Coupled Electron–Proton Transport in Flavolipid Functionalized Liposome

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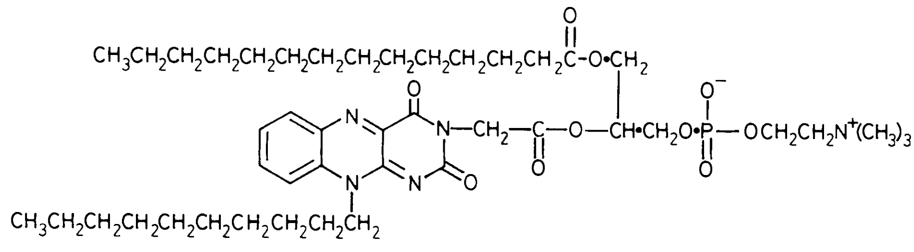
A pH gradient was generated across the liposomal membrane on coupling with channel electron transport through the flavolipid-containing artificial bilayer membrane.

A proton gradient across the membrane is a biological energy source for ATP synthesis. The gradient is generated by the coupling of proton flow with vectorial electron flow through the respiratory chain arranged in the membrane for oxidative phosphorylation. For deeper understanding, and possible applications, of bioenergetic transformation systems it seems desirable to construct a simple artificial cell which mimics the biological coupling between electron and proton fluxes across the membrane. We have recently reported an electron channel composed of the redox centre fixed appropriately in



(1)

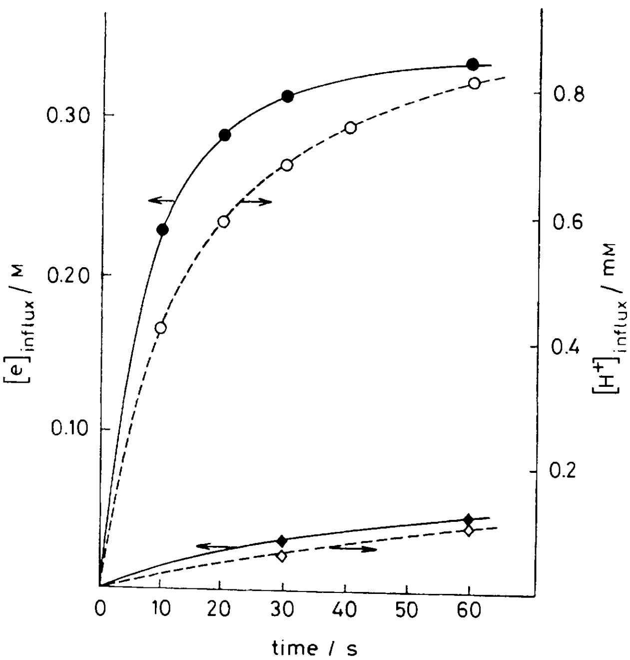
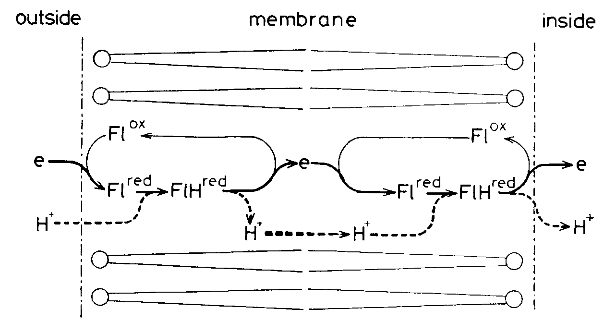


Figure 1. Proton–electron flux coupling across the membrane. The amount of electron transport across the membrane modified (—●—) and unmodified (—◆—) with (**1**), and the amount of H^+ transport across the membrane modified (---○---) and unmodified (---◇---) with (**1**) at 25 °C under Ar.



Scheme 1

the liposomal membrane.[1] In this Communication, we report a new observation that a totally artificial system successfully generated a proton gradient by coupling with the electron flux across the membrane. The pH gradient generated would be sufficient to synthesize ATP.

