PROGRESS IN BIOPHYSICS

VOLUME 27

PROGRESS IN
BIOPHYSICS
AND MOLECULAR BIOLOGY

27

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PERGAMON PRESS
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THE ANALYSIS OF CIRCULAR DICHROISM OF BIOMOLECULES

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1. INTRODUCTION

Optical rotatory dispersion (ORD) and circular dichroism (CD) properties of molecules are determined by their conformation, i.e., their specific three-dimensional organization. The importance of conformation in the function of biological molecules is now an accepted fact, and measurement of ORD and CD spectra has become a routine means of studying molecular conformation in solution. The interpretation of these spectra is often complicated. The aim of this article is to give a simple but functional description of some of the less familiar spectroscopic principles involved, and to show how an understanding of the origins of optical activity in biomolecules can help in deriving structural information from CD spectra.

Two broad approaches to the analysis of CD are discussed.

(i) In the *phenomenological* approach, the spectra of complex molecules are analysed in terms of the sum of contributions from specific structures such as helices, coils, and sheets. This multicomponent analysis depends upon comparison of experimental spectra with those of the well-characterized structures taken alone and in simple combinations. This procedure is partially successful, but is limited by the variability of the reference states and by the degree of correspondence of these with the actual conformations present. An understanding of the origins of optical activity helps to set the limits to the interpretation of these analyses.

(ii) In the *theoretical* approach, optical rotation theory is used to predict the properties of proposed structures. For well-characterized chromophores, such as peptide, aromatic, and nucleotide, the optical properties may be computed for a variety of systems, e.g. for dimers as a function of the conformational variables; for small specific structures with a limited number of chromophores; and for large, effectively infinite, polymeric systems of regular structure, embodying the elements of helical symmetry. In addition, the irregular, yet fully specified regions of biological macromolecules can be treated, and the limitations of the multicomponent analysis can be assessed quantitatively.

The second approach with which this article is mainly concerned emphasizes that it is the total conformation which determines the optical properties. Rotational strength is, ideally, the parameter by which theory and experiment are to be compared. This quantity, which is the factor common to ORD and CD, is in general readily obtained from CD spectra, and may be derived theoretically. In all the theoretical mechanisms involved, the sign and magnitude of the rotational strength are dependent upon conformation. The methods apply to assemblies of all chromophores; particular examples will be taken from proteins and polypeptides.

Finally, applications of the theoretical ideas are discussed and examples from current experimental work are presented.

2. BASIC DEFINITIONS

Details of the basic principles may be found in the articles by Moscowitz (1960), Schellman and Schellman (1964), and Fasman (1963) and the books of Velluz and Legrand (1965) and Crabbé (1965). Principles of electronic absorption spectroscopy are treated at an intermediate level in the books by Sandorfy (1964) and Murrell (1963). Only a brief survey of principles is given here.

(i) *Absorption*. In the visible and ultraviolet regions of the electromagnetic spectrum (600–180 nm), the interaction of radiation with the polarizable electrons of the molecule causes a redistribution of the electron density, and a reduction of the velocity of the radiation (relative to velocity *in vacuo*) by the factor n, the refractive index. At certain frequencies ν, the energy of the incident radiation will satisfy the condition $\Delta E = h\nu$, where ΔE is the energy difference between the ground state and an electronic excited state, and the electron will undergo a transition into the excited state. This transition is characterized by the frequency, or wavelength at which it occurs, and the magnitude of the electronic displacement involved. In the vapour state, absorption spectra approximate to a collection of discrete
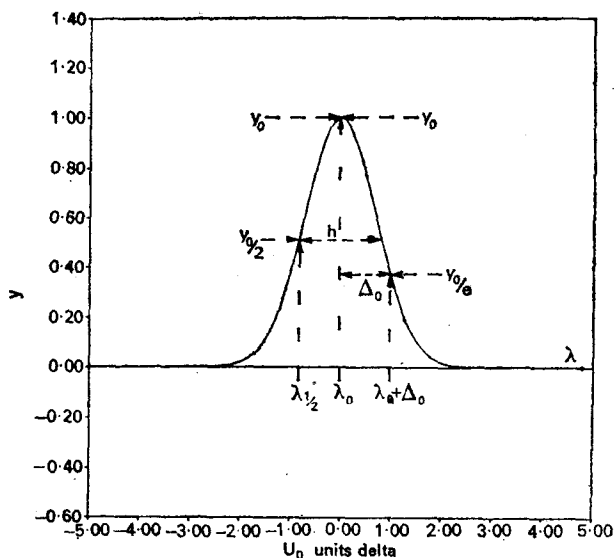
FIG. 1. Parameters of a Gaussian curve. The curve $y = y_0 \exp - u_0^2$

