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Circular Dichroism of Biological Macromolecules

Circular dichroism spectra of proteins and nucleic acids provide insights into solution conformations.

Sherman Beychok

In the continuing search for relationships between structure of biological macromolecules and their function, the study of conformation in solution must play a leading role. For many years, there was simply no objective method of discerning the three-dimensional arrangements of molecular groups within the macromolecular substance, and solution investigations were largely restricted to description of gross hydrodynamic properties. It is only within the last 10 years that investigators have succeeded in establishing experimental techniques for estimating, with a high degree of confidence, the number of amino acid residues in a protein which are present in the highly ordered periodic arrangement of the α-helix. This outstanding feat was accomplished, in large measure, by the analysis of the optical rotatory dispersion spectra of polypeptides and proteins (*1*), just prior to the dramatic and pioneering elucidation of the crystal structure of myoglobin (*2*). On the basis of correlation with the structure established by x-ray analysis (*3*), it later became possible to assign the sense of helix (right-handed in myoglobin), as well as content of helix, in other proteins and polypeptides. Not the least interesting aspect of these studies was the demonstration that helix content of myoglobin (secondary structure) was essentially preserved on passing from the wet crystalline state to a solution of the protein at high dilution (*3*).

There is, today, much interest in a still more particular aspect of structure-function relationship—namely, the possible conformational alterations which accompany interaction of the biological macromolecules with other cellular constituents. Studies of changes in optical activity appear to offer much hope of detection of change in the fraction of residues within α-helical segments which occurs consequent to a particular interaction (one such case is described below). In addition, circular dichroism spectra appear to shed light on structural alterations in proteins which are not primarily due to overall change in helix content, but rather are due to local, confined changes at particular sites which may not importantly affect any periodic or nonperiodic arrangement of peptide bonds. Clearly, studies of enzyme complexes, organized protein structures (subunit structures), polypeptide hormone interactions, and antibody-antigen complexes are examples of cases in which detailed studies of optical activity may be valuable aids in gaining the understanding we seek. In nucleic acids and polysaccharides in solution, elements of order—or periodicity—are well discerned by measurements of optical activity, and it may be that more subtle aspects of structure, too, will yield to sensitive measurements of optical activity. Finally, all the biological macromolecules have the characteristic ability of inducing asymmetry in small bound molecules which are symmetric in the unbound condition. The nature of the induced asymmetry depends on the asymmetry

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of the site in the macromolecule and it can, thus, serve as a probe in the study of binding sites.

In this article I briefly describe the phenomenon of circular dichroism, indicate possible origins of some of the bands which occur in circular dichroism spectra, and give a few examples of the applications of measurements of circular dichroism spectra to elucidation of aspects of structure in solution of proteins, nucleic acids, and certain polysaccharides.

Relation between Circular Dichroism and Optical Rotatory Dispersion

The closely related phenomena of circular dichroism and optical rotation are both manifestations of the same property of molecules which have asymmetric or disymmetric groupings of atoms. Optical rotation, or its variation with wavelength—optical rotatory dispersion—is, by far, the more familiar of these two measurements because a simple instrument has been available with which chemists have characterized their compounds according to specific rotation at the sodium D line or mercury green line for more than 100 years. It is only recently that instruments have been developed for the measurement of circular dichroism over a wide spectral range. For reasons which will be discussed presently, circular dichroism spectra have certain inherent advantages for studying optical activity, relative to optical rotatory dispersion spectra. Furthermore, by virtue of entirely general mathematical and physical relations connecting the two kinds of spectra, it is possible to transform experimental circular dichroism into optical rotatory dispersion curves. It is thus to be expected that in the next decade circular dichroism spectra will gradually replace optical rotatory dispersion spectra in studies of optical activity of several classes of compounds of chemical and biochemical interest.

Both optical rotatory dispersion and circular dichroism spectra have their origins in the absorption bands of optically active compounds. It is thus not surprising that these two phenomena are intimately related. The nature of this relationship was the subject of early papers by Kuhn (*4*), and the detailed theoretical formulation given later by Moffitt and Moscowitz (*5*) drew heavily on Kuhn's work while adding much that was novel and that depended on more recent developments in molecular spectroscopy. Since it is possible to transform either kind of data to the other, what is the value of separate circular dichroism measurements, especially in view of the far wider availability of recording spectropolarimeters capable of measurements over at least as broad a spectral interval as the best circular dichroism instruments now can achieve? The answer is that circular dichroism is an absorptive phenomenon, observable only in the frequency intervals where absorption occurs. In practice, this means that it is possible to locate the positions and signs (since the bands of circular dichroism spectra may be positive or negative, as discussed below) of the bands in circular dichroism spectra with far less equivocation than is possible in optical rotatory dispersion spectra. Optical rotatory dispersion is a dispersive phenomenon and is exhibited at frequencies (wavelengths) far from—as well as near—those characteristic of the electronic transitions which are responsible for the optical activity. The structural information which we seek in such spectra and the identification of the optically active chromophores depend on precise knowledge of frequency, shapes, and amplitudes of the bands in the spectra.

In the vicinity of an isolated absorption band in an optically active material, the optical rotation varies with the wavelength in a manner shown in Fig. 1A. The negative "Cotton effect" shown in the figure comprises two extrema and an inflection point. It should be noted that rotation persists at wavelengths far from the Cotton effect. It is because of this persistence that transparent materials exhibit optical activity in the visible spectrum. If the smooth rotatory dispersion curve of a colorless material is continued into the ultraviolet, it will somewhere have the appearance shown in Fig. 1A, usually in the vicinity of the longest wavelength absorption band. At the next absorption band another Cotton effect may occur, and so on through the entire spectrum. Thus in every optically active material, there are several such Cotton effects, some positive and some negative; each is set upon the background provided by the "tails" of all the other Cotton effects. The background rotation may be sufficiently large, or change sufficiently rapidly, that a small Cotton effect set upon it is

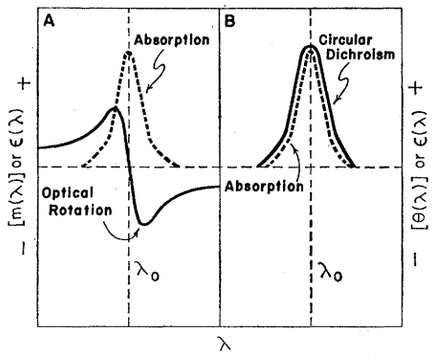


Fig. 1. Isolated absorption bands with associated negative Cotton effect in the optical rotatory dispersion spectrum (A) and positive ellipticity band in the circular dichroism spectrum (B). The symbol λ is a wavelength designation, increasing in value toward the right and decreasing toward the left in each half of the figure.

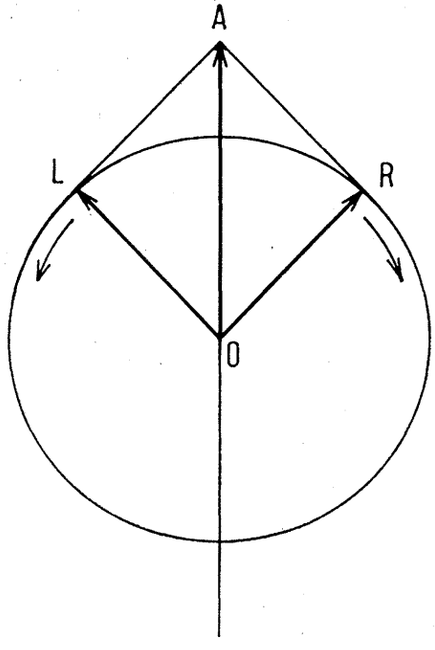


Fig. 2. Resolution of electric vector of plane polarized light into electric vectors of right and left circularly polarized light.

