NET ELECTRIC CHARGE ON PHOTOPIGMENT MOLECULES AND FROG RETINAL RECEPTOR DISK MEMBRANE STRUCTURE

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ABSTRACT The photopigment molecules in frog retinal receptor disk membranes protude some 50–65% of their molecular diameter (~42 A) into the aqueous surface layer of the disk membrane, depending on whether the photopigment is bleached, while the remainder is embedded in the lipid core of the membrane. In order to determine whether the presumably polar groups covering this surface protruding into the aqueous phase possessed net electric charge, we collected X-ray diffraction data from the photopigment molecules in wet pellets of oriented disk membranes as a function of the pH and ionic strength of the sedimentation medium. The Fourier analysis applied to this data provided average nearest neighbor separations for the photopigment molecules for their planar arrangement in the disk membranes. Changes in the average separation of photopigment molecule nearest neighbors as a function of pH, ionic strength, and photopigment bleaching indicated that photopigment molecules possess negative net electric charge, that this net electric charge occurs in the aqueous surface layer of the disk membrane, and that this net charge is reduced on photopigment bleaching. This polar portion of the photopigment molecule may thereby determine the location of the photopigment molecules relative to the lipid core and other photopigment molecules in the disk membrane. In addition, the orientation (dichroism) of the photopigment relative to an axis normal to the plane of the disk membrane and the bleaching-dependent "sinking" of the photopigment molecule into the lipid core of the disk membrane may be accounted for.

1. INTRODUCTION

In another paper (1), we showed that the photopigment molecules in frog retinal receptor disk membrane protrude some 50–65% of their diameter (~42 A) into the aqueous surface layer of the disk membrane, depending on whether the photopigment is bleached or unbleached, respectively. The remainder of the photopigment molecule is embedded in the lipid hydrocarbon core of the disk membrane.

The surface of the photopigment molecule which protrudes into this aqueous surface layer would be expected to consist of primarily polar residues of the protein. These polar residues could give the photopigment molecules net electric charge. Resulting electrostatic interaction among the photopigment's nearest neighbors and with other components of the disk membrane, primarily phospholipids (2), might then play an important role in determining the structure of the disk membrane.

The experiments reported in this paper show that (*a*) the photopigment molecules bear net electric charge, (*b*) this electric charge occurs in the aqueous surface layer of the disk membrane, (*c*) electrostatic interaction determines the degree of order in the planar arrangement of photopigment molecules in the disk membrane, and (*d*) reorganization of this electric charge may be responsible for the sinking of the photopigment molecules some 7 A further into the lipid hydrocarbon core of the disk membrane with bleaching of the photopigment. These results were determined from the effect of the pH and/or ionic strength of the sedimentation medium on X-ray diffraction arising from the photopigment molecules in wet pellets of oriented frog retinal receptor disk membranes.

2. MATERIALS AND METHODS

Ultracentrifugal pellets of isolated frog (*Rana pipiens*) retinal receptor disk membranes were prepared in a manner identical with that described previously (1, 3, 4) except for the composition of the final sedimentation medium. The final sedimentation media were 0.115 M NaCl in 0.01 M phosphate buffer at pH 6.0, 7.0, and 8.0, and 0.01 M phosphate buffer, pH 7.0, in 0.115 M NaCl, 0.230 M NaCl, 0.460 M NaCl, 0.920 M NaCl, and 1.840 M NaCl.

The pellets were mounted in the diffraction camera, maintained wet at 25 ± 0.2°C for the duration of the exposure (5 hr) in all cases as before (1, 3, 4). The diffraction camera setup was identical to that described (3, 4), and the X-ray beam was incident along the sedimentation axis. Slit-smearing corrections and background scattering for this camera setup and these specimens has been discussed previously (4).

The diffracted intensities corrected for background were used for the calculations in section 4. Errors in the measurement of the diffracted intensities are indicated in each figure and were regularly less than ±5% as judged by the noise level in the microdensitometer tracings. Each experiment was repeated with a different specimen and the diffracted intensity data from two identical experiments generally coincided within the error of measurement and the ratios r_2^*/r_1^* (Table I) were always reproducible. The diffraction theory for these calculations appears in section 4. All nontrivial calculations were performed on a PDP-6 computer (Medical School Computer Facility, University of Pennsylvania).

