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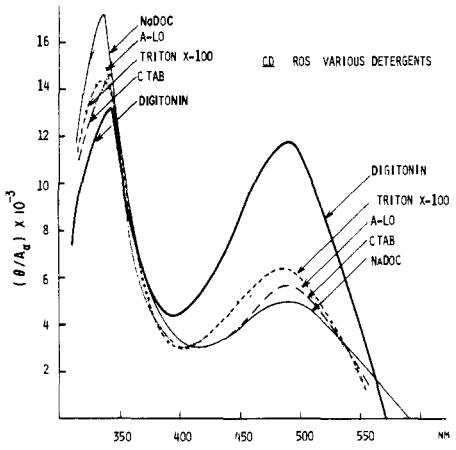

Figure 1. Circular dichroism spectra of bovine rod outer segments (ROS) in various detergents, room temperature. Wavelength is plotted against $(\theta/A_\alpha) \times 10^{-3}$, or ellipticity in millidegrees of α (or β) band/absorption of α band.

Table I. Absorption and Circular Dichroism Spectra

Detergent[a]	λ_{max}	Cotton effects[b] β	Cotton effects[b] α	θ_{max}/A_α[c,d] β	θ_{max}/A_α[c,d] α	R_α/R_β[e]
Digitonin[f]	498	340	490	12.6	11.7	0.87
Triton X-100	498	335	485	14.4	6.8	0.44
A-LO	498	330	490	16.1	6.3	0.42
CTAB	497	340	490	14.1	5.7	0.46
NaDOC	497	335	485	17.0	4.9	0.36

[a] Triton X-100, poly(oxyethylene (9∿10)octylphenol); A-LO, 2:1 mixture of lauryldimethylamine *N*-oxide (LDAO) and tetradecyldimethylamine *N*-oxide; CTAB, cetyltrimethylammonium bromide; NaDOC, sodium desoxycholate. All detergent concentrations were 50 mM, 67 mM phosphate buffer, pH 8.0. [b] Error ±3 nm. [c] Average of several runs. [d] θ, ellipticity in millidegrees of α (or β) CD band maximum; A, absorption of α band maximum. [e] R = rotational strength. [f] 2% w/v.

Micellar Effects on the Photochemistry of Rhodopsin

Sir:

The visual pigment rhodopsin is composed of an 11-cis retinyl chromophore[1,2] bound covalently via a protonated Schiff base linkage[3] to the ϵ-amino group of a lysine[4-6] in the apoprotein ospin. Rhodopsin has absorption band maxima at 498 (ϵ 40 600), 350, and 280 nm, and positive circular dichroism bands at 490 (α-band), 340 (β-band), and 270 nm.[7-9] Upon absorption of a photon of light by rhodopsin, the 11-cis chromophore is isomerized to the all-trans form with a quantum efficiency of 0.67.[10] At room temperature, all-trans retinal and opsin are the reported products of this bleaching process; however, a series of thermal intermediates has been characterized at lower temperatures.[11]

During our studies on visual pigments[12,13] it was observed that cis retinals could be isolated from the bleaching of rhodopsin and that the amount of cis retinal formed varied with detergents as well as with the irradiation time. Since most of the spectral and photochemical studies of rhodopsin have been carried out in various detergents, it appeared important to study the spectral and photochemical properties of rhodopsin in several detergents in order to determine the extent that these properties are altered. Thus we have examined the circular dichroism spectra and photochemistry (resulting from pulsed laser excitation) of rhodopsin in several detergents.

Rod outer segments were obtained from bovine retinas (Hormel-Austin, Minn.) by the sucrose flotation method.[14] The rhodopsin solutions were in 67 mM phosphate buffer, pH 8.0 with detergent concentrations of 50 mM, with the exception of digitonin which was 2% (w/v). All solutions were prepared at 0°C under a dim red light.

The electronic absorption spectra (Table I), Cary 17, of the extracted rod outer segments were identical in all detergents. The circular dichroism (CD) spectra, JASCO J-40, scan rate 50 nm/min, optical density of 0.5–1.0 at 500 nm, 1-cm path length, were recorded twice in succession to verify that bleaching had not occurred. Although positions of the α- and β-bands are quite close in the five detergents (Table I, Figure 1) large variations were present in the values of θ_{max}/A_α (θ is molar ellipticity, A is optical density) and R_α/R_β (R is rotational strength) calculated from these spectra. The CD results are interesting since, regardless of whether the origin of rhodopsin optical activity is due to a twisted chromophore[7,8] and/or a dipole-dipole interaction,[15,16] they provide a sensitive measure of the secondary chromophore-protein interactions. The fact that the θ_{max}/A_α and R_α/R_β values vary with detergents shows that the micelle formed from these detergents affect the shape of rhodopsin, thus giving rise to different steric and/or electronic interactions with the chromophore.