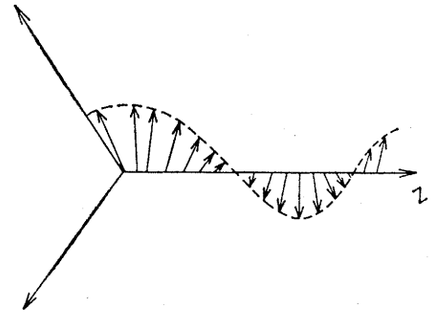


Fig. 3. Electric vector of right circularly polarized light at constant time and variable distance from light source.

obscured. This may lead both to erroneous assignment of position, sign, and amplitude of the Cotton effect and to uncertainty in assignment of the chromophoric transition which is optically active.

An isolated band (positive ellipticity band) in a circular dichroism spectrum is shown in Fig. 1B. The band is of the same shape as the absorption band and, with certain exceptions noted later, the maxima occur at the same wavelength. Ellipticity bands are thus often set upon a background which is essentially zero. Even when there is overlap, computer analysis may allow resolution of the bands and largely unequivocal assignment of their numbers, positions, signs, and amplitudes in complex spectra. Such resolution is feasible, in principle, in optical rotatory dispersion spectra also, but serious complications occur because major contributions to background rotation may arise from large Cotton effects at wavelengths hundreds of millimicrons from the region of measurement, and dominant contributions may come from transitions at inaccessible ultraviolet frequencies. Several examples of the importance of these distinctions between the optical rotatory dispersion and circular dichroism spectra to the experimentalist and theoretician are discussed below.

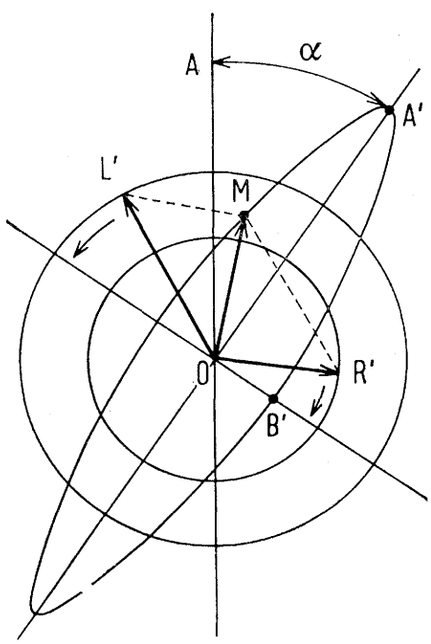


Fig. 4. Action of optically active medium on plane polarized light. Right circularly polarized light absorbed to a greater extent than left and index of refraction for left greater than that for right, as depicted [after Velluz *et al.* (*11*)].

While the electronic absorption bands and the circular dichroic bands (or Cotton effects) in the spectra of optically active compounds are intimately connected, there are distinctions which must be recognized, for there exists no simple one-to-one relationship. These distinctions will now be briefly described.

Conditions for the Production of Optical Activity

Light, or electromagnetic radiation, comprises oscillating electric and magnetic fields. In linearly polarized light, the sinusoidally varying electric field is confined to a plane, as is the magnetic field, and these planes are perpendicular to one another. When light impinges on a molecule, the electrons respond to, and are displaced from, their unperturbed ground-state configurations by the electric field of the light. If the frequency of the linearly polarized light corresponds to one of the proper exciting frequencies, then an electron will be promoted to an upper state, and dissipation of the increased energy into heat will lead to ordinary absorption. However, this is not a sufficient condition for optical activity. In order that a Cotton effect (Fig. 1A) or circular dichroic band (Fig. 1B) be observed it is necessary that the displacement of electronic charge have a circular component as well as a linear component in the direction of the applied field. This will induce a magnetic moment in the direction of the electric field of the light wave, and an electric moment perpendicular to that direction. As an example, we may consider an electron in an asymmetric static field (provided, say, by an asymmetric distribution of static charges in the vicinity of the chromophore) which is constrained to move in a helical path. An electric field in the direction of the helix axis tends to move the electron in a direction opposite to the field and parallel to it. However, the electron cannot be so displaced without at the same time undergoing a rotational displacement, and this leads to the required induced magnetic moment. The intensity of absorption thus depends only on the electric dipole transition moment, whereas the intensity of circular dichroism depends both on the electric and magnetic dipole transition moments. We may, then, speak of optically active absorption bands and nonoptically active absorption bands (*6*).

Several qualitative, but highly important, results follow from this simplified presentation. The most familiar result is that a very weak absorption band may exhibit intense optical activity. The absorption band may be only weakly allowed or "symmetry forbidden" and barely discernible or nondiscernible in the absorption spectrum, but the associated optical activity may be great. This case is of practical importance for the theoretician as well as for the experimentalist. An outstanding example is furnished by one of the prominent bands in the optical rotatory dispersion and circular dichroism spectra of α-helical polypeptides with a very weak—barely discerned—associated absorption band (*7, 8*). Similarly, very strong absorption bands may generate little or no optical activity, and all intermediate cases between these extremes may be encountered.

A second result of immediate value is that the sense of the electronic displacement (in the case described above, right- or left-hand helical path) determines whether the observed Cotton effect or ellipticity band is positive or negative. Kauzmann *et al.* demonstrated in their classical paper in 1940 (*9*) that a chromophoric electron moving in a right-handed helical path on excitation should generate a positive Cotton effect and ellipticity band. This result has been experimentally confirmed (*10*).

The last point in this connection is particularly germane to the understanding of optical activity of the biological macromolecules which always have asymmetric carbon atoms. Under different conditions, different absorption bands are optically active. This comes about largely because the chromophoric groups which generate the absorption bands are often not bonded directly to the asymmetric carbons. For example, the well-known absorption of tyrosine residues in proteins near 275 mμ is due to the promotion of one of the electrons in a π bonding orbital to an antibonding π^* orbital. The electron is associated with the ring, which is separated by a β carbon from the asymmetric carbon. If rotation occurs about the α-β carbon bond or about that from the β carbon to the ring carbon, then the ring is free to assume many orientations relative to the neighboring charges or dipoles, and the optical activity will

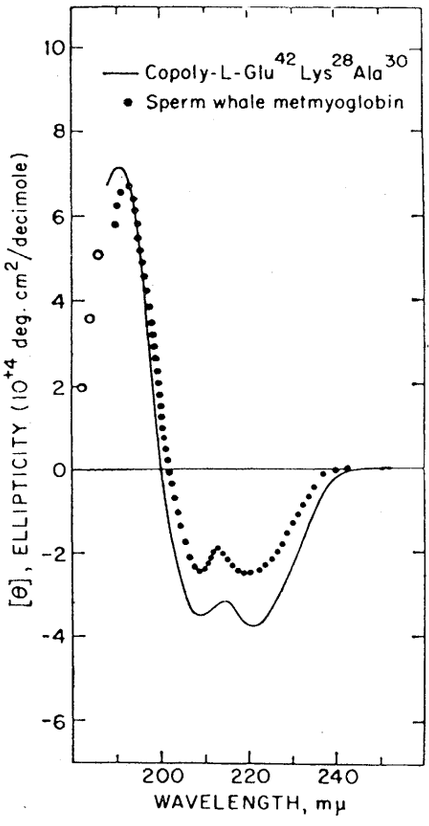


Fig. 5. Ultraviolet circular dichroism spectrum of a right-handed helical copolymer and of metmyoglobin [after Holzwarth and Doty *(13)*].

cancel or vanish. Thus, while the spectral absorption band may change only slightly when the structure of the protein is altered from an ordered to a disordered one (even if only near a particular tyrosine residue), the associated optical activity may be drastically reduced or eliminated. The situation may be somewhat less dramatic in the case of a nucleotide purine or pyrimidine transition because the base is directly attached to an asymmetric carbon in the sugar. Still, it is well to realize that many of the characteristic absorption bands are due to electronic excitations largely confined to particular atoms in the bases and that optical activity in these absorption bands also may diminish enormously when the fixed base orientations of ordered polynucleotides are destroyed by virtue of conformational alterations brought about by any number of chemical or physical alterations.

These remarks should emphasize the conformational dependence of ellipticity bands even while configuration about asymmetric carbon atoms is preserved.

