Protein–Phospholipid–Cholesterol Interaction in the Photolysis of Invertebrate Rhodopsin†

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ABSTRACT: Three aspects of protein–phospholipid–cholesterol interaction in microvillar membranes of octopus photoreceptor cells were studied: (1) the effect of hydrophobic environment on the kinetics of thermal transformation in the photolysis of rhodopsin; (2) effects of cholesterol in microvillar membranes on protein–lipid interaction; (3) the effect of membrane proteins on the dynamic properties of lipids in membranes. Dynamic properties of hydrophobic environments were observed with electron spin resonance spectroscopy with various lipid spin-labels. The kinetics of thermal transformation of mesorhodopsin to metarhodopsin was followed by flash photolysis experiments. The thermal stability of mesorhodopsin was followed by low-temperature spectrophotometry. The rate of transformation is influenced by the properties of both hydrophobic environments and temperature. The effect of the former is prominent at physiological temperatures, while the latter becomes a key factor at lower temperatures. The effect of cholesterol, which accounts for 19 mol % of total lipid in microvillar membranes, on the properties of the membranes was observed by comparing the temperature profiles of the order parameter of spin-labeled stearic acid in liposomes formed of either the total lipid or the polar lipid extracted from microvillar membranes. These experiments demonstrate that cholesterol has a large effect on the dynamic properties of membranes, lowering and broadening the temperature region of the gross phase change in the total lipid liposomes. In contrast, the temperature profile of the order parameter of the fatty acid spin-label in microvillar membranes shows only small differences from that in total-lipid liposomes. The minor effects of proteins on the dynamic properties of the membrane lipid are ascribed to the presence of cholesterol in the microvillar membranes.

A number of functions of proteins are greatly facilitated by the internal fluctuation of protein conformation [for a recent review, see Careri et al. (1979) and Karplus & McCammon (1981)]. Oosawa (1973, 1975) emphasized the importance of the environmental fluctuation of electric potential around proteins with long correlation times and long correlation lengths[1] in determining the reaction rate of enzymes. This type of spontaneous environmental fluctuation occurs relatively rarely but supplies sufficient free energy, coupled with internal protein fluctuation, to enhance enzymatic activity. The hydrophobic portion of biological membranes is of particular interest in this respect because the spectrum of spontaneous fluctuations is expected to shift to longer correlation times and lengths due to the higher viscosity and ordered structure of hydrophobic loci in membranes. One of the aims of this work is to study the influence of the environment on the function of the protein. The thermal conversion of the photochemical intermediates of rhodopsin (a membrane protein) after photolysis gives an ideal model for the study of the relationship between the function of the protein and the environment, since the transformation of the intermediates (hypso–batho–lumi–meso–meta) is strictly thermally driven and the photochemical process is well established (Yoshizawa, 1972; Tsuda et al., 1980; Tsuda, 1982a).

The study of the influence of hydrophobic environment on rhodopsin after photolysis is also significant in clarifying how absorption of a photon by rhodopsin leads to visual excitation. Intense effort is in progress in many laboratories to gain an understanding of the linkage between the photochemical event in rhodopsin and excitation of plasma membrane of photoreceptor cells. Light-activated enzymes in photoreceptor cells such as GTPase and phosphodiesterase have been proposed to be involved in this process. Apparently, one of the photochemical intermediates is able to activate these enzymes since neither rhodopsin nor its final photoproduct, opsin plus retinal (vertebrate) or metarhodopsin (invertebrate), can (Calhoon et al., 1981). Since rhodopsin is a membrane protein and the light activation of the enzyme cascade seems to be initiated at membranous loci, it is important to understand rhodopsin–lipid interaction during the photolysis of rhodopsin.

