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PURIFICATION OF SQUID RHODOPSIN AND REASSEMBLY INTO LIPID BILAYERS

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Rhodopsin from squid photoreceptor membranes was solubilized in octyl glucoside and purified to a single band on SDS-polyacrylamide gels of M_r 46000. Purified rhodopsin was recombined with phospholipids to form vesicles by detergent dialysis. Spectroscopic analysis of the rhodopsin-lipid vesicles showed that the interconversion between acid and basic metarhodopsin had a pK of 8. Furthermore, rhodopsin in the vesicles could be photoregenerated from metarhodopsin in solutions of either neutral or alkaline pH. These two spectroscopic properties are comparable to those for rhodopsin in photoreceptor membranes. The results indicate that the native conformation of rhodopsin is preserved during purification and after recombination with phospholipids into vesicles. This preparation is, therefore, an active starting point for functional reconstitution studies.

Introduction

A useful means for gaining an understanding of cellular processes has been to purify the constituents of a system and examine the interactions of the elements when they are recombined. This allows a functional assessment of the recombined system compared to the native cell and permits a description of the role of individual components in creating a functional whole. This method has been applied successfully to study the interaction of vertebrate rhodopsin with elements of rod phosphodiesterase [1–3], and also for the study of permeability properties of vertebrate rhodopsin in vesicles (for review, see Ref. 4).

Squid rhodopsin has been studied spectroscopically in detergents after partial purification [5–14]. However, because the functional properties of invertebrate rhodopsin in the photoreceptor were not known, methods were not extended to include studies of purified rhodopsin in the membrane. Recently, we showed that squid rhodopsin activates GTPase activity, guanine nucleotide binding and cholera toxin-catalyzed labeling of a 44 kDa protein after illumination [15]. Furthermore, rhodopsin phosphorylation and phosphinositide turnover also are influenced by light in squid photoreceptors [16,17]. As a step towards examining the properties of purified rhodopsin with these enzyme systems in the natural environment of the lipid bilayer, we describe a method for purification of squid rhodopsin using the dialyzable detergent octyl glucoside. We show that recombined lipid vesicles containing purified squid rhodopsin exhibit spectroscopic properties indicative of rhodopsin in a native conformation.

Experimental Procedures

Materials

Octyl β-D-glucoside was purchased from Calbiochem-Behring Corp. (La Jolla, CA), di-

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Abbreviation: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

phytanoylphosphatidylcholine from Avanti Biochemicals (Birmingham, AL), DEAE-cellulose DE 32 from Whatman (Clifton, NJ); gel electrophoresis reagents from Bio-Rad (Richmond, CA); and [U-^{14}C]glucose-labeled octyl β-D-glucoside from New England Nuclear (Boston, MA).

Photoreceptor preparation

Squid (*Loligo opalescens*; Sea Life Supply, Monterey, CA) were dark adapted for 2 h, after which the eyecups were dissected under dim red light (Kodak Wratten 2) and then stored at −80°C. All procedures except gel electrophoresis were carried out at 4°C in the dark or under dim red light unless stated otherwise. Solutions were bubbled with argon prior to use to protect the membranes from peroxidation. For purification of the photoreceptors, 50–70 eyecups were thawed and then gently shaken in 680 mM NaCl to dislodge the photoreceptors from the retina. The suspension was centrifuged (35 000 × *g*, 30 min) and the pellet resuspended by homogenization with 10 slow passes of a motor-driven teflon-on-glass homogenizer in 120 ml of 40% (w/v) sucrose/300 mM NaCl/100 mM sodium phosphate/2 mM EDTA, pH 6.8. 20 ml aliquots of this suspension were placed in a sucrose step gradient overlayed with 13 ml 33% (w/v) sucrose and 6 ml 10% (w/v) sucrose both containing 300 mm NaCl, 100 mM sodium phosphate and 2 mM EDTA, pH 6.8. The gradient was centrifuged in a Beckman SW 27 rotor at 23 000 rpm for 90 min. The bright pink band of membranes at the interface between the 33% and 10% sucrose layers was collected from each gradient, pooled and washed three times with 35 ml 820 mM NaCl (35 000 × *g*, 20 min). The membranes were further washed once with 35 ml 1 mM EDTA, pH 7.0 (35 000 × *g*, 40 min), four times with 35 ml 820 mM NaCl (35 000 × *g*, 20 min), once with 35 ml 1 mM EDTA, pH 7.0 (35 000 × *g*, 35 min) and finally resuspended in 10 mM imidazole/1 mM EDTA, pH 7.0. The membranes were divided into aliquots containing about 0.1 μmol rhodopsin, centrifuged (35 000 × *g*, 30 min) and the membrane pellets frozen in liquid nitrogen for storage at −80°C.

