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T-637-1

OPTICAL PROPERTIES OF BIOPOLYMERS IN THE RANGE OF ELECTRON ABSORPTION BANDS

[Following is the translation of an article by Yu. N. Chirgadze and V. A. Lobachev, Institute of Biological Physics, USSR Academy of Sciences, Pushchino-na-Oke, published in the Russian-language periodical Biofizika (Biophysics), Vol XIII, No 1, 1968, pages 5-19. It was submitted on 10 Jun 1967.]

The study of the optical properties of molecules produces a considerable amount of information concerning their structure. During the interaction of light with a substance we are dealing simultaneously with a number of phenomena in which the optical properties of molecules are manifested. At the present time many of these phenomena have been studied sufficiently and are widely used in various sensitive analytical methods. Such phenomena include absorption and emission of light by molecules, refraction of light wave, deflection of plane of polarization during the transmission of light through a substance, and others /1/. A simple interpretation may be provided only in ranges of the spectrum where a substance is relatively transparent. However without a doubt more information about the properties of the molecules under study can be obtained by analyzing the ranges of strong resonance absorption, since it is namely here that the source of effects is found. Absorption in the visible and ultraviolet ranges of the spectrum is conditioned by the interaction of the light wave with electrons which are found in the molecules; implied are the so-called valence electrons which take part in the formation of chemical bonds.

The purpose of the present work is to bring the attention of investigators who are studying the structure of biopolymers to the peculiarities in the manifestation of the optical properties of these molecules in the ranges of main absorption of recurrent links in the polymer chain. In connection with this it is necessary to examine the characteristics of transitions and their manifestations in the spectra of absorption and especially in the spectra of optical rotation. This manifestation depends essentially both on the spatial distribution of transitions in the molecule and on the nature of their interaction. We did not aim to give a review on the theory and effect of the optical properties of biopolymers, therefore the illustrations cited relate mainly to well studied helical systems. Of course in hoping to point out the basic points

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In [illegible] the interaction of molecules with the [illegible] a light wave it is sufficient to consider the inter[illegible] the electrical vector of the wave. Being accelerated by the [illegible] field the valence electrons of a molecule may transfer into excited states. This takes place only at a specific frequency of light oscillations and a specific, in respect to the particular molecule, position of the plane in which the electrical vector is oscillating; then we observe strong absorption of light in a substance. The probability of the transition in a unit of time, W_{ab} of a molecule from one energy condition a into another b with absorption or emission of a quantum of light is proportional to the square of the matrix element of the dipole moment μ_{ab} (it is also called the moment of transition), to the square of the electrical field intensity of the light wave E, and also depends on the degree of population of the levels between which the transition takes place [5]. During the study of a molecule with the help of spectroscopy for us it is important to know that the degree of absorption is proportional to the value

$$W_{ab} \sim |\mu_{ab} E|^2 = |\mu_{ab}|^2 \cdot |E|^2 \cdot \cos^2 a$$

Here a - the angle between the vector of moment of transition μ_{ab} and vector E.

In a physical sense the moment of transition corresponds to the change of the vector of electrical dipole moment during the given transition. The dependence of absorption on the direction of polarization of the electrical vector of the field makes it possible to determine the spatial distribution of individual groups of atoms in the molecule. For this it is necessary to know the direction of the moment of transition in the structure and the linear dichroism of the corresponding band of absorption. The presence in the spectrum of a band of absorption reflects the fact that the moment of the given transition does not equal zero (under the condition that the vector of the field E is not perpendicular to the moment of transition); such a transition is called allowed or active in the spectrum of absorption. In the general case of an assymetrical molecule all transitions are active.

The speed of light in a medium is different from the speed of light in a vacuum, reaching extreme values close to resonanse absorption, which is reflected in the dispersion of the refractive index (Fig. 1).

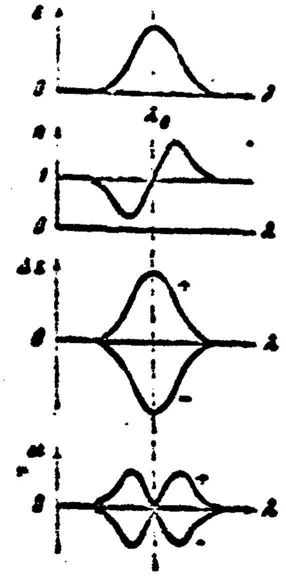

Fig. 1. Changes in the coefficient of absorption ε, refractive index n, coefficient of circular dichroic absorption $\Delta\varepsilon$, and optical rotation α in the area of an isolated band of absorption (ideal case). "+" - positive Cotton effect, "-" - negative Cotton effect.

Just as the absorption coefficient, the refractive index in the case of an anisotropic medium takes various values along various directions (double refractions), and the corresponding difference $\Delta n = n_1 - n_2$ may serve as a measure of the asymmetry of molecules and their orientation relative to the distinguished directions. Between the phenomena of absorption and dispersion of the refractive index there is a unique bond making it possible, based on the coefficient of absorption, to find the dependence of the refractive index on the frequency and conversely. This bond is determined by the expressions of Kronig-Cramer [2,3]. In ordinary practice it is more convenient to measure the spectra of absorption, although sometimes it is also necessary to know the refractive index.