The influence of lipid environment on rhodopsin has been studied mostly with vertebrate rhodopsin (Applebury et al., 1974; O'Brien et al., 1977; Davoust et al., 1979; Kusumi et al., 1980b, 1982). However, invertebrate photoreceptors have a number of advantages such as the following in studying the mechanism of visual transduction: (1) both rhodopsin and the light-dependent ion channel(s) are located in the same membrane (microvilli); (2) the thermal transformation of invertebrate rhodopsin after photolysis is simpler than that of vertebrate rhodopsin (no complicated transformation after metarhodopsin); (3) metarhodopsin, which is the final photoproduct of rhodopsin in the invertebrate case, is stable at physiological temperatures; (4) invertebrate rhodopsin can be photoregenerated from metarhodopsin. In particular, octopus rhodopsin can be almost completely photoregenerated from metarhodopsin upon irradiation of orange light (Tsuda, 1979a). This offers a good test of the reversibility of photoinduced phenomena and facilitates signal averaging in photolysis experiments. Consequently, invertebrate photoreceptor membranes have become an active subject of investigation in recent

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[1] Correlation time refers to the time correlation of random phenomena while correlation length is a concept that comments on the spatial correlation of random phenomena.

0006-2960/83/0422-1165$01.50/0

years (Tsuda, 1982b): Light activation of enzyme systems associated with microvillar membranes has been studied (Calhoon et al., 1980); Akino & Tsuda (1979) characterized the chemical composition of lipids and alkyl chains of phospholipids in microvillar membranes of octopus photoreceptor cells; Kusmi et al. (1980a) found conformational changes of proteins in the microvillar membranes after photolysis of rhodopsin by using the spin-label method; and Tsuda & Akino (1981) studied the rate of photochemical processes of octopus rhodopsin in detergent extracts of both native and variously treated membranes. In the present work, we further extended this line of studies. The effect of hydrophobic environment on the transformation of mesorhodopsin to metarhodopsin (octopus) has been examined. Dynamic properties of membranes and micelles have been monitored with lipid spin-labels. The kinetics of thermal transformation in the photolysis of rhodopsin has been followed by both flash photolysis experiments and the thermal stability by low-temperature spectrophotometry. The results of these measurements showed that the rate of the transformation of mesorhodopsin to metarhodopsin increases in the fluid hydrophobic environment, although the fluid environment is not a prerequisite for the transformation.

The physiological content of cholesterol significantly affects the phase behavior of membranes, as is shown by comparing liposomes made of total lipid with those of polar lipid extracted from microvillar membranes. Mobility of spin-labels in microvillar membranes is very similar to that in total lipid liposomes, suggesting that microvillar proteins have little effect on the dynamics of bulk lipid.

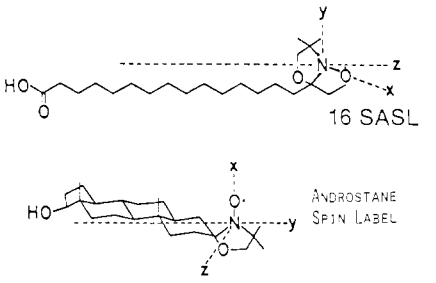
Experimental Procedures

Materials. Digitonin was obtained from Calbiochem (La Jolla, CA). The "soluble" part of digitonin was prepared by the method of Bridges (1977). Sucrose laurate was a gift from Ryoto Co., Tokyo, Japan. The following buffers were utilized in this work: 0.2 M acetate–sodium acetate for pH 5.6 and 6.0; 0.2 M *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid for pH 6.8 and 7.6; 0.1 M borate buffer for pH 9.5. All buffers were deoxygenated by a bubbling with nitrogen gas to prevent peroxidation of lipids.

All procedures involving rhodopsin were carried out under dim red light or total darkness. Microvillar membranes of octopus (Mizudako, *Paroctopus defleini*) photoreceptor cells were isolated as described previously (Tsuda, 1979b; Kusumi et al., 1980a,b). In some experiments, the microvillar membranes were solubilized with 2% digitonin or 2% sucrose laurate in a selected buffer. The solution was then centrifuged at 16000*g* for 30 min, and the clear supernatant was used for further experiments. Sucrose laurate solubilized the membrane almost completely. Solubilization with digitonin is less efficient. The supernatant after centrifugation contained 54% of protein, 40% of cholesterol, and 46% of phospholipid of the initial membrane preparation (all numbers in reference to starting amount). No selective extraction of specific phospholipid classes was observed.

