Communications to the Editor

Absorption Spectra of Polypeptide Films in the Vacuum Ultraviolet

The lack of experimental information on the energies, oscillator strengths, and transition moment directions of electronic transitions in the peptide chromophore at wavelengths shorter than 185 mμ severely limits the development of the theories of optical rotatory power and of hypo- and hyperchromism. This is because optical rotatory and absorption properties of bands at wavelengths greater than 185 mμ are derived in large part from orientation-dependent interactions with transitions further in the ultraviolet. A sound theoretical basis for the optical properties of proteins and polypeptides is necessary in order to deduce conformation of proteins in solutions and in order to interpret changes in circular dichroism, optical rotatory dispersion, and absorption patterns in terms of changes in the relative orientation of groups within the protein. We have begun a study of the polarized absorption spectra of polypeptide films in the vacuum ultraviolet. At this stage we wish to communicate the absorption spectrum of poly-L-alanine films in the 240–130-mμ range, the polarized absorption spectrum of an oriented film of poly-γ-methyl-L-glutamate in the same wavelength interval, and the absorption curve of N-methylacetamide in the condensed phase.

The data were obtained on a McPherson Model 218 scanning monochromator equipped with a McPherson Model 665 double beam attachment and on McPherson Models 781 and 782 dual channel ratio recording systems and confirmed on a similarly equipped Model 225 scanning monochromator. The polarizers were of the type described by Robin, Kuebler, and Pao[1] and were purchased from McPherson Instruments. Lithium fluoride disks were mounted on a substrate holder which could be rotated on the beam axis without opening the vacuum chamber.

The poly-γ-methyl-L-glutamate film was cast on a lithium fluoride disk from a trifluoroethanol solution and oriented by stroking with a fine wire or by rolling with a glass rod. The poly-L-alanine film was cast from a solution of 10% trifluoroacetic acid in trifluoroethanol. The coated lithium fluoride disk was mounted immediately before the phosphor detector such that essentially all forward scatter was captured. Extension of the curves from 300 to 250 mμ allowed a small correction for light scattering. Base lines were run on the lithium fluoride disk before coating and after removal of the film by washing with the respective solvent. The before and after base lines were essentially identical till 150 mμ; reproducibility was approximately ±3% transmittance below 150 mμ. The facility with which films are removed from the LiF disk and the original base line regained demonstrates that the solvents do not adhere to the disk and do not result in absorption. On the contrary, sonication in trifluoroethanol is an excellent way of improving the transmission properties of lithium fluoride. One cannot be as satisfied that solvent molecules do not remain associated with the polypeptide in the film. However, the films are dried under an infrared lamp and then placed in the vacuum sample compartment. Curves run immediately after obtaining a sufficient vacuum (10^{-5} mm) were essentially identical with those run after 12 hr in the continuously pumped vacuum chamber. Absorption errors due to irregular film thickness were minimized by working at low absorbances. N-Methylacetamide was studied in a very short path length cell with LiF windows.

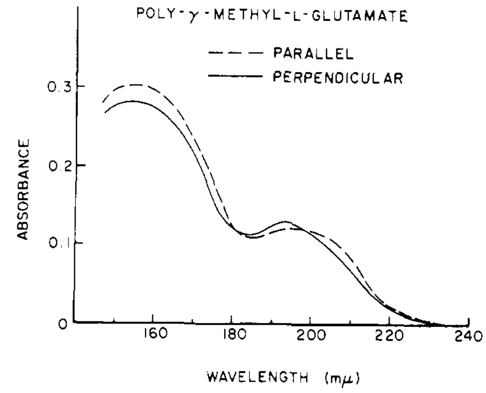

Figure 1. Polarized absorption curve of poly-γ-methyl-L-glutamate film case from a trifluoroethanol solution and oriented by unidirectional stroking with a wire or by rolling with a glass rod. The band between 200 and 210 mμ and the band peaking near 190 mμ are seen to be polarized parallel and perpendicular to the helix axis, respectively. The absorptions at shorter wavelengths between 130 and 170 mμ exhibit a preferred parallel absorption.

Gratzer, Holzwarth, and Doty[2] determined the polarized absorption spectra of both poly-γ-methyl-L-glutamate and poly-L-alanine to 185 mμ; they observed a parallel polarized band in the 200–210-mμ wavelength interval and a perpendicularly polarized band at 190 mμ. The qualitative splitting features of the monomer N–V_1 transition had been predicted for the α helix by Moffitt.[3,4] Later Holzwarth and Doty[5] showed a band near 220 mμ to have a perpendicular polarization. Figure 1 contains the polarized absorption spectra of poly-γ-methyl-L-glutamate (PγMG). The bands and polarizations reported by Gratzer, Holzwarth, and Doty are observed in this study. In addition a broad absorption at shorter wavelengths (130–175 mμ) is seen to have a parallel polarization. PγMG has the advantages of being soluble in trifluoroethanol and of making a well-oriented film with minimal scatter, but it has the disadvantage of containing the ester chromophore in the side chain. Poly-L-alanine, on the other hand, does not contain a highly absorbing side chain but neither does it make a well-oriented film with low light scattering. The latter point may derive

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(1) M. B. Robin, N. A. Kuebler, and Y-H. Pao, *Rev. Sci. Instr.*, **37**, 922 (1966).

