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USE OF ELECTRIC DICHROISM TO STUDY POLYMER CONFORMATION*

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Abstract

A new, highly sensitive method to measure the linear dichroism of polymer solutions is described. It can provide information about the permanent dipole moment and about the orientation, mobility and electronic properties of side chains of helical polymers. Preliminary results are reported for helical poly-L-tyrosine in dioxane solution, oriented by a static electric field.

Introduction

Electric dichroism (ED), which is a form of linear dichroism in which molecules are (partially) oriented by an electric field, is one of many methods which utilize the anisotropic nature of the absorption process (to the first order, absorption depends on the interaction of the electric vector of the light with the electric dipole transition vector) to provide direct information about the vector properties of the absorber, e.g., polarization of transitions and orientation of chromophores. In addition, the permanent dipole moment, $\underline{\mu}$, is determinable, since the molecules are oriented because of the interaction

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of the permanent dipole with the electric field. We report here a new technique for studying ED [the electric dichroic rotation of a medium in a static electric field ($\underline{E}$)], in which very small effects can be measured, and which can yield information about $\underline{\mu}$, side-chain orientation and mobility, and polarization of transitions in polyamino acids and other polymers.

Summary of Theory[¶]

The rotation (α) of a linearly polarized light beam by an oriented medium depends on four properties: 1) ED: $\epsilon_{\parallel} - \epsilon_{\perp}$ (the one of interest here); 2) electric birefringence (EB): $n_{\parallel} - n_{\perp}$; 3) circular dichroism (CD): $\epsilon_L - \epsilon_R$; and 4) circular birefringence (ORD): $n_L - n_R$; where ϵ = molar extinction coefficient, n = refractive index, $\parallel$ and $\perp$ indicate linearily polarized light parallel and perpendicular to $\underline{E}$, respectively, and L and R indicate left- and right-circularly polarized light, respectively. Of course, CD and ORD are found in isotropic media. The general change in the polarization of a light beam can be treated by the Mueller matrix method[1-3], in which the medium is a linear operator acting on the incident polarized light. For an electrically ordered medium in which $\underline{E}$ is perpendicular to the direction of propagation of the beam, the matrix method (which can greatly simplify the analysis of many optical devices used to study anisotropic media) yields

$$\alpha(\text{degs}) = 180\left\{\frac{\ln 10}{4\pi}(\epsilon_{\parallel} - \epsilon_{\perp})\ell c \sin 2\theta - \frac{\pi}{2}\left[(n_{\parallel} - n_{\perp})\frac{\ell}{\lambda}\right]^2 \sin 4\theta + (n_L - n_R)\frac{\ell}{\lambda}\right\} + \text{negligible terms of higher order, for } |\alpha| \leq 1^\circ \quad (1)$$

[¶] Details of the theory, method and results will be presented elsewhere.

where λ = wavelength of light in vacuum, ℓ = path length in medium, c = molar concentration, and θ = angle between major axis of oncoming emergent beam and $\underline{E}$ (clockwise rotation from $\underline{E}$ being positive). For $\theta = 45°$, and assuming the field dependence of $n_L - n_R$ to be negligible,

$$\alpha_{ED,45°} = \left(\alpha_{E\neq 0} - \alpha_{E=0}\right)_{45°} = 33\left(\epsilon_{\parallel} - \epsilon_{\perp}\right)\ell c \qquad (2)$$

Since the permanent dipoles are oriented by $\underline{E}$, the chromophores (with electric dipole transition moments, $\underline{\mu}_{oi}$ at angles ξ_{oi} to $\underline{\mu}$) are also oriented. By averaging over all orientations of the permanent dipole with respect to $\underline{E}$, the electric dichroic absorption is[4]

$$\epsilon_{\parallel} - \epsilon_{\perp} = 3f\left(\frac{\mu E}{kT}\right) \sum_i g(\xi_{oi})\epsilon_{oi,E=0} \qquad (3)$$

$$\begin{aligned} f\left(\frac{\mu E}{kT}\right) &= 1 - \frac{3kT}{\mu E}\coth\left(\frac{\mu E}{kT}\right) + 3\left(\frac{kT}{\mu E}\right)^2 \\ &= \frac{1}{15}\left[\left(\frac{\mu E}{kT}\right)^2 - \frac{2}{21}\left(\frac{\mu E}{kT}\right)^4 + \cdots\right] \end{aligned} \qquad (4)$$

$$g(\xi_{oi}) = \frac{3\cos^2\xi_{oi} - 1}{2} \qquad (5)$$

where k = Boltzmann constant, T = absolute temperature, and o and i indicate the ground and i^{th} excited states, respectively. For a helical homopolyamino acid, the symmetry requires that $\underline{\mu}$ be parallel to the helix axis. The function $g(\xi)$, which ranges between +1 at $\xi = 0°$ and -0.5 at $\xi = 90°$ (and is zero for a random distribution of $\underline{\mu}_{oi}$'s relative to $\underline{\mu}$), contains the important conformational information, and also enables ED to be used to help resolve overlapping absorption bands into those from single transitions. By determining the ξ_{oi}'s, and knowing the directions of the $\underline{\mu}_{oi}$'s within the chromophores, we can establish the orientation of the side-chain chromophore relative to the helical backbone. Other considerations¶ important in the interpretation of $(\epsilon_{\parallel} - \epsilon_{\perp})$ are: the shape of the macromolecule, the contribution of the polarizibility to the orientation distribution, molecular-weight heterogeneity, distribution of the side-chain conformation (degree of randomness), exciton coupling, overlapping absorption bands, and form dichroism. For the conditions of eq. 2, $\alpha_{ED,45°}$ becomes

$$\alpha_{ED,45°} = 99f\left(\frac{\mu E}{kT}\right)\ell c \sum_{i} g(\xi_{oi})\epsilon_{oi,E=0} \qquad (6)$$

Method

A Cary model 60 spectropolarimeter was used to measure α. The cell was constructed from Kel-F, with parallel stainless steel electrodes and strain-free quartz end windows, and mounted in a rotatable (θ being adjustable) insulating holder, which was placed in the cell compartment. The voltage was generated by a Spellman (Model 2040) variable DC high voltage power supply, and was measured (to a precision of < 1%) with a high voltage potential divider and potentiometer arrangement. In this way, the Cary model 60 spectropolarimeter becomes a highly sensitive ED instrument to measure $(\epsilon_{\parallel} - \epsilon_{\perp})\ell c$ as low as 3×10^{-5} with minimal auxiliary apparatus.