Flash Photolysis Experiments. Change of transmittance following flash excitation was measured with a fast kinetic spectrometer. A blue pulse flash (blue-4, Phase-R) with a half-width of 0.5 μs from a dye laser (Phase-R DL1000) excited the sample in a 1 × 1 cm fluorescence-type curvette. The measuring beam, which passed through a monochromator, transversed the sample perpendicular to the excitation light and was detected by a photomultiplier after passing through a second monochromator. The preamplified signal was digitized with a Biomation 802 transient recorder. The recorder

Chart I



was interfaced with a Digital PDP MINC 11 computer for signal averaging.

Low-Temperature Spectrometry. Absorption spectra at lower temperatures were measured on a Cary 118C spectrophotometer with a specially designed glass cryostat similar to that described by Yoshizawa (1972). The temperature of the sample was monitored with a copper–constantan thermocouple. The sample was irradiated by light from a slide projector with a glass cut-off filter and an interference filter.

Preparation of Liposomes of Total and Polar Lipids from Microvillar Membranes. Lipids of microvillar membranes were extracted by the method of Bligh & Dyer (1959). The total-lipid extract was separated by column chromatography on silicic acid into a neutral lipid fraction eluted with chloroform and a polar lipid fraction eluted with methanol. Each total and polar lipid fraction was mixed with spin-label and then placed under a nitrogen stream. The residue was further dried under vacuum. Liposomes were formed by adding selected buffer solutions to the dried lipids and vortexing the mixture. Liposomes were centrifuged briefly, and the resultant loose pellet was used for electron spin resonance (ESR) measurements.

ESR Measurements. The structures of the spin-labels used in this work are shown in Chart I. Lipid-type spin-labels were incorporated into microvillar membranes and detergent extracts by the following method. A very thin film of the spin-label was formed at the bottom of the test tube by evaporating solvent from a chloroform solution of the spin-label first under a nitrogen stream and then under vacuum (ca. 0.1 mmHg). Membrane suspensions or detergent extracts were poured on the film and incubated under nitrogen at 4 °C for 10 h. For measurement of ESR spectra, the spin-labeled microvillar membranes were loosely pelleted. The samples were taken in a capillary (0.9-mm i.d.) made of a gas-permeable methylpentene polymer known as TPX (Popp & Hyde, 1981). This plastic is permeable to nitrogen, oxygen, and carbon dioxide and is substantially impermeable to water. Samples were placed inside the dewar and equilibrated with nitrogen gas that was used for temperature control. ESR spectra were obtained with a Varian E-109 X-band spectrometer with Varian temperature-control accessories and E-231 Varian multipurpose cavity (rectangular TE_{102} mode). The sample was thoroughly deoxygenated, allowing prolonged measurements at physiological temperatures (9–15 °C in the case of the octopus). Order parameters of 16 SASL were determined according to Gaffney (1976).

Results and Discussion

Effect of Hydrophobic Environment on the Kinetics of the Transformation of Mesorhodopsin to Metarhodopsin. Flash photolysis data of octopus rhodopsin at 15 °C are shown in Figure 1. The time course of the transformation of mesorhodopsin to metarhodopsin in sucrose laurate extracts of the membrane and in the intact membrane is fast ($\tau_{1/e}$ less than

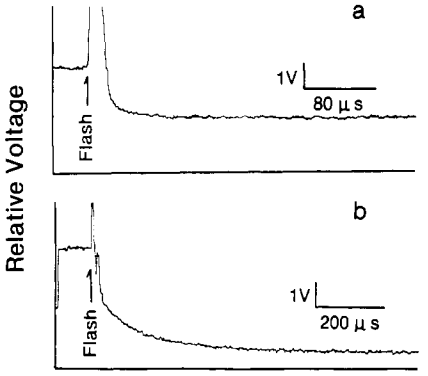


FIGURE 1: Transformation from mesorhodopsin to metarhodopsin in 0.2 M acetate–sodium acetate buffer (pH 5.6) observed at 540 nm after a blue flash (λ = 460 nm) at 15 °C. (a) Microvillar membranes. The decay in sucrose monolaurate is almost identical. (b) Digitonin extract. Notice that the scale of the abscissa is larger in (b).

