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Circular Dichroism

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CIRCULAR DICHROISM

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Optical rotation (ORD) and circular dichroism (CD) are two inseparable properties which result from the interaction of polarized light with "optically active" samples. In order to be considered "optically active" a sample must be dissymmetric, i.e. lacking a rotation-reflection axis S_p (or $\tilde{p}$ = 360°/rotation angle). Of course an asymmetric molecule, like those possessing a carbon atom saturated by four different groups, will be also optically active. Another definition of a dissymmetric molecule is that of a molecule which is not superimposable to its mirror image.

a. Interaction of polarized light with an optically active substance.

A polarized light is composed of two circularly polarized waves traveling at the same speed (fig.1).

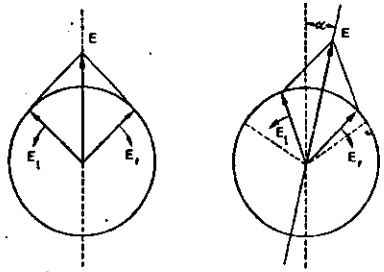

Fig. 1 Projection of the electric field vectors (the light moves towards the observer) showing composition of linearly polarized light.
Left: Two equal components of left and right circularly polarized light.
Right: The situation after light at a wavelength which is not absorbed has left an optically active medium

When the light (at a wavelength outside an absorption region) impinges an optically active medium the two component waves will be differently retarded because of the different refractive indices of the medium ($n_L \neq n_R$) (n=c/v). If the light travels through a layer of pathlength d the time-delay of the two waves will be

$$\Delta t = \frac{n_L - n_R}{c} d \qquad c = \text{light speed}$$

This will result in a phase difference $\phi = 2\pi\nu\Delta t = \frac{2\pi\nu}{c}(n_L - n_R)d =$

$$= \frac{2\pi}{\lambda_{vac}}(n_L - n_R)d \ (rad)$$

After emerging from the sample the two components will travel again at the same speed but the polarization plane will be rotated with respect to the original one of an angle (fig.1)

$$\alpha = \frac{\phi}{2} = \frac{180}{\lambda_{vac}}(n_L - n_R)d \ (deg)$$

A specific rotation is defined for the substance (at defined T and λ values)

$$[\alpha]^T_\lambda = \frac{\alpha}{cd} \qquad \frac{deg\ cm^3}{g\ dm} \qquad c = \text{concentration in g cm}^{-3}$$

On molar basis

$$[\phi]^T_\lambda = \frac{M}{100}[\alpha]^T_\lambda = \frac{M}{100}\frac{\alpha}{cd} \ \left(\frac{deg\ cm^2}{decimol}\right)$$

M = molecular weight of the substance or average molecular weight of the repeating unit in the case of polymers.

When the impinging light is in a wavelength range of an absorption band, in addition to a differential retardation also a differential absorption of the two circularly polarized components is occurring ($\varepsilon_L \neq \varepsilon_R$) (circular dichroism) The projections of the two vectors not only differ in angular velocity (fig.1) but also in length (fig.2). The resulting wave is no more plane-polarized but

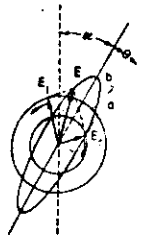

Fig. 2 Elliptical polarization of light upon leaving an optically active medium in the absorption range of an optically active transition: circular dichroism (Projection as in Fig.

it is elliptically polarized. The ellipticity θ is defined as the arctangent of the ratio of the minor to the major axis of the ellipse (fig.2).

It is easy to demonstrate that $\theta = 33\Delta A$. θ and ΔA are in general extremely small quantities.
As in the case of rotation a specific and a molar ellipticity are defined

$$[\theta]^T_\lambda = \frac{\theta}{cd} \ ; \quad [\theta_M]^T_\lambda = \frac{M}{100}\frac{\theta}{cd}$$

Sometimes circular dichroism is reported as $\Delta\varepsilon = \varepsilon_L - \varepsilon_R = \frac{[\theta]}{3300}$.
Both optical rotation and circular dichroism are dependent upon the wavelength. Fig.3 shows this dependence.