Much more minute interactions are manifested in the optical activity of separate transitions in a molecule [4-7]. Optical activity is expressed in circular dichroism (circular double refraction), i. e., in different absorption of light waves, polarized clockwise (R) and counterclockwise (L), on the one hand, or in the rotation of the plane of polarization of plane polarized light on the other (Fig. 1).

All the phenomena analyzed by us are presented in Table 1, where on each line are recorded the corresponding optical properties of molecules.

Table 1

Relationship between optical properties of molecules

① Характер эффекта	② Обозначение		③ Характер эффекта
(a) Поглощение света, коэффициент поглощения	[illegible]	[illegible]	Преломление света, коэффициент преломления (e)
(b) Линейный дихроизм, дихроичное отношение	$\varepsilon_\parallel/\varepsilon_\perp$	$\Delta n = n_\parallel - n_\perp$	Двулучепреломление, разность главных показателей преломления (f)
(c) Круговой дихроизм			Вращение плоскости поляризации (g)
(d) [illegible] или эллиптичность	$\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ $\theta \sim \Delta\varepsilon$	[illegible]	угол вращения (h)

1) Nature of effect; a) Absorption of light, coefficient of absorption; b) Linear dichroism, dichroic ratio; c) Circular dichroism; d) Circular dichroic absorption of ellipticity; 2) Designations; 3) Nature of effect; e) Refraction of light, coefficient of refraction; f) Double refraction, difference in main indices of refraction; g) Rotation of plane of polarization; h) angle of rotation.

A measure of circular dichroism is the circular dichroic absorption $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ or the ellipticity θ, expressing the capability of the medium for transformation of linear polarized light into elliptically polarized with the corresponding ratio of the large and small axes of ellipse [5]. The degree of optical rotation is the angle of turn of the plane of polarization φ. In any optically active medium both effects are manifested simultaneously. Therefore, speaking accurately the question is of the rotation of the large axis of the ellipse and not of rotation of the plane of polarization. Particularly large values of ellipticity may be manifested in the ends of the absorption bands of the substance.

Under specific conditions it may even turn out that plane-polarized light converts into light which is polarized circularly. The reader will find the general expression for ellipticity in work [4]. Frequently the ellipticity encountered in practice (even in the ends of the absorption band of a specimen) is minor and in this case ellipticity θ is expressed simply through the coefficient of absorption for counterclockwise and clockwise circular-polarized light. It is convenient to use the so-called molecular ellipticity [illegible]:

$$[\theta] = 3300(\varepsilon_L - \varepsilon_R) = 3300\,\Delta\varepsilon.$$

where ε_L and ε_R are the molecular coefficients of absorption correspondingly for light which is polarized counterclockwise and clockwise.

Circular dichroism and optical rotation are connected by interreversible relations which are analogous to the relations of Kronig-Cramer for the values of ε and n. Therefore for the values of α and θ, both for all the areas of the spectrum and for the individual isolated band of absorption within which α and θ are carried, which are named correspondingly particular optical rotation and particular circular dichroism, it is possible to write [3, 4, 5]:

$$\alpha(\nu) = \frac{2\nu^2}{\pi}\int_0^\infty \frac{\theta(\nu')}{\nu'(\nu'^2 - \nu^2)}\,d\nu',$$

$$\theta(\nu) = -\frac{2\nu^3}{\pi}\int_0^\infty \frac{\alpha(\nu')}{\nu'^2(\nu'^2 - \nu^2)}\,d\nu',$$

where ν and ν' - the frequency of the light wave.

At the present time in practice equal success is being achieved in measuring circular dichroism on dichrographs or optical rotation on polarimeters. The optical activity of the absorption band, connected with transition $a \rightarrow b$, is characterized by strong rotation R_{ba}:

$$R_{ba} = Im(\mu_{ab} \cdot m_{ba}) = \text{const} \cdot \int_0^\infty \frac{\theta(\nu)}{\nu}\,d\nu.$$

Here μ_{ab} and m_{ba} are the electrical and magnetic moments of transition correspondingly. Im indicates that it is necessary to take the minimum part of the scaler product of the moments.

In the majority of cases the prediction of the force of rotation bears only a qualitative nature. The calculation of transition moments requires knowledge of the wave functions of a molecule which is excited by the field of the light wave; an accurate expression of them is obtained quite complexly. In the works [6-11] one can become acquainted with examples of the theoretical calculation of the force of rotation.

Polypeptides, proteins. In a recurrent link - the peptide group p - electrons of atoms of oxygen, carbon, and nitrogen form a collective system of π-electrons (Fig. 2). Thus the chemical bonds of CO and CN have a multiplicity close to 1.5, conditioned by electrons of the π-system apart from single valences of firmly bound σ-electrons. The absorption bands corresponding to transitions of σ-electrons onto excited levels are found in the far ultraviolet range of the spectrum. During excitation of the π-electron system the transitions have less energy and the longest wave ones correspond to bands of absorption of around 200-220 nm. Several of the amino acid radicals have aromatic systems, the transitions in which take place at long waves of around 260-280 nm. However this is not peptide absorption and we will not consider it.

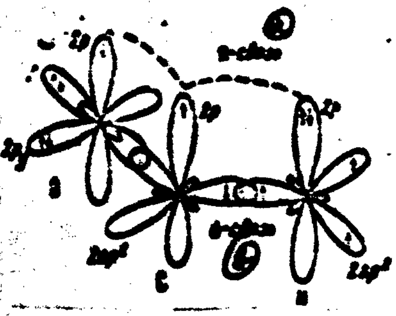

Figure 2. Electron configurations of atoms of a peptide group. Key: (a) π-bond; (b) σ-bond.

