Optical Rotation

Classical Interpretation

Optically active substances can rotate the plane of polarization of light that passes through them. From the classical viewpoint this requires (1) that the oscillating magnetic field of the incident light induce an electric dipole moment in the molecule parallel to the incident magnet field and (2) that the electric field of the incident light must induce a corresponding magnetic moment parallel to the incident electric field. Thus,

$$\mathbf{m} = -\beta/c\ \partial\mathbf{H}/\partial t \text{ and } \mu = \gamma/c\ \partial\mathbf{E}/\partial t$$

where $\mathbf{m}$ and μ are the induced electric and magnetic dipole moments and $\mathbf{H}$ and $\mathbf{E}$ are the magnetic and electric fields of the incident light.

There is a simple qualitative interpretation of the significance of these requirements, as shown in Figures 15-24 and 15-25 from Kauzmann's Quantum Chemistry, below. Consider a molecule in which the electrons are constrained to move along a helical path. Suppose plane polarized light is incident upon the molecule such that the oscillating magnetic field is parallel to the helix axis. Then according to Faraday's laws the changing magnetic field induces an EMF in the helical conducting path, and so causes a resultant oscillating electric dipole parallel to the helix axis, i.e., parallel to the magnetic field of the incident light. This oscillating electric dipole produces an oscillating electric field which is perpendicular to the electric field of the incident light. Since the incident and induced fields are in phase, they can be combined by vector addition, leading to a resultant electric field which is rotated relative to the incident field. The same argument holds for the magnetic field induced by the alternating electric field of the light.

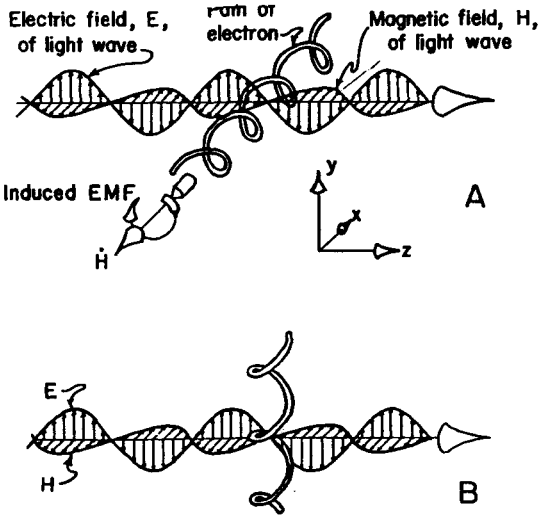


FIG. 15-24.
A. Mechanism of inducing an electric dipole moment in a helix by the changing magnetic field of a light wave.
B. Mechanism of inducing a magnetic dipole moment in a helix by the changing electric field in a light wave (see page 619 for explanation).

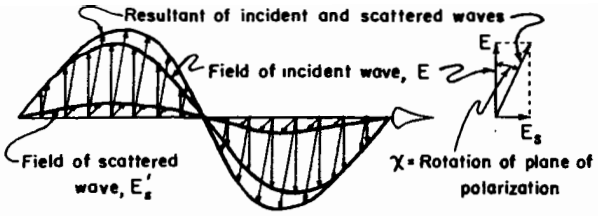


FIG 15-25. Origin of optical rotatory power from light scattered by an electric dipole that has been induced by the changing magnetic field of a light wave.

Quantum Interpretation

The problem is to calculate β and γ. The quantity β is calculated by taking account of the perturbation of the electronic states of the optically active molecule by the magnetic field of the incident light. The perturbation energy is $H' = -\mu \bullet \mathbf{H}$, which leads to a magnetic transition dipole moment:

$$\langle a|\mu|b\rangle = \int \psi_b^*(r)\, \mu(\mathbf{r})\, \psi(r)\, d^3 r$$

The theory similarly yields $\langle a|\mathbf{m}|b\rangle$. Eventually one obtains

$$\beta = -4\pi c/3h \sum_k \mathrm{Im}(\langle k|\mathbf{m}|0\rangle \bullet \langle 0|\mu|k\rangle)/(\omega_{ko}^2 - \omega^2)$$

and $\gamma = \beta$. Hence, the quantity governing the amount of rotation is the rotatory strength $R = \mathrm{Im}(\langle k|\mathbf{m}|0\rangle \bullet \langle 0|\mu|k\rangle)$.

