ANISOTROPIC ABSORPTION OF LINEARLY POLARIZED LIGHT BY CYLINDRICAL MOLECULES

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ABSTRACT A general theory relating the orientation of the transition moment in a chromophore to incident light, linearly polarized at an arbitrary angle, is discussed for cylindrical molecules. Experimental verification of this theory is presented for molecules of tobacco mosaic virus (TMV), T_2 DNA, and polyadenylic acid (poly-A).

The ultimate purpose of dichroic studies of biological molecules is to determine the orientations of the transition moments associated with various chromophoric groups with respect to some fixed reference. This fixed reference is usually an orthogonal coordinate system with at least one axis along a distinguishing dimension of the molecule, with the transition moment being a vector characteristic of a particular absorption transition of a chromophore. In order to assign a chromophore a unique position within a molecule, it is necessary to have knowledge of the direction of the transition moment within the chromophore itself for the absorption band of interest. This information is usually obtained separately, either experimentally as, for example, Stewart and Davidson (1963) have shown for several purines and pyrimidines, or theoretically, for example, as Takashima (1969) has shown for adenine and uracil. Also, the more transition moments that can be spatially related to both a fixed coordinate system and the chromophore, the more uniquely can the chromophoric orientation be defined within the molecule. Dichroic data, then, yield a specific type of information which, when combined with other physical-chemical data, can help determine the intricate internal structure of biological molecules.

Cylindrically shaped molecules are particularly well suited for dichroic measurements because of their asymmetry, as they can be oriented in a velocity gradient. These oriented molecules are usually irradiated with light polarized parallel and perpendicular, respectively, to the flow lines, and the dichroic ratio calculated by dividing parallel by perpendicular absorbance. The theory for this special case of anisotropic absorption has been described by Fraser (1953), and Mayfield and

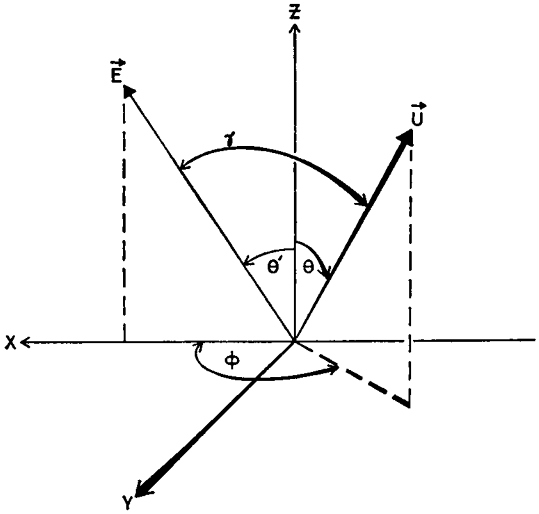

FIGURE 1 Geometric relationship of radiation incident on a rod perfectly oriented along the Z axis with transition moment $\bar{U}$ at angles θ, ϕ. The direction of propagation of the light wave is the Y axis; $\bar{E}$ is polarized at an arbitrary angle θ'. γ is the angle between $\bar{E}$ and $\bar{U}$.

Bendet (1970). We present here a quantitative extension of anisotropic absorption theory for cylindrically symmetric molecules relating the angle of the transition moment in the chromophore to an arbitrary angle of incident linearly polarized light.

Following Fraser's semiclassical approach (1953), the absorption of a chromophore is proportional to $(\bar{E}\cdot\bar{U})^2$, where $\bar{E}$ is the electric vector of the incident linearly polarized light and $\bar{U}$ is the transition moment of the chromophore, or to $\cos^2\gamma$, where γ is the angle between $\bar{E}$ and $\bar{U}$. If our chromophore has a linear transition moment oriented at angles θ, ϕ with respect to an external spherical coordinate system (see Fig. 1), and $\bar{E}$ is incident at an arbitrary angle θ' ($\phi' \equiv 0$), then it can be shown by trigonometry that

$$\cos\gamma = \cos\theta\cos\theta' + \sin\theta\sin\theta'\cos(\phi - \phi').^1 \qquad (1)$$

The case of cylindrical symmetry is illustrated by rotating $\bar{U}$ in Fig. 1 about the Z axis so that it describes a cone of constant half-angle θ and takes on all values of ϕ, $0 \leq \phi \leq 2\pi$.