There are still very few experimental works on the determination of parameters of transitions in a peptide group. This is explained by the purely technical difficulties of spectroscopy in the short-wave range. However it is known that with π^* transitions (the asterisk indicates excitation) the following bands are connected /13-15/:

strong band around 160 nm - $\pi \rightarrow \pi^*$,
strong band around 190 nm - $\pi \rightarrow \pi^*$,
weak band around 220 nm - $n \rightarrow \pi^*$.

The first two transitions are polarized in the plane of the peptide group, since it is namely in this plane, which passes through atoms of O, C, and N that redistribution of the density of π-electrons takes place with the given excitation. The last transition of unshared electrons (n) of the $2p_y$-orbit of the atom of oxygen should be perpendicular, however an experimental study of dichroism of the corresponding band in a crystal with a known structure shows that there is a quite strong component directed in the plane of the peptide group. This is connected with induction of the $\pi\pi^*$-transition. In comparison with the $\pi\pi^*$-transitions the $n\pi^*$-transition is manifested very weakly in the spectrum of absorption. A theoretical consideration shows that a transition of this [illegible] type is forbidden as an electrical dipole, then allowed as a "magnetic dipole," that is, there is a magnetic moment of transition which is not vanishing.

In the presently accessible region of the spectrum from 180 nm and higher polypeptides and proteins should thus give two bands of absorption with a maximum of around 190 and 220 nm. The latter band is quite weak and is not always manifested. In Figure 3 are given the spectra of poly-L-lysine hydrochloride in aqueous solution under various conditions, depending on which the molecule has a various structure [19].

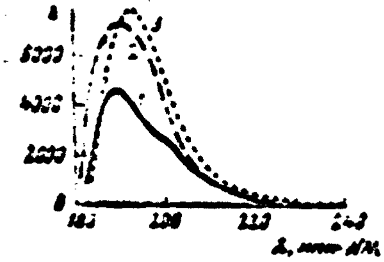

Figure 3. Ultraviolet spectra of absorption of poly-L-lysine hydrochloride in aqueous solution [19]. 1 - a-helix, pH 10.8, 25° C, 2 - irregular coil, pH 6.8, 25° C, 3 - β-form, pH 10.8, 52° C.

Each of the structures: stretched - β, coiled in a helix - α, or irregular has a corresponding spectral curve with specific characteristics. The considerable lessening of absorption in the helical structure is called the hypochromic effect [20, 21] and reflects the interaction of the given moment of transition with other moments, in our case with a transition having a length of wave around 150 nm. The stated interaction of dipoles depends both on the mutual location in space of the groups in which the transitions under consideration take place and on the force of the transition and their frequency (length of wave). A calculation of the hypochromic effect shows [20] that a structure of the a-helix type should have approximately the same magnitude of hypochromic effect that is observed in experiment. They assume that the increase in absorption for the a-helix in the range of transition with a length of wave of around 220 nm (hyperchromism) is conditioned in the helical structure by the interaction (by a borrowing of intensity) with a strong transition of around 190 nm. All these specific interactions are averaged to a considerable degree in the irregular conformation.

The detected differences may be used in principle for a study of the complex structure of molecules of globular proteins, however, here it is necessary to introduce corrections for absorption of different side groups of amino acid radicals. This was mentioned above.

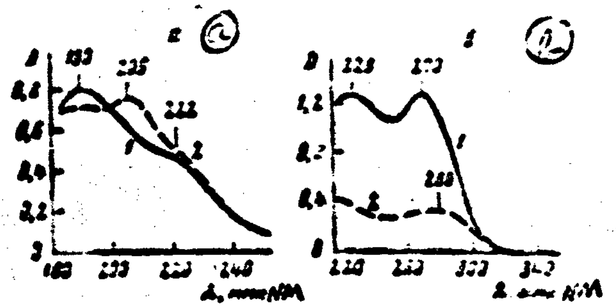

Figure 4. Absorption spectra of oriented films of poly-γ-methyl-L-glutamic acid (a) and polycytidylic acid (b); data from works /18/ and /25/ respectively.
1 - electrical vector of light wave E perpendicular to the direction of orientation, 2 - parallel. Axis of ordinates - D - optical density.

In ordered structures resonance interaction of excited conditions of identical groups may lead to splitting of the absorption band corresponding to the given transition, however the overall intensity of all the components of the band remains constant. Such a splitting should be manifested in the structure of the α-helix /11, 20/ and is easily observed in the polarization spectrum of oriented film /18/, as this is apparent from Figure 4,a. The weak transition of around 220 nm is not split. The short-wave component of the $\pi\pi^*$-transition in a molecule (the completely symmetrical oscillation of type A is polarised along the axis of the helix) corresponds to the simultaneous cophasal transitions in individual peptide groups, and the long-wave component (twice the degenerated oscillation of type E is polarized across the axis of the helix) - to transitions with a phase difference $2\pi/n$, where n - the number of monomer units in one turn of the helix /22/. The dichroic ratio, calculated for the given transition in the structure of the α-helix, is found in agreement with experiment /20/.

