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Melvin Calvin

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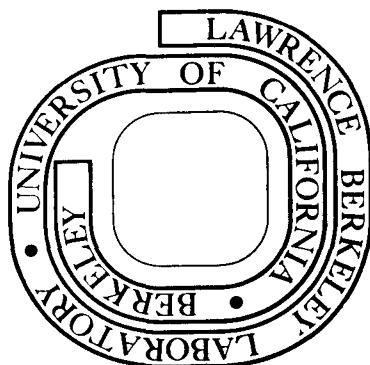

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Photo-sensitized Electron Transport Across Phospholipid Vesicle Walls

As a means of solar energy conversion we are interested in the photosensitized decomposition of water with pigmented lipid bilayer membranes that have O_2 and H_2 evolving catalysts at opposing membrane surfaces. Our present work toward this goal involves the investigation of light-induced electron transfer reactions between aqueous solutions of electron donors and acceptors that are on opposite sides of single-lamella liposomes (vesicles) with incorporated surfactant photosensitizers. Related experiments with planar bilayer membranes[1] or liposomes[2-4] using chlorophyll as the photosensitizer have been reported, but it is not clear that energy storage has been achieved in these systems. A possible exception is the photooxidation of water with vesicles that contain chlorophyll and trapped aqueous ferricyanide.[4] We report here that we have observed the photosensitized reduction of methylviologen (1,1'-dimethyl-4,4'-bipyridinium^{2+}, abbreviated to MV^{2+}) dissolved in the exterior aqueous phase of a phospholipid vesicle dispersion containing a surfactant analogue of tris(2,2'-bipyridine)ruthenium^{2+} when ethylenediamine-N,N,N',N'-tetraacetate (EDTA) is dissolved in the enclosed aqueous phase of the vesicles. The net reaction, shown in Figure 1, involves the irreversible oxidation[5] of EDTA; the reaction is endoergic by approximately 20 kcal per mole of EDTA consumed. This reaction is sensitized in aqueous solutions by the photoexcited triplet state of the tris(2,2-bipyridine)-ruthenium^{2+} ion.[6] Representative experiments are illustrated in Figure 2.

The vesicles described here contained phylloquinone (vitamin K_1, a hydrogen carrier[7]), hexadecylviologen (1,1'-dihexadecyl-4,4'-bipyridinium^{2+}), and decachloro-m-carborane (a proton carrier[8]) in addition to egg yolk phosphatidylcholine and (N,N'-di(1-hexadecyl)-2,2'-bipyridine-4,4'-dicarboxamide)-bis(2,2'-bipyridine)ruthenium^{2+} (abbreviated to Ru). The vesicle dispersions were prepared (see Figure 3) by rapidly injecting[9] (via syringe) 0.3 ml of an ethanol-dimethylformamide solution of the vesicle components into 6.0 ml of a vigorously stirred aqueous solution composed of buffered 0.5 M EDTA (as its ammonium salt). The buffer was 1 M ammonium acetate, 0.1 M NaCl, and 0.1 M KCl, pH 7. The composition of the organic solution of the vesicle components is listed in the legend of Figure 3. Untrapped EDTA was removed by gel filtration of 2 ml of the vesicle dispersion over a column (11 x 185 mm) of Sephadex G-25 that had been equilibrated with buffer. 2.5 ml of the eluted vesicle dispersion was transferred to a rubber serum stoppered glass tube that contained a teflon-coated magnet, 12.5 μl of 0.20 M aqueous methylviologen dichloride, and 25 μl of 1.0 M zinc acetate. Air in the magnetically stirred sample was replaced with argon by four cycles of evacuation followed by bubbling with argon. The vesicle dispersion was transferred (via syringe) to a glass curette with 1 cm pathlength and bubbled briefly with argon before the stopcock at the top of the cuvette was closed. The cuvette was immersed in a glass container with 10 ml of water and irradiated with a collimated beam from a 900 W xenon arc lamp. Light with wavelengths longer than 600 nm and shorter than 420 nm was removed by the use of aqueous cupric sulfate and Corning #3-73 filters. Absorption spectra of the sample were recorded before illumination and after intervals of steady illumination. The optical density of the vesicle dispersion at the visible absorption maximum

of Ru (477 nm) was 0.8. The concentration of viologen radical produced was estimated by using the value of 12,400 M^{-1} cm^{-1} for its extinction coefficient at 603 nm[10].

