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Publication Date

1979-05-01



Lawrence Berkeley Laboratory

UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

Submitted to Proceedings of the National Academy of
Sciences of the USA

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May 1979

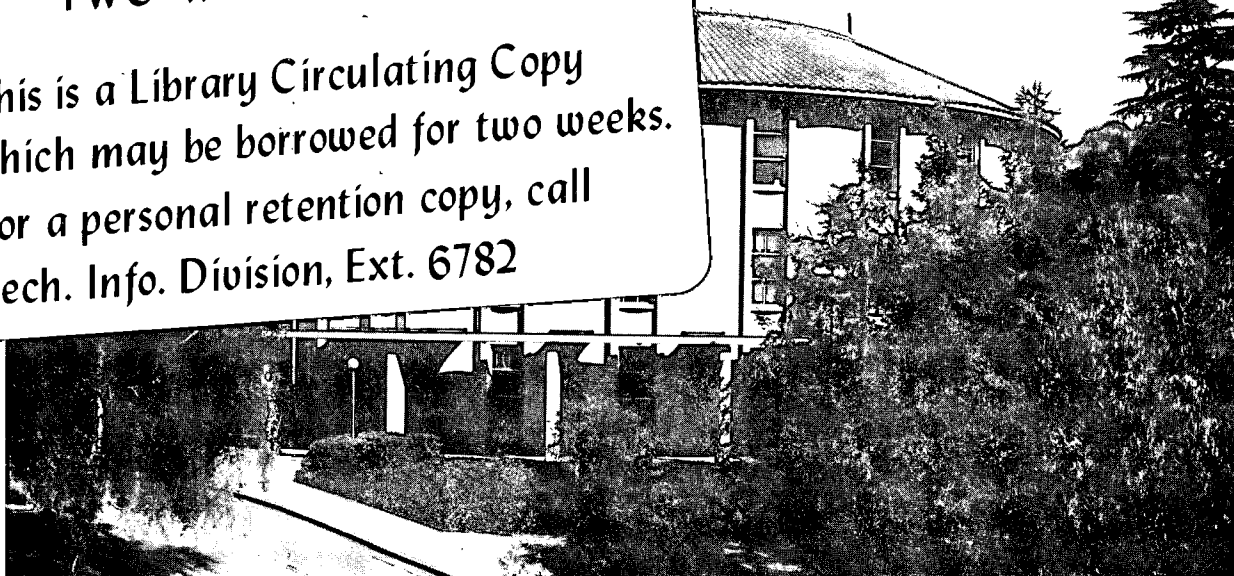
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LBL-9195c.2

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Chemistry

Photosensitized electron transport across lipid vesicle walls: Quantum yield dependence on sensitizer concentration

(solar energy/membrane/ruthenium complex/electron exchange)

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Abbreviations: C_7V^{2+} , 1,1'-diheptyl-4,4'-bipyridinium(2+); EDTA, ethylenediamine-N,N,N',N'-tetraacetate(3-); PC, egg yolk phosphatidylcholine; $RH\cdot$, EDTA with hydrogen atom abstracted; Ru^{2+} , (N,N'-di(1-hexadecyl)-2,2'-bipyridine-4,4'-dicarboxamide)-bis(2,2'-bipyridine)-ruthenium(2+); $*Ru^{2+}$, sensitizing excited state of Ru^{2+} ; $Ru(bipy)_3^{2+}$, tris(2,2'-bipyridine)ruthenium(2+).

ABSTRACT An amphiphilic tris(2,2'-bipyridine)ruthenium(2+) derivative that is incorporated into the walls of phosphatidylcholine vesicles photo-sensitizes the irreversible oxidation of ethylenediaminetetraacetate(3-), dissolved in the inner aqueous compartments of the vesicle suspension, and one-electron reduction of heptylviologen(2+), dissolved in the continuous aqueous phase. The quantum yield of viologen radical production depends on the phospholipid-to-ruthenium complex mole ratio. A kinetic model is used to derive an order-of-magnitude estimate for the rate constant of electron transport across the vesicle walls. The results are inconsistent with a diffusional mechanism for electron transport and are interpreted in terms of electron exchange.

