Electron transfer through vesicle membranes: Mechanistic ambiguities

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Abstract. We have studied transmembrane electron transfer mediated by an amphiphilic viologen ($C_{16,1}, V^{2+}$) in lecithin vesicles. There is no long-range electron transfer between viologens bound to opposite interfaces of the vesicle membrane, as is often proposed. Instead we proposed a mechanism where the rate-determining step is the disproportionation of two viologen radical cations ($2C_{16,1}, V^+ \rightleftharpoons C_{16,1}, V^{2+} + C_{16,1}, V^0$) forming a doubly reduced, uncharged viologen that transfers electrons by rapid transmembrane diffusion and subsequent reduction of electron acceptors. Studies on other redox mediators provided further information. A brief background to the field is given, including comments on work done by others in relevant systems.

Keywords. Electron transfer; membrane; viologen; disproportionation mechanism.

1. Introduction

The separation of electron transfer products is the primary step in both natural and artificial photosynthesis. There are basically two approaches to separation of the initially produced “electron/hole” -pair: one is by using microheterogeneous media (micelles, vesicles etc.) and the other is by using a sequence of electron transfer steps between species in a relatively ordered array, as for example in covalently linked donor–acceptor systems (CLDA) (Balzani and Scandola 1991). There are only few examples of artificial systems combining both approaches (see e.g. Matsuo 1985, Moore *et al* 1987, Krueger *et al* 1988, Nango *et al* 1988, Momenteau *et al* 1989, Kobuke *et al* 1991, Kugimiya *et al* 1991), as natural photosystems do. Using, for example, a CLDA in a vesicle is an attractive extension of the artificial systems. However, it is necessary to control and understand the simple vesicular systems better before trying to incorporate more complicated molecules. Despite the great number of articles describing transmembrane electron transfer in vesicles (for recent reviews, see Hurst 1990, Robinson and Cole-Hamilton 1991, Lymar *et al* 1991), the general understanding of these systems is still very limited, especially concerning the mechanism of transmembrane electron transfer.

We have studied a system where an amphiphilic viologen ($C_{16,1}, V$) acts as redox mediator in vesicles of egg lecithin. We have found that there is no long-range electron transfer through the vesicle membrane between viologens bound to opposite interfaces,

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as is often proposed. Instead, we propose a mechanism where two singly reduced viologen radical-cations exchange an electron in a disproportionation reaction, (1).

The doubly reduced, uncharged species thus formed diffuses rapidly through the membrane and reduces a secondary acceptor. By comparing with similar redox mediators we have been able to examine detailed reaction steps and draw further conclusions.

This article will summarize our results (Hammarström *et al* 1992, 1993) and comment briefly on the results of others in similar or otherwise relevant systems.

2. Transmembrane electron transfer in vesicular systems

2.1 Vesicle characteristics

A very common type of vesicle is that composed of phosphatidylcholine lipids. Some of its properties are summarized in figure 1. The zwitterionic head-group is rather large and the two acyl chains usually contain 16 or 18 carbons. Natural lecithin is

Figure 1. A schematic cross-section of a small, unilamellar vesicle of lecithin. There are typically ≈ 3000 monomers; the diameter is 200–300 Å and the thickness of the membrane bilayer is ≈ 40 Å. Also shown are the structures of a typical phosphatidylcholine lipid, a monomer of DHP, and of DODAX (see text).

a mixture with many mono-(or poly-) unsaturated chains. Because of the mixture and presence of unsaturated chains the lecithin vesicles are in a liquid state instead of a gel state at room temperature. Lateral diffusion is thus rapid, but exchange of monomers between the different monolayers in the same vesicle or between different vesicles is extremely slow (of the order of one day).

The most important types of synthetic vesicles are composed of dihexadecylphosphate (DHP) or dioctadecyldimethylammoniumhalide (DODAX, figure 1). They are, of course, highly charged and the effective size of the monomer head group is strongly dependent on the counter-ions present. They are therefore more sensitive to perturbations from di- or multivalent ions – and also from complex-forming additives – than lecithin vesicles and are thus known to fuse or to be destroyed upon addition of several species (Carmona-Ribeiro 1992; Hurst *et al* 1983; Robinson *et al* 1990). Vesicles of DHP and DODAX are larger than the small, unilamellar vesicles of egg lecithin, but the thickness of the bilayer is about the same (≈ 40 Å). As for vesicles of non-mixed saturated phosphatidyl choline (e.g. pure DPPC), they are in a gel-state at room temperature.

