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THE TRANS-MEMBRANE ELECTRON TRANSPORT COUPLED WITH DYE REDOX CYCLE IN THE LIPOSOME SYSTEM

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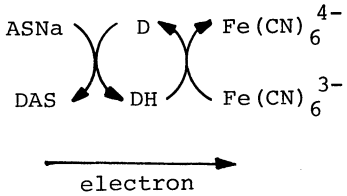
The trans-membrane electron transport reaction was studied in the single wall liposome system in the presence of various redox dyes both in the irradiated and non-irradiated system. The reaction proceed through the steps coupled with the dye redox cycle, and was completely controlled by the redox property of dye.

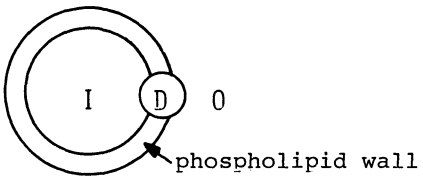
The trans-membrane electron transport system coupled with the redox cycle plays very important roles in the bioenergetic system. It is very important to make the model of the electron transport not only for making a new method of solar energy conversion but also for simulating the bioenergetic system. Though many groups of workers reported the electron transport model across the model membrane recently[1,2,3,4,5,6], little has been reported about dye sensitized electron transport in the liposome system. We have previously reported that electron can be transported across the phospholipid wall of single wall liposome coupled with methylene blue photoredox reaction [7]. In this system, the photosensitized electron transport was observed in the absence of quinones and and carotenes as an electron carrier. As a part of our recent work, we have carried out the electron transport in the liposome system using various redox dyes, whose redox potential(Em) is different from that of methylene blue, both in the irradiated system and non-irradiated system. The electron transport was observed in the presence of thionine in the non-irradiated system, though it was observed in the presence of methylene blue only in the irradiated system. The electron transport in the non-irradiated system may become one of the important aproaches to the vital staining method.[8] We would like to report here the result of our recent work, the electron transport model using various redox dyes, and the further discussion of our dye



sensitized electron transport model from the standpoint of the redox potential of redox dyes.

The reaction was carried out in the single lamella liposome system (Fig.1). The liposome system, containing sodium ascorbate(ASNa) only in the inner aqueous phase of each vesicle and potassium ferricyanide(FCN) only in the outer aqueous phase, was prepared by the following way.

Fig.1



System A)

I= ASNa , O= FCN

Sytem B)

I= FCN , O= ASNa

Lecithin (isolated from egg-york[9]) was dispersed in 1M ASNa aqueous solution (buffered with 1M Tris-Cl and 0.1M KCl, at pH 7.5) by ultrasonication. Untrapped ASNa was removed by gel-filtration over a column of Sephadex G-50. Then the solution of thionine(Th) and FCN was added (System A). The reaction was also carried out in the liposome system, containing ASNa only in the outer aqueous phase and FCN only in the inner aqueous phase in the presence of various dyes (System B). The preparation method of system B was almost the same as the previous report.[6,7]

Reduction of FCN, which is caused by the trans-membrane electron transport across the phospholipid wall from ASNa to FCN, was determined by measuring the decrease in the absorption of FCN at 420nm (A420).

Fig.2 shows the change in the absorption of FCN at 420nm and that of Th at 600nm (A600) in the dark (System A). Th absorbs light of wavelength at 600nm, but leuco thionine(LTh) does not absorb. Though A420 rapidly decreased due to the reduction of FCN, A600 was almost constant until all FCN was reduced (a -- b). After all FCN was reduced, the decrease in A600 due to the reduction of Th to LTh

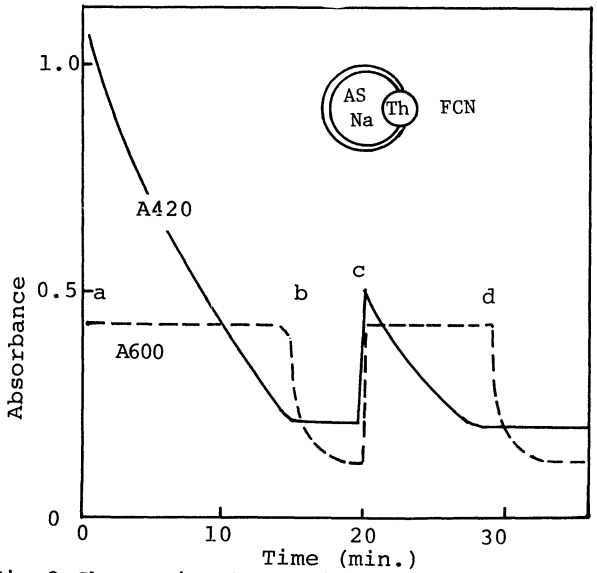


Fig.2 Change in absorption at 420 nm and 600 nm in the presence of Thionine.
[Th]=0.86 x10^{-5} M, [FCN]=1 mM
(at c FCN was added again)

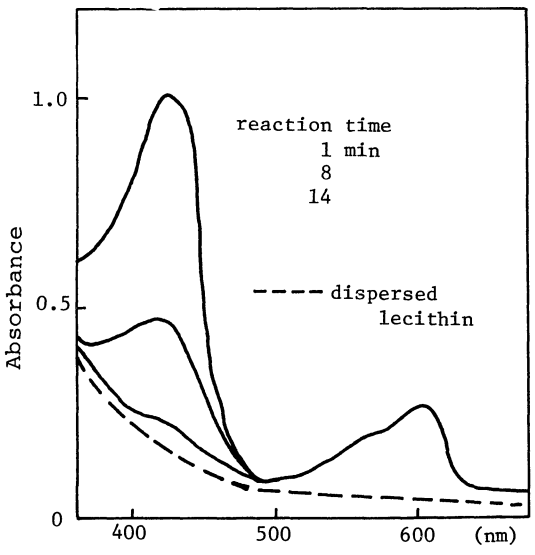


Fig.3 Progressive spectral changes in the presence of thionine
[Th]=0.86 x10^{-5} M, [FCN]=1 mM

started (b -- c). If the solution of FCN was added to this solution after all Th turned to LTh, A600 rapidly increased again and was almost constant until added FCN was reduced completely (c -- d). This continuous reduction of FCN and the change in A600 like a saw tooth continued until all ASNa was oxidised to dehydroascorbate (DAS).