Circular Dichroism and the Origin of Ellipticity Bands

Plane-polarized light can be resolved into two circularly polarized components by passage of the monochromatic polarized beam through an appropriate quarter wave plate. A birefringent plate has two axes along which the indices of refraction are different. Two vibrations which are in phase on incidence emerge from the plate with a phase difference, δ, which is proportional to the thickness d and to the difference in refractive index along the axes:

$$\delta = 2\pi d\,(n_1 - n_2)/\lambda$$

A quarter wave plate introduces a phase difference $\delta = \pi/2$.

If an observer stands on the z-axis of a right-hand coordinate system facing the light source and fixes his attention on the electric field vector at the point where he is standing, then for light polarized in the yz plane and propagated in the positive z direction, he will see the vector extremity move up and down on the y-axis, alternately positive then negative. The circularly polarized components which this light comprises would appear to him as vectors (electric field) moving in a circle — clockwise for the right-handed component and counterclockwise for the left-handed component (Fig. 2). The loci of the electric-field vector extremities as a function of distance along the z axis, at any moment, describe a helix which is right-handed for the right and left-handed for the left circularly polarized light (Fig. 3). When combined, these two helices resolve to give the time- and distance-dependent electric field which we recognize as plane-polarized light. The corresponding magnetic fields in the light beam can be treated similarly.

Thus, we shall consider plane-polarized light as the sum of two coherent circularly polarized components. The plane-polarized vibration OA (Fig. 4) has an amplitude A_0 and is the vector sum of A_l and A_r, the amplitudes of the circularly polarized vibrations. When the light passes through an optically active material it will be partly absorbed if its wavelength is close to one of the natural frequencies. More particularly, one of the circularly polarized components will be absorbed to a greater extent than the other. The consequence of this unequal absorption is that, on resolution, the vibration is no longer linearly polarized, but rather elliptically polarized (Fig. 4). The ellipticity per unit length is defined as the angle whose tangent is the ratio of the minor to the major axis. The relation between the ellipticity and the difference in extinction coefficient for left and right circularly polarized light is not difficult to derive and has been given in detail by Velluz *et al.* *(11)*.

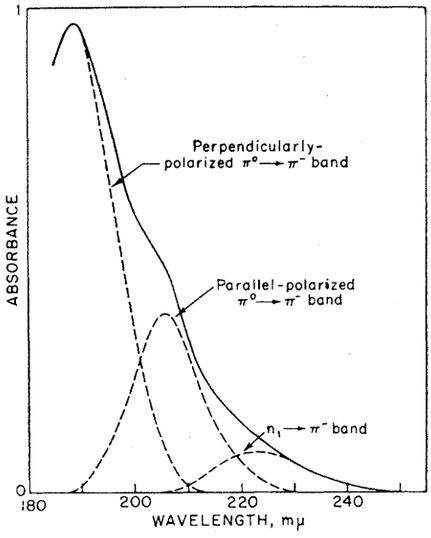


Fig. 6. Absorption spectrum of α-helical poly-γ-methyl-L-glutamate in trifluoroethanol and its proposed resolution into component transitions [after Holzwarth and Doty *(13)*].

In practice, the difference in optical density for the two circularly polarized components is measured directly. This difference is then converted to a difference in extinction coefficient. The final expression relating the molecular ellipticity $[\theta]$ to this difference in extinction coefficient is *(11)*

$$[\theta]_\lambda = 2.303 \cdot \frac{4500}{\pi} \cdot (\epsilon_l - \epsilon_r) = 3300 \cdot (\epsilon_l - \epsilon_r) \qquad (2)$$

Equation 2 is the quantitative expression of the finding of Kauzmann *et al.* *(8)*, alluded to above. If the left circularly polarized light is absorbed to a greater extent than the right, a positive ellipticity band will occur. Thus, if an electron undergoes a helical-like displacement in an excitation, the sense of this displacement is known from the sign of $[\theta]$ of the ellipticity band associated with the electronic transition.

The instrument used in our laboratory for the production of circularly polarized light and for measurement of unequal absorption of the circularly polarized components is manufactured by the Societé Jouan in Paris and was modified by us for tenfold greater

Table 1. Ellipticity band of α-helical polypeptides.

Wavelength ($m\mu$)		$10^{40} \cdot R_k$(cgs)	
Theoretical	Exp.	Theoretical	Exp.
	Band n_1-π^*(n-π^*)		
210–230 (*13*)	222 (*13, 19*)	−3.4 (*46*)	−22 (*13*)
	224 (*17*)		−19 (*19*)
			−15 (*17*)
	Band π^0-π^*(π-π^*) parallel		
198 (*13*)	206 (*13*)	−126 (*47*)	−29 (*13*)
	204 (*17*)		−29 (*17*)
	Band π^0-π^*(π-π^*) perpendicular		
191 (*13*)	190 (*13*)	+242 (*47*)	+81 (*13*)
	192 (*17*)		+51 (*17*)

sensitivity. The instrument and modifications have been described in detail (*11, 12*). Examination of Eq. 1 reveals that, in principle, the designation "quarter-wave plate" refers to light of a particular wavelength for a fixed thickness plate. For scanning over a broad-wavelength interval, the Jouan dichrograph makes use of an electro-optic shutter. In this application, the shutter is a z-cut plate of ammonium dihydrogen phosphate, across the faces of which is imposed an alternating electric field. The field makes the plate birefringent (Pockels effect). With appropriately chosen voltages, it is possible to convert linearly polarized light to light which passes from left circularly to right circularly polarized in one alternation of the field. The impressed voltage is varied with the wavelength in such a manner that the ammonium dihydrogen phosphate crystal acts as a quarter-wave plate at all wavelengths. The detection and electronic systems are thoroughly discussed by Velluz *et al.* (*11*).

With this instrument, the highest optical densities which can be used are about 2.4. Quite commonly, for many protein and nucleic acid absorption bands, values of molar extinction coefficients lie between 1000 and 10,000. The difference in extinction coefficients (ϵ_l-ϵ_r) may be of the order of 1 to 10, and often lower. Thus it is necessary to measure optical density differences of the order of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ in solutions whose mean optical densities may be between 1 and 2.

Characterization of Ellipticity Bands

An isolated ellipticity band is characterized by three parameters: $[\theta]_k^0$, which is the ellipticity at the wavelength of the band maximum; λ_k^0, which is the wavelength of maximum ellipticity; and Δ_k^0, which is an expression of band width and is defined as the wavelength interval between λ_k^0 and the wavelength at which the ellipticity is e^{-1} times $[\theta]_k^0$. If the band is Gaussian, then

$$[\theta] = [\theta]_k^0 \exp[-(\lambda - \lambda_k^0)^2/(\Delta_k^0)^2] \quad (3)$$

The rotational intensity of the transition is gauged by a signed quantity called the rotational strength of the kth transition:

$$R_k = \frac{3hc}{8\pi N} \int [\theta]_k \frac{d\lambda}{\lambda} \quad (4)$$

in which h is Planck's constant, c is the velocity of light and N is the number of absorbing molecules per cubic centimeter. If the band is Gaussian, then, by substituting Eq. 3 into the integrand and evaluating the integral,

$$R_k \approx 0.696 \cdot 10^{-42} \cdot \sqrt{\pi} \cdot [\theta]_k^0 \cdot \frac{\Delta_k^0}{\lambda_k^0} \quad (5)$$

The rotational strength of the transition is an important quantity in the quantum theory of optical activity. It is a scalar product of two vectors which represent, respectively, the light-induced electric and magnetic moments associated with excitation of the electron from the ground to the excited state (*9*).

In a complex circular dichroic spectrum comprising overlapping bands it sometimes occurs that the apparent positions and intensities of the several bands are different from the positions and intensities of the isolated bands. Computer analysis often leads to resolution of the isolated bands, and such resolution is important if the position of the band is to be used in identifying the chromophore which is optically active.