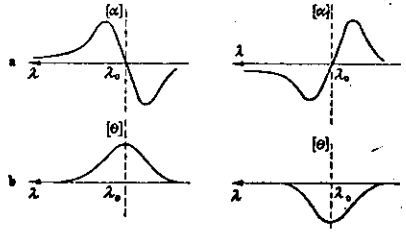

Fig. 3 a, b. Cotton effects of absorption bands at λ_0. *a* [α] as a function of λ: ORD spectrum; *b* [θ] as a function of λ: CD spectrum. *Left*: positive, *right*: negative Cotton effect

As the two phenomena are coupled it is possible to calculate one from the other by using the so called Kronig-Kramers transforms.
The different behavior upon the wavelength delineates the advantages of one technique over the other. When the sample absorbs in a wavelength range accessible to available instruments CD is by far the most convenient technique to be used. ORD becomes useful in case the absorption bands are below 190 nm or when the solvent is not transparent in the wavelength range of sample absorption.

b. Molecular origin

A CD band has generally the same shape as the corresponding absorption band. The area of the CD band is a measure of the rotational strength of the corresponding electronic transition $o \rightarrow i$

$$R_{oi} = \frac{3hc}{8\pi^3 N} \int \frac{[\theta]_\lambda}{\lambda} d\lambda \qquad N = \text{Avogadro number}$$

For a gaussian band

$$R_{oi} = 4.118 \cdot 10^{-57} [\theta]_{oi} \frac{\Delta_{oi}}{\lambda_{oi}} \quad (Am^3s)$$

where λ_{oi} and $[\theta]_{oi}$ are values corresponding to the maximum of the band and Δ_{oi} is the wavelength interval where $[\theta]$ is $\frac{[\theta]_{oi}}{e}$.

From a quanto-mechanical point of view

$R_{oi} = \mathrm{Im}\, \mu_{oi} \cdot m_{oi}$

where $\mu_{oi} = \langle \psi_o | \underline{\mu} | \psi_i \rangle$ (electric transition dipole) and $m_{oi} = \langle \psi_i | \underline{m} | \psi_o \rangle$ (magnetic transition dipole). $\underline{\mu}$ is the electric dipole operator, $\underline{m}$ is the magnetic dipole operator (imaginary).

For a non-zero rotational strength the two transition dipoles must be non-perpendicular to each other. This is equivalent to say that the charge displacement comprises a traslatory as well as a rotatory component with a resultant screw or helical motion (fig.4).

Figure 4
Schematic diagram of light-induced charge displacement in a molecule. (a) Pure electronic absorption. (b) Pure magnetic absorption. (c) Optical activity.

The chromophores which give rise to a CD spectrum can be divided in two broadly different groups:

a) intrinsically dissymmetric chromophores
b) intrinsically symmetric chromophores dissymmetrically perturbed.

An example of the first group is hexalicene (fig.5), an aromatic

fig.5

molecule which for steric reasons possesses one of the possible helical conformations. As the π-π^* transitions spread over the entire molecule they are intrinsically dissymmetric, in other words the electrons are forced to move in a helical motion.

Much more frequent are in nature the molecules which possess chromophores of the second class. Among them the molecule of biological interest. Nucleic acids, proteins and polysaccharides (the most important biopolymers) have chromophores which are intrinsically symmetric but are perturbed dissymmetrically by the fields generated by the neighbors groups. Very often these groups are themselves dissymmetric (like the carbon atoms of the sugar residue in nucleic acids and polysaccharides, or the α-carbon atom in the polypetide chain), but a further source of dissymmetry is the helical conformation that such molecules may assume in appropriate conditions.

In the last fifty years or so a great deal of effort has been devoted to the teoretical calculations of CD spectra of ordered polymers (or any other aggregate of interacting chromophores). One example of such kind of calculations involves the subdivision of the polymer in subunits (monomeric units, base-pairs etc.)

