Lipids and the Molecular Structure of Photoreceptors

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Abstract

A knowledge of the molecular structure and the chemistry of photoreceptors is essential in order to understand how they function. Chloroplasts and the outer segments of the retinal rods and cones of the eye are photoreceptors; they are able to receive, convert and transfer light energy to chemical and to electrical energy in the processes of photosynthesis, vision and nervous excitation. Chemically, their major constituents are pigments, lipids and proteins. Structurally, they are an ordered system of tightly-packed plates, discs, or tubes. Their fine structure in electron micrographs appears as electron dense double layers (lamellae) with a total thickness of the order of 250Å; each lamella or membrane at the interface is of the order of 50Å.

To discover how the pigment molecules are associated with these lamellae in the photoreceptors, the geometry (that is, the length, diameter, and number of lamellae) was measured and the pigment concentration determined spectroscopically. These data were then used to calculate the area that each pigment molecule would occupy if spread as a monolayer on the lamellar surfaces. In addition, pigment-lipid protein micelle structures have also been studied.

On the basis of these physical-chemical studies, molecular models for the photoreceptors have been proposed.

Introduction

LIPIDS ARE IMPORTANT components of cell membranes, myelin, the nerve axon, and brain cells. Cell organelles, e.g. mitochondria (Fig. 1 d,e,f), chloroplasts (Fig. 2 a,b,c), retinal rods and cones (Fig. 1 a,b,c), also possess high concentrations of lipids. These organelles are concerned with energy transfer in the metabolism of the cell with photosynthesis and vision. The questions then are—what is their composition—their chemistry—their molecular structure— and how are these related to their physiological functioning.

In order to approach these problems I would like to summarize some experimental studies on the photoreceptors of plant and animal cells. The chloroplasts of plant cells are the light receptors for the photochemistry of these cells in the process of photosynthesis, and the retinal rods and cones of the eye are the light receptors where the visual process is initiated.

Structural data obtained by electron microscopy for the chloroplasts in a variety of plants and for the retinal rods and cones in a variety of animal eyes show an ordered *fine structure* in molecular dimensions of stacked plates or discs of the order of 250 Å in thickness (Figs. 1c; 2 b, c). These are double membranes (lamellae); the thickness of these lamellae is of the order of 50–100 Å (9,13). The electron-dense lamellae are associated with the lipids, and the less dense interspaces with aqueous proteins. These assumptions are based on the staining and chemical reactions of fixing agents within these structures.

Chemical analysis of isolated chloroplasts from a variety of plants shows that they contain from 35 to 55% protein, 18 to 37% lipids (2), and 5 to 8% inorganic material. The chloroplast pigments, the chlorophylls, average about 6% and the carotenoids about 2%. For isolated retinal rod outer segments, the proteins constitute 40 to 50%, the lipids 20 to 40%, and the visual pigment, rhodopsin, 4 to 10%. Rhodopsin is a complex of retinene (vitamin A aldehyde) with opsin, a protein or lipoprotein. The resemblance in structure of the chlorophyll phytol to the retinene molecule and to half the β-carotene molecule is to be noted (see Figs. 3 and 4). These pigments are polyenes which can also be considered part of the lipids of the photoreceptors.

The photochemistry of the pigment-complex within the chloroplast or the retinal receptors can now be studied in situ by microspectrophotometry. An ideal organism for study of the plant and animal lipids and their relationship to the photoreceptors is *Euglena* (9). Spectral shifts during the light <—> dark reactions can be followed. An estimate of the pigment concentration in a single photoreceptor can be made; e.g. there are 1.3×10^9 chlorophyll molecules per *Euglena* chloroplast (9) and 3.2×10^9 rhodopsin molecules per frog retinal rod (12).

