Facilitated Electron Transfer from NADH to Flavolipid Bound in Ammonium Bilayer

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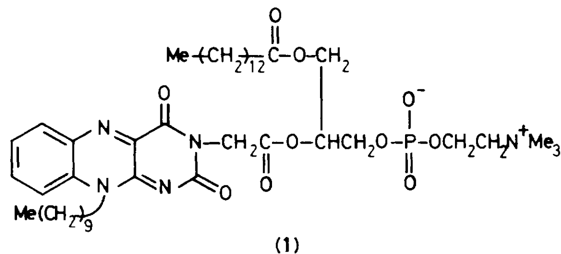
Cationic ammonium liposome bound NADH at the surface to induce an open conformation, favourable for the electron transfer to flavolipid in the membrane phase.

NADH (dihydronicotinamide adenine dinucleotide) is one of the main electron donors to flavoproteins in many biological oxido-reduction processes such as in the respiratory chain and in cytochrome P-450 reductase. Although nonenzymatic reductions of flavins by NADH model compounds have been investigated, only a few reports utilize NADH itself as the electron donor. This is mainly because the oxidation of NADH by flavin is about 10—10^2 times slower than those of NADH model compounds.[1]

Here, we report that the artificial flavolipid (**1**), bound to a cationic liposome, showed a large rate acceleration for the NADH oxidation, compared with (**1**) located in a zwitterionic liposome. The system represents a simplified NADH dehydrogenase model of high efficiency.

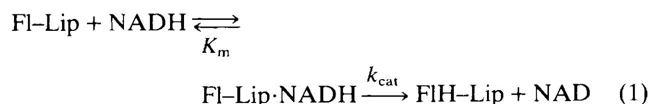
In order to obtain an assembled cationic surface of the bilayer membrane, dioctadecyldimethylammonium chloride (DODAC) was used and the artifical flavolipid (**1**) was incorporated stably into the bilayer membrane of DODAC by sonication.[2] Dynamic light scattering measurements indicated that the diameters of the present flavoliposomes were in the range 110—190 nm with a maximum at 130 nm. These values are similar to those of DODAC liposomes without flavolipid



(1)

(100—150 nm).[3] The flavin chromophore in the DODAC liposome showed an absorption maximum at 444 nm with distinct shoulders at 418 and 472 nm. The maximum appeared at almost the same position in chloroform solution with characteristic shoulders in the above region. On the other hand, the absorption maximum of flavin in aqueous and tris(hydroxymethyl)aminomethane (Tris) buffer solutions appeared at a significantly shorter wavelength of 433 nm without any shoulders. Therefore, it is concluded that the flavin unit in the DODAC liposome is tightly bound in a hydrophobic region of the DODAC bilayer membrane.

The oxidation of NADH by the flavolipid in the bilayer membrane was examined under pseudo-first-order conditions with respect to the flavolipid concentration at 30 °C, pH 7.0 (5 mM Tris HCl) under argon. We have previously reported that electron transfer from the flavin in the outer half layer of the bilayer membrane to that in the inner layer was relatively fast compared to the interphasic electron transfer from NADH to the flavin.[4] The kinetic data were fitted to a Michaelis–Menten saturation scheme (1). Double reciprocal plots of k_{obs} *vs.* [NADH] (Lineweaver–Burk plot) gave $k_{cat} = 1.2\ s^{-1}$, and $K_m = 6 \times 10^{-4}$ M and therefore, the overall catalytic efficiency (k_{cat}/K_m) is $2 \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$.



$$\text{Fl–Lip} + \text{NADH} \underset{K_m}{\rightleftharpoons} \text{Fl–Lip·NADH} \xrightarrow{k_{cat}} \text{FlH–Lip} + \text{NAD} \quad (1)$$

In contrast to the kinetic behaviour of the flavolipid (**1**) in a DODAC liposome, the oxidation of NADH by (**1**) in the egg lecithin liposome did not show any saturation kinetics and gave a simple second order rate constant (k_2) of 0.043 $dm^3\ mol^{-1}\ s^{-1}$. The oxidation rate of NADH by (**1**) in the DODAC liposome having the ammonium head group is calculated to be accelerated markedly, by a factor of 4.6×10^4, compared with that in the egg lecithin liposome having the zwitterionic phosphatidylcholine head group. When the ionic strength was increased from 0.005 to 0.1, the saturation kinetics disappeared to give a simple decelerated second order rate constant of 20 $dm^3\ mol^{-1}\ s^{-1}$.

