Study of Packing State in Lipid Bilayers with the Aid of Ultraviolet Spectroscopy

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Synopsis. The packing states of lipid bilayers of three phosphocholines were investigated based on the density dependent UV shifts of probes incorporated into bilayers. As UV probes, two retinal Schiff bases were used. It was confirmed that the packing state of the fatty acyl chain region is well probed by UV spectroscopy.

Lipids are prerequisite for an activation of membrane enzymes.[1] Lipid bilayers would contribute the stabilization of enzyme conformation required for an activation. In many cases, fluidity of lipid is also necessary for the enzyme activity.[2-4] For these reasons, many spectroscopic methods have been applied, for example, NMR,[5-11] ESR,[12-15] fluorescence,[16-19] IR,[20-22] etc. Up to now, an investigation of the state of lipid bilayers using an UV spectroscopy notwithstanding its high sensitivity has not been reported. In this note, we will show that the UV shifts could provide the information on packing states of lipid bilayers.

Experimental

Three types of phosphocholine(PC) were used; egg yolk phosphocholine(EPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine(DMPC), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine(DPPC). As UV probes, retinal Schiff bases, i.e., *N*-retinylidene-butylamine(NRB) and α-(*t*-Butyloxycarbonyl)-ε-*N*-retinylidene-L-lysyl-L-phenylalanyl-L-tyrosine methyl ester(PEP) were used (Fig. 1). Methods of syntheses and characterization of these compounds were described elsewhere.[23,24] The molar ratio of probe to lipid was almost 1/10. Homogeneous vesicle dispersion in distilled water containing 100 mM NaCl (1 M=1 mol dm^{-3}) is prepared by sonication with TOMY model UR-200P sonicator at frequency 20 kHz and output power 200 W under cooling by ice/water until translucence (about 20 min). It has confirmed that the incorporation of retinal less than 20 mol% causes the slight expansion of EPC vesicle size but dose not induce the obvious disruption.[25] UV spectra were observed on a Beckman model 25 spectrometer with a probe concentration of ca. 5×10^{-2} mol dm^{-3}.

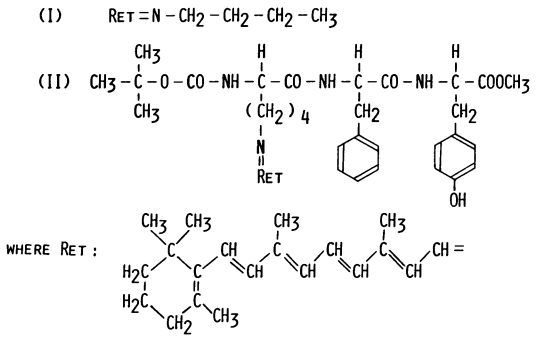

Fig. 1. Chemical structure of UV probes. (I) NRB and (II) PEP(peptide probe).

Results and Discussion

Retinal moiety of the peptide probe PEP is located in the region of fatty acyl chains in the bilayers.[24] NRB is also seemed to locate in the same region, but not near the polar head group, because of its higher hydrophobicity. PEP in EPC vesicle shows UV absorption maximum λ_{max} of retinal moiety at 380 nm, which is more bathochromic than in EPC inverted micell (in hexane, λ_{max}=360 nm).[24] This shift is mainly due to the van der Waals effect which depends on the density of medium. Similar shift has been observed in normal hydrocarbons as solvents, where the λ_{max} value of retinal Schiff base shifts to more bathochromic as the density of the solvent becomes larger.[26] Fatty acyl chains of EPC in inverted micell are not so densely packed compared with those in EPC bilayer vesicle. The λ_{max} value in vesicle is, therefore, in longer wavelength region than that in inverted micell. It suggests that by using the λ_{max} shift we can study the packing state of fatty acyl chain region of lipid bilayers.