5 μs), and it was difficult to obtain reliable estimates of the decay time with the present experimental setting. However, it is established that the rate of the transformation in digitonin extract is much slower ($\tau_{1/e} \approx 140$ μs) as is shown in Figure 1b.

Dynamic properties of the hydrophobic moiety of intact membranes, sucrose laurate extracts, and digitonin extracts were monitored by ESR with 16 SASL. Spectra of 16 SASL in microvillar membranes and sucrose laurate extracts show much sharper lines than those in digitonin extracts, indicating greater mobility of the spin-label in the two former systems (Figure 2a–d). These results suggest that the rate of the transformation from mesorhodopsin to metarhodopsin increases as the fluidity of hydrophobic environment increases around rhodopsin. It is possible that the structural variations among membranes and the micelles of different detergents are responsible for the observed difference in transformation kinetics. The difference in curvature or interaction with water, for example, could affect the rate of the transformation. These detergents were selected because of the extensive use of them in invertebrate rhodopsin research. Basic amphipathic structure of rhodopsin appears to be preserved in these micelles of detergents chosen in this work, since rhodopsin is stable at physiological temperatures (10–15 °C) and retains reversible photosensitivity. The existing literature suggests that rhodopsin conformation is largely conserved in nonionic detergents (Shichi & Shelton, 1974; Pober & Stryer, 1975; Pontus & Delmelle, 1975; Osborne, 1975). Therefore, it is concluded that the rate of thermal transformation of mesorhodopsin increases in the fluid environment at physiological temperatures.

In digitonin extracts, 16 SASL spectra show two components above −15 °C (Figure 2c) that correspond to strongly and weakly immobilized spin-labels. The ratio of the two components changes when the initial ratio of digitonin and microvillar membrane is changed. When the amount of membrane is increased, the weakly immobilized component increases (Figure 2c,d). The 16 SASL spectra in micelles composed of only digitonin consist of a single, strongly immobilized component (data not shown). It is not clear whether the two spectral components arise from microheterogeneity in digitonin micelles or the existence of two types of micelles. A brief description of the chemical composition of the detergent (mixed micelles) is given under Experimental Procedures. Further characterization of the digitonin extract will be published elsewhere.

Effect of Hydrophobic Environment on the Transformation of Mesorhodopsin to Metarhodopsin at Low Temperatures.