Figure 1 shows the fluorescence titration of NADH by the DODAC liposome, where the emission maximum of NADH (excitation 360 nm) is shifted from 458 nm in the bulk aqueous phase to a saturation value of 450 nm at a DODAC concentration of >1 mM. The blue shift of the emission maximum suggests that the dihydropyridine ring of NADH is located in the membrane (hydrophobic) phase rather than exposed in the aqueous phase.[5] Moreover, the fluorescence arising from intramolecular energy transfer from the adenyl to the dihydropyridine rings, observable in a closed conformation of NADH (450 nm, excitation 260 nm),[5] was quenched to about 55% of that in the bulk solution. The combined evidence indicates that the conformation of NADH, on electrostatic binding to the DODAC liposome, is altered to the open form from the closed one and that the dihydropyridine ring is extended into the membrane phase.

It is of interest to compare the result with the oxidation rates of NADH by various flavoenzyme models. The present

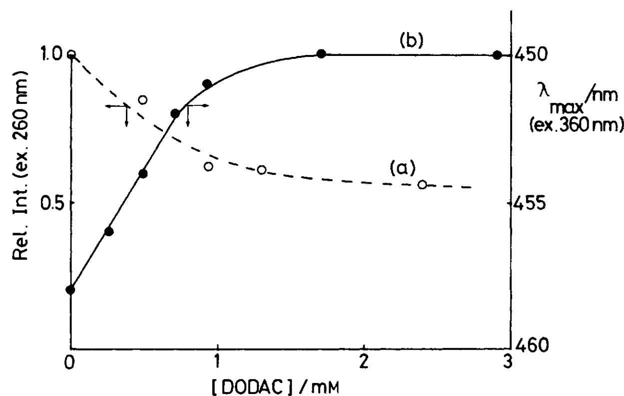


Figure 1. Change of the fluoresence spectrum of NADH by addition of the DODAC liposome at pH 7.0, 30 °C in 5 mM Tris buffer: (a) emission intensity of 450 nm excited at 260 nm, ○; (b) shift of emission maximum excited at 360 nm, ●.

flavolipid in a DODAC liposome shows a rate acceleration of about 2200-fold, compared with the homogeneous NADH oxidation by 3,10-dimethylisoalloxazine ($k_2 \simeq 0.9\ dm^3\ mol^{-1}\ s^{-1}$).[6] In a nonenzymatic oxidation of NADH by flavins, Shinkai obtained rate accelerations of 460—4600 by the flavin immobilized in the cationic polymers. This acceleration was claimed to be due to electrostatic interaction.[7]

In the continuing efforts to prepare semisynthetic flavoenzymes, Kaiser reported flavopapain[8] and recently flavo-GAPDH,[9] which was capable of producing a catalytic rate acceleration of 5800 for NADH as the best substrate. It is interesting that *X*-ray analysis shows that NADH is bound to the GAPDH in an open form. The k_{cat} in the structural arrangement reported here is the best among flavoenzyme models, being 1.2 s^{-1}. The corresponding values are 0.06—0.37 for flavin immobilized in polymer,[7] 0.007 for flavopapain,[8] and 0.0647 for flavo-GAPDH.[9] It may suggest that the nicotinamide moiety released from the closed conformation can interact favourably with the flavin unit extended into the membrane phase.

In conclusion, the positively charged liposome surface bound NADH and induced the conformational change of NADH to the open form. The proximity of NADH to the flavolipid as well as the induced conformational change produced a large rate enhancement for the oxidation of native NADH by the flavolipid in the liposomal membrane.

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