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A CONCERTED TWO-STEP ACTIVATION OF PHOTOINDUCED ELECTRON-TRANSPORT ACROSS LIPID MEMBRANE[1)]

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Photosensitized reduction of disodium 9,10-anthraquinonedisulfonate was carried out by the use of vesicles incorporating an amphipathic zinc porphinato complex ($ZnC_{12}TPyP$) and electron mediators. An extremely rapid electron-transport across the vesicle wall was observed with 1,3-dibutylalloxazine as the electron mediator. Two-step activations of the sensitizer ($ZnC_{12}TPyP$) at the inner- and outer surfaces of vesicles were suggested to account for the electron-transport.

Photoinduced electron-transports across phospholipid vesicle walls have been studied by several groups in order to construct a photosynthetic model system for solar energy conversion.[2,3)] The reported rates of electron-transport across the interfaces of vesicles, however, are not so rapid as to be used for practical purposes. The present authors have found that an extremely fast electron-transport becomes possible in the presence of appropriate electron mediator incorporated into the vesicle wall as described here. In addition, two photoredox reactions at the inner- and outer interfaces of vesicles could be coupled by the mediators so that two-step photoactivation of electron-transport proceeded in a concerted manner.

The rates of electron-transport in the presence of mediators, such as alloxazines and naphthoquinones, were compared with that in the absence of mediator, where an electron-exchange across the interfaces is expected to take place as pointed out by Calvin and his associates.[2c)] The photoelectron was delivered to mediators by the use of an amphipathic derivative of zinc porphinato complex, as the sensitizer, or via photoreduction of 3,6-diamino-10-methylacridinium chloride (acriflavin, AF) as in the system reported by Lehn.[4)] In either case, the final source of the electron was EDTA, which was dissolved in the inner aqueous phase of vesicles.

The vesicles were prepared by sonicating (Bransonic 12, 50 W) dipalmitoyl-D,L-α-phosphatidylcholine (DPPC) dispersion in buffered aqueous solutions (1 M CH_3COONH_4, 0.1 M NaCl, and 0.1 M KCl) of EDTA (0.4 M) at 50-60 °C until the solution became almost clear (*ca.* 40 min). The vesicle solution was purified by gel-filtration (Sephadex G50). The following materials were used as the electron mediator: vitamin K_1 (VK_1), 1,3-dibutylalloxazine (DBA), and 1,3-didodecylalloxazine (DDA). DBA and DDA were prepared by alkylation of alloxazine, while VK_1 was a commercial reagent of a

guaranteed grade. Ligands for the amphipathic zinc porphinato complex was obtained by treating meso-tetra(4-pyridyl)porphine with dodecyl iodide. The monododecyl-substituted pyridinium salt was easily converted into zinc complex (abbreviated to $ZnC_{12}TPyP$) and smoothly incorporated into vesicle wall.[5] Disodium 9,10-anthraquinone-2,6-disulfonate (AQDS) was added into the outer aqueous phase of the vesicle and the reduction was followed at the characteristic absorption (383 nm) of the corresponding hydroquinone ($AQDSH_2$) under irradiation (above 500 nm). Inside of the vesicle was filled with EDTA solution (local conc., 0.4 M; pH 7). Zinc acetate was added to the bulk water in order to deactivate the remaining EDTA at the outside of the vesicle.[2b] The deaerated sample solution in an optical cell (1 cm pathlength) was irradiated in argon atmosphere at room temperature, with a collimated light beam from a 500 W high-pressure mercury lamp passing through an appropriate cut off filter so that the sensitizer (AF or $ZnC_{12}TPyP$) alone was photoexcited.

At first, the rates of photoelectron transfer to the mediators in the vesicle wall were investigated (Type I exp.).[6] In the case of DBA (5×10^{-5} M), for example, smooth reduction to the corresponding dihydro derivative ($DBAH_2$) was detected by the difference absorption on the photoirradiation of either AF (5×10^{-3} M) or $ZnC_{12}TPyP$ (2.5×10^{-5} M). Under these conditions, the rate of photoreduction of DBA in ZnC_{12}-TPyP-sensitized reaction (1.5×10^{-6} M/min) was almost as fast as that in AF-system (0.5×10^{-6} M/min) at the initial stage of the photoirradiation.