TABLE I

	$-h\nu$			$+h\nu$		
	pH 6.0	pH 7.0	pH 8.0	pH 6.0	pH 7.0	pH 8.0
r_1^*	2.35	2.30	2.20	2.38	—	2.30
r_2^*	3.35	3.35	3.35	3.35	—	3.35
r_2^*/r_1^*	1.43	1.46	1.53	1.41	—	1.46

r_1^*, r_2^* in arbitrary units.

3. RESULTS

We have shown in earlier papers (1, 3, 4) that X-ray diffraction from wet ultracentrifugal pellets of isolated frog retinal receptor disk membranes is cylindrically symmetric about the sedimentation axis. With the beam incident along the sedimentation axis, the beam is incident normal to the planes of the disk membranes and we obtain diffraction arising from electron density contrast projected in the plane of the disk membrane. X-ray diffraction obtained with the beam incident normal to the sedimentation axis arises from electron density contrast in the direction normal to the planes of the disk membranes providing reflections from the lamellar disk-to-disk repeat.

Fig. 1 shows diffraction obtained with the beam incident along the sedimentation axis from disk membrane pellets (in which the photopigment is unbleached) in phosphate-buffered NaCl at pH 6.0 and 8.0. For cylindrical coordinates r, ϕ, z for the disk membrane, where z is normal to the disk membrane, the corresponding reciprocal space coordinates are r^*, ϕ^*, z^* where $\mathbf{z} \cdot \mathbf{z}^* / |\mathbf{z}||\mathbf{z}^*| = 1$. With the disk membrane pellet in this orientation with respect to the X-ray beam, we obtain the diffracted intensity $I(r^*, z^* = 0)$ defined here as $I_{obs}(r^*)$ where $r^* = (2 \sin \theta)/\lambda$. We have previously shown (3, 4) that the intensity maxima for $r^* \leq 1/45 \text{ A} = r_0^*$ arise from the planar arrangement of the photopigment molecules, i.e., the correlation of photopigment molecules with each other in the plane of the disk membrane and the diffracted intensity in this region is denoted by $i_{pp}(r^*)$. Fig. 2 shows similar data where the photopigment is bleached and the pH of the buffered NaCl is 6.0 and 8.0.

These data are tabulated in Table I where r_1^* and r_2^* denote the reciprocal space coordinate of the first and second intensity maxima $i_{pp}(r_1^*)$ and $i_{pp}(r_2^*)$ of the diffracted intensity $i_{pp}(r^*)$. It is clear from Table I that X-ray diffraction arising from

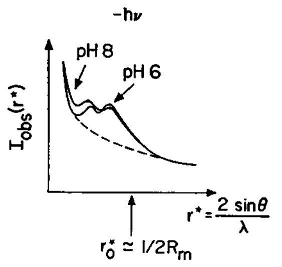

FIGURE 1

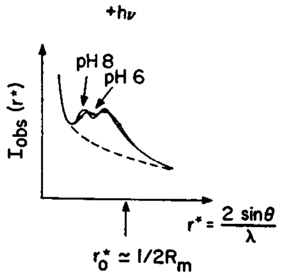

FIGURE 2

FIGURE 1 Observed X-ray intensity diffracted by unbleached photopigment molecules in wet disk membrane pellets at pH 6 (—) and pH 8 (curve with points) with the beam incident along the sedimentation axis. The dashed curve represents the background scattering for our cameras and these specimens. The measurement error in the diffracted intensity is indicated by the size of the dots marking the pH 8 data.

FIGURE 2 Same as Fig. 1, except the photopigment molecules have been bleached. pH 6, curve with points; pH 8, —. The measurement error in the diffracted intensity is indicated by the size of the dots marking the pH 6 data.

the photopigment molecules in wet isolated disk membranes depends on the pH of the sedimentation medium and on whether the photopigment is unbleached or bleached. In addition, Figs. 1 and 2 show that i_{pp} (r^*) for 0.020 $\text{A}^{-1} \leq r^* \leq 0.036$ A^{-1} is unaffected by pH and bleaching of the photopigment.

Fig. 3 shows diffraction obtained with the beam incident along the sedimentation axis from disk membrane pellets (in which the photopigment is unbleached) in phosphate-buffered NaCl at pH 7.0 and varying concentrations of NaCl. These data clearly show that the diffracted intensity from the photopigment molecules in wet isolated disk membranes depends on the ionic strength of the sedimentation medium. Again, $i_{pp}(r^*)$ is relavitely unaffected by ionic strength variation in the region 0.020 $\text{A}^{-1} \leq r^* \leq 0.036$ A^{-1}.