In any case, whether resolution is achieved or not, it is possible, by the use of the general Kramers-Kronig dispersion relations, to transform the entire circular dichroism spectrum into an optical rotatory despersion spectrum (*5*). If the molar rotation is $[m(\lambda)]$ and the molar ellipticity is $[\theta(\lambda)]$, then

$$[m(\lambda)] = \frac{2}{\pi} \int_0^\infty [\theta(\lambda')] \cdot \frac{\lambda'}{(\lambda')^2 - \lambda^2} d\lambda' \quad (6)$$

The integral can be evaluated by summation with a small interval—1 $m\mu$ or less—and the point at $\lambda = \lambda'$ is eliminated. Without any assumptions about the number or rotational strengths of individual bands, the entire circular dichroism spectrum can be transformed, or any part may be transformed for the purpose of seeing what contribution this portion of the spectrum makes to the observed optical rotatory dispersion curve.

Determination of Helix Content in Proteins

Studies of optical activity of proteins and polypeptides, both by optical rotatory dispersion and circular dichroism, have been most notably successful in estimating the presence and content of α-helix. Figure 5 shows the ultraviolet circular dichroism spectra of a fully helical polypeptide and of metmyoglobin which, in the crystal state, has about 75 percent of its residues in segments of α-helix (*13*). The circular dichroism spectrum of the α-helix in this wavelength interval comprises three bands, all due to spectral transitions associated with peptide bonds arranged in right-handed helical array. The spectrum of the helical polypeptide is shown in Fig. 6, as is its resolution into three absorption bands. The longest wavelength band is termed n_1-π^- (or, more commonly, n - π^*) and is due to the promotion of an electron from an oxygen nonbonding atomic orbital to an antibonding molecular orbital involving the oxygen, carbon, and nitrogen atoms (*14*). This spectral band is characterized by a very low extinction coefficient and is ordinarily not distinctly perceived in protein or polypeptide spectra. The two shorter wavelength transitions result from splitting of a single absorption band (observed in an isolated peptide bond). The splitting is a direct consequence of the helical arrangement of the peptide bonds and was predicted by Moffitt (*15*). The longer wavelength member of this pair is polarized parallel to the helix axis; the shorter wavelength band is polar-

ized perpendicular to the helix axis. All three bands are optically active. It is especially important to note that the parallel and perpendicular π^0-π^- ellipticity bands are of opposite sign (*16*).

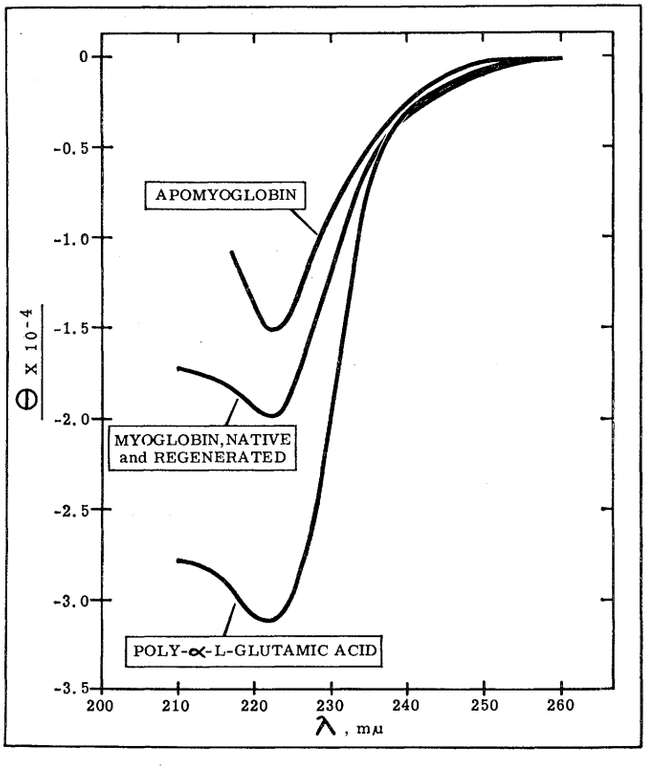
Table 1 gives the calculated rotational and spectral characteristics of these three bands and lists also the results of some theoretical calculations. The experimental values disagree somewhat from different laboratories but, all in all, it may be concluded that the major features of the peptide absorption and rotational bands in this spectral region have now been satisfactorily established.

The curve in Fig. 5 for myoglobin is clearly very closely related to that of the helical polypeptide. In order to calculate helix content, it is necessary to assume that those portions of the molecule which are not helical have the rotatory characteristics of a random-coil polypeptide. The ellipticity bands of random coil polypeptides have been described (*12, 13, 17, 18*). In the spectral region 210 to 230 mμ, random poly-α, L-glutamate and poly-L-lysine each show a small positive band. If one calculates helix content for myoglobin from the ellipticity value at 222 mμ (n_1-π^- maximum in the helix), a value of about 66 to 68 percent is obtained (*19*). If the full circular dichroism spectrum in this region is compared to that of the fully helical polymer, a higher value—between 70 and 80 percent—is obtained (*13*). The uncertainty is due to the relatively greater ellipticity at 200 mμ compared to higher wavelengths, but the uncertainty of the measurement is also greater at the shorter wavelength. Hemoglobin gives virtually identical results (*20*). Helix contents of other proteins determined from circular dichroism spectra in this wavelength interval are given in Table 2.

Table 2. Helix content estimated from n_1-π^0 ellipticity band. The random coil value at 222 mμ is taken as +3000 deg cm^2/decimole. Estimates of the helix content of a large number of proteins by analyses of optical rotatory dispersion have been reviewed (*1*).

$\theta_{222\ m\mu}$ (deg cm^2/decimole)	Percentage right-handed helix	Ref.
	Beef insulin (pH 2)	
−8100	32	(*48*)
	Lysozyme (pH 7.0)	
−8000	30	My data
	Ferrihemoglobin (*horse*, pH 6.6)	
−21000	68–70	(*20*)
	Ovalbumin (pH 6)	
−9400	35	My data
	Glutamic dehydrogenase (*bovine liver*, pH 7)	
−9800	37	My data

An interesting example of detection of change of helix content by circular dichroism changes resulting from an important interaction is illustrated in Fig. 7. There, the n_1 - π^- ellipticity band of myoglobin is shown, along with the curve of apomyoglobin, that is, the apoprotein remaining after removal of the prosthetic group heme. The residue ellipticity is reduced by about 5000 deg cm^2/decimole. When exactly one equivalent of hemin is added to the apomyoglobin, the ellipticity is restored. Breslow *et al.* (*19*) interpreted this to mean that the removal of heme from the globin is accompanied by a conformational alteration, one important feature of which is the change in the number of residues in segments of α-helix. Apparently, 10 to 15 residues which are in helical segments in myoglobin are not in such an array when the heme is removed. A comparable result is observed with hemoglobin, although the situation is a good deal more complex because of cooperative effects among the individual chains in recovery of helix content (*20*).



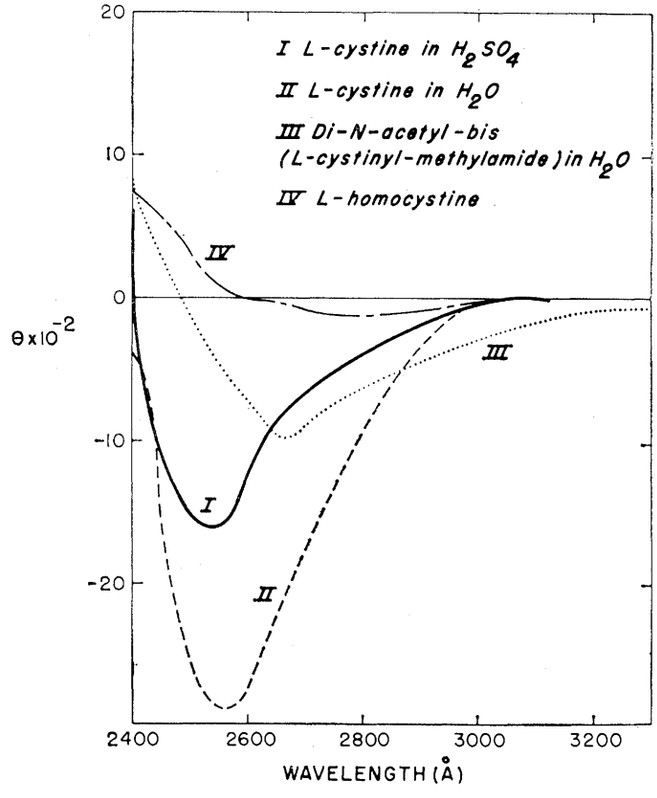


Fig. 7 (left). Ultraviolet circular dichroism spectra of native metmyoglobin, apomyoglobin, and regenerated metmyoglobin [after Breslow *et al.* (*19*)]. Fig. 8 (right). Near-ultraviolet circular dichroism spectra of L-cystine, di-*N*-acetyl-bis(L-cystinylmethylamide), and L-homocystine in water.