It is possible to trap water-soluble species in the internal water phase of the vesicles since the membrane allows only very slow diffusion of most ions, and the same species can be removed from the bulk (external) water phase by gel exclusion chromatography resulting in an asymmetric arrangement. It is also possible to dissolve hydrophobic species in the hydrocarbon core of the vesicle and to bind amphiphilic molecules to the interfaces. Thus, vesicular systems allow manifold arrangements of redox-active species.

2.2 Mechanistic principles

There are two types of mechanisms possible for electron transfer through vesicle membranes mediated by water soluble or interfacially bound species (figure 2). One is diffusive, where the redox-mediator is reduced on one side and oxidized on the other, and at least one redox form diffuses through the membrane. More than one electron can be carried by each mediator and protonation/deprotonation can also be involved. The diffusion can be facilitated by a carrier, for example, and the reduction/

Figure 2. Schematic picture of the two types of mechanistic principles for transmembrane electron transfer discussed in the text: diffusional mechanism (top) and self-exchange mechanism (bottom).

oxidation steps of the mediator can be very complicated. The important feature is that the diffusive mechanism does not involve long-range electron transfer but requires transmembrane molecular motion, reversible or not.

The other type of mechanism is self-exchange between a reduced mediator on one side of the vesicle and an oxidized mediator on the other (figure 2). In the self-exchange reaction, the electron is supposed to be transferred via tunnelling, perhaps in several steps if there are mediators distributed at different depths in the membrane. A super-exchange mechanism where the hydrocarbon chains of the vesicle surfactants would facilitate electron transfer has also been suggested. Direct self-exchange between interfacially bound mediators, i.e. over a distance of at least 30 Å, is not possible. Simple calculations on both barrier penetration and super-exchange result in predicted rates that are several orders of magnitude slower than the observed ones. Sometimes it is argued that the actual transfer distance is diminished by simultaneous, momentary and partial penetration of the membrane by two mediators on opposite interfaces prior to their self-exchange reaction, thus depending on a sequence of coincident events. The ideas of self-exchange mechanisms over long distances has of course been inspired by the increasing awareness of the importance of long-range electron transfer in biological systems (Johnson *et al* 1990) and of the successful work on artificial covalently linked donor-acceptor systems (CLDA; Balzani and Scandola 1991). The term "long-range" in those systems usually refers to 15 Å or less, while the thickness of the hydrophobic part of a vesicle membrane is ≥ 30 Å. Furthermore, in the vesicular systems the donors and acceptors are (usually) not linked either to each other or to the surfactants of the vesicle, and the hydrocarbon chains of the latter are flexible and not linked across the bilayer. Thus there are substantial differences between the systems that make long-range electron transfer via tunnelling or super-exchange across the entire membrane of the vesicles much less plausible.

The self-exchange type of mechanism is electrogenic, i.e. if there is no charge compensating ionic flow a transmembrane electric potential will rapidly build up, thus terminating the electron transfer reaction. The diffusive type of mechanism is also electrogenic if the diffusing species is charged. This complicates interpretation further since the charge compensating ions are often not identified. These could be H^+/OH^- , one of the redox-active species themselves or any other ions present. Usually the rates of transmembrane diffusion of those species are not known. Thus it is often possible to explain unexpected results by suggesting that there are different rate-limiting steps under different circumstances. Very rapid (in the order of 1 ms) rates of proton transfer through lecithin vesicle membranes have been reported by several workers (Cevc and Marsh 1987, and references therein). If this is correct, the charge compensation is usually not a problem since the observed rates are much slower in most cases.

2.3 Systems with biological redox mediators

This section contains a few examples of systems relevant to the question of the mechanism of transmembrane electron transfer. For a more complete list the reader is referred to the reviews on this topic (*vide supra*).

Many biological redox mediators are large compared to the membrane thickness, and are also, at least partially, hydrophobic. For example in a system studied by Tabushi and coworkers (Tabushi and Nishiya 1981) cytochrome-*c*3 was bound to both

interfaces of lecithin vesicles. Due to their large size ($\approx 30 \text{ \AA}$) and partial penetration of the membrane by cytochromes on different sides of the membrane, they could form an aggregate acting as an electron channel through the membrane allowing for electron transfer between the cytochromes.

