive frequency for a transition to an excited state in group *i*

$(V_{ij})_{aia'j;0} = \int \phi_{ai}^* \phi_{aj}^* V_{ij} \phi_{oi} \phi_{oj} d\tau$   
 $V_{ij}$  = potential energy between the instantaneous charge distributions of the *i*-th and *j*-th residue

We approximate  $V_{ij}$  by

$$V_{ij} = \mathbf{u}_i \cdot \mathbf{T}_{ij} \cdot \mathbf{u}_j$$

$$T_{ij} = \frac{1}{r_{ij}^3} \left[ 1 - \frac{3\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2} \right]$$

$$\mathbf{u}_i = e \sum_s \mathbf{r}_s^{-1}$$

The sum over *a'* includes all excited states in groups *j*.

The correct zero order wave functions for the excited state *a* of the polymer are perturbed by interactions with other singly excited states.

(20) Other possible phenomena are discussed in ref. 1-10 and are critically reviewed by Haselkorn.<sup>21</sup>

(21) R. Haselkorn, Ph.D. Thesis, Harvard University, 1959.

(22) A. M. Michelson, *Nature*, **182**, 1502 (1958).

$$\psi_{aK}^1 = \sum_{i=1}^N C_{aKi} \left\{ \psi_{a1} - \sum_{a' \neq a} \left[ \sum_{j=1}^N \frac{(V_{ij})_{a'j; ai} \psi_{a'j}^1}{\hbar(\nu_{oa'} - \nu_{oa})} \right] \right\} \quad (A6)$$

The subscript  $i$  has been omitted from  $\nu_{oa}$  as the residues are identical.

$$(V_{ij})_{a'j; ai} = \int \phi_{a'j}^* \phi_{oi}^* V_{ij} \phi_{ai} \phi_{oj} d\tau$$

The sum over  $j$  and  $a'$  includes all groups not  $i$  and all states not  $a$  in the solution.

To obtain the polymer absorption corresponding to the  $o \rightarrow a$  transition in the monomer we need the total electric dipole transition moment  $\mathbf{u}_{T_{oa}}$  for the polymer. The oscillator strength of the transition which is a measure of this absorption (see eq. 2) is

$$\begin{aligned} NF_{oa} &= \frac{8\pi^2 m \nu_{oa}}{3\hbar e^2} \mathbf{u}_{T_{oa}} \cdot \mathbf{u}_{T_{oa}} \\ f_{oa} &= \frac{8\pi^2 m \nu_{oa}}{3\hbar e^2} \mu_{oa}^2 \\ \mathbf{u}_{T_{oa}} &= \sum_{K=1}^N \int (\psi_o^1)^* \sum_{i=1}^N \mathbf{u}_i \psi_{aK}^1 d\tau \\ \mathbf{u}_{T_{oa}} &= \sum_{K=1}^N \sum_{i=1}^N C_{aKi} \left[ \mathbf{u}_{ioa} - \sum_{j \neq i}^N \frac{(V_{ij})_{ai; aj} \mathbf{u}_{j oa}}{2\hbar \nu_{oa}} - \right. \\ &\quad \left. 2 \sum_{a' \neq a} \sum_{j \neq i}^N \frac{(V_{ij})_{ai; a'j} \mathbf{u}_{j oa} \nu_{oa'}}{\hbar(\nu_{oa'}^2 - \nu_{oa}^2)} \right] \quad (A7) \\ \mathbf{u}_{ioa} &= \int \phi_{io}^* \mathbf{u} \phi_{ia} d\tau \end{aligned}$$

This is equation 10 of Moffitt, *et al.*<sup>15</sup> Omitting terms in  $\hbar^{-2}$  we have

$$\begin{aligned} \mathbf{u}_{T_{oa}} \cdot \mathbf{u}_{T_{oa}} &= \sum_{K=1}^N \sum_{i=1}^N \sum_{l=1}^N C_{aKi} C_{aKl}^* \mathbf{u}_{ioa} \cdot \mathbf{u}_{l oa} - \\ &\quad \sum_{K=1}^N \sum_{i=1}^N \sum_{l=1}^N \sum_{j \neq i}^N \frac{(V_{ij})_{ai; aj} C_{aKi} C_{aKl}^* \mathbf{u}_{j oa} \cdot \mathbf{u}_{l oa}}{\hbar \nu_{oa}} - \\ &\quad 4 \sum_{K=1}^N \sum_{i=1}^N \sum_{l=1}^N \sum_{a' \neq a} \sum_{j \neq i}^N \frac{(V_{ij})_{ai; a'j} C_{aKi} C_{aKl}^* \mathbf{u}_{j oa} \cdot \mathbf{u}_{l oa} \nu_{oa'}}{\hbar(\nu_{oa'}^2 - \nu_{oa}^2)} \quad (A8) \end{aligned}$$