In this reaction, electron was transported from inside (ASNa) to the outside (FCN). This result shows that Th completely recycled in the course of the electron transport reaction in the presence of electron acceptor (FCN). Saw tooth like change in A600 proved that the destruction of the membrane did not occur and electron was transported by Th.

The relative initial reduction rate of FCN are summerized in Tab.1 (System B). The reduction of FCN was observed when Em of dye was higher than that of ASNa (Em=0.06V, pH 7), though it was not observed when Em of dye was lower. In other words, the electron transport occurred only when AsNa could reduced dye.

From these results, it is clear that this reaction completely coupled with the redox cycle of dye even when the solution was not irradiated. The electron might be transported by the diffusion of dyes.

The reaction in the irradiated system showed a little difference from the reaction in the non-irradiated system. Photoreaction was carried out by irradiating the system B with a super-high pressure mercury lamp under nitrogen atmosphere. The light of wavelength shorter than 460nm was cut off by UV-46 filter. Dyes used in the photoreaction was Phenosafranin(PhS) and Neutral Red (NR).

The reduction of FCN was observed both in the presence of PhS and in the presence of NR, though it was not observed in the non-irradiated system. The typical result in the presence of PhS is shown in Fig. 4. The reduction of FCN was not observed in the absence of dyes.

From these results, the following steps are proposed to this electron transport.

Tab.1 The relative reduction rates of FCN in the liposome system (System B)

	Relative Rate	Concentration*	Em_7 (mV)
Neutral Red	0.01	1	-325
Pheno Safranine	0.01	1	-252
	0.01	10	
Indigo-disulfonate	0.01	1	-125
Thionine	1.0	0.8	+ 64
	2.0	1.6	
Toluylene Blue	2.9	1	+115
none	0.01	——	——

*) x 10^{-5} M

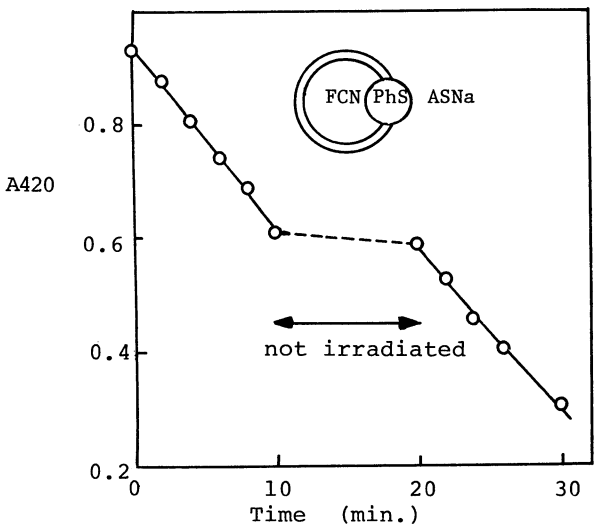


Fig. 4 Change in the absorption at 420nm in the presence of PhS during irradiation. [PhS]=0.054 mM , [ASNa]=0.01 M

(1) $Do + ASNao \longrightarrow DHo + DASo$ (2) $DASo + DHo \longrightarrow Do + ASNao$

(3) $Do + h\nu \longrightarrow D^*o$ (4) $D^*o + ASNao \longrightarrow DHo + DASo$

(5) $DHo \longrightarrow DHi$ (6) $Di \longrightarrow Do$

(7) $DHi + 2Fe(CN)_6^{3-}i \longrightarrow Di + 2Fe(CN)_6^{4-}\bar{i} + 2H^+$

where D=dye in the oxidised form, DH=dye in the reduced form, D*=dye in the photo-excited state, *o*=located in the outside, and =located in the inside

When the reaction in the non-irradiated system is concerned, Step(3) and Step (4) do not take part in the reaction. For the reaction to occur in the non-irradiated system, dye must satisfy following properties. Firstly, Em of dye is higher than that of ASNa and lower than that of FCN. Secondly, dye can move in the lipid phase and penetrate the phospholipid wall easily. Most dyes, except its surfactant-like derivatives, can satisfy the latter conditions because most of them are both water soluble and lipophillic. Diffusion of D*i* and DH*o* (Step(5) and Step(6)) occurs in order to balance the amount of D*o* and DH*i* with amount of D*i* and DH*o* . From the reason described above, this electron transport in the non-irradiated system was strongly controlled by the redox property of dye.

When the reaction in the irradiated system is concerned, Step(3) and Step(4) are more important than Step(1) and Step(2). It is well-known that Em of D* is generaly higher than that of D. Actually, in the case of PhS and NR, Step(4) can occur in the irradiated system, though it cannot occur in the non-irradiated system. This is the reason why the reduction of FCN, the trans membrane electron transport, was observed during irradiation in the presence of PhS and NR.

Following conclusion can be reached on the basis of these results. The electron transport of this system, ASNa-dye-FCN in the liposome, occurred through the step completly coupled with dye redox cycle both in the irradiated and non-irradiated system. The electron transport is cotrolled by Em of dye and (probably) the mobility of dye. Photoirradiation may only play the part in the trigger of this reaction.

The energy storage type reaction utilizing this system is now undertaken.

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