There is evidence (Ford and Tollin 1982) that chlorophyll-*a* (chl-*a*) binds in a heterogeneous manner to lecithin vesicles so that there are chl-*a* bound interfacially as well as in the hydrophobic part of the membrane. The rapid transmembrane electron transfer observed in this system ($k_{\text{obs}} \geq 10^4 \text{ s}^{-1}$) can be explained by a sequence of electron transfer steps between chl-*a* molecules distributed at different depths in the membrane. The distance in each step can then be small or of moderate size (a few Ångströms).

Transmembrane electron transfer between hydrophobic Mg-porphyrins linked by spacers of different lengths to hydrophilic "anchors" on different interfaces of the membrane has been studied (Dannhauser *et al* 1986). No electron transfer was observed until the edge-to-edge distance between the porphyrins was 4 Å or less.

There are several systems with porphyrins that exhibit transmembrane electron transfer. For photoinduced reactions in many systems, it has been observed that the transmembrane electron transfer is a two-photon process. This has sometimes been taken as evidence for a long-range self-exchange reaction between a photo-oxidized porphyrin on one side of the membrane and a photo-excited or photo-reduced porphyrin on the other (Katagi *et al* 1981, 1982; Lyman *et al* 1991). However, in other systems there was a carrier for the electron acting as a mediator via diffusion between the porphyrins (Matsuo *et al* 1980). A two-photon ionization of a porphyrin dimer on one side of the membrane has also been proposed as the initial reaction step, explaining the quadratic dependence on the number of photons (Jusupov *et al* 1985). It seems possible that the relatively hydrophobic porphyrins could be distributed in the membrane in the same way as the chl-*a* (*vide supra*) allowing for short-range, multi-step electron transfer, even if some results indicate that most porphyrins are located closer to the interface of the membrane than to the middle (Lyman *et al* 1991). In the latter case the observed two-photon dependence could be explained by the dimer-process according to Jusupov *et al* (1985).

2.4 Systems with viologens as mediators

Viologens constitute a very important class of redox mediators (Bird and Kuhn 1981). Usually the substituents on the nitrogens of the bipyridine rings are simple alkyl groups (figure 3), but several compounds with, for example, charged substituents have been synthesized (Summers 1991). The alkyl substituted viologens are in the following abbreviated $C_{n,n'}V$ where n and n' are the number of carbons in the substituents. The air-stable form is a dication which can be reduced to the radical cation, which under certain circumstances can be further reduced to the electro-neutral, doubly reduced form. The redox steps are reversible and an equilibrium constant, K_d , for disproportionation, (1), can be defined as below:

$$K_d = ([C_{n,n'}V^{2+}][C_{n,n'}V^0])/[C_{n,n'}V^+]^2. \quad (2)$$

This equilibrium has been studied in several homogenous media (Bird and Kuhn 1981; Mohammad *et al* 1981). Viologens are also known to form dimers; in particular the radical dimer has been studied, but mixed dimers are also known to exist (Bird

Figure 3. The redox mediators used (from top): $C_{16,1}V^{2+}$, $C_{16}B^+$, $C_{16}NA^+$.

Figure 4. The system of Tricot and coworkers. Shown is the configuration where both CdS and MV^{2+} are in the interior of the DHP vesicles. The curved lines represent the interfaces of the membrane where the exterior is to the left and the interior to the right of the lines.

et al 1981). The different redox forms are spectroscopically distinct and can thus be detected by absorption spectroscopy.

Tricot and coworkers (Tricot and Manassen 1988; Tricot *et al* 1991) have used colloidal particles of CdS adsorbed to DHP-vesicles as photoexcited electron donors with water soluble methylviologen (MV^{2+}) as acceptor (figure 4). By combining absorption spectroscopy and electrochemical detection they were able to distinguish between viologen radicals in the internal water phase of the vesicles and those in the bulk phase. They concluded that there was no photostimulated diffusion of oxidized MV^{2+} through the membrane, but upon reduction of internal viologen the radical (MV^+) diffused through the membrane. Since no viologen was initially present outside the vesicles this excludes the possibility of the self-exchange type of mechanism (*vide supra*). Note that it is not possible to conclude from these experiments alone in what form the reduced viologen is diffusing, e.g. if it is MV^+ or MV^0 that is actually transverse the membrane. Electron transfer in this system also occurred between CdS on the outside and MV^{2+} on the inside of the vesicles, but only at high loadings of CdS on the vesicle and at high light-intensities. It might be suspected that under those conditions there is some critical perturbation of the vesicle that induces transmembrane diffusion and/or partial penetration of the membrane by species

otherwise membrane impermeable/impenetrable. Especially in steady-state illuminated systems, photostimulated diffusion has proved to be a problem as pointed out by the authors (Tricot and Manassen 1988). Tricot and coworkers found that the CdS particles penetrate the membrane to some extent and this must perturb the membrane locally due to the large size of the CdS particles.