To relate the composition of the photoreceptors back to their structure and to more precisely determine how the chlorophyll may be associated within the lamellae of the chloroplast, the geometry of the chloroplast (the length, diameter, and number of lamellae) was measured and statistically evaluated. These data together with the average chlorophyll concentration per chloroplast was used to calculate the area that each chlorophyll molecule would occupy if spread as a monolayer on the lamellar surfaces (assuming that each disc is a double-layer). The calculated cross-sectional area that a chlorophyll molecule would occupy was found to be about 225 Å^2, which is the cross-sectional area for a porphyrin molecule when spread on a water-air interface (7).

A simplified structural molecular model for the chloroplast was proposed based on these data, in which four chlorophyll molecules are united to form tetrads, with the reactive isocyclic rings turned toward each other (Fig. 3). Interaction between the phytol tails is eliminated in the model by arranging the tetrads in such a way that only one of the phytol tails is located at each intersection in the rectangular network. If the chlorophylls are arranged in a monolayer as shown in the schematic molecular network, space is available for the carotenoid molecules. If these spaces are occupied as shown, there will be one carotenoid molecule for at least every three (or up to six) chlorophyll molecules in the network. This kind of close packing of the chlorophyll and carotenoid molecules in the monolayer would permit energetic interaction between them.

Similarly, for the retinal rods, the geometry and pigment concentration was used to calculate the cross-sectional area of the rhodopsin molecule (8). The cross-sectional area, A, which would be associated with each rhodopsin molecule, is $A = \frac{\pi D^2}{4P}$, where D

