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Photochemical Electron Transfer across Surfactant Bilayers mediated by 2,1,3-Benzothiadiazole-4,7-dicarbonitrile

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2,1,3-Benzothiadiazole-4,7-dicarbonitrile (BTDN) has been photochemically reduced by 4-morpholine-ethanesulphonic acid (MES) in the presence of vesicles of dioctadecyldimethylammonium bromide (DODAB) or egg yolk phosphatidylcholine containing DODAB. Using asymmetrical vesicles, electron transfer can occur from MES entrapped within the inner water pools to suitable anthraquinonesulphonates (1; 1,5; 2,6 but not 2) in the bulk water mediated by BTDN. Evidence is presented that these reactions involve the genuine transport of the electron across the bilayer and not leakage of either donor or acceptor across the bilayer. Kinetic and flash photolysis studies show that the rate-determining step of the reaction is electron transport across the vesicle, that this occurs by diffusion of $BTDN^{\bullet -}$ and that the rate of this diffusion reaction is in turn controlled by the charge-compensating diffusion of OH^- in the opposite direction across the bilayer.

The transfer of electrons across membranes or vesicle bilayers is of considerable interest not only because of the biological importance, but also because it allows the separation of the formation sites of oxidation and reduction products, *e.g.* in artificial solar energy conversion.[1]

Although the transfer of electrons across biological membranes has been extensively studied, fewer investigations involving synthetic membranes or bilayers have been reported.[1,2] Early studies involved the use of phospholipid liposomes and, for example, ferrocene was found to transfer electrons from external ascorbate ion to entrapped ferricyanide.[3] A marked increase in the rate of this electron-transfer reaction on addition of *p*-trifluoromethoxyphenylhydrazone, a proton carrier, was attributed to the ability of this substance to increase proton transfer and hence neutralise any charge accumulation generated by electron transfer across the bilayer. Biological cytochrome c[4,5] or synthetic [4-dodecenylpyridinepentammineruthenium(III)] mediators can promote similar reactions.[6]

Photochemical systems generally include a chromophore, electron donor and electron acceptor. Chlorophyll has proved to be an important chromophore and, being membrane soluble, can transport electrons across phospholipid liposomes from *e.g.* external ethylenediaminetetraacetate (EDTA) to *N*,*N*′-dimethyl-4,4′-bipyridinium (MV^{2+}) ion[7] or from external glutathione to entrapped *S*-(2-methyl-1,4-naphthoquinonyl)-3-glutathione.[8] In both cases, it is believed that the electron transfer occurs *via* self-exchange of $[chlorophyll]^{\bullet +}$ on the inner surface with chlorophyll on the outer surface of the liposome, rather than by electron tunnelling or diffusion of $[chlorophyll]^{\bullet +}$ across the membrane.

Photochemical electron transfer across similar liposomes from ascorbate ion to MV^{2+} is observed using chlorophyllin, a water-soluble chlorophyll derivative,[9] or various zinc porphyrins.[10] If these are water soluble or amphiphilic, high rates of electron transport are observed only if a mediator, 1,3-dibutylalloxazine or 1,3-didodecylalloxazine, is incorporated.[11] It is proposed that some of these reactions require biphotonic processes, a photon being absorbed on each side of the liposome.[12] Neutral zinc tetraphenylporphyrin acts both as a chromophore and as a mediator for the transfer of electrons from internal EDTA or NADH to external MV^{2+}.[13] In the latter case, higher quantum yields are obtained if $[Ru(bipy)_3]^{2+}$ is entrapped in the inner water pools as an additional chromophore.[14] Surfactant derivatives of $[Ru(bipy)_3]^{2+}$ incorporated into the bilayer promote rapid (several orders of magnitude faster than diffusion) electron transfer from internal EDTA to external MV^{2+}, and the rate is not further enhanced by addition of electron transfer agents such as vitamin K, quinone, or decachlorocarborane.[15] A monophotonic electron-exchange mechanism, similar to that for chlorophyll, has been proposed,[15] but questioned by others.[16]

Although completely synthetic vesicle bilayers have been known since 1977, unambiguous demonstration of mediated electron transport across them has been hampered by the ready diffusion, sometimes photochemically assisted[17] of even quite large molecules. It has been claimed that at high concentrations of CdS embedded in the bilayer, electrons can be transferred to MV^{2+} inside dihexadecylphosphate vesicles,[18] but even in this case the interpretation is complicated by diffusion of $MV^{\bullet +}$ through the vesicle.

Following our discoveries that 2,1,3-benzothiadiazole-4,7-dicarbonitrile (BTDN) can be photoreduced by suitable electron donors [EDTA, triethanolamine (TEOA) or 4-morpholine-ethanesulphonic acid (MES)] in the presence of cationic [cetyltrimethylammonium bromide (CTAB)] micelles[19] and that its radical anion can be stabilised by incorporation in the micelle for subsequent reduction of *e.g.* anthraquinonedisulphonates (AQDS),[20] we have investigated the potential of BTDN for the transfer of electrons across synthetic positively charged vesicles. These studies have produced the first unequivocal demonstration of electron transport across a vesicle bilayer or across any bilayer with positive head groups. A preliminary report of these results has appeared.[21]

Experimental

UV–VIS spectra were measured on a Pye-Unicam SP8-150 spectrometer using pure buffer or water solution as blank,

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and the pH was measured using a Pye-Unicam model 292 pH meter with Russell Electrodes glass electrode. The water was triple distilled.