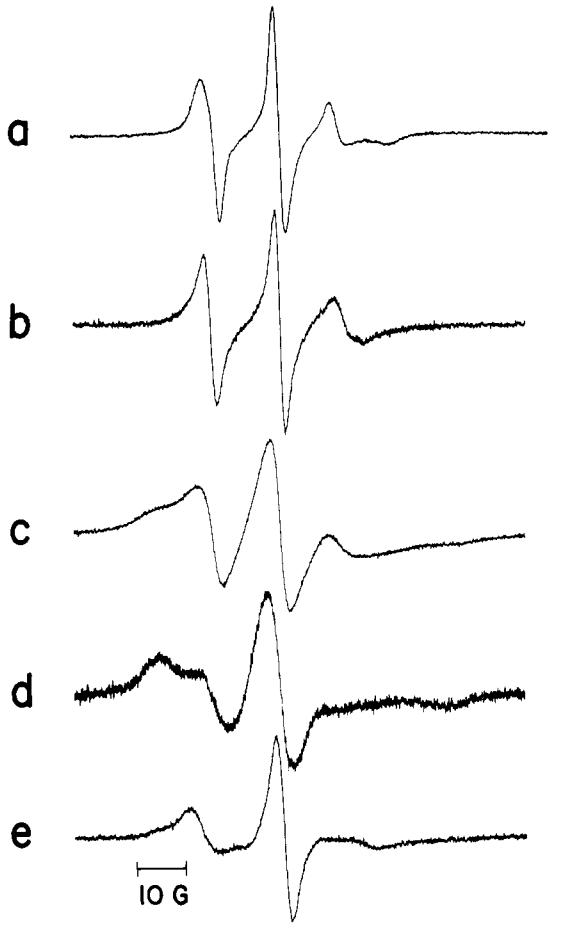


FIGURE 2: ESR spectra of 16 SASL at 10 °C in (a) microvillar membranes, (b) sucrose laurate, (c) digitonin extract, and (d) digitonin extract from one-fifth the amount of the membrane used in (c); (e) ESR spectrum of ASL in microvillar membranes at 12.6 °C. These spectra are all obtained in 0.2 M *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid, pH 7.6. The pH dependence of the spectrum is small as shown in Figure 5. The field modulation width was 0.5 G, and the frequency 100 kHz. Incident microwave power was 0.5 mW.

Microvillar membranes and digitonin extracts of the membranes were irradiated with blue light (wavelength around 440 nm) at −197 °C in the presence of 67% (v/v) glycerol. Bathorhodopsin was formed under these conditions. The temperature was gradually raised and mesorhodopsin was formed by thermal decay from lumirhodopsin. The thermal transformation from mesorhodopsin to metarhodopsin was observed by measuring absorption spectra. The wavelengths at maximum absorbance (λ_{max}) are plotted against temperature in Figure 3. λ_{max} of mesorhodopsin is 480 and 476 nm in microvillar membranes and in digitonin extracts, respectively. λ_{max} of metarhodopsin is 494 and 491 nm in microvillar membranes and in digitonin extracts, respectively. Although the overall shape of the spectrum in microvillar membranes is very similar to that in digitonin extracts for both mesorhodopsin and metarhodopsin, the small shift in λ_{max} (3–4 nm) between microvillar membranes and digitonin extracts would reflect a difference in the *all-trans*-retinal–protein interaction in these two environments. The transformation of mesorhodopsin to metarhodopsin starts around −40 °C in microvillar membranes and −30 °C in digitonin extract.

The temperature profile of the dynamic properties of the hydrophobic environment of rhodopsin in these systems was again monitored with 16 SASL (in the dark). Order parameters estimated from maximum splitting values of the ESR

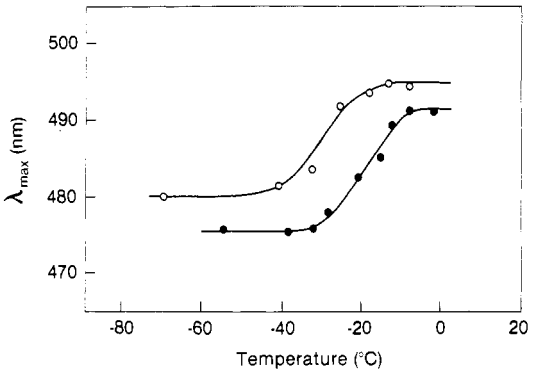


FIGURE 3: Thermal transformation of mesorhodopsin to metarhodopsin as measured by a shift in the λ_{max} at low temperatures. Each sample with glycerol (67% v/v buffered with 67 mM acetate–sodium acetate at pH 5.6) was irradiated with blue light (~440 nm) at −197 °C for 10 min, warmed up to −74 °C, and then further warmed very gradually with intermittent measurements of absorption spectrum. (○) Microvillar membranes; (●) digitonin extract.

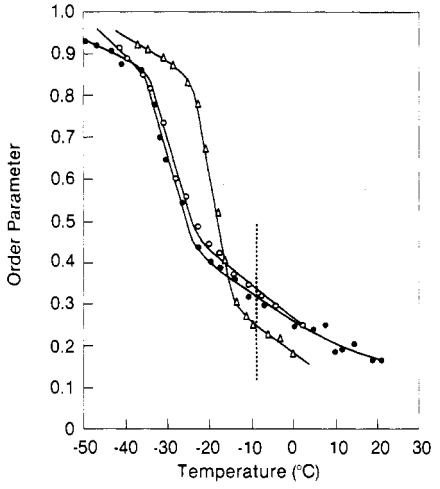


FIGURE 4: Temperature profile of the order parameter of 16 SASL in microvillar membranes (●), liposomes formed with extracted total lipid (○), and liposomes formed with extracted polar lipid (△) at pH 6.0. The dotted line indicates freezing of bulk water.

spectrum (Gaffney, 1976) of this probe in microvillar membranes are plotted as a function of temperature (Figure 4). Order parameters reflect the alkyl chain order in membranes, larger values corresponding to more trans and less gauche structure in the chain. Gross phase change of membrane lipids is seen between −36 and −23 °C. This change closely correlates to the transformation of mesorhodopsin to metarhodopsin.