Approximately 10^{-4} M of viologen radical was present in the vesicle dispersion after 80 min of illumination (Figure 2, bottom left). Since the concentration of the hexadecylviologen was ca. 10^{-5}M, it is clear that MV^{2+}, added after vesicle formation, was being reduced. The concentration of Ru (ca. 8 x 10^{-5} M) changed by less than 5%, so the complex was acting as a photo-catalytic agent. No reduced viologen was observed after 60 min of illumination in the control experiment without EDTA.

In order to insure that EDTA trapped inside the vesicles was the source of electrons for the MV^{2+} reduction, 10 mM of zinc ion had been added to the gel-filtered vesicles. The inhibitory effect of zinc ion on the photosensitized reaction in aqueous solution was demonstrated using tris(2,2'-bipyridine)-ruthenium^{2+} as the photosensitizer (Figure 2, top). The rate of $MV^{\dot{+}}$ formation in the presence of 1 mM EDTA and 2 mM Zn^{2+} was no more than 1% of the rate with 1 mM EDTA alone. The total concentration of EDTA, trapped as its 0.5 M aqueous solution in phosphatidylcholine vesicles that had been gel filtered and dissolved with detergent was estimated to be 1.5 $\pm$ 0.3 mM, as determined spectrofluorometrically using the cupric ion complex of calcein blue as indicator. Therefore the amount of zinc ion in the vesicle dispersion was well in excess over the amount of EDTA outside the vesicle walls that was not removed by gel filtration or that may have escaped from the vesicle interiors. Finally, the impermeability of phospholipid vesicles to MV^{2+} was demonstrated by comparing the concentration of MV^{2+} (determined spectrophotometrically) in vesicle dispersions with trapped 0.20 M MV^{2+}

that had been gel filtered once, and then a second time 5 minutes or 2 hours after the first gel filtration.

Thus, we have performed a photosensitized oxidation-reduction reaction, up a free energy gradient, with the reactants contained in separate compartments joined by a vesicle wall through which a net transfer of electrons and protons has taken place.

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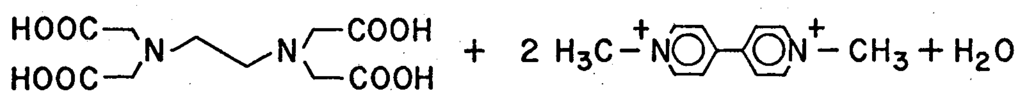
FIGURE LEGENDS

Figure 1. Net reaction for the photo-sensitized reduction of methylviologen and oxidation of EDTA.

Figure 2. The change in methylviologen radical cation ($MV^{\dot{+}}$) concentration with time of exposure to high intensity visible light in homogeneous (top) and micellar (bottom) aqueous solutions flushed with argon. The concentration of $MV^{\dot{+}}$ was calculated from the increase in absorbance at 603 nm (ε = 12,400 M^{-1} cm^{-1}). Top left: The buffered (pH 7) aqueous solution contained 0.05 mM tris(2,2'-bipyridine)ruthenium^{2+}, 0.5 mM methyl viologen (MV^{2+}), and 1mM EDTA. Top right: The solution had the same composition as in the top left plot except that 2 mM Zn^{2+} was also present. No $MV^{\dot{+}}$ was detected after 15 min of illumination. Bottom left: 0.5 M aqueous EDTA was trapped in phospholipid vesicles that contained a surfactant analogue of tris(2,2'-bipyridine)ruthenium^{2+}. 1 mM MV^{2+} and 10 mM Zn^{2+} were added to the vesicles after the removal of untrapped EDTA by gel filtration. See the text for details. Bottom right: Vesicles with trapped 0.5 M EDTA were prepared as for the bottom left plot, but in this case 18 mM 3-(dimethylhexadecylammonio)propane-1-sulfonate was added with 1mM MV^{2+} and 10 mM Zn^{2+} in order to solubilize the vesicles and release the trapped EDTA.