Nucleotides and nucleic acids. The structural frame of molecules consists of nitrogen bases of two types - purine and pyrimidine, attached by chemical bonds to a phosphosaccharic chain. The system of conjugated π-electrons in nitrogen bases is more branched than in a peptide group, however, qualitatively these systems are very similar: namely the same atoms of nitrogen, carbon, and oxygen, in principle the same electron structure of conjugate p-orbits and unshared pairs of electrons. Consequently the spectra also will have an analogous interpretation: a number of strong bands with maxima of around 190 and 260 nm correspond to $\pi\pi^*$-transitions, and weak bands of around 280 nm to $n\pi^*$-transitions with unshared orbits found in specific atoms of nitrogen or oxygen /23, 24/. An analysis [illegible] by the fact that each of the four [illegible] bases - adenine, thymine, guanine, or cytosine - [illegible] of absorption bands [illegible] 2[illegible]], as this can be [illegible] the spectra of various nucleosides in Fig. 5.

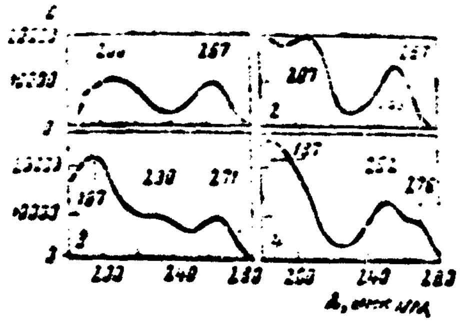

Figure 5. Spectra of absorption of deoxynucleosides [illegible]. 1 - thymidine; 2 - deoxyadenosine; 3 - deoxycytidine; 4 - deoxyguanosine; ε - molecular coefficient of absorption.

All the $\pi\pi^*$-transitions are polarized in the plane of bases, while the $n\pi^*$-transition is perpendicular to this plane. According to the Watson-Crick model a molecule of DNA in native state consists of two helical polynucleotide chains connected by transverse hydrogen bonds, and the plain of the nitrogen bases is approximately perpendicular to the long axis of the molecule (under various conditions the angle of inclination of these planes to the axis of the molecule can change). In this case the electron spectra in the areas of 190-260 nm should have perpendicular polarization, and the weak band of around 230 nm - parallel. This was in fact detected [illegible] and thus confirmed the perpendicular arrangement of the planes of nitrogen bases in the structure of DNA. The behavior of synthetic polynucleotide chains in a number of cases is analogous to the behavior of a molecule of DNA. Here the spectra of the polynucleotides are more distinct and dichroism in oriented specimens is manifested more strongly. As an example Figure 4.b shows the polarization spectrum of polycytidylic acid [illegible]. It is understood that changes of conditions, conditioning the development of other structural modifications, reflect very sharply on the dichroism of the absorption bands.

During transition of the regular structure into random the spectrum of DNA changes: the intensity of bands is increased. The hypochromic effect of the native structure for $\pi\pi^*$-transitions is conditioned by the stack-form arrangement of nitrogen bases in the double helix [25, 27, 28]; it may be used as a measure of helicity in the structures of DNA or RNA. In regards to the $n\pi^*$-transition in the double helix, then here the theory is expressed concerning the increase in absorption (hyperchromism), which was also actually detected [illegible].

Dispersion of Optical Activity in Circular Dichroism of Biopolymers

Different interactions of transition moments are reflected in the optical activity of ultraviolet absorption bands considerably more strongly than in intensities and frequencies, therefore the method of dispersion of optical activity is exceedingly sensitive even to small changes in the structure of molecules.

We will examine the optical activity of $\pi\pi^*$ type transitions with a strong dipole electrical moment. In a polymeric molecule the optical activity consists of the following main segments.

1. Inherent optical activity of monomeric chromophores - a peptide group in proteins and nitrogen bases in nucleic acids.
2. Conformation optical activity, developing due to the specific location of the mutually interacting chromophore groups.

Optical activity of chromophores may manifest itself during a study of low molecular compounds. The curve of dispersion of optical activity or circular dichroism will have the form of a curve which is depicted in Fig. 1. Such a dependence is named the simple Cotton effect.

The type of circular activity for regular structures made up of identical groups is considerably more complex, based on the principle that in the electron spectrum of the absorption band there is usally a splitting into two or several components. Such a dependence is conveniently called the complex Cotton effect. The force of rotation, and consequently the contour of the band of circular dichroism or curve of dispersion of optical activity, in these cases is determined by the interection of transition moments (primarily electrical dipole moments) in addition to the inherent optical activity of chromophores. Complex problems of direct calculations are discussed in detail in the special theoretical papers [8-11, 29-33]. We are interested mainly in the conformation optical activity. For a qualitative exposure of the effects caused only by conformation optical activity of strong absorption bands which are conditioned by $\pi\pi^*$-transitions, it is possible to assume the following conditions.

1. A molecule of a polymer consists of identical additive chemical groups with π-electrons localized within a group. This localization is also preserved during excitation.

2. The band of absorption corresponding to the individual chromophore is not optically active, i. e., the magnetic dipole transition moment is equal to zero.

3. We will limit ourselves only that absorption band which is of interest to us, since although optical rotation depends on the properties of all the absorption bands, the dispersion of rotation close to a given band of absorption is determined mainly by this band.

4. Finally only resonance interactions are analyzed and not interactions of transitions with a various wave length, i. e., the influence of hypo- and hyperchromism is ignored.

In this approximation the development of conformation optical activity of a polymer may be explained by the interaction of electrical dipole transition moments in the identical groups from which the molecule is made up [52].

