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The Packing of Lipid Chains Changes the Character of Bacteriorhodopsin Reconstituted in a Model Membrane

Hirotaka Sasaki, Mayumi Araki, Seketsu Fukuzawa* and Kazuo Tachibana*

Department of Chemistry, Graduate School of Science, The University of Tokyo and CREST, Japan Science and Technology Corporation (JST), Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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Abstract—The packing of lipid chains in bicelles was 5–9% less than that in mixed micelles at temperatures between 298 and 318 K. This reduction of packing that accompanied the formation of bicelles changed the spectroscopic character of reconstituted bacteriorhodopsin, as indicated by static absorption measurements.

The function of living biological systems (cells, cell organelles and whole organisms) is essentially connected to the occurrence and structure of fluid biological membranes.[1] Biological membranes act as highly selective permeability barriers separating the contents of the cell from its environment. Moreover, membranes provide membrane proteins with a suitable environment for exhibiting their functions, and they control a variety of indispensable processes for life, such as passive and active transport. As biological membranes are composed of various kinds of lipids that assemble with each other noncovalently, their shapes are flexible and can be changed quite readily. The packing of hydrocarbon chains in the hydrophobic region of the membrane, which depends upon the shape of the membrane, is thought to induce changes in the membrane function. Booth et al. reported that a membrane protein embedded in the bilayer experiences changes in the lateral pressure of lipid chains, which are closely related to packing, as the environment of the membrane is altered, and in turn can change the folding and behavior of the embedded protein.[2]

Bicelles are bilayered, discoidal lipid-detergent assemblies in which the lipid-rich bilayer planes are stabilized at their edges by the detergent component (Fig. 1).[3,4] The morphology of bicelles changes according to the 1,2-dimyristoyl-3-*sn*-phosphatidylcholine (DMPC)/1,2-dihexanoyl-3-*sn*-phosphatidylcholine (DHPC) molar ratio, which is known as the q value.[5] When the total lipid concentration is less than ca. 10% (w/v), transition between spherical mixed micelles (Fig. 1) and discoidal bicelles occurs around the gel-to-liquid crystalline transition temperature (T_m) of DMPC in the cases where $q > \sim 1.8$; mixed micelles and bicelles are formed below and above the T_m, respectively. However, in the cases where $q < \sim 1.8$ bicelle formation does not occur, and only mixed micelles can be observed at temperatures between 293 and 318 K.[6]

In this investigation, we compared the packing of bicelles and mixed micelles, and investigated effects of the packing of lipid assemblies upon the character of reconstituted bacteriorhodopsin. Changes in the packing of lipid chains in bicelles and mixed micelles were estimated by incorporating the pyrene-labeled lipid, 1,2-bis-(1-pyrenedecanoyl)-3-*sn*-phosphatidylcholine (10dipyPC) (Fig. 1), into the DMPC/DHPC mixtures. This attachment of the pyrene at position C10 of the lipid chains means that the pyrenes are within the membrane in the region from C10 down to the bilayer midpoint. They can thus be used to monitor packing changes around the center of the bilayer. The ratio of the pyrene excimer to excited monomer (E/M_1) fluorescence is an effective measure of the packing of lipid chains.[7] The kinetic constant of pyrene excimer formation is proportional to the collision frequency between pyrene molecules. Therefore, the greater E/M_1 value in the DMPC/DHPC mixtures means that more collisions occurred at the pyrene moieties (on the same lipid chain) of 10dipyPC,

*Corresponding author. Tel./fax: +81-3-5841-8059; e-mail: seketsuf@chem.s.u-tokyo.ac.jp