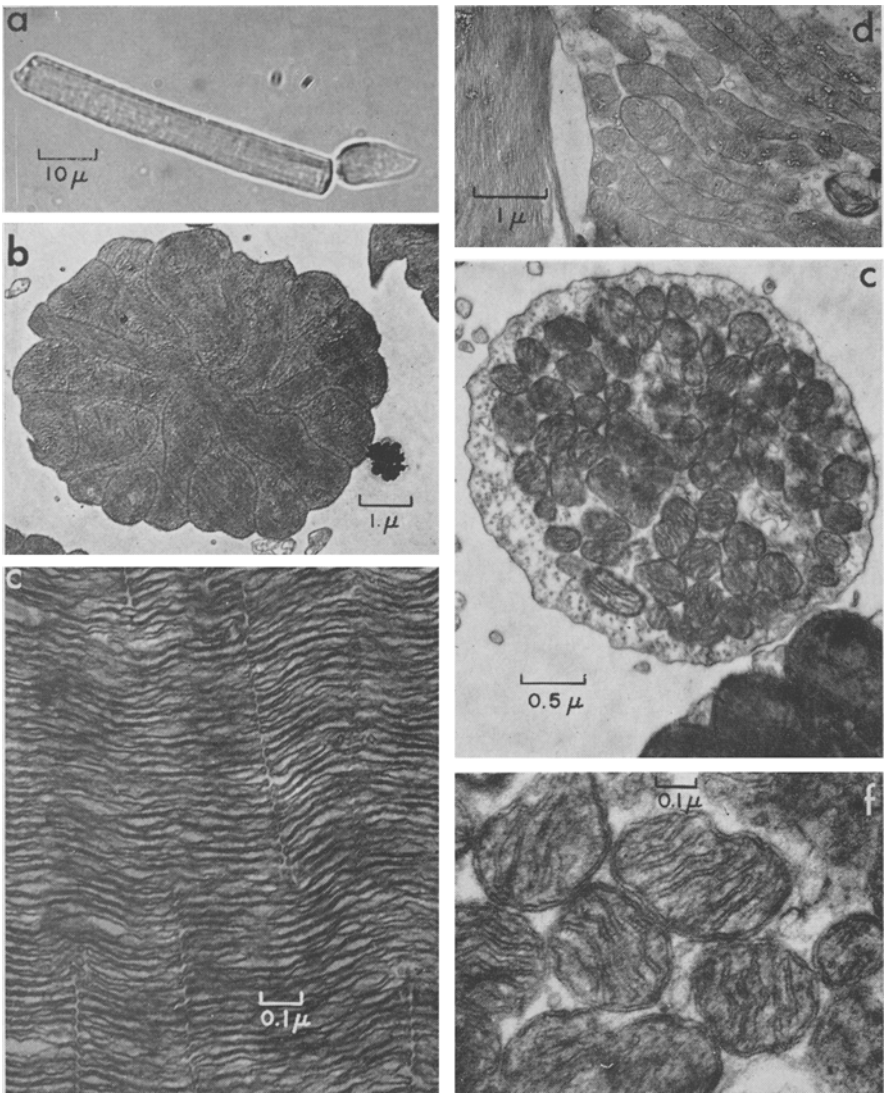

FIG. 1. Part *a*, freshly isolated frog retinal rod; electron micrographs of frog retinal rod; *b*, cross-section; *c*, longitudinal section; *d*, longitudinal section showing areas of outer segment and inner segment (note numerous mitochondria in inner segment); *e*, cross-section of inner segment; *f*, mitochondria of inner segment.

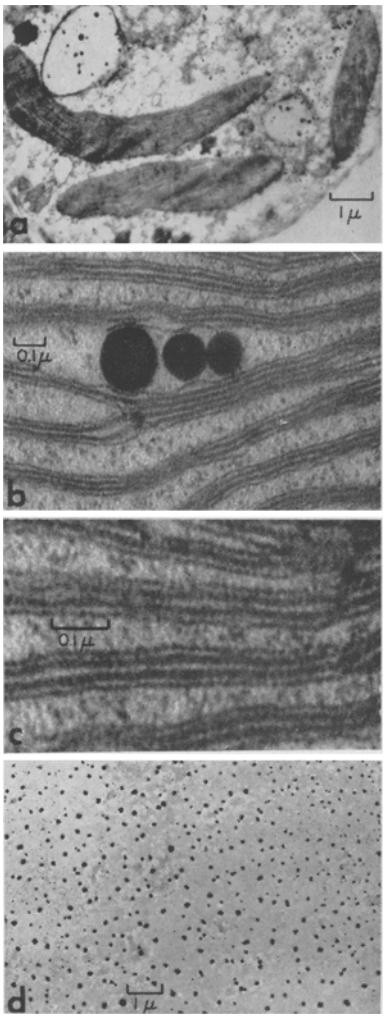

FIG. 2. Chloroplast, *Euglena gracitis*, electron micrographs: *a*, cross-section showing chloroplasts; *b*, an area of the chloroplast; note electron dense granules in inner spaces and the electron-dense lamellae; *c*, an enlarged small area of b, showing that the lamellae consist of particles; *d*, particles of chloroplastin (solubilized chloroplast in 1.8% digitonin).

is the diameter of the photoreceptor, and P is the number of rhodopsin molecules in a single monomolecular layer. The maximum cross-sectional area, A, associated with each rhodopsin molecule can be derived from the above equation where P is replaced by N/2n in which N is the pigment concentration in molecules per retinal rod and *n* is the number of electron dense lamellae, surfaces available per outer segment, then $A = \frac{\pi D^2 n}{2N}$. The cross-sectional area calculated from this equation for cattle and frog rhodopsin was found to be 2500 Å^2 and 2620 Å^2, respectively. This would indicate that the diameter of the rhodopsin molecule would be of the order of 50 Å (Fig. 4). This is the right order of magnitude if rhodopsin is a globular, spherical molecule.

The pigment-lipid-protein complexes are intimately tied to the structure of the photoreceptor, and both retinal rods and chloroplasts are dependent on the pigment, in the right molecular form and shape, for stabilization of their structure. For if there are any physical or chemical forces, i.e., heat, light, or drugs, which interfere with the synthesis of the photoreceptor pigments, the fine structure as shown in Figures 1 c, and 2 b and c, of the photoreceptors is disrupted (3,8,13).