Nonpeptide Optical Activity

One of the serious difficulties involved in estimation of helix content by the method just described is that proteins contain other chromophores which absorb in the 200 to 230 mμ region of the spectrum. If any of these are optically active they, too, will contribute to the observed optical rotatory dispersion and circular dichroism spectra and complicate calculation of helix content. Optical rotatory dispersion studies are considerably more subject to this uncertainty because of the background contributions, inherent in the method, which may have spectral origins well removed in frequency from the peptide-bond absorption frequencies. However, circular dichroism is not free of this complication when side-chain chromophores absorbing between 200 and 230 mμ are optically active. While this is a drawback in one sense, it provides at the same time a new tool which is highly valuable in our understanding of protein structure.

There are several chromophores which absorb not only below 230 mμ, but at longer wavelengths as well. Above 240 mμ, any optical activity cannot be directly due to the peptide bond. Rather, specific side-chain chromophores may be optically active by virtue of their local environment. This optical activity is very sensitive to aspects of conformation involving these side chains. Thus the study of such optical activity affords a means of examining details of tertiary structure.

Any optical activity in this spectral interval (240 to 310 mμ—for proteins containing no prosthetic groups) can be due only to the aromatic residues or to cystine. In an investigation by the circular dichroism method of the optical activity of aromatic residues in polypeptides, Beychok and Fasman (*21*) analyzed the complicated ultraviolet circular dichroism spectrum of poly-L-tyrosine in helical and nonhelical conformations. That study showed that tyrosine residues in the helical conformation exhibit ellipticity bands associated with the well-known near-ultraviolet absorption bands of tyrosine. Furthermore, it was demonstrated that even in the free amino acid the bands could be detected. The spectral shift which accompanies ionization of tyrosine is paralleled by shift in the ellipticity bands. It was noted that a nonhelical conformation of poly-L-tyrosine did not exhibit these long wavelength ellipticity bands.

The complexity of the long wavelength (> 240 mμ) bands in the aromatic amino acids and their simple derivatives is apparent in Table 3. The unsubstituted amino acid tyrosine shows bands at 275 and 295 mμ in the phenol and phenolate forms, respectively, and the shift in wavelength follows the phenol titration curve. The same is observed with tyrosine ethyl ester. However, *N*-acetyltyrosine ethyl ester shows a small negative band at 272 mμ in acid, but exhibits a positive band at 285 mμ in alkali. The shorter wavelength ellipticity bands are, however, observed in acid as well as in alkali, but L-tyrosine ethyl ester shows no band in alkali between 210 and 260 mμ. A final apparent complexity is that the ionized form of tyrosine and of helical and random coil poly-L-tyrosine exhibit ellipticity bands near 245 mμ associated with the well-known absorption band of ionized tyrosine at this wavelength, but neither the *N*-acetyl-L-tyrosine ethyl ester nor tyrosine ethyl ester exhibit an ellipticity band at this wavelength, but rather at 235 mμ.

Partly on the basis of studies of amino acids and partly on the basis of our experience with certain proteins, we would suspect that tryptophan residues can generate ellipticity bands in the spectral interval 240 to 320 mμ at wavelengths as short as 265 mμ and as long as 300 mμ. Tyrosine residues in protonated form may generate bands at 275 to 280 mμ and in the ionized form at 235 to 245 and 295 mμ. Phenylalanine residues appear to generate only exceedingly weak bands at wavelengths longer than 240 mμ [see, especially, Moscowitz *et al.* (*22*)].

Table 4 is a partial list of proteins whose circular dichroism spectra show bands at wavelengths longer than 240 mμ. Except for two cases described below, it has thus far been impossible to assign the bands of kinds of chromophores, much less to specific residues. It is interesting that ovalbumin, which is, among all proteins, most notable for inaccessibility of tyrosine residues, shows no optical activity in the near-ultraviolet which is associated with the aromatic residues. Carbonic anhydrase (human, B and C) exhibits a highly complex spectrum in which one or more of the bands is probably due to tyrosine, because a red shift is observed as the *p*H is raised above 10.8 (*23*). The long wavelength band in ribonuclease moves to approximately 292 mμ when the *p*H is raised to 11.5. In both native proteins, a number of tyrosines are unavailable for titration, and presumably the optical activity, if due to tyrosine, is contributed to by accessible tyrosines. There is, however, an uncertainty in the assignment in ribonuclease because of the presence of disulfides (as discussed below) which can generate ellipticity bands in this spectral interval. The influence of *p*H and charge on these bands is still uncertain, and it cannot yet be stated unequivocally that the bands in ribonuclease arise from tyrosine residues. In carbonic anhydrase there are no cystine residues.

Table 3. Molar ellipticity of aromatic amino acids.

*p*H 1 to 2		*p*H 6 to 8		*p*H 13		Ref.
λ_{max}	θ	λ_{max}	θ	λ_{max}	θ	
			L-Tyrosine			
200	+			210	+ 7260	(*49*)
226	+ 8315			230	— 2310	(*49*)
274	+ 1320			293	+ 1155	(*49*)
			L-Tyrosine ethyl ester			
270–225	+ 960			289–290	+ 1320	My data
	+ 7600			*		
			N-acetyl-L-tyrosine ethyl ester			
270–280 (negative band)†				285‡	+ 890	My data
	— 500					
226	+17,300			235–238§	+13,000	My data
			N-acetyl-L-tyrosine amide			
222	+19,000			235	+15,200	My data
			L-Tryptophan			
207	—13,860	195	—30,360			(*49*)
225	+18,810	223	+26,070	275	+19,470	(*49*)
265	+ 1980	266	+ 1155	270	+ 1155	(*49*)
222	+17,400	222	+ 8,300	227	+19,900	My data
265	+ 2600	265	+ 1900	265–270	+ 2000	My data

* No band discernible between 210 and 260 mμ. † Negative band also observed with *N*-acetyl-L-tyrosine amide in acid; positive band in alkali. ‡ Spectral maximum at 295 mμ. § Spectral maximum at 245 mμ in all alkaline tyrosine derivatives.

Optically Active Disulfide Transitions

Near-ultraviolet optical activity of cystine residues in proteins and small peptides is of special interest, if only because circular dichroism spectra may yield information not readily available by other techniques applicable to proteins in solution.

In a simple aliphatic disulfide, the dihedral angle (the angle between planes defined by R_1-S-S and S-S-R_2) is close to 90°, and the barrier to rotation about the S-S bond is very high, having variously been estimated as between 5 and 15 kcal/mole (*24*). In principle, then, any such disulfide can exist in one of two rotamer forms, that is, either a right-handed or left-handed screw. In this sense, it is proper to speak of the conformation, or configuration, of a disulfide apart from other centers of asymmetry which may occur in the molecule. The disulfide sense in hexagonal cystine crystals is right-handed; that in cystine hydrochloride, glycylcystine and cystine hydrobromide is left-handed (*25*). Apart from these crystals and the very recent assignment of "screw sense" to all four disulfides in lysozyme crystals (*26*), nothing is known about disulfide "screw sense" in peptides or proteins. Furthermore, there is no information about disulfide dihedral angles in proteins or even in small peptides. It has been known for some time that alkyldisulfides exhibit an absorption band or shoulder near 250 mμ and that in cylic disulfides, in which the dihedral angle may be reduced to 25 degress or less, the band is shifted well to the red, to wavelengths near 330 mμ. A good deal of work has been done in the theoretical aspects of this shift and in the nature of the transition responsible for the band. Thus, the location of the absorption maxima of disulfides in proteins should give much useful information.