Pigmented membranes that separate aqueous compartments are promising as components in artificial photosynthetic devices (1-3). Solar energy can be captured and stored by photosensitizing complimentary redox reactions at opposing membrane-water interfaces that result in decomposition of water to hydrogen and oxygen. This process requires transport of electrons and protons across the membrane.

Lipid bilayer vesicles (4,5) are attractive as membrane substrates for a number of reasons, including: (i) vesicle compositions can be widely varied simply by dispersing different lipid mixtures, (ii) the lipids are in a liquid crystalline state, which provides organization, and (iii) the membranes are highly dispersed, with specific surface areas of several hundred square meters per gram.

Design of efficient energy-converting devices using vesicles requires knowledge of factors that control rates of charge transport across lipid membranes. The walls of bilayer vesicles are quite thin (typically about 50 Å thick), and several electron transport mechanisms are possible, which have been considered in discussions of electron transport chains of biological systems (6,7). Photosensitized electron transport across lipid bilayer membranes is a relatively new field of research (1-3,8); the ability of lipid layers to transmit electrons is established, although the electron transport mechanism is not always certain.

As a model for studying photosensitized charge transport across vesicle walls, we have used a tris(2,2'-bipyridyl)ruthenium(2+) derivative with two n-hexadecyl substituents (abbreviated to Ru²⁺) to mediate transfer of

electrons from ethylenediamine-N,N,N',N'-tetraacetate (EDTA) dissolved in the encapsulated aqueous compartments of phosphatidylcholine (PC) vesicles, to viologens dissolved in the continuous aqueous phase of the vesicle suspension (9). Initially the vesicles contained hexadecylviologen, vitamin K₁ quinone, and decachlorocarborane to assist charge transport across the membrane-water interface and membrane interior. We have since demonstrated that these additional membrane components are not required: the vesicle walls are composed of PC and Ru²⁺ only. Our preliminary results were interpreted to mean that electron transport through the membrane occurs by electron exchange between Ru²⁺ and Ru³⁺ in opposing lipid monolayers of the vesicles (10). In this paper we show that the quantum yield dependence on PC-to-Ru²⁺ mole ratio is consistent with the proposed electron exchange mechanism, and we estimate the rate constant for transmembrane electron transport, under our conditions, to be of the order of 10⁴ to 10⁵ s⁻¹, which is several orders of magnitude faster than that for transmembrane diffusion of lipids (11).

MATERIALS AND METHODS

Materials.* PC, from hens' egg yolks, is purified by the method of Singleton, et al. (12). 1,1'-Diheptyl-4,4'-bipyridinium dibromide (heptylviologen, C₇V²⁺) is purchased from Aldrich, ethylenediaminetetraacetic acid from Mallinckrodt, and Reinecke salt from Eastman. The Reinecke salt is converted to the potassium form and recrystallized (13).

*The synthesis of Ru²⁺ will be described elsewhere.

Vesicle Preparation. For 200:10 (mole PC:mole Ru^{2+}) vesicles, stock solutions of PC (38 mM) in ethanol, and perchlorate salt of Ru^{2+} (10 mM) in dimethylformamide, are mixed and added via syringe (14, 9) to 3.0 ml of vortex-stirred .30 M $(\text{NH}_4)_3\text{EDTA}$ (pH 8.6 ± 1), giving 2 mM PC and .1 mM Ru^{2+} . The vesicle suspension is stored refrigerated, under N_2 , for about six hours before gel filtration and illumination. For a suspension with the same amount of PC and $2.8 \pm .2$ times as much Ru^{2+} (200:28 mole ratio), a 28 mM stock solution of Ru^{2+} is used.

Gel Filtration and Illumination. Sephadex G-25 column (10 x 175 mm) chromatography is used to replace the EDTA solution outside the vesicles with .95 M ammonium acetate-hydroxide solution containing .018 M zinc acetate (pH $8.5 \pm .1$), with about 10% dilution of the vesicle suspension. A concentrated aqueous solution of C_7V^{2+} is added to give .001 M C_7V^{2+} . The suspension (3.1 ml) is transferred to a 1 x 1 cm glass cuvette and deaerated using either scrubbed N_2 or Ar.

