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LIPID-PROTEIN INTERACTION IN THE PHOTOLYSIS OF OCTOPUS RHODOPSIN

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Summary

Microvillar membranes of octopus photoreceptor cells were treated with phospholipase A_2, phospholipase C, hexane, or their combinations. By these means, various membrane preparations containing qualitatively and quantitatively different lipids were obtained. The lipid composition and phospholipid content of the membrane preparations obtained by the above methods were determined.

Photochemical processes in the digitonin extract of the native and treated membranes have been studied by flash photometry. The results suggest that several different variations in the lipids can affect the rates of the photochemical transformations; these are: the content of phospholipid, the amount of unsaturated hydrocarbon chains and free fatty acids.

Introduction

The molecular photoreceptor in both the vertebrate and invertebrate retina is the chromoprotein rhodopsin, which consists of 11-*cis*-retinal covalently attached to the glycolipoprotein opsin. When rhodopsin is irradiated in vitro there is an initial photoisomerization of the 11-*cis*-retinal to the all-*trans* form followed by a series of thermal transformations leading to the final photoproduct [1,2]. One or more of these thermal reactions, presumably initiate the electrical activity in the photoreceptor cell which is then transmitted to higher-order neurons [3,4].

Rhodopsin is an integral protein of the photoreceptor membrane (rod outer segment for vertebrates and microvillar tube for invertebrates) and is in contact with the hydrophobic interior of the membrane. Thus, to describe the excita-

tion mechanism in molecular terms it requires a greater understanding of the conformational changes of rhodopsin upon light absorption as well as a greater understanding of the interaction between rhodopsin and lipid in its native membrane environment.

The chemistry of many aspects of the vertebrate photoreceptor membrane has been carefully studied (reviewed in Ref. 5). In particular, investigations of the lipid-rhodopsin interaction have been limited to the vertebrate pigment [6—9]. We wish to extend these studies to invertebrate photoreceptor membranes. Recently, a detailed study of the lipid composition of the octopus photoreceptor (Mizudako, *Paroctopus defleini*) was done by us [10]. The major lipid is phospholipid, but the cholesterol content is relatively high (9.7%). Phosphatidylethanolamine and phosphatidylcholine are the major phospholipids. The phosphatidylserine content is lower in octopus retina compared with the vertebrate retina. The predominant fatty acids of phosphatidylethanolamine and phosphatidylcholine are highly unsaturated. By treatment with phospholipase A_2, phospholipase C, hexane, or a combination of these agents, we have obtained several different preparations of octopus photoreceptor membranes which have a qualitatively and quantitatively different lipid composition from the native membrane. This paper reports the development of a method for the study of the functional interaction between rhodopsin and components of its membrane environment as 'assayed' by observing the rate of two of the thermal transformations in the photolysis of octopus rhodopsin: lumirhodopsin to mesorhodopsin and mesorhodopsin to acid metarhodopsin.

Experimental

Material. Octopuses (Mizudako, *Paroctopus defleini*) were collected at Oshidomari, Rishiri Island, northern Hokkaido, Japan. The preparation of microvillar membranes of octopus photoreceptor cells was carried out as previously described [11]. The dissected eyes were shaken in 10 mM imidazole hydrochloride buffer (pH 7.2) containing 1 mM $MgCl_2$ and 500 mM NaCl. The microvillar membranes were isolated by repeated discontinuous sucrose gradient centrifugation. An orange band which appeared at the 1.12/1.15 g/ml interface was collected and washed repeatedly with 100 mM imidazole hydrochloride (pH 7.2). The membrane was treated with phospholipase A_2, phospholipase C and/or hexane and lipid analysis was performed. These preparations were solubilized with 2% digitonin with 100 mM imidazole hydrochloride (pH 5.7) for flash photolysis experiment.