Table 4. Ellipticity bands observed in several proteins between 240 and 310 mμ.

*p*H	λ_{max} (mμ)	Sign	θ_{max}*	Ref.
		Avidin		
6.8	270–275	Pos.	+360	(*50*)
		Strepavidin		
9.0	265–270	Pos.	+360	(*50*)
		Pepsinogen		
7.76	277–279	Neg.	− 50	(*51*)
9.9	No detectable band			
10.6	275 (broad)	Pos.	+ 50	
11.6	265–280 (plateau)	Pos.	+ 65	
		Pepsin		
4.6	280–281	Pos.	+ 75	(*51*)
		Aldolase		
6.	280	Pos.		My data
		Chymotrypsinogen		
6.8	350–310	Neg.	−11 to −18	
	285–295	Pos.	+ 27	
	255–258 (shoulder)	Neg.	− 135	(*48*)
		Chymotrypsin		
6.8	307–308	Neg.	− 11	
	295	Pos.	+ 54	(*48*)
	255 (shoulder)	Neg.	− 271	
		Carbonic anhydrase C		
7.0	270	Neg.	−165	(*23*)
	245	Pos.	+115 to +160	
		Trypsin		
8.13	280	Neg.		My data
		Trypsinogen		
3.	260–280 (possibly two bands)	Neg.		My data
		Bovine albumin		
5.15	260 (shoulder)	Neg.	− 120	My data
		Ovalbumin		
6.06	No detectable band			My data
		β–Lactoglobulin		
6.5	285	Neg.	75	My data

* Mean residue weight basis.

The near-ultraviolet circular dichroism spectra of L-cystine and some related small molecules are shown in Fig. 2. It is very interesting that even L-homocystine shows a band, albeit a very small one. The smallness of the band may be an indication of rotation about carbons β and γ, with consequent averaging of the asymmetric fields. It is likely that the structure of homocystine, whether because of rotation about carbon-carbon, carbon-sulfur, or sulfur-sulfur bonds, is less rigid than that of L-cystine and its peptides.

Except for cystine in acid, the negative maxima occur at wavelengths longer than the characteristic 243 to 248 mμ absorption maximum shown by aliphatic disulfides and by cystine itself (whose absorption curve exhibits a shoulder). Near neutrality, both the circular dichroism and absorption maxima move to longer wavelengths. My colleagues and I are currently investigating this shift, which is complicated and which may depend on the crystal form in which the cystine was prepared. Of those peptides which we have examined and in which a long wavelength disulfide ellipticity band is present and identified, only oxytocin, lysine vasopressin, and their derivatives

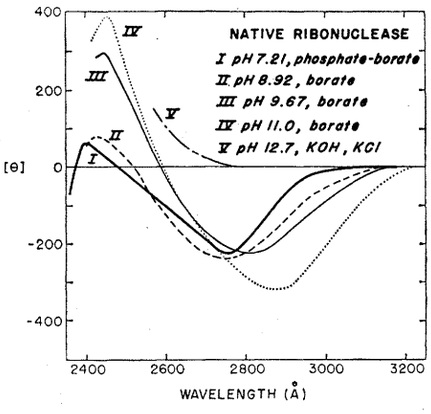


Fig. 9. Near-ultraviolet circular dichroism spectra of ribonuclease at various *p*H [after Pflumm and Beychok (*31*)].

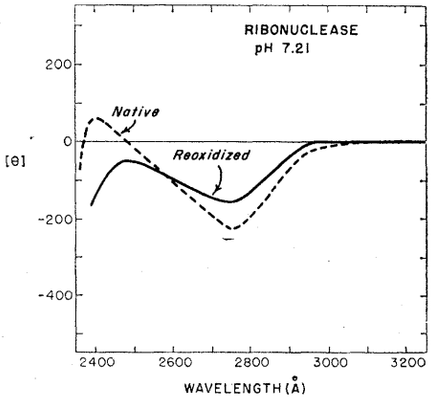


Fig. 10. Near-ultraviolet circular dichroism spectrum of native and reoxidized ribonuclease [from previously unpublished results of Pflumm and Beychok (*31*)].

exhibit a positive band. In glycine oxytocin, there is no aromatic residue at all and the positive band shown at 250 mμ is unequivocally associated with a disulfide transition. The position of the band strongly indicates that the dihedral angle is close to 90° (*27*).

Ribonuclease, Native and Reoxidized

The near-ultraviolet optical activity of native ribonuclease has been described with optical rotatory dispersion spectra and circular dichroism spectra. Glazer and Simmons (*28*) observed a small Cotton effect in the vicinity of 275 mμ and ascribed it to the titratable tyrosines of ribonuclease. Beychok (*29*) had previously noted two oppositely signed ellipticity bands; a negative band is centered at 275 mμ and a smaller positive band occurs at 238 to 240 mμ. The near-ultraviolet circular dichroism spectra of native ribonuclease at several *p*H values nicely illustrate the great advantage that circular dichroism enjoys by virtue of the absence of a background everywhere due to transitions at much shorter wavelengths (Fig. 9). The small positive band may be enhanced relative to its intensity at neutral *p*H by working at mild acid (*p*H 3) near 0°C. The wavelength maximum does not shift with this change of *p*H, in contrast to the change effected by increasing alkalinity.

Resolution of the complex spectrum into component bands will doubtless reveal that the 240 mμ band is more intense than observed in the composite spectrum because it is sandwiched between two negative bands, one of which, at the shorter wavelength, is very intense. It is difficult at present to make a secure assignment of the chromophores responsible for either of the bands.

Aside from the general interest in assigning the chromophores, there is a particular interest because this spectrum is highly sensitive to conformational alteration. The spectra of native ribonuclease and that of ribonuclease which has been reduced with mercaptoethanol, then reoxidized according to the methods of White and of Anfinsen and Haber (*30*) are compared in Fig. 10. The same spectrum is obtained from ribonuclease that has been subjected to reoxidization conditions for 100 hours directly measured or after column separation and crystallization, with native ribonuclease crystals

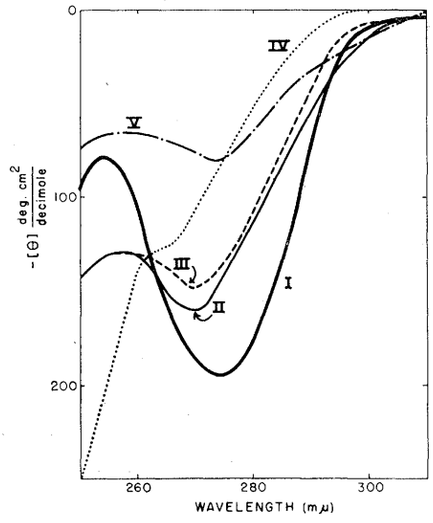


Fig. 11. Circular dichroism spectra between 250 and 310 mμ of insulin at several *p*H values and in 8*M* urea. Curve I, *p*H 8.4 to 8.6, average of seven spectra; curve II, *p*H 10.55, average of three spectra; curve III, *p*H 10.96, average of three spectra; curve IV, *p*H 11.52, average of five spectra; curve V, *p*H 8.5 in 8*M* urea. All at 0.1 ionic strength in KCl, KOH solutions. For curve I, the signal-to-noise ratio at 273 mμ is 8:1; 25°C [after Beychok (*29*)].

being used for seeding (*31*). The enzymatic activity against RNA varies somewhat from preparation to preparation but it is always 85 percent or more of the enzymatic activity shown by native ribonuclease.

The band near 275 mμ is diminished in intensity, and the band near 240 mμ appears to be absent altogether. The proteins are in other critical ways quite similar. For example, the absorption spectra of the proteins are virtually (though not exactly) identical, a finding reported also by White (*30*). The alkaline spectrophotometric titration curves (which reveal the inaccessibility of tyrosyl residues) are very similar in both proteins. Reoxidized ribonuclease, also, undergoes in acid the thermal transition described for native ribonuclease (*32*); but, again, the circular dichroism spectra of both the low- and high-temperature states are different in the two proteins. Clearly, the identification of that chromophore which is optically active in the native protein and inactive in the reduced-reoxidized protein would be highly relevant to our understanding of ribonuclease structure.