Note that even bands with relatively weak absorption (small $\langle k|\mathbf{m}|0\rangle$) can have a substantial rotatory power if $\langle 0|\mu|k\rangle$ is big enough.

Phenomenology of Optical Rotation

Plane polarized light can be decomposed into two opposite circularly polarized components. **Optical rotation** results from circular birefringence, i.e., from a difference between the indices of refraction for right and left circularly polarized light, n_R and n_L. This in turn implies that the extinction coefficients for the right and left circularly polarized components are different; $\varepsilon_L(\lambda) \neq \varepsilon_R(\lambda)$. This difference between the extinction coefficients for right and left circularly polarized components leads to **circular dichroism**. Because the R and L components are absorbed to different extents, the beam which emerges from the sample is elliptically polarized. There are many different ways to characterize optical rotation and circularly dichroism, as illustrated in the following table.

Table 10.6 Summary of experimental parameters for optical rotation and circular dichroism*

Optical rotation

$$\text{Specific rotation} = [\alpha] = \frac{\alpha}{dc} \tag{10.39}$$

$$\text{Molar rotation} = [\phi] = \frac{M[\alpha]}{100} = \frac{100\alpha}{lm} \tag{10.40}$$

Circular dichroism

$$\text{Specific ellipticity} = [\psi] = \frac{\psi}{dc} \tag{10.41}$$

$$\text{Molar ellipticity } [\theta] = \frac{M[\psi]}{100} = \frac{100\psi}{lm} \tag{10.42}$$

$$\text{Circular dichroism} = \Delta\varepsilon = (\varepsilon_L - \varepsilon_R) = \frac{A_L - A_R}{lm} \tag{10.43}$$

$$[\theta] = 3298(\varepsilon_L - \varepsilon_R) \tag{10.44}$$

* α = rotation angle, degrees; d = path length, dm
ψ = ellipticity, degrees; l = path length, cm
ε = molar absorptivity; c = concentration, g cm^{-3}
M = molecular weight; m = concentration, mol ℓ^{-1}

Applications

We can determine the representation of different secondary structures in a protein molecule from their contributions to the CD spectrum of the protein. For simplicity suppose that we are concerned only with α-helical, β-sheet, and "random" conformations. We could express the molar ellipticity as :

$$[\theta(\lambda)] = X_\alpha [\theta_\alpha(\lambda)] + X_\beta [\theta_\beta(\lambda)] + X_r [\theta_r(\lambda)]$$

where X_α, X_β, and X_r represent the fractional compositions of the designated structures in the protein and the $[\theta_i(\lambda)]$ are reference CD spectra for α, β, and r structures (see Figure 3, below). The $[\theta_i(\lambda)]$ can be obtained from measurements of polypeptides in the appropriate conformation and/or from CD spectra of proteins of known structure. If the $[\theta_i(\lambda)]$ are well known and if $[\theta(\lambda)]$ is measured at a number of λ's, then it should be possible to invert the above equations, i.e., to determine the X_i from the measurements of $[\theta(\lambda)]$. This is the basis for the determination of the secondary structure components of a protein from CD measurements. See W. Curtis Johnson (1998) Ann. Rev. Biophys. Biophys. Chem. 17:145-166.

In fact there are more than three elementary secondary structures to consider: α-helix, β-sheet, many (11) types of β-turns, and contributions from chromophores, supertwists, aromatic side chains, etc. One maximizes the discrimination of the method by extending the spectral measurements to the region of 180 nm. It has been shown from an analysis of proteins of known structure that only 4 independent variables can be extracted from the data.

