BIOORGANIC CHEMISTRY 11, 412–419 (1982)

Coenzyme Model Reaction in Lipid Bilayers

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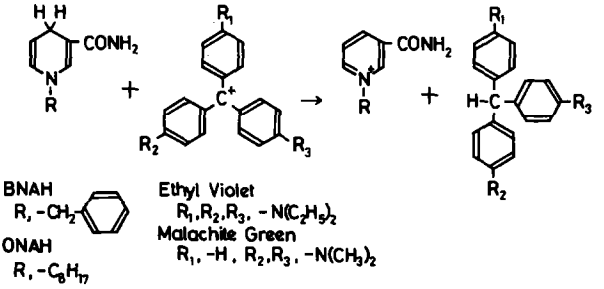
Received March 2, 1982

Coenzyme model reactions, such as the H^- ($H^+ + 2e^-$) transfer from NADH models to triphenyl methane dyes, were investigated in the presence of lipid bilayers, for example, L-α-dimyristoyl phosphatidyl choline and egg yolk lecithin. In the temperature dependence of the acceleration effect by the lipid bilayer, discontinuous points were observed, corresponding to the phase transition point such as gel–liquid crystal (T_c) or the segregation point (T_s). The T_c and T_s values of the bilayers varied with the reactant as a result of the difference of perturbing effect on the structure of the bilayers. The pressure effect on the transition point was also studied. Transition points such as T_c or T_s became higher with increasing pressure, and dT_c/dP or dT_s/dP was different for various bilayers. In the gel phase of the membrane, stereospecific reduction of malachite green was observed by chiral nicotinamide: the difference in the catalytic effect on the reduction rate between (*R*)- and (*S*)-dihydronicotinamides was larger in the gel phase than that in the liquid crystal phase or in the phase separated state, which suggests that the gel-state molecule can recognize the molecular structure better than the liquid-crystal state molecule.

INTRODUCTION

Among many kinds of reactions occurring in the biomembrane, energy transfer reactions utilizing NAD(P)H are particularly important. For example, the cytochrome b_5 system is maintained by electron transfer such as NADH → FAD-reductase → cytochrome b_5 → desaturase (*1, 2*). In this paper we examined the influence of a model biomembrane of various phases and structures on coenzyme model reactions such as direct transfer of H^- ($H^+ + 2e^-$) from NADH model compounds to triphenyl methane dyes (*3*) (Scheme 1). Furthermore, we investigated the pressure effect on the phase transition of a model membrane, which



SCHEME 1

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could be reflected in the change in the catalytic effect by the membrane or the change in the turbidity of the membrane. From dT/dP values (T is a phase transition point at P atm) we wished to learn the important physical properties of the membrane; for example, the rigidity of membrane. Lipid bilayers and polymeric bilayers have recently been studied as drug carrier (*4–6*) and solar energy storage systems (*7, 8*).

We also examined the stereospecific acceleration effect of a model membrane on the H^- transfer reaction between chiral coenzyme models and dyes. An ordered or oriented environment is expected to enhance the recognition of chirality of reactants in our catalytic reaction system.

EXPERIMENTAL

Materials. L-α-Dipalmitoyl phosphatidyl choline (DPPC), L-α-dimyristoyl phosphatidyl choline (DMPC), and egg yolk lecithin (EPC) were purchased from Sigma. 1-Benzyl-1,4-dihydronicotinamide (BNAH) was prepared according to the method by Lindsey *et al.* (*9*). (*R*)- and (*S*)-*N*-α-phenethyl-1-propyl-1,4-dihydronicotinamides ((*R*)- and (*S*)-PNAH) were synthesized according to the method of Ohno *et al.* (*10*). 1-Octyl-1,4-dihydronicotinamide (ONAH) was prepared as follows: 13 g of nicotinamide and 50 g of octyl bromide were coupled in CH_3CN at 50° for 2 days. 1-Octylnicotinamide (ONA) was precipitated by acetone and washed with acetone several times (*11*). The ONA obtained (21 g) was dissolved in water and 27 g of K_2CO_3 and 46 g of $Na_2S_2O_3$ were added. After 2 hr, viscous orange supernatant was purified several times by ethanol solubilization, dehydration, and recrystalization.