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[1] Equation 1 is derived by equating two equivalent forms of the scalar (dot) product between $\bar{E}$ and $\bar{U}$:

$$(\bar{E}\cdot\bar{U}) = EU\cos\gamma = E_xU_x + E_yU_y + E_zU_z,$$

where

$$\bar{U} = U\sin\theta\cos\phi\hat{\imath} + U\sin\theta\sin\phi\hat{\jmath} + U\cos\theta\hat{k},$$

and

$$\bar{E} = E\sin\theta'\cos\phi'\hat{\imath} + E\sin\theta'\sin\phi'\hat{\jmath} + E\cos\theta'\hat{k},$$

($\hat{\imath}$, $\hat{\jmath}$, $\hat{k}$, are unit vectors along the X, Y, Z axes, respectively). We then obtain

$$\cos\gamma = \sin\theta\sin\theta'(\cos\phi\cos\phi' + \sin\phi\sin\phi') + \cos\theta\cos\theta',$$

which yields equation 1 by trigonometric identity.

To derive an expression proportional to absorption, one can then integrate:

$$\int A(\theta', \theta, \phi)\, d\phi = \frac{K}{2\pi} \int_0^{2\pi} \cos^2 \gamma\, d\phi,$$

where the factor $1/(2\pi)$ is incorporated for normalization purposes and K is a constant of proportionality. Then

$$A(\theta', \theta) = K[\cos^2 \theta \cos^2 \theta' + \tfrac{1}{2} \sin^2 \theta \sin^2 \theta']. \tag{2}$$

For perfectly oriented rods, equation 2 describes the absorption as a function of incident polarization angle θ' and transition moment angle θ.

Setting the derivative of $A(\theta', \theta)$, with respect to θ', equal to zero gives

$$\frac{\partial A(\theta', \theta)}{\partial \theta'} = 0 = K \sin \theta' \cos \theta' (\sin^2 \theta - 2 \cos^2 \theta), \tag{3}$$

which indicates an extreme (maximum or minimum) absorption at $\theta' = 0$ and 90° *regardless of the transition moment angle θ.*

The second derivative,

$$\frac{\partial^2 A(\theta', \theta)}{\partial (\theta')^2} = K (\sin^2 \theta - 2 \cos^2 \theta)(\cos^2 \theta' - \sin^2 \theta'), \tag{4}$$

indicates whether a maximum or minimum will occur at $\theta' = 0$ or 90° for a particular angle θ. For $0° \leq \theta < 54°44'$, $\theta' = 0°$ corresponds to a maximum absorption, while for $54°44' < \theta \leq 90°$ a minimum absorption occurs. This is reflected by the dichroic ratio $A(\theta' = 0°, \theta)/A(\theta' = 90°, \theta)$ being greater than 1 for $\theta < 54°44'$ and less than 1 for $\theta > 54°44'$. A dichroic ratio greater than 1, then, does not necessarily imply a transition moment angle "more parallel than perpendicular to the long axis of the molecule," as has been previously indicated for TMV (Schachter, Bendet, and Lauffer, 1966; Gabler, 1967). The special case of $\theta = 54°44'$ represents the transition moment angle at which no dichroism occurs for cylindrically symmetric molecules. Equation 4 also indicates that a point of inflection will occur at $\theta' = 45°$ when $A(\theta', \theta)$ is plotted as a function of θ'.

If one plots the empirically formulated expression $[A(\theta', \theta) - A_u]/A_u$ as a function of θ', where A_u represents the absorption of randomly oriented particles, one obtains a family of curves (see Fig. 2) to which experimental data may be fitted, for a particular wavelength, to obtain θ.