The cuvette is immersed in a water bath ($23 \pm 1^\circ \text{C}$) in a blackened plexiglass box with a 2x1 cm window, and the vesicle suspension is stirred magnetically during illumination. The light source is a 900-W xenon arc lamp whose beam is collimated and filtered through cupric sulfate solution and two glass filters (Corning #3-72 and #5-57) to isolate blue light (60% maximum transmittance at 460 nm). The incident photon flux is $(2.9 \pm .4) \times 10^{-5}$ einstein $\text{min}^{-1}\text{cm}^{-2}$, determined by Reinecke salt actinometry (13). The production of viologen radical (C_7V^+) after intervals of illumination is monitored by its absorbance at 602 nm; the concentration of C_7V^+ is calculated assuming the extinction coefficient of the radical is the same as for methylviologen radical, $12,400 \text{ M}^{-1}\text{cm}^{-1}$ (15).

RESULTS AND DISCUSSION

The composition of the vesicle suspension is visualized in Fig. 1, which illustrates the cross-section of a single vesicle whose wall contains PC and Ru^{2+} in a 200:10 mole ratio.

Illumination of the vesicle suspensions results in the appearance of C_7V^+ after a pronounced induction period (Fig. 2). The quantum yield of radical formation maximizes after about 1% of the viologen is reduced, and falls to about one-fifth the maximum value after about 10% reduction. The maximum quantum yield for C_7V^+ production in the 200:10 (PC: Ru^{2+}) mole ratio sample is $(3.8 \pm .7) \times 10^{-4}$. The maximum yield in the 200:28 mole ratio sample is $2.2 \pm .3$ times that in the 200:10 ratio sample. In the latter case, air is admitted to the cuvette after about thirty minutes of illumination, and the sample visible absorption spectrum is compared to that before illumination; the concentration of Ru^{2+} changes by less than 2%. The final pH is $8.5 \pm .1$.

The origin of the induction period is not known. We have observed no correlation between the length of the induction period and the rate of viologen reduction in these and other experiments with different compositions, which suggests that residual oxygen in the cuvette or oxidizing impurities in the materials used are not the primary cause. Saturation of the reaction is probably due to increased probability for back transfer of electrons from C_7V^+ to Ru^{3+} (see below). Light attenuation by C_7V^+ , and possibly quenching of the excited state of Ru^{2+} by energy transfer to C_7V^+ , also

contribute to saturation. C_7V^+ is only slightly soluble in water (16), so a significant fraction of the radical may be associated with the vesicles, which would cause the observed saturation at relatively low C_7V^+ concentrations.

To assist interpretation of the results, the kinetic scheme of Fig. 3 is helpful. The vertical lines represent the inner (EDTA side) and outer (C_7V^{2+} side) vesicle surfaces. The five states, labeled A to E, can be considered to interconvert with the first-order rate constants k_0 to k_4 . The constancy of the k 's implies that the concentrations of the reaction partners on either side of the vesicle wall do not vary significantly. Values for these rate constants consistent with the conditions of our experiments are included; their derivation is described below. The processes envisaged are as follows: The sensitizing excited state of Ru^{2+} ($*Ru^{2+}$) is assumed to be produced with nearly unity quantum yield (A \rightarrow B), as is the case for tris(2,2'-bipyridine)ruthenium(2+) ($Ru(bipy)_3^{2+}$) (17). $*Ru^{2+}$ decays to the ground state (B \rightarrow A) or is oxidatively quenched by C_7V^{2+} (B \rightarrow C). Back transfer of electrons from C_7V^+ to Ru^{3+} (C \rightarrow A) competes with electron transport across the membrane (C \rightarrow D), which is assumed to be reversible (D \rightarrow C). Oxidation of EDTA by Ru^{3+} (D \rightarrow E) is irreversible because of fragmentation and addition of water, with likely (18) products being ethylenediaminetriacetate, formaldehyde, bicarbonate, and protons. We assume that protons are transported in the same direction as electrons.

With the steady-state approximation that the concentrations of $*Ru^{2+}$ and Ru^{3+} are very small, and the assumption that k_3 equals k_{-3} , the overall quantum yield (sequence A \rightarrow E) is given by:

$$\phi_{AE} = \phi_{AC} \cdot \phi_{CE} = \frac{k_1}{k_0 + k_1} \cdot \frac{k_3 k_4}{k_3 k_2 + k_2 k_4 + k_3 k_4}, \quad [1]$$

where ϕ_{AC} and ϕ_{CE} are the yields for sequences $A \rightarrow C$ and $C \rightarrow E$.