Figure 2 shows the temperature dependence of the λ_{max} values of two probes in three different PC vesicles. At 5—10 °C, the λ_{max} values of probes in DPPC are larger than those in DMPC and EPC. This result suggests that DPPC bilayers are more densely packed than the other two PC bilayers. The bilayers of pure DPPC, DMPC, and EPC show phase transition at

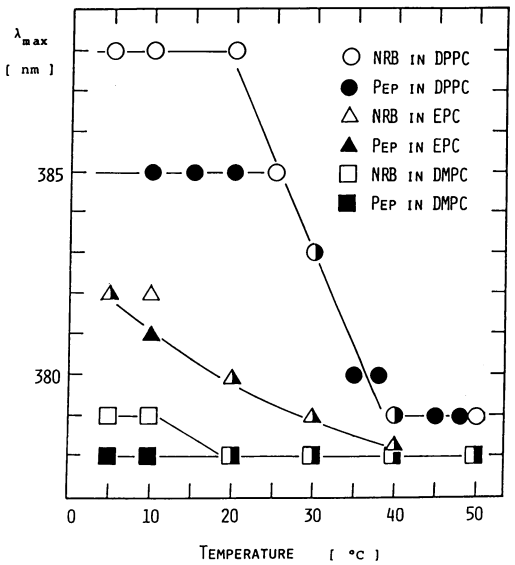

Fig. 2. Temperature dependence of λ_{max} values of UV probes in phosphocholine(PC) bilayers. Errors in temperature and λ_{max} are ±2.0 °C and ±1 nm, respectively.

temperatures (T_c) of 41.3,[27] 23.8, and −15.0 °C,[28] respectively. At the temperature range 5—10 °C, DMPC and DPPC would be in the gel state, while EPC in the fluid state. When the fatty acyl chains consist of saturated carbon-carbon bonds and the bilayers are in the gel state, the packing effect depends on the length of the acyl chains. Raman studies in the gel state showed that DMPC is more perturbed and its fatty acyl chains are more rich in gauche conformers than the case of DPPC.[27,29,30] The packing strength is influenced by the perturbation which becomes larger with a decreasing of the chain length.[30] Our UV result in the gel state parallel that from Raman study. Almost all acyl chains of EPC are longer than those of DPPC, but they contain a lot of unsaturated bonds,[28] loosening the packing of the bilayers. In DPPC bilayers, the λ_{max} value of NRB is larger than that of peptide probe PEP, indicating that the packing of DPPC acyl chains is affected by the nature of the probes. The smaller perturbation of NRB compared with PEP reflects the better compatibility of the former with the lipid bilayers.

On the other hand, the differences between the λ_{max} values of NRB and PEP are very small in DMPC and EPC bilayers. The packing of these bilayers may be not so dense by nature to be affected significantly by NRB and PEP.

At 40—50 °C, the bilayers of three PC types containing the UV probes would have almost the same packing density, since the λ_{max} values are almost the same and constant. The probes in EPC bilayers show small blue shift with an increase of temperature. EPC bilayers are in the fluid state in this temperature range and their packing becomes looser as temperature rises.

The λ_{max} values of both UV probes in the DPPC bilayers show large blue shifts with an increase in temperature from about 25 to 35 °C, indicating a large change in the packing state of bilayers. IR studies have showed that the acyl chain packing of DPPC changes from an orthorhombic to a hexagonal subcell between 36—38 °C (pretransition), which is lower than a main phase transition temperature T_c.[31,32] The UV λ_{max} shifts may correspond to this change in packing state. Thus this change would accompany the density change. For PC with saturated acyl chains, more gauche isomers exist in the fluid state than in the gel state.[30,31] The structural disorder of the fatty acyl chains would cause the loosening of the packing.

On the other hand, λ_{max} value in DMPC bilayers is almost constant both below and above T_c. For DMPC, consisting of shorter acyl chains, the alignment of acyl chains in the gel state are originally much disturbed[30] and the density would not change so much with phase transition. In this case, the packing would not be so strong even in the gel state.

From these UV results, it is confirmed that the packing strength of acyl chain region of bilayers is affected mainly by the length and the chemical structure of the fatty acyl chains when the polar head group is identical, and it also depends on the order or fluidity of bilayers. Further it is confirmed that UV spectroscopy is one of the best methods for the study of the packing state of lipid bilayers. Although the probe is used in this work at a relatively high concentration, it is easy to lower its concentration because of its high UV sensitivity. By using an UV probe bound to a protein, such as retinal which can bind to a protein through a lysine side chain by forming a Schiff base linkage as found in rhodopsin[33] and bacteriorhodopsin,[34] it would be possible to obtain the information on the state of lipid surrounding the protein and on the lipid-protein interaction.

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