Phospholipase A_2 treatment. Snake venom phospholipase (*Naja naja*, Sigma) was prepared by heat treatment following the method of Shichi [12]. An aqueous solution of venom (1.5 mg/ml) at pH 6.0 (100 mM imidazole hydrochloride buffer containing 10 mM $CaCl_2$) was heated for 10 min at 75°C and centrifuged at 10 000 × *g* for 15 min. The supernatant was diluted to a concentration of 0.3 mg/ml with 100 mM imidazole buffer (pH 5.7) and used as the phospholipase A_2 preparation; it showed no proteolytic activity as assayed with bovine serum albumin as substrate and with ninhydrin for the detection of liberated amino acids. The enzyme solution was mixed with a suspension of microvillar membranes (1 mM of enzyme per 1 unit membrane (8 mg/ml)) and

the mixture was incubated at 36°C for 60 min. After the incubation with enzyme, suspensions were immediately cooled to 4°C and then centrifuged for 10 min at 20 000 × *g*. The sediment was washed twice with distilled water. As a control, an aliquot of the same suspension was incubated without enzyme. Incubation of the control suspension under these conditions did not cause any appreciable degree of thermal bleaching of rhodopsin or change in the lipid composition.

Phosholipase C treatment. Microvillar membrane suspensions (8 mg/ml) in 0.1 M imidazole hydrochloride buffer (pH 7.2) containing 10 mM $CaCl_2$ were incubated at 36°C for 1 h under N_2 with units of phospholipase C (*Bacillus cereus*, Sigma). The enzyme reaction was stopped by cooling the preparation to 4°C and the enzyme was washed out with the same method as for phospholipase A_2 treatment.

Extraction of the outer segment with hexane. Intact microvillar membranes or microvillar membranes treated with phospholipase A_2 or phospholipase C were lyophilized, and the dried orange powder was extracted with 1 ml hexane per 8.0 mg dry wt. of membrane at −20°C for 1 h under N_2 gas. The material was centrifuged at 1200 × *g* for 15 min at 4°C and the supernatant was removed. The pellet was exposed to a stream of N_2 to remove hexane completely.

Lipid analysis. All solvents were deoxygenated with N_2 prior to use. Analytical procedures for the lipids were generally the same as described in our previous paper [10]. The microvillar membrane preparations, treated with phospholipases, by hexane extractions, or their combination, were homogenized with Tris-HCl buffer (pH 7.4) and aliquots were taken for protein determination. Lipids were then extracted from the homogenates of the microvillar membrane preparations according to the method of Bligh and Dyer [13]. The total lipid was separated by column chromatography on silicic acid into neutral lipids and phospholipids. The amounts of cholesterol, acylglycerol and free fatty acid were determined in the neutral lipid fraction after elution from the column with chloroform. After elution with methanol, aliquots were taken from the phospholipid fraction for the determination of lipid phosphorus. The remaining phospholipid fraction was employed for two-dimensional thin-layer chromatography with a 0.25 mm layer of silica gel G. The following solvent systems were used: chloroform/methanol/28% aqueous ammonia (65 : 35 : 5, v/v) for the *y* dimension and chloroform/acetone/methanol/acetic acid/water (5 : 2 : 1 : 1 : 0.5, v/v) for the *x* dimension. The pattern of the separation in these solvent systems and qualitative identification of the phospholipids has been given in our previous paper [10]. Lysophosphatidylethanolamine and an unidentified phospholipid, which has been shown as spot 6 in the previous paper [10], were not separated from each other in this solvent system. After development, the spots on the plates were detected by iodine vapor. Each spot was scraped off and transferred into test tubes. Lipid phosphorus of the spots was then determined. The phospholipids from the digitonin extract were separated in the same way as mentioned above, after removing digitonin by precipitation with added free cholesterol.