(2) W. B. Gratzer, G. M. Holzwarth, and P. Doty, *Proc. Nat. Acad. Sci. U.S.*, **47**, 1785 (1961).

(3) W. Moffitt, *ibid.*, **42**, 736 (1956).

(4) W. Moffitt, *J. Chem. Phys*, **25**, 467 (1956).

(5) G. Holzwarth and P. Doty, *J. Amer. Chem. Soc.*, **87**, 218 (1965).

from the requirement of trifluoroacetic acid to achieve an adequate concentration of high molecular weight poly-L-alanine.[6] One can make a nonoriented film with minimal scattering. Accordingly a nonpolarized absorption spectrum of poly-L-alanine was obtained. It is also possible to obtain a low concentration of low molecular weight poly-L-alanine without addition of trifluoroacetic acid. The polypeptide in these solutions is still highly helical although the molar ellipticities are slightly lower than for the high molecular weight polymer.[6] Films of low molecular weight poly-L-alanine cast from a trifluoroethanol solution were also studied.

The absorption spectra of poly-L-alanine (film) and N-methylacetamide (pure liquid) in the wavelength interval 240–120 mμ are reported in Figure 2. High molecular weight poly-L-alanine, cast from a solution in which it is helical, exhibits two major absorptions near 190 and 165 mμ, an increasing absorbance at shorter wavelengths and a shoulder on the long wavelength edge. The oscillator strength of the band near 165 mμ is approximately 0.1. Low molecular weight poly-L-alanine cast from a trifluoroethanol solution exhibits the band near 165 mμ but with a lower intensity relative to the 190-mμ band.

The differences between the absorption of poly-L-alanine and N-methylacetamide in the 170–150-mμ wavelength range are of particular interest. The absorption band near 165 mμ does not occur in the disordered peptide chromophore; it is lower with low molecular weight poly-L-alanine in accord with lower molar ellipticities of the solution circular dichroism spectra, and therefore it would appear that the band near 165 mμ arises from orientation-dependent interactions between identical peptide units in helical array. Recently Barnes and Rhodes[7] have calculated the optical absorption properties of helical polypeptides and obtained an intense, parallel polarized[8] band near 160 mμ which arises from a constructive interference between bands at shorter and longer wavelengths.[9] To our knowledge the data reported above provide the first evidence of such a resonance band in polymers.

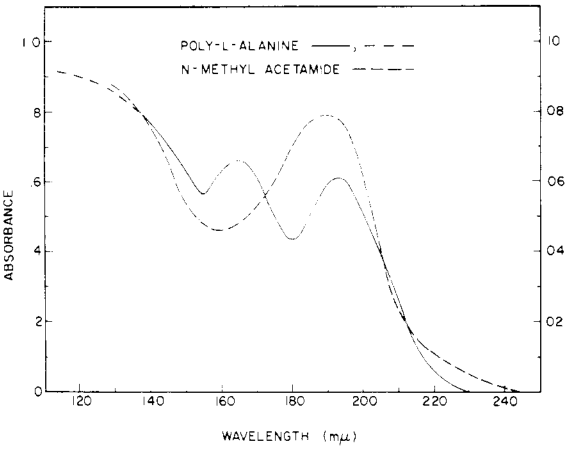

Figure 2. Absorption spectrum of a poly-L-alanine film cast from a solution of 10% trifluoroacetic acid in trifluoroethanol (right-hand ordinate) and of a liquid sample of N-methylacetamide (left-hand ordinate). Note the differences in absorption near 160 mμ.

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(6) F. Quadrifoglio and D. W. Urry, *J. Amer. Chem. Soc.*, **90**, 2755 (1968).

(7) D. G. Barnes and W. Rhodes, *J. Chem. Phys.*, **48**, 817 (1968).

(8) The necessary condition for preferred parallel absorption at wavelengths less than 180 mμ is observed for oriented films of poly-γ-methyl-L-glutamate. However, since the ester chromophore also absorbs in this region and since these groups may assume preferred conformations, one cannot exclude the possibility that the observed parallel polarization may arise in part (or in total) from the ester groups.

(9) W. Rhodes, private communication.

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