Electron Conduction across Vesicular Bilayer Membranes induced by a Caroviologen Molecular Wire

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The zwitterionic caroviologen $1^{2\pm}$, incorporated into vesicle bilayer membranes, functions as a molecular wire mediating electron transfer from an external reducing phase to an internal oxidising phase.

The development of molecular electronic devices requires the design of functional components capable of handling electrons and their incorporation in an organised fashion into supramolecular architectures. Such devices should perform their function at the molecular and supramolecular levels, as distinct from the level of the bulk material.[1,2]

Among the basic components of molecular electronic circuitry (rectifiers, transistors, switches and photodiodes) a molecular wire (W) is of crucial importance since it is required

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for connecting the components of the system and for allowing electron flow between them.

Our first approach towards the design of molecular wires was based on the caroviologens CV^{2+} such as $\mathbf{1}^{2+}$, that combine the features of the carotenoids and of the viologens: an extended conjugated polyenic chain for electron conduction, fitted with terminal electroactive pyridinium groups for reversible electron exchange. They must be long enough to be able to span a typical organised supporting unit such as a lipid membrane. Caroviologens CV^{2+} were synthesised and incorporated into dihexadecyl phosphate (DHDP) vesicles.[3] Linear dichroism measurements on model lipid membranes confirmed the transmembrane orientation for CV^{2+} of length compatible with the bilayer thickness.[4]

Electron conduction by such species may be investigated by following transmembrane redox processes between a reductive and an oxidative aqueous phase (Fig. 1). Oxidation–reduction reactions across membranes of vesicles containing various redox species have been studied (see for instance refs. 5 and 6). We have performed electron transfer experiments with CV^{2+} compounds of different lengths, but mainly with $\mathbf{1}^{2+}$ and $\mathbf{1}^{2\pm}$ containing nine conjugated double bonds. They were incorporated in lipid vesicles that contained an oxidant, potassium ferricyanide (FIC), $K_3Fe(CN)_6$ ($E'_0 = +0.36$ V *vs.* NHE[7,8]) (NHE = normal hydrogen electrode), and that were suspended in an aqueous solution of a reductant, sodium dithionite (DT), $Na_2S_2O_4$ ($E'_0 = -0.66$ V *vs.* NHE[7,8]). These agents are able to reduce $\mathbf{1}^{2+}$ or to reoxidise reduced $\mathbf{1}^{2+}$ ($E_0 \sim -0.60$ V *vs.* SCE)[3] (SCE = standard calomel electrode).

Electron transfer experiments employing the initial $\mathbf{1}^{2+}$–DHDP vesicle system[3] were unsuccessful. This may be due to the high negative charge of the membrane surface which, by electrostatic repulsion with the anionic reductant and oxidant and strong attraction with the terminal pyridinium groups of $\mathbf{1}^{2+}$,[9] can markedly decrease electron-transfer rates and modify the interfacial redox potential of incorporated $\mathbf{1}^{2+}$ by stabilising the unreduced state.[10] Also the DHDP vesicles were not sufficiently stable to the high concentrations of FIC required for its detection by electronic absorption spectroscopy. We then turned to phospholipid vesicles which allow the inclusion of up to 0.5 mol dm^{-3} FIC[6] and are impermeable to DT.[11,12] However, the cationic species CV^{2+} were found not to be incorporated into these membranes.

Zwitterionic caroviologens $CV^{2\pm}$ such as $\mathbf{1}^{2\pm}$ were synthesised by reaction of the parent α,ω-bispyridyl polyene with 1,4-butanesultone [$-(CH_2)_4OSO_2-$].[13] The proton NMR spectrum indicated that the compound $\mathbf{1}^{2\pm}$ obtained was >95% in the all-*trans* configuration. The caroviologen $\mathbf{1}^{2\pm}$ was incorporated, in the presence of 0.33 mol dm^{-3} FIC, into the membrane of vesicles formed from a 9 : 1 mixture of dimyristoyl phosphatidylcholine (DMPC) and egg lecithin (EPC) as shown by coelution from a Sephadex column.‡ The DMPC membranes are thinner (~30 Å in the fluid state[14]) than the DPPC (dipalmitoyl phosphatidylcholine) or DHDP ones and thus correspond better to the length of the W $\mathbf{1}^{2\pm}$. EPC was introduced in order to stabilise the DMPC membranes. The absorption spectrum of $\mathbf{1}^{2\pm}$ in DMPC bilayers indicated that it was in the monomeric state (λ_{max} = 540 nm) at all temperatures explored. By analogy with $\mathbf{1}^{2+}$,[3,4] one may assume that $\mathbf{1}^{2\pm}$ is oriented in a transmembrane fashion.