Rhodopsin purification

Rhodopsin was purified by extraction into octyl glucoside followed by batch-wise chromatography on DEAE-cellulose. An aliquot of sucrose-density purified photoreceptor membranes with 0.15–0.2 μmol rhodopsin was extracted into 6 ml of 50 mM octyl glucoside/10 mM imidazole/1 mM EDTA, pH 7.0 containing 0.5 mg/ml diphytanoylphosphatidylcholine (membrane solubilizing solution) for 30 min, and then centrifuged to remove insoluble material (35 000 × *g*, 40 min). The detergent-solubilized rhodopsin was incubated for 15 min with 1 ml of DEAE-cellulose that had been equilibrated in the membrane-solubilizing solution. Before use, the DEAE-cellulose was prepared by washing in distilled water until the supernatant was clear, then washing in 0.5 M HCl for 1 h, washing in water, then washing in 0.5 M NaOH 1 h, and then rinsing extensively with water until the pH of the supernatant was neutral. At low ionic strength, rhodopsin does not bind to the DEAE-cellulose, and it can be separated from other photoreceptor proteins which adhere to the DEAE-cellulose. The rhodopsin-containing solution was removed after centrifugation (1 min, clinical centrifuge), and the DEAE-cellulose pellet was extracted once more with 2 ml of membrane-solubilizing buffer to remove rhodopsin remaining in the pellet volume. The rhodopsin concentration of octyl glucoside-solubilized samples was determined spectroscopically at 8°C with a Perkin-Elmer 550 ultraviolet/visible absorption spectrophotometer using an extinction coefficient at 490 nm of 41 700 $M^{-1} \cdot cm^{-1}$ [18]. The yield of rhodopsin after purification using DEAE-cellulose was 73–89% of the amount solubilized in octyl glucoside.

Reconstitution procedure

The rhodopsin-containing supernatants from the DEAE-cellulose were pooled, combined with 1 ml membrane-solubilizing buffer containing 18 mg diphytanoylphosphatidylcholine, and dialyzed against 2 liters of 100 mM NaCl/10 mM imidazole/1 mM EDTA, pH 7.0. After 1 day the dialysate was replaced with fresh solution, and dialysis continued overnight. The rhodopsin-containing vesicles were frozen in liquid nitrogen and stored at −80°C.

Spectroscopic measurements

Absorption spectra of the rhodopsin-containing

vesicles were recorded in the absence of detergent with a modified Cary 14 spectrophotometer on line with a PDP-8 minicomputer (Digital Equipment Corp, Marlboro, MA) [19]. All spectra were recorded from 650 nm to 390 nm and stored as 650 digital values in the minicomputer. The absorbance was quantitated using calibrated neutral density filters. Absorbance is expressed relative to the absorbance at 650 nm. To minimize the effect of light scattering on the spectrum, the photomultiplier tube was located next to the sample. In addition, a scattering background was imposed on the vesicle suspension and then subtracted to obtain the absolute spectrum. The measuring beam of the spectrophotometer passed vertically through a cylindrical cuvette containing the vesicles and a disk of Whatman 1 filter paper at the bottom of the cuvette. The absolute spectrum was generated by subtracting the baseline recorded with the filter paper and buffer solution in the absence of vesicles. When buffer additions were made between spectra, the increase in sample volume was compensated for by an increase in the pathlength through the sample due to the vertical path of the measuring beam, so the spectra did not need to be corrected for the effects of dilution. Measurements of the pH of the sample were made in the cuvette with a Corning semi-micro combination electrode (No. 476050) immediately before measurement of the corresponding spectrum.