Figure 3. Schematic diagram of the method used for preparing the vesicle dispersions. The composition of the injected organic solution was as follows: phosphatidylcholine, 42 mM; (N,N'-di(1-hexadecyl)-2,2'-bipyridine-4,4'-dicarboxamide)bis(2,2'-bipyridine)ruthenium^{2+}, 2.0 mM; phylloquinone, 2.0 mM; hexadecylviologen, 0.20 mM; decachloro-m-carborane, 0.002 mM; 80% ethanol, 20% dimethylformamide (V/V). The volume of the injected solution was 5% of the aqueous solution volume. Other details are given in the text.

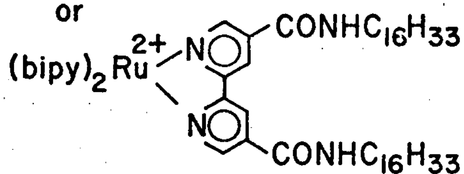


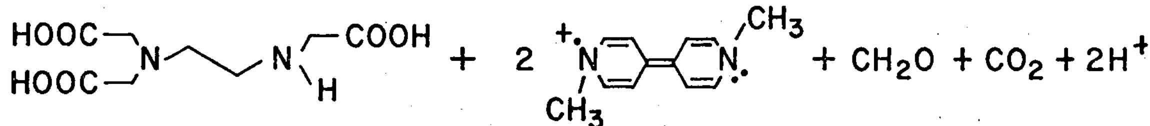
λ 450

or

λ 480

or





$\Delta F° \sim +20$ Kcal per mole of EDTA

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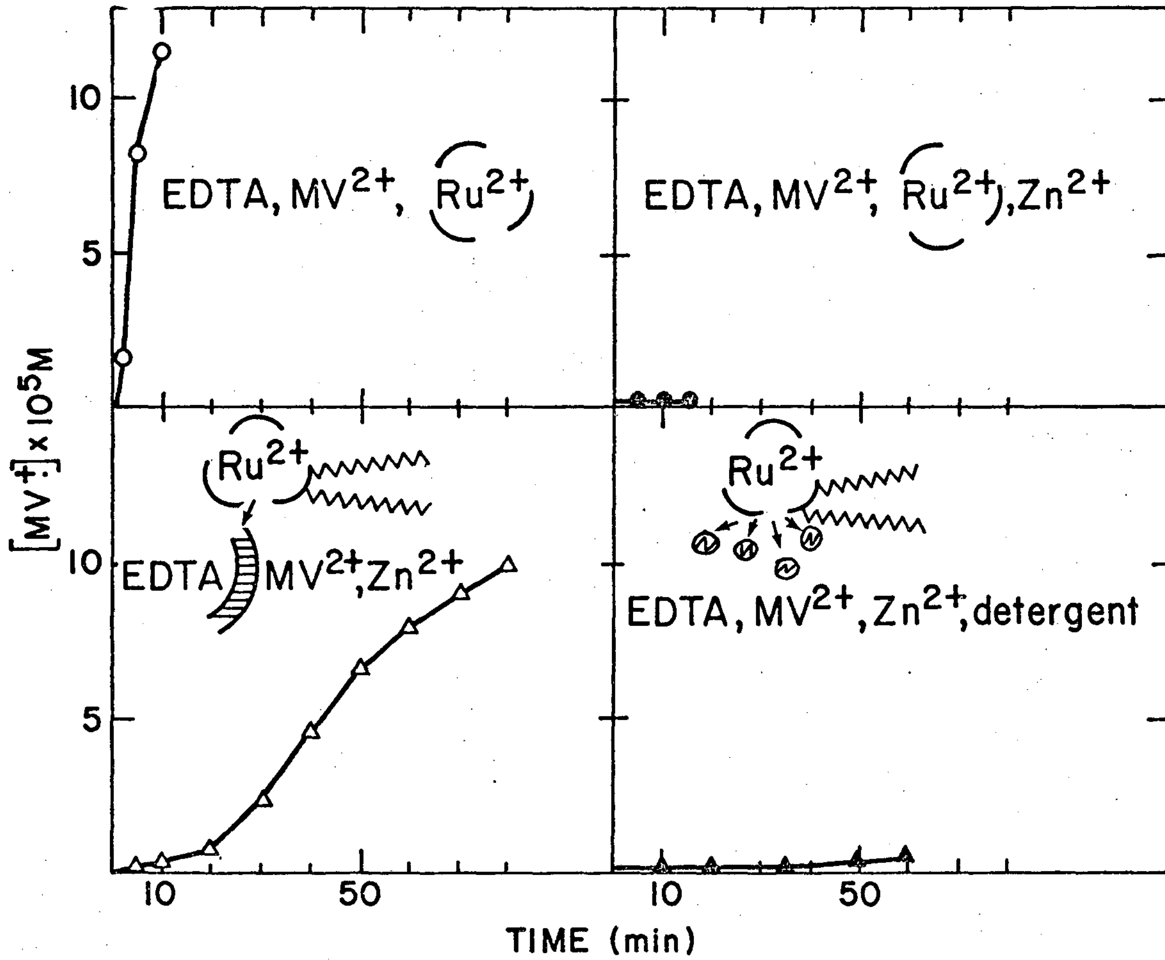