Two mechanisms for electron transport across the vesicle walls ($C \rightarrow D$) will be considered: i) there is net diffusion of Ru^{3+} to the EDTA side of the membrane, and Ru^{2+} to the C_7V^{2+} side, and ii) Ru^{2+} on the EDTA side transfers an electron to Ru^{3+} on the C_7V^{2+} side (electron exchange). Consider the ratio of quantum yields ϕ'_{AE}/ϕ_{AE} for the two experiments with different mole ratios (PC: Ru^{2+}). If electron transport across the membrane is by diffusion of the ruthenium complex, then k_3 is the first order rate constant for diffusion, so the quantum yield is independent of Ru^{2+} concentration, and

$$\frac{\phi'_{AE}}{\phi_{AE}} = 1.$$

If the transport mechanism is electron exchange, then k_3 equals the exchange rate constant times the concentration of Ru^{2+} , and the ratio of quantum yields (R) equals the ratio of Ru^{2+} concentrations (r) times a fraction:

$$R = \frac{\phi'_{AE}}{\phi_{AE}} = r \cdot \frac{1}{1 + (r - 1) \phi_{CE} \left[1 + \frac{k_2}{k_4} \right]}, \quad r = \frac{[Ru^{2+}]'}{[Ru^{2+}]} > 1. \quad [2]$$

Thus the result of Fig. 2 is consistent with Eq. 2 when the fraction is $.79 \pm .16$ (estimated uncertainty).

With the assumption that the mechanism is electron exchange, we obtain from Eqs. 1 and 2 expressions for k_2 and k_3 in terms of the ratios R and r, ϕ_{CE} , and k_4 :

$$\frac{k_2}{k_4} = \frac{r - R}{R \phi_{CE} (r - 1)} - 1 \quad [3]$$

$$\frac{k_3}{k_4} = \frac{r (1 - R \phi_{CE}) + R (\phi_{CE} - 1)}{r (R - 1)} \quad [4]$$

The value of k_4 is estimated using the bimolecular rate constant for reduction of $\text{Ru}(\text{bipy})_3^{3+}$ by EDTA (pH 8.2) (19) in aqueous solution:

$$k_4 \sim (2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}) [\text{EDTA}],$$

where concentration is the local concentration, that is, at the vesicle surface. The local concentration of EDTA is approximately its concentration inside the vesicles, .3 M, so $k_4 \sim .6 \times 10^{-6} \text{ s}^{-1}$.

From our experiments we find $R = 2.2 \pm .3$ and $r = 2.8 \pm .2$. For the 200:10 mole ratio vesicles, the value of ϕ_{CE} is estimated by dividing the maximum overall quantum yield, $\phi_{AE} = (3.8 \pm .7) \times 10^{-4}$, by ϕ_{AC} , whose upper limit is $.09 \pm .02$ as determined by standard luminescence quenching techniques. Only about 25% of quenching events between photoexcited $\text{Ru}(\text{bipy})_3^{2+}$ and methylviologen in aqueous solution result in detectable electron transfer (20), so the extreme values of ϕ_{CE} are $.0046 \pm .0018$ and $.019 \pm .008$. Thus ϕ_{CE} is probably between .02 and .004.

Using these figures and eqs. 3 and 4, we estimate k_2 and k_3 to be $2 \times 10^7 \text{ s}^{-1}$ and $1 \times 10^5 \text{ s}^{-1}$ if all quenching events result in electron transfer. If only one-quarter result in electron transfer, estimates for k_2 and k_3 are $4 \times 10^6 \text{ s}^{-1}$ and $9 \times 10^4 \text{ s}^{-1}$. Within the limits of uncertainty, however, k_2 varies from 1×10^5 to $5 \times 10^7 \text{ s}^{-1}$; k_3 varies from 3×10^3 to $3 \times 10^5 \text{ s}^{-1}$. To complete the list of rates in the kinetic scheme, we

assume $k_0 \sim 2 \times 10^6 \text{ s}^{-1}$, the luminescence decay rate of $\text{Ru}(\text{bipy})_3^{2+}$ in aqueous solution (21), so $k_1 \sim 1.3 \times 10^5 \text{ s}^{-1}$.