Other materials were reagent grade and used as supplied: DODAB (Kodak); MES (Aldrich); 1,5-AQDS (Aldrich); 2,6-AQDS (Aldrich); 1-anthraquinonecarboxylic acid (Aldrich); L-α phosphatidylchloline (Sigma); *N*-octyl-β-D-glucopyranoside (Sigma); choline chloride (Fluka); Sephadex G-50 (fine and medium) (Pharmacia); Sephadex G-100 (Pharmacia); cellulose (Fluka); Sephacryl S-1000 (Pharmacia). BTDN was prepared as previously described.[20] The acetate buffer solutions were prepared by dissolving sodium ethanoate (19.1 g) in water (1 dm^3) and adding ethanoic acid to give the desired pH.

Vesicle Preparation

Sonication

Aqueous solutions (10 cm^3) containing DODAB (8×10^{-3} mol dm^{-3}) were sonicated with MES (0.05 mol dm^{-3}) at pH 6.5 for 15 min at 70 °C with an MSE ultrasonic probe of 5 μm, operating at maximum power from a 4 mm titanium head.

Injection

A solution (2×10^{-5} dm^3) of DODAB in ethanol (0.87 mol dm^{-3}) was slowly injected into a vigorously stirred aqueous solution containing other required reagents (2 cm^3).

Dialysis

DODAB (2×10^{-3} mol dm^{-3}), *N*-octyl-β-D-glucopyranoside, BTDN (3.3×10^{-4} mol dm^{-3}) and TEOA (0.01 mol dm^{-3}) were dissolved in acetate buffer (5 cm^3, pH 7). The solution was dialysed at 65 °C in the dark, by placing in a tube of 'Visking 9-36/32' with a buffer solution (250 cm^3, pH 7) containing BTDN (3.3×10^{-4} mol dm^{-3}) and TEOA (0.01 mol dm^{-3}). After 12 h, the dialysing solution was replaced with another solution at room temperature (250 cm^3) containing only buffer and BTDN (3.3×10^{-4} mol dm^{-3}). Finally, after 12 h, the dialysing solution was again replaced with a solution (250 cm^3) containing only buffer and BTDN (3.3×10^{-4} mol dm^{-3}).

Column Separation of Vesicles

The vesicles (in 2 cm^3 of solution), generally formed by sonication, were separated from the bulk aqueous solution and its contents by passage down a freshly prepared and degassed column of Sephadex G-50 (10 cm long, 2 cm diameter). The vesicles were collected from the column with an eluted volume of 6–8 cm^3, whilst the other components of the mixture passed from the column with an eluted volume of 24–28 cm^3.

Photolysis

Samples (4 cm^3) containing separated vesicles, BTDN and 1,5-AQDS were placed in a 4 cm^3 quartz UV cell (1 cm pathlength) fitted with a vacuum-tight tap and wrapped on three sides with aluminium foil. They were degassed by bubbling N_2 for 0.5 h. The resulting solutions were photolysed using white light from a Thorn EMI 500 W tungsten halogen lamp (M40) focussed onto the sample through a quartz lens. The sample was held at 13 °C by holding it in a circulating water bath. Yields of 1,5-$AQDSH_2$ were determined from absorption at 383 nm ($\varepsilon = 8500$ dm^3 mol^{-1} cm^{-1}).[20]

Check for Leakage across Vesicles

DODAB vesicles containing MES (0.05 mol dm^{-3}) were prepared by sonication and separated from the outer water pools on a Sephadex G-50 column. BTDN (1.65×10^{-3} mol dm^{-3}) and 1,5-AQDS (1.65×10^{-3} mol dm^{-3}) were added and the resulting solution was photolysed until *ca.* 50% conversion to 1,5 $AQDSH_2$ (*ca.* 45 min). The solution was eluted through a Sephadex column and the fractions containing both the vesicles and the 'bulk' solution were collected. UV–VIS spectra showed that 1,5-AQDS was in both samples (it binds electrostatically to the vesicles), but that BTDN was largely in the bulk water. Both fractions were photolysed after degassing and no 1,5-$AQDSH_2$ was produced. BTDN (1.65×10^{-3} mol dm^{-3}) was added to the vesicle-containing fractions and the solution rephotolysed. 1,5-$AQDSH_2$ was again produced at *ca.* 50% of the original rate.

Laser Flash Photolysis

Flash photolysis studies were carried out with a Lambda Physik EMG-50 excimer laser operating on XeCl (308 nm). The pulse duration was *ca.* 20 ns and had a maximum measured output of 40 mJ per pulse. Absorbance measurements were made at 525 nm with an Applied Photophysics Kinetic spectrometer with a 250 W Xenon arc lamp together with a model 7300 monochromator and photomultiplier. Measurements of absorbance were made at 4 ms intervals over 800 ms.