The polymer Hamiltonian is the sum of subunits Hamiltonians plus an interaction potential V_{ij} (the Coulomb law for all the particles in the subunit i and j)

$$H = \sum_i H_i + \sum_i \sum_{j>i} V_{ij}$$

The polymer electronic wavefunction, ψ, is expanded in a basis set of products of subunit electronic wavefunctions, ϕ_{ia}, where i designes the subunit 1,2,3... and a labels the state (ground, first excited...). Thus one obtains the energy matrix, whose eigenvalues and eigenvectors characterize the polymer spectrum. Usually the interaction potentials are calculated either by a dipole-dipole approximation or by a monopole-monopole approximation. In the first case one must know the permanent electronic dipole moment in the ground state, μ_{oo}, the permanent dipole in the excited state, μ_{aa} and the transition electri dipole moment μ_{oa}. In the monopole-monopole approximation the wavefunctions of the subunits are needed so that a permanent charge or a transition charge can be assigned to each atom.

Usually in a polymer with strong interactions between chromophores the calculated rotational strength is the sum of three contributions:

a) the one-electron term, which is the contribution of each monomer and is generally small

b) the electric-magnetic coupling, which is also small except in the case of interactions between chromophores one of which has a small magnetic transition dipole and the other a small electric transition dipole

c) the coupled oscillator term (exciton term) which depends on the magnitude of the electric transition dipoles (usually large) of the monomers, on their relative distance and orientation. This contribution, which is very sensitive to conformation, generally dominates the rotatory strength and gives rise to two opposite bands, whose separation depends upon the interaction term. Note that the coupled oscillator term can be generated also by non-optically active monomers when they interact in a dissymmetric, fixed geometry (e.g. Dyes plus charged polymers).

A good example of calculated optical properties is that reported by Woody (fig.6) who derived the CD spectrum of the α-helical poly-L-alanine (no chromophores in the side-chains). The agreement with the experimental results is indeed good.

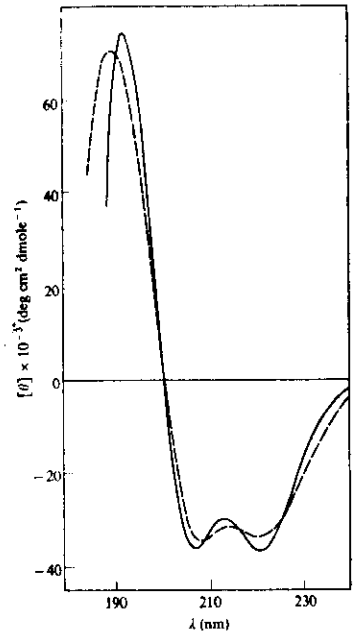

Figure 6
CD spectrum of poly-L-alanine in an α-helical conformation. Calculated (*dashed line*) and observed (*solid line*) spectra are shown. [After R. W. Woody, *J. Chem. Phys.* 49:4797 (1968).]

Although some success has been obtained in calculating the CD spectrum of different homopolymers, the application of theoretical methods to natural biopolymers is still far from reaching reliable and conclusive results.

In the field of proteins one possible application of CD spectroscopy is that of deriving from the measured CD spectrum of a given protein the relative amounts of different secondary structures like α-helix, β-form and disordered form. In practice this is done taking as standards the CD spectra recorded for poly-L-lysine in the three limit conformations (fig.7).

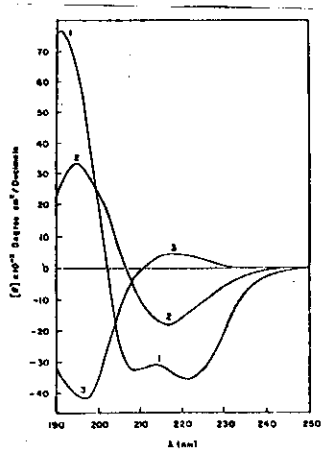

Fig. 7. Circular dichroism spectra of poly-L-lysine in the 100% α-helical (curve 1), β (curve 2), and random coil (curve 3) conformations. [From N. J. Greenfield and G. D. Fasman, *Biochemistry* 8, 1108 (1969).]