In some cases, phosphatidylcholine and phosphatidylethanolamine were isolated from the phospholipid fraction by thin-layer chromatography (TLC) on

silica gel G plates with a chloroform/methanol/water (70 : 30 : 5, v/v) solvent system. Each band on the plates was scraped and eluted with chloroform/methanol/acetic acid/water (50 : 39 : 1 : 10, v/v) as described by Arvidson [14], and the extract was washed with 4 N ammonia and 50% methanol. The fatty acid methyl esters of the phospholipids prepared with boron trifluoride/methanol [15] were analyzed on a 2 m × 4 mm outer diameter pyrex column packed with 10% diethyleneglycol succinate on Chromosorb W at 185°C. Identification of fatty acid methyl esters was carried out as described previously [10]. Lipid phosphorus was determined by the method of Aalbers and Bieber [16], and of Bartlett [17]. Glycerol was determined by the method of van Handel and Zilversmit [18], total cholesterol by the method of Zak et al. [19] and free fatty acid by the method of Itaya and Ui [20]. Protein was determined by the method of Lowry et al. [21] with bovine serum albumin as the standard.

Flash photometry. The flash photolysis apparatus which was used in these experiments was similar to that described in a previous paper [22]. The flash lamp had a half duration of about 200 μs. The beam was focused on the sample cell using an elliptical mirror; a thermal filter and a blue color glass filter (VV 42, Toshiba) were placed on the light path. Measurements were made at a temperature of 1.2°C (±0.1°C) in a thermostatically controlled double-jacketed cylindrical optical cell (6 mm × 50 mm). The kinetic record is digitized with 8-bit resolution and stored in 8-bit memory. The data fluctuations could be minimized by accumulation of traces up to 255 times, if necessary. Analogue replica could be recorded on an X-Y recorder as changes in absorbance (ΔA) as a function of time. Typical data are shown in Fig. 1.

Results

Lipid content of treated microvillar membranes

In the present study, the microvillar membranes of octopus photoreceptor cells were treated with phospholipase A_2, phospholipase C, hexane, or their combination. By these means, various membrane preparations containing qualitatively and quantitatively different lipids were obtained. Results of the lipid anlysis of the native membranes have already been reported [10].

Table I shows the phospholipids present in the hexane extract and in the hexane-extracted residue of the microvillar membranes. By extraction of the lyophilized membranes with hexane for 1 h at −20°C, 70% of phospholipids originally present were removed from the membranes. Higher percentages of phosphatidylethanolamine and ceramide 2-aminoethylphosphonate were found in the hexane extract than in the hexane-extracted residue. About 80% of phosphatidylethanolamine and 81% of ceramide 2-aminoethylphosphonate were extracted from the intact membranes, whereas 50% of phosphatidylcholine originally present was found in the hexane extract. These findings indicate that phosphatidylethanolamine and ceramide 2-aminoethylphosphonate were more readily removed by the hexane extraction than other phospholipids such as phosphatidylcholine. However, no significant difference was found in the fatty acid profiles of phosphatidylcholine and phosphatidylethanolamine in the hexane extract and the hexane-extracted residue. This result shows that the

TABLE I

PHOSPHOLIPIDS PRESENT IN THE HEXANE EXTRACT AND IN THE HEXANE-EXTRACTED RESIDUE OF MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

Values are means ± S.D. Values in parentheses represent percentages of each phospholipid present in the original membranes.

	Hexane extract (n = 3)	Hexane-extracted residue (n = 3)
Distribution of phospholipid (%)	70.0	30.0
Phospholipid composition (%)		
Phosphatidylethanolamine	53.2 ± 1.9 (80.0)	31.1 ± 1.7 (20.0)
Phosphatidylcholine	30.0 ± 1.2 (57.7)	51.3 ± 2.4 (42.3)
Ceramide 2-aminoethylphosphonate	6.8 ± 0.7 (81.4)	3.5 ± 0.7 (18.6)
Sphingomyelin	3.0 ± 0.6 (53.8)	6.1 ± 0.6 (46.2)
Phosphatidylserine	3.6 ± 0.2 (61.8)	5.2 ± 1.8 (38.2)
Others	3.4 ± 0.3 (73.9)	2.8 ± 0.9 (26.1)

preferential extraction by hexane of a specific molecular species of a phospholipid does not occur.

Table II shows the results of the quantitative decrease of phospholipids in treated membranes compared to native membranes. This table also gives the lipid composition in the membrane preparations. The membranes originally contained 39% phosphatidylcholine and 45% phosphatidylethanolamine. After hexane extraction, the phospholipid content decreased by 70%.