Similar measurements were made at various pH values and in the presence of 67% (v/v) glycerol (Figure 5). This is because ESR spectra of 16 SASL are pH dependent (Sanson et al., 1976; Egret-Charlier et al., 1978; Kusumi et al., 1982), and optical measurements at lower temperatures were made in the presence of glycerol. Changes in the maximum splitting value occur about the same temperature range under all these conditions.

In contrast to the results of microvillar membranes, 16 SASL in digitonin extracts does not show any abrupt change around the transformation temperature of mesorhodopsin to metarhodopsin (Figure 5). This result indicates that the mobility of the 16 SASL chain deep inside the digitonin micelles is very restricted and has little temperature dependence at the transformation temperatures.

There may be some complexity because 16 SASL may not "experience" the same environment as rhodopsin and also because 16 SASL may not be sensitive to the mode of environmental fluctuation that affects rhodopsin. However, the clear difference between microvillar membranes and digitonin extracts is that the former has a marked *gross* phase change that would influence both rhodopsin and 16 SASL.

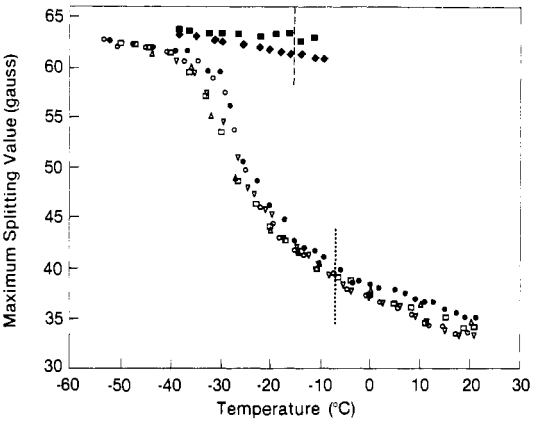


FIGURE 5: Temperature profiles of maximum splitting values of 16 SASL in microvillar membranes at pH 4.5 (○), 6.0 (□), 7.6 (△), and 9.5 (●). The effect of glycerol (67% v/v) was examined (▽) at pH 7.6. Maximum splitting values in digitonin extract in the presence of 67% glycerol are also shown: pH 5.6 (♦) and 7.5 (■). At a temperature above that shown by the dashed line, weakly immobilized peaks appear. The dotted line indicates freezing of the bulk water.

These data imply that the transformation of mesorhodopsin was facilitated by a more fluid hydrophobic environment, as is seen in the coincidence of the onset temperatures between the gross phase change of the membrane and the transformation of mesorhodopsin in the membrane. It should also be noted that this thermal conversion of mesorhodopsin occurs at lower temperatures in microvillar membranes than in the less fluid digitonin micelles. However, a fluid hydrophobic environment is not a prerequisite for the transformation as is seen in digitonin extracts. The results in this and in the previous section show that a thermal transformation of a rhodopsin intermediate is dependent on both temperature (environment-independent thermal fluctuation of protein conformation) and the hydrophobic environment (Beece et al., 1980). It would be interesting to compare these results with the vertebrate rhodopsin data (Applebury et al., 1974; O'Brien et al., 1977). Applebury et al. (1974) observed the transformation of metrarhodopsin I to metarhodopsin II (bovine) and found that the rate of transformation is influenced greatly by the lipid environment, which is consistent with our observation. O'Brien et al. (1977) also studied kinetics of the decay of metarhodopsin I and found the requirement of unsaturated phospholipids for the decay rate in reconstituted membranes comparable to that in rod outer segment membranes. Since they found that the decay is very slow in dimyristoylphosphatidylcholine even above the phase transition temperature, fluidity, which can be increased in the presence of unsaturated chains, is not the only determinant of the decay constant.