However

$$\sum_{K=1}^N \sum_{i=1}^N C_{aKi} C_{aKi}^* = N \quad (A9)$$

$$\sum_{K=1}^N \sum_{i=1}^N \sum_{l \neq i}^N C_{aKi} C_{aKl}^* = 0$$

Equations A9 follow from the orthonormality of the  $\psi_{aK}^0$  and the fact that the interaction of degenerate levels does not change their center of gravity. We now have

$$\begin{aligned} \mu_{T_{oa}}^2 &= N \mu_{oa}^2 - \sum_{i=1}^N \sum_{j \neq i}^N \frac{(V_{ij})_{ai; aj} \mathbf{u}_{ioa} \cdot \mathbf{u}_{j oa}}{\hbar \nu_{oa}} - \\ &\quad 4 \sum_{a' \neq a} \sum_{i=1}^N \sum_{j \neq i}^N \frac{(V_{ij})_{ai; a'j} \mathbf{u}_{ioa} \cdot \mathbf{u}_{j oa} \nu_{oa'}}{\hbar(\nu_{oa'}^2 - \nu_{oa}^2)} \quad (A10) \end{aligned}$$

If we write  $\mathbf{u}_{ioa} = \mu_{oa} \mathbf{e}_i$ ,  $\mathbf{u}_{j oa} = \mu_{oa} \mathbf{e}_j$ , etc., and  $(V_{ij})_{ai; aj} = \mathbf{u}_{ioa} \cdot \mathbf{T}_{ij} \cdot \mathbf{u}_{j oa} = \mu_{oa}^2 \mathbf{e}_i \cdot \mathbf{T}_{ij} \cdot \mathbf{e}_j = \mu_{oa}^2 G_{ij}$ , we obtain eq. 1 by using the definition of an oscillator strength.

$$\begin{aligned} \frac{F_{oa}}{f_{oa}} &= 1 - \frac{K \lambda_{oa}^2 f_{oa}}{N} \sum_{i=1}^N \sum_{j \neq i}^N G_{ij} \mathbf{e}_i \cdot \mathbf{e}_j - \\ &\quad \frac{4K \lambda_{oa}^2}{N} \sum_{a' \neq a} \sum_{i=1}^N \sum_{j \neq i}^N \frac{G_{ij} \mathbf{e}_i \cdot \mathbf{e}_j f_{oa} \lambda_{oa'}^2}{\lambda_{oa}^2 - \lambda_{oa'}^2} \quad (A11) \end{aligned}$$

For the actual calculation for DNA we ignored end effects; this has the effect of removing the  $\frac{1}{N} \sum_{i=1}^N$  from eq. A11.

The distinction between identical transitions which belong in the second term of eq. A11 and distinct transitions which

belong in the third term is an operational one. All transitions within the absorption band we are measuring are by definition identical and therefore to be included in the second term. Their wave length maxima  $\lambda_{oa}$  and their oscillator strengths  $f_{oa}$  may be different; therefore appropriate averages are used.

**Energy Levels.**—Each wave function has associated with it an energy; therefore in general we expect a change in electronic structure to be mirrored both by a change in the intensity of absorption and also by a change in the absorption maximum. In a polymer with induced dipole interaction, hypochromism may be associated with a shift in  $\lambda_{max}$  to shorter wave lengths (blue shift) while hyperchromism implies a red shift. However, Simpson and Peterson<sup>23</sup> have shown that unless the expected shift (expected on the basis of an infinitely sharp absorption line) in  $\lambda_{max}$  is substantially greater than the width of the actual absorption band then no shift occurs. That is, if the coupling between chromophores is not strong,<sup>23</sup> then the vibrational structure of the band must be considered. We will first calculate the expected shift on the assumption of strong<sup>23</sup> coupling and then discuss the reasons for experimentally finding weak or intermediate coupling.

