Tetrahedron Letters,Vol.23,No.26,pp 2661-2664,1982 0040-4039/82/262661-04$03.00/0
Printed in Great Britain

BASIC PRINCIPLE OF COUPLING BETWEEN OXIDATION AND pH GRADIENT GENERATION. ARTIFICIAL LIPOSOME DIGESTING H_2

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Concept of coupling between oxidation and generation of pH gradient across membrane is presented by use of artificial liposome modified with electron transport catalysts, cyt c_3 or C_4V^{++}. The presented coupling mechanism based on facilitated "down-hill" electron flow, electroneutrality preservation and permeability control is confirmed by independent and direct measurements.

Although most of complex and sophisticated mechanisms of the biological reactions in the present living systems may be well understood on the basis of chemical principles, still some biological mechanisms seem to have interesting and quite unusual characteristics, difficult to understand on the chemical viewpoints.

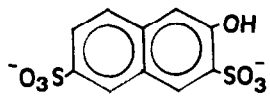
One such unusual characteristics is the "coupling" process connecting the oxidation-reduction reactions with phosphoric acid anhydride formation in *neutral aqueous solution* which plays a key-role in *respiration* or *photosynthesis*. The authors have currently been interested in the oxidation-dehydration coupling and doing a series of experiments in order to gain a fundamental chemical concept[1,2]. Now the authors wish to present a simple chemical concept based on facilitated "down-hill" electron flow, electroneutrality rule and permeability control.

Several successful preparations of the artificial liposome modified with the efficient electron transport catalysts have been reported[1-5], where the fastest electron flow was observed by us for the artificial lecithin liposome modified with cyt-c_3 on the membrane and with $K_3Fe(CN)_6$ in the interior (abbreviation, $K_3Fe(CN)_6$ (i)|Lip$^-$·cyt c_3, is used)[1]. And it was also shown by us that the rapid electron flow (negative charge flow) caused by the down-hill oxidation-reduction reaction (1) was perfectly "coupled" with the cotransport of H^+ from

$$Red^{-n} + Fe^{III} \longrightarrow Red^{-(n-1)} + Fe^{II} \qquad (1)$$

outside to inside the liposome and/or with the counter-transport to OH^-. In other words, the total amount of electrons flowing in the interior was equal to the total amount of ion trans-

ported, $\Delta = \Delta^{o\rightarrow i}_{H^+} + \Delta^{i\rightarrow o}_{OH^-} \approx \Delta^{o\rightarrow i}_{H^+} - \Delta^{o\rightarrow i}_{OH^-}$, at the early stages of the electron-transport[2,6], in order to keep so-called "electroneutrality rule". This discriminating H^+ and/or OH^- transport is resulted simply from their larger permeabilities compared with other ions. The pH gradient thus generated was directly measured by the absorbance change of an indicator[2] incorporated in the interior of the modified artificial liposome. However, the *actual* pH gradient thus observed was very small to be 0.01, when the electron transport started from pH 9 which is the most appropriate pH for **2** (pK_a = 9.5). Obviously, an electron transport starting from pH 7 or slightly above in the presence of least amount of an indicator of appropriate pK_a will generate the largest pH gradient across the membrane by the certain amount of Δ, which is "coupled" with the down-hill electron transport. Thus, we have prepared a new indicator 3-chloro-4-naphthol-1,6-disulfonate, **3**, having an appropriate pK_a value (6.2) for this pH range. The present new indicator is very stable toward



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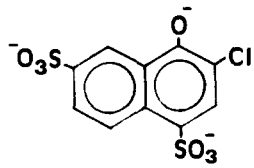

3

CNDS

λ_{max} (nm) in H_2O	
364,345	(pH 11)
300	(pH 4)

usual oxidizing and/or reducing reagents, very soluble in water, not incorporated in the hydrophobic area of membrane at all and has remarkable absorbance change in the pH range of 5—7 (pK_a = 6.2).

The lipid film of egg-lecithin 80 mg and cardiolipin 20 mg was suspended in the 5 ml of solution (1mM Tris-HCl pH 7.0) containing 270 mg of $K_3Fe(CN)_6$ and CNDS (9 mg - 51 mg) and this solution was sonicated and treated on a Sepharose 4B column as described previously[1,2]. To this artificial liposome, solution of cyt c_3 or C_4V^{++} was added to give the modified artificial liposome; $K_3Fe(CN)_6$ (i)·CNDS (i)$|Lip^-\cdot$cyt c_3 (4) or $K_3Fe(CN)_6$ (i)·CNDS (i)$|Lip^-\cdot C_4V^{++}$ (5) for the pH measurment during the electron transport. An electron-supplying system used was H_2 gas in the presence of collidal Pt supported on PVA[2]. The pH gradients generated across the membrane were shown in Table 1, demonstrating that the Δ value is independent of the indicator concentration. In the presence of 3.4×10^{-4} M of the indicator where ca. 99.8% of Δ was still consumed for the protonation of the indicator anion and the buffer, the pH gradient generated already reached to 1.05. It is very reasorable to extrpolate the pH gradient to the zero indicator and buffer concentration based on the above discussion. This extrapolation gives the pH gradient as large as 3.8 when started from pH 7. This proton gradient is large enough for ATP synthesis by using ATPase (or ATP synthetase)[7]. The observed Δ value increased

