A NATURAL CD LABEL TO PROBE THE STRUCTURE OF THE PURPLE MEMBRANE FROM HALOBACTERIUM HALOBIUM BY MEANS OF EXCITON COUPLING EFFECTS.

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SUMMARY

Coupling between retinal chromophores on adjacent bacteriorhodopsin molecules in the hexagonal surface lattice of the purple membrane from Halobacterium halobium R_1 leads to exciton circular dichroism (CD) spectra in the 567 nm absorption band. Uncoupling by solubilization of bacteriorhodopsin in Triton X-100 results in a loss of this couplet. In dimethylsulfoxide (DMSO)/water mixtures, both exciton peaks disappear and a positive CD band develops at 460 nm. The change is reversible. It is suggested that DMSO increases the protein mobility in the membrane. Bleaching causes the concerted disappearance of both exciton peaks around 567 nm, and the appearance of optical activity in the 412 nm band. Because of its strong dependence on geometry, this CD effect appears to be a sensitive probe to study changes in protein mobility and in protein-protein interactions.

INTRODUCTION

Both rhodopsin and bacteriorhodopsin contain a single molecule of retinal covalently bound to the protein. In the visible region the CD spectrum of rhodopsin in suspensions of disc membranes as well as in detergents consists of a single positive band centered approximately at the absorption maximum (1). In contrast to disc membranes, in which the rhodopsin molecules are highly mobile (2), the bacteriorhodopsin molecules in the purple membrane are immobilized and form a regular hexagonal surface lattice (3, 4, 5, 6, 7). The close packing and the restricted mobility of the protein molecules are necessary conditions for the occurrence of exciton coupling effects between retinal chromophores of neighboring bacteriorhodopsin molecules. A brief description of the CD spectra of purple membrane suspensions in water, mentioning possible exciton interactions, was recently presented (8). We present strong evidence that such coupling effects occur and show how this natural CD signal may be used to study the protein arrangement in the purple membrane.

MATERIALS AND METHODS

Suspensions of purple membranes were prepared from Halobacterium halobium R_1 following the standard method (9). CD measurements were carried out with a Cary 61. To study bleaching effects, the cell compartment and cell holder assembly were modified to allow illumination in a direction perpendicular to that of the measuring beam. A Schott KL 150 lamp with a light conductor system was used for this purpose. Tests with various substances showed that with these modifications CD spectra could be recorded under photostationary conditions.

RESULTS AND DISCUSSION

CD spectra of purple membrane suspensions. Fig. 1 shows the CD spectra of purple membrane suspensions in phosphate buffer pH 6.88 from 250 to 700 nm. Note the asymmetry in amplitude of the two bands of opposite sign with crossover at 574 nm. We will restrict the discussion to this unique feature of the spectrum, for which there are at least two possible cxplanations: (1) a superposition of a broad positive band centered at the absorption maximum (567 nm), which is due to the interaction of the retinyl residue with the protein part (analogous to the band observed for rhodopsin at 500 nm), and an exciton band with a positive short wavelength lobe and an equal negative long wavelength lobe, which is due to the interaction between retinal chromophores on adjacent bacteriorhodopsin molecules in the membrane; (2) a combination of two different transitions with opposite circular dichroism. There is no evidence for the second interpretation in the absorption spectrum.

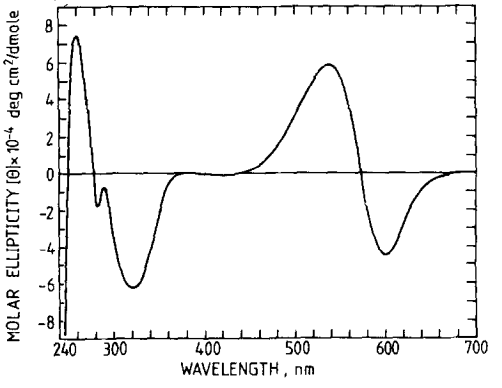

Fig. 1. The CD spectrum of a light adapted suspension of purple membranes from H. halobium in 0.025 M phosphate buffer (pH 6.88). Molar ellipticities in this and other figures are based on spectrophotometrically determined chromophore concentrations. Temperature 20° C.