Although uncertainties are rather large, the values for k_3 consistent with our data and kinetic model are several orders of magnitude greater than diffusion ("flipping") rate constants for amphiphilic molecules across lipid bilayer membranes: $\sim 10^{-6} \text{ s}^{-1}$ for PC (11), $\sim 10^{-3} \text{ s}^{-1}$ for cholesterol (22), and $\sim 1 \text{ s}^{-1}$ for fatty acids (5). Thus both the rate of membrane charge transport and its dependence on Ru^{2+} are inconsistent with a mechanism that depends on "flipping" of the ruthenium complex, while the dependence of Ru^{2+} concentration is consistent with an electron exchange mechanism.

An estimate for the bimolecular rate constant for electron exchange (k_{exch}) is obtained by dividing k_3 by the local concentration of Ru^{2+} , about .1 M. Therefore k_{exch} is about $1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, with a range of 3×10^4 to $3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$. For comparison, the rate constant for electron exchange between $\text{Ru}(\text{bipy})_3^{2+}$ and $\text{Ru}(\text{bipy})_3^{3+}$ is $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ in acidic aqueous solution (23), and $8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ in acetonitrile solution (24) at 25°C.

CONCLUSION

We conclude that our results add support to evidence (1-3, 8) that pigmented lipid bilayer membranes can transmit electrons. Also, it is apparent that diffusional electron-transporting molecules are not required for photosensitized electron transport across vesicle walls. We have interpreted the results in terms of electron exchange. The facility

with which tris-bipyridyl metal ion complexes undergo electron exchange (19) is important in this model. Since the membrane thickness is about three or four times the diameter of the Ru^{2+} chromophore, the exchanged electrons may have to tunnel (3,6,7,25) through part of the hydrocarbon-like core of the membrane.

Transport of protons (or other cations) must accompany electron transport for charge neutralization. Our interpretation of the quantum yield dependence on Ru^{2+} concentration implies that electron transport is the rate-determining step, since Ru^{2+} is not expected to affect proton transport.

The model presented assumes that the physical properties of the vesicles are not significantly different when PC: Ru^{2+} mole ratios are 200:10 and 200:28. However, two properties that could differ are membrane fluidity and degree of lateral phase separation, both a result of chemical differences between PC and Ru^{2+} . Fluidity could affect the average distance between ruthenium chromophores in opposing monolayers, thereby affecting the probability for electron exchange. Lateral phase separation (5), favored by increasing compositional heterogeneity, could cause the appearance of Ru^{2+} aggregates, which is predicted (26,27) to make electron transport across the membrane more probable. Although we do not expect either effect to play an important role in the present case, further investigation will be required to determine the actual charge transport mechanism, and its dependence on membrane parameters.

This work was supported, in part, by the Basic Energy Sciences Division of the Department of Energy under Contract no. W-7405-ENG-48.