A diffusional model for transmembrane electron transfer with a conjugation of inward flux of $C_{n,n'}V^+$ and outward flux of $C_{n,n'}V^{2+}$ for alkylviologens in lecithin vesicles has been developed (Tabushi and Kugimiya 1984, 1985). The authors divided the reaction in steps of entrance of viologen radicals into the membrane and exit to the opposite water phase. They claimed that, in stopped-flow experiments, a steady-state was obtained where the viologen fluxes and the rates of reduction by external dithionite and oxidation by an internal electron acceptor were the same. It has been shown that the oxidized $C_{n,n'}V^{2+}$ does not permeate the membrane of a lecithin vesicle (Zamaraev *et al* 1988; Hammarström *et al* 1992). Also, the kinetic trace shown (Tabushi and Kugimiya 1985) for the fastest mediator used, $C_{4,4}V^{2+}$, exhibits almost no change in concentration of viologen radical between 20 and 30 s (taken as evidence for steady-state kinetics) which is not consistent with later results (Zamaraev *et al* 1988) where the observed rate in an identical experiment with $C_{16}V^{2+}$ was of the order of one second. According to the figure caption (Tabushi and Kugimiya 1985), the amount of internal, secondary acceptor was too small to be able to re-oxidize all viologen since two equivalents are needed (*vide infra*), which explains the long-time stability of the viologen radical. Considering the discrepancies with the results of other groups as indicated above, this model of flux conjugation can be rejected.

$Ru(bpy)_3^{2+}$ -sensitized transmembrane electron transfer mediated by alkylviologen in phosphatidylcholine vesicles was studied by flash-photolysis (Khannanov *et al* 1987). The authors found no dependence of the observed rate on the ratio of viologen to lipid, which is an argument against self-exchange mechanisms since they depend on the probability of two viologens diffusing to adjacent positions on opposite sides of the membrane. Note that the increase in viologen/lipid ratio does not necessarily result in more viologen radical produced by each laser flash if there is already an efficient quenching of the excited $Ru(bpy)_3^{2+}$. Thus, it was not shown how the observed rate varies with the surface concentration of viologen radical. The authors suggested that diffusion of viologen radical was responsible for transmembrane electron transfer, and their interpretation of the temperature-dependence supported this suggestion. However, they unexpectedly found that the observed rate was independent of the length of the alkyl chains of the viologens, contrary to what was found in stopped-flow experiments by others (Thompson *et al* 1987; Zamaraev *et al* 1988).

A combination of self-exchange and viologen radical diffusion was proposed by Zamaraev and co-workers in a system where $C_{16,16}V^{2+}$ mediated electron transfer from dithionite (stopped-flow) or excited $Ru(bpy)_3^{2+}$ (flash-photolysis) on one side of lecithin vesicle membranes to $Fe(CN)_6^{3-}$ or $C_{n,n'}V^{2+}$ on the other (figures 5 and 6, Lymar *et al* 1991, and references therein). If, in the stopped-flow experiments, $Fe(CN)_6^{3-}$ was absent the observed rate was proportional to the viologen/lipid ratio and electron tunnelling between viologens on different interfaces of the membrane was proposed to be rate-determining. If $Fe(CN)_6^{3-}$ was present the observed rate was somewhat slower and independent of the viologen/lipid ratio, in which case it was proposed that a charge-compensating transmembrane diffusion of viologen

Figure 5. The system of Zamaraev and coworkers. Shown is the configuration where dithionite is used as reductant (SO_2^- reduced the viologen after the equilibrium $\text{SO}_2\text{O}_4^{2-} \rightleftharpoons 2\text{SO}_2^-$). When excited $\text{Ru}(\text{bpy})_3^{2+}$ is used as a reductant it is dissolved in the vesicle interior and $\text{Fe}(\text{CN})_6^{3-}$ is in the exterior.