Results and Discussion

Demonstration of electron transfer across a vesicle bilayer requires that the contents of the inner water pools of the vesicles differ in composition from those of the surrounding bulk solution, since otherwise homogeneous reactions would complicate the recognition of such transfer processes. Preparation of these 'unsymmetrical' vesicles first requires the formation of symmetrical ones. The composition of the external bulk solution can then be altered either by dialysis[22] or by gel permeation chromatography (GPC).[23] Unsymmetrical vesicles, by definition, have different concentrations of (different) species on their two sides and it is important that these concentration gradients should be maintained, at least for the electron donor and acceptor, throughout the course of any experiment.

Vesicle Preparation

Initial attempts to prepare positively charged vesicles of dioctadecydimethylammonium bromide (DODAB) in acetate buffer (0.2 mol dm^{-3}) containing triethanolamine and BTDN by sonication,[23] injection,[24] or dialytic detergent removal all led to cloudy suspensions which only contained small amounts of vesicles, as indicated by their failure to elute from a gel column [Sephadex G-50 (fine or medium), Sephacryl S-1000, cellulose or fluorosil]. Only in the case of dialytic detergent removal were significant amounts of vesicles formed, and photolysis following addition of 1,5-AQDS to the outer water pool after removal of TEOA led to the production of 1,5-$AQDSH_2$, suggesting possible trans-bilayer electron transfer. However, the likely solubility of TEOA in the lipid bilayer led us to suspect that it might be leaking from the inner water pools during the course of the experiments. We, therefore sought other, less lipid-soluble, electron donors and, since EDTA at the concentration that we wished to use caused coagulation of the vesicles, chose MES which has suitable redox properties,[25] and is mononegative. Thus, it will neither be lipid soluble nor cause coagulation of the vesicles. We also dispensed with the acetate buffer since it had previously been shown that clear vesicle suspensions cannot be obtained in solutions of high ionic strength.[26]

Vesicles were thus routinely prepared by sonication of DODAB in the presence of MES in redistilled water. If

unsymmetrical vesicles were required, the sonicated solution containing the vesicles, which are known to have low internal volume but are almost entirely unilamellar,[23] with entrapped MES was then separated from the MES in free solution by elution through a freshly prepared Sephadex G-50 column. BTDN was added either during the initial sonication or after the column separation. If it was added during the initial sonication, much of the BTDN was lost on the column. This is because, although the BTDN has a partition coefficient of 9 : 1 for octanol : water, the volume of the bulk water is so much greater than that of the vesicle bilayers or the entrapped water pools that most of the BTDN is in the bulk water and the BTDN in the vesicle bilayer is continually leaking during passage down the column. The lost BTDN was replaced before the photolysis experiments.

The electron acceptor, 1,5-AQDS, was added after the column separation, at concentrations that were not sufficient to cause vesicle coagulation, to give a final system that contained 1,5-AQDS in the bulk water, MES in the inner water pools and BTDN, if present, distributed through the vesicle bilayers, the bulk water and the inner water pools.

Photolysis Studies

Symmetrical Vesicles

Photolysis of an almost colourless solution containing DODAB vesicles, BTDN and MES at pH 6.5 produced a red–brown coloration from $BTDN^{\bullet -}$ (UV and EPR evidence), see fig. 1. Based on the known[27] size of the DODAB vesicles and the concentration of DODAB, we have estimated that only *ca.* 8% of the BTDN resides in the vesicle (assuming the partition coefficient between the vesicles and water is similar to that between octanol and water, which we have measured as 9). Examination of fig. 1 shows that *ca.* 50% conversion to $BTDN^{\bullet -}$, which must be incorporated in the vesicle since it is not stable in aqueous solution at this pH,[20] is produced. This suggests a much higher solubility for $BTDN^{\bullet -}$ than for BTDN in the vesicles. We have previously observed[26] a similar concentrating effect when generating $BTDN^{\bullet -}$ in the presence of CTAB micelles, suggesting that in this symmetrical system the function of the vesicles is very similar to that already established for the micelles.

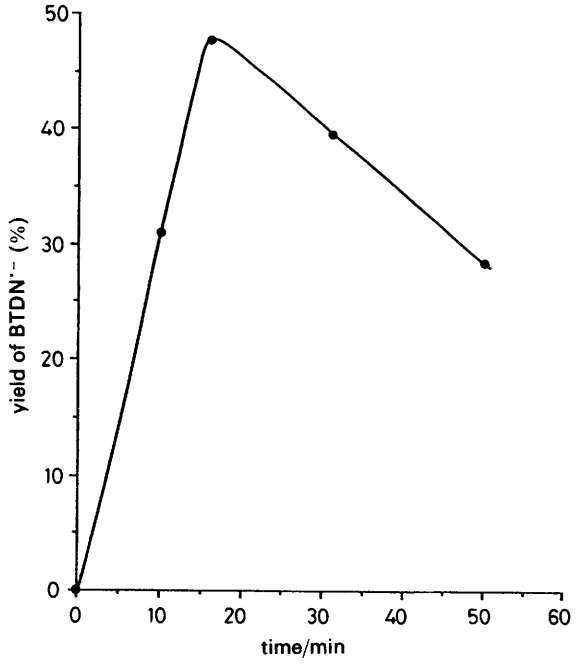


Fig. 1. Photogeneration of $BTDN^{\bullet -}$ from BTDN (1.65×10^{-4} mol dm^{-3}), MES (0.05 mol dm^{-3}) and symmetrical DODAB (4×10^{-3} mol dm^{-3} vesicles).