By phospholipase A_2 treatment, glycerophospholipids in the native membranes were completely hydrolyzed to their corresponding lyso compounds and free fatty acids. The amount of lipid phosphorus did not decrease significantly, but the kind of lipids in the phospholipase A_2-treated membranes were free fatty acid (43%), lysophosphatidylethanolamine plus X (25%) and lysophosphatidylcholine (21%). Lysophosphatidylserine was not clearly detected by TLC. 'X' is as unidentified phospholipid discussed in Ref. 10.

Phosphatidylcholine and phosphatidylethanolamine, the major lipids originally present, were almost completely degraded to diacylglycerol by phospholipase C treatment. Accordingly, the predominant lipid in phospholipase C-treated membranes was diacylglycerol (71%). Ceramide 2-aminoethylphosphonate, sphingomyelin and X appeared to remain intact upon phospholipase C treatment, whereas about half of phosphatidylserine seemed to be hydrolyzed.

Marked decreases of phospholipids were found to be in membranes subjected to the combination of hexane extraction and phospholipase C treatment. As compared to the native membranes, 9.9 and 6.1% of phospholipids were recovered in the hexane-extracted, phospholipase C-treated and phospholipase C-treated and hexane-extracted membranes, respectively. However, the lipid composition was considerably different from each other. The former (hexane-extracted and phospholipase C-treated membranes) contained diacylglycerol as the main lipid, but the major lipids of the latter (phospholipase C-treated and hexane-extracted membranes) were phospholipids, mainly composed of sphingomyelin and ceramide 2-aminoethylphosphonate.

The main lipid constituents of the membranes prepared by the combination

TABLE II

DECREASE OF PHOSPHOLIPID AND LIPID COMPOSITION IN THE VARIOUS PREPARATIONS OF MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

	Membrane preparation							
	Native	Hexane-extracted	Phospholipase A_2-treated	Phospholipase C-treated	Hexane-extracted, Phospholipase A_2-treated	Hexane-extracted, Phospholipase C-treated	Phospholipase A_2-treated, hexane-extracted	Phospholipase C-treated, hexane-extracted
Amount of phospholipid from native membranes remaining (%)	100	32.8	92.4	22.1	29.8	9.9	63.3	6.1
Lipid composition (wt. %)								
Cholesterol	10	5	6	9	3	6	4	5
Acylglycerols *	2	—	1	71	—	81	—	14
Free fatty acid	4	1	43	3	46	2	16	1
Phospholipid	84	94	50	17	51	11	80	79
Phospholipid composition (mol %)								
Phosphatidylethanolamine	45	31	—	4	—	—	—	—
Phosphatidylcholine	39	51	—	2	—	—	—	—
Ceramide 2-aminoethylphosphonate	5	4	5	47	7	24	2	27
Sphingomyelin	2	5	2	21	4	40	3	41
Phosphatidylserine	4	6	—	15	—	17	—	21
Lysophosphatidylethanolamine + X **	4	3	51	11	39	19	52	10
Lysophosphatidylcholine	—	—	42	—	50	—	43	—

* Acylglycerols include triacylglycerol and diacylglycerol

** X is an unidentified phospholipid, which migrates with lysophosphatidylethanolamine on two-dimensional thin-layer chromatography, and is alkali and acid stable as described in a previous paper [10].

of hexane extraction and phospholipase A_2 treatment were lysophospholipids. The amount of phospholipids in the hexane-extracted, phospholipase A_2-treated membranes decreased to about 30% of the phospholipids originally present, but the lipid composition was almost identical with the phospholipase A_2-treated membranes. Upon hexane extraction of the phospholipase A_2-treated membranes, about 70% of free fatty acids were obtained, and consequently the major lipids were lysophosphatidylcholine and lysophosphatidylethanolamine.