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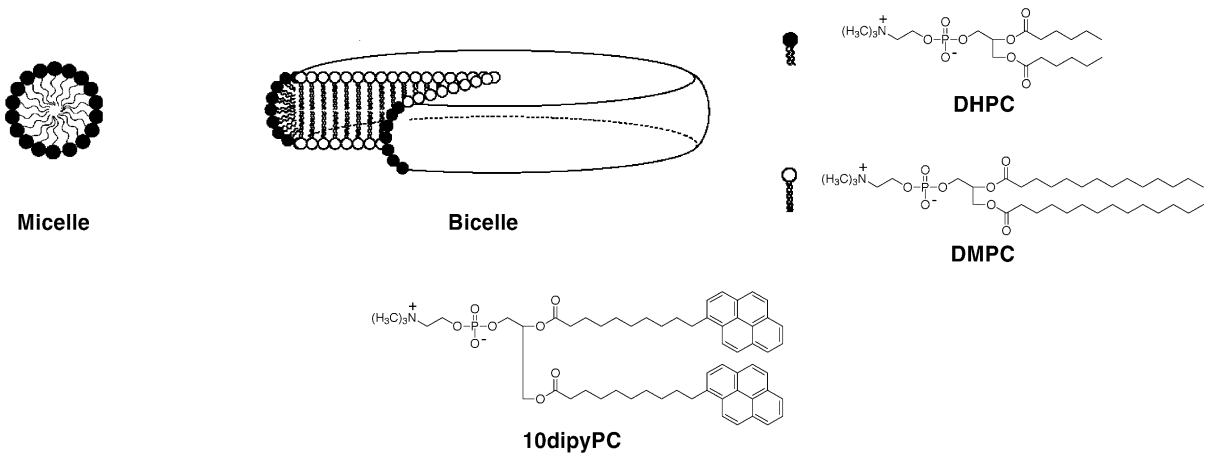

Figure 1. Structures of micelle, bicelle, DHPC, DMPC, and 10dipyPC.

which reflects a tighter packing of the lipid chains. Excimer formation between pyrenes on different labeled lipids is dependent on the pyrene concentration as well as on the lipid packing. To avoid such intermolecular excimers, the mole fraction of the pyrene-labeled lipid was adjusted to 0.001 against DMPC.[8] The excimer lifetime is absolutely temperature-independent above the T_m of lipids.[9,10] Therefore, we carried out all fluorescence measurements above the T_m of DMPC (298–318 K).

The fluorescence intensity is the intensity at a particular wavelength: 377 nm for M_1 excited monomer fluorescence and 475 nm for excimer fluorescence (E). The pyrene excimer to excited monomer (E/M_1) fluorescence is related to the temperature, T, according to eq 1:[10]

$$E/M = AT\ \exp(-E_a/RT) \tag{1}$$

where A, E_a and R have their usual thermodynamic meanings, that is, frequency factor, activation energy and gas constant, respectively. The frequency factor can be considered dependent on the temperature as well as on the lipid packing. Therefore, eq 1 can be expressed as:

$$E/M_1 = BP(T)T\ \exp(-E_a/RT) \tag{2}$$

where B is a constant and $P(T)$ is a temperature dependent value related to the packing around the pyrene molecules. Arrhenius plots of the 10dipyPC E/M_1 ratio in DMPC/DHPC bicelles and mixed micelles are shown in Figure 2. The points fit straight lines with correlation coefficients of $r = 0.92$ and 0.98 for the bicelles and mixed micelles, respectively. This allows us to write the following equation:

$$P(T) = P_0\ \exp(C/T) \tag{3}$$

where both P_0 and C are constant values. Therefore, the ratio of the bicelle $P(T)$ to that of mixed micelles, as determined from Figure 2, can be written as follows:

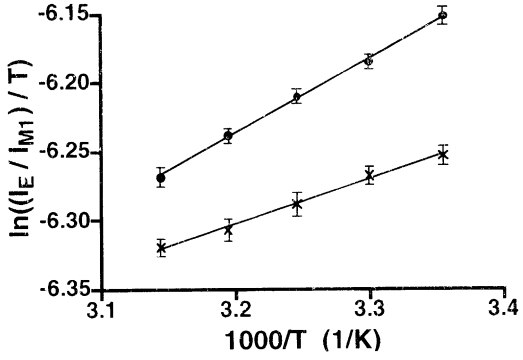

Figure 2. Arrhenius plot of the excimer to excited monomer fluorescence intensity ratio E/M_1 of 10dipyPC in bicelles (×, DMPC/DHPC molar ratio was 2.2) and mixed micelles (●, DMPC/DHPC molar ratio was 1.4). Both samples contained 56 mM of DMPC, 56 μM of 10dipyPC, and the 1.7 mM of cholesterol. Each sample contained 5.0% (w/v) and 5.6% total lipid (DMPC + DHPC), respectively. Fluorescence measurements were carried out above the T_m of DMPC (298–318 K).

$$P(T)_{q=2.2}/P(T)_{q=1.4} = 1.87\ \exp(-216/T) \tag{4}$$

where $P(T)_{q=2.2}$ and $P(T)_{q=1.4}$ are $P(T)$ values of bicelles and mixed micelles, respectively. Based on this equation, the packing of bicelles is 5–9% less than that of mixed micelles at temperatures between 298 and 318 K.