Unsymmetrical Vesicles

Photolysis of unsymmetrical vesicles, prepared as described above, with entrapped MES and 1,5-AQDS in the bulk water gave substantial amounts of reduced 1,5-AQDS when BTDN was present but very little when it was absent (fig. 2), suggesting that neither 1,5-AQDS nor MES leak through the vesicle bilayer to a great extent and that BTDN is an efficient electron-transfer catalyst. In the presence of BTDN, 1,5-AQDS is reduced rapidly until it is almost all consumed (see fig. 2) and this occurs even if BTDN is present at a lower total concentration than that of 1,5-AQDS (fig. 2), suggesting that BTDN is being recycled and acting catalytically. Significantly, the initial rate of formation of $1,5\text{-AQDSH}_2$, however, was only slightly lower for the lower BTDN concentration (fig. 2), suggesting a considerably less than first-order dependence on [BTDN]. Similarly, the dependence on the incident light intensity was much less than first-order (fig. 2).

Although the studies described above suggest that neither MES nor 1,5-AQDS leak across the bilayer, they do not rule out the possibility that BTDN provides some photochemical transport mechanism for one or other of the electron donor or acceptor. In order to rule out this possibility, we have carried out a photolysis of the type of system described above, in the presence of BTDN until the conversion of 1,5-AQDS to $1,5\text{-AQDSH}_2$ (the observed product) was 50% of the optimum yield. The solution was then chromatographed on a Sephadex column in air (air oxidises the $1,5\text{-AQDSH}_2$ back to 1,5-AQDS) and the fractions containing the bulk water and the vesicles collected. UV–VIS spectroscopy showed that 1,5-AQDS was present in both fractions,

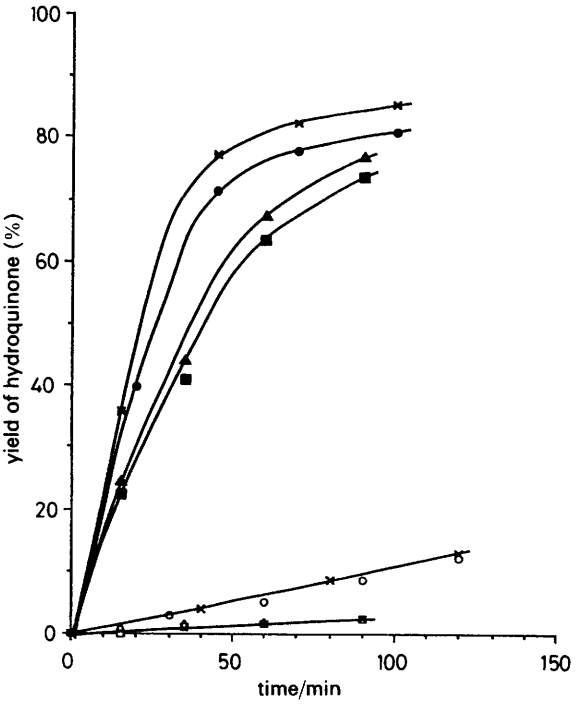


Fig. 2. Plots of % yield of $1,5\text{-AQDSH}_2$ against time for photochemical transfer of electrons from MES to 1,5-AQDS (1.65×10^{-4} mol dm^{-3}) separated by DODAB (4×10^{-3} mol dm^{-3}) using BTDN as electron carrier. * [BTDN] = 1.65×10^{-4} mol dm^{-3}, [MES] = 0.1 mol dm^{-3} (in inner water pools), full light intensity; × as * but with light off; ● as * but [BTDN] = 8.25×10^{-5} mol dm^{-3}; ○ as ● but with light off; ▲ as * but [MES] = 0.05 mol dm^{-3} (in inner water pools); △ as ▲ but with light off; ■ as ▲ but light intensity was halved; □ as ■ but with light off.

although it was mostly adsorbed onto the vesicles,† and BTDN was largely present in the bulk water. Reduction of 1,5-AQDS was not observed on photolysis of either of these fractions showing (i) that MES had not escaped into the bulk water and (ii) that 1,5-AQDS was adsorbed only on the external surface of the vesicles and had not passed through the bilayer. Addition of fresh BTDN to the fraction containing the vesicles followed by photolysis caused 1,5-AQDS to be reduced once again, confirming that the vesicles had retained their integrity (as also indicated by their retention time on the column), and that MES remained entrapped within them.