As the next step, AQDS was added to the outer aqueous phase of DPPC vesicle incorporating $ZnC_{12}TPyP$, and the electron-transport from the inner aqueous phase to the bulk water was followed by monitoring the increase of the absorption due to $AQDSH_2$ at 383 nm (Type II exp.). The rate of formation of $AQDSH_2$ at the initial stage of the reaction was determined with various concentrations of DBA. The rate of reaction linearly increases with the concentration of DBA at first, and decreases after reaching a maximum around 10^{-5} M of DBA. The sensitized reduction of AQDS was almost completely suppressed, when the vesicles were raptured by sonicating the solution in the presence of Triton X-100, a nonionic surfactant, above the cmc. In other words, EDTA is effectively deactivated by Zn^{2+} ions, as expected, if they are in the same phase. Then, it is clear that the sensitized reduction of AQDS, proceeds via electron-transport from EDTA across the vesicle wall by the aid of electron mediators.

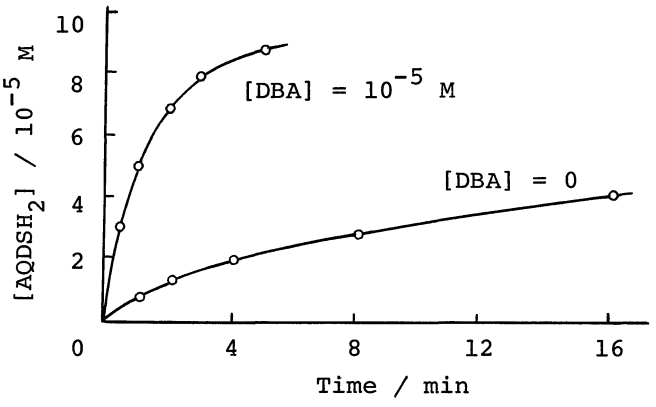

Fig. 1 Photosensitized reduction of AQDS (1×10^{-4} M) in the outer aqueous phase of vesicle carrying $ZnC_{12}TPyP$ (3.2×10^{-5} M) and electron mediators (1×10^{-5} M). Reducing agent (EDTA, 0.4 M in local conc.) is enclosed inside of the vesicle and Zn^{2+} (5×10^{-3} M) is added to the outer aqueous phase to deactivate EDTA at the outside of the vesicle. [DPPC] = 2×10^{-3} M.

The effect of various electron mediators on the photoreduction rate of AQDS was examined with the mediator concentration (10^{-5} M), which was the most appropriate for DBA. The results are shown in Fig. 1. Remarkable

enhancement effects were observed with both DBA and DDA as it is shown in the rate of formation of $AQDSH_2$ at the initial stage of the photoirradiation: DBA system, 5.20×10^{-5}; DDA system, 2.65×10^{-5}; VK_1, 0.38×10^{-5}; and without mediator, 0.81×10^{-5} M/min. It is also interesting that rather small enhancement effect was observed with VK_1, which has been frequently used as the electron mediator in various membrane systems.[2,4] At the higher concentration of VK_1 (5×10^{-5} M), the reaction was even considerably retarded. The rate enhancement of the electron-transport, as observed in Fig. 1, appears to be strongly related to the redox potential (vs. NHE) of the mediator which increases in the following order:

$$\text{DBA } (-0.55\text{ V}) < \text{DDA } (-0.49\text{ V}) < VK_1\ (-0.39\text{ V})$$

Of course, the mobility of the mediator in the lipid bilayer should also be taken into consideration in order to account for the relative rate of electron-transport across the vesicle wall. The difference between DBA and DDA as observed above however, is rather small in comparison with the large difference in the reported mobility of these mediators.[7] Thus, the redox potential of the mediator is considered to be the dominant factor to determine the rate of electron-transport.

Mechanistic details of the electron-transport were examined as the final step. To start with, AQDS was added to the bulk water phase of the AF/EDTA/DBA-system as used in Type I experiment and the rate of formation of $AQDSH_2$ was measured. The obtained value (2.5×10^{-7} M/min) was less than one percent of that (5.2×10^{-5} M/min) in $ZnC_{12}TPyP$/EDTA/DBA-system in Fig. 1. Thus, it is clear that the direct electron transfer from the photoreduced DBA in the vesicle to AQDS is not good enough to account for the increased rate of formation of $AQDSH_2$ in Type II experiment. The electron-transport in Type II experiment must be enhanced by some new factors which may be ascribed to the presence of AQDS in the bulk water. It has been well established that AQDS oxidizes photoexcited zinc tetraphenylporphyrin (ZnTPP) incorporated to micelles.[8] In the case of $ZnC_{12}TPyP$, formation of the cation radical and AQDS anion was also verified by the flash photolysis of the Triton X-100 micellar solution. Then the relevant processes to be taken into considerations are as the following:

$$ZnC_{12}TPyP_{in/out} \xrightarrow{h\nu} {}^*ZnC_{12}TPyP_{in/out} \quad (1)$$

$${}^*ZnC_{12}TPyP_{in} + DBA \longrightarrow ZnC_{12}TPyP^+_{in} + DBA^- \quad (2)$$

$$ZnC_{12}TPyP^+_{in} + DBA^- \longrightarrow ZnC_{12}TPyP_{in} + DBA \quad (3)$$

$$ZnC_{12}TPyP^+_{in} + EDTA \longrightarrow ZnC_{12}TPyP_{in} + EDTA_{oxidized} \quad (4)$$

$$DBA^- + H^+ \longrightarrow DBAH \quad (5)$$

$$ZnC_{12}TPyP^+_{in} + DBAH \longrightarrow ZnC_{12}TPyP_{in} + DBA + H^+ \quad (6)$$

$$2\ DBAH \longrightarrow DBAH_2 + DBA \quad (7)$$

$$DBA^- + AQDS \longrightarrow AQDS^- + DBA \quad (8)$$

$$DBAH + AQDS \longrightarrow AQDSH + DBA \quad (9)$$

$$DBAH_2 + AQDS \longrightarrow AQDSH + DBAH \quad (10)$$

$${}^*ZnC_{12}TPyP_{out} + AQDS \longrightarrow ZnC_{12}TPyP^+_{out} + AQDS^- \quad (11)$$

$$ZnC_{12}TPyP^+_{out} + AQDS^- \longrightarrow ZnC_{12}TPyP_{out} + AQDS \quad (12)$$

$$ZnC_{12}TPyP^+_{out} + DBA^- \longrightarrow ZnC_{12}TPyP_{out} + DBA \quad (13)$$

$$ZnC_{12}TPyP^+_{out} + DBAH \longrightarrow ZnC_{12}TPyP_{out} + DBA + H^+ \quad (14)$$

$$ZnC_{12}TPyP^+_{out} + DBAH_2 \longrightarrow ZnC_{12}TPyP_{out} + DBAH + H^+ \quad (15)$$

(The subscripts, in and out, stand for the inner- and outer surfaces of a

vesicle, respectively.)

The sensitized reduction of AQDS at the outer surface (Eq. 11) is suggested to be responsible to the enhancement of the electron transport in comparison with the AF/EDTA/DBA-system, where the electron must be directly transferred across the interface from either one of the three species (DBA^-, DBAH and $DBAH_2$) as shown by Eqs. 8-10. In the case of $ZnC_{12}TPyP$/EDTA/DBA-system, on the other hand, the oxidized sensitizer at the outer surface will be quickly reduced back to the original form by the above three reduced species of DBA. In other words, the electron-transfer across the interface is mediated by the photoexcited $ZnC_{12}TPyP$. In a summary, the electron transport in Type II experiment is concluded to proceed via two step activation of $ZnC_{12}TPyP$, one at the inner- and the other at the outer surface of vesicles, as given by Eqs. 2 and 11 in cooperation with processes 13 and 14 (Fig. 2).

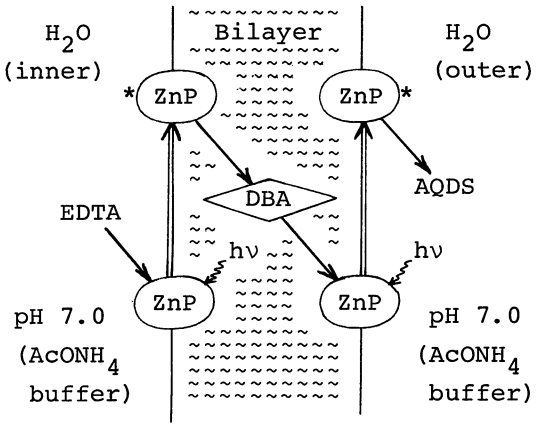

Fig. 2 Schematic diagram for two-step activation of photoinduced electron-transport across lipid membrane. ZnP stands for $ZnC_{12}TPyP$.

NOTES AND REFERENCES

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3. Y. Sudo and F. Toda, Nature, 279, 807 (1979).
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5. The substance to be incorporated into the vesicle was added to DPPC, at the beginning of the sample preparation, as the methanol solution. The solution was evaporated, dispersed in water, and then sonicated to get clear solutions.
6. Prior to sonication, AF was added to the buffered aqueous solution to be enclosed in the vesicle. The excess AF in the bulk phase was removed by gel-filtration. The mediators were incorporated into the vesicle wall by the above-described procedure.
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