Through linear combination of the three spectra one can simulate the CD spectrum of a given protein and therefore derive the ratios at which the three limit structures are present. This method, however, suffers of many limitations:
1) the choice of the standard spectra. Whereas the α-helical CD curve is almost invariant (fig.8), this is not so for β-structures or for random coil form (figs.9 and 10)

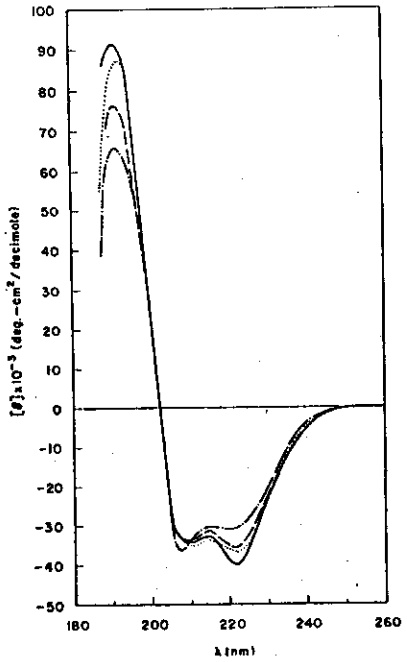

Fig. 8. The circular dichroism of the α-helix in various polypeptides: Poly-L-glutamic acid in water, pH 4.1 ——— [from G. M. Holzwarth and P. Doty, *J. Amer. Chem. Soc.* **87**, 218 (1965)]. Poly-L-lysine in water, pH 11.0, - - - - - - [from R. Townend, T. F. Kumosinski, S. N. Timasheff, G. D. Fasman, and B. Davidson, *Biochem. Biophys. Res. Commun.* **23**, 163 (1966)]. Poly-[N^5-(2-hydroxyethyl)-L-glutamine] in methanol:water, 8:2, · · · · (G. D. Fasman, unpublished data). Poly-L-alanine in trifluoroethanol:trifluoroacetic acid, 98.5:1.5, - - - - - - [from F. Quadrifoglio and D. W. Urry, *J. Amer. Chem. Soc.* **90**, 2755 (1968)].

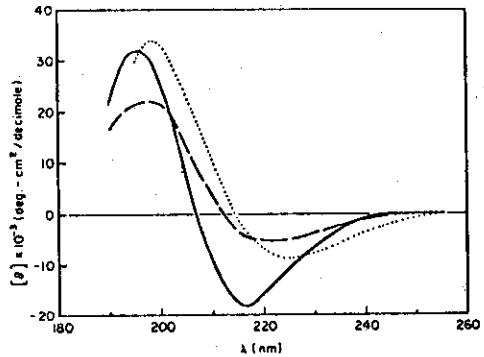

Fig. 9. The circular dichroism of the β form of various polypeptides: Poly-L-lysine in water, pH 11, ——— [from N. J. Greenfield and G. D. Fasman, *Biochemistry* **8**, 4108 (1969)]. Poly-L-serine in water, - - - - [from F. Quadrifoglio and D. W. Urry, *J. Amer. Chem. Soc.* **90**, 2760 (1968)]. Poly-*S*-carboxymethyl-L-cysteine in water, pH 4.3, · · · · · · (G. D. Fasman, unpublished data).

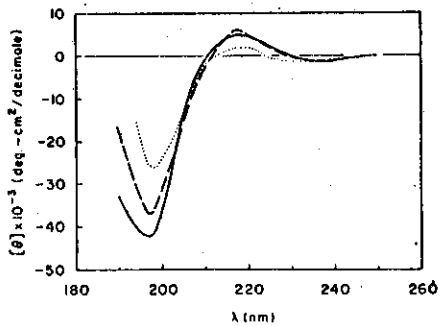

Fig. 10. The circular dichroism of the random coil form of various polypeptides: Poly-L-glutamic acid in water pH 7.5, ——— [from A. J. Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.* **90**, 4736 (1968)]. Poly-L-lysine in water, pH 5.7, - - - - [from N. J. Greenfield and G. D. Fasman, *Biochemistry* **8**, 4108 (1969)]. Poly-[N^5-(2-hydroxyethyl)-L-glutamine in water, · · · · · · [from A. J. Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.* **90**, 4736 (1968)].