The relative permeability of synthetic surfactant vesicles has previously hindered the recognition of vectorial, mediated electron transport across the bilayers of these assemblies.[17] However, by constructing a vesicle system in which the concentration gradients of electron donor and acceptor can be maintained throughout the duration of our experiments, we have been able to demonstrate mediated electron transport across a synthetic surfactant vesicle for the first time.

Alternative Electron Acceptors

As with the results that we have previously reported[20] for the photochemical reduction of AQDS mediated by BTDN in the presence of micelles, a range of other anthraquinones can be used as electron acceptor in the trans-bilayer electron transfer. These include, the 1-sulphonate, the 2,6-disulphonate and the 1-carboxylate, but not the 2-sulphonate derivative. We have presented evidence[20] that these observations are consistent with the known redox potentials, given that these are modified in the micellar or vesicular systems. We have not carried out such detailed studies of these acceptors since their absorption bands often overlap with those of the reduced product and hence quantitative studies are difficult, but we note that the 1-sulphonate and the 1-carboxylate, being mononegative, may not cause problems of vesicle coagulation and could perhaps be employed at higher concentrations than 1,5-AQDS.

Mixed Vesicle Systems

In order to study the effect of surface charge on the efficiency of electron transport across the vesicle bilayer, we have studied the use of vesicles made up of phosphatidylcholine (PC) for which the zwitterionic head groups hold an overall neutral charge, as well as mixed vesicles of phosphatidylcholine and DODAB. In the absence of DODAB, BTDN-mediated electron transfer does not occur from MES in the inner-water pools to 1,5-AQDS in bulk solution. As DODAB is added to the phosphatidylcholine vesicles, the rate of leakage across the vesicles increases (see fig. 3). This is to be expected in view of the known increased permeability of surfactant bilayers relative to their biological counterparts.[23,27] This leakage does not, however, account for the great increase of the rate of formation of 1,5-$AQDSH_2$, which must arise from trans-bilayer electron transfer once the DODAB renders the vesicle surface positively charged.

A positive surface charge could facilitate the transfer of an electron from MES to 1,5-AQDS in several ways: (*a*) by locally concentrating MES and/or AQDS, both of which are negatively charged, near the inner and outer surfaces of the bilayer respectively; (*b*) by facilitating charge separation after the transfer of an electron between MES and BTDN through the electrostatic potential gradient at the inner surface of the bilayer; (*c*) by rapidly entrapping and hence stabilising $BTDN^{\bullet -}$ immediately it is formed.

† The 1,5-$AQDSH_2$ formed was also adsorbed onto the surface of the vesicles giving a similar bathochromic shift for the absorption bands as already reported[20] to occur in the presence of CTAB vesicles and thought to be the result of electrostatic binding to the positive head groups.

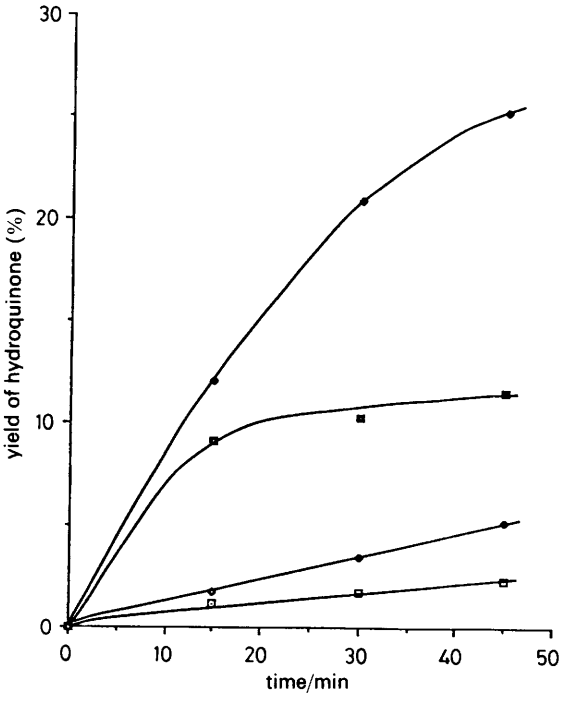
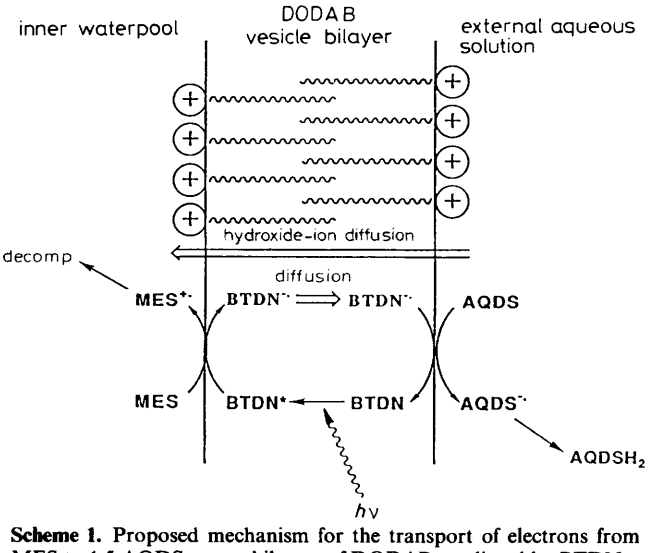


Fig. 3. Effect of [DODAB] in PC vesicles on the photochemical electron transfer from MES (0.05 mol dm^{-3}) in the inner water pools to 1,5-AQDS (1.65×10^{-4} mol dm^{-3}) in the bulk water across vesicles, promoted by BTDN (1.65×10^{-4} mol dm^{-3}). Total vesicle precursor concentration 4×10^{-3} mol dm^{-3} (◆) PC : DODAB = 2 : 1; ◇ as ◆ but with light off; ■ PC : DODAB = 20.1; □ as ■ but with light off.