Owing to the sensitivity of $\mathbf{1}^{2\pm}$ to high FIC concentrations and despite the short time, low temperature and low power employed during vesicle formation by sonication, a large amount of the W was destroyed in the process. The remaining active W was estimated from the absorption at 540 nm (ε = 58 000 dm^3 mol^{-1} cm^{-1}) by subtracting the spectra obtained for vesicles (0.33 mol dm^{-3} internal FIC) without and with $\mathbf{1}^{2\pm}$. The concentration of phospholipids was determined following the published procedure.[15] The concentrations of lipids and $\mathbf{1}^{2\pm}$ in an experiment using 1 mole% $\mathbf{1}^{2\pm}$ at the start of vesicle preparation, were found to be respectively 1.6×10^{-3} mol dm^{-3} and 1.7×10^{-6} mol dm^{-3}, amounting to about 0.1% incorporated intact W $\mathbf{1}^{2\pm}$ after gel filtration.

The number of intact $\mathbf{1}^{2\pm}$ molecules per vesicle was estimated from the mean vesicle diameter (~120 nm) by assuming the vesicle to be spherical and the surface occupied by a lipid at room temperature to be about 60 $Å^2$.[14] This yields

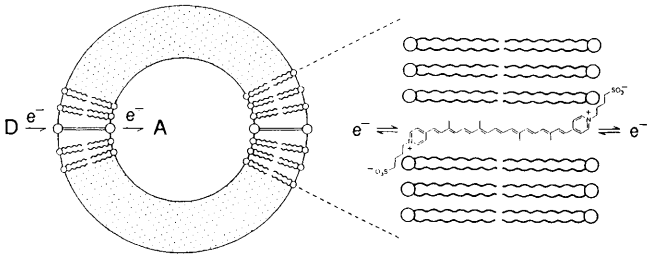
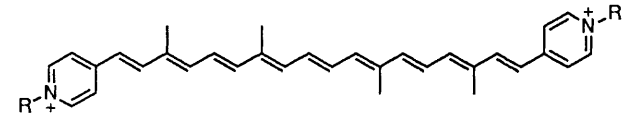
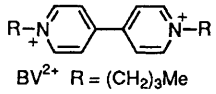


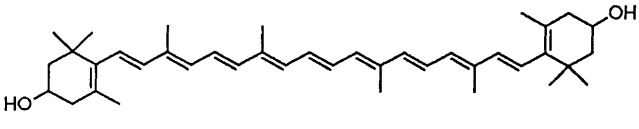
Fig. 1 Schematic representation of electron conduction through the zwitterionic, caroviologen molecular wire $\mathbf{1}^{2\pm}$ incorporated in a phospholipid (DMPC + EPC) vesicle membrane, from an outside reducing agent D (sodium dithionite) to an internal electron acceptor A (potassium ferricyanide); see also text