Further Characterization of Protein–Phospholipid–Cholesterol Interaction in Microvillar Membranes. The spectrum of androstane spin-label, a sterol analogue, possesses two distinct components, more immobilized and less immobilized, at physiological temperatures for octopus (Figure 2e). The more immobilized component may be due to the spin-label in direct contact with proteins, and the less immobilized component can be ascribed to the probe off the protein surface. Androstan spin-label was utilized to mimic the mobility of cholesterol. The cholesterol content in microvillar membranes of octopus photoreceptors (Akino & Tsuda, 1979) is much

higher than that in vertebrate disc membranes of rod outer segments. It is important to notice that the more immobilized component appears under physiological conditions. In most cases reported so far, this immobilized component was observed at high protein-to-lipid ratios and/or at lower temperatures (Davoust et al., 1979).

The interaction of proteins, polar lipids, and neutral lipids was studied by observing the temperature profile of the order parameter of 16 SASL in the intact membranes and liposomes formed with either total or polar lipids extracted from microvillar membranes. More than 90 mol % of the neutral lipid is cholesterol. The amount of cholesterol is 19 mol % of the total lipid (Akino & Tsuda, 1979). 16 SASL failed to show a distinctly more immobilized component. As was mentioned previously, microvillar membranes showed a large phase change between −36 and −23 °C (Figure 4). This result is in agreement with the endothermic transition of the compositionally similar squid photoreceptor membranes (Anderson et al., 1978; Akino & Tsuda, 1979) measured by differential scanning calorimetry (Mason & Abrahamson, 1974). The liposome formed with extracted total lipid showed almost the same temperature profile as intact microvillar membranes. Little difference between intact membrane and the liposomes made of extracted total lipid has been reported on other systems (Casal et al., 1980; Fretten et al., 1980), suggesting that proteins have little effect on the rotational mobilities of lipids in each of the systems studied. Liposomes formed with polar lipids showed higher inflection temperatures and a more abrupt phase change (Figure 4). These results demonstrate that cholesterol *lowers* the inflection temperatures and broadens the gross phase change. These data also imply that the effect of membrane proteins on the alkyl chain flexibility as detected by 16 SASL can be moderated in the presence of cholesterol, because it is shown that vertebrate rhodopsin affects the phase transition of reconstituted phosphatidylcholine membranes (Kusumi et al., 1980a,b; Kusumi & Hyde, 1982). Cholesterol acts as a "fluidity buffer" so that small changes in the amount and arrangement of membrane proteins may not affect the overall properties of the membranes. The effect of cholesterol may be the result of the induction of protein aggregation (Rousselet et al., 1981), which reduces the influence of proteins on membrane lipids. This point needs more study under better defined systems such as reconstituted membranes.

Summary

Effects of the properties of hydrophobic environment on the function of the membrane protein were studied by using the thermal decay of a photochemical intermediate of octopus rhodopsin as a convenient model system. Experiments at physiological temperatures showed that a more fluid hydrophobic environment increases the rate of the thermal transformation of mesorhodopsin. At lower temperatures, however, temperature rather than fluidity is a key factor for the transformation of mesorhodopsin. The transformation took place at ca. −30 °C in digitonin micelles in which 16 SASL indicated little mobility.

Cholesterol, which amounts to 19 mol % of total lipid, produces a large effect on the dynamic properties of the membranes. Temperature profiles of the mobility of 16 SASL were compared in the liposomes formed from total lipid or polar lipid extracted from microvillar membranes. The presence of cholesterol lowers and broadens the temperature region of the gross phase change of the total-lipid liposomes compared with that of polar-lipid liposomes. The temperature profile of 16 SASL mobility in the microvillar membranes showed only minor difference from that in total-lipid liposomes. The existence of the more immobilized androstane spin-label population shows that sterol molecules can be in the boundary region of membrane proteins. Taken cumulatively, cholesterol decreases the effect of intercalation of proteins on the gross physical properties of the bulk membrane lipid, thereby serving as a fluidity buffer in the membrane. Preserving the fluidity of microvillar membranes is physiologically important because, as has been shown in this work, the kinetics of thermal transformation of photolyzed rhodopsin is dependent on the fluidity of hydrophobic environment at physiological temperatures.