Results and Discussion

Purification of squid rhodopsin

Squid photoreceptors were purified by sucrose density centrifugation using conditions that eliminated rhodopsin proteolysis and minimized lipid peroxidation. Previous studies of squid photoreceptors have reported a variety of molecular weights for rhodopsin, due to the action of proteases which rapidly degrade rhodopsin [20]. Exclusion of calcium from the solution and the addition of EDTA or EGTA to chelate endogenous calcium eliminated proteolysis of the photoreceptor proteins. In some species of squid, such as *L. pealei*, this precaution was not necessary because proteolysis did not occur. The presence of EDTA and deoxygenation of solutions by bubbling with argon reduced oxidation of the membrane. Squid photoreceptor lipids were reported to have the highest polyunsaturated fatty acid content ever observed in either vertebrate or invertebrate photoreceptors, amounting to 60–90% in the various lipid classes [21]. When precautions were not exercised to reduce oxidation, rhodopsin became covalently crosslinked, and rhodopsin dimers and trimers were observed on SDS-polyacrylamide gels even in the presence of reducing agents.

Rhodopsin was purified by chromatography with DEAE-cellulose after extraction into octyl glucoside. Previously reported methods [22,23] re-

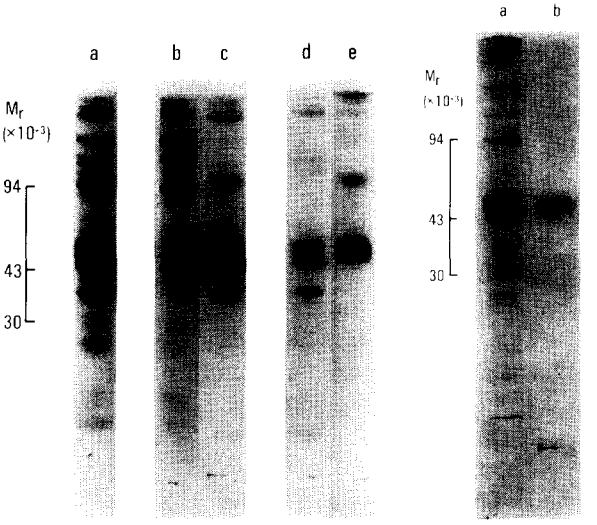

Fig. 1. (Left-hand). Polyacrylamide gels of the rhodopsin preparation during purification. Rhodopsin was purified as described in Experimental Procedure. Aliquots of the preparation at various stages of the purification were prepared for electrophoresis by the addition of 2.5% sodium dodecyl sulfate, 240 mM dithiothreitol, 3% (v/v) β mercaptoethanol, 10% (v/v) glycerol. Samples were subjected to electrophoresis on 7% SDS-polyacrylamide gels [27], and were then stained with Coomassie brilliant blue [28]. a, Photoreceptor membranes after sucrose density gradient centrifugation; b, material not solubilized by 50 mM octyl glucoside; c, proteins solubilized by 50 mM octyl glucoside; d, proteins adhering to the DEAE-cellulose; e, rhodopsin, and oligomers of rhodopsin, not bound to DEAE-cellulose in the presence of 50 mM octyl glucoside, 10 mM imidazole, 1 mM EDTA, pH 7.0.

Fig. 2. (Right-hand). Polyacrylamide gel of sucrose-density purified photoreceptors and DEAE-cellulose purified rhodopsin. Aliquots of the preparation were prepared for electrophoresis as described in Fig. 1. a, Sucrose-density purified photoreceptors; b, DEAE-cellulose purified rhodopsin.