4. INTERPRETATION

These results are interpreted in terms of the planar pair correlation function representation for the planar arrangement of photopigment molecules in the disk membranes. The Fourier analysis of the diffracted intensities, $i_{pp}(r^*)$, which gives this representation for the photopigment molecules is discussed in detail in the Appendices of our earlier work (4). The planar radial distribution function $2\pi r \rho_{pp}(r)$ describing the planar arrangement of photopigment molecules in the disk membrane is given in terms of the observed diffracted intensity $i_{pp}(r^*)$ from these molecules by:

$$2\pi r \rho_{pp}(r) = 2\pi r \rho_0 + 2\pi r \int_0^\infty 2\pi r^* \left\{ \frac{K i_{pp}(r^*)}{N f_p^2(r^*)} - 1 \right\} J_0(2\pi r r^*) \, dr^*. \quad (1)$$

K is the normalization constant which normalizes the observed relative intensity

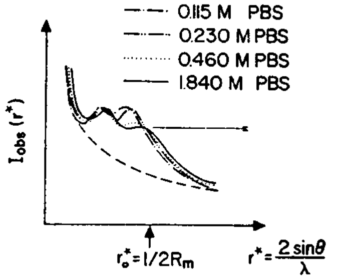

FIGURE 3

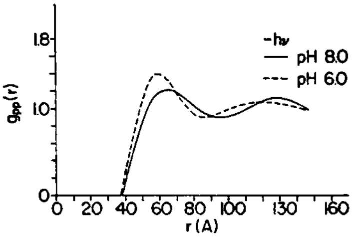

FIGURE 4

FIGURE 3 Observed X-ray intensity diffracted by unbleached photopigment molecules in wet disk membrane pellets at varying concentrations of phosphate-buffered NaCl (PBS) with the beam incident along the sedimentation axis. The measurement error in the diffracted intensity data is indicated by the error bar and is very nearly that indicated for all four sets of data.

FIGURE 4 Pair correlation functions $g_{pp}(r)$ for the planar arrangement of unbleached photopigment molecules at pH 6 and pH 8. The average separation of first nearest neighbors in a particular planar arrangement of photopigment molecules is denoted by r_1, the radial position of the first maximum in $g_{pp}(r)$.

$i_{pp}(r^*)$ to the molecular scattering factor $f_p(r^*)$ for the photopigment molecules, N is the number of photopigment molecules in the planar arrangement, J_0 is the zero-order Bessel function, and ρ_0 is the average density of photopigment molecules in the plane of the disk membrane. K/N was determined as previously (4), $\rho_0 = 1/4900$ A^2, and $f_p(r^*)$ is based on the Fourier transform of a sphere of uniform electron density 42 A in diameter (see reference 4, Appendices, for details).

The first maximum in the planar pair correlation function, $g_{pp}(r) = \rho_{pp}(r)/\rho_0$, occurs at the most probable average separation of nearest neighbors in the planar arrangement of photopigment molecules in the disk membrane (5). The area under the first maximum in the planar radial distribution function corresponds to the average number of nearest neighbors for an arbitrary photopigment molecule in the planar arrangement (5). For the purposes of this paper, we shall concern ourselves primarily with the average separation of nearest neighbors for the planar arrangement of photopigment molecules since the radial position of the first maximum in the pair correlation function $g_{pp}(r)$ is expected to be determined primarily by the radial position of the potential energy minimum in the effective interaction potential for the photopigment molecules (5, 6).

The planar pair correlation functions calculated for the photopigment molecules in sedimentation media of different pH are shown in Fig. 4, while those in sedimentation media of different ionic strengths are shown in Fig. 5.

The radial position of the first nearest neighbor maximum r_1 for these pH conditions is tabulated in Fig. 6. The small shifts in the radial position of this maximum as a function of pH are resolvable under our transform truncation conditions for the Fourier integral in equation 1 (4). As shown in Fig. 6, the average separation of nearest neighbors for the planar arrangement of photopigment molecules increases with increasing pH for both unbleached and bleached photopigment. At a given pH, the average separation of nearest neighbors is less when the photopigment is bleached and all nearest neighbor separations for bleached photopigment molecules are less than the smallest nearest neighbor separation for unbleached photopigment in the pH range investigated.

