CATION-DEPENDENT LIGHT-INDUCED STRUCTURAL CHANGES IN VISUAL RECEPTOR MEMBRANES

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SUMMARY

A stearamide spin probe was used to study the light-induced structural changes in Rod Outer Segment Membranes in the presence of sodium and calcium ions. The correlation time (τ_c) for the reorientation of the probe was calculated in the dark and light. In the presence of sodium ions, $\tau_c = 3.3 \times 10^{-9}$ sec in the dark, and 2.7×10^{-9} sec in the light while the opposite was noticed in the presence of calcium ions, $\tau_c = 2.9 \times 10^{-9}$ sec in the dark and 3.6×10^{-9} sec in the light. The correlation times for reorientation of the probe were also calculated in aqueous glycerol solutions of varying viscosities at 20°C. Comparison of the values of τ_c (dark and light) suggests a change in local mobility in the ROS corresponding to a macroscopic viscosity difference of approximately 150 cp. The significance of calcium ion interaction with negatively charged groups and the formation of a Schiff base is emphasized.

The role of ions in the excitatory process of Rod Outer Segment (ROS) membranes is not precisely understood. Rhodopsin, a lipoprotein and a complex of opsin and 11-cis retinal, is the light-absorbing pigment associated with ROS membranes. It is well known that rhodopsin goes through various intermediates upon bleaching[1] and is believed to be associated with the electrical events in the membrane, which in turn excite the optic nerves. From x-ray data Blasie[2] concluded that rhodopsin sinks deeper into the hydrophobic phase on bleaching. Brown[3] and Cone[4] have shown by measuring photo-induced dichroism in visual receptor membranes that rhodopsin exhibits a rotational motion about an axis perpendicular to

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the ROS membranes. Yoshikami and Hagins[5] have proposed an excitation model for rods and cones in which exposure to light initiates a flow of calcium ions through the disk membranes and the increased calcium concentration reduces the permeability to sodium ions of the plasma membrane. Verma et al.[6] using spin probe techniques have reported light-induced structural changes in phospholipid multibilayer model membranes containing light-sensitive pigments. From the present spin probe data we propose that structural changes occur in rhodopsin in the visual receptor membrane upon illumination and that the nature of the changes depends on whether sodium or calcium ions are present.

The structural changes were monitored using a spin probe which is very sensitive to changes in the local environment,[7-10] the stearamide spin probe (SSP) (4-stearamide-1-oxyl-2,2,6,6-tetramethyl piperidine), prepared by published methods.[11] ROS were isolated from dark-adapted bovine retina (Hormel Institute, Austin, Minn.) as reported by Heller[12] in sucrose solution. (density 1.139). Approximately 0.1 mg of SSP was dissolved in 5 ml of alcohol and was mixed with 20 ml of 0.066 M sodium phosphate buffer (pH 7.0). The mixture was shaken for at least 40 min and then centrifuged at 11,000 x g (Sorvall RC-3) in order to remove the precipitated SSP. The supernatant was mixed with the ROS suspension in 0.066 M sodium phosphate buffer for about 2 hr. The suspension was centrifuged at 12,000 x g for 10 min. The ROS pellets were collected from the bottom of the centrifuge tubes and then washed several times with phosphate buffer until the supernatant showed no electron spin resonance signal from free SSP. The labelled pellets were then resuspended in solutions of the different ions under investigation. All of these preparations were carried out at 4°C under a dim red light, or in the dark. Electron spin resonance (ESR) spectra were recorded at 20°C with a Varian E-9 spectrometer operating at 9 GHz. An unfiltered Hg lamp (H4AB, G.E., 100 W) was used as a light source. The beam was first passed through

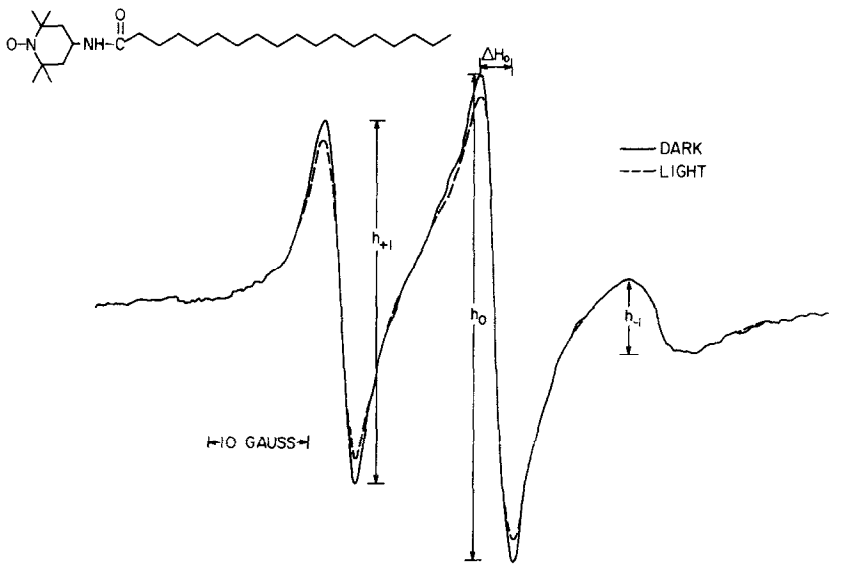

Figure 1. ESR spectra of the stearamide spin probe in ROS membranes in the presence of 0.1 M Na^+.