lied on a similar purification procedure, but rhodopsin was solubilized in digitonin or sucrose lauryl ester L-1690, detergents that could not be removed subsequently from rhodopsin by dialysis. Octyl glucoside, while not a suitable detergent for spectroscopic studies because of metarhodopsin instability, was a useful detergent for purification. Octyl glucoside was found to solubilize rhodopsin effectively, and if maintained at temperatures below 10°C in the dark, the rhodopsin spectrum was unaltered. The addition of phospholipid further stabilized the rhodopsin spectrum to prevent denaturation. Preservation of function by the addition of lipids during protein solubilization in detergents also has been reported for the acetylcholine receptor [24,25] and sodium channel [26]. Other detergents commonly used to solubilize vertebrate rhodopsin, such as sodium cholate, cetyltrimethylammonium bromide, and dodecyldimethylamine *N*-oxide, were not suitable for squid rhodopsin because rhodopsin was substantially denatured following solubilization. The scheme described here for rhodopsin purification and reassembly into membranes also takes advantage of the selective extraction of rhodopsin into octyl glucoside, with many retinal proteins remaining insoluble.

Figs. 1 and 2 show SDS-polyacrylamide gel electrophoresis patterns of the preparation at various stages of rhodopsin purification. The photoreceptor membranes after the sucrose density gradient contained many proteins other than rhodopsin (Fig. 1 lane a, and Fig. 2 lane a). Retinochrome (M_r 24000) was absent from the membranes, and less than a 5% decrease in the absorption spectrum was recorded after the addition of 10 mM NH_2OH. Rhodopsin was solubilized by the addition of octyl glucoside (Fig. 1 lane c) and was purified substantially from other proteins which remained insoluble (lane b). Removal of the remaining proteins was effected with DEAE-cellulose. At low ionic strength other photoreceptor proteins adhered to the DEAE-cellulose (lane d) and rhodopsin was separated readily. Rhodopsin formed a single band on SDS-polyacrylamide gels of apparent M_r 46000 (Fig. 1 lane e, and Fig. 2 lane b) which occasionally formed oligomers (Fig. 1 lane e). The rhodopsin band, and oligomers of rhodopsin when present, exhibited fluorescence under long wave ultraviolet light when rhodopsin was reduced to *N*-retinyl-opsin by treatment with sodium borohydride [18]. This preparation had an absorbance ratio 280 nm/490 nm of 3.0–3.4. The best preparations had an absorbance ratio of 3.0 which is comparable to the value of 2.5 reported by Nashima et al. [20] for delipidated rhodopsin which was partially proteolyzed.

Squid rhodopsin in phospholipid vesicles

Diphytanoylphosphatidylcholine, a synthetic lipid, was used to form vesicles containing rhodopsin because it is not oxidizable and yet exhibits a high degree of fluidity [29], usually associated with unsaturated lipids. The recombinant vesicles were formed by detergent dialysis. The octyl glucoside concentration was measured following dialysis using ^{14}C-labeled octyl glucoside and was found to be 26 μM, a 2000-fold reduction in concentration. The composition of the vesicle population was 160–220 mol diphytanoylphosphatidylcholine/mol rhodopsin, and 1.3–1.7 mol octyl glucoside/mol rhodopsin.

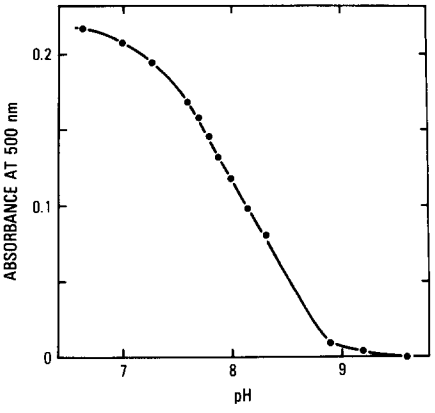

Fig. 3. pH dependence of the formation of metarhodopsin acid from metarhodopsin basic in rhodopsin-lipid vesicles. Vesicles containing purified rhodopsin and diphytanoylphosphatidylcholine in 2.5 ml 20 mM NaCl/6 mM Na_2CO_3/2 mM imidazole/0.2 mM EDTA, pH 9.6, were illuminated to produce metahodopsin basic using light filtered through a narrow band interference filter with a λ_{max} at 499 nm until there was no further change in the absorption spectrum. The pH was titrated to lower values in increments by the addition of 1 M Tris-HCl, pH 8.3, then with 1 M imidazole pH 7.0, and finally with 2 M HCl. After each addition the pH of the vesicle suspension was measured, and the absorption spectrum was recorded. Spectra exhibited an isosbestic point at 415 nm. Temperature 0°C.