Ethyl violet (EV) and malachite green (MG) were purchased from Schmidt & Company and Merck, respectively, and used without further purifications.

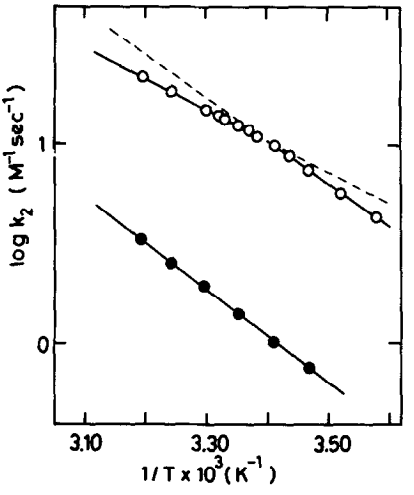


FIG. 1. Effect of EPC on the reduction of EV by BNAH. ○, EPC; ●, spontaneous; [BNAH] = 2.1×10^{-3} *M*; [EV] = 2.0×10^{-5} *M*, pH 7.7.

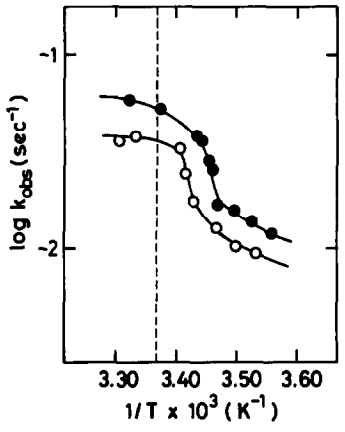


FIG. 2. Effect of DMPC on the reduction of MG by ONAH. ●, [ONAH] = 7.5×10^{-4} *M*, [MG] = 5×10^{-6} *M*; O, [ONAH] = 3.8×10^{-4} *M*, [MG] = 2.5×10^{-4} *M*; ---, transition point of DMPC in BNAH–MG system.

Kinetic measurements. Reductions of EV or MG at 1 atm were followed under a pseudo-first-order condition [dihydronicotinamide] ≫ [dye] by the decrease in the absorbance at 600 or 620 nm, respectively, using a Union high-sensitivity spectrophotometer SM 401 (Union Engineering, Osaka, Japan). At high pressures, the reaction was followed using a Union high-pressure spectrophotometer with a Drickamer-type optical cell with sapphire windows. (*12*).

Differential scanning calorimeter (DSC) measurements. A differential scanning calorimeter (SSC 560, Daini-Seikosha, Tokyo) was used to investigate the effect of ONAH on the transition temperature of DMPC bilayers at a scan rate of 1.5°C/min. The concentration of DMPC bilayer in the DSC measurement was 6 m*M*.

Preparation of bilayers. Preparations of lipid bilayers were done by the conventional method (*13*). Ultrasonication was done using a Kontes Ultrasonic cell disrupter at 23.5 kHz for 100 min under N_2 atmosphere. The solution was filtered using a Millipore filter (pore size 0.45 μm) to remove insoluble materials. All lipids were suspended in pH 7.7, 0.05 *M* Tris–HCl buffer solution.