Our experiments on the other hand strongly support the first interpretation. Since the crossover of a conservative CD exciton spectrum occurs at the absorption maximum (567 nm) and the two extrema are located symmetrically with respect to this maximum both in amplitude and wavelength, the proposed superposition in (1) accounts for the asymmetry in amplitude and for the fact that the crossover occurs at 574 nm rather than 567 nm. This interpretation also predicts that changes in exciton band amplitude should be equal in both lobes and that shifts in the wavelength of the absorption maximum should be reflected in equal shifts in both exciton lobes. Upon light adaptation we observed indeed in both peaks the same redshift of about 8 nm as in the absorption spectrum. At the same time both peaks increased by approximately equal amounts. Moreover, raising the temperature resulted in an equal reduction in amplitude of both bands. The two peaks are 65 nm apart, which is approximately to be expected for a simple coupled dimer model. For exciton coupling effects to occur, a number of conditions have to be satisfied. A rather rigid arrangement of the bacteriorhodopsin molecules is required. Interchromophore distances, estimated from the 7 Å map (6, 7), range from 12 Å to 45 Å. Since exciton CD amplitudes are proportional to the square of the extinction coefficient maximum (10), the high extinction

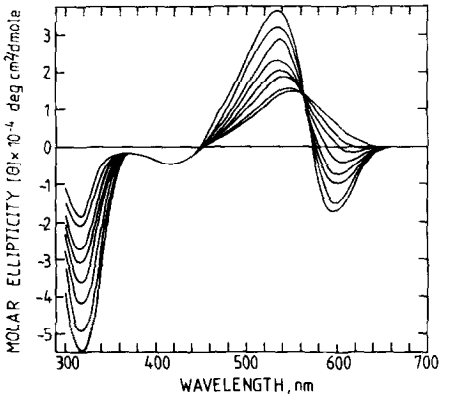

Fig. 2. The effect of solubilization in a 2 °/o (v/v) mixture of Triton X-100 and 0.025 M phosphate buffer (pH 6.88) on the CD spectra of purple membranes from H. halobium. With increasing time after mixing with Triton X-100, the CD amplitudes decrease. Recording of the spectra was started at the following times after mixing: 2, 13, 30, 46, 60, 80, 125 and 200 minutes. Temperature 20° C.

coefficient at 567 nm (63 000 M^{-1} cm^{-1} (12)) makes coupling over long distances possible. The transition dipoles cannot be parallel to the plane of the membrane, since this would cause the exciton effect to vanish.

Decoupling by solubilization in Triton X-100. One of the simplest tests for the tentative interpretation of the CD spectrum presented above would be to disassemble the surface lattice by solubilization of the protein in a detergent. If this can be done without serious denaturation or loss of the chromophore, the exciton coupling bands are expected to disappear with the positive band due to the isolated molecules remaining. Solubilization in Triton X-100 is accompanied by a small decrease in extinction and a shift of the absorption maximum to 550 nm. Depending on conditions, solubilization may take from several minutes to several hours, and can be monitored by following the decrease in light scattering. At room temperature and pH 6.88 protein-micelle formation occurs over a period of about two hours. We observed (Fig. 2) that during this time both exciton peaks decreased by approximately equal amounts and that at the end of this period a broad positive band centered at 550 nm remained. This is clearly in agreement with our interpretation of the spectrum. Absorption spectra which were measured simultaneously with the CD spectra showed that up to this time no denaturation or chromophore

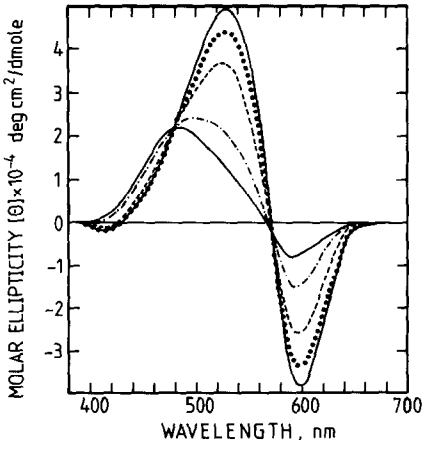

Fig. 3. The effect of temperature on the CD spectrum of a suspension of purple membranes from H. halobium in a 36.7 % (v/v) mixture of dimethylsulfoxide and 0.025 M phosphate buffer (pH 6.88). ____ : 29.0°C; ···· : 34.8°C; ---- : 40.0°C; -·-·- : 45.0°C; ____ : 50.0°C. Upon cooling from 50.0°C to 29.0°C the original spectrum at 29.0°C was obtained.

loss had occurred. Eventually, however, denaturation sets in, manifesting itself by an extinction decrease at 550 nm and the appearance of a 380 nm absorption band.