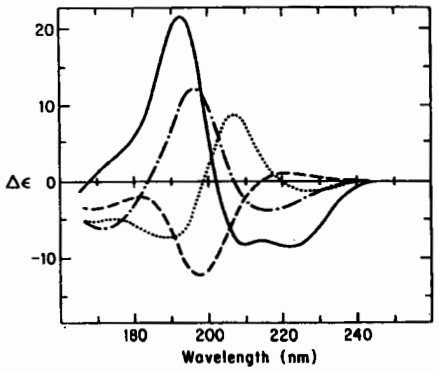


Figure 3 The CD spectra for the α-helix (*solid line*), β-sheet (*dots and dashes*), β-turn (*dotted line*), and random coil (*dashed line*), redrawn from Brahms & Brahms (14).

Reference CD spectra are provided by measurements of synthetic polypeptides which have only a single type of secondary structure. Nevertheless, the CD of an α-helix depends on the helix length. This can be taken into account by measuring the CD spectra of proteins with helices of known length. More generally, there are procedures for extracting a set of "basis vectors" which give the needed $[\theta_i(\lambda)]$ from CD spectra of proteins of known structure.

Nucleic acid helices have high rotational strength. Nearest neighbor sequences influence the CD spectra.

Fluorescence Spectroscopy

We denote the excited state of a molecule M by M^*: $M + h\nu_1 \rightarrow M^*$. M^* can give up its energy by emitting fluorescence, $M^* \rightarrow M + h\nu_2$ where $\nu_2 < \nu_1$. These processes can be described as shown below.

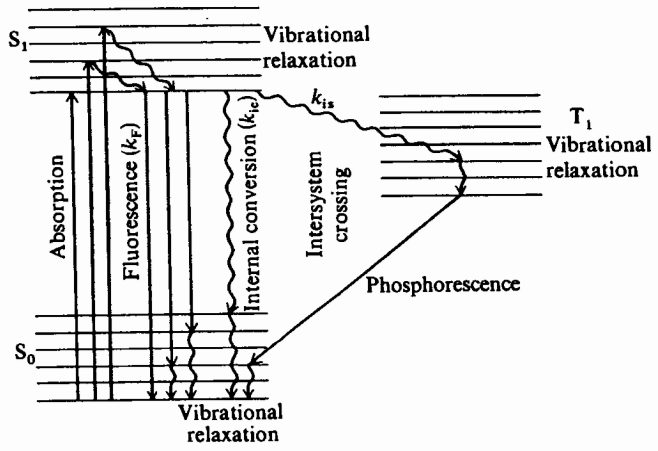


Figure 8-11

Pathways for production and deexcitation of an excited state. [After C. R. Cantor and T. Tao, in *Procedures in Nucleic Acid Research*, vol. 2 (New York: Harper & Row, 1971), p. 31.]

The fluorescence intensity is proportional to the rate of deexcitation:

$$f \propto dM^*/dt = -k_d M^*$$

where the fluorescence decay time is given as $\tau = 1/k_d$. If deexcitation can occur only by emission of fluorescence, then the excited state life time has a maximum value, τ_0, and the decay rate is a minimum, k_f: $k_f = 1/\tau_0$. But there can be many other nonradiative deexcitation pathways, each with a characteristic rate constant:

k_t , the rate constant for thermal deactivation;
k_p , the rate constant for photochemistry;
$k_Q[Q]$, the pseudo first order rate constant for quenching.

Then, $-dM^*/dt = \{k_f + k_t + k_p + k_Q[Q]\}(M^*) = k_d(M^*)$. In the presence of all of these pathways to
deexcitation, the fluorescence lifetime will be

$$\tau = \{k_f + k_t + k_p + k_Q[Q]\}^{-1} = k_d^{-1}$$

The quantum yield for fluorescence is defined as

ϕ_f = number of emitted fluorescence photons/number of photons absorbed

At steady state $\phi_f = k_f(M^*)/k_d(M^*) = \tau/\tau_0$.