Fig. 7 shows the manner in which the average separation of nearest neighbors for

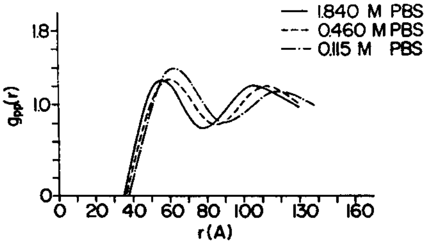

FIGURE 5 Pair correlation functions $g_{pp}(r)$ for the planar arrangement of unbleached photopigment molecules at varying concentrations of PBS.

the planar arrangement of photopigment molecules decreases with increasing ionic strength at constant pH.

We note that for the calculation of the planar radial distribution functions we assumed ρ_0 to be independent of pH in the range 6.0–8.0 and to be independent of ionic strength at pH 7.0 in the range 0.115–1.840 M for NaCl. Since the surface potential (7) is constant for the known lipid components (2) of the disk membrane in this pH range, we would not expect the structure of the lipid core of the disk membrane (8, 9), hence its area and, subsequently, ρ_0, to be significantly affected by pH in the same range. With regard to variation of ionic strength, 94% of the lipids in the disk membrane would be zwitter ionic at pH 7.0 and, hence, would not be expected to be sensitive to changes in ionic strength. In addition, the position and form of the intensity maximum at $r^* \simeq 1/4.5$ A, which presumably arises from the correlation of lipid hydrocarbon chains with each other (10), is unaffected by variation of ionic strength over this range. Thus, it seems reasonable that the lipid core of the disk membrane, hence its area and, subsequently, ρ_0, are unaffected by ionic strength variation in this range at pH 7.0.

We expect that pH changes in the range 6.0–8.0 and ionic strength changes at pH 7.0 in the range 0.115–1.840 M for NaCl directly affect the photopigment molecules since the other major components of the disk membranes, the lipids, should be unaffected by these same changes (see preceding paragraph). This idea is supported by the effect of pH on nearest neighbor separations for the photopigment depends on whether the photopigment molecules are unbleached or bleached. Bleaching should add positive charge to the photopigment under these conditions (11, 12).

We have shown that $Ki_{pp}(r^*) \rightarrow Nf_p^2(r^*)$ for $r^* > (2R_m)^{-1}$. Thus, the result that $K/N\ i_{pp}(r^*)$ for $0.020\ A^{-1} \leq r^* \leq 0.036^{-1}$ is unaffected by pH changes in the range 6.0–8.0 and ionic strength changes at pH 7.0 in the range 0.115–1.840 M for NaCl would indicate that the molecular structure of the photopigment molecules is rela-

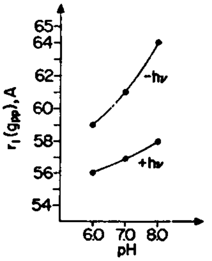

FIGURE 6

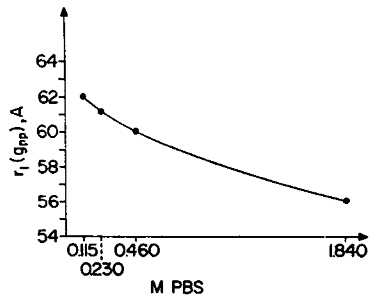

FIGURE 7

FIGURE 6 Average nearest neighbor separations for the planar arrangement of photopigment molecules as a function of pH and bleaching.

FIGURE 7 Average nearest neighbor separation for the planar arrangement of unbleached photopigment molecules as a function of NaCl concentration.

tively unaltered by these changes at least to low resolution (i.e., for the photopigment molecules treated here as hard spheres of uniform electron density, their radius R_m is unaffected to within experimental accuracy of ±1 A [4]). For identical reasons, the molecular radius of the photopigment molecules R_m is unaltered by bleaching within ±1 A.

5. DISCUSSION

If the surface of the photopigment molecules which protrudes into the aqueous surface layer of the disk membranes consists primarily of polar groups and these polar groups give the molecule a net electric charge, we would expect the following:

(*a*) An increase in this net electric charge would increase the range of the repulsion term in the interaction potential for the photopigment molecules and shift the potential energy minimum to larger intermolecular separation (13). This would make larger separations for the photopigment molecules more probable, shifting their average nearest neighbor separation to larger values (6). The converse would be expected for decreased net electric charge.