2) the presence of aromatic side-chais. These chromophores can strongly couple with peptide electronic transitions and with themselves giving rise to substantially different CD curves (fig.11)

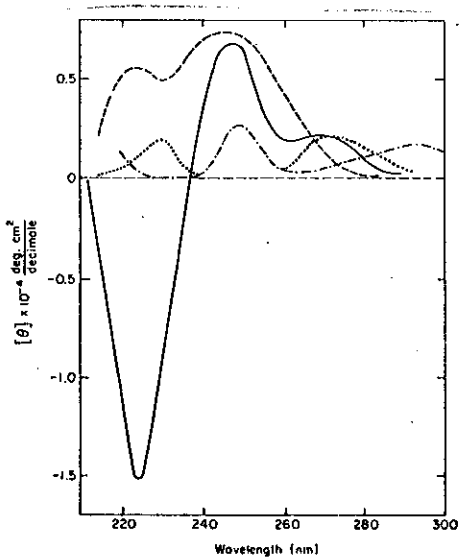

Fig. 11. Circular dichroism of helical poly-L-tyrosine (——), random poly-L-tyrosine (– – –) (both at pH 11.2), and L-tyrosine at pH 8 (· · · ·) and at pH 12 (–·–·) [from S. Beychok and G. D. Fasman, *Biochemistry* 3, 1675 (1964)]. The helical form of the polymer was prepared by direct dissolution into water at pH 11.2; the random-coil form was first brought to pH > 12 and then back-titrated to pH 11.2.

3) the presence of other basic structures. Many proteins, in fact, contain different secondary structures, in addition to the usual ones, like 3_{10} helices and, more often, β -turns. Different CD curves have been recently obtained with several β -turns.

Despite of these limitations the method has been applied to several proteins with fairly good results (Table 1).

Table 1
Estimates of protein secondary structure from CD measurements

Method	Structure	Protein			
		Carboxypeptidase	α-Chymotrypsin	Myoglobin	Lysozyme
X-ray structure	α Helix	23	8	~68	28
	β Sheet	18	22	0	10
	Random plus other	59	70	~32	62
CD calculation from poly-L-lysine basis set	α Helix	13	12	68	29
	β Sheet	31	23	5	11
	Random	56	65	27	60
CD calculation from protein basis set	α Helix	26	20	—[a]	—[a]
	β Sheet	18	20	—[a]	—[a]
	Random	56	60	—[a]	—[a]

[a] Prediction in these cases is not a fair test, because these proteins were included in the original basis set.

Though CD spectroscopy has been applied to proteins mostly with the aim of deriving secondary structure contributions, many attempts (with contradictory results) have been devoted to characterize tertiary and quaternary structures using the CD signals of aromatic side-chains or of prosthetic groups.

In the DNA field CD spectroscopy has been used essentially to derive the conformational state of the nucleic acid chains. Typical CD spectra are obtained with the basic conformations (fig. 12) so that any conformational change induced by a number of factors like temperature, solvent, salts, pH etc. can be conveniently studied.

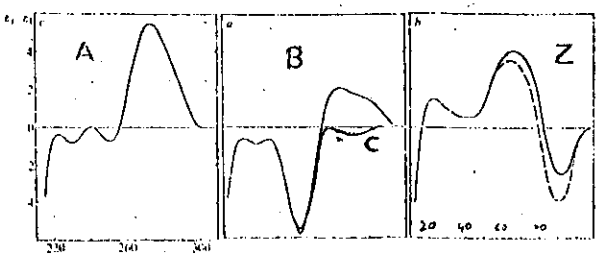

Fig. 12

As the CD spectrum of a DNA should depend on its base sequence, due to different orientation of electronic transition dipole moments of each base-pair, one should in principle be able, for a fixed conformation, to derive the base sequence of a given DNA from its CD spectrum. This is not possible in practice either because a long polymer averages all the single contributions, either because the same conformation is locally somewhat dependent on base sequence and the prerequisite of fixed conformation is therefore lost.