$$\text{where } u_0 = \left\{\frac{\lambda - \lambda_0}{\Delta_0}\right\}:$$

the area is given in terms of Δ_0, the half-width at $y_{0/e}$ by

$$\text{area} = \sqrt{(\pi)} \times y_0 \times \Delta_0$$

or, in terms of h, the width at $y_{0/2}$ by

$$\text{area} = 1.06 \times y_0 \times h$$

Δ_0 and h are related by $\Delta_0 = 0.598 h$.

lines corresponding to such transitions, with fine structure deriving from different vibrational levels of the two electronic states. In solution, the spectra are broadened by interaction of the chromophore with the solvent, vibrational structure may be lost entirely, and smooth curves are observed with defined band shape, e.g. Gaussian, log-normal (Siano and Metzler, 1969), etc. The parameters of a Gaussian curve are given in Fig. 1 and all derivations will be made for this band shape. The integrated area under the absorption band of the Kth transition yields the quantity f_K, the oscillator strength of classical theory, or its quantum-mechanical analogue, the dipole strength D_K. This quantity can be calculated from the computed electron densities of ground and excited states. The absorption spectrum may be expressed as the sum of a series of transitions, characterized by intensity D_K, located at λ_K and with Δ_K (Gaussian) bandwidth. For practical purposes it is more convenient to use the maximum absorption ϵ_{max}, λ_K and Δ_K, where ϵ is molar absorptivity (molecular extinction coefficient). Useful relationships are $f_K = 4.6 \times 10^{-9} \times \epsilon_{max} \times h$ (cm^{-1})

$$\text{and} \quad f = 1.085 \times 10^2 \times D_K/\lambda_K \text{ (nm)}$$

(ii) *Optical activity* comprises the phenomena of optical rotation and circular dichroism both of which derive from the unequal interaction of the right- and left-handed circularly polarized components of plane polarized light with asymmetric molecules. Optical activity is characterized by the rotational strength of a transition R_K, which may be positive or negative. ORD and CD spectra may therefore be represented as the sum of a series of transitions, characterized by intensity R_K, located at λ_K, and with Δ_K (Gaussian) bandwidth.

(iii) *Circularly polarized light* may be thought of as an electrical vector rotating with the appropriate handedness while simultaneously propagating linearly towards an observer. The sense of hand is that seen by the observer.

(iv) *Optical rotation.* The rotation of the plane of polarization of plane polarized light was formerly known as circular birefringence. This more descriptive title indicated the origin of the effect as the difference in n_L and n_R, the refractive indices for left- and right-handed circularly polarized light. The rotation is given by $\alpha = \pi/\lambda \, (n_L - n_R)$, with units radians per centimetre of path. $[\alpha]$, the specific rotation of a solution, concentration C g ml^{-1} is given by $[\alpha] = \alpha 1800/C\pi$, with units degree/decimetre path for 1 g/cc: it is this quantity which is measured experimentally. For a solution C g ml^{-1}, path length L decimetres, observed rotation in degrees (α_{obs}),

$$[\alpha] = \frac{(\alpha_{obs})}{CL}$$

$[\phi]$, the molecular rotation of this solution, solute molecular weight M is given by $[\phi] = [\alpha]M/100$, with units degree cm^2 decimole^{-1}.

For polymers, a mean residue molecular weight is generally taken for M, giving $[m]$ the mean residue rotation. Correction for solvent refractive index gives $[m'] = [m] \, 3/(n^2 + 2)$.

(v) *Circular dichroism* is the difference in extinction coefficient for left- and right-handed circularly polarized light.

$\Delta\epsilon = (\epsilon_L - \epsilon_R)$ where all ϵ refer to molecular extinctions and units, as for ϵ, are mole^{-1} cm^2 or M^{-1} cm^{-1}, i.e. 1 cm path of 1 molar solution.

(vi) *Ellipticity* is an alternative means of expressing circular dichroism: the differential absorptivity for left- and right-handed circularly polarized light causes the transmitted light to be elliptically polarized to a degree directly proportional to the circular dichroism.

Molecular ellipticity is given by $[\theta] = 3299$. $\Delta\epsilon$; units are degree cm^2 decimole^{-1}.