(*b*) This net electric charge protruding into the aqueous surface layer should be shielded by counterions in that aqueous phase. Increasing this counterion concentration would increase this shielding and reduce the range of the repulsion term in the interaction potential for the photopigment molecules. Subsequently, the potential energy minimum would be shifted to smaller intermolecular separations (13), making smaller average nearest neighbor separations for the photopigment molecules more probable.

These predictions and the effect of pH and ionic strength on average nearest neighbor separations for the photopigment molecules presented in section 4 are consistent with the following:

(*a*) The photopigment molecules bear net electric charge since their average nearest neighbor separations depend on pH in a range where the disk membrane's lipids are unaffected and this pH dependence is affected by bleaching the photopigment.

(*b*) The net electric charge on unbleached and bleached photopigment molecules increased with increasing pH in the range 6.0–8.0 since their average nearest neighbor separation increases with increasing pH. In view of the usual amino acid composition of protein molecules (14), this indicates that this net electric charge is negative. This is consistent with the known amino acid composition of photopigment molecules (15).

(*c*) The net negative electric charge is reduced when the photopigment molecules are bleached since average nearest neighbor separations for bleached photopigment are smaller than those for unbleached photopigment at a given pH. This is consistent with the fact that bleaching would expose two polar groups which would bear positive electric charge for our bleaching conditions (11, 12).

(*d*) The polar groups of the photopigment molecules providing this net electric charge occur in the aqueous surface layer of the disk membrane since counterions in the aqueous phase can effectively shield this electric charge.

Hence, these results are consistent with the idea that the portion of the photopigment molecule which protrudes into the aqueous surface layer of the disk membrane (1) is composed of polar groups possessing net negative electric charge and that this electric charge is reduced by the exposure of positively charged polar groups on total bleaching of the photopigment.

The area on the surface of the photopigment molecule occupied by these polar groups would be expected to determine the degree to which the photopigment molecules protrude into the aqueous surface layer of the disk membrane since the surface of the molecule embedded in the lipid hydrocarbon core of the disk membrane would presumably be composed primarily of nonpolar groups. The reduction in net electric charge on the photopigment molecule with bleaching may result in a decreased area occupied by the polar groups on the surface of the photopigment molecule protruding into the aqueous surface layer of the disk membrane. This may allow the photopigment molecules to sink further into the lipid hydrocarbon core of the disk membrane on bleaching, as reported by us (1).

In addition, the net electric charge on the polar portion of the photopigment molecule clearly affects the planar arrangement of the photopigment molecules in the disk membranes in terms of the average separation of their nearest neighbors. Through electrostatic interactions among photopigment molecule nearest neighbors, their planar arrangement is dependent on bleaching of the photopigment.

We note that the X-ray exposure times necessary for these experiments necessitated the comparison of diffraction from unbleached vs. totally bleached photopigment. The effects of total bleaching discussed in the two preceding paragraphs, however, should occur in a similar fashion, but perhaps to a lesser extent, at the meta I → meta II stage of photopigment bleaching since a positively charged group is exposed at that step in the bleaching process (11).

We also note that the polar portion of the photopigment molecule protruding into the aqueous surface layer of the disk membrane bearing net electric charge is presumably responsible for maintaining the orientation of the photopigment molecule relative to the axis normal to the plane of the disk membrane. These groups would prevent tumbling of the photopigment molecule about any axis parallel to the plane of the disk membrane and thereby explain the highly dichroic nature of the photopigment in retinal receptor outer segments (16).

It is easy to suspect that a change in the location of a photopigment molecule on bleaching relative to the lipid hydrocarbon core and/or relative to the location of nearby photopigment molecules could affect the macroscopic properties of the disk membrane. The precise manner in which the changes in these locations affect these properties is more difficult to predict.

6. CONCLUSIONS

Analysis of X-ray intensity diffracted from photopigment molecules in wet pellets of oriented frog retinal receptor disk membranes as a function of pH and ionic strength of the sedimentation medium indicates the following:

(*a*) That portion of the photopigment molecules which we showed to protrude into the aqueous surface layer of the disk membranes (1) possesses negative net electric charge.

(*b*) This net electric charge on the polar portion of the photopigment molecule is reduced when the photopigment is bleached.

(*c*) This net electric charge gives rise to a bleaching-dependent electrostatic interaction in the planar arrangement of photopigment molecules.

(*d*) The decrease in this net electric charge on bleaching of the photopigment may give rise to the sinking of the photopigment molecule into the lipid core of the disk membrane which accompanies bleaching (1).

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