Reversible changes in protein mobility induced by dimethylsulfoxide. Dimethylsulfoxide (DMSO)/water mixtures between 30 °/o and 60 °/o induce a reversible change in the purple membrane absorption spectrum, with the 567 nm band shifting to 460 nm (11). In parallel with these changes in absorbance, we observed major changes in the CD spectra. With increasing DMSO concentration, the exciton peaks disappear and a positive CD band develops centered at 460 nm. Complete reversibility of this effect was established. At fixed DMSO concentrations, the same reversible effect can be produced by altering the temperature. Fig. 3 shows the change in the CD spectra of purple membrane suspensions in a 36.7 °/o DMSO/water mixture, when the temperature is raised from 29° C to 50° C. In a concerted way, both exciton peaks decrease by equal amounts, as expected for a conservative band structure. Positive CD amplitudes develop from 410 to 490 nm. At 55 °/o DMSO the exciton bands have disappeared almost completely, and a positive band centered at 460 nm can be clearly identified. These findings can be most easily explained by assuming that DMSO increases the protein mobility in the membrane, leading to a more fluid state. Increased rotational and translational mobility would lead to uncoupling, to larger average distances between proteins and to angular averaging of the CD amplitude. Together with the simultaneous band shift, this could easily lead to the complete disappearance of the exciton bands and to the appearance of a positive band at 460 nm due to isolated uncoupled bacteriorhodopsin molecules. This interpretation is in agreement with the fact that the diffraction rings from the surface lattice disappear upon the addition of DMSO (R. Henderson, private communication).

Bleaching effects at low temperatures. Under photostationary conditions and at temperatures ranging from - 10° C to - 30° C, CD spectra were recorded of purple membrane suspensions in 60 °/o glycerol/water mixtures. At room temperature the CD spectra in this solvent system (Fig. 4) are quite similar to those observed in water. Depending on the light intensity and the temperature, the photo-equilibrium between the 567 nm and 412 nm states, which at room temperature lies on the side of the 567 nm state, can

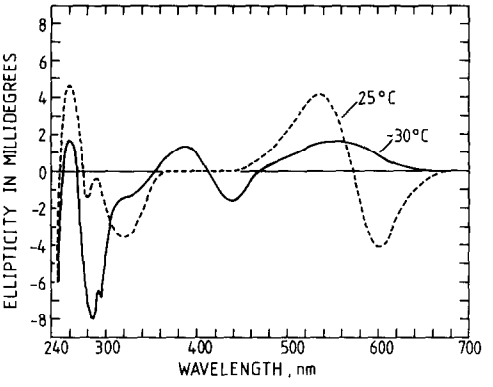

Fig. 4. The effect of bleaching on the CD spectra of a suspension of purple membranes from H. halobium in a 60 °/o (V/v) mixture of glycerol and 0.025 M phosphate buffer (pH 6.88). The spectrum at - 30°C was recorded under photostationary conditions with most of the bacteriorhodopsin molecules bleached. The spectrum at + 25°C refers to the unbleached light adapted state.

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be shifted towards the 412 nm state (12). Although several other shorter lived intermediates exist (13), it is sufficient for the purposes of this discussion to consider only these two states. At low light intensities, the amplitude of the exciton bands near 567 nm is reduced and at the same time optical activity develops at 412 nm. Raising the light level further leads to the appearance of an exciton type band at 412 nm, whereas at 567 nm the exciton bands are replaced by a weak positive band (Fig. 4). These observations can be explained as follows. At low light intensities, the exciton bands at 567 nm are reduced, since some molecules are bleached. The bleached molecules, absorbing at 412 nm, can only have a single sign CD band and cannot lead to exciton CD bands, since they have on the average no bleached neighbors. At high light intensities on the other hand, the probability of a bleached molecule having a bleached neighbor becomes high enough to allow for the observation of exciton effects at 412 nm. The remaining isolated unbleached molecules will lead to the broad band at 567 nm, since even at - 30°C and high light levels not all molecules are bleached. Although the experimental data shown in Fig. 4 appear to be in agreement with this interpretation, further experiments on the intensity and temperature dependence are needed. There can be no doubt however, that bleaching leads to a coordinated disappearance of both exciton peaks, and to the appearance of

optical activity in the bleached state. The underlying positive band at 567 nm has been well exposed in the bleaching process, supporting our interpretation of the CD spectrum.

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