The only significant shifts in energy levels due to dipolar interaction come from the interaction among the degenerate levels in the polymer. Although interactions between non-degenerate levels contribute to the change in intensity, they do not affect the energy levels appreciably. The energy of the perturbed degenerate level  $\Delta E_{aK}$  measured from the unperturbed monomer level is

$$\begin{aligned} \Delta E_{aK} &= \sum_{l=1}^N \sum_{j \neq i}^N \int (\psi_{aK}^0)^* V_{ij} \psi_{aK}^0 d\tau \\ \Delta E_{aK} &= \sum_{l=1}^N \sum_{j \neq l}^N C_{aKi}^* C_{aKj} \int \psi_a^0 V_{oj} \psi_{aj} d\tau \quad (A12) \\ \Delta E_{aKl} &= \mu_{oa}^2 \sum_{l=1}^N \sum_{j \neq l}^N C_{aKl}^* C_{aKj} G_{lj} \end{aligned}$$

Moffitt<sup>24</sup> has found that for a very long helix  $C_{aKl}$  can be written as<sup>25</sup>

$$C_{aKl} = \frac{1}{\sqrt{N}} e^{2\pi i l K / N}$$

Therefore the  $N$  polymer energy levels corresponding to the monomer level  $a$  are

$$\Delta E_{aK} = \frac{2\mu_{oa}^2}{N} \sum_{l=1}^N \sum_{j < l}^N \cos[2\pi(j-l)K/N] G_{jl} \quad (A13)$$

For a long helix the transition dipole moment is not zero only for transitions to the three levels  $K = N/P$ ,  $N(1 - 1/P)$ , and  $N$ , where  $P$  is the number of residues per turn. The first two transitions are degenerate and polarized perpendicular to the helix axis. The third level ( $K=N$ ) is polarized parallel to the helix axis and is zero for native DNA because all the residue transitions are perpendicular to this axis. Therefore, for native DNA the one important energy level is

$$\begin{aligned} \Delta E_a &= \frac{2\mu_{oa}^2}{N} \sum_{l=1}^N \sum_{j < l}^N \cos \gamma_{jl} G_{jl} \\ \gamma_{jl} &= (2\pi/P)(j-l) \quad (A14) \end{aligned}$$

Except for a constant and a change in sign, eq. A14 is identical to the second term in eq. 1 or A11; it can be written (with  $\lambda$  and  $G$  in Å.) as

$$\Delta E_a (\text{cm.}^{-1}) = 112 \lambda_{oa} (f_{oa}/N) \sum_{i=1}^N \sum_{j \neq i}^N G_{ij} \mathbf{e}_i \cdot \mathbf{e}_j \quad (A15)$$

Using the same approximations as in the intensity calculation we obtain

$$\Delta E_a = (2842 - 1048 \cos^2 \phi) \text{ cm.}^{-1} \quad (A16)$$

The crucial assumption for this result is that the degenerate levels that are interacting are the electronic ones. This is

(23) W. T. Simpson and D. L. Peterson, *J. Chem. Phys.*, **26**, 588 (1957).

(24) W. Moffitt, *ibid.*, **25**, 467 (1956).

(25) Although this approximation is not valid for treatment of optical activity,<sup>14</sup> it is useful for discussion of absorption.

only true if the interaction is strong. For weak or intermediate coupling we must consider the shift of each vibrational level and substitute in eq. A15 the much smaller oscillator strength for a single vibronic transition. This causes the calculated shift in energy to be negligible. The criterion<sup>23</sup> for weak or intermediate coupling is that the calculated energy shift, eq. A16, be less than the band width of the absorption. For DNA polymer or monomers the width of the band at half maximum is about 6500 cm.<sup>-1</sup>. This is larger than the values obtained from eq. A16, and therefore it is consistent with the absence of a significant shift in the absorption maximum.