Rotational strength is given as the integrated intensity beneath a single band in CD spectra:

$$R_K \propto \int \frac{\Delta \epsilon}{\lambda} \, d\lambda$$

If the band is Gaussian,

$$\Delta \epsilon_K = \Delta \epsilon_K^\circ \exp - \left(\frac{\lambda - \lambda_K}{\Delta_K} \right)^2$$

and then

$$R_K = \frac{1}{2.28} \frac{\Delta \epsilon_K^\circ \Delta_K}{\lambda_K}$$

The units are Debye magnetons (1 DM $= 0.927 \times 10^{-38}$ c.g.s. units). The derivation of R_K from optical rotation data is discussed later (§ 6).

(vii) *The relationship of ORD and CD.* The concept of plane-polarized light as the sum of left- and right-handed circularly polarized light and the interrelationship of ORD and CD are clearly illustrated in Moscowitz (1960), Velluz and Legrand (1965), and Crabbé (1965). It is important to emphasize that there is only one physical process involved, namely the interaction of the components of the plane-polarized electromagnetic radiation (light) with the electrons of the molecule. Either a refractive property (ORD) or an absorptive property (CD) may be observed, depending upon the experimental arrangement. The optical rotation varies non-linearly (i.e. disperses) throughout the spectrum; by contrast CD occurs only in the region of absorption bands, and will be closely related to the absorption spectrum, to which reference is generally made in assigning the optically active electronic transitions of the chromophore. The analogy of [α] and Δε to refractive index and absorption coefficient for unpolarized light is close. Differences are that in the case of ORD and CD the spectra may take on both positive and negative values and that optical activity may be associated with bands which are only weak in absorption. The relationships between absorptive and refractive properties are governed by the Krönig–Kramers transform which allows either property to be computed from the other. Details are given in Lowry (1935), Moscowitz (1961), and Emeis *et al.* (1967). The general relations between intensive and extensive properties of this kind are discussed by Schellman and Schellman (1964) and qualitatively by Foss (1963).

(viii) *Conformation, configuration, and asymmetry.* Optical activity is shown by molecules which do not possess either a centre of symmetry or a mirror plane: a simple test is that the molecule is not superposable upon its mirror image. Although different usages exist, for this article the term configuration will be restricted to the spatial distribution about a single point, e.g. an asymmetric tetrahedral carbon atom (cf. IUPAC, 1970). Thus proteins are in general made up of L-amino acids; polynucleotides are based on the D-sugars. The polymers must themselves be asymmetric, being based on asymmetric units, and in addition they are capable of a variety of spatial arrangements of the constituent units. The term conformation is used to cover the full three-dimensional organization. This may involve a regular geometric transformation between units leading to a helical structure. Alternatively, if successive transformations are unrelated, a fully specified but *irregular* structure is generated.

It may now be seen why asymmetric molecules show differential properties to left- and right-handed circularly polarized light. These polarizations have helical symmetry and an asymmetric molecule, e.g. a helical molecule of defined handedness presents a differently

polarizable medium to right- and left-handed circularly polarized light. It may be noted that a helix maintains its handedness when viewed from either end. The properties in question are therefore due to the intrinsic asymmetry of the molecular structure and persist in solution. In non-helical asymmetric molecules, the polarizability similarly but less obviously has a defined handedness.

(ix) *Chromophores*. Reference will frequently be made to the properties of individual chromophores: these are groups identifiable both structurally and spectroscopically, e.g. peptide, aromatic, purine, and pyrimidine. The macromolecule exhibits properties related to its constituent chromophores. To a first approximation, a principle of additivity holds in absorption properties allowing identification of component chromophores, e.g. aromatic groups in proteins, purines, and pyrimidines in nucleic acids. Additivity clearly does not hold when electron interchange occurs, e.g. a conjugated polyene is not the sum of its constituent ethylenic groups, see, for example, Murrell (1963), but would be treated as a new chromophore itself.