Photochemical processes in treated preparations of microvillar membrane

Delipidated and/or digested microvillar membranes were solubilized by 2% digitonin (10 mM imidazole hydrochloride, pH 5.7) and used for kinetic studies. As shown in Table III, the phospholipid composition and the fatty acid composition of total lipid of the digitonin extracts are qualitatively identical with those in the membrane preparations. About 55% of phospholipids were solubilized by the digitonin extraction of the membrane preparations. The ratios of phospholipid to protein were almost the same in the case of the original membranes and the digitonin extracts. The intermediates in the photolysis of octopus rhodopsin have been reported in previous papers [11,23–25]. Of the photochemical processes, the transformation of lumirhodopsin → mesorhodopsin and mesorhodopsin → acid metarhodopsin are illustrated in Figs. 1A and 2A for native membrane solubilized in 2% digitonin at pH 5.6 (0.1 M imidazole hydrochloride) and 1.2°C. The curves in Figs. 1A and 2A show the transient change in absorbance at 480 and 460 nm, respectively, after a blue light flash (Toshiba VV42). These changes are associated with the formation and decay of mesorhodopsin.

TABLE III

EFFECTS OF DIGITONIN EXTRACTION ON PHOSPHOLIPID COMPOSITION AND FATTY ACID COMPOSITION OF TOTAL LIPID OF THE MICROVILLAR MEMBRANES OF OCTOPUS PHOTORECEPTOR CELLS

The values represent means of duplicate analyses.

	Native membranes	Digitonin extract
Phospholipid composition (%)		
Phosphatidylethanolamine	45.2	44.5
Phosphatidylcholine	39.0	38.2
Ceramide 2-aminoethylphosphonate	5.4	6.4
Sphingomyelin	2.6	2.2
Phosphatidylserine	3.8	4.8
Others	4.0	3.9
Fatty acid composition (%) of total lipid		
14 : 0	0.7	0.4
16 : 0	16.8	15.0
18 : 0	2.5	1.9
18 : 1	7.5	6.4
18 : 2	2.4	1.8
20 : 4	22.0	19.6
20 : 5	13.5	15.7
22 : 6	32.8	36.6
Others	1.8	2.6

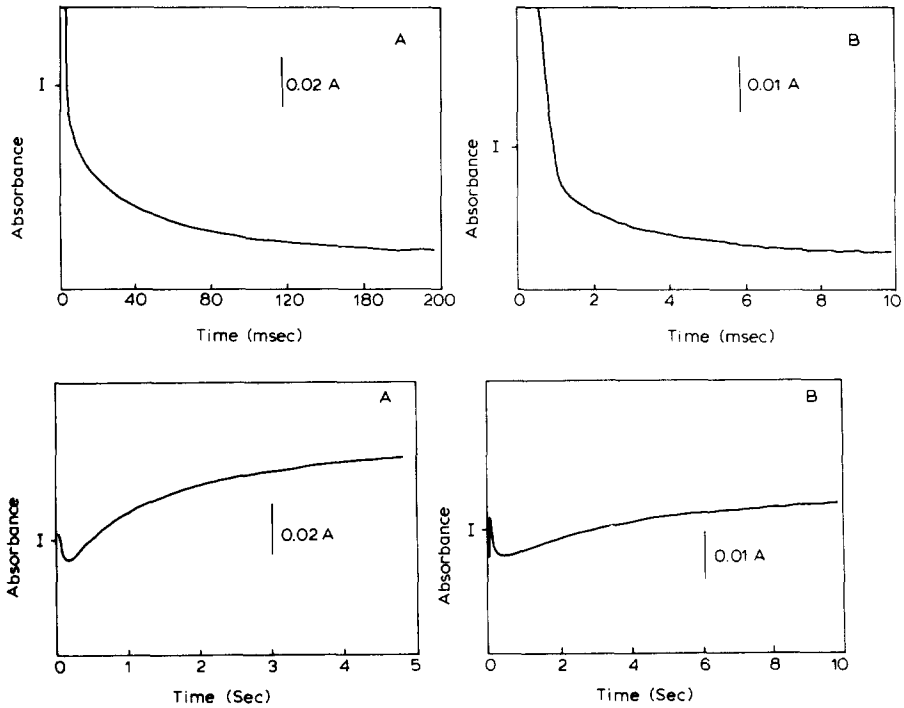

Fig. 1. (Upper figures.) Transformation of lumirhodopsin to mesorhodopsin observed at 480 nm after a blue flash (Toshiba VV42) at 1.2°C. Native membrane (A) and phospholipase C-hexane-treated membrane (B) solubilized in 2% digitonin at pH 5.6 (0.1 M imidazole hydrochloride).