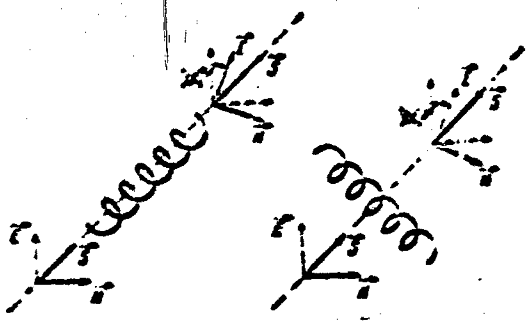

Figure 6. Optical rotation in the case when the light flux falls parallel $[\alpha]^{\parallel}$ to the helical axis of the molecule (electrical vector $\vec{E}$ will always be perpendicular to the axis of the helix) and when the light flux falls perpendicular $[\alpha]\perp$ to the helical axis (here the electrical vector $\vec{E}$ may be at any angle to the axis of the helix, by this being perpendicular to the given direction of propagation of the light wave).

$\vec{S}$ - direction of propagation of light wave, $\vec{E}$ - electrical vector of light wave lying in the plane of polarization of linear-polarized light, $\vec{H}$ - magnetic vector of light wave.

Knowing the location of these dipole moments in space and their magnitude, it is possible to calculate the force of rotation R_k of each k split component and then circular dichroism or, for example, specific rotation based on the formula

$$[\alpha] = \text{const} \cdot \nu^2 \sum_{k=1}^{N} \frac{R_k(\nu_k^2-\nu^2)}{(\nu_k^2-\nu^2)^2+\nu^2\Gamma^2}.$$

Here R_k and ν_k - force of rotation and frequency of the individual components into which the transition is split; Γ - factor of damping, equal in magnitude to the half thickness of the absorption band; N - number of identical groups.

We note that the expression standing under the sum sign describes the curve of rotation for a simple Cotton effect (Fig. 1).

It turns out that the basic characteristics of optical rotation of a polymer may be predicted based on the results of such calculations.

At the present time experimental data on optical activity in the range of absorption of polypeptides have been obtained and discussed only for molecules with helical conformation [34-42], though without a doubt all the other structural modifications are of no less interest.

During the passage of a light ray through a cuvette with a solution there may be two cases of interaction of the light wave with a helical molecule. Those are depicted schematically in Fig. 6. In the first of them the pencil of light is parallel to the long axis of the molecule, and in the second - perpendicular. In the first case the electrical vector of light wave excites only the perpendicular compone[illegible] E oscillations, while in the second - both perpend[illegible] parallel (types E and A). If rotation for these two cases is designated correspondingly as $[\alpha]^{\parallel}$ and $[\alpha]^{\perp}$, then total rotation during random distribution of molecules in a solution will equal

$$[\alpha] = \frac{1}{3}[\alpha]^{\parallel} + \frac{2}{3}[\alpha]^{\perp}.$$

For a further determination of values $[\alpha]^{\parallel}$ and $[\alpha]^{\perp}$ it is convenient to use one of the so-called rules of conservation. In application to our case it may be formulated as follows. "If the rotational force of transition in an individual chromophore is equal to zero, then the total of rotational forces of all transitions of this type, resulting due to resonance interaction, will also remain equal to zero."

From here it follows that

$$\text{for } [\alpha]^{\parallel} \quad \sum_{k=1}^{N} R_{Ek} = 0,$$

$$\text{for } [\alpha]^{\perp} \quad \sum_{k=1}^{N} (R_{Ek} + R_{Ak}) = 0.$$

Here N - the number of radicals in the polymer. Summation for k is necessary since each of the two basic helical levels (A and E) is N-times degenerated. In carrying out the summation it is possible within the extent for an infinite polymer to obtain the following expression [33]:

$$[\alpha]^{\parallel} = C_1 \nu^2 \nu_0 \nu_E \frac{(\nu_E^2-\nu^2)^2 - \Gamma^2\nu^2}{(\nu_E^2-\nu^2)^2 + \Gamma^2\nu^2}.$$

$$[\alpha]^{\perp} = C_1 \nu^2 \nu_0 \left[\frac{\nu_A^2-\nu^2}{(\nu_A^2-\nu^2)^2+\Gamma^2\nu^2} - \frac{\nu_E^2-\nu^2}{(\nu_E^2-\nu^2)^2+\Gamma^2\nu^2}\right].$$

In these formulas ν_0 - value for the frequency of the individual chromophore. The sign and value of constant coefficients $C_{\parallel}$ and $C_{\perp}$ depend on many specific properties of the molecule: geometry of the helix, number of radicals per turn, polarization of the transition moment in the chromophore, and also the extent of the interaction. Each of the members in the formulas for $[\alpha]_{\parallel}$ and $[\alpha]_{\perp}$, depending on frequency, has the form of a bell-shaped curve. The first curve is characterized by a maximum at ν_E, and the second with a certain value of frequency included between ν_E and ν_A (Fig. 7). Total curve of rotation $[\alpha]$ will also have a bell-shaped contour in the event the signs of the coefficients $C_{\parallel}$ and $C_{\perp}$ conform and a more complex contour if these signs are different.