(x) *Non-additivity of chromophores*. Strong interactions between component chromophores may lead to non-additive properties where the non-additivity is dependent upon conformation. The property of the assembly may be less than or greater than the sum of its parts. In absorption, hypochromism, and hyperchromism of the Kth transition, taken over n groups in the assembly, may be defined in terms of the oscillator strengths as:

$$f_{K_{obs}} < \sum_{i=1}^{n} f_{K,i}: \text{ hypochromism;}$$

$$f_{K_{obs}} > \sum_{i=1}^{n} f_{K,i}: \text{ hyperchromism.}$$

Since, for any single group, the Kuhn–Thomas sum rule (see Kauzmann, 1957) requires conservation of total oscillator strength, i.e.

$$\sum_{i=1}^{n} \sum_{K=1}^{N} f_K = \text{constant,}$$

where the sum is over all transitions, it follows that hypochromism in one part of the spectrum must be balanced by hyperchromism elsewhere, and vice versa. It may also be noted that hyper- and hypochromism may occur in situations of high symmetry and are therefore not necessarily related to optical activity.

(xi) *Optically active chromophores*. For single chromophores a useful distinction has been made by Moffit and Moscowitz (1959) and Moscowitz (1961) in terms of (a) chromophores which are intrinsically symmetrical (e.g. a planar ketone) but disymmetrically perturbed by the environment, and (b) chromophores which are intrinsically disymmetric (e.g. the isomers of the screw type of molecule hexahelicene, in which the identifiable chromophore itself has a helical sense) and in which the optical activity is larger by several orders of magnitude.

In macromolecules containing many chromophores, such mechanisms for the generation of optical activity rarely occur in isolation. The occurrence of chromophore–chromophore interactions in an asymmetric conformation gives rise to optical activity by mechanisms distinct from those above. In order to establish the common ground between these mechanisms, the electronic origins of rotational strength must be examined.

3. THE ELECTRONIC ORIGINS OF ROTATIONAL STRENGTH

The rotational strength of a transition is given by the Rosenfeld equation

$$R_K = \mathrm{Im}(\mu_e \cdot \mu_m).$$

This equation says that the rotational strength of the Kth transition is given by the imaginary part of the dot or scalar product of the electric and magnetic transition dipoles, μ_e and μ_m respectively. "Imaginary" refers to the presence of the quantity $i = \sqrt{-1}$. Thus in order to show optical activity the transition must have both electric and magnetic transition dipole properties, and since these quantities are vectors, they must have directional components in common.

The properties of electric and magnetic transition dipole derive from the nature of the redistribution of electron density between ground and excited state associated with the electronic transition. Where these two states can be accurately represented by wavefunctions for the molecule (e.g. by combination of simpler atomic orbitals) a variety of properties of the states themselves and for transitions between the states can be evaluated theoretically. Two particularly notable transition properties may be distinguished: the transition may involve the linear displacement of electronic charge, or it may involve the rotation of electronic charge about an axis. The linear translation of charge generates an *electric dipole*: in polyatomic systems the redistribution of charge over many centres may be represented as the vector sum of the individual dipoles, and the net effect is again a single electric dipole. The rotation of charge about an axis generates a magnetic moment in the direction of the axis, predicted by the right-hand rule and represented by a *magnetic dipole*. These properties are generated by the electronic transition, and are consequently known as the *electric* and *magnetic transition dipoles*. They constitute the basis of the selection rules for absorption spectroscopy:

$$\mu_e \neq 0; \quad \mu_m \simeq 0 \quad \text{electrically allowed transition (magnetically forbidden);}$$

$$\mu_e = 0; \quad \mu_m \neq 0 \quad \text{magnetically allowed transition (electrically forbidden).}$$

The dipole strength (the integrated intensity beneath an absorption band) is given by

$$D_K = \mu_e \cdot \mu_e;$$

putting $\mu_e = e\mathbf{r}$, where e ($= 4.8 \times 10^{-10}$ e.s.u.) is taken as unit charge and D_K is then expressed as Å^2. It is the electrically allowed transitions which are readily observed in absorption spectroscopy, e.g. adenine for which $\epsilon_{max} = 1.5 \times 10^4$ at $\lambda_{max} = 260$ nm and $\lambda_{\frac{1}{2}} = 280$ nm; hence $f = 0.37$, $D = 0.89$ Å^2, and $\mu_e = 4.52$ Debyes. This is equivalent to the translation of one electron over approximately 1 Å. The most common source of bands of this type are transitions of π electrons into the lowest unoccupied π^* orbital. An example of the allowed $\pi-\pi^*$ transitions is the 190 nm transition of the peptide chromophore. Electronically this transition is mainly accounted for as the displacement of π electron density from the region of the nitrogen atom into the π^* orbital when it is located mainly on the carbon and oxygen atoms (Schellman and Nielsen, 1967a). Consequently, $\mu_e \simeq 3$ Debyes, and the direction is found experimentally to be in plane at $+9°$ towards the carbon atom from the O–N line (Peterson and Simpson, 1957). The principal bands of purines and pyrimidines in the region of 260 nm have intensities of the order of 5×10^3–10^4. The electronic character of these bands has been correlated with $\pi-\pi^*$ transitions of benzene (Clark and Tinoco, 1965): the