Figure 6. The mechanism for viologen-mediated transmembrane electron transfer proposed by Zamaraev and coworkers and by Hurst and coworkers.

radical was rate limiting. The independence of the rate on the lipid/viologen ratio is a surprising observation which is not consistent with the proposed mechanism. Any charge-compensating transmembrane diffusion of ions in the case without $\text{Fe}(\text{CN})_6^{3-}$ would be active in the case with $\text{Fe}(\text{CN})_6^{3-}$ and would give the same observed rate and rate-dependences. A proportional dependence of the observed rate on the viologen/lipid-ratio was later observed in identical experiments with $\text{C}_{16,1}\text{V}^{2+}$ and internal $\text{Fe}(\text{CN})_6^{3-}$ (Hammarström *et al* 1992).

An important finding was that, with an excess of both external (membrane impermeable) dithionite and internal $\text{Fe}(\text{CN})_6^{3-}$, all viologen on the vesicle exterior migrated to the interior and was trapped there as the membrane impermeable dication (Zamaraev *et al* 1988). This was explained by transmembrane diffusion of the viologen radical and its subsequent reoxidation by internal $\text{Fe}(\text{CN})_6^{3-}$. In flash-photolysis, the observed kinetic trace deviated from a single exponential and could be accounted for by a model where the created transmembrane electric potential following tunnelling of one electron slows down the tunnelling of the next (Lymar *et al* 1991). This model assumed that the rate of the charge-compensating ionic flow was slower than the electron tunnelling. The observed rate at large number (≥ 8) of transferred electrons per vesicle agreed with the observed rate in the stopped-flow experiments. In this case the rate of electron tunnelling was supposed to be retarded so that it was equal to the rate of charge-compensating diffusion of ions. However, the observed rate constant in stopped-flow experiments varied between 1 and 6 s^{-1} when the viologen/lipid ratio was varied (Khramov *et al* 1987), while charge-compensating diffusion would not be dependent upon that ratio. Note also that second-order kinetics, as is predicted by our mechanism (*vide infra*), result in qualitatively the same behaviour, i.e. the kinetic trace can be divided in parts, each of which can be fitted to one single exponential with a rate constant that is somewhat different from the others. Unfortunately, no value was given for the observed rate constant when less than eight viologen radicals were produced per vesicle. In case of second-order kinetics the rate would be slower when the surface concentration of viologen radical is lower, as observed

in pulse-radiolysis (*vide infra*), while the model of Zamaraev and co-workers predicts that the rates would be the same so long as an equal number of electrons has been transferred per vesicle. It is very difficult to discriminate between different kinetic models by the shape of the kinetic traces, especially considering the low signal-to-noise ratio usually obtained in flash-photolysis experiments in vesicular systems.

Transmembrane electron transfer mediated by viologens with alkyl chains of different lengths has been extensively studied in DHP vesicles (Hurst 1990 and references therein; Lymar and Hurst 1992). The authors proposed basically the same mechanism as Zamaraev and co-workers (figure 6), based on several experimental indications. The major experimental support comes from the kinetics and stoichiometry of the redistribution of viologen between the outside and the inside of the vesicle upon reduction by external dithionite. Interesting differences in behaviour between $C_{n,1} \cdot V^{2+}$ with $n \geq 12$ and $n \leq 10$ was observed in several types of experiments, indicating that there are at least two binding sites available at the external interface of the vesicle for viologens with $n \leq 12$. For $n \leq 10$, all viologen reduced by dithionite on the outside migrated to the inside of the vesicle if there was an excess of $C_{n,1} \cdot V^{2+}$ acting as electron acceptor in the vesicle interior. When $n \geq 12$ not all external viologen moved to the interior (Hurst 1990; Lymar and Hurst 1992). The authors explained this by proposing that some of the viologens with longer chains were bound to the outer interface of the vesicle in a way that they could not diffuse through the membrane as radical cations ($C_{n,1} \cdot V^+$). The proposed electron tunneling pathway should therefore be blocked due to lack of charge-compensating ion flow. As a consequence, addition of lipophilic cations, allowing for charge compensation, would increase the amount of reduced acceptor (internal $C_{n,1} \cdot V^{2+}$) in the vesicle interior, as was indeed observed. The same effect of lipophilic cations was observed when the external viologen had extra charged groups attached (Patterson and Hurst 1990). However, N-methyl-4,4'-bipyridine (MB^+) was used as lipophilic cation, and the authors have later shown that the oxidized form (MB^+) does not permeate the membrane of vesicles composed of DHP (Lymar and Hurst 1992). Reduced N-cetyl-4,4'-bipyridine ($C_{16}B^0$, figure 3) has been shown to rapidly permeate lecithin vesicles (Hammarström *et al* 1993, *vide infra*). It seems probable that the dithionite in the system of Hurst and coworkers was able to partially reduce MB^+ to MB^0 which rapidly diffused across the membrane and reduced viologen in the vesicle interior, in which case it was not a question of charge-compensation. Note also that the same redistribution stoichiometry is expected from the mechanism of Zamaraev and others and Hurst and others and the disproportionation mechanism proposed by Hammarström and coworkers (*vide infra*).