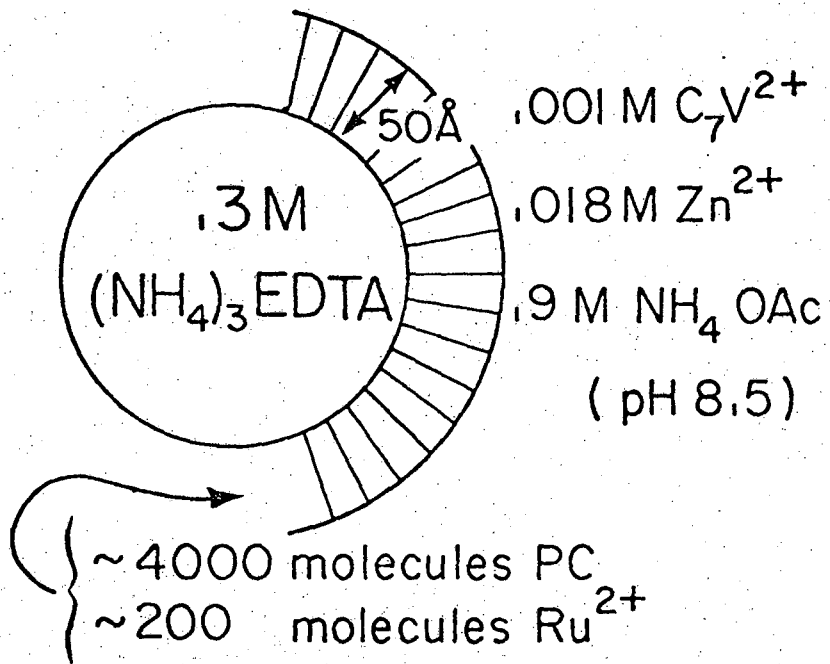
1. Berns, D. S. (1976) Photochem. Photobiol. 24, 117-139.
2. Almgren, M. (1977) in Solar Energy--Photochemical Conversion and Storage, eds. Claesson, S. & Engström, L. (National Swedish Board for Energy Source Development, LiberTryck, Stockholm), NE 1977:6, pp. VII: 1-88.
3. Kuhn, H. (1979) J. Photochem. 10, 111-132.
4. Papahadjopoulos, D. & Kimelberg, H. K. (1974) in Progress in Surface Science, ed. Davison, S. G. (Pergamon Press, Oxford), Vol. 4, pp. 141-232.
5. Sackmann, E. (1978) Ber. Bunsenges. Phys. Chem. 82, 891-909.
6. Van Huevelen, A. (1974) J. Biol. Phys. 1, 215-243.
7. Moore, G. R. & Williams, R. J. P. (1976) Coord. Chem. Rev. 18, 125-197.
8. Tien, H. T. (1979) in Topics in Photosynthesis--Photosynthesis in Relation to Model Systems, ed. Barber, J. (Elsevier/North-Holland Biomedical Press, Amsterdam), Vol. 3, pp. 116-173.
9. Ford, W. E., Otvos, J. W. & Calvin, M. (1978) Nature 274, 507-508.
10. Calvin, M. (1979) Int. J. Energy Res. 3, 73-87.
11. Rothman, J. E. & Lenard, J. (1977) Science 195, 743-753.
12. Singleton, W. S., Gray, M. S., Brown, M. L. & White, J. L. (1965) J. Am. Oil Chem. Soc. 42, 53-56.
13. Wegner, E. E. & Adamson, A. W. (1966) J. Am. Chem. Soc. 88, 394-404.
14. Batzri, S. & Korn, E. D. (1973) Biochim. Biophys. Acta 196, 221-234.
15. Steckhan, E. & Kuwana, T. (1974) Ber. Bunsenges. 78, 253-259.
16. Van Dam, H. T. & Ponjée, J. J. (1974) J. Electrochem. Soc. 121, 1555-1558.
17. Bolletta, F., Maestri, M. & Balzani, V. (1976) J. Phys. Chem. 80, 2499-2503.

18. Massey, V., Stankovich, M. & Hemmerich, P. (1978) Biochem. 17, 1-8.
19. Sutin, N. (1979) J. Photochem. 10, 19-40.
20. Kiwi, J. & Gräetzel, M. (1978) Helv. Chim. Acta 61, 2720-2730.
21. Van Houten, J. & Watts, R. J. (1976) J. Am. Chem. Soc. 98, 4853-4858.
22. Backer, J. M. & Dawidowicz, E. A. (1979) Biochim. Biophys. Acta 551, 260-270.
23. Young, R. C., Keene, F. R. & Meyer, T. J. (1977) J. Am. Chem. Soc. 99, 2468-2473.
24. Chan, M.-S. & Wahl, A. C. (1978) J. Phys. Chem. 82, 2542-2549.
25. Zamaraev, K. I. & Kharirutdinov, R. F. (1974) Chem. Phys. 4, 181-195.
26. Ilani, A. & Berns, D. S. (1973) Biophysik 9, 209-224.
27. Mangel, M. (1976) Biochim. Biophys. Acta 430, 459-466.

FIG. 1. Schematic of bilayer vesicle cross-section illustrating the composition of the aqueous phases and vesicle wall for 200:10 (PC:Ru²⁺) mole ratio vesicles. The composition of the membrane phase is calculated for vesicles with $\sim 250 \text{ \AA}$ outer diameters.