RESULTS AND DISCUSSION

A. Effect of Phase Transition of the Membrane on the Rate of the Coenzyme Model Reaction

First, we examined the effect of EPC bilayers on the reduction of EV by BNAH. The reduction was accelerated by addition of EPC because of the hydrophobic interaction between lipid bilayer and EV, and between lipid bilayer and BNAH. A similar effect was observed with the addition of a surfactant (*14*). We examined the temperature effect on the acceleration by EPC (Fig. 1). A clear bending point was observed at 20–21°C in the presence of EPC. Similar tendencies were observed in the proton abstraction and the decarboxylation in the syn-

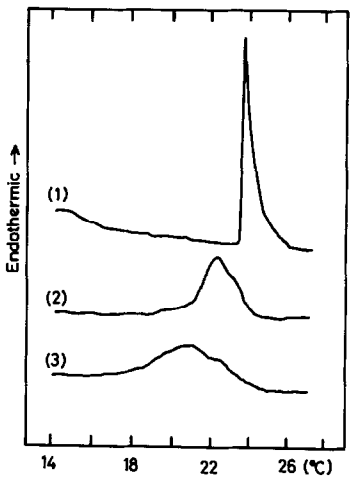


FIG. Effect of ONAH on the transition of DMPC. Curve 1, none; curve 2, [ONAH] = 3.8×10^{-4} *M*; curve 3, [ONAH] = 7.5×10^{-4} *M*; pH 7.7.

thetic bilayers by Kunitake *et al.* (*15, 16*). EPC is considered to contain fatty acids of 50% saturated and 50% unsaturated hydrocarbons (C-16 and C-18) (*17*) and is known to undergo a phase change at −15°C (*18*). At this point, all alkyl chains of the lipid were considered to change from gel to liquid-crystal states in a cooperative way. The presence of a bending point observed at about 20°C in Fig. 1, however, suggests that a portion of the long saturated alkyl chains such as stearyl groups might still exist in the gel state (segregated phase) between T_c (−15°C) (*18*) and 20°C, as observed by Verma *et al.* using Raman spectroscopy (*19*). We call this bending point a segregation point (T_s) of EPC. Recently Sano *et al.* also

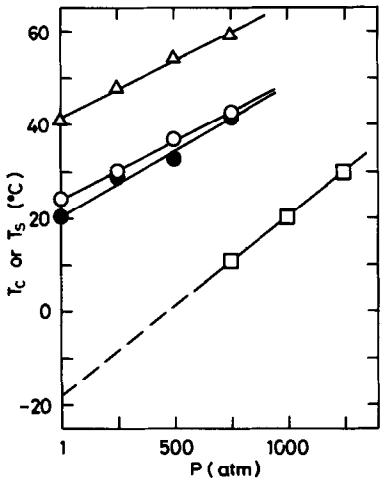


FIG. 4. Pressure effect on the transition points of EPC (□), DMPC (○), and DPPC (△) and the segregation point of EPC (●).

