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# Circular Dichroism of Oriented Photoreceptor Membrane Film

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Summary: The circular dichroism of photoreceptor membrane oriented in multilamellar films has been measured. In the visible region the 500 nm alpha-band increases 4-fold and the 340 nm beta-band is absent compared to unoriented membrane. In the near ultraviolet region, the appearance of a large negative peak at 275 nm, most likely due to tyrosine, is found. These results may be useful for elucidation of the origin of the optical activity in rhodopsin.

Introduction: The optical activity of visual pigments, such as rhodopsin, is of considerable interest since it can provide clues to the nature of the protein-chromophore interaction in rhodopsin as well as the conformational changes which occur subsequent to light absorption. However, despite numerous experimental and theoretical studies (1-10), the molecular basis for visual pigment optical activity is poorly understood. While native retinal is optically inactive, the retinylidene chromophore of rhodopsin has strong optical activity near 500 nm (alpha-band) and 340 nm (beta-band) (1,2). In addition, there are weaker bands in the near-ultraviolet which may be due either to the chromophore or the protein part of rhodopsin (3-4). Three different models have been proposed to account for the optical activity of the rhodopsin chromophore (5-10) i) In solution, optically active enantiomers of retinal with opposite chirality cancel each other, whereas opsin binds preferentially only one enantiomer and is thus optically active. ii) The retinylidene chromophore is strained by binding with opsin thus inducing optical activity. iii) There exists dipole-dipole interactions between opsin and retinal which gives rise to optical activity.

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Additional information about the molecular basis of optical activity can in principle be found by measuring the circular dichroism of oriented molecules (11-13). This approach has been applied recently to purple membrane fragments which assume a uniaxial orientation upon drying (14). It would be desirable to study the optical activity of oriented photoreceptor membrane. In this regard, it has been shown that uniform multilamellar films of photoreceptor membrane can be produced by using a new method based on isopotential spin-drying (15). Linear dichroism of the alpha absorption band in these films indicates that the retinylidene chromophore of rhodopsin maintains a 17 $^{0}$ angle with the membrane plane (15), in agreement with studies on intact ROS (16). Futhermore, the similarity of visible, UV and infrared spectra of photoreceptor films and suspended ROS along with the ability of the films to fully regenerate in the presence of 11-cis retinal indicates that rhodopsin in the film state maintains its structural integrity (15,17). We report here the results of the first circular dichroism study of photoreceptor membrane films. Further studies on the effects of bleaching as well as membrane modification will be reported elsewhere.

Materials and Methods: Rod outer segments were isolated from cattle retinas (Hormel Inc., Austin, Min.) by the method of DeGrip et al.. (18). This resulted in samples with a 280nm/500nm absorption ratio of less than 2.1. Photoreceptor membrane films were prepared by isopotential spin-drying, which is fully described elsewhere (19). This method consists of slowly evaporating solvent from a suspension of membrane fragments while simulaneously ultracentrifuging the fragments onto an isopotential surface. Typically a 1 ml suspension of osmotically lysed ROS with OD at 500 nm of 0.05 was spun at 11,000 g for up to 17 hours at 4$^{0}$C in an isopotential centrifugation cell (ICC) (19) using .15 mm thick fused quartz (Esco, Oak Ridge, N.J.) as a substrate. This resulted in uniform films less than 5 microns in thickness and 1 cm $^{2}$ area.

CD measurements were made on a Cary model 61 spectropolarimeter which was calibrated using a standard solution of d-10-camphorsulfonic acid in water (20). CD spectra of photoreceptor membrane films were recorded with light incident perpendicular to the film plane. In the case of thin films with absorbances of less than 0.1 at 498 nm, we found a linear relationship between the absorbance and ellipticity. In addition, the light scattering of the films was not greater than that of the solution as determined by absorption spectroscopy at 650 nm. In order to check for possible artifacts due to linear dichroism from photoselection of an anisotropic CD beam (21-22), we rotated the film plane of the sample 90 degree about the beam axis after a fast single scan (about 5 minutes). No change outside of the noise-level of the spectrum was detected. In addition, irradiation of photoreceptor membrane film with a nonpolarized light flash produced the same effects as exposure to the CD beam after several scans indicating that there

does not exist noticeable artifacts due to photoselection under these conditions.