Fluorescence Quenching

One nonradiative path for deexcitation is by collision of the excited state molecule with another molecule to which the excitation energy is transferred and then converted to heat. For example, O_2 is an excellent quencher. In the absence of quencher, $\phi_f^0 = k_f/(k_f + k_i + k_p)$. In the presence of quencher at concentration [Q], $\phi_f = k_f/(k_f + k_i + k_p + k_Q[Q])$. Thus,

$$\phi_f^0/\phi_f = 1 + k_Q[Q]/ (k_f + k_i + k_p) = 1 + K_Q[Q]$$

where $K_Q = k_Q/(k_f + k_i + k_p)$. This is known as the Stern-Volmer equation. It can be used to test the exposure of fluorophores to interaction with solute (quencher) molecules.

The Intrinsic Lifetime

According to the classical theory the lifetime of an excited state is $\tau = 2m_e/(\eta + \mu)$, where η and μ are the "viscous" and radiation damping constants, respectively. The maximum lifetime is obtained when $\eta = 0$ so that deexcitation can occur only by radiation damping. Then,

$$\tau_0 = 2m_e/\mu \cong 3m_e c^3/e^2\omega_0^2,$$

where $\omega_0 = \sqrt{(k/m_e)}$. Hence, the higher the natural frequency, the shorter the lifetime. According to quantum mechanics,

$$\tau_0 = 1/A_{ba}$$

where $A_{ba} = (64\pi^4\nu^3)/(3c^3h)\, D_{ab}$ and $D_{ab} = |\langle b|\mu|a\rangle|^2 = 9.18\times10^{-3} \int (\varepsilon/\nu)\, d\nu$ (in Debyes), as indicated in a previous lecture.

Since D_{ab} and A_{ba} are proportional, we conclude that the stronger the absorption, the more rapid the rate of fluorescence emission, i.e., the shorter the maximum or "radiative" lifetime, τ_0.

Fluorescence Resonance Energy Transfer (FRET)

Consider two molecules, the donor, D, and the acceptor, A, such that the emission band of the donor overlaps the absorbance band of the acceptor. If the donor and acceptor molecules are sufficiently close and if the emission transition dipole moment of the donor is oriented properly with respect to the absorbance transition dipole moment of the

acceptor, then excitation energy can be transferred from donor to acceptor in a radiationless process.

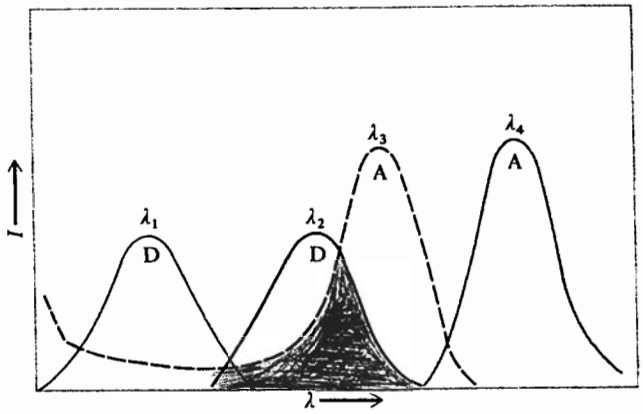


Figure 8-18

Schematic spectra for a donor–acceptor pair suitable for singlet–singlet energy-transfer measurements. Shown are the absorption spectra for donor (*colored line*) and acceptor (*dashed line*), and the emission spectra for donor (*black line*) and acceptor (*gray line*). The spectral overlap (neglecting the factor of ν^{-4}) is shaded.

