EFFECT OF THE NATURE OF THE LIPID AND VIOLGEN ON TRANSMEMBRANE ELECTRON TRANSFER IN THE VESICULAR PHOTOSYSTEM: ELECTRON DONOR–Ru(bipy)$^3^+\text{–VIOLGEN–OXIDANT}$

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The rate constant of transmembrane electron transfer $k_{tr}$ and the viologen radical cation are the basic parameters which determine the efficiency of spatial separation of the charges formed in the primary photochemical reaction of an excited molecule of a photosensitizer ($^\ast\text{Ru(bipy)}_3^{2+}$) with an electron acceptor, viologen [1].

The rate of transmembrane electron transfer is apparently more easily susceptible to a deliberate change in comparison to the rate of the recombination process. An attempt was made in the present study to act on the rate of transmembrane electron transfer by altering the structure of the lipid and viologen and the temperature of the solution.

The following phospholipids were used: D,L-dipalmitoyl-$\alpha$-phosphatidylcholine (DPPC); D,L-dimyristoyl-$\alpha$-phosphatidylcholine (DMPC); D,α-dioleoyl-$\alpha$-phosphatidylcholine (DOPC); D,L-dioctadecyl-$\alpha$-phosphatidylcholine (DODPC); egg lecithin and viologens: methylviologen MV$^2+$, propylviologen C$_3$V$^2+$, trimethylammonium propylviologen TMAPV$^4+$ (water-soluble), octadecylviologen C$_{18}$V$^2+$, hexadecylviologen C$_{16}$V$^2+$, cetylviologen C$_{14}$V$^2+$, and dodecylviologen C$_{12}$V$^2+$ (virtually soluble in the lipid bilayer alone).

EXPERIMENTAL

Cp or analytically pure phosphatidylcholines from Fluka: DPPC, DMPC, DSPC, and DOPC, and egg lecithin from the Kharkov Bacterial Preparation Plant were used without additional purification. Ru(bipy)$_3$Cl$_2\cdot$6H$_2$O, Co(NH$_3$)$_5$Cl$\cdot$Cl$_2$, C$_{20}$VCl$_2$, C$_{16}$VCl$_3$, C$_{14}$VCl$_2$, C$_{12}$VCl$_2$, C$_9$VCl$_2$, TMAPVCl$_4$ and DODPC were prepared according to [2-6]. The viologens with a hydrocarbon chain length from C$_{12}$ to C$_{18}$, almost insoluble in water (the solubility at 20°C is 4.3$\times$10$^{-5}$, 1.1$\times$10$^{-5}$, 4.4$\times$10$^{-6}$ and <10$^{-7}$ mole/liter for C$_{12}$V$^2+$, C$_{14}$V$^2+$, C$_{16}$V$^2+$, and C$_{18}$V$^2+$) were incorporated in the wall of the vesicle during ultrasound dispersion of the lipid film. The water-soluble viologens MV$^2+$, C$_9$V$^2+$, and TMAPV$^4+$ were incorporated inside the lipid vesicles in ultrasound dispersion of DPPC in an aqueous solution of the viologen. Vesicles did not form when a solution of heptylviologen was used. Heptylviologen apparently behaves as a detergent and prevents the formation of stable vesicles.

The vesicles were prepared by 30-min ultrasound dispersion of the lipid in 0.04 M acetate buffer solution (pH 5.0) at a temperature 15-20°C above the lipid phase transition temperature.

Fig. 1. Dependence of the transmembrane electron transfer rate constant $k_{tr}$ on the temperature for the phospholipids: 1) DODPC; 2) DSPC; 3) DPPC; 4) DMPC; 5) egg lecithin; 6) DOPC. [Phospholipid] = 5·10^{-3} M; $C_{18}V^{2+}$/phospholipid = 1/50; [Ru(bipy)$_3^{2+}$] = 2·10^{-2} M; [EDTA] = 0.1 M; pH 5.0 [DODPC vesicles begin to decompose at a temperature above the phase transition (58-62°C)].

Fig. 2. Dependence of the transmembrane electron transfer rate constant $k_{tr}$ on the temperature for the viologens: 1) MV$_2^+$; 2) $C_{14}V^{2+}$; 3) $C_{16}V^{2+}$; 4) TMPAV$_4^+$; 5) $C_{12}V^{2+}$; 6) $C_{16}V^{2+}$; 7) $C_3V^{2+}$. [DPPC] = 5·10^{-3} M; $C_{18}V^{2+}$, $C_{16}V^{2+}$, $C_{12}V^{2+}$, or $C_{12}V^{2+}$/DPPC = 1/50; [Ru(bipy)$_3^{2+}$] = 2·10^{-2} M; [EDTA] = 0.1 M; [MV$_2^+$] = $C_3V^{2+}$ = [TMPAV$_4^+$] = 5·10^{-2} M; pH 5.0.

The kinetics of quenching of radicals of the viologens were recorded on a pulsed photolysis setup (150 J pulse energy, ~10 μsec duration) based on the change in the optical density of the solution at $\lambda = 600$ nm ($\varepsilon = 1.2·10^4$ liter/mole·cm). A correction for absorption of Ru(bipy)$_3^{3+}$ at 600 nm ($\varepsilon = 243$ liter/mole·cm), calculated with the concentration of Ru(bipy)$_3^{3+}$ measured with the value of the optical density of the solution at 730 nm ($\varepsilon = 291$), was introduced in processing the kinetic curves, and this permitted controlling the stability of the Ru(bipy)$_3^{3+}$ in the experimental conditions. The purity of the lipids was such that the lifetime of Ru(bipy)$_3^{3+}$ was at least 1.5-2 orders of magnitude greater than the characteristic time of the transmembrane electron transfer reaction. Photoexcitation of the solutions was conducted in the absorption band of Ru(bipy)$_3^{2+}$, and ZhS-11+SS-5 glass light filters were used to separate the 400-500 nm region.

Gel filtration of the vesicles was conducted on Sephadex G-150, and the concentration of the viologen was measured spectrophotometrically ($\lambda = 603$ nm, $\varepsilon = 1.24·10^4$ liter/mole·cm) on a Specord M-40 after reduction with an excess of Na dithionite in borate buffer solution (pH 9.0).

DISCUSSION OF THE RESULTS

On addition of a sufficient amount of oxidant ($A_2$) to vesicles containing Ru(bipy)$_3^{2+}$, EDTA (internal volume), and viologen (membrane), the effective pseudo-first order rate constant of quenching of the viologen radical attains the limit value of $k_{tr}$ which is not dependent on $[A_2]$ and the nature of the oxidant [1]. Oxidation of the viologen radical by the oxidant thus takes place in the nonlimiting stage and $k_{tr}$ can be interpreted as the effective rate constant of transmembrane electron transfer.

It is convenient to use the Ru(bipy)$_3^{3+}$ formed during pulsed irradiation of the solution in the presence of Co(NH$_3$)$_5$Cl$^{2+}$ as the external oxidant

$$^{*}\text{Ru(bipy)}_3^{2+} + \text{Co(NH}_3)_5\text{Cl}^{2+} \xrightarrow{k_{tr}} \text{Ru(bipy)}_3^{3+} + \text{Co}^{2+} + 5\text{NH}_3 + \text{Cl}^-$$

Since the quantum yield of Ru(bipy)$_3^{3+}$ in reaction (1) is relatively high ($k_q = 9.3·10^8$ liter/mole·sec, $\phi_C = 1$ [7]), an ~30-fold excess of oxidant with respect to the radical can be obtained in pulsed irradiation of vesicles in the presence of 2·10^{-3} M Ru(bipy)$_3^{2+}$ and...