Experimental confirmation of the shape of the curves in Fig. 2 appears in Fig. 3. Solutions of TMV, poly-A, and T_2 DNA were oriented in the flow apparatus of Schachter et al. (1966), their absorbance measured at $\lambda = 260$ nm, since an absorption maximum is located here for nucleic acids, and the quantity $[A(\theta', \theta) - A_u]/A_u$

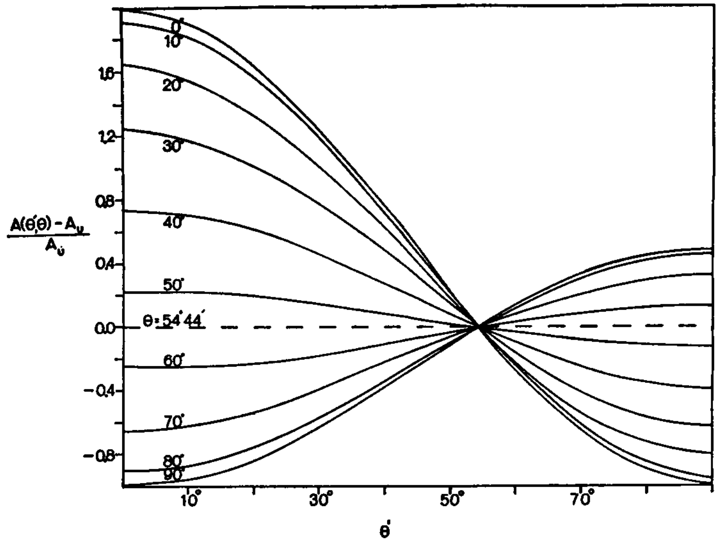

FIGURE 2 Theoretical curves for the quantity $[A(\theta', \theta) - A_u]/A_u$ vs. θ', where $A(\theta', \theta)$ and A_u are the absorbances of partially oriented and randomly oriented molecules, respectively. A_u is calculated to be 0.333.

calculated as a function of θ'. All three curves represent the results obtained from essentially the same velocity gradient which, however, does not produce perfect orientation of the molecules.

In order to correct for this lack of perfect orientation, θ must be related to the over-all orientation of the molecule in the flow gradient. For rigid molecules, Mayfield and Bendet (1970) have related the angle that the particle makes with the Z axis to a relationship which describes the distribution of orientations of the particles about this Z axis as a function of the flow gradient. Also, by measuring the anisotropic turbidity of a solution of rigid cylindrical particles, such as TMV, the above authors showed that it is possible to calculate the extent of orientation of the particles and, hence, to extrapolate dichroic ratio measurements to perfect orientation. In addition, this theory indicates that for imperfectly oriented particles, the value of θ as calculated from the experimental results will be closer to 54°44′ than the true value. Fig. 2 also indicates that resolution of dichroic measurements is best for $20° \leq \theta \leq 70°$. This reduced resolution for $70° \leq \theta \leq 90°$ and $0° \leq \theta \leq 20°$ is inherent in the measurement on cylindrical molecules, and conceivably could account for Gray and Rubenstein's (1968) difficulty in distinguishing the A and B forms of DNA by dichroism.

Flexible macromolecules, as DNA is assumed to be, present a somewhat different and more complex problem, in that the molecular conformation, and hence the orientation of the transition moments, depends on the flow gradient. Considering models of equivalent ellipsoids of revolution for such molecules, Wada (1964) calcu-