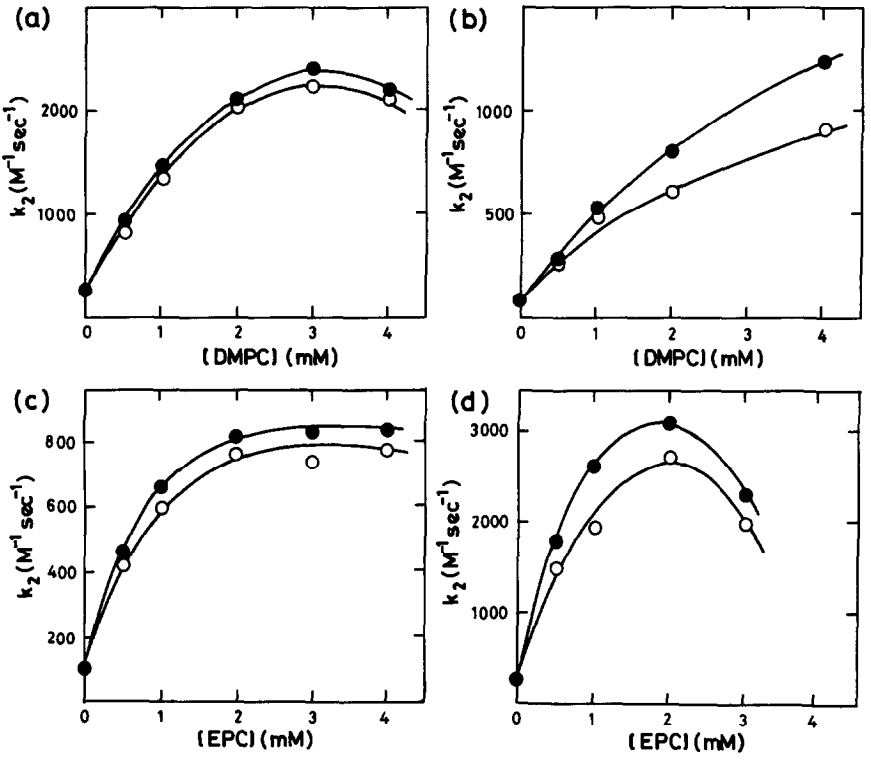


FIG. 5. Effect of DMPC or EPC on the reduction of MG by (*R*)- or (*S*)-PNAH. ○, (R)-PNAH; ●, (S)-PNAH. (a) DMPC 1 atm at 28.3°C, (b) DMPC 1 atm at 18°C, (c) EPC 1 atm at 19.0°C, (d) EPC 1250 atm at 19.0°C. [PNAH] = 5×10^{-5} *M*; [MG] = 5.0×10^{-6} *M*.

observed a clear bending point at about 16°C in the Arrhenius plot of intra- and intermolecular excimer formation in EPC liposomes (*20*). Their results also support our data.

Similar experiments were examined in the DMPC systems. In the multilamellar DMPC membrane, the phase transition point observed in the BNAH–MG system (24°C) agreed well with the literature value of T_c (23.9°C) (*21*).

We also examined the reduction of MG by ONAH in the presence of DMPC multilamellar membrane at two different concentrations of the substrates concentration (Fig. 2). As the substrate concentration became higher, the transition point was shifted to lower temperature. This behavior was confirmed by the differential scanning calorimetry (DSC) (Fig. 3). With an increase in ONAH concentration, T_c of DMPC dropped from 24 to 20.5°C and at the same time the endothermic region was broadened. The reason the transition point of ONAH was lower than that of BNAH would be that ONAH tends to perturb the membrane structure, whereas BNAH does not. In addition, the fact that the molecular shapes of reactants strongly influence both the catalytic activity and the bending point of bilayers suggests that these reactions occur in the immediate vicinity of or inside the membrane.

B. Pressure Effect on the Phase Transition of Membrane

Next we observed the pressure effect on the transition point of EPC, DMPC, and DPPC in order to get information necessary to carry out a stereoselective coenzyme model reaction in various phases of the membrane, which will be discussed later. The main transition point (T_c) and the segregation point (T_s) of EPC were determined from the catalytic effect on the coenzyme model reaction in a way similar to that shown in the previous sections. The T_c of DMPC and DPPC was determined from the change in turbidity. The results are shown in Fig. 4. As seen from the figure, T_c and T_s became higher with increasing pressure. The phase transition points of cholesterol esters were also observed by Osugi *et al.* (*22*) to become higher with pressure.

For EPC, T_c at 1 atm was shown by extrapolation to be −17°C, and this value roughly agreed with the literature value (−15°C) (*18*). From the results of Fig. 4, we could easily estimate dT_c/dP or dT_s/dP, which are listed in Table 1. The value for dT_c/dP of EPC was larger than those of DMPC and DPPC, probably because the unsaturated lipid facilitates thermal motion of hydrocarbon chain in the membrane. The dT_s/dP for EPC was nearly the same as dT_c/dP (not dT_s/dP) of DMPC and DPPC, which supports the assumption that the segregation for EPC is the gel–liquid crystal transition of saturated alkyl chain.