3. Results and discussion

We have studied transmembrane electron transfer mediated by cetylmethylviologen ($C_{16,1} \cdot V^{2+}$) in lecithin vesicles, mainly by pulse radiolysis and stopped-flow techniques (Hammarström *et al* 1992, 1993). The advantage of these methods is the rapid reduction of viologen to a great extent compared to photoinduced methods. This allowed us to study the kinetic dependence on various parameters. In photoinduced experiments the slow rate and/or small extent of reaction may allow background processes to be significant and photostimulated diffusion might occur.

3.1 Kinetic studies

We have used different arrangements of the system as described in figure 7. In vesicles of type I the amphiphilic viologen was bound to both interfaces of the vesicles, while in type II it was bound only to the outside. In vesicles of type III and IV, $\text{Fe}(\text{CN})_6^{3-}$ was present in the internal water phase as an acceptor. The kinetic traces from dithionite-induced reduction in stopped-flow are shown in figure 8. In all types of vesicles a fast reduction of external viologen by dithionite was observed, which obeyed pseudo-first order kinetics since dithionite was in excess. In vesicles of type II (figure 8b) the absorption signal from the viologen radical was quite stable after that, while in type I (figure 8a) a slower reduction step was observed. In vesicles of types III and IV, identical kinetic traces were observed (figure 8c) where the initial fast reduction was followed by a slower reoxidation of viologen by the internal $\text{Fe}(\text{CN})_6^{3-}$. Figure 8d shows the trace of the absorbance at 420 nm where the extinction coefficients for $\text{C}_{16,1}\text{V}^+$ and $\text{Fe}(\text{CN})_6^{3-}$ are 2600 and 1000 $\text{M}^{-1} \text{cm}^{-1}$, respectively. It can be seen that the initial, rapidly produced signal from the viologen radical decayed simultaneously with bleaching of $\text{Fe}(\text{CN})_6^{3-}$, and from the amplitudes of the phases it can be shown that two $\text{Fe}(\text{CN})_6^{3-}$ were reduced for each viologen initially reduced on the outside of the membrane. Even though dithionite was in excess, all viologen was in oxidized form at the end of the reaction. The stoichiometry and the complete migration of reduced viologen to the vesicle interior was also confirmed by careful measurements in a spectrophotometer, and is consistent with the earlier observations for $\text{C}_{16,16}\text{V}^{2+}$ (Lyman *et al* 1991; *vide supra*). The kinetics and observed rates for

Figure 7. The types of vesicle configurations used by Hammarström and coworkers. The curved lines represent the interfaces of the membrane and the "ball-and-stick"-structures represent the amphiphilic viologen. The figure shows viologen distributed on both interfaces (types I and III) or only on the outer (types II and IV). In types III and IV, $\text{Fe}(\text{CN})_6^{3-}$ is present in the interior. The buffer used was 50 mM phosphate (pH = 8).

Figure 8. (a–c) (Caption on next page.)

Figure 8. Kinetic traces from stopped-flow reduction of vesicles. The absorbance at 602 nm was followed: (a) type I, (b) II, (c) III and IV; (d) the absorbance followed at 420 for the same solution as in 8c. In c and d the residuals from a second-order fit for the part of the curve between the arrows are shown. Note that in 8a–b solutions were less concentrated than in 8c–d, but the viologen/lipid ratio was higher. (Reprinted with permission from Hammarström *et al* 1992, © 1992, *Am. Chem. Soc.*)

the fast reduction step were the same for all types of solutions if the concentration of dithionite was the same. The slower step could in all cases be fitted to a second-order kinetic model, and the rate was the same for types III and IV (figure 8c, decaying signal) and also for type I (figure 8a, growing signal) if the viologen/lipid ratio was the same. This is the transmembrane electron transfer step where external viologen mediates reduction to internal viologen (type I) or $\text{Fe}(\text{CN})_6^{3-}$ (types III and IV). The identical behaviour of vesicles of types III and IV shows that transmembrane electron transfer proceeds in the same way irrespective of the presence or absence of viologen on the internal interface of the membrane, an observation excluding self-exchange types of mechanisms.