Bleaching was carried out using one 460-nm excitation pulse from a tunable dye laser, Phase-R DL 2100 B, $t_{1/2} \sim$ 300 ns. The opsin was denatured and the chromophore was extracted[17] by addition of an equal volume of cold methylene chloride to the laser flashed rhodopsin solution through a no. 14 gauge syringe and emulsification by repeated suction-protrusion in the dark. The emulsion was centrifuged and the methylene chloride layer was separated, dried over sodium sulfate, and concentrated to 20–30 μl,

Table II. Pulsed Laser Excitation of Rhodopsin

Detergent[a]	Φ[b]	Bleaching products[c,d]
Triton X-100	0.64	Trans
A-LO	0.62	Trans, 2% 9-cis
CTAB	0.69	Trans, 10% 9-cis, 3% 13-cis
NaDOC	0.55	Trans, 5% 9-cis, 15% 13-cis

[a] See Table I. Digitonin was not studied because the chromophore could not be extracted with methylene chloride from a digitonin solution. [b] Reproducibility ±5%, 50 mM $NH_2OH \cdot HCl$. The relative bleaching rates were the same in the absence of $NH_2OH \cdot HCl$. [c] Conversion yields are 50–75%, 23°C. The percent are approximate because the detergents broadened the HPLC peaks. The 11-cis isomer, the chromophore in rhodopsin, is omitted in the estimations of product distribution. [d] The methylene chloride extraction procedure does not induce isomerization of retinals as shown by the liberations of only 11-cis and 9-cis retinals, respectively, from rhodopsin and isorhodopsin (ref 13).

and products were analyzed by high pressure liquid chromatography (HPLC), Waters ALC-100, 6000 psi pump, two 1-ft μ-porasil columns,[18] 1% ether in hexane, 1.5 ml/min, 350-nm detector. The HPLC peak areas were corrected for detector response by dividing by $\epsilon_{350\,nm}$ of the respective retinal isomer. Quantum yields were determined by monochromatic excitation at 500 ± 5 nm and monitoring the 500-nm rhodopsin absorption in the presence of 50 mM hydroxylamine hydrochloride. The lamp flux was calibrated by ferrioxalate actinometry.[19]

Extraction of nonirradiated rhodopsin via the methylene chloride process afforded exclusively the 11-cis retinal,[13] while all-trans retinal was the only product when rhodopsin is bleached by pulsed laser excitation in Triton X-100 (Table II). In contrast to the nonionic micelles (Triton X-100, A-LO), excitation of rhodopsin in the cationic detergents (CTAB, NaDOC) gave relatively large amounts of cis retinals, 9-cis and 13-cis, as primary photoproducts resulting from simultaneous isomerization about two double bonds. It should be noted that 9,13-isorhodopsin (isorhodopsin II),[13] upon pulsed excitation, also yields primary products resulting from a one-photon two-bond isomerization, i.e., a 15:1 mixture of all-trans and 11-cis retinals.[20] Although the question of multiple photon events is a relevant one since rhodopsin is isomerized within 6 ps of photon irradiation,[21] it should be pointed out that the number of absorbing molecules was approximately equal to the photons per flash, and that rhodopsin gave no cis retinal products when flash irradiation was carried out under identical conditions in Triton X-100; however, upon prolonged excitation in Triton X-100 some cis products were formed.

Of the detergents used in this study, only CTAB has been thoroughly investigated. Molecules such as benzene, *N,N*-dimethylaniline, and hexanol are solubilized at the micelle-water interface, while cyclohexane is located in the micelle interior.[22] The location of rhodopsin in CTAB is unknown, although ultracentrifugation studies conclude that three rhodopsin-opsin molecules can be contained in a single CTAB micelle.[23] Even though we cannot elaborate upon specifics of the protein-micelle interaction, we know that the location of the protein in the micelles, interior or interface, and the shape of the micelles are important,[24] due to the structural and electronic features of the different detergents and the fact that rhodopsin is thought to have an elongated shape.[25]

It is evident that the protein-chromophore interactions alter the photochemical properties of the polyene. These photochemical variations may be caused either by different twists of the chromophore in the different micelles, and/or different electronic interactions between the protein and chromophore due to varying protein conformations. These would affect: (i) the energy levels of the 11-cis chromophore and its photoproducts, or (ii) the pathway of photoisomerization, i.e., singlet or triplet. Multiple bond photoisomerization in polyenes usually occur via a triplet state pathway.[26,27] Since interactions with the protein could alter the photochemistry of the chromophore and thus result in two-bond isomerizations via the singlet, it is not possible to determine the isomerizing state from the nature of the photoproducts.

Finally, in view of the differences in the circular dichroism spectra, quantum yields of bleaching, and bleaching products of rhodopsin in the various detergents, caution should be exercised in comparing the spectral and photochemical properties of rhodopsin in different micelle environments where some consideration of the chromophore microenvironment must be made.

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A Rotation-Controlled Excited-State Reaction. The Photoenolization of Ortho Alkyl Phenyl Ketones[1]

Sir:

We wish to report evidence that the well-known and much studied photoenolization of ortho methyl ketones[2-5] is dominated by hitherto unsuspected conformational factors,