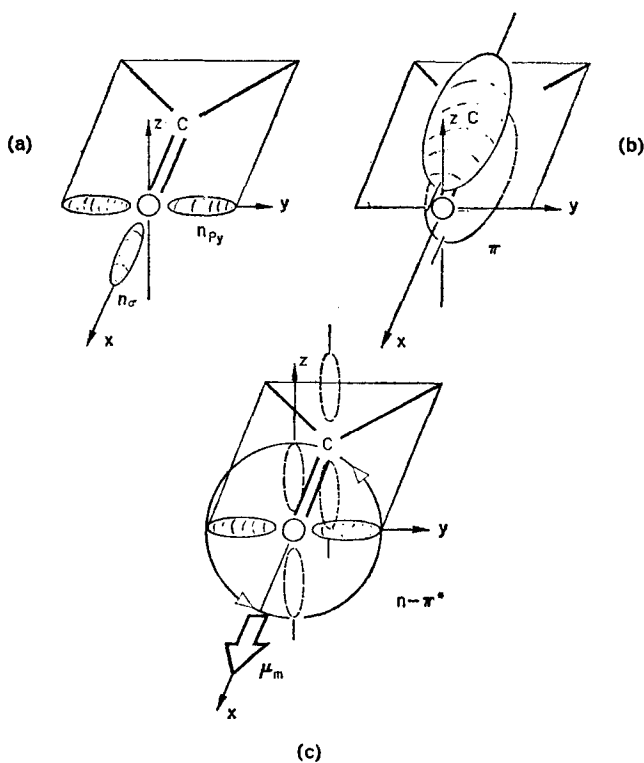FIG. 2. The carbonyl chromophore. (a) n orbitals: n_{P_y} is the optical electron, n_σ is the (linear) H-bonding electron. (b) π orbital: molecular orbital formed from C_{2P_z} and O_{2P_z} atomic orbitals in bonding combination. (c) π^* orbital (dotted): ($2P_z$ atomic orbitals in antibonding combination). The transition from n_{P_y} to π^* is magnetically allowed, generating μ_m axially.

greatly increased intensity, relative to benzene, is due to the lower symmetry of these hetero-cycles, and the substitution by N and O functions. Not all π–π^* transitions are necessarily electrically allowed: for the 260 nm transition of benzene itself, the symmetries of the ground state π and excited state π^* orbitals are such that $\mu_e \sim 0$. The transition is effectively for-bidden, the relatively low intensity ($\epsilon_{max} = 10^2$) deriving from vibrational modes which violate the strict symmetry considerations ' $\vdots$e, for example, Murrell, 1963).

The classic example of the magnetically allowed transition is the n–π^* transition of ketones (e.g. acetone $\epsilon_{278} = 15$) (Stern and Timmons, 1970) and peptides (e.g. the amide of acet-amide derivatives $\epsilon_{210} = 100$) (Nielsen and Schellman, 1967). The transition involves the displacement of an n electron (non-bonding, or inert-pair electron) into an orbital of π^* symmetry. Transitions of non-bonding electrons have been discussed in detail by Kasha (1961). Of the two non-bonding orbitals associated with C=O, it is the axial n_σ orbital which acts as hydrogen-bond acceptor, and the n_{P_y} orbital which contains the optical electron (Fig. 2a). The transition from P_y symmetry to the P_z symmetry of the π^* orbital constitutes a rotation of charge about the carbonyl axis and consequently generates a magnetic transi-tion dipole axially along the C=O bond (Fig. 2b, c). The lack of translation is reflected in $\mu_e \sim 0$, and the band is very weak in absorption: in the case of peptides it is observed only as a tail to the strong π–π^* transition.

As described here, the electrically allowed and magnetically allowed transitions constitute mutually exclusive groups. Yet the Rosenfeld equation requires the simultaneous existence of non-zero μ_e and μ_m for the generation of optical activity. This is achieved by the breakdown of the above exclusion under suitable conditions of asymmetry and is the feature common to the examples of optically active chromophores cited in the previous section. Thus while the phenomenon of optical activity is relatively commonplace, its electronic origins depend upon rather subtle effects, involving the principle of non-superposition of asymmetric isomers. This will be illustrated in § 4 with reference to specific models.