In the overlap region of the spectra we can consider that the donor emission band and the acceptor absorbance band are in resonance. Typically, resonance transfer occurs to a vibrationally excited level of the acceptor absorbance band. The vibrational relaxation of the acceptor occurs rapidly to a spectral region which overlaps little with the donor and so transfer back to the donor is rare. FRET can be detected either as a quenching of the fluorescence of the donor or as a sensitized emission of the acceptor. In the former case both the quantum yield and the fluorescence lifetime of the donor are decreased. We can determine the efficiency of transfer, E, from these changes in lifetime and quantum yield. Suppose that the rate constant for energy transfer is k_R. Then E is defined as the fraction of donor excitation energy that is lost by transfer:

$$E = k_R/(k_R + k_f + k_i + k_p)$$

where we have neglected collisional quenching for simplicity. Now compare the quantum yield of the donor in the presence and absence of the acceptor,

$$\phi_{D+A}/\phi_D = [k_f/(k_R + k_f + k_i + k_p)]/ [k_f/(k_f + k_i + k_p)] = 1 - E$$

The rate of transfer, k_R, depends on the distance between and relative orientations of the donor emission and acceptor absorbance transition dipole moments. Theory shows that

$$E = R_0^6/(R_0^6 + R^6)$$

where R = distance separating the donor and the acceptor transition dipoles and

$$R_0 = 8.79 \times 10^{-5} (J\kappa^2 n^{-4} \phi_D)^{1/6} \text{ in Angstrom units}$$

and $J = \int \varepsilon_A(\lambda) f_D(\lambda) \lambda^4 \, d\lambda$;
κ^2 is a factor which depends on the angle between the donor and acceptor transition dipole moments;
n is the refractive index;
ϕ_D is the quantum yield of the donor;
$\varepsilon_A(\lambda)$ is the absorbance coefficient of the acceptor at wavelength λ; and
$f_D(\lambda)$ is the fraction of donor fluorescence at λ

Hence, measurement of the efficiency of transfer yields an estimate of the distance between donor and acceptor transition moments.

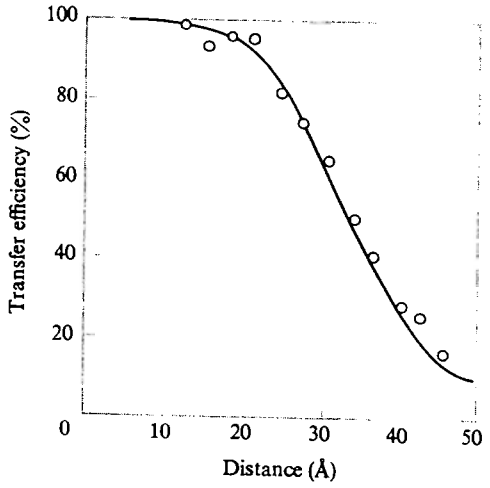
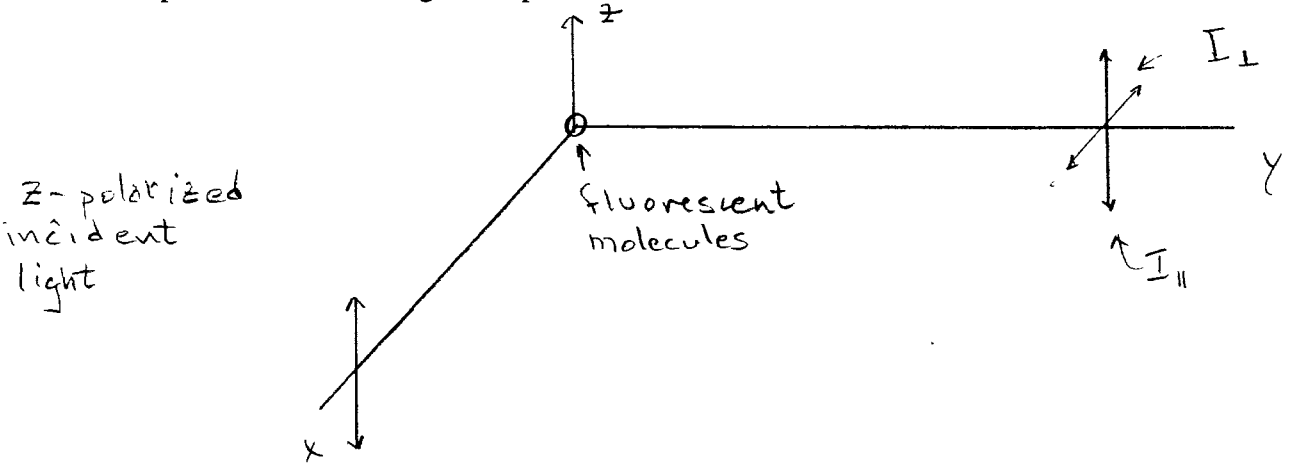