On the contrary CD spectroscopy has been widely used for the study of DNA-drug interactions. Most of the drugs are symmetric molecules which become optically active through interaction with nucleic acids. Intercalation and external binding can be sometimes distinguished by CD measurements.

EXPERIMENTAL APPARATUS

Fig. 13 shows the basic optical system of a typical CD instrument.

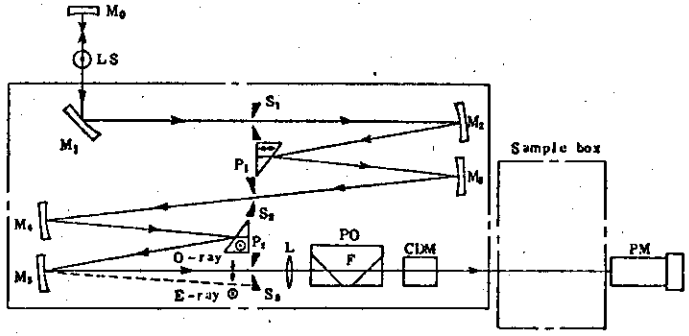

Fig.13 Optical system

M_0, M_1, M_2, M_3, M_4, M_5: mirror
LS: Light source
S_1, S_2, S_3: Slit
P_1 : First prism (Horizontal axis)
P_2 : Second prism (Vertical axis)
O-ray: Ordinary rary
E-ray: Extra-ordinary ray
L: Lens
F: Filter
PO: Rochon prism (J-500C)
CDM: CD Modulator
PM: Photomultiplier tube

The light beam from the light source, a 450 W Xenon lamp, is focused by the spherical mirror M_1 on to the entrance slit S_1. A segment of the optical system from the entrance slit S_1 to the intermediate slit S_2 is designated as the first monochromator, and that from S_2 to the exit slit S_3 is the second monochromator.

Together they constitute a double monochromator which is indispensable in the CD measurements owing to its reduced stray light. In same model P_1 and P_2, the crystal quartz prisms, have different axial direction with respect to each other, so that the light beam in S_3 is monochromatic and also linearly polarized, oscillating in the horizontal direction. If fused quartz is used for P_1 and P_2 a Rochon prism is necessary after S_3 to polarize the light. The linearly polarized light is modulated to right and left circularly polarized light by CD modulator which works on the principle of the"piezo effect".

When the sample is inserted into the beam the intensity I of the light transmitted through the sample varies as shown in fig.14.

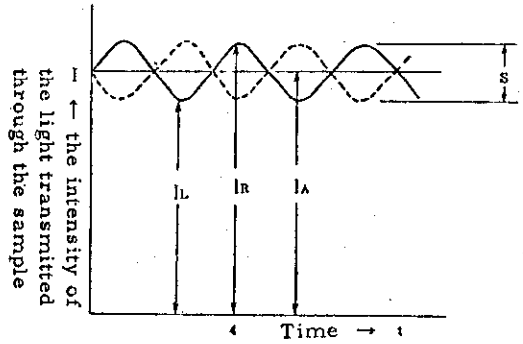

fig. 14

The maximum and minimum intensities appear as the applied voltage passes through its peak values (both the plus and minus peaks) and correspond to the intensities of the transmitted right (or left) and left (or right) circularly polarized light depending upon the values of ε_L and ε_R. In fig. 14 the solid and dashed curves correspond to the cases $\varepsilon_R > \varepsilon_L$ and $\varepsilon_R < \varepsilon_L$, respectively. Whel the light of fig.14 impinges upon the photomultiplier its output signal consists of a DC component proportional to I_A and an AC component proportional to S.

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