On a purely theoretical basis, if ψ_G and ψ_S are wavefunctions for the ground and excited states of a molecule, transition properties μ_e and μ_m may be evaluated using the quantum-mechanical operators for electric or magnetic dipole (see Kauzmann, 1957). μ_e and μ_m are then given by integrals between the states of the type $\mu_e = <\psi_G \mid \mathbf{E} \mid \psi_S>$, equivalent to $\int \psi_G \, \mathbf{E} \, \psi_S \, d\tau$ and $\mu_m = \psi_G \mid \mathbf{M} \mid \psi_S$ equivalent to $\int \psi_G \, \mathbf{M} \, \psi_S \, d\tau$.

For $(\mu_e)_x$, the electric dipole in the x direction, the electric dipole operator $|\mathbf{E}|$ is given by $|ex|$: this is an odd-function of x, i.e. $f(x) = -f(-x)$ and for $(\mu_e)_x \neq 0$ the transition from ψ_G to ψ_S must involve a change from odd to even function (or vice versa). For $(\mu_m)_x$, the magnetic dipole in the x direction, the magnetic dipole operator $|\mathbf{M}|$ includes the term

$$\left(y \frac{d}{dz} - z \frac{d}{dy} \right)$$

which operates on y to give $-z$ and on z to give y: thus for $(\mu_m)_x \neq 0$, ψ_G as a function of y with ψ_S as a function of z (or vice versa) is a suitable combination (Fig. 2). The selection rules for a transition may be obtained relatively simply: the validity of quantitative predictions, however, depends on the quality of the functions taken for ψ_G and ϕ_S. Such *ab initio* calculations are possibly only for the simplest systems, and consequently all calculations of rotational strength represent approximate treatments. Assumptions may be inherent at all levels, e.g. wavefunctions, the basis states or the properties of individual chromophores. Nevertheless, certain mechanisms have become well established and subjected to experimental test. It is instructive to see how individual models can account for rotational strength before considering in detail the results of a more general model.

4. MODELS FOR OPTICAL ACTIVITY AND ITS CONFORMATIONAL DEPENDENCE

Optical activity requires a transition to have both electric and magnetic transition dipoles associated with it. The mechanism for the simultaneous generation of μ_e and μ_m for a helix is shown in Fig. 3. An electron travelling in a right-handed helical path generates μ_m along the axis ($+z$) by the right-hand rule; the translational component also along $+z$ generates μ_e. In this case, R is positive. For the mirror-image situation (Fig. 3b), the sign of μ_m is reversed and R would be negative.

Helical systems are therefore amongst the simplest prototypes for optical activity. Only in certain rare disymmetric molecules are both the ground and excited *states* extended over a helical pathway as in Fig. 3a and b, when enormous values for R_K are found. Examples of this sort are hexahelicene, urobilin, an asymmetric dipyrrylmethene, and skewed conjugated dienes (Moscowitz *et al.*, 1964; Lightner *et al.*, 1970). Bilirubin bound to albumin (Blauer and King, 1970) and certain cyclic polyene antibiotics (§ 11.3; Bayley and Calam, 1972) are even more intense (cf. $R_K \sim 1.5$ DM for L-urobilin, ~ 20 DM for bilirubin-BSA, and ~ 70
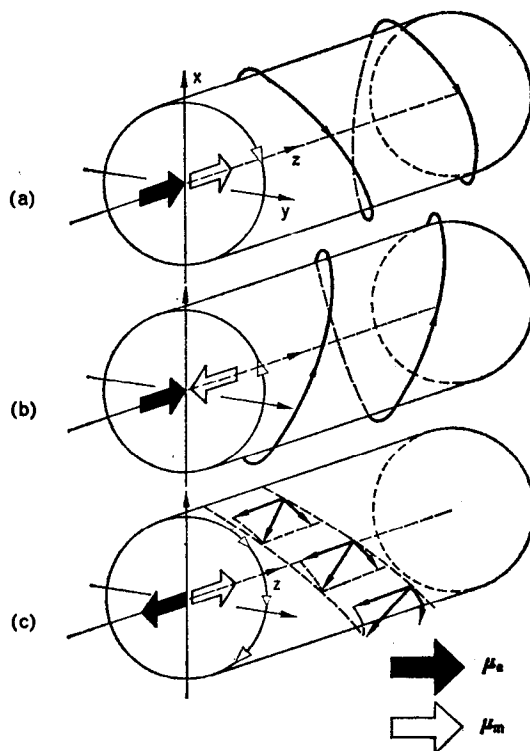
FIG. 3. Helical excitations. The generation of μ_e and μ_m by an electron travelling in (a) right-handed, (b) left-handed helical path: μ_m derives from application of the "right-hand rule": μ_e by the translation along the axis: $R = \mu_e \cdot \mu_m$ is (a) positive, (b) negative; (c) the sense of handedness of the structural helix is right-handed: the relative orientation of the (coupled) monomer μ_e (solid arrows) gives a polymer μ_e in $-z$ and μ_m in $+z$, for the fully in-phase transition. Hence $R = \mu_e \cdot \mu_m$ is *negative*.