Fig. 2. Transformation of mesorhodopsin to acid metarhodopsin observed at 460 nm after a blue flash (Toshiba VV42) at 1.2°C. Native membrane (A) and phospholipase A_2-hexane-treated membrane (B) solubilized in 2% digitonin at pH 5.6 (0.1 M imidazole hydrochloride).

From these data, we can calculate the rate constants as 23.5 ± 0.8 s^{-1} for the lumirhodopsin → mesorhodopsin transformation and 0.29 ± 0.02 s^{-1} for the mesorhodopsin → acid metarhodopsin transformation. The rate of these intermediate processes for preparations treated with phospholipases or hexane was different from that of the native preparation. Typical kinetic data of native and treated preparations are shown in Fig. 1A and B. The rate of the lumirhodopsin → mesorhodopsin transformation of the digitonin extract of phospholipase C-treated and hexane-extracted membranes increased by 7-times compared with the native preparation. On the other hand, the rate of the mesorhodopsin → acid metarhodopsin transformation of the digitonin extract of phospholipase A_2-treated and hexane-extracted membranes decreased 2-fold. The kinetic data for the preparations described in previous sections are gathered in Table IV. The effects of treatment on the rate constant of the two kinetic transformations of rhodopsin photolysis are expressed as:

$$\alpha_{a\text{-}b} = \frac{k^{T}_{a\text{-}b}}{k^{N}_{a\text{-}b}}$$

TABLE IV

KINETIC PARAMETERS OF TREATED PREPARATIONS

pH = 5.7; temperature = 1.2°C.

Membrane preparation	Lumirhodopsin → mesorhodopsin			Mesorhodopsin → acid metarhodopsin		
	k (s^{-1})	α	β	k (s^{-1})	α	β
Native	24 ± 0.8	1.0		0.29 ± 0.02	1.0	
Hexane-extracted	65 ± 3	2.7	1.0	0.26 ± 0.01	0.9	1.0
Phospholipase A_2-treated	120 ± 7	5.0		0.86 ± 0.03	3.0	
Phospholipase C-treated	32 ± 2	1.3		0.33 ± 0.02	1.1	
Hexane-extracted, phospholipase A_2-treated	153 ± 6	6.4	2.4	0.30 ± 0.03	1.0	1.3
Hexane-extracted, phospholipase C-treated	65 ± 3	2.7	1.0	0.31 ± 0.02	1.0	1.2
Phospholipase A_2-treated, hexane-extracted	22 ± 3	0.9		0.14 ± 0.06	0.48	
Phospholipase C-treated, hexane-extracted	159 ± 4	6.6		0.75 ± 0.20	2.6	

where $k^{T}_{a\text{-}b}$ and $k^{N}_{a\text{-}b}$ show the rate constant of a → b transformation of treated and native preparations, respectively.

Phospholipase C series

The rate constant of mesorhodopsin → acid metarhodopsin transformation was not greatly affected by the treatment with phospholipase C. On the other hand, the rate of lumirhodopsin → mesorhodopsin transformation ($\alpha_{l\text{-}m}$) increased by 1.3. Further treatment of the phospholipase C preparation with hexane greatly affected the rates of both transformations, that is, for lumirhodopsin → mesorhodopsin transformation ($\alpha_{l\text{-}m} = 6.6$) and for mesorhodopsin → acid metarhodopsin transformation ($\alpha_{m\text{-}a} = 2.6$).