From the dT_c/dP values of DMPC and DPPC, we could estimate the volume change (ΔV) for the phase transition of lipid membrane using a Clausius–Clapeyron equation $dT_c/dP = T_c\,\Delta V/\Delta H$ (Table 1). The ΔH used for DMPC (6.6 kcal mol^{-1}) and DPPC (8.7 kcal mol^{-1}) were taken from literature (*25*). The ΔV for DPPC approximately agreed with the literature value. Melchior *et al.* (*27*) examined the dilatometry of lecithin aggregate. Though the values of ΔV were slightly different from ours, ΔV for DPPC in the two measurements was larger than that for DMPC, because ΔV is the volume change from gel to liquid-crystal states, mainly reflecting the difference in their hydrocarbon chains.

C. Stereoselective Acceleration of a Coenzyme Model Reaction in Various Phases of Membrane

As shown in the preceding sections we can easily produce various phases such

TABLE 1

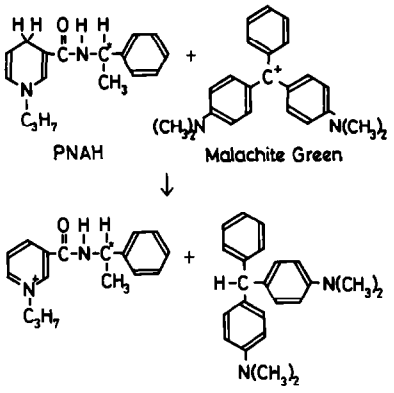
PHYSICAL PROPERTIES OF DMPC, DPPC, AND EPC

	T_c (°C) (at 1 atm)	T_s (°C) (at 1 atm)	dT_c/dP (°C/atm)	dT_s/dP (°C/atm)	ΔV (cm^3 mol^{-1})
DMPC	24.0	—	0.024 ± 0.001	—	22 ± 1
DPPC	41.0	—	0.026 ± 0.001[b]	—	30 ± 1[c]
EPC	−15.0[a]	20	0.038 ± 0.001	0.030 ± 0.004	—

[a] From Ref. (*18*).

[b] Literature values are 0.024 and 0.022°C/atm (*23, 24*).

[c] Literature value is 27.0 ml mol^{-1} (*26*).



SCHEME 2

as gel, gel–liquid crystal mixture, and liquid crystal by varying the pressure or temperature of the reacting solution. Therefore, we were able to study the influence of the reaction phases on coenzyme model reactions. As a first step, we tried to realize stereospecific recognition by bilayers. *N*-α-Phenethyl-1-propyl-1,4-dihydronicotinamide (PNAH) was reported to reduce ethyl benzoylformate into chiral ethyl mandelate (*10, 28, 29*). We examined here the reduction of malachite green (MG) by chiral (*R*)- or (*S*)-PNAH (Scheme 2) in the presence of egg yolk lecithin (EPC, L-form) or L-α-dimyristoyl phosphatidyl choline (DMPC) at various temperatures and pressures. The results obtained are shown in Figs. 5a–d. From the figures, it is apparent that chiral recognition was slightly better in the gel phase than in the liquid crystal phase.

Substrates might enter a site deeper in the bilayers of the liquid-crystal phase than in those of the gel phase. Since the asymmetric carbon of lipid exists near the surface of the bilayer, deeper penetration lowered the recognition between chiral carbons of bilayer and nicotinamide, decreasing the difference in reaction rate between (*R*)- and (*S*)-dihydronicotinamides.

Asymmetric synthesis in the liquid crystal was reported by several researchers (*30*). As for micellar reaction, Moss *et al.* reported that the steric course of deamination of alkyl amine changes from 22.9% inversion ($C_2H_5CH(NH_2)CH_3$) to 11.8% retention (n-$C_8H_{17}CH(NH_2)CH_3$) with an increase in the length of alkyl chain (*31*). They attributed their results to the formation of a micelle of the substrate, that is, the enhanced orientation of reacting molecules. Since the solvents used in asymmetric syntheses were organic in many cases, our results suggest the utility of oriented bilayers for the reaction medium of organic synthesis in aqueous solution.

ACKNOWLEDGMENTS

Differential scanning calorimeter measurements were performed in the Research Institute for Food Science, Kyoto University. We thank Professor M. Kito and Dr. M. Ishinaga for their kindness.

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