Spectroscopic properties of purified rhodopsin-containing vesicles

The spectral properties of purified rhodopsin in diphytanoylphosphatidylcholine vesicles were similar to those of rhodopsin solubilized in digitonin [12–14,30] or sucrose lauryl ester L-1690 [31], both mild detergents in which rhodopsin can be maintained for long periods of time. Upon absorption of light, rhodopsin (λ_{max} = 490 nm) is converted to metarhodopsin. Depending on the pH, metarhodopsin is in an acid (λ_{max} = 500 nm) or basic (λ_{max} = 380 nm) form. The pK of the metarhodopsin acid to basic transition is dependent on the environment of the protein, both the nature and concentration of detergents and phospholipid produce variations in the pK [31]. Fig. 3 shows the titration curve of metarhodopsin basic to metarhodopsin acid with a pK of about 8.1 in the vesicles. The native membrane of *L. opalescens* shows a similar pK of 8.3 (not shown).

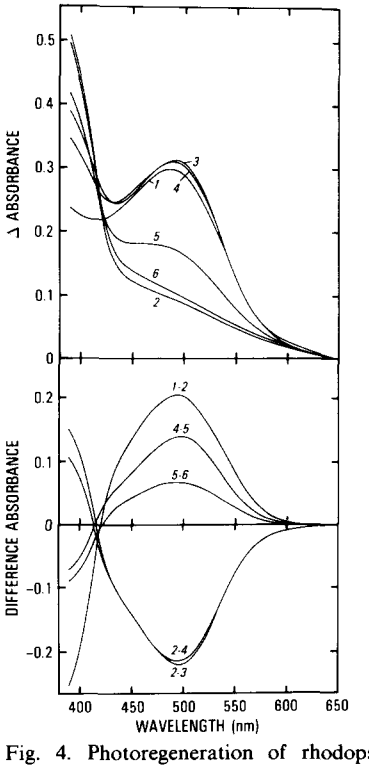

Fig. 4. Photoregeneration of rhodopsin at neutral pH in rhodopsin-lipid vesicles. A suspension of rhodopsin-containing vesicles was subjected sequentially to illumination and changes of pH demonstrating rhodopsin regeneration from metarhodopsin acid at neutral pH. Temperature 0°C. Upper panel: absorption spectra (expressed relative to the absorbance at 650 nm). Lower panel: difference spectra of the indicated curves. (1) Rhodopsin spectrum. Vesicles containing purified rhodopsin and diphytanoylphosphatidylcholine in 2.5 ml 20 mM NaCl/6 mM Na_2CO_3/2 mM imidazole/0.2 mM EDTA, pH 9.5. (2) Metarhodopsin basic. Vesicles were illuminated with light filtered through a narrow-band interference filter with λ_{max} at 499 nm. (3) Metarhodopsin acid. The pH was changed to 7.45 by the addition of imidazole to a final concentration of 32 mM. (4) Mixture of metarhodopsin acid and rhodopsin. The sample was illuminated with light filtered at 499 nm to regenerate rhodopsin from acid metarhodopsin. (5) Mixture of metarhodopsin basic and rhodopsin. The pH was changed to 9.4 by the addition of Na_2CO_3 to a final concentration of 115 mM. (6) Metarhodopsin basic. Vesicles were illuminated with light of λ_{max} 499 nm.