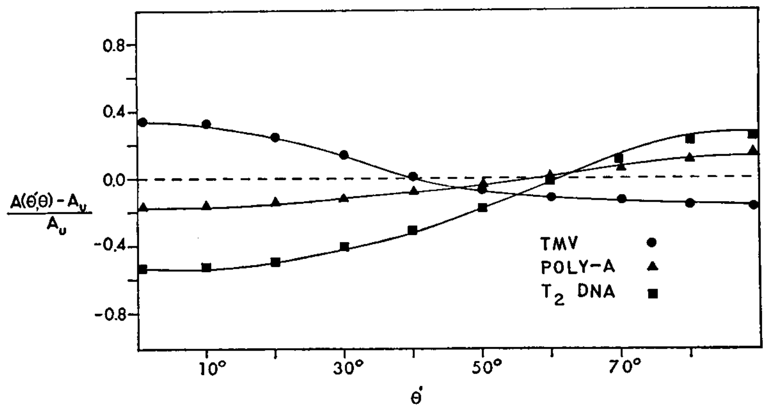

FIGURE 3 Experimental results for $[A(\theta', \theta) - A_u]/A_u$ vs. θ' at $\gamma = 260$ nm for solutions of TMV, poly-A (both in phosphate buffer, pH 5.15, 0.1 μ), and T_2 DNA (in 0.1 M NaCl). A_u is experimentally determined by a separate measurement. The TMV spectrum has been corrected for scattering by extrapolation from a nonabsorbing region (Leach and Scheraga, 1960), while the poly-A and DNA were not corrected since they exhibit little or no scattering. The TMV was prepared by the method of Boedtker and Simmons (1958) while the T_2 DNA was prepared according to the method of Mandell and Hershey (1960). The poly-A, obtained from Miles Laboratories, Elkhart, Ind., was dissolved in pH 7.0 phosphate buffer and then dialyzed to pH 5.15.

lated an "external" (particle) orientation function related to the rotary diffusion coefficient and velocity gradient. He then superposed experimental curves of reduced dichroism, $\Delta\epsilon/\epsilon = [\epsilon_{\parallel}(\lambda) - \epsilon_{\perp}(\lambda)]/\epsilon(\lambda)$ [where $\epsilon_{\parallel}(\lambda)$ and $\epsilon_{\perp}(\lambda)$ are the molecular absorptivities for partially oriented particles with $\bar{E}$ polarized parallel and perpendicular, respectively, to the flow lines, and $\epsilon(\lambda)$ is the absorptivity for unoriented molecules irradiated by unpolarized light] vs. velocity gradient on this theoretical plot to obtain θ as well as the rotary diffusion coefficient.

Fig. 3 suggests that for $\lambda = 260$ nm TMV fits the theoretical curves for $45° \leq \theta \leq 50°$, T_2 DNA for $65° \leq \theta \leq 70°$, and poly-A for $55° \leq \theta \leq 60°$. For the special case of $\theta' = 0°$, Mayfield (1968), after applying his orientation correction, predicted a value of $45° \leq \theta \leq 50°$ for TMV. Wada (1964) and Gray and Rubenstein (1968) obtained experimental results for $\theta' = 0°$ similar to those presented in Fig. 3 for T_2 DNA. When Wada's orientation correction was applied to his own work, θ was calculated to be 90°. Using this same orientation correction, Gray and Rubenstein obtained a value of $\theta \geq 77°$. The value of θ obtained for poly-A is somewhat smaller than the minimum of 70° which would be predicted by the model of Rich, Davies, Crick, and Watson (1961), but this is thought to be due to the fact that poly-A is a smaller molecule than either T_2 DNA or TMV, and hence oriented less perfectly in the flow gradient.

The analysis of dichroic measurements to obtain transition moment orientations need not be restricted to only one wavelength, as has been done here, but may be extended to any number of different absorption bands (and associated transition moments) in the molecular spectrum. Meaningful information about individual transition moments, and hence, chromophore orientations, however, can be obtained only when one chromophore dominates an absorption band. Overlapping absorption bands due to different transition moments yield average transition moment orientations, and for complicated absorbing molecules such as TMV, this presents a rather formidable problem in interpretation. Schachter et al. (1966) have discussed this problem for TMV, and work has recently been completed[2] to isolate individual chromophoric contributions in TMV-like molecules.

For systems other than those considered here, it is possible that values of θ' other than 0 or 90° will lead to additional information about the transition moment angle. In particular, for a crystalline system in which all the transition moments are parallel, the absorption is proportional to $\cos^2\gamma$. For the cylindrically symmetric systems considered here, however, we have shown that in an anisotropic absorption measurement, values of θ' other than 0 or 90° also can lead to values of the transition moment angle θ, although the greatest effects (maximum or minimum absorption of polarized light) will always occur at $\theta' = 0$ or 90° for oriented molecules.

Thus measurements of absorption at any value of θ' and a knowledge of the overall particle orientation are sufficient to allow one to calculate values of the transition moment angle with respect to some fixed axis. In order, however, to determine the exact relation of the chromophore to this same axis, it is also necessary to know the geometric relationship between the transition moment and the chromophore itself.

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