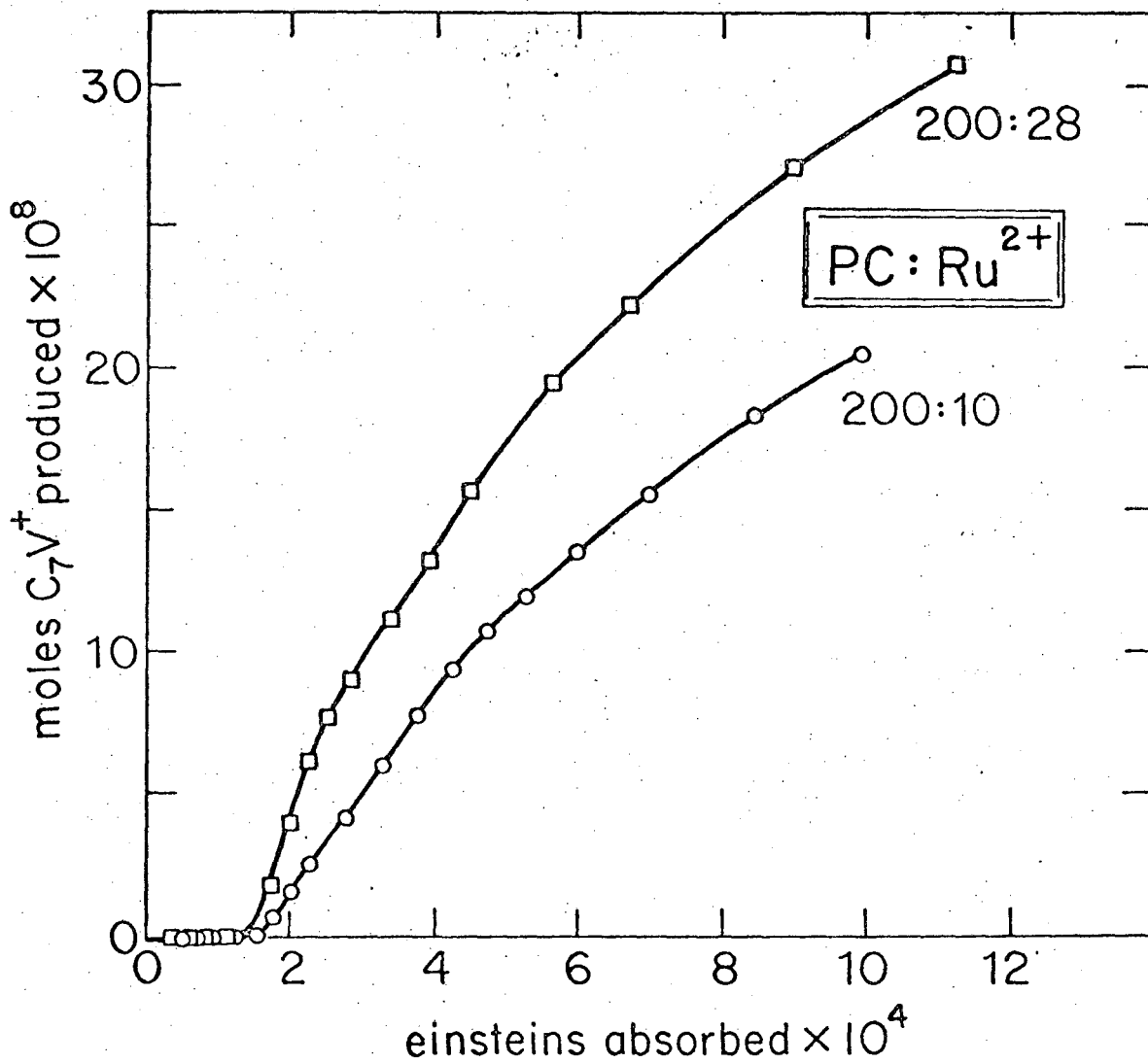
FIG. 2. Production of heptylviologen radical as a function of cumulative number of absorbed photons for two vesicle wall compositions. Vesicles composed of PC and Ru²⁺, with PC:Ru²⁺ mole ratios 200:10 and 200:28, are illuminated with blue light.