Finally, there are several other ways to abolish the positive band in the native enzyme. Elevation of temperature in acid, to 60°C, is one way. In 8*M* urea, the band is eliminated. In both these cases, the negative band near 275 mμ persists, though it is diminished in intensity. Reoxidation of reduced ribonuclease in urea also results in a negative band at 275 mμ, with no positive band at 240 mμ. In all these cases, however, the far ultraviolet circular dichroism spectra near 215 mμ are very different from that of reoxidized ribonuclease, which is only slightly altered by reduction and subsequent reoxidation. It may be that a single disulfide is somehow locked into a different conformation in the reoxidized protein, but this is far from certain.

Insulin

The near-ultraviolet circular dichroism spectrum of insulin has been reported (*29, 33*) and is shown at several *p*H's in Fig. 11. The negative band at 272 mμ does not shift to longer wavelengths with increasing *p*H, in contrast to the spectral absorption bands in this region. The latter, of course, arise mainly from tyrosine residues, which are freely titratable in insulin and which account for the well-known red shift of the insulin absorption spectrum that accompanies ionization of the tyrosine residues (*34*). The failure of the ellipticity band to move to longer wavelengths indicates that the chromophores responsible for the band are not the aromatic tyrosine residues. Indeed, increase of *p*H does not lead to any increase of magnitude at all near 295 mμ but, rather, to a decrease as the band shifts to shorter wavelength and changes in character.

Thus the band arises from cystine transitions, and it is interesting to see what, if any, treatment of the protein alters the band. Exposure of the protein to alkali for long periods of time brings about an elimination of this band. When the solution is restored to *p*H 8.4, a band of much reduced magnitude appears, although the helix content (as judged by the far-ultraviolet circular dichroism spectrum) is not restored at all. The presence of the band is not dependent on the presence of the fully native structure nor on the full complement of helix, both of which are apparently irreversibly destroyed on long exposure to alkali. This phenomenon may be connected with slow disulfide interchange in alkali and consequent reformation of incorrect disulfides. Addition at *p*H 8.5 of 0.001*M* cysteine

to solutions of 0.1 percent insulin results in a very complicated sequence of events with an initial shift of the band to shorter wavelengths and concomitant decrease in intensity, followed by restoration of the band (in several hours) to its initial position and intensity. Addition of 0.01*M* mercaptoethanol to 0.1 percent insulin at *p*H 8.5, in the absence of urea, leads to very rapid reduction of the band maximum by about 85 percent. When mercaptoethanol is added to insulin at *p*H 5, analysis after 4 hours indicates that 2 moles of cysteine (per 5734 molecular weight) have appeared. In analogy with the finding of Lindley (*35*), we believe that the intrachain disulfide may have been reduced. When this product is separated from the reagents at *p*H 3 on Sephadex, the circular dichroism spectrum reveals a negative band whose intensity is two-thirds that of native insulin. The helix band, in the far ultraviolet, is slightly greater (~ 5 percent) than that in native insulin. Restoration of the solution to *p*H 8.5 and exposure to air restores part, but not all, of the difference at 272 mμ.

The foregoing results indicate that all three disulfides contribute to the large negative ellipticity band at 272 mμ in native insulin. The position and intensity of the band (particularly the latter) depend on the native structure and on the intactness of all three disulfides. Interchange, partial reduction, and formation of mixed disulfides alter the position always in the direction of shorter wavelengths and the intensity to less negative values. Finally, the ellipticity band center (also, presumably, the absorption maximum, a difficult decision if tyrosine is present) is at a very long wavelength, a possible indication that the disulfide dihedral angles are altered by as much as 25° from the characteristic 90° value. There are, however, other possible explanations for this wavelength anomaly. The disulfide is a good nucleophile, and it presumably reacts with aqueous solvent when exposed to it. The spectral transition responsible for the band is thought to be an excitation of one of the nonbonding electrons to an antibonding σ^* orbital (*24*). One would expect that a polar solvent would stabilize the ground state and shift the band to shorter wavelengths. If the disulfides were prevented, by virtue of their location in the protein, from coming into contact with water, this might lead to an anomalous red-shifted (lower energy) transition.

If either of these two explanations were applicable to insulin, it would follow that the observed blue shift in insulin at alkaline *p*H, before any irreversible changes have occurred, representing a "normalization" of the disulfide state which may allow the subsequent irreversible changes to take place.

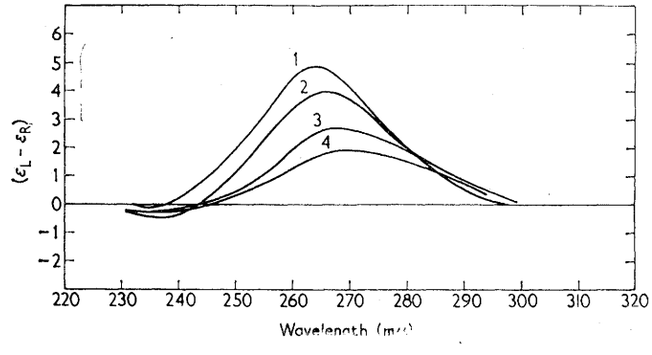
Moffitt and Moscowitz (*5*) showed that ellipticity bands and absorption bands might not exactly coincide when the extinction coefficient of the absorption band is very low (<100), because of the dominance of particular vibration states in the circular dichroism spectra. We do not believe this is the primary explanation of the position of the insulin bands, first because the observed displacement is greater than Moffitt and Moscowitz suggested even for extreme cases and, second, because disulfide rotational bands in many compounds have a very close correspondence with the absorption bands.

Table 5. Rotational strength (R_{ba}) of nucleic acids and polyadenylic acid (polyA). The data is taken from the work of Brahms and Mommaerts (*37*).

Material tested	Circular dichroism, maximum (mμ)	$10^{-40}R_{ba}$ (cgs)	Circular dichroism, minimum (mμ)	$10^{-40}R_{ba}$ (cgs)
DNA aqueous solution	273	+ 5	243	− 5
DNA in 80% alcohol	273	+16		
RNA aqueous solution	265	+15		− 0.5
PolyA, *p*H 7, 10°C	264	+35	247	−15
PolyA, *p*H 4·86	262	+64	242	−13

Nucleic Acids and Polynucleotides

Several studies of the circular dichroism spectra of polynucleotides and nucleic acids have been made (*36, 37*). The spectra of soluble RNA (sodium salt) (Fig. 12) and of calf thymus DNA (Fig. 13) show a diminution of the intensity of the bands with elevation of temperature. The most striking aspect of the figures, however, is that the DNA spectra show two oppositely signed overlapping bands while, in the same spectral interval, only a single positive band is observed with RNA. Brahms and Mommaerts (*37*) reported that this difference was present in all RNA's and DNA's they examined (Table 5). That this difference is not simply a result of the difference in sugars is evident from the fact that



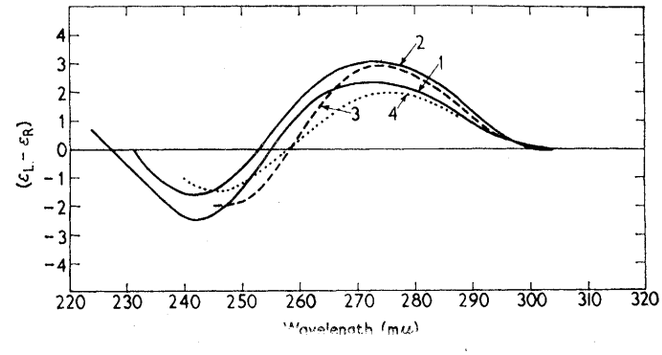


Fig. 12 (left). Circular dichroism curves of soluble RNA (sodium salt) in 0.15*M* NaCl, 0.01*M* tris buffer *p*H 7.4, 0.001*M* EDTA, measured at different temperatures as follows: curve 1 at 22°C; curve 2 at 45°C; curve 3 at 70°C; curve 4 at 80°C [after Brahms and Mommaerts (*37*)]. Fig. 13 (right). Circular dichroism curves of DNA (calf thymus) in 0.01*M* NaCl, 0.01*M* tris buffer, 0.001*M* EDTA measured at different temperatures as follows: curve 1, native DNA at 20°C; curve 2 at 45°C; curve 4 at 80°C; curve 3, DNA heated to boiling temperature for 10 to 15 minutes, followed by rapid cooling [after Brahms and Mommaerts (*37*)].

homoribopolynucleotides display under certain circumstances spectra which are qualitatively similar to those of the DNA's (as discussed below). Furthermore, all homopolynucleotides display a positive circular dichroism band in the region 260 to 280 mμ, even though monodeoxyribonucleotides show opposite-sign Cotton effects in this spectral region for purine and pyrimidine nucleotides (*38*).