Bacteriorhodopsin (bR) is an integral membrane protein that consists of one polypeptide chain of 248 amino acids, in which a retinal molecule that serves as a chromophore is bound to a lysine residue in a Schiff's base. Bacteriorhodopsin was dissolved in DMPC/DHPC/cholesterol mixtures and its character in bicelles was compared with that in mixed micelles. UV–vis absorption spectra acquired around the lipid T_m (ca. 297 K)

Table 1. λ_{max} (±0.3 nm) of bacteriorhodopsin in various media

q	λ_{max} at 295 K (nm)	λ_{max} at 299 K (nm)
3.3 (bicelles)	552.4	560.4
1.4 (micelles)	552.8	552.2
bR in PM	—	559.8

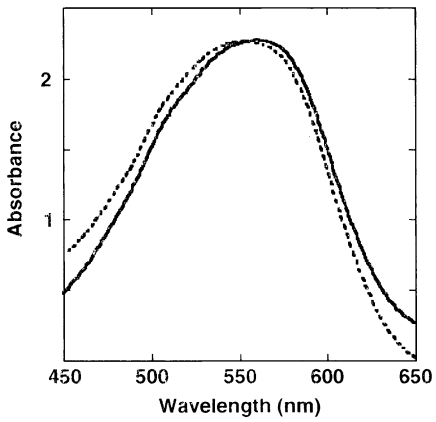

Figure 3. Absorption spectra of cholesterol doped bicelles (3.0 mol% vs DMPC) containing bR. The spectra were acquired at 295 K (···) and 299 K (—). The concentration of bR was 0.48 mM. The sample contained 7.0% (w/v) total lipid (DMPC + DHPC), and the DMPC/DHPC molar ratio was 3.3.

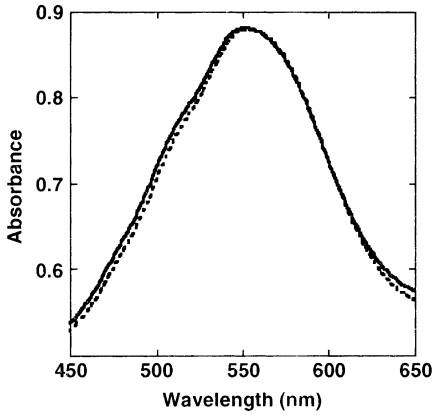

Figure 4. Absorption spectra of cholesterol doped micelles (3.0 mol% vs DMPC) containing bR. The spectra were acquired at 295 K (···) and 299 K (—). The concentration of bR was 0.28 mM. The sample contained 5.4% (w/v) total lipid (DMPC + DHPC), and the DMPC/DHPC molar ratio was 1.4.

revealed that the λ_{max} of the chromophore was red-shifted from 552.4 nm to 560.4 nm, which was absolutely identical to the λ_{max} found in purple membranes (PM) (559.8 nm) (Table 1), and this shift accompanied bicelle formation (Fig. 3).[11] On the other hand, when bicelle formation did not occur, λ_{max} was constant at 295 and 299 K (Fig. 4), suggesting that the spectral change observed in Figure 3 was induced exclusively by the formation of bicelles.

It was reported that an increase in the lateral pressure within the bilayer was responsible for the slowing of a rate-limiting folding step for bacteriorhodopsin when experiments were carried out using mixed micelles or vesicles.[2,8] Our results imply that decreased packing around the hydrophobic lipid assemblies that accompanies formation of bicelles changes the static parameters of the folding of bacteriorhodopsin. Therefore, it can be considered that bicelles have a great potential as a medium for reconstitution of membrane proteins.

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