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TEMPERATURE-DEPENDENT SPECTRAL CHANGES OF A HEMIN-LIPOPHILIC IMIDAZOLE COMPLEX INCORPORATED INTO LIPOSOMES PREPARED FROM DIPALMITOYL-, DIMYRISTOYL- AND EGG YOLK-PHOSPHATIDYLCHOLINE

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SUMMARY

The optical spectra of the hemin-lipophilic imidazole complex incorporated into liposomes prepared from three types of phosphatidylcholine were studied at various temperatures. The Soret peak of the system with dipalmitoyl- and dimyristoyl-phosphatidylcholine liposomes showed a marked bathochromic shift at the phase transition temperature, but in egg yolk phosphatidylcholine liposomes no spectral change was observed in the temperature range of 10° to 50°.

The importance of the fluidity of membrane lipids in regulating membrane functions has been suggested by a number of recent studies (1-3). It is known that lipid molecules in lipid bilayers undergo phase transitions and phase separations (4,5). Recently, the incorporations of purified preparations of membrane-bound heme proteins, such as cytochrome P-450 and cytochrome b_5, into artificial lipid bilayer membranes have been reported and alteration of enzyme reactions at the phase transition temperature have been investigated (6).

Previously, we reported the incorporation of the hemin-lipophilic imidazole complex into liposomes prepared from egg yolk phosphatidylcholine and proposed that this system, especially in the six-coordinating ferrous heme form, can be used as a model of heme protein in the membrane-bound state (7).

This paper reports studies on the temperature-induced spectral changes of the hemin-lipophilic imidazole complex incorporated into liposomes, showing that with liposomes prepared from synthetic saturated phosphatidylcholines

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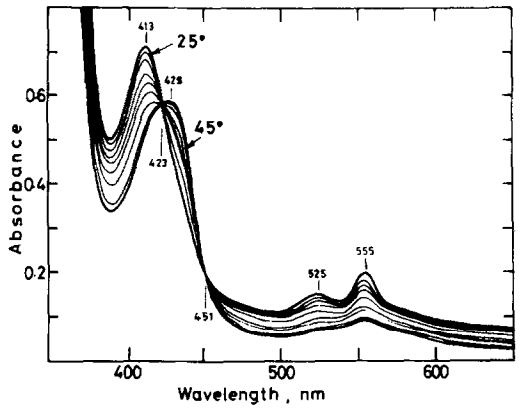


Figure 1. Temperature-induced spectral changes of an imidazole-binding, six-coordinating ferrous heme-lipophilic imidazole complex incorporated into dipalmitoylphosphatidylcholine vesicles.
The mixture (1 ml) consisted of 0.1 M phosphate buffer (pH 7.4), 67 mM imidazole and liposomes containing 800 nmoles of dipalmitoylphosphatidylcholine, 100 nmoles of 2-undecylimidazole and 10 nmoles of hemin chloride.

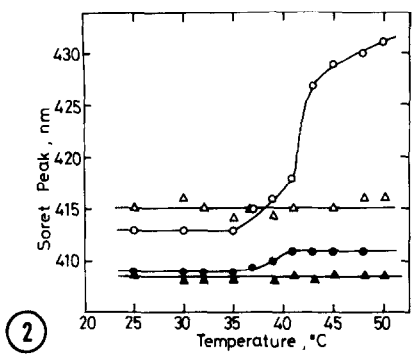
(dipalmitoyl- and dimyristoyl-phosphatidylcholine) the optical spectrum of heme changes abruptly at the phase transition temperature.

Materials and Methods

Dipalmitoyl- and dimyristoyl-phosphatidylcholine and hemin chloride were obtained from Sigma Chemical Co. 2-Undecylimidazole was a generous gift from Shikoku Fine Chemicals, Co. Egg yolk phosphatidylcholine was prepared by the method of Pangborn (8). Stock solutions of the phosphatidylcholines were prepared in chloroform and stored at -20°. Concentrations of phosphatidylcholines were determined by the method of Chalvardjian et al. (9). The hemin-2-undecylimidazole complex was incorporated into liposomes as reported previously (7). The optical spectra of the complexes were measured with a Union Giken SM-302 spectrometer. Changes in temperature were achieved with a circulating water bath attached to the cuvette chamber of the spectrometer and the temperature in the cuvette was measured directly. Before measurement of the spectrum of the reduced form of the hemin complex, the solution in the cuvette was treated with a little sodium dithionite.

Results

The temperature-induced spectral change of the imidazole-binding, six-coordinate heme(Fe^{II})-lipophilic imidazole complex incorporated into dipalmitoylphosphatidylcholine vesicles is shown in Fig. 1. The Soret peak shifted from 413 nm to 430 nm with increase in temperature, and two isosbestic points at 423 and 451 nm were observed, indicating that the temperature-induced spectral change is reversible and the two chemical species are in equilibrium.