with time at first generating the pH gradient, which then drove passive H^+ transport and finally Δ decreased by passing a broad maximum (Fig. 1). The maximum proton gradient discussed above is, therefore, determined by a ratio R given by (6).

$$R = \frac{(d\Delta/dt) \text{ coupled with electron flow}}{(d\Delta/dt) \text{ driven by pH gradient}} \qquad (6)$$

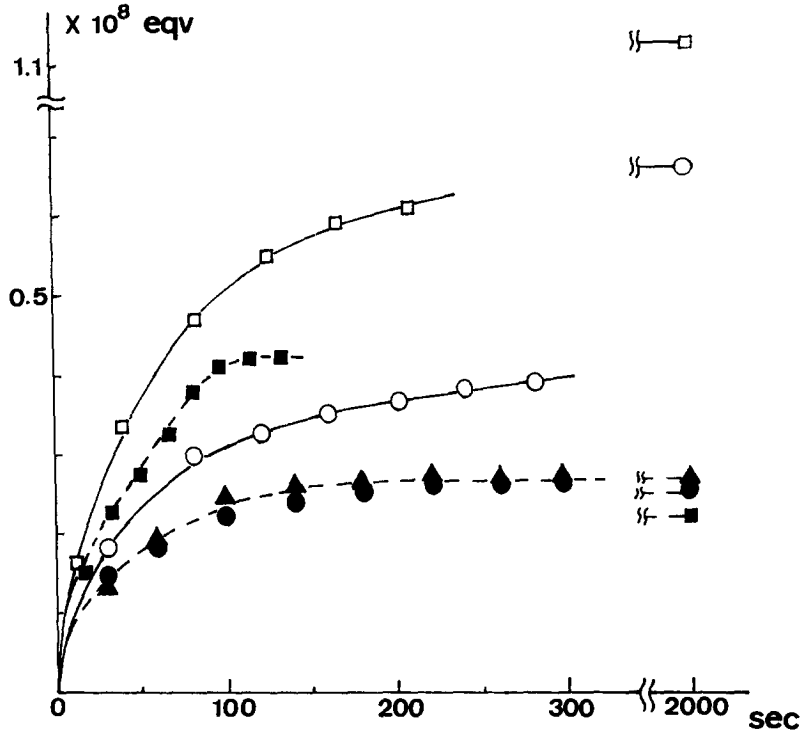

Fig. 1. Δ value coupled with electron transport.

Δ Value; -■- for C_3-liposome (CNDS) = 1.64×10^{-3}
-●- for C_4V^{++}-liposome (CNDS) = 2.04×10^{-3}
-▲- for C_4V^{++}-liposome (CNDS) = 0.34×10^{-3}
Transport of electron; —□— for C_3-liposome
—○— for C_4V^{++}-liposome

The passive H^+ transport rates directly measured for HCl (i) CNDS (i)$|\text{Lip}^-$ cyt c_3 or HCl (i)·CNDS (i)$|\text{Lip}^-\cdot C_4V^{++}$ by applying the initial pH gradient of 1.0 or 2.0 were much slower than those driven by the electron flow observed for the present liposome having large hydrophobic barrier in the membrane. The present situation of large $(d\Delta/dt)_{coupled}$ and small

Table. 1. Mamimum pH Gradient Generated Across the Membrane

	C_4V^{++}-Liposome		Cty a_3-Liposome
conc. of CNDS	2.04×10^{-3}	0.34×10^{-3}	1.64×10^{-3}
Δ to generate max. pH gradient (eqv)	0.26×10^{-8}	0.27×10^{-8}	0.42×10^{-8}
pH i	6.5	5.95	6.25

$(d\Delta/dt)_{passive}$ gives promise of the generation of the large and long-lived pH gradient.

In conclusion, facilitated (by an appropriate electron transport system) electron flow driven by the large oxidation potential difference is one of the best ways to generate the large proton gradient across the satisfactorily hydrophobic membrane, most conveniently in a *neutral aqueous* solution. Preparation of an artificial system to prepare ATP from ADP by applying pH gradient is now under investigation.

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(Received in Japan 12 March 1982)