Acknowledgments

We thank Professor J. S. Hyde and Dr. J. B. Feix, National Biomedical ESR Center, The Medical College of Wisconsin, and Professor T. G. Ebrey, Department of Physiology and Biophysics, University of Illinois, for useful discussions and critical reading of the manuscript, Professor B. J. Gaffney, Department of Chemistry, Johns Hopkins University, for helpful discussions, and T. Sakaki, Department of Biophysics, Kyoto University, for helping with the preliminary ESR measurements. Optical experiments were made possible by the cooperation of Professor Ebrey and performed in his laboratory. We are indebted to Professor M. Takahashi, The Marine Institute of Medicine, Sapporo Medical College, for the supply of octopus eyeballs.

Registry No. Cholesterol, 57-88-5.

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Interaction of Dipalmitoylphosphatidylcholine and Dimyristoylphosphatidylcholine-d_{54} Mixtures with Glycophorin. A Fourier Transform Infrared Investigation†

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ABSTRACT: Glycophorin from the human erythrocyte membrane has been isolated in pure form and reconstituted into large unilamellar vesicles comprised of binary mixtures of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) and chain perdeuterated 1,2-dimyristoyl-3-*sn*-phosphatidylcholine (DMPC-d_{54}). The effect of temperature and protein on lipid structure and mixing was monitored by using Fourier transform infrared spectroscopy; deuteration of one of the components of the mixture permits observation of the protein interaction with each lipid species. The melting curves were analyzed by assuming that each lipid chain can exist in one of two physical states (i.e., gel or liquid crystalline), characterized by a temperature-dependent Lorentzian distribution for the line shape of the C-H or C-D stretching vibrations. The fraction of each lipid component melted at temperatures within the two-phase region of the phase diagram was calculated and approximate phase diagrams were constructed. Addition of protein lowers the liquidus line of the phase diagram while leaving the solidus line essentially unchanged. No lipid phase separation is observed. The effect of protein is more pronounced on the DPPC component than on the DMPC-d_{54}. The former is significantly more disordered and/or fluidized at all lipid mole fractions in the ternary system than in the binary phospholipid mixture.

Structural studies of lipid-protein interaction in reconstituted systems have provided basic information about the organization of biological membranes. Although many experiments have focused on the interaction of membrane proteins with a single lipid component [for a current overview, see Parsegian (1982)], less information is available for ternary systems consisting of a binary phospholipid mixture along with protein. Such experiments provide an opportunity to determine whether membrane proteins partition into regions of particular chemical structure or physical order in a complex lipid environment. The related question of lipid control of membrane protein function may also be investigated.

Several structural studies of ternary systems have appeared. Differential scanning calorimetry (DSC)[1] has been used to detect phase separation induced by lipophilin in mixtures of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) and phosphatidylserine (Boggs et al., 1977a,b), while Chapman et al. (1977) have investigated the partitioning of gramicidin A into 1,2-dilauroyl-3-*sn*-phosphatidylcholine-DPPC mixtures. At low concentrations, the polypeptide preferentially associated with the lower melting region of the bilayer whereas at higher levels a mixing of the two lipids was induced. Kleeman et al. (1974) used freeze-fracture electron microscopy to investigate the partitioning of glycophorin into two binary phosphatidylcholine mixtures. More recent spectroscopic studies include those of Verma et al. (1980) and Jonas & Mason (1981).

Fourier transform infrared (FT-IR) spectroscopy offers several advantages for the study of ternary systems. Vibrational spectra are sensitive to alterations in the conformation

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DMPC-d_{54}, chain-perdeuterated 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; Tris, tris(hydroxymethyl)aminomethane; DEGS, diethylene glycol succinate; DSC, differential scanning calorimetry; NANA, *N*-acetylneuraminic acid; FT-IR, Fourier transform infrared; DPPE, 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine; T_m, temperature of the gel to liquid-crystal phase transition.

0006-2960/83/0422-1170$01.50/0