Results and Discussion: Figure 1 compares the absorption spectrum of photoreceptor membrane suspended in 1% digitonin and in a multilayer film. It is found that the absorption spectra are similar except for a drop in the 280/500 nm absorption ratio in the film which can be accounted for by the preferential orientation of the retinylidene chromophore in the film plane. A slight narrowing of the 500 nm absorption is also noted. In contrast, major changes are found in the circular dichroism spectrum of photoreceptor membrane film compared to suspended rhodopsin as shown in Figs. 2 and 3. The visible and near-ultraviolet regions are discussed separately below.

Visible Region, 600-300 nm: As shown in Figure 2, there are major differences between the CD spectrum of digitonin suspended rhodopsin and photoreceptor membrane film. In particular, there is a 4-fold increase in the 500 nm alpha-band and an apparent absence of the 340 nm beta-band in the film. Since the ratio of the alpha to beta CD bands has been found to vary for different detergents (3,6), the changes in the film could reflect

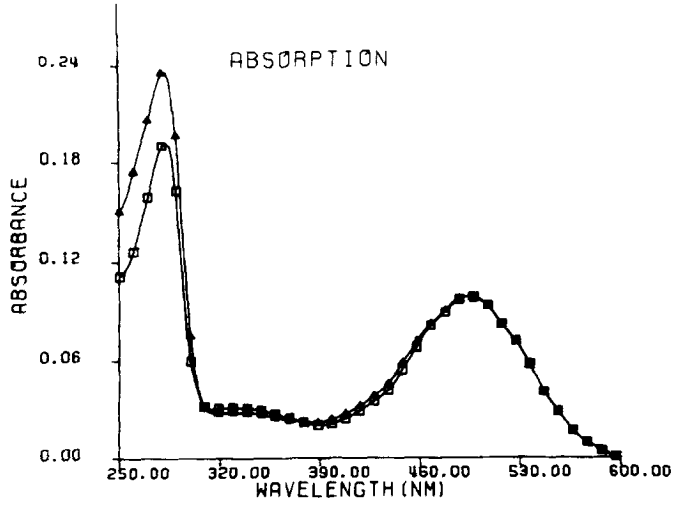


Figure 1. Absorption spectrum of photoreceptor membrane in (-▲--▲-) digitonin solution, (-□-□) oriented film. The optical pathlength was 1.0 cm for the solution. Absorption measurements were made on a Cary 219 UV-Vis spectrophotometer, slit width .1 nm, scanning speed 2 nm/sec. Spectra were digitized every 10 nm in the visible region and 2.5 nm in the ultraviolet region and computer smoothed using a three-point fit.

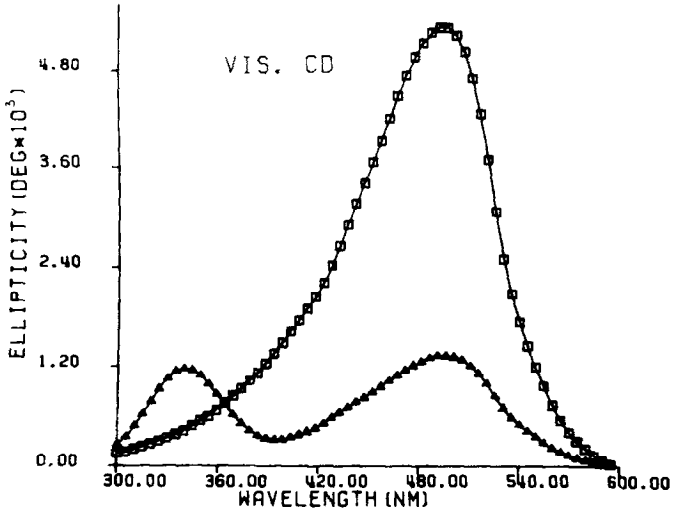


Figure 2. Visible circular dichroism of photoreceptor membrane in (-▲-▲-) digitonin solution, (-□-□-) oriented film. The CD of the film has been increased by 1.5 from measured spectrum, since OD of both samples was 0.1 . This correction allows comparison of the samples at equivalent concentration and takes into account preferential orientation of the chromophore in the film plane. The film samples were equilibrated at room humidity. CD measurements were made at 23 ℃, the dynode voltage was always less than 0.4 KV, the period 10 seconds and the recording speed 30 nm/min. Spectra were digitized and smoothed as in Fig. 1.