Figure 8-20

Efficiency of energy transfer as a function of distance in dansyl-(L-prolyl)$_n$-α-naphthyl semicarbazide oligomers with $n = 1$ to 12. The curve was fit to the data with Equation 8-57. [From L. Stryer and R. P. Haugland, *Proc. Natl. Acad. Sci. USA* 98:719 (1967).]

Fluorescence Polarization

The degree of polarization of the fluorescence light emitted by molecules excited by polarized incident light can provide an indicator of molecular rotation rate.



Suppose the angle between the absorbance transition dipole and the z-axis is θ. Then the probability of absorbing a photon is proportional to $\cos^2\theta$. Hence, only molecules with transition dipole moments parallel or nearly parallel to the z-axis absorb excitation light with electric vector polarized along z (principle of "photoselection"). If these molecules do not move during the fluorescence lifetime and if the absorbance and transition dipole moments are parallel, the fluorescence emission will also be polarized parallel to z, i.e. $I = I_{total}$ and $I_\perp = 0$. If the molecules rotate rapidly so that their orientations are randomized during the fluorescence lifetime, then the emitted fluorescence is completely depolarized. If the rotation is slower but still significant during the lifetime, then the emitted fluorescence is partially depolarized. The extent of depolarization provides a measure of the rate of rotation. Two indices of polarization are used:

the polarization, $P = (I_{//} - I_\perp)/(I_{//} + I_\perp)$ and the anisotropy, $A = (I_{//} - I_\perp)/(I_{//} + 2I_\perp)$. For molecules which do not rotate, $P_0 = (3\cos^2\xi - 1)/(\cos^2\xi + 3)$ and $A_0 = (3\cos^2\xi - 1)/5$, where ξ is the angle between the absorption and emission dipoles.
Hence, $1/2 \geq P_0 \geq -1/3$ and $2/5 \geq A_0 \geq -1/5$. For molecules rotating rapidly, so that their orientations are randomized during the fluorescence lifetime, $I = I_\perp$, and so $A = P = 0$.

Consider a spherical molecule. Its diffusion rate is determined by a rotational diffusion coefficient, $D_r = k_B T/f_r = k_B T/6V_h\eta$ where

k_B is Boltzmann's constant;
T is the absolute temperature;
V_h is the hydrodynamic volume; and
η is the solvent viscosity.

A rotational correlation time is defined as $\tau_c = 1/6D_r = V_h\eta/k_B T$.

It can be shown that $A(t) = (1/5)\exp(-t/\tau_c)[3\cos^2\xi - 1]$. Hence measurement of the anisotropy decay rate yields τ_c and therefore V_h.

Because fluorescence lifetimes are typically in the range 10^{-9} to 10^{-8} seconds, a sophisticated experimental setup is needed for this measurement. It is also possible, however, to use measurements of steady state fluorescence polarization or anisotropy to derive information about rotation rates.

$$1/\langle P\rangle - 1/3 = (1/\langle P_0\rangle - 1/3)(1 + \tau_F/\tau_c) = (1/\langle P_0\rangle - 1/3)[1 + k_B T\tau_F/(V_h\eta)]$$

and

$$\langle A\rangle^{-1} = \langle A_0\rangle^{-1}(1 + \tau_F/\tau_c) = \langle A_0\rangle^{-1}[1 + k_B T\tau_F/(V_h\eta)]$$

where τ_F is the fluorescence lifetime;
$\langle P\rangle$ and $\langle A\rangle$ are the steady-state fluorescence polarization and anisotropy, respectively;
and $\langle P_0\rangle$ and $\langle A_0\rangle$ are limiting values as $T/\eta \rightarrow 0$.