Phospholipase A_2 series

Phospholipase A_2 treatment increased rates of both kinetic transformations, that is, for lumirhodopsin → mesorhodopsin ($\alpha_{l\text{-}m} = 5.0$) and for mesorhodopsin → acid metarhodopsin ($\alpha_{m\text{-}a} = 3.0$). Furthermore, when the phospholipase A_2-treated preparation was extracted with hexane in order to remove the free fatty acid released, the rate of both transformations was influenced. However, the trends were in opposite directions; that is, the rate of the lumirhodopsin → mesorhodopsin transformation of the digitonin extract of phospholipase A_2-treated and hexane-extracted membranes was 5.5-times smaller than that of phospholipase A_2-treated membranes, and the rate of the mesorhodopsin → acid metarhodopsin transformation of the former preparation was 6-times smaller than that of the latter preparation, that is $\alpha_{l\text{-}m} = 0.9$ for lumirhodopsin → mesorhodopsin transformation and $\alpha_{m\text{-}a} = 0.48$ for mesorhodopsin → acid metarhodopsin transformation.

Hexane treatment series

The rate of the mesorhodopsin → acid metarhodopsin transformation for the digitonin extract of hexane-treated membranes was less than that for the native

preparation. On the other hand, the rate of lumirhodopsin → mesorhodopsin transformation for the digitonin extract of the hexane-extracted membranes was 3-times larger than that for the native preparation, that is $\alpha_{l\text{-}m} = 2.7$. Enzymic hydrolysis of phospholipid in a hexane-treated preparation affects the rate of both transformations (Table IV). In order to clarify the effect of phospholipase treatment on the rate of each transformation, it may be convenient to compare the parameter, described previously, to a new parameter β. β is the ratio of the rate of the transformation of hexane-extracted and phospholipase-treated preparation to that of hexane-extracted preparation. For the phospholipase C treatment, the β values for both lumirhodopsin → mesorhodopsin and mesorhodopsin → acid metarhodopsin were near unity. These results are consistent with the fact that the α values for these transformations are also about unity. In the case of phospholipase A_2 treatment, the β value for lumirhodopsin → mesorhodopsin is 2.4 and that for mesorhodopsin → acid metarhodopsin is 1.3. The α values for corresponding transformations are slightly lower, but the trend of α and β for both transformations was consistent.

Discussion

The technique of manipulating the interacting components of the photoreceptor membrane is a powerful method of studying its structure and function. One technique often used is the reconstitution of the membrane from purified components [6—8]. Another method is the removal and/or modification of lipids in the membrane [11,26,27]. Purified bovine rhodopsin has been successfully incorporated into various kinds of phospholipid bilayers and these reconstituted membranes have provided valuable information about rhodopsin-lipid interactions [6—8]. So far we have been unable to obtain completely purified octopus rhodopsin although a variety of techniques, such as concanavalin-A affinity chromatography, have been tried [28]. In the present paper, we used the second method of studying protein-lipid interactions noted above. The treatment of microvillar membranes of octopus photoreceptor cells by phospholipase A_2 and C, hexane extraction, or their combination qualitatively and quantitatively alters the lipid composition of the membranes. Our results suggest that several different variations in the lipids can affect the rates of the photochemical transformations; these are: the content of phospholipid, the amount of unsaturated hydrocarbon chains and free fatty acids.

Among our preparations, native membranes, hexane-extracted membranes and phospholipase C-treated, hexane-extracted membranes contain diacylphospholipids as their major lipids, but the amount of phospholipids varies. The hexane-extracted membranes and phospholipase C-treated and hexane-extracted membranes contain only about 33 and 6%, respectively, of the phospholipid originally present in the native membranes. There is no significant difference in the fatty acid profiles of the phospholipids of either the native or treated membranes (digitonin extract of native membranes and of hexane-extracted membranes). The results of Table II and IV show that the rate of the lumirhodopsin → mesorhodopsin and the mesorhodopsin → acid metarhodopsin transformations seems to increase with decreasing diacylphospholipid content. Moreover, the rate of the lumirhodopsin → mesorhodopsin transformation

is much more sensitive to the phospholipid content than the mesorhodopsin → acid metarhodopsin transformation, that is, $\alpha_{l\text{-}m}$ and $\alpha_{m\text{-}a}$ for the digitonin extract of hexane-extracted membranes is 2.8 and 1.1, respectively, for the digitonin extract of phospholipase C-treated and hexane-extracted membranes is 6.8 and 2.5, respectively. These results suggest that a large change in rhodopsin-lipid interaction in the photolysis of octopus rhodopsin takes place during the lumirhodopsin → mesorhodopsin transformation.