The ability to regenerate rhodopsin from metarhodopsin is a critical test of the native conformation of purified rhodopsin. In the recombined vesicles, rhodopsin may be regenerated photochemically from metarhodopsin acid at both neutral and basic pH values. Fig. 4 demonstrates rhodopsin regeneration at a pH near neutrality. Rhodopsin, with a λ_{max} of 490 nm (curve 1), was first illuminated at pH 9.5 to convert it to metarhodopsin (curve 2). At this pH the predominant form of metarhodopsin is metarhodopsin basic, with a λ_{max} of 380 nm. A change in the pH to 7.45 resulted in an increase in the visible absorption (curve 3) corresponding to the formation of metarhodopsin acid, with a λ_{max} of 500 nm. This procedure is the only means for preparing pure acid metarhodopsin, because illumination of rhodopsin in an acid solution produces an equilibrium mixture containing both rhodopsin and acid metarhodopsin. When the vesicles containing acid metarhodopsin were illuminated further with green light, rhodopsin was photoregenerated partially, as evidenced by a slight decrease in absorbance and shift of the λ_{max} to shorter wavelength (curve 4). Because there is extensive overlap of the rhodopsin and acid metarhodopsin spectra, they are readily interconverted by absorption of light, and a photochemical equilibrium results with approximately equal concentrations of rhodopsin and acid metarhodopsin. The extent of rhodopsin regeneration was examined by changing the pH to 9.4 (curve 5) so that the basic form of metarhodopsin predominated and did not contribute substantially to the visible absorbance. The photoregenerated rhodopsin could be bleached to basic metarhodopsin by illumination with green light (curve 6).

The difference spectra in Fig. 4 (lower panel) illustrate the differences between rhodopsin and basic metarhodopsin (curve 1–2), the subsequent increase in visible absorbance due to the difference between acid metarhodopsin and basic metarhodopsin (curve 2–3), and the shift in the visible absorption maximum caused by the difference between regenerated rhodopsin plus acid metarhodopsin compared to basic metarhodopsin (curve 2–4). The subsequent losses in visible absorbance are shown by the difference between acid metarhodopsin and basic metarhodopsin obtained after increasing the pH (curve 4–5), followed by the bleaching of regenerated rhodopsin to basic metarhodopsin (curve 5–6).

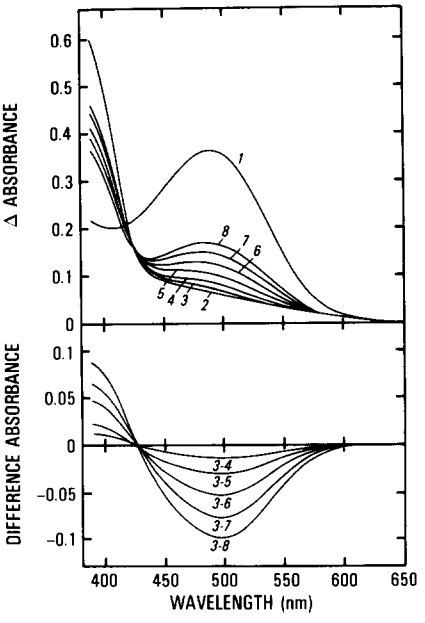

Fig. 5. Regeneration of rhodopsin at alkaline pH in rhodopsin-lipid vesicles. Spectra of rhodopsin-lipid vesicles at pH 10.0 demonstrate the dark regeneration of rhodopsin after illumination. All spectra at 15°C. Upper panel: absorption spectra. Lower panel: difference spectra. (1) Rhodopsin spectrum. Vesicles containing purified rhodopsin and diphytanoylphosphatidylcholine in 1.2 ml 83 mM $Na_2B_4O_7$/17 mM NaCl/1.7 mM imidazole/0.17 mM EDTA, pH 10.0. (2) Metarhodopsin basic. Vesicles were illuminated with light filtered by a sharp cut-off yellow Corning CS 3-69 filter that passes light of $\lambda \geqslant 520$ nm. (3–8) regeneration of rhodopsin in the dark after illumination by a 150 watt high pressure xenon lamp for 1 h at 0°C through a Corning CS 3-69 filter. (3) Immediately after returning to 15°C; (4) 2 min after the end of illumination, (5) 7 min after the end of illumination; (6) 17 min after the end of illumination, (7) 32 min after the end of illumination; (8) 182 min after the end of illumination.

Unlike regeneration of rhodopsin from acid metarhodopsin, Hara and Hara [30] reported that illumination of basic metarhodopsin with near ultraviolet light yields variable results depending on the species of squid, producing a mixture of rhodopsin, isorhodopsin, and/or a photostable pigment with a λ_{max} similar to that of rhodopsin. With *L. opalescens* photoreceptors or using purified rhodopsin in lipid vesicles, this method of rhodopsin regeneration did not prove satisfactory because most of the product was an unidentified photostable pigment with a λ_{max} of 490 nm that subsequently could not be bleached with green light.