The quantities $\underline{\mu}$ and $g(\xi)$ are computed by two different methods: (1) from the initial slope and intercept (ordinate) of an $\alpha_{ED,45°}/E^2$ vs E^2 plot and (2) from the slope and intercept (abscissa) of the tangent at the inflection point of an

$\alpha_{ED,45°}$ vs E plot. The experimental value of $g(\xi)$ is

$$g(\xi)_\lambda = \sum_i g(\xi_{oi})\epsilon_{oi,E=0}/\epsilon_{E=0} \tag{7}$$

The absorption, CD and ORD of poly-L-tyrosine (PT) (DP~200) was studied in dioxane (D), methanol (MeOH), and dimethylformamide (DMF), and the ED in dioxane. The CD and ORD data indicated that the polymer is helical in these solvents.

Results and Discussion

The absorption and ED spectra of PT in D are shown in Fig. 1. In other ED measurements, α exhibited a sin 2θ behavior at 278 mμ for E = 20.7 KV/cm., suggesting that the sin 4θ term in eq. 1 is negligible. From the data of Fig. 1, a value of <u>μ</u> of

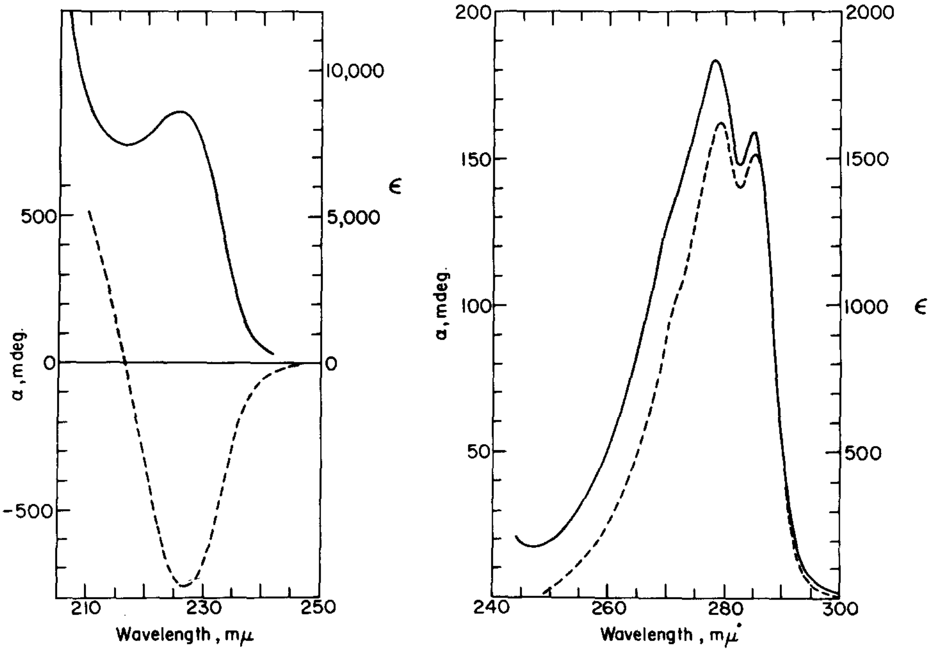

Fig. 1. The spectra of PT in D at room temperature: solid curves, molar extinction coefficient; dashed curves, ED [$\alpha_{ED,45°}$] in millidegrees, for $c = 1.46 \times 10^{-4}$ M, E = 21.3 KV/cm, ℓ = 1.05 cm.

1090 Debyes is obtained; this is consistent with the conclusion that the polymer is highly helical. The computed values of g(ξ) are small, being +0.042 at 279.6 mμ and -0.043 at 227 mμ.

In interpreting the values of g(ξ) in terms of the orientation of the side chain, it must be recalled that the directions of the transition moments within the tyrosyl group are along the in-plane short axis for the 278 mμ transition and along the in-plane long axis for the 226 mμ transition[5,#]. From conformational energy calculations[6-8], it appears that only about 15% of the area of a χ_2 vs χ_1 contour map (where the χ's are the side-chain dihedral angles) for an α-helical backbone is energetically allowable; hence, the small values of g(ξ) cannot be due to a <u>random</u> distribution over <u>all</u> values of χ_1 and χ_2. <u>Some</u> of the energetically allowed values[6-8] of χ_1 and χ_2 would yield values of g(ξ), which are much higher than those observed here, and therefore can be eliminated from consideration. In the 15% energetically allowed area, the side chain may either be restricted to a specific, fairly rigidly fixed conformation, or may be randomly distributed over this <u>restricted</u> region of χ_1 and χ_2; this possibility, and similar studies on other polymers, are currently being explored.

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[#] Below 226 mμ, it is not yet clear how to assign the polarizations of the transitions of the side chain. Nevertheless, it is likely that the large increase in $\alpha_{ED,45°}$ below 225 mμ arises from the contribution from the parallel-polarized (g = +1) component of the exciton split π→π* amide backbone transition of the α-helix.

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