In the case of bovine rhodopsin, it has been reported that the lifetime of the metarhodopsin I to metarhodopsin II transformation depends upon the phospholipid content [7,27].

One of the representative preparations whose lipid composition was different from the native photoreceptor membranes is the phospholipase A_2-treated and hexane-treated membrane. Lysophospholipids are obtained by phospholipase A_2 treatment, which removes the fatty acid from the 2-position. Lysophosphatidylcholine and lysophosphatidylethanolamine are the major lipids found. For the kinetic data the digitonin extract of phospholipase A_2 and hexane-extracted membranes has the lowest rates for both of the transformations ($\alpha_{l\text{-}m}$, $\alpha_{m\text{-}a}$). As shown in a previous paper [10], approx. 70% and 90% of the fatty acids in the 2-position of the phosphatidylcholine and phosphatidylethanolamine in octopus photoreceptor membranes are highly polyunsaturated fatty acids such as docosahexaenoic acid (22 : 6), arachidonic acid (20 : 4) and eicosapentaenoic acid (20 : 5). On the other hand, 52% of the fatty acids in the 1-position of phosphatidylcholine are palmitic acid (16 : 0), although the fatty acids in the 1-position of phosphatidylethanolamine are mainly unsaturated. These facts may indicate that the phospholipase A_2-treated and hexane-extracted membranes containing lysophospholipids as the major lipids are much less unsaturated as compared to the original membranes. It may therefore be that the reduced lipid fluidity of the preparation which results from the treatment with phospholipase A_2 inhibits the transformation of the intermediates. In the case of bovine rhodopsin, recombination of rhodopsin with saturated phospholipids inhibited the transformation from metarhodopsin I to metarhodopsin II [8].

In contrast to the digitonin extract of phospholipase A_2-treated and hexane-extracted membranes, the rates of both transformations of the photolytic sequence for the digitonin extract of phospholipase A_2-treated membranes were much higher than those of the native preparation ($\alpha_{l\text{-}m} = 5.0$; $\alpha_{m\text{-}a} = 3.0$). The major difference between the phospholipase A_2-treated membranes and the phospholipase A_2-treated and hexane-extracted membranes is that the former includes much more free fatty acid than the latter. It is possible that the amount of free fatty acid, which includes a lot of unsaturated chains, can control the rate of transformation of intermediates.

Treatment of microvillar membranes with phospholipase C leads to a largely phospholipid-depleted membrane (22% of original phospholipids) whose predominant lipids are diacylglycerols (71%) and phospholipids (17%). However, this treatment only slightly affects the rates of the lumirhodopsin → mesorhodopsin transformation ($\alpha_{m\text{-}a} = 1.1$). The fact that kinetic parameters of the digitonin extract of phospholipase C-treated membranes are similar to those of the native preparation and are different from those of digitonin extract suggests

that the rhodopsin-lipid interactions in these two preparations are similar one to the other. This result also suggests that diacylglycerol in the digitonin extract of phospholipase C-treated membranes interacts with rhodopsin as acyl chain of phospholipid of that of native preparation does, though diacylglycerol in phospholipase C-treated membrane may aggregate itself to form an oil drop as shown by Olive et al. [30].

Though phospholipids found in the digitonin-solubilized octopus photoreceptor membrane are qualitatively identical to those in native membrane as shown in Table III, the rhodopsin-lipid interaction may be disturbed by detergent. However, Stubbs and Litman [9] and Shichi et al. [29] observed that different rhodopsin/phospholipid ratio in detergent-solubilized bovine rod outer segment gave the different rates for the metarhodopsin I to metarhodopsin II transformation. Thus, kinetic data of the present preparations provide useful information on the role of lipid in the photolytic sequence of octopus rhodopsin.

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