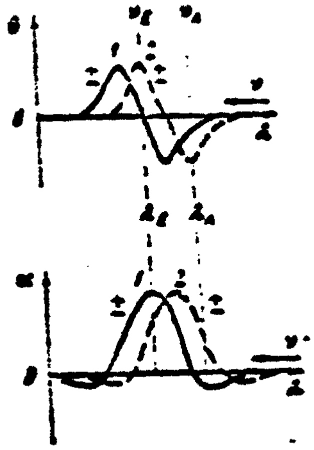

Figure 7. Curves of circular dichroism and dispersion of optical rotation, explaining the appearance of a complex Cotton effect for an electrically resolved transition in a helical polymer.
1 - Effect for light falling along the axis of the helix; 2 - for light falling perpendicular to the axis of the helix. The curves qualitatively represent the behavior of frequency dependent particles in the formulas for $[\alpha]_{\parallel}$ and $[\alpha]_{\perp}$, i. e., coefficients $C_{\parallel}$ and $C_{\perp}$ are taken equal to an unit. Under specific conditions these coefficients have a different value and sign. The case depicted in the drawing can qualitatively explain experimental data for polypeptides in the α-helical conformation.

It is necessary to speak separately about the optical activity of "magnetic" $n\pi^*$-transitions [41, 42]. Being of low intensity in the absorption spectrum, they make a large contribution in the spectra of circular dichroism and rotation.

The optical activity of this transition is created by the static field of charges of the separate groups surrounding the given one, and not due to the interaction of the electrical dipole moments as in $\pi\pi^*$-transitions. Here the near groups play the main role. It is important to note that a significant contribution in the optical activity of an $n\pi^*$-transition should be provided by the inductive component of the electrical moment of a strong $\pi\pi^*$-transition which is parallel to the magnetic moment of the $n\pi^*$-transition. Due to the small magnitude of the electrical dipole moment of the $n\pi^*$-transition the resonance interaction is not great, therefore it is possible to disregard the splitting of the $n\pi^*$-transition, and in this way the Cotton effect should be simple.

The relative contributions of the $n\pi^*$- and π. [illegible] ions in optical density of the inherent electron absorption bands of helical molecules depend on the geometry of these structures. Therefore the form of the curves of rotation and circular dichroism in the range of 180-230 nm will be different for different helical structures of the polypeptine chain, as an example the α-helix, the 3_{10} helix, and structures of collagen and polyproline. The same is in regards to the manifestation of spectral properties by various structural forms of double helical molecules of DNA and RNA in the range of 230-330 nm.

Knowing now the origin of optical activity in helical polymers we will switch to a discussion of experimental results obtained in the ultraviolet range of the spectrum, beginning with a wave length of 180 nm.

Figure 8 depicts the spectra of absorption, circular dichroism and dispersion of optical rotation of synthetic polypeptide in the helical form and the form of an irregular coil /20, 34, 40/. The difference in optical activity of these two forms is apparent. An irregular coil is characterized by a simple Cotton effect of relatively small scope, and,as this is expected, the passage of the curve of rotation through zero coincides with the maximum of the curve of absorption. Optical activity in the irregular form can have different explanations. On the one hand the peptide group in the polymer, strictly speaking, is asymmetrical, i. e., local symmetry selection, if we consider only atoms which are found in the plane of the peptide group, is not fulfilled. On the other hand it is possible that there are two or three chromophores which preserve in an "irregular" conformation some middle effective structure which conditions the optical activity observed.

The helical structure corresponds to a complex Cotton effect, the amplitude of which at the maximum reaches a great value.

The theory of the origin of this effect [illegible], which was presented above, shows that a direct relation between individual components in the spectrum of absorption and the contour in the spectrum of dichroism or rotation is absent, i. e., the two main peaks - positive and negative - cannot be directly compared to components of absorption with frequency ν_1 and ν_A . The theoretical curves depicted in Fig. 7 make it possible to qualitatively explain the course [illegible] the experimental curves, and the sign and the value of [illegible] [illegible]ces of rotation of the corresponding transitions are in [illegible]ment with those predicted [illegible].

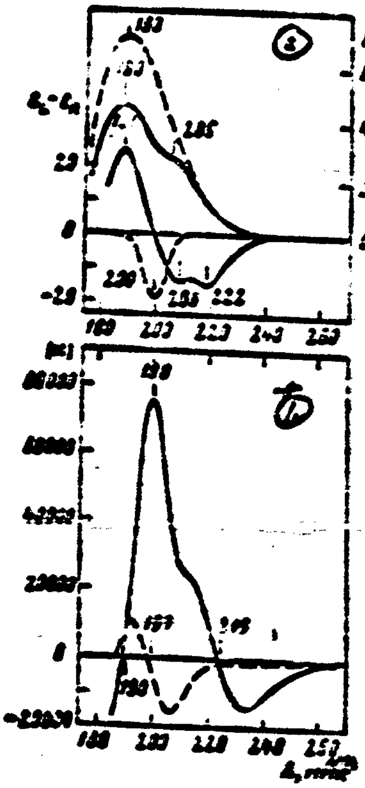

Figure 8. Spectra of absorption, circular dichroism (a), and dispersion of optical activity (b) of poly-L-glutamic acid in an aqueous solution.
In all cases the solid curve relates to α-helical conformation (pH 4.3), and the dotted curve to irregular conformation (pH 7.3). Concentration is within the limits of 0.0176-0.04%. Spectra of absorption, curves of circular dichroism and dispersion of optical rotation (b) are taken respectively from the works [20; 35 and 40; 3[illegible]]. Corrections for refractive index of the solution and numerical values of specific rotation are not introduced. ε - molar coefficient of rotation, [α] - specific rotation.