DM for amphotericin B). These examples of disymmetry, though illuminating, are clearly exceptional.

The more general case of helical structures involves chromophores which are not conjugated but are arranged in a helical array. The interaction of chromophores in the excited states generates a set of exciton states (McRae and Kasha, 1964) in which the *excitation* is effectively delocalized through the array. Consequently the helical geometry confers simultaneous non-zero values for the μ_e and μ_m of certain exciton states. This concept is further discussed in § 8.1. It may be noted that the handedness of the structure and the handedness of the electron path on excitation are not necessarily identical (Fig. 3c).

In simpler systems involving a small finite number of symmetric (generally planar) chromophores oriented in a structure which is itself asymmetric, two principle means of generating μ_e and μ_m simultaneously will be distinguished: (i) the mixing of transitions within a chromophore, and (ii) the coupling of transitions between chromophores.

These mechanisms involve the interaction of the excited states of chromophores; ground-state electron exchange (e.g. conjugation) between chromophores is not involved. The formulation used is the matrix formulation introduced by Schellman (Schellman and Nielsen,

1967b; Bayley *et al.*, 1969) and Woody (1968) which has the advantage of relative simplicity of notation. There is a close formal resemblance to the secular matrix method for deriving molecular orbitals by linear combination of atomic orbitals (see, for example, Streitweiser, 1961). In this case, it is electronic *states* which are being combined and hence may be described as configuration interaction. The results are in accord with the general perturbation theory described by Tinoco (1962).

4.1. *The Mixing of States*

For the peptide chromophore, the $n-\pi^*$ transition (210 nm) and the $\pi-\pi^*$ transition (190 nm) correspond to transitions from ground state $|0>$ to excited states $|a>$ and $|\beta>$ respectively (Schellman and Nielsen, 1967a). The wavefunctions corresponding to these basis states are ψ_0, ψ_α, and ψ_β respectively. The orientation of μ_m of the $n-\pi^*$ and μ_e of the $\pi-\pi^*$ transitions are shown in Fig. 4a and the relative energies in Fig. 4b.
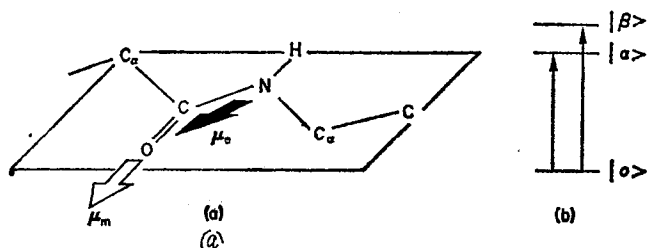

FIG. 4. The peptide chromophore. (a) The relative orientations (in plane) of μ_m ($n-\pi^*$) and μ_e ($\pi-\pi^*$) of the monomer. (b) relative energies of the states $|0>$, $|a>$ and $|\beta>$, where $|0> \rightarrow |a>$ is the $n-\pi^*$ and $|0> \rightarrow |\beta>$ the $\pi-\pi^*$ transition.

In the quantum-mechanical representation of electronic states, where some mechanism of interaction exists between two states, a better representation of physical reality is given by a linear combination of the two (cf. the LCAO method). Condon *et al.* (1937) showed that mixing of certain states can occur in the presence of an asymmetric electrostatic field. Thus for the peptide, in the presence of an asymmetric distribution of the bonding, nonbonding electrons, formal charges, and ionized groups, the excited states $|a>$ and $|\beta>$ undergo mixing. New transitions $|\phi> - |\phi_1>$ and $|\phi> - |\phi_2>$ are produced, where