Since the photoreceptors contain large quantities of lipid, it is difficult to solubilize them in water. Colloidal suspensions can also be prepared of the chloroplasts and retinal rods by ultrasonics. The photoreceptors can be solubilized with detergents, e.g., 1–2% digitonin. Such solubilized photoreceptors are referred to as *chloroplastin* and *rhodopsin* and have such properties as absorption spectra, composition, and photochemistry similar to isolated chloroplasts and retinal rods (10,11).

In the photochemistry of rhodopsin, a light-dependent reaction analogous to that occurring in the retinal rods can be followed spectroscopically (6). Retinene, depending on the organic solvent, has an absorption maximum near 380 mμ, but when it is complexed with opsin to form rhodopsin, the shift in absorption maximum is toward the red to around 500 mμ. Retinene can exist in a number of different configurations corresponding to the possible *cis-trans* isomerization around the different double bonds of these molecules (Fig. 4). The 11-*cis* (neo-b) isomer is the most easily formed upon irradiation and the

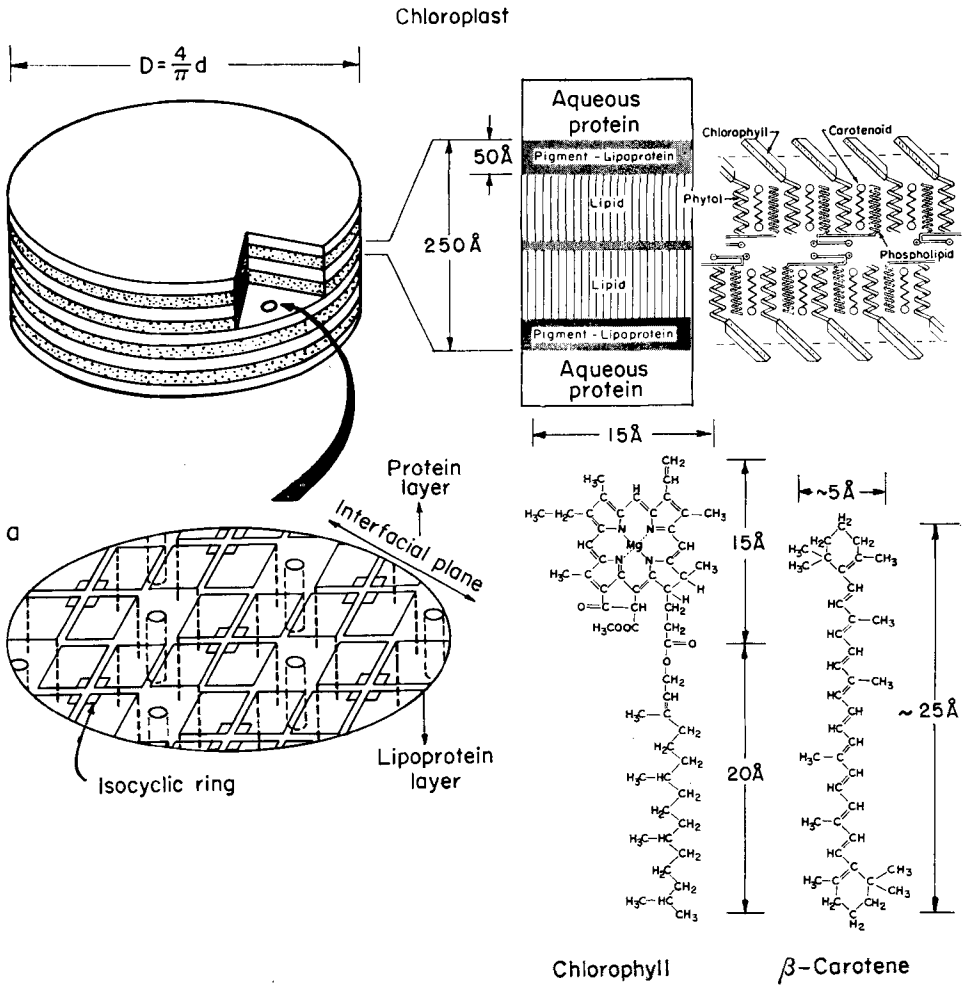

FIG. 3. Schematic molecular model for the chloroplast (or granum). Note relationship of the phytol of chlorophyll to β-carotene and all-trans retinene molecules in Figure 4.