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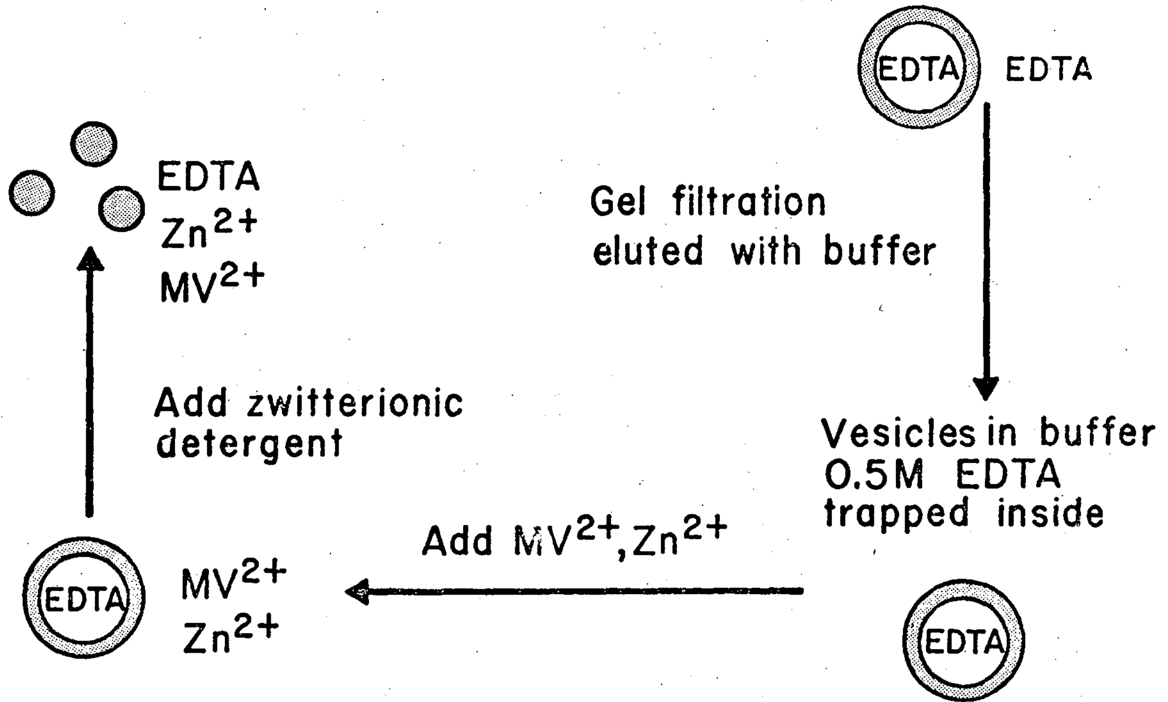

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