$$|\phi_1> = C_{11}\psi_\alpha + C_{12}\psi_\beta,$$

$$|\phi_2> = C_{21}\psi_\alpha + C_{22}\psi_\beta.$$

The coefficients derive from the diagonalization of the matrix

$$\begin{vmatrix} E_\alpha & V_{\alpha\beta} \\ V_{\alpha\beta} & E_\beta \end{vmatrix} \quad \text{and} \quad C_{11} = C_{22} = C_1$$

$$\text{and} \quad -C_{12} = C_{21} = -C_2$$

equivalent to the solution of the simultaneous equations

$$C_1(E_\alpha - \lambda) + C_2 V_{\alpha\beta} = 0,$$

$$-C_2 V_{\alpha\beta} + C_1(E_\beta - \lambda) = 0.$$

In these expressions, E_α and E_β are the energies of states $|\alpha>$, $|\beta>$ relative to $|0>$. $V_{\alpha\beta}$ is the term representing the interaction between states $|\alpha>$ and $|\beta>$ in the asymmetric electrostatic field. It may be evaluated explicitly in terms of the electronic formulation of the basis states. In general, $V_{\alpha\beta} \ll E_\alpha, E_\beta$, and C_2 is small. The eigenvalues are $\lambda_1 \sim E_\alpha$ and $\lambda_2 \sim E_\beta$. $|\phi_1>$ is predominantly $|\alpha>$ and $|\phi_2>$ predominantly $|\beta>$. The non-zero values of C_1 and C_2 indicate that ϕ_1 also contains some $|\beta>$ character and ϕ_2 some $|\alpha>$ character. Since $|\alpha>$ and $|\beta>$ have μ_m and μ_e associated with them respectively, the states $|\phi_1>$ and $|\phi_2>$ satisfy the conditions for optical activity. These results are summarized in Table 1.

TABLE 1. GENERATION OF ROTATIONAL STRENGTH BY THE MIXING OF $|\alpha>$ AND $|\beta>$

State	Eigenfunction	Transition dipole		Rotational strength
ϕ_1	$C_1\psi_\alpha + C_2\psi_\beta$	Magnetic Electric	$C_1\mu_m$ $C_2\mu_e$	$R_1 = C_2\mu_e \cdot C_1\mu_m$ $= C_1C_2\,\mu_e \cdot \mu_m$
ϕ_2	$-C_2\psi_\alpha + C_1\psi_\beta$	Magnetic Electric	$-C_2\mu_m$ $C_1\mu_e$	$R_2 = C_1\mu_e \cdot -C_2\mu_m$ $= -C_1C_2\mu_e \cdot \mu_m$

It is seen that $R_2 = -R_1$ and hence

$$\sum_{i=1}^{2} R_i = 0.$$

This is implicit in the unitary nature of the secular matrix. The conservation rule $\sum R_i = 0$ is obeyed over this limited set of two transitions.

The unique feature of this mechanism involves the transition of an n electron in the presence of an asymmetric field. It is known as the "one-electron" mechanism. Two points are notable.

(i) The development of rotational strength R_1 in the state $|\phi_1>$ (which is principally the n–π^* transition since $C_1 \sim 1$) is accompanied by the simultaneous development of R_2 $(= -R_1)$ in $|\phi_2>$, the state from which electric dipole moment is "borrowed" (which is principally the π–π^* transition); thus more than one state is involved, and consequently the name is not to be taken too literally. (It will be referred to as the CAE mechanism, Schellman, 1968.)

(ii) If μ_e and μ_m had been perpendicular to one another, no rotational strength would have developed by this mechanism even in the presence of an asymmetric field; this situation is likely to be encountered in heterocycles with in-plane π–π^* and perpendicular n–π^* transitions (Rich and Kasha, 1960).

A more sophisticated treatment would include interactions with higher energy electrically allowed transitions β_j assuming explicit forms were available for computing the corresponding terms $V_{\alpha\beta_j}$; the conservation law would then hold over the set of (α, β_j) interactions.

The conformational dependence of this mechanism resides in the nature of the asymmetric perturbation. This determines the sign of $V_{\alpha\beta}$, hence the phase relations of C_1 and C_2 and the ultimate absolute sign of R_1 and R_2. The symmetry rule for the peptide follows a quadrant rule based on the C=O atoms (Schellman, 1966a). For carbonyl alone, an octant rule is predicted (Moffitt et al., 1961). The symmetry rules for optical rotation have been described in detail (Schellman, 1968).

In the case of polypeptides, the contribution of this CAE mechanism to the n–π^* rotational