10 cm of water to eliminate sample heating and then was focused on the cavity of the ESR machine with an ordinary glass lens.

ESR spectra of ROS containing SSP in the presence of Na^+ are given in Figure 1. The correlation time (τ_c) for reorientation of the probe was calculated using the equation

$$\tau_c = 6.82 \times 10^{-10} (\Delta H_0) \left(\sqrt{\frac{h_0}{h_{-1}}} - \sqrt{\frac{h_0}{h_{+1}}} \right)$$

where ΔH_0 is the line width in Hz of the central ESR line, and h_{+1}, h_0 and h_{-1} are the amplitudes of the low field, center, and high field ESR lines, respectively. In the presence of 0.1 M Na^+, τ_c was 3.3×10^{-9} sec in the dark, and 2.7×10^{-9} sec in the light. The opposite behavior was noticed in 1 mM Ca^{2+}, $\tau_c = 2.9 \times 10^{-9}$ sec in the dark and 3.6×10^{-9} sec in the light. The correlation times reported above in the presence of light are after bleaching ROS containing SSP for 10 min and are the mean values obtained from several sets of experiments. The change in the correlation times

noticed in the dark and light is essentially constant for different ROS preparations.

Although the changes observed in the correlation times of SSP in the dark and light are small, they are significant when compared with the correlation times calculated for SSP in aqueous glycerol solutions of varying viscosities at 20°C. By comparing the correlation times of SSP in ROS and in aqueous glycerol solution, it can be stated that upon illumination in the presence of sodium, SSP in ROS experiences an increased fluidity, whereas the opposite occurs in the presence of calcium ions. Upon bleaching the ROS the increase (decrease) in fluidity around the probe in the presence of sodium ions (calcium ions) is approximately equivalent to that experienced in glycerol solutions whose macroscopic viscosities differ by 150 cp.

Two important questions arise -- what causes this change in fluidity of the SSP environment upon illumination and why are the effects in the presence of Na^+ opposite to those in the presence of Ca^{2+}? The possible mechanisms which we suggest at the moment are partially derived from our observations with visual pigment-doped phospholipid multibilayer model membranes intercalated with a cholestane spin probe. Their preparation has been reported elsewhere.[6,7] The results can be summarized as follows.

The addition of all-trans retinal and/or retinol (when present in more than 30 weight percent) to phospholipid (egg lecithin, phosphatidyl ethanolamine, phosphatidyl serine, and/or brain lipid[13]) multibilayers alter their organization in the dark as well as upon illumination.[6] In the hydrated phosphatidyl ethanolamine - retinal films, the ESR spectra of the cholestane spin probe are the same when the applied magnetic field is parallel or perpendicular to the plane of the film. The hyperfine splitting is 16-17 G and the correlation time is 2.0×10^{-9} sec, demonstrating a fluid environment around the probe. A possible reason for this change is that the bilayer

arrays of phosphatidyl ethanolamine, retinal and the probe have been converted to the liposomic or a globular micelle[14] type of structure in which spin probe experiences a more mobile environment.

This change in the mobility of the probe in ROS could be due to Schiff base formation[15] of the amino groups in phosphatidyl ethanolamine with the aldehyde group of all-trans retinal. The formation of a Schiff base destroys the zwitterionic character of head groups by confirming on them a net negative charge. Consequently, calcium ions experience a greater electrostatic attraction towards the phosphate group. This interaction is responsible for the decreased fluidity of the film. A similar possibility has been mentioned earlier by Bonting et al.[15] and such rigidifying effects of Ca^{2+} on negatively-charged lipid films have been detected.[7,16] The nitroxide of the spin probe should be near the surface of rhodopsin molecules in the membrane since it has been demonstrated to prefer a polar environment (unpublished results of S. Schreier-Muccillo and I.C.P. Smith).

In the presence of Na^{+} ions, which have been shown to be less effective in ordering negatively-charged lipids,[7,16] the increased head group repulsion due to Schiff base formation could result in greater lipid-lipid spacing and hence increased fluidity of the lipids. The differences in response to ions are significant, especially in view of the behavior of cations in the optic response.

Quenching of the ESR signal was also noticed on illumination. This is undoubtedly due to interaction of the photo-induced free radicals[17] with the nitroxide of the spin probe, which has been observed in phospholipid multibilayers containing light-sensitive pigments[6] as well as in ROS membranes. The quenching of the ESR signal has been observed to depend on the visual pigment concentration present in the phospholipid multibilayers and on the maximum absorption wavelength for the pigment (350-375 nm).[6] The rate of decay in the signal intensity upon illumination does not depend on the presence of ions

like Na^+, K^+ or Ca^{2+} but increases in the presence of ions like Fe^{3+}. This indicates that the signal destruction depends on the presence of electron acceptor ions. Although the present data do not allow us to furnish a suitable mechanism at the moment, the results are significant in indicating light-induced structural (fluidity) changes in ROS in the presence of Na^+ and Ca^{2+} ions. Structural changes induced by sterols have been correlated[7,18] with changes in permeability.[19] The present data suggest that the observed light-induced structural changes could alter the permeability properties of ROS, and that the nature of the alteration depends heavily on the presence of Na^+ and Ca^{2+} ions.

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