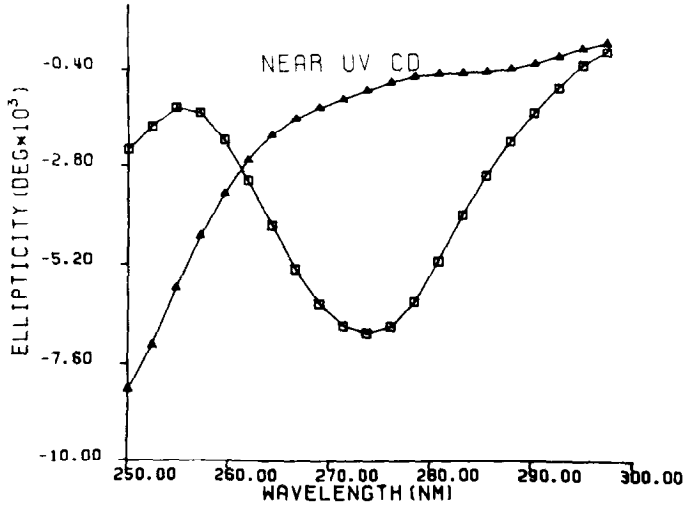


Figure 3. Near ultraviolet circular dichroism of photoreceptor membrane in (-▲-▲-) digitonin solution, (-□-□-) oriented film. The conditions are the same a described in Figure 2 except that the recording speed is 10 nm/min. Spectra were digitized and smoothed as in Fig. 1.

conformational changes of rhodopsin (6). This is unlikely, however, since rhodopsin is found to be regenerable in the film state in contrast to most detergent suspensions of rhodopsin, thus indicating little structural alteration. In addition, most detergents cause a blue shift of the alpha-band and an increase in the beta-band relative to sonicated photoreceptor membrane (6). A second, more likely explanation for the altered CD of photoreceptor membrane film is related to the uniaxial orientation of the rhodopsin and retinylidene chromophore about the normal to the film plane and incident radiation. Such effects have been observed in a variety of oriented molecular systems. For example, 4-Cholesten-3-one, when dissolved in a nematic liquid crystal and measured with light along the optic axis displays a complete reversal in the sign of the circular dichroism of the major band near 330 nm compared to the molecule in an isotropic phase (23). In contrast, a similar molecule, 3 β-Acetoxy-5-cholesten-7-one, which contains a chromophoric group which makes a larger angle with the optic axis, displays a slight increase in the circular dichroism in the nematic phase (23). These results demonstrate the extreme sensitivity of the optical activity to the orientation of chromophore relative to the incident circularly polarized light. It is interesting to note that the CD spectrum of oriented purple membrane films also exhibit major changes in this region which are attributed to loss of predominantly out-of-plane (11) exitonic contributions.

Near-UV Region, 300-250 nm: There appears in the CD spectrum of photoreceptor membrane film a large negative band centered at 275 nm which is absent in rhodopsin-digitonin solution. A similar band is observed in numerous proteins and polypeptides containing tyrosine and phenylalanine (24,25). Since phenyalanine produces only weak CD activity, these bands are usually attributed to tyrosine. A less intense peak or shoulder at 275 nm has also been previously reported in the spectra of rhodopsins and porphyropsins (4), and has been attributed either to an aromatic group of the protein or to

the chromophore. Since this band is still present upon bleaching of the film in hydroxylamine, whereas all optical activity above 300 nm disappears, it is probable that its origin is due to aromatic residues (most likely tyrosine). It is also notable that the molar ellipticity of this band per tyrosine in rhodoposin is much larger than normally found in proteins by at least a factor of 10.

Differences in the far-UV region (170-250 nm) have also been reported by us for photoreceptor membrane films (26). These changes involve a loss of activity at 190 and 210 nm, normally attributed to the alpha-helix Moffitt bands (27,28). Theory and experiments on oriented alpha-helices (12-14,27-30) both predict a disappearance of these bands when the light beam is parallel to the alpha-helix axis, thus strongly indicating a predominantly perpendicular orientation of rhodopsin alpha-helices relative to the membrane plane.

Since the visible and near ultraviolet CD spectra of photoreceptor membrane also exhibit changes due to orientation, it may be possible to relate these changes to the absolute chromophore configuration and/or specific protein-chromophore interactions. For example, the disappearance of the beta-band raises the possibility that its origin is due to some type of in-plane interaction which is optically inactive for light incident normal to the membrane plane. The present results also place new constraints on models of rhodopsin optical activity. It will be important to test whether other visual pigments exhibit similar optical activity when oriented.

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