An alternate method for the photochemical regeneration of rhodopsin is to illuminate metarhodopsin with green light at an alkaline pH in the presence of borate ion [12–14]. Rhodopsin is formed subsequently from an intermediate, P380, in the dark. Since acid metarhodopsin is the form of metarhodopsin that absorbs at these wavelengths, presumably the regeneration proceeds through acid metarhodopsin [12]. The high pH and the presence of borate is reported to slow the reaction so that an appreciable quantity of the dark intermediate P380 can accumulate before rhodopsin is formed [12]. If rhodopsin were created rapidly, then it too would absorb light to generate metarhodopsin, and no net rhodopsin accumulation would result. The reaction rate was reported to be influenced by the concentration of the detergent (digitonin) that was in the solution [12]. Low detergent concentrations increased the rate of rhodopsin regeneration so that little rhodopsin accumulated. Fig. 5 shows that also in the absence of detergent rhodopsin could be photoregenerated at alkaline pH. First, rhodopsin (curve 1) was bleached to metarhodopsin (curve 2) at pH 10.0. At this pH less than 2% of the metarhodopsin is expected to be in the acid form. Then the sample was illuminated at 0°C with a very high intensity light filtered to pass wavelengths of at least 520 nm so that acid metarhodopsin would absorb light but basic metarhodopsin would not be photoactivated. The vesicle suspension was returned to 15°C and spectra measured as a function of time. Initially the absorbance at 390 nm decreased

markedly (curve 3), corresponding to the formation of P380 which has a lower extinction coefficient than basic metarhodopsin. With time the absorbance in the visible region increased (curves 3–8). The difference spectra (lower panel of Fig. 4) show an increase in the visible absorbance with a λ_{max} at 490 nm due to the formation of rhodopsin, and an isosbestic point at about 425 nm. Using this procedure, 35% of the rhodopsin was regenerated. The rhodopsin which was photoregenerated could be rebleached to metarhodopsin with visible light (not shown).

Conclusion

We describe a method for the rapid purification of squid rhodopsin using octyl glucoside. Vesicles containing purified squid rhodopsin were formed by dialysis of octyl glucoside and exhibited spectroscopic properties indicating that the native conformation of rhodopsin was preserved. Upon illumination, rhodopsin was converted to metarhodopsin which was in an acid or basic form depending on the pH of the solution. The pK of the interconversion of acid and basic metarhodopsin was similar in the recombined vesicles and in the native photoreceptor membrane. This suggests that the method for purification and recombination with lipid preserved the rhodopsin conformation. Our studies also showed that rhodopsin could be photoregenerated from metarhodopsin in either neutral or alkaline pH.

It is anticipated that these squid rhodopsin vesicles will provide a basis for further studies of the function of light-regulated processes in the photoreceptor, particularly with respect to the interaction of invertebrate rhodopsin with light-dependent enzyme systems [15,16]. This approach has proved valuable in the vertebrate photoreceptor [1,3,32] as well as in the hormone-activated adenylate cyclase [32,33].

Of general interest to the study of enzyme cascades in signal transduction is the question of the rate of information transfer through a cascade mechanism. The use of invertebrate photoreceptor enzymes may permit measurement of the kinetics of rhodopsin/G protein interaction. Photoreceptors offer a unique advantage in the study of receptor/G protein interactions in that rhodopsin molecules can be synchronously activated by light. Invertebrate rhodopsin provides the additional advantage that rhodopsin can be photoregenerated from metarhodopsin, allowing for kinetic study of the turn-off of the G protein in response to metarhodopsin depletion. This endeavor could serve to answer the questions of whether the activation of an enzyme cascade is of the appropriate time scale to be considered in the main pathway of visual transduction in invertebrate as well as vertebrate photoreceptors. It is anticipated that the convergence of biochemical and physiological approaches will lead to a detailed and quantitative description of the primary events in vision.