The increase in the rate of 1,5-$AQDSH_2$ formation was not proportional to the amount of DODAB added (see fig. 3); without knowledge of the detailed architecture of these mixed vesicles, it is not possible to rationalise this behaviour.

Mechanistic Studies

The mechanism by which we believe this electron transfer occurs is shown in scheme 1. We believe that BTDN acts as combined chromophore and electron transfer catalyst and that, whilst its initial reduction probably occurs in free solution within the entrapped water pools, the $BTDN^{\bullet -}$ so formed is immediately stabilized by the surface potential of



Scheme 1. Proposed mechanism for the transport of electrons from MES to 1,5-AQDS across bilayers of DODAB, mediated by BTDN.

the bilayer. The electron is then transported across the bilayer before being released to 1,5-AQDS in the bulk water. From other studies, 1,5-AQDS$^{\bullet -}$ is known rapidly to disproportionate and protonate to give 1,5-AQDSH$_2$ and 1,5-AQDS under the reaction conditions that we employ.[20]

Three of the events described above could, in principle, be rate determining for the trans-bilayer reduction of 1,5-AQDS by MES. These are: (*a*) the transfer of an electron to BTDN mediated photochemically near the inner surface of the bilayer; (*b*) the transport of the electron across the vesicle bilayer; (*c*) the transfer of the electron from BTDN$^{\bullet -}$ within the bilayer to 1,5-AQDS close to the outer surface of the bilayer. We can rule out (*c*), the transfer of an electron from BTDN$^{\bullet -}$ to 1,5-AQDS as being rate-determining since increasing the concentration of 1,5-AQDS by a factor of 10 (fig. 4) hardly alters the rate† of formation of 1,5-AQDSH$_2$. We have shown that this is not a saturation effect since even at the higher concentration, gel permeation chromatography shows that almost all the 1,5-AQDS is electrostatically bound to the vesicles. In addition, the failure to observe a build-up (steady-state concentration) of BTDN$^{\bullet -}$ during photolysis of this system suggests that the rate-determining step does not involve electron transfer from this species.

The initial formation of BTDN$^{\bullet -}$ can also be ruled out as rate-determining since laser flash photolysis studies show that a significant amount of this species was formed within the duration of a 20 ns pulse, but that it did not decay to a significant extent over the next 200 μs.

Somewhat surprisingly, doubling the concentration of entrapped MES in our asymmetric DODAB system had relatively little effect on the rate of formation of 1,5-AQDSH$_2$ (see fig. 2) above the background level, which notably was twice the level previously observed. We return to an explanation of this phenomenon below.

Having ruled out the two electron transfer events as being rate-determining, we can conclude that electron transport across the vesicle bilayer is the rate-determining step. Once again there are several possible mechanisms for the transport of the electron across the vesicle bilayer. These include: (*a*) the exchange of a DODAB molecule with an electrostatically attached BTDN$^{\bullet -}$ from the inner surface to the outer surface of the vesicle, the so-called 'flip-flop' mechanism;[28,29] (*b*) self-exchange of the electron from BTDN$^{\bullet -}$ bound near the inner surface of the bilayer to BTDN near the outer surface, possibly *via* a series of BTDN molecules within the vesicle bilayer; (*c*) electron tunnelling across the vesicle bilayer; and (*d*) diffusion of BTDN$^{\bullet -}$ across the bilayer.

In order to try to distinguish between these four possibilities, we have performed laser flash photolysis experiments in which we recorded the absorbance at 525 nm (an absorption maximum for BTDN$^{\bullet -}$ but not for 1,5-AQDSH$_2$) during and after a *ca.* 20 ns flash at 308 nm, within the absorption envelope of BTDN. After an initial rapid increase in the absorption at 525 nm, the signal decayed exponentially during the next 850 ms with a first-order rate constant k_{obs} of 2.82 s^{-1} at 25 °C.