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## Effect of Light Scattering on Ultraviolet Difference Spectra<sup>1</sup>

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Ultraviolet difference spectra obtained with protein solutions may contain a contribution not only from a shift in the spectrum of the tyrosyl group but also from Rayleigh light scattering when there are large differences in the state of molecular aggregation. The scattering contribution may be subtracted out by extending spectral measurements into the visible region. When this procedure is used for insulin solutions, anomalies near the *pH* region of insolubility disappear and the difference spectra show a *pH* dependence which is consistent with the existence of a tyrosyl-carboxylate ion hydrogen bond in which the carboxylate acceptor group has an observed *pK* of 3.5 to 3.6.

### Introduction

Previous work on the *pH*-dependence of the ultraviolet difference spectrum of insulin<sup>2</sup> indicated an apparently anomalous increase in the optical density in the *pH* regions 3.5 to 4 and 7.0 to 8.0 (see Fig. 6 of ref. 2). Furthermore, in this *pH* range optical density differences were finite though small even at say 310 m $\mu$ , where insulin solutions are not expected to show light absorption. It seemed possible that large molecular aggregates, present in solution near the region of insolubility (*pH* 4 to 7), gave rise to an increase in the optical density due to light scattering. The present work was therefore carried out to assess the influence of light scattering on the *pH*-dependence of the ultraviolet difference spectrum in a solution where the state of aggregation varies markedly with *pH*.

### Experimental

All materials (including Eli Lilly crystalline beef zinc insulin, batch No. 535, 664), solutions, method of measurement, etc., were similar to those previously described.<sup>2</sup> The only difference was that a Beckman model DK-2 ratio-recording spectrophotometer was used instead of the model DU. The concentration of the insulin solutions was 0.5% and matched silica cells of 0.5 cm. light path were used. Owing to the high optical densities of these solutions it was necessary to use the photomultiplier, and slit-widths did not exceed 0.71. For comparison with previous results,<sup>2</sup> the data were in some cases converted to a standard basis for a 1 cm. cell. Insulin solutions at *pH* 1.5 were used as the reference and the optical densities,  $\Delta D$ , of matched solutions<sup>2</sup> at higher *pH*'s were determined over the wave length range of 240–600 m $\mu$  with a hydrogen lamp source for the whole range.

(1) This investigation was supported, in part, by Research Grant G-6461 from the National Science Foundation.

(2) M. Laskowski, Jr., S. J. Leach and H. A. Scheraga, THIS JOURNAL, **82**, 571 (1960).

### Theory

The value of  $\Delta D$ , due to different absorbances at each *pH*, may be augmented by a contribution from light scattering if the state of aggregation is different from that at the reference *pH*. The light scattering effect may be represented in terms of the turbidity  $\tau$  by the approximate equation at finite concentration

$$\frac{I_t}{I_0} = e^{-\tau x} \quad (1)$$

where  $I_t/I_0$  is the fraction of the light transmitted and  $x$  is the length of the scattering medium, taken here as 1 cm. The quantity  $\tau$  may be related to the molecular weight  $M$  and concentration  $c$  (in g./cc.) of the solute by equation 2, which is an approximation at finite concentration.

$$\tau = HcM \quad (2)$$

where

$$H = \frac{32\pi^3}{3N\lambda^4} n_0^2 \left(\frac{dn}{dc}\right)^2 \quad (3)$$

and  $\lambda$  is the wave length in cm.,  $N$  is Avogadro's number,  $n_0$  is the index of refraction of the solvent and  $dn/dc$  is the refractive increment. From eqs. 1 and 2 the transmittances at two *pH*'s, say 1.5 and 3.5, may be related.

$$\frac{(I_t/I_0)_{pH 1.5}}{(I_t/I_0)_{pH 3.5}} = \frac{(e^{-HcM})_{pH 1.5}}{(e^{-HcM})_{pH 3.5}} \quad (4)$$

Hence, defining  $\Delta D_{286}$  for scattering as

$$\Delta D_{286} = \log \left(\frac{I_0}{I_t}\right)_{pH 3.5} - \log \left(\frac{I_0}{I_t}\right)_{pH 1.5} \quad (5)$$

we obtain for the scattering contribution to  $\Delta D_{286}$

$$\Delta D_{286} = Hc (M_{pH 3.5} - M_{pH 1.5})/2.303 = Hc\Delta M/2.303 \quad (6)$$