FIG. 3. Kinetic model for photosensitized electron transport across vesicle wall, with first-order rate constants. Vertical lines represent membrane-water interfaces. RH* stands for EDTA minus hydrogen atom.



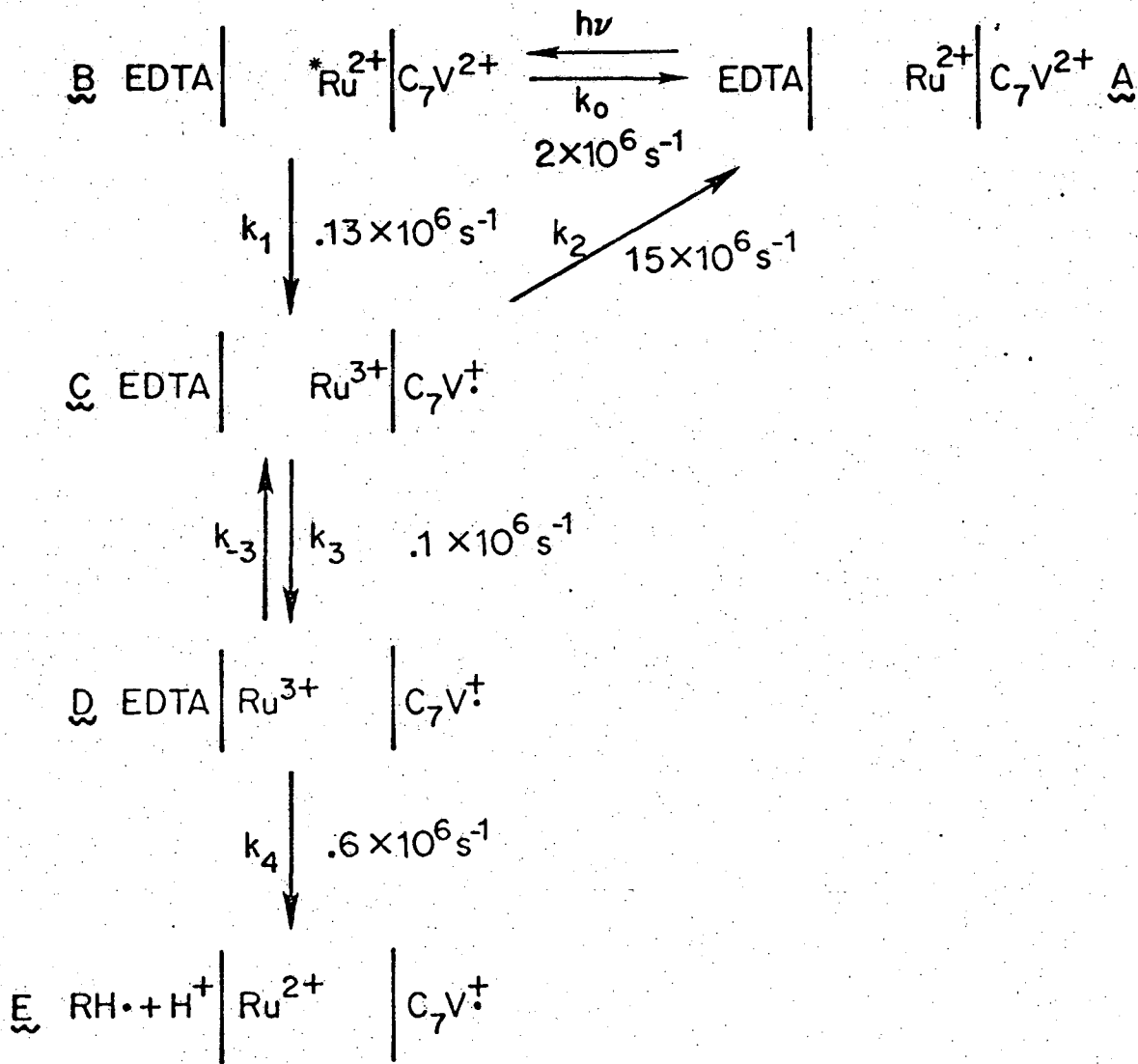
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FIG. 1



XBL794-4767

FIG. 2



XBL794-4751

FIG. 3

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