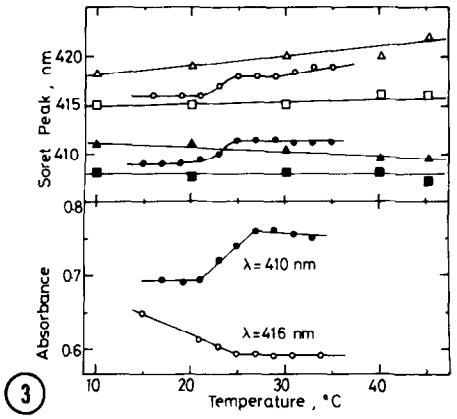


Figure 2. Temperature-dependent change in position of the Soret peak of the imidazole-binding, six-coordinating hemin-lipophilic imidazole complex incorporated into dipalmitoylphosphatidylcholine vesicles.
The complete system was as described for Fig. 1. Complete system : —●— oxidized form, —○— reduced form. Complete system without 2-undecylimidazole : —▲— oxidized form, —△— reduced form.

Figure 3. Temperature-dependent changes in position (top) and absorbance (bottom) of the Soret peak of the imidazole-binding hemin-2-undecylimidazole complex incorporated into various types of liposomes.
The amounts of each component were described for Fig. 1. Dimyristoylphosphatidylcholine vesicles : —●— oxidized form, —○— reduced form. Egg yolk phosphatidylcholine vesicles : —▲— oxidized form, —△— reduced form. Hemin-bisimidazole complex without liposomes : —■— oxidized form, —□— reduced form.

The positions of the Soret peak of the oxidized and reduced forms were plotted against temperature (Fig. 2). The peak of the reduced form shifted gradually in the temperature range of 35° to 41° and changed abruptly between 41° and 43°. The latter temperature range is close to the phase transition temperature(41°) for dipalmitoylphosphatidylcholine measured by differential thermal analysis and X-ray diffraction (10). The oxidized form also showed a temperature-dependent spectral change. However, in the system without lipophilic imidazole, no temperature-induced spectral change was observed. A similar spectral change depending on temperature was observed with liposomes prepared from dimyristoylphosphatidylcholine (Fig. 3, top). In the oxidized and reduced forms of the hemin complex, the Soret peaks changed in the temperature range of 21° to 25°, corresponding to the phase transition temperature of dimyristoylphosphatidylcholine (23°) (10), although the changes were not so marked as in liposomes prepared from dipalmit-

oylphosphatidylcholine. Thus, the absorbances of each Soret peak were monitored against temperature (Fig. 3, bottom). Clear temperature-dependencies were observed for both forms, and especially the reduced form, which showed increase in absorbance between 21° to 27°, with a center at 24°.

On the other hand, in liposomes prepared from egg yolk phosphatidylcholine, whose phase transition temperature is reported to be -7° to -15° (11), no spectral change was observed in either form on increase in temperature from 10° to 50°. Furthermore, a hemin-low molecular bisimidazole complex without liposomes did not show any spectrum change in the temperature range examined.

Discussion

In this work we showed that the optical spectrum characteristic of heme incorporated into liposomes with saturated phosphatidylcholine (dipalmitoyl- and dimyristiyl-phosphatidylcholine) changed markedly at the phase transition temperature, but that of heme in liposomes with unsaturated egg yolk phosphatidylcholine did not change in the temperature range from 10°-50°. Furthermore, the Soret peak of the ferrous heme complex showed a bathochromic shift with increase in temperature, especially at the phase transition temperature. At temperatures below the phase transition, the lipid hydrocarbon chains are relatively rigid and extended, whereas above the phase transition they are flexible. Thus increase in thermal mobility of the chains in the lipid barrier seems to influence the optical properties of the heme group.

In connection with our findings, it is interesting to note that temperature-dependent spectral changes have been observed in cholesterol binding to cytochrome P-450 of rat adrenal (12), in the substrate-induced difference spectrum of rat liver microsomes (13) and in the difference-spectrum of purified cytochrome P-450_{CAM} in the absence of substrate (14). These observed perturbations of the optical properties of heme proteins by temperature can generally be correlated with alteration of the spin state of heme iron (14). It is also wellknown that the Soret peaks of myoglobin and hemoglobin in the ferrous form show bathochromic shifts with transition from low to high spin state (15).

Moreover it was reported that equilibrium between the high and low spin states of ferric cytochrome P-450$_{CAM}$ was shifted toward the low spin state with decreasing temperature (14). From these observations we can explain the spectral changes observed in this work as due to spin transition of heme iron: presumably the low molecular imidazole in the six-coordinating complex (low spin state) is removed from its proximal (sixth) position with increasing fluidity of the liposomes and the five-coordinating complex (high spin state) is formed.

Another possible explanation of the spectral changes is formation of heme-heme interaction. However, this possibility may be ruled out because relative change of the concentration of hemin and lipophilic imidazole did not affect the optical spectrum. At present, the reason why alteration of membrane-fluidity changes the coordination entity of heme is unknown.

It is very interesting with regard to the functions of membrane-bound heme proteins that spectral change of a heme compound incorporated into liposomes was observed on alteration of the membrane-fluidity at the phase transition temperature.

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