For the concentration regimen of the flash photolysis experiments, electron transfer from BTDN$^{\bullet -}$ to AQDS is apparently rate-determining (see below). This means that the electron transfer across the vesicle, which is rate-determining during continuous photolysis experiments, must have a first-order rate constant significantly >2.82 s^{-1}. The observed rate constant immediately excludes the 'flip-flop' exchange mechanism since the rate constant for this type of process has been measured *ca.* 10^{-5} s^{-1} in both phospholipid and vesicle bilayers.[28,29] The concentration of BTDN$^{\bullet -}$ produced during the flash was *ca.* 10% of that of the BTDN estimated to be present in the bilayer (1.6×10^{-4} mol dm^{-3}). It must be greater than this during continuous photolysis. In this case, a pseudo-first-order decay should be observed for a self-exchange mechanism. The second-order rate constant for this process would then be >2.82/[BTDN] *i.e.* $\gg 1.4 \times 10^4$ dm^3 mol^{-1} s^{-1}.

We have measured the self-exchange rate constant between BTDN$^{\bullet -}$ and BTDN in acetonitrile using an electrochemical method[30,31] as 2.6×10^{-3} dm^3 mol^{-1} s^{-1}, many orders of magnitude lower than the second-order rate constant calculated from our experimental data. The need to separate the electron from the electrostatic potential close to the bilayer surface probably means that self-exchange will be slower in the vesicle bilayer than in the free MeCN solution so that our observed rate (*ca.* 7 orders of magnitude higher than self-exchange in free solution) rules out self-exchange as the electron-transport mechanism.

In order to test whether an electron-tunnelling mechanism is compatible with the observed rate of electron transport across the vesicle, we have used a previously published[32] method to estimate the rate that would be expected for this process. Assuming that intralayer trapping sites are available at the intersection of the hydrocarbon tails (half-way across the bilayer), that the bilayer thickness is 4 nm[23] and that π^0 for BTDN/BTDN$^{\bullet -}$ in the presence of DODAB is −0.333 V (ref. SHE), a value that we have measured electrochemically

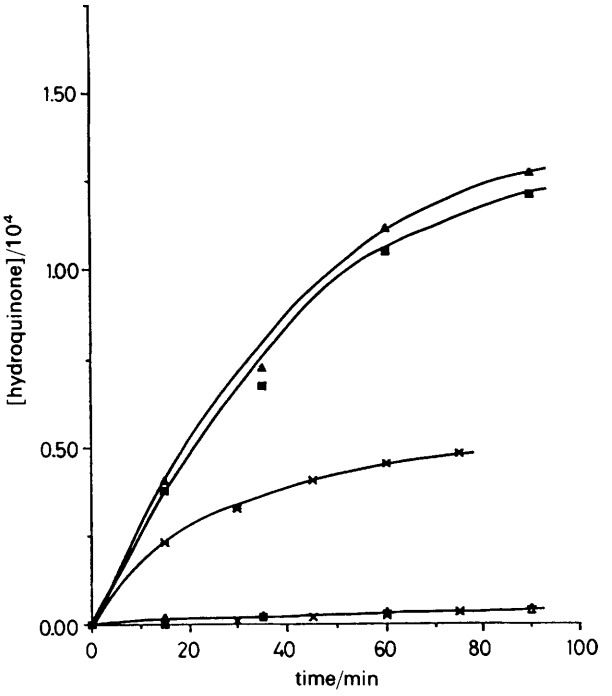


Fig. 4. Plots of yields of 1,5-AQDSH$_2$ against time for photochemical electron transfer from MES (0.05 mol dm^{-3}) in the inner water pools to 1,5-AQDS in bulk water separated by DODAB (4×10^{-3} mol dm^{-3}) vesicles using BTDN (1.65×10^{-4} mol dm^{-3}) as electron carrier. ▲ [1,5-AQDS] = 1.65×10^{-3} mol dm^{-3}; △ as ▲ but with light off; ■ [1,5-AQDS] = 1.65×10^{-4} mol dm^{-3}; □ as ■ but with light off; * [1,5-AQDS] = 8.25×10^{-5} mol dm^{-3}, × as *, but with light off.

† The rather lower total percentage yield of 1,5-AQDSH$_2$ observed when it is at the higher concentration arises because of exhaustion of the MES in the inner water pools; at concentrations lower than those generally employed, the rate shows an approximately first order dependence on [1,5-AQDS], suggesting that the electron transfer from BTDN$^{\bullet -}$ to 1,5-AQDS may become rate-determining at these low 1,5-AQDS concentrations.

and which is consistent with the reducing power of $BTDN^{\bullet -}$ (see above), the estimated tunnelling rate constant is *ca.* 10^{-3} s^{-1}. Once again, this is very much less than the observed rate constant, thus effectively ruling out tunnelling as the electron-transport mechanism.

It appears, then, that diffusion of $BTDN^{\bullet -}$ is the mechanism by which electrons are transported across the vesicle, although the rate constant for this electron transport is probably lower than that measured for *e.g.* the diffusion of a K^+ (valinonycin) complex across bilayers.[33] In part, this slower rate may be attributable to electrostatic attraction between the positive head groups of the vesicle wall and the negative $BTDN^{\bullet -}$, but other observations suggest a different explanation.

Clearly, if the rate of production of $AQDSH_2$ is independent of all the parameters [AQDS], [BTDN], [MES] and light intensity, zeroth-order kinetics apply and the actual rate of the reaction is measured at 5.6×10^{-8} mol s^{-1} dm^{-3}, equivalent to a rate of transport of electrons across the vesicle of 1.12×10^{-7} mol s^{-1} dm^{-3}.