The liposomal membrane, made of egg yolk lecithin, was functionalized with 0.037 mM of the flavolipid (**1**) as the redox centre. This concentration level has already been established to be effective for electron transport through the membrane.[1] No structural change of the liposome was induced by the incorporation of the flavolipid (**1**); this was proven by gel permeation behaviour, dynamic light scattering measurements, and the stability of the liposome on standing.† This artificial lipid which contains a flavin unit as the redox active centre, at a fixed distance of 10 Å [by Corey–Pauling–Koltun (CPK) model] inwards both from the outer and inner membrane surfaces, affords a model of membrane-bound flavoprotein in Complex I at the mitochondrial inner membrane. An electron from the externally added $Na_2S_2O_4$ (1.1 mM) is accepted by one flavolipid in the outer membrane layer and transferred to the other in the inner layer *via* a rate determining electron transfer mechanism.[1] The electron is finally accepted by 0.34 M (inner local concentration) $K_3[Fe(CN)_6]$, in the 1.0 mM Tris buffer [Tris = Tris(hydroxymethyl)aminomethane] (initial pH 7.0), in the internal aqueous phase of the artificial cell. The time course is shown in Figure 1 (—●—), where more than 90% of ferricyanide was reduced within 60 s after mixing with $Na_2S_2O_4$.

The reduction of ferri- to ferro-cyanide is expected to give rise to the formation of an electromotive force across the membrane, which may be compensated for by the influx of cationic and/or the efflux of anionic species through the membrane. Among transferable ionic species, proton and hydroxide are the most likely, because of their large permeabilities across the bilayer lipid membrane.[2] Trisodium 8-hydroxy-1,3,6-pyranetrisulphonate, pyranine (4.0 mM), was introduced into the internal aqueous phase of the artificial cell to detect possible counter transport of proton and/or cotransport of hydroxide (these two transports cannot be differentiated from each other and hereafter are simply called proton transport), since the logarithmic fluorescence intensity ratios of pyranine at 510 nm, excited at 450 and 400 nm, were correlated linearly with the pH of the medium[3] in the range pH 4.1—7.0. According to this monitoring system, the apparent pH of the liposomal interior was observed to change from 7.0 to 5.9 accompanied by the electron flux. Considering proton equilibria with pyranine (pK_a 7.22) and the Tris buffer (pK_a 8.10), the amount of proton transport into the liposome, $\Delta[H^+]$, was calculated and plotted as a function of time in Figure 1 (---○---).

The active proton influx started immediately after the addition of $Na_2S_2O_4$ and the total influx, $\Delta[H^+]$, reached 0.82 mM after 60 s. This amount should give a hypothetical, (*i.e.* obtainable in the absence of the indicator and the Tris buffer), value of 3.09 for the inner pH. Therefore, the pH gradient across the membrane should reach a value as high as 3.9, which would be, in principle, sufficient to synthesize ATP. This proton influx was associated with the electron influx into

† The retention volume of the flavolipid-functionalized liposome was almost the same as that of egg lecithin liposome. A dynamic light scattering study demonstrated that the mean diameter of the liposome was *ca.* 25 nm. No precipitation was observed within seven days on standing at 4 °C.

the liposomal interior, suggesting the coupling of active proton transport with facilitated electron transport. The coupling efficiency of the proton to the electron flux, expressed by $\Delta[H^+]/\Delta[e^-]$, was determined to be 1/400 for this system.

The rate of the proton flow driven by the electron flow, P_{H^+},[2] was estimated to be 7×10^{-7} s^{-1} cm^{-2} from the initial 10 s time profile. Without the incorporation of flavolipid, very slow electron (—◆—) and proton (---◇---) influxes, which were ascribable to the passive leakage of neutral species of dithionite $H_2S_2O_4$ or HSO_2,[4] were observed through the lecithin bilayer membrane. This proton flux was estimated to be 3×10^{-8} s^{-1} cm^{-2}, demonstrating an enhancement factor of coupling transport of more than 10. Since the membrane containing flavolipid in the oxidized form shows P_{H^+} of 10^{-8} s^{-1} cm^{-2} by a separate experiment,‡ it is clear that half and/or fully reduced flavin units facilitate proton transport significantly. It is known that reduced flavins can accept protons easily upon reduction by use of their favourable protonating sites, pK_a being 6.5 and 6.3 for half and fully reduced flavins, respectively.[5] Therefore, the electron–proton coupling may be illustrated as shown in Scheme 1. Accompanied by the reduction of outer flavolipid, the proton is incorporated into the membrane phase and liberated there on its oxidation. It is then transferred to inner flavolipid, again accompanied by its reduction, and liberated again on its oxidation. According to such a vectorial electron influx, the overall proton flow goes from the external to the internal aqueous phase so as to generate a proton gradient across the membrane. Detailed mechanistic studies on this coupling transport by flavolipids are under way in our laboratory.

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‡ The passive H^+ transport rate across the membrane with oxidized flavolipid driven by the pH gradient was measured in conditions similar to the coupled transport, initial pH of the internal and external phases being 7.0 and 3.5, respectively.

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