most sensitive to temperature and light (6). Light "bleaches" rhodopsin and results in a shift of the absorption peaks through a series of transients to retinene and opsin. When chloroplastin is bleached by light, no similar shifts in spectra are observed; there is a steady decrease in optical density, and the chlorophyll absorption maximum at 675 mμ eventually disappears. However, with chloroplastin during the Hill reaction with the dye (dichlorobenzenoneindophenol), it was found that an absorption peak at 488 mμ (one of the carotenoid absorption peaks) increases in optical density in the light and then decreases in the dark to its original density (4). This reaction can be repeated by placing the reactants alternately in light and darkness without further addition of dye, indicating that there is increase in the amount of carotenoid absorption at 488 mμ. This reaction has analogies in some ways to the light bleaching and dark regeneration of the rhodopsin solution.

The structure of the photoreceptors, as revealed by electron microscopy, may be associated with a unique property of the lipids; that is, their capacity to form fairly uniform thin layers. This is due to the presence of hydrophobic, water-soluble groups at one end of the molecule and to hydrophilic, fat-soluble groups at the other end of the molecule. This allows the lipids then to form molecular sheets. In a way, this can serve in separating one part of a reaction from another. These lipid sheets can be folded and behave like *liquid crystal* structures. The question then is, Are the lipids a necessary part of the structural network, or do they play a more functional role? Extraction of the lipids from the photoreceptors with organic solvents inhibits the photochemistry.

The ordered structure for the photoreceptors has led to the idea that they have a close relation to a

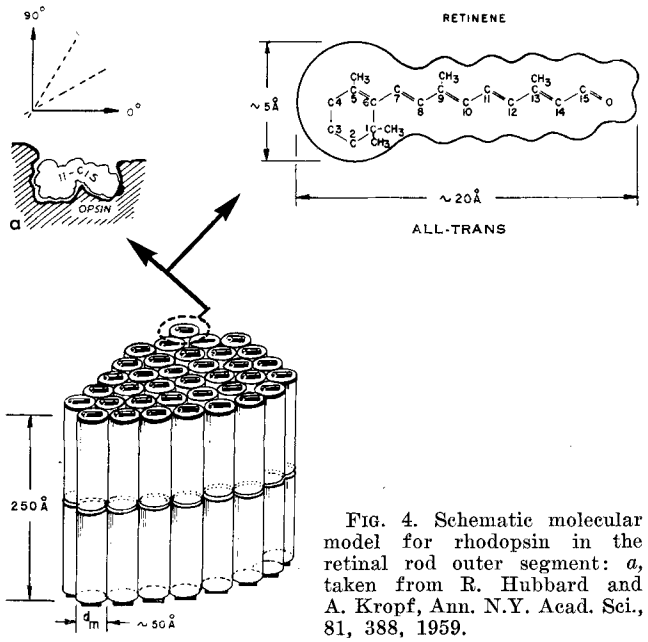

FIG. 4. Schematic molecular model for rhodopsin in the retinal rod outer segment: *a*, taken from R. Hubbard and A. Kropf, Ann. N.Y. Acad. Sci., 81, 388, 1959.