$\mathbf{1}^{2+}$ R = Me
$\mathbf{1}^{2\pm}$ R = $(CH_2)_4SO_3^-$



BV^{2+} R = $(CH_2)_3Me$



ZX

‡ Incorporation was performed by the following two methods. Method A: A solution of DMPC (9.5 mg), EPC (1.5 mg) and $\mathbf{1}^{2\pm}$ (0.114 mg, 1% by mole with respect to the lipids) in chloroform–methanol (1 : 1) was slowly evaporated under reduced pressure under argon to give a film which was taken up in Tris buffer (50 mmol dm^{-3}, 1 ml, pH 7) and stirred for 30 min. The suspension obtained was sonicated three times [Branson B50 sonicator equipped with a titanium ultrasound probe (power 1.5)] for 5 min at 3 min intervals under argon in an ice bath. Then $K_3Fe(CN)_6$ (2 ml of 0.5 mol dm^{-3} solution in Tris buffer) was added and the suspension sonicated for 5 min (power 2.5). Method B: The film obtained in method A was taken up in $K_3Fe(CN)_6$ (3 ml of 0.33 mol dm^{-3} solution in Tris buffer) and kept under argon at room temperature for 30 min. The suspension was then sonicated three times for 5 min at 3 min intervals in an ice bath (power 3.0). Absorption measurements indicated that method A gave a better conservation of the polyene $\mathbf{1}^{2\pm}$ (less contact with FIC). The vesicle suspension was then filtered over Sephadex G 25M (5 cm column) at 4 °C eluting with Tris buffer (50 mmol dm^{-3}) containing KCl (1 mol dm^{-3}). The separation of internal and external FIC was complete. The vesicles have been characterized by quasi-elastic light scattering and by electron microscopy. Freeze fracture pictures clearly indicated that the bilayer structure was conserved for vesicles containing 0.33 mol dm^{-3} internal FIC and showed fractures at the monolayer level. After filtration (0.2 μm polycarbonate filter) they had an average diameter of about 120 nm and an acceptable polydipersity (variance ~0.3) both in the absence and in the presence of 0.33 mol dm^{-3} FIC (1.0 mol dm^{-3} KCl outside). Whereas incorporation experiments performed in the absence of FIC left most of the wire intact, this was not the case in presence of FIC (see text).

Table 1 Observed rate constants k_{obs} for the reduction of internal potassium ferricyanide by external sodium dithionite in phospholipid vesicles containing additives $1^{2\pm}$, BV^{2+} or ZX[a]

Run	$[Fe(CN_6)^{3+}]_i$ /mol dm^{-3}	$[KCl)_0$ /mol dm^{-3}	$1^{2\pm}$: lipids (%)[b]	BV^{2+} : lipids (%)	ZX : lipids (%)	k_{obs} 10^{+4} s^{-1}	Ratios[d]
a	0.5	1.5	0	0	0	4	
b	0.33	1.0	0.24	0	0	14	b : a = 3.5
c	0.5	1.5	0	0	0	23	
d	0.33	1.0	0.24	0	0	86	d : c = 3.8
e	0.33	1.0	0	0	0	()[d]	
f	0.33	1.0	1(0.1)[c]	0	0	8.9 (1.1)[d]	f : e = 8
g	0.33	1.0	0	1	0	50.0 (6.0)[d]	g : e = 8
h	0.33	1.0	0	0	1	3.9 (17)[d]	h : e = 0.25

[a] DMPC–EPC 9 : 1 vesicles prepared by method A; runs b, d, e–h; by method B: runs a, c (but with 0.5 mol dm^{-3} FIC); experiments a and b at 15 °C all others at 30 °C; BV^{2+}: butyl viologen; ZX: zeaxanthine. [b] Amounts of initially added $1^{2\pm}$. [c] Amount of intact $1^{2\pm}$ after vesicle preparation: 0.1%; initially added $1^{2\pm}$: 1%; see text. [d] Since different vesicle preparations gave markedly different rates of electron transfer, a reference run e was conducted in parallel with each run f, g or h. The k_{obs} values ( ) obtained for f, g or h should only be compared to the e values of the same runs; these are given in parentheses. The ratios of k_{obs} were reproducible within ±10%. Furthermore, since for b : a and d : c the reference experiments a and c had a higher internal concentration of FIC, the ratios calculated are minimum values.

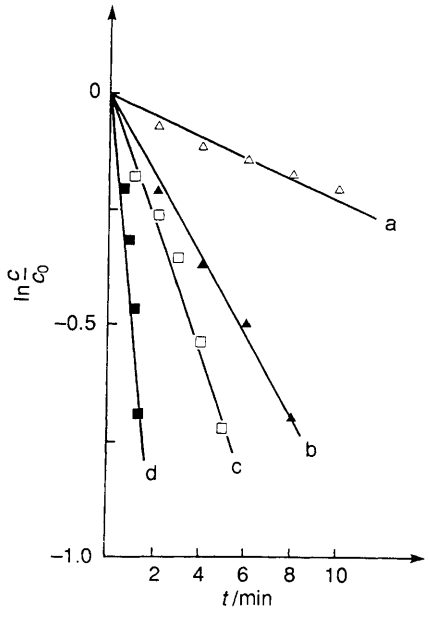


Fig. 2 Observation of the rate of potassium ferricyanide reduction in the system of Fig. 1 as a function of time at 15 °C (a, b) and at 30 °C (c, d); c, c_0: respectively concentration at a given time and initial concentration of internal ferricyanide; for conditions see Table 1 (runs a–d), Fig. 1 and text

an average number of 150 000 phospholipid molecules and approximately 150 intact Ws $1^{2\pm}$ per vesicle prepared in the presence of 0.33 mol dm^{-3} FIC.