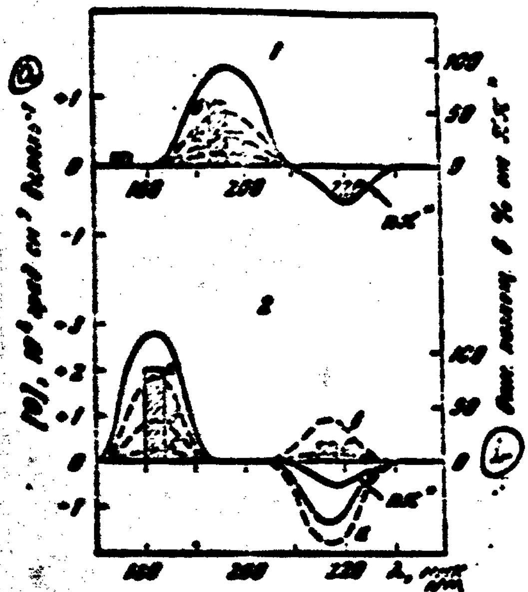

Fig. 9

Fig. 9. Calculated spectra of absorption and circular dichroism for antiparallel (1) and parallel (2) folded layers of the polypeptide chain [43].
The columns depict the forces of oscillators of individual components of the transition in percentages in respect to the force of the oscillator of the $\pi\pi^*$-transition as a whole. The solid curve represents the curve of ellipticity for the band with a width near 2500 cm^{-1}. Dotted lines a, b, c - corresponding contributions in optical activity caused by resonance (exciton) interaction, the interaction of connected oscillators, and the influence of a static field. The length of wave of the $n\pi^*$-transition in both cases is taken as 218 nm, and its intensity is negligibly small.
Key: (a) degree · cm^2 · dimole^{-1}; (b) Relative absorption in % from the $\pi\pi^*$.

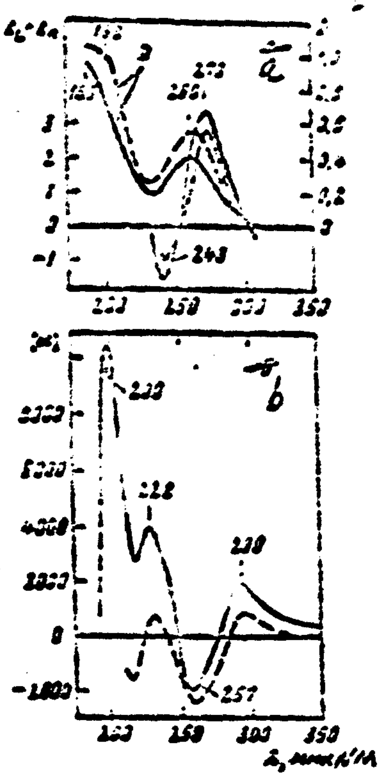

Fig. 10

Fig. 10. Spectra of absorption, circular dichroism (a) and dispersion of optical rotation (b) of aqueous solutions of nuclei acids.
In all cases the solid line relates to native helical conformatio (+20°), and the dotted to the denatured state, obtained by means of heating (+90°).
Spectra of absorption are given for a solution of DNA from calf thymus in heavy water, length of cuvette 1 cm, concentration 0.002% /47/. Curves of circular dichroism - for DNA from calf thymus /48/. Curves of dispersion of optical rotation - for DNA from salmon sperm /48/. Corrections for refractive index of the solution in numerical values of specific rotation are not brought in.

Recently a theoretical calculation was made of the optical activity of a polypeptide chain in the β-conformation [illegible] and also of the helical structures of polyproline I and II [illegible]. It turned out that in these cases one could not be limited to an examination which was suitable for the β-helical model of polypeptides. [illegible] from the resonance interaction it is necessary to consider the interaction of the given [illegible] transition with other electron transitions in the more remote ultraviolet range of the spectrum, and also to keep in mind the influence of the electrical field which is created mainly by the static dipole moments of the peptide groups (Fig. 9). It is necessary to note that at the present time there is still insufficient experimental data on the β-form of polypeptides [illegible] a quantitative comparison with the results of theoretical computations.

Table 2

Main parameters of dispersion of optical rotation (DOR) and circular dichroism of polypeptides in various conformations[1] [illegible].

	(a) [illegible][2]		(b) [illegible][3]		(c) [illegible][4]	
(d) ДОВ	λ, mμ	[m']	λ, mμ	[m']	λ, mμ	[m']
f. [illegible]	232—233	−15000	229—230	(−[illegible])	238	[illegible] j.
g. [illegible]	198—199	+65000	205	([illegible])	228	[illegible] j.
f. [illegible]	182—184	—	190	(−17000)	205	−[illegible]
g. [illegible]					190	+[illegible]
(e) КД	λ, mμ	[θ]	λ, mμ	[θ]	λ, mμ	[θ]
h. [illegible]	222	−[illegible]			238	−[illegible]
h. [illegible]	206—210	−[illegible]	218	(−[illegible])		
i. [illegible]	191	+[illegible]	193—197	(+[illegible])	218	+[illegible]
h. [illegible]					198	−[illegible]

1. The cited values for extremes are preliminary. Designations are as follows: [m'] - average rotation of radical, degree • cm^2 • decimole^{-1}, [θ] - uncorrected, based on refractive index, average ellipticity of radical, degree • cm^2 • decimole^{-1}.
2. Poly-L-glutamic acid in water, pH 4.75.
3. Silk fibroin in a 50% aqueous solution of methanol. Values are given in parentheses since they are obtained by extrapolation to a 100% β-form. These values are approximate and depend on the composition of the solvent.
4. Poly-L-glutamic acid in water, pH 7.32.