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References

1 Shinozawa, T., Uchida, S., Martin, E., Cafiso, D., Hubbell, W. and Bitensky, M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1408–1411
2 Fung, B.K.-K. and Stryer, L. (1980) Proc. Natl. Acad. Sci. U.S.A. 78, 152–156
3 Kuhn, H., Bennett, N., Michel-Villaz, M. and Chabre, M. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 6873–6877
4 Montal, M. (1979) Biochim. Biophys. Acta 559, 231–257
5 Hubbard, R. and St. George, R.C.C. (1958) J. Gen. Physiol. 41, 501–528
6 Kropf, A., Brown, P.K. and Hubbard, R. (1959) Nature (London) 183, 10–17
7 Yoshizawa, T. and Wald, G. (1964) Nature (London) 201, 340–345
8 Azuma, K., Azuma, M. and Suzuki, T. (1975) Biochim. Biophys. Acta 393, 520–530
9 Tokunga, F., Shichida, Y. and Yoshizawa, T. (1975) FEBS Lett. 55, 229–232
10 Sulkes, M., Lewis, A. and Marcus, M.A. (1978) Biochemistry 17, 4712–4722
11 Schichida, Y., Kobayashi, T., Ohtani, H., Yoshizawa, T. and Nagakura, S. (1978) Photochem. Photobiol. 27, 335–341
12 Suzuki, T., Sugahara, M. and Kito, Y. (1972) Biochim. Biophys. Acta 275, 260–270
13 Suzuki, T., Sugahara, M., Azuma, K., Azuma, M., Saimi, Y. and Kito, Y. (1973) Biochim. Biophys. Acta 333, 149–160
14 Tsuda, M. (1978) Biochim. Biophys. Acta 502, 495–506
15 Vandenberg, C.A. and Montal, M. (1984) Biochemistry, in the press
16 Vandenberg, C.A. and Montal, M. (1984) Biochemistry, in the press

17 Yoshioka, T., Inoue, H., Inomata, K., Hayashi, F., Takagi, M. and Tanekaka, T. (1981) Proc. Jap. Acad. 57, Ser. B, 309–313
18 Hagins, F.M. (1973) J. Biol. Chem. 248, 3298–3304
19 Butler, W.L. and Hopkins, D.W. (1970) Photochem. Photobiol. 12, 439–450
20 Nashima, K., Mitsudo, M. and Kito, Y. (1979) Biochim. Biophys. Acta 579, 155–168
21 Anderson, R.E., Benolken, R.M., Kelleher, P.A., Maude, M.B. and Weigand, R.D. (1978) Biochim. Biophys. Acta 316, 316–326
22 Kito, Y., Suzuki, T., Sugahara, M., Azuma, K. and Mishima, K. (1973) Nature (New Biol.) 243, 53–54
23 Nashima, K., Mitsudo, M. and Kito, Y. (1978) Biochim. Biophys. Acta 536, 78–87
24 Epstein, M. and Racker, E. (1978) J. Biol. Chem. 253, 660–662
25 Anholt, R., Lindstrom, J. and Montal, M. (1981) J. Biol. Chem. 256, 4377–4387
26 Agnew, W.S. and Raftery, M.A. (1979) Biochemistry 18, 1912–1919
27 Weber, K. and Osborne, M. (1969) J. Biol. Chem. 244, 4406–4412
28 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617
29 Lindsey, H., Petersen, N.O. and Chan, S.I. (1979) Biochim. Biophys. Acta 555, 147–167
30 Hara, T. and Hara, R. (1972) Handbook of Sensory Physiology: Photochemistry of Vision (Dartnall, H.J.A., ed.), Vol. VII/1, pp. 720–746, Springer Verlag, Berlin
31 Nashima, K., Kawase, N. and Kito, Y. (1980) Biochim. Biophys. Acta 626, 390–396
32 Bitensky, M.N., Wheeler, M.A., Rasenick, M.M., Yamazaki, A., Stein, P., Haliday, K.R. and Wheeler, G.L. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 3408–3412
33 Pedersen, S.E. and Ross, E.M. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 7228–7232