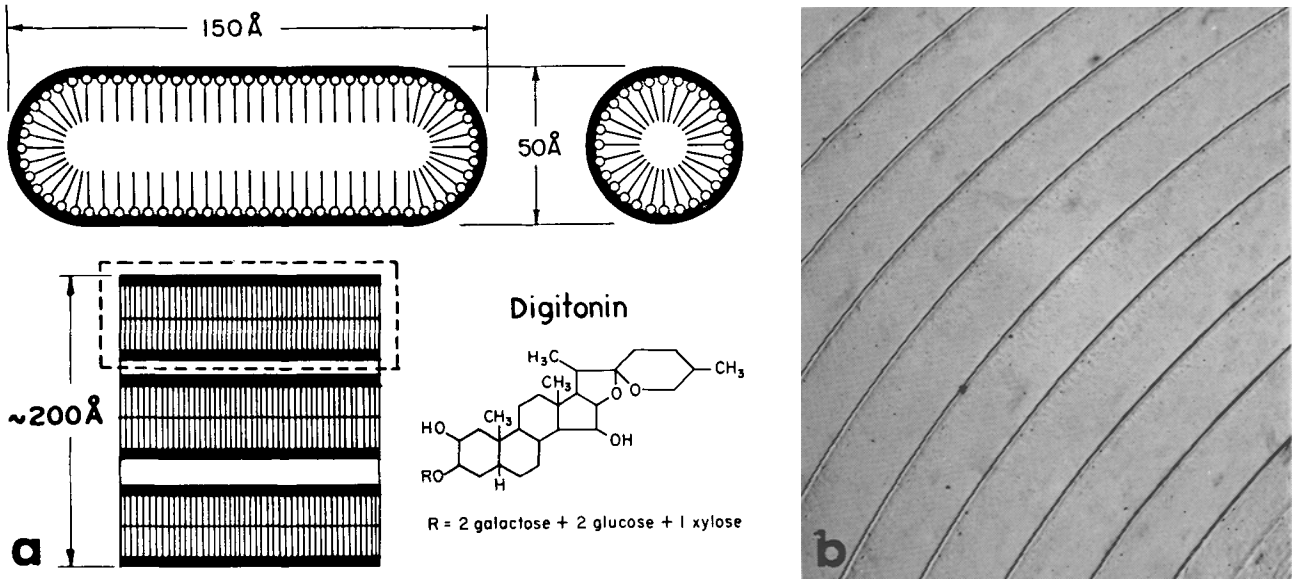

FIG. 5: Letter *a*, schematic molecular model of the dye digitonin micelle; *b*, chloroplastin film.

solid state system. That is, that they should exhibit such properties of the solid state as electronic energy transfer or electronic charge transfer. Experiments have been carried out with chloroplasts and films of extracted chlorophyll, chlorophyll plus β-carotene alone, spread on various surfaces. Such models have demonstrated that there is a photoconductive mechanism of energy transfer (1,5). The results for dark-adapted chloroplastin samples show an activation energy of 2.4 ev; after 30 min illumination in white light, the activation is 0.95 ev. This seems to indicate that the energy received from illumination was lost by some process which reduced the number of charge carriers available for conduction. Similar measurements made on rhodopsin in the dark show an activation of 0.45 ev (13).

In addition, we have examined the kinds of chemical systems that give rise to periodic ordered structures. One such model is the Liesegang phenomenon, in which the impregnation of potassium dichromate and silver nitrate in proteins forms colored precipitated complexes in periodic layers. Light can modify these periodic structures if the precipitated molecules are light-sensitive. Another model is that of cholesteric-type liquid crystals. Digitonin, a 1–2% aqueous solution used for extracting the pigment-complex from the photoreceptors, has a very strong attraction for many complex dye molecules (e.g., chlorophyll and retinene) and also has properties of a cholesteric-type molecules. (Fig. 5a,b).

In the chloroplastin micelles we have chlorophyll, carotenoids, cytochrome, lipids, and protein, essentially in the same relative concentration and orientation as in the chloroplast; similarly, in the rhodopsin micelles we have retinene, opsin, and lipid in the same relative concentration and orientation as in the retinal rod outer segment. Therefore, the micelle structures provide for the orientation of the right number of pigment molecules for light absorption and for the necessary photochemical reactions. Perhaps this is a mechanism for initiating the processes of photosynthesis and vision.

Lipids and their synthesis, therefore, have acquired an exciting new interest in biological investigations in the quest to understand their role in these highly ordered, energy-transferring, cellular organelles.

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