For vesicles of type IV the viologen/lipid ratio was varied in stopped-flow experiments and the observed rate was found to be proportional to this ratio (figure 9). This shows that the transmembrane electron transfer is second-order in viologen radical on the outer interface of the vesicle ($[\text{C}_{16,1}, \text{V}^+]_{\text{out}}$). Such a dependence was earlier found in vesicles equivalent to type I, i.e. with $\text{C}_{16,16}, \text{V}^{2+}$ present on both interfaces and no $\text{Fe}(\text{CN})_6^{3-}$, but was interpreted as a first-order dependence on both $[\text{C}_{16,16}, \text{V}^+]_{\text{out}}$ and $[\text{C}_{16,16}, \text{V}^{2+}]_{\text{in}}$ (Khramov *et al* 1987).

We also varied the extent of reduction of viologen on the outer interface of vesicles of type IV by pulse radiolysis (figure 10). The rate was found to be proportional to the surface concentration of viologen radical produced by the radiation pulse. If the line fitted to the data is extrapolated to 100% reduction of viologen ($= 24 \mu\text{M}$) the observed rate is $\approx 5 \text{ s}^{-1}$, which is in excellent agreement with the rate obtained in the stopped-flow experiments (100% reduction by dithionite in excess) for the same viologen/lipid ratio (1:100). Together this shows that the rate of transmembrane electron transfer is second-order in $[\text{C}_{16,1}, \text{V}^+]_{\text{out}}$ and independent of $[\text{C}_{16,1}, \text{V}^{2+}]_{\text{out}}$

Figure 9. The rate (inverse half-life) versus the viologen/lipid ratio for stopped-flow reduction of vesicles of type IV (100% reduction). Each point is an average of 4–8 individual determinations for the same vesicle preparation. The standard deviation (not shown) was less than the size of the points. The line is a least-squares fit to the data. (Reprinted with permission from Hammarström *et al* 1992, © 1992, *Am. Chem. Soc.*)

Figure 10. The rate (inverse half-life) versus the concentration of external viologen radical immediately after the pulse for pulse radiolytic reduction of vesicles of type IV (partial reduction). All data are individual determinations for the same vesicle preparation. The line is a least-squares fit through the origin.

and $[C_{16,1}, V^{2+}]_{in}$. This excludes both self exchange types of mechanisms and simple diffusions of viologen radicals.

3.2 Mechanism

The mechanism we propose for the transmembrane electron transfer mediated by viologen is shown schematically in figure 11. The rate-determining step is the disproportionation, (1), of two viologen radicals forming one oxidized $C_{16,1}, V^{2+}$ and one doubly reduced $C_{16,1}, V^0$. This uncharged viologen diffuses rapidly through the membrane and is re-oxidized by $Fe(CN)_6^{3-}$ in two steps. There is never any detectable

Figure 11. Reduction scheme for the mechanism proposed by Hammarström and coworkers. The scheme depicts the situation in pulse radiolysis where there is no external reductant after the first 100 μ s. The viologen used ($C_{16,1}V^{2+}$) is denoted CMV^{2+} . The individual steps are marked by their rate constants: k_d , k_t , k_i and k_{ii} for the disproportionation, transmembrane diffusion and for the two steps of re-oxidation by $Fe(CN)_6^{3-}$, respectively.

amount of $C_{16,1}V^0$ present. The rate of conproportionation, i.e. the reverse of (1), is slower than the transmembrane diffusion and re-oxidation of $C_{16,1}V^0$. This explains the observed independence on $[C_{16,1}V^{2+}]_{out}$, while second-order dependence on $[C_{16,1}V^+]_{out}$ is explained by the rate-determining bimolecular disproportionation. We have found no other mechanism explaining the same rate-dependencies and stoichiometry. Formation of radical dimers $(C_{16,1}V^+)_2$ is a bimolecular reaction between viologen radicals, but the dimers are larger and more highly charged than the radical monomers, so there is no reason why transmembrane diffusion of the former would be faster than that of the latter. Also, diffusion of radical dimers is not consistent with the stoichiometry in the re-oxidation by $Fe(CN)_6^{3-}$. Note that, since the external reductants produced in pulse radiolysis (alcohol radicals) recombine within 100 μ s, disproportionation is the only possibility of forming significant amounts of doubly reduced $C_{16,1}V^0$.