Thus, as Brahms and Mommaerts suggest, this dramatic difference of DNA and RNA as shown by circular dichroism spectra resides primarily in structural features. Reovirus RNA, which is known to be double-stranded in the solid state and believed to be double-stranded in solution (*39*), exhibits a circular dichroism spectrum very similar to the typical RNA spectrum. The x-ray diffraction analysis of reovirus RNA shows that it is similar in structure to the A form (low relative humidity) of DNA in which the bases are inclined at about 70° to the helix axis (*39*). In the B form of DNA at high relative humidity (presumably this is the structure of DNA in solution), the bases are at 90° to the helix axis. Perhaps this difference of geometry is responsible for the difference in the circular dichroism spectra. It is also unclear how the circular dichroism spectra of stacked arrangements (single stranded) compare with double-stranded helices. Tinoco *et al.* (*40*) and Tinoco (*41*) have discussed the subject.

In this connection, two observations made in my laboratory may be relevant. Polycytidylic acid at *p*H 4.6, where it is thought to be double-stranded, shows the "DNA" circular dichroism spectrum; at *p*H 7, where it is thought to be an ordered single-strand structure, polycytidylic acid exhibits the "RNA" circular dichroism spectrum. Double-stranded MS2-specific RNA (*42*) (supplied by C. Weissmann) shows a circular dichroism spectrum close to that given by other RNA's but differing in one important detail. Near 295 mμ, a negative band is exhibited, closely overlapped with an intense positive band with a peak at 260 mμ.

From all these observations it appears that the circular dichroism spectra of nucleic acids are highly sensitive to details of geometry and structural arrangement and that optical activity measurements in solution will provide critical tests of comparability and distinctions of closely related nucleic acids and polynucleotides.

Polysaccharides

Polysaccharides comprising unsubstituted linked monosaccharides have their longest wavelength absorption bands at wavelengths shorter than 190 mμ and thus exhibit no circular dichroism above 200 mμ. In many biological polysaccharides, however, carbon No. 2 in the sugars is substituted with an acetamido group (*N*-acetyl) which is optically active near 220 mμ. Recently, Beychok and Kabat (*43*) reported the optical rotatory dispersion spectra of a variety of immunochemically reactive amino sugars in free and polymeric states. They were able to isolate the contribution to this spectrum of the 2-acetamido group and to identify a Cotton effect characteristically associated with the n-π^* transition of this *N*-acetyl moiety. From the effects of substitution of other sugars on the ring containing the *N*-acetyl substituent, and from studies of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine, these authors proposed the existence of a strongly preferred conformation of the planar amide group with respect to the sugar ring. The preferred orientation was elucidated by application of the octant rule (*44*).

Studies of the ellipticity bands associated with this 2-acetamido group confirm the identification of the chromophore and indicate that it is conformation-dependent in various biologically active polysaccharides. Furthermore, combination of optical rotatory dispersion and circular dichroism data allows decisions about the configuration of the anomeric carbon (α- or β-linkages) in cases where analytical and other chemical techniques fail or are imprecise (*45*). The circular dichroism spectra of many oligo- and polysaccharides have been studied in detail (*45*).

Summary

Circular dichroism, the unequal absorption of right and left circularly polarized light, is a manifestation of optical activity in the vicinity of absorption bands. To the experimental scientist interested in the conformation of macromolecules and in the sensitive response of optical activity to conformational alteration, it offers a relatively new and powerful means of understanding the environment of chromophoric residues. As a tool in the elucidation of electronic spectra, it should be useful to the theoretical scientist in identifying weakly allowed absorption bands as well as in providing rotational parameters which can be compared with the developing theory of optical activity. I have stressed application of circular dichroism to experimental aspects of protein and nucleic acid conformation in solution. Much is still uncertain in particular quantitative details. However, even these early results shed new light and yield new information on the conformation of these molecules.

References and Notes

1. An extensive literature exists describing the innovations and advances in the application of ORD to elucidation of protein structure. We may refer the reader, in particular, to several excellent articles: E. R. Blout, in *Optical Rotatory Dispersion*, C. Djerassi, Ed. (McGraw-Hill, New York, 1960); P. J. Urnes and P. Doty, *Advance. Protein Chem.* **16**, 401 (1961); G. D. Fasman, *Methods Enzymol.* **6**, 928 (1963). Pioneering work was carried on in the laboratories of E. R. Blout, P. Doty, E. Katchalski, W. Moffitt, and J. Schellman.
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ting is obviously of importance in many ordered molecules, of which the α-helix is one example and a nucleotide dimer another. A. S. Davydov, *Theory of Molecular Excitons*, (McGraw-Hill, New York, 1962); see also I. Tinoco, Jr., *Radiation Res.* **20**, 133 (1963); W. Moffitt, *Proc. Nat. Acad. Sci. U.S.* **42**, 736 (1956).
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52. Supported in part by PHS grants GM 10576 and 5-K3-GM 21, 933, and NSF grant GB 4850. I thank Dr. Weissman for the sample of double-stranded MS-2 specific RNA.

National Document-Handling Systems in Science and Technology

A study team has outlined the federal government's responsibilities and ways in which they can be met.

Launor F. Carter

Concern over the adequacy of this nation's document-handling and information system is far from new. Since World War II there have been at least 15 major proposals for the establishment of some sort of national system. Critics often point to the centralized Russian abstracting and announcing service VINITI (*1*) as a prototype we might well follow. Two recent reports, precursors to the study discussed in this article, have been given wide attention. In 1962 the "Crawford Report" (*2*), sponsored by the President's Science Advisor, advocated a major reorientation of organizational responsibilities for scientific and technical information within the federal government. The following year marked the appearance of the "Weinberg Report" (*3*), sponsored by the President's Science Advisory Committee. Although each of these reports received high-level governmental consideration, their recommendations were not widely adopted; rather, certain portions were incorporated into the existing structure. One innovation was the formation of the Committee on Scientific and Technical Information (COSATI) of the Federal Council for Science and Technology. The Director of the Office of Science and Technology asked COSATI to undertake a comprehensive study which would lead to the formulation of recommendations for a national document-handling system in science and technology. The results of the COSATI study, supported by a team from the System Development Corporation (SDC), are contained in an extensive report (*4*), the highlights of which are reviewed here.

The Problem

Each of the studies mentioned above views the problems of handling scientific and technical information and documents from a somewhat different point of view, largely because the problems have not been addressed as a single multifaceted problem. There are many different problems which, when summed, indicate that current practices and institutions need major revision. Among the problems which must be considered by a comprehensive design are the following:

1) Federal responsibility for scientific and technical documentation must be clarified and formalized. It is stated as a basic proposition that the federal government should be responsible for assuring the existence within the United States of at least one accessible copy of every significant publication in the worldwide scientific and technical literature. This has not heretofore been explicitly accepted as a responsibility of the federal government. As a result many government agencies are not oriented toward implementing such a policy, nor could they do so readily if the policy were formally adopted.

2) The number of users, and their requirements, are increasing. In 1960 there were 2,370,000 scientists, engineers, and technicians in the United States; it is estimated there will be 4,000,000 by 1970 (*5*). The documen-

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