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CONTROL OF ELECTRON TRANSPORT BY THERMALLY INDUCED PHASE TRANSITION OF LIPOSOMAL MEMBRANE

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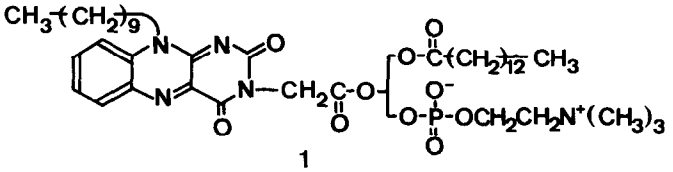
The facilitated electron transport rate via the flavolipid half-channel in the bilayer membrane was remarkably controlled by thermally induced phase transition.

Electron transporting systems in biological membrane play significant roles in energy metabolisms as demonstrated in photosynthetic charge separation as well as oxidative phosphorylation in respiratory systems. These electron transducing devices locate in the membrane as a coupled entity of several elements, to make the understanding of the nature of these biological systems very difficult. Therefore as a simplified model, the development of artificial systems may contribute greatly to the elucidation of the mechanism and to the construction of biomimicking function. According to this line, we have developed an artificial flavolipid molecule which was demonstrated to facilitate efficiently the transmembrane electron movement[2].

The target is now focused on the control of electron transport rate. A successful demonstration of this idea will also be important for the future development of molecular switching in ordered matrix. In this communication, we report that the electron transport rate across the bilayer membrane modified with the flavolipid (1) is controlled by the thermally induced phase transition of the flavoliposome.



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Dipalmitoyl phosphatidylcholine (DPPC) was used in place of egg lecithin to prepare liposomes having a definite Tc. This was functionalized with flavolipid (DPPC : (1) = 97 : 3 (mole/mole)), in a manner similar to the procedure for the egg lecithin liposome[2,3]. The electronic spectrum of the flavoliposome (λ_{max} = 336, 443 nm) resembled that in chloroform (λ_{max} = 336, 442 nm) significantly shifted from the flavin chromophore in bulk water (λ_{max}= 347, 433 nm). Such spectral change suggests that the flavin chromophore is incorporated into the inner bilayer membrane phase rather than exposed to the bilayer interface. The DPPC liposome containing flavolipid in 3 mole % quantity was stable for at least four days at 4°C. This liposome is less

stable than the corresponding egg lecithin liposome having 7 days stability. All kinetic studies and the differential scanning calorimetry (DSC) measurements were performed within two days after preparation.

The phase transition temperature (Tc) of the present flavoliposome was determined to be 38° - 39°C by DSC (Fig. 1) and there observed also a pretransition peak at 35°C. These data are in the range of Tc's observed for the parent DPPC liposome[4]. No other peaks corresponding to phase transitions of the flavolipid domain were detected.

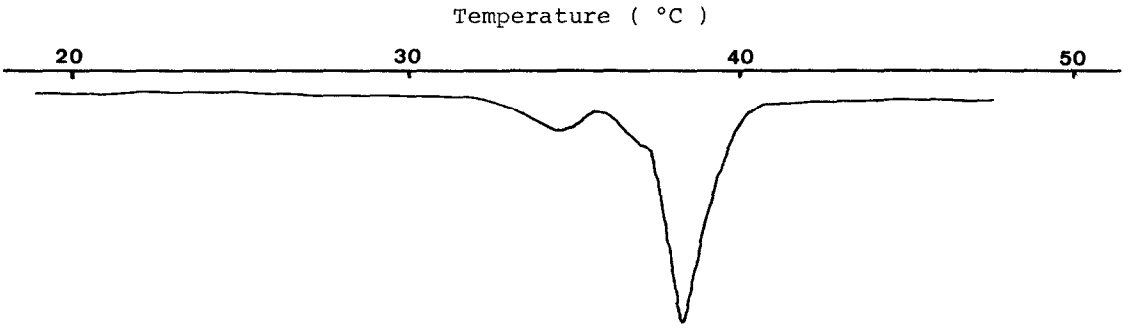

Fig.1 DSC curve of the flavoliposome
The flavoliposome was purified through centrifugation at 4°C after sonication.

Kinetic measurements were performed by the syringe stopped-flow method[3] after purification of the flavoliposomes through gel filtration. The exterior ferricyanide was removed by this treatment and its very rapid reduction, $k > 10\ s^{-1}$ in the homogeneous state, was never observed in the runs.