Under the experimental conditions, it was not possible to directly produce measurable amounts of $C_{16,1}V^0$, hence N-cetyl-4,4'-bipyridine ($C_{16}B^+$, figure 3) was used as a model compound for the viologen. The singly reduced form ($C_{16}B^0$) is structurally very similar to the doubly reduced $C_{16,1}V^0$ and both species are uncharged. Thus, the rates of transmembrane diffusion for the two species is expected to be very similar. By pulse radiolysis we were able to study transmembrane electron transfer mediated by $C_{16}B^+$ in vesicles equivalent to type IV (Hammarström *et al* 1993). The observed rate ($1.5 \times 10^3 \text{ s}^{-1}$) was independent of the extent of reduction of $C_{16}B^+$. The mechanism for the reaction was simple reduction of $C_{16}B^+$ to $C_{16}B^0$ by the radiation pulse. The uncharged form diffused through the membrane and was re-oxidized by $Fe(CN)_6^{3-}$ in the vesicle interior. Assuming that the rate of transmembrane diffusion of $C_{16,1}V^0$ is the same as for $C_{16}B^0$, this observed rate is probably large enough, as compared to the conproportionation, to explain the negligible influence of the latter on the overall mechanism (*vide infra*).

N-cetyl-nicotinamide ($C_{16}NA^+$, figure 3) was shown to mediate transmembrane electron transfer by the same simple mechanism as $C_{16}B^+$, i.e. by diffusion of the singly reduced $C_{16}NA^0$, but the observed rate was much lower (3 s^{-1}), probably due to the relatively high polarity of the amide group (Hammarström *et al* 1993). This shows the sensitivity of the transmembrane diffusion to differences in structure and polarity.

3.3 Disproportionation equilibrium

The equilibrium constant of disproportionation can be calculated according to (3), since both redox steps are reversible;

$$\log_{10} K_d = (E^0_2 - E^0_1)/0.060. \quad (3)$$

In homogeneous media it is rather low. For methylviologen in water the first and second reduction potentials are reported as -0.45 V and $-0.8 \pm 0.1\text{ V}$ vs NHE, respectively (Bird and Kuhn 1981) yielding a value of $K_d = 10^{-5}$ – 10^{-8} . The low solubility of the doubly reduced MV^0 in water is the cause of the large scatter in the data for the second reduction step. Heterogeneous media, e.g. vesicles, will increase the solubility of all redox forms of $C_{n,n'}V$ due to the variety of environments prevalent. As a consequence, the disproportionation must be much more favourable in such systems. Attempts to measure the redox potentials of amphiphilic viologens in membranes have been made by several authors and the potentials reported for $C_{16,1}V$ in lecithin vesicles are -0.38 V and -0.66 V vs NHE respectively, i.e. $\Delta E^0 = 280\text{ mV}$ (Lei and Hurst 1991). This yields a value of $K_d = 2 \times 10^{-5}$. However, the measured redox potentials for a microheterogeneous system must be corrected for the different distributions of all redox forms over all phases present, and the different responses at the tool of measurement (the electrode, redox dye etc.) from species in different phases must be considered. In principle, this situation could be handled if all relevant distribution constants and relative measurement responses were known. However, in practice this is not the case, especially since there are a variety of interfacial equilibrium sites for the different redox forms. If the "true" ΔE^0 i.e. the kinetically relevant ΔE^0 in (3) equals -180 mV a rate of transmembrane diffusion of $[C_{16,1}V^0] \approx 1.5 \times 10^3\text{ s}^{-1}$ (*vide supra*) is sufficient to explain the negligible effects of disproportionation (Hammarström *et al* 1993).

4. Conclusion

A common feature of the mechanism of transmembrane electron transfer in the systems we have studied is that the transfer step is the diffusion of an uncharged form of the redox mediator. For $C_{16}B^+$ and $C_{16}NA^+$ there is a simple one-step reduction preceding the transmembrane step, but for $C_{16,1}V^{2+}$ the formation of $C_{16,1}V^0$ proceeds through a rate-determining disproportionation. We have shown that there is no long-range self-exchange (electron tunnelling) between viologens as is often supposed. A disproportionation mechanism has been proposed earlier in water-in-oil microemulsions (Goren and Willner 1983) and we suggest that it might be general for viologen in microheterogeneous media.

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