Key: (a) - α-helix[2]; (b) - β-form[3]; (c) - coil[4]; (d) DOR; (e) - Circular Dichroism; (f) - trough; (g) - peak; (h) - minimum; (i) - maximum; (j) - little.

The main parameters for the curves of dispersion of optical rotation and circular dichroism for various conformations of the polypeptide chain, including the β-form of the antiparallel folded layer, are presented in Table 2 [45].

Data of polarimetric measurements for nucleic acids are cited in Fig. 10 [41-47]. In principle ribonucleic acids produce an analogous range of curves of optical rotation. In comparison with polypeptides nucleic acids have a more complex spectrum of absorption, however in view of the peculiarities in the structure of a molecule of nucleic acid a parallel component with frequency $\nu_{\parallel}$ for $\pi\pi^*$-transitions is lacking. Therefore the corresponding optical activity, manifested only in the case when the wave falls along the axis of the molecule, should have a form corresponding to a form 1a which is analogous to the formula for [46] // or the curve depicted in Fig. 7 with the solid line [47]. Thus the curve of optical activity for each of the strong bands of absorption in nucleic acids should have a bell-shaped form. Apparently this main conclusion of the theory is in agreement with experimental data for $\pi\pi^*$-transitions.

In actuality the main maxima in the spectrum of absorption (around 260 and 185 nm) correspond to the maxima in the spectrum of rotation (around 257 and 200 nm). The curve of circular dichroism, measured only in the range from 230 nm [48], passes through zero close to the length of the wave of the maximum of absorption. On the other hand its positive shoulder is considerably larger based on the magnitude of the negative one. It is possible that this peculiarity is connected with the manifestation of optical activity of the transition, which has a maximum of absorption of around 260 nm. Besides this the molecule of nucleic acid in addition to the helical conformation activity should also possess activity which is conditioned by the mutual influence of the two bases, found one against the other in various unary helixes. It can be seen from Fig. 11 that the pairs which contain guanine and cytosine have a very large positive peak of optical rotation with a maximum of around 290 nm [50]. From here it is understandable why the magnitude of optical activity at this length of wave is proportional to the content of these radicals in the molecule of DNA [46]. Thus the contribution of dimers may be quite large, which introduces the corresponding distortion in the curve of conformation optical activity, This circumstance may explain to some degree the incomplete elimination of the optical activity of DNA during transition from a native state to denatured. The disposition of the nearest neighbors in the denatured state may also play an important role

Without a doubt a study of synthetic polynucleotides with a regular structure [51] may shed light on an understanding of several peculiarities in the dispersion of optical rotation and circular dichroism of nucleic acids. However, in view of the fact that molecules of polynucleotides in a number of cases have a conformation which is different from the conformation of DNA, an analysis of their optical activity requires special attention.

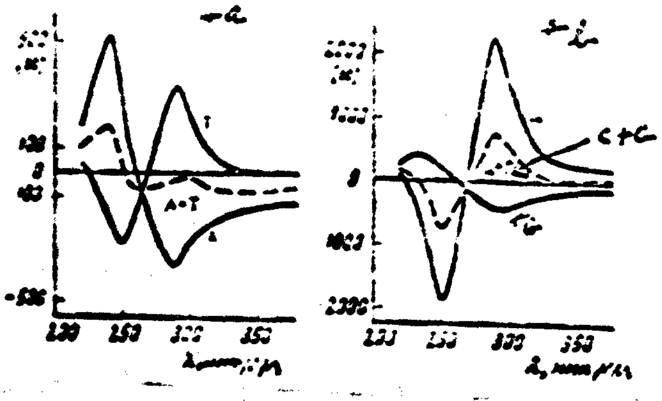

Fig. 11. Dispersion of optical rotation of deoxyribonucleotides in solution (pH 7.7).
The nitrogen base of each of the compounds is designated by the letters: A - adenine, T - thymine, (a), C - cytosine, G - guanine (b). The dotted lines show the curves of dispersion of optical activity of dimers with complementary bases, calculated by the formula $f_1[\alpha]_1 + f_2[\alpha]_2$, where f_1 and f_2 are the molecular parts of the compounds. Data cited from work [30].

Conclusion

In analyzing the main optical properties of polypeptides and nucleic acids ~~we have turned~~ attention to the essence of the origin of effects in the area of absorption bands. Only the experimental results for long helical molecules were discussed. Certain problems which are secondary in respect to the main effects, such as the influence of the solvent or manifestation in transparent areas of the spectrum, were not touched upon ~~in this article~~, though they undoubtedly have great significance in analytical work. Nevertheless, ~~we hope~~ that an analysis of dispersion of rotation in the visible range may produce supplementary information in an analysis of a mixture of structural forms, [56], though at the present time this interpretation bears an empirical nature to a considerable degree. A comparison of theory and experimental data for the area of absorption shows that there is qualitative agreement between them. In a quantitative comparison the problem arises of the accuracy of the spectral characteristics used during calculation on the one hand, and consideration of the contribution of various effects of a second order of smallness on the other.

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