Zeroth-order dependence on the various parameters listed above indicates that the overall reaction rate must be dependent upon some external physical process. Since trans-bilayer electron transfer is electrogenic, a substantial trans-bilayer electrical potential would soon develop, increasingly opposing and ultimately limiting the trans-bilayer redox reaction. Since this did not occur in our system, it is apparent that some compensating charge transfer must be occurring *via* ion diffusion across the bilayer. Since neither AQDS nor MES penetrate the bilayer and since diffusion of Na^+ or Br^- across the bilayer are both known to be very slow,[34,35] it seems likely that charge compensation occurs *via* trans-bilayer transport of H^+ or OH^-. Support for this proposal comes from the observation that the pH of the solution changes very little during the production of $AQDSH_2$ (6.5–6.1), whereas the pH of the external water pools would be expected to rise to >11 if all the AQDS is converted to $AQDSH_2$ and there is no diffusion of OH^- or H^+ across the bilayer.[1]

Kinetic studies have been carried out on the transport of OH^-† across DODAC vesicles‡ and a rate constant of 4–20×10^{-4} s^{-1} determined.[29,34,36] This increases slightly (3.3×10^{-3} s^{-1}) in the presence of excess Br^- (*i.e.* when the vesicle is largely present at DODAB).[34] This last rate constant gives a rate for OH^- transport at pH 6.5 of 10^{-10} mol s^{-1} dm^{-3}. The initial rate of electron transport in our system (= 2 × rate of formation of $AQDSH_2$) is *ca.* 1.1×10^{-7} mol s^{-1} dm^{-3}. However, other studies[37] on OH^- transport have shown that having different anions attached to the inner and outer surfaces of the vesicle, as is the case in our studies (MES and Br^- on the inner surface, AQDS and Br^- on the outer) can markedly increase the rate of transport of anions (Br^- in the case reported) across the vesicle bilayer. Another difference between our system and those in which the rate of OH^- transport has been measured is that in the reported systems OH^- transport must be accompanied by transport of another ion across the vesicle in order to prevent charge accumulation. Often, it is the rate of transport of this ion (Na^+, K^+, Cl^- *etc.*) that actually determines the reaction rate.[29,38] In our system, the diffusion of OH^- is compensating for the charge transfer by $BTDN^{\bullet -}$ and no ions with high charge-to-radius ratio (and hence low diffusion rates through the bilayer) other than H^+ and OH^- are involved.

Studies on liposomes derived from *desulpho-vibrio vulgaris* containing cytochrome c have shown[39] that the rate of 'active' H^+/OH^- transport which compensates for electron transfer across the vesicle is *ca.* 10^3 times the rate for passive H^+ transport to neutralise a pH gradient. Although this system is very different from the one that we have studied, it does show the dramatic differences in rate of H^+/OH^- transport that can be caused by having different ions carrying out the charge compensation.

Apparently, in contradiction to the above discussion, flash photolysis studies carried out on our unsymmetrical vesicle system show that little or no decay of the signal from generated $BTDN^{\bullet -}$ occurs in the μs time domain but that a first-order decay (*i.e.* dependent on $[BTDN^{\bullet -}]$) of $BTDN^{\bullet -}$ occurs over several hundred ms with a first-order rate constant of 2.82 s^{-1}. We can, however, rationalise this behaviour, since the concentration of $[BTDN^{\bullet -}]$ is very low compared with the continuous photolysis studies. This concentration has been measured from the extinction coefficient immediately after the pulse as *ca.* 2.4×10^{-4} mol dm^{-3} within the vesicle. An absolute maximum for the rate of electron transfer can be estimated, assuming that all of the $BTDN^{\bullet -}$ is available for electron transfer and all of the AQDS is available for accepting the electron as 1.1×10^{-7} mol dm^{-3} s^{-1}. Clearly, neither of these assumptions is valid and the actual rate will be very much less than this. Since the actual rate will then be very much lower than that of compensating diffusion of OH^- or H^+, a different step will be rate-determining, in this case probably electron transfer from $BTDN^{\bullet -}$ to AQDS and this should show first-order kinetics in $[BTDN^{\bullet -}]$. The rate constant measured for electron transfer from $BTDN^{\bullet -}$ to AQDS is then 2.82 s^{-1}.

We tentatively conclude that, in continuous photolysis experiments, the electron is transported across the vesicle bilayer by diffusion of $BTDN^{\bullet -}$ but that the overall rate of this electron transfer is determined by diffusion of H^+ or OH^- across the vesicle to prevent charge build-up. This is presented in scheme 1 assuming that OH^- is the charge-compensating species. H^+ could equally migrate in the reverse direction.

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† In reality, it is very difficult to distinguish between H^+ transport in one direction and OH^- in the other. Often this is referred to as H^+/OH^- transport. We shall refer to it as 'OH^- transport' although we note that similar arguments apply if the actual transporting species is H^+. ‡ DODAC differs from DODAB in that the counterions are chloride not bromide.

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