The rate of transmembrane electron transport was estimated from the reduction rate of ferricyanide in the interior aq. phase by the addition of excess sodium dithionite in the exterior. As described previously, rapid reduction of the flavolipid ($fl^{ox} \rightarrow fl^{red}$) which was monitered by the change of absorbance at 472 nm was followed by a slow decrease of interior ferricyanide which was monitored by the change at 425 nm. Under this condition, the rate followed the pseudo first order relation with respect to the ferricyanide concentration:

$$\frac{-d[Fe(CN)_6^{3-}]}{dt} = k_{obs}[Fe(CN)_6^{3-}]$$

The pseudo first order rate constants were obtained at temperatures ranging from 10°C to 50°C and plotted in Fig. 2. Below Tc the electron transport rate ($k_{obs} = 0.02\ sec^{-1}$) was very slow and increased dramatically above Tc to give the largest rate ($k_{obs} = 1.5\ sec^{-1}$ at 40°C) ever reported. Thus the rate difference below and above Tc amounted to ca 10^2. The pseudo first order rate constant here obtained corresponds roughly to that of lecithin liposome modified with the same flavolipid (1), 1.3 sec^{-1} at 50°C.

Therefore the dramatic change of the electron transport observed here can be ascribed to the suppression of electron transport at the temperature region lower than Tc. The electron transport rate across the parent DPPC liposome was observed to be as small as 10^{-3} sec^{-1} in the whole temperature range examined (Fig. 2).

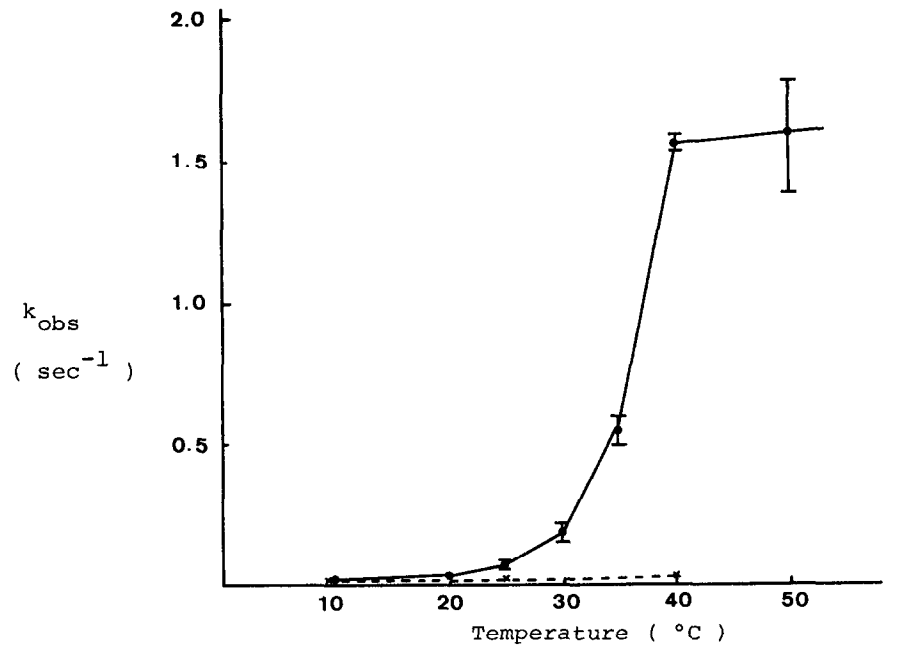

Fig.2 Temperature dependence of the first order rate (k_{obs}) for the reduction of interior $K_3Fe(CN)_6$ at pH 7.0. Analytical concentration of $K_3Fe(CN)_6$, fl-Lip. and $Na_2S_2O_4$ were 0.25 mM, 0.02 mM, and 4.2 mM respectively. DPPC liposomes with (——) and without (---) flavolipid.

The artificial flavolipid (1), an efficient electron transport catalyst in the artificial liposome system, has the following characteristics : (i) The molecular length is designed to fit to that of the lipid molecule, i.e. a half of the thickness of the bilayer membrane of liposome. (ii) The carrier transport mechanism through membrane is improbable because the transmembrane diffusion rate of the flavolipid is estimated to be in the order 10^{-5} sec^{-1} and obviously too slow to account for the present rapid electron transport. (iii) In contrast, the lateral diffusion remains relatively free for the artificial lipid molecule. (iv) A significant contribution of flavolipid by 2nd powers of its concentration was observed in the interior ferricyanide reduction of the egg lecithin liposome modified with this flavolipid[5]. These combined characteristics will allow us to propose that the present flavolipid provides a half-channel as an electron transporting unit in the bilayer membrane.

The dramatic enhancement of the electron transport rate here observed can reasonably be related to the increase in the lateral diffusion rate in the phospholipid bilayer. Fluorescence recovery after photobleaching tells us that the lateral diffusion rate of phospholipids in the bilayer membrane increases by a factor of (1.4 - 5.5) $\times 10^2$ between temperatures below and above Tc [6]. Therefore the transmembrane electron transport mediated by a half-channel is concluded to be controlled by the lateral diffusion rate of the half-channel. This suggests that the electron is passing through a "transient channel" extending through the whole membrane.

It has been reported previously that the permeability of inorganic as well as organic materials through the liposomal membrane is controlled by the temperature. However, all of these permeations were interpreted[7] by a simple "leakage" process due to the disorder of the membrane. In contrast with them, the present system provides the first demonstration that the facilitated electron transport from the exterior electron donor to the interior acceptor via the electron channel was successfully controlled by the gel-liquid crystal phase transition.

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