The vesicle suspension (1 ml) was made 5 mmol dm^{-3} in DT by addition of a freshly prepared concentrated aqueous DT solution (0.1 ml). Electron transfer was studied by following the reduction of internal FIC monitored by the absorption at λ_{max} = 420 nm (ε = 1000 dm^3 mol^{-1} cm^{-1}) as a function of time at two temperatures, in the presence and in the absence of incorporated $1^{2\pm}$. The experiments were repeated several times in order to confirm the observations and to check their reproducibility. Since an excess of DT was used, pseudo-first-order rate constants could be determined from the plots of FIC disappearance with time (Fig. 2). Table 1 lists the conditions of the experiments and the observed rate constants k_{obs}. Comparison experiments without and with additive were always run on simultaneously prepared vesicles.

When $1^{2\pm}$ was present the rate of reduction increased significantly, by a factor of four or eight (Table 1, runs a–f) depending on the concentration of intact wire in the lipid membrane, both below and above the transition temperature (20.9 °C[16]) of the lipid membrane. The largest acceleration (runs f and g) corresponds to the highest concentration of intact polyene. In this latter case, the turnover was estimated to be about 50 electrons per wire per minute on the basis of the number of intact wires per vesicle (see above).

The rate increase may seem small, but one must realize that it corresponds to a rather small number of $1^{2\pm}$ molecules per vesicle and that the total process involves two second-order electron transfer reactions, one at each interface: a DT unit must reduce a given molecule dispersed in a sea of lipids and an FIC complex must on the internal interface find a reduced molecule to reoxidise. The reduction observed in the absence of W may be ascribed to species diffusion§ and/or vesicle destruction. Ideally one may wish to study the operation of a single W per vesicle, but this is precluded by background reduction in the present conditions.

In order to compare the efficiency of the process effected by $1^{2\pm}$ to a free redox carrier, experiments were performed with butyl viologen (BV^{2+}) iodide, the most efficient alkyl viologen type carrier.[17] Electron transport by methylviologen (MV^{2+}) involves transmembrane diffusion of $MV^{+\bullet}$.[12] The acceleration factor was about eight (Table 1, run g) for an amount of active carrier about ten times that of intact $1^{2\pm}$. The latter is therefore markedly more efficient.

Finally, experiments were conducted with zeaxanthine ZX which has a polyene chain similar to that of $1^{2\pm}$ but no electroactive endgroups and is known to be incorporated in DMPC or EPC vesicles in a transmembrane fashion.[18] ZX decreased the rate of FIC reduction by a factor of about four (Table 1, run h). ZX is known to lower the permeability of DMPC membranes to water[19] and may in the present case decrease the leakage of the species involved.§ This indicates that the effect produced by $1^{2\pm}$ is probably not due to increased leakage *via* a perturbation of the membrane structure and that it could in fact be larger by a factor of about four than that calculated by comparison with membranes without additive [ratio (f : e)/(h : e) ~ 32, Table 1].

Phenylenediamine bridged dimers containing two bixin polyene molecules have been incorporated into a DPPC vesicle redox system. They do not function as electron-transfer agents but render the membranes permeable to ionic reductants.[20]

The present results lead to the conclusion that the caroviologen $1^{2\pm}$ indeed functions as a transmembrane molecular wire connecting the external reducing and the internal oxidising

§ Probably diffusion of decomposition products of dithionite (thiosulphate, bisulphite) into the vesicle rather than leakage of the highly charged FIC out of the vesicle.

phases, and operating in an electron channel mode. The electronic conduction probably proceeds *via* monoelectronic reduction of $\mathbf{1}^{2\pm}$ to a delocalised radical entity $[\mathbf{1}^{2\pm}, e^-]^{-\cdot}$ of the type of the $CV^{+\cdot}$ species observed when CV^{2+} caroviologens are reduced by pulse radiolysis.[21] The soliton width of 30 carbons reported for charge delocalisation in polyenyl anions[22] is larger than the length of molecule $\mathbf{1}^{2\pm}$. Electrochemical reduction of $\mathbf{1}^{2+}$ is irreversible in aqueous solution and affords a doubly reduced species.[3,13] This indicates that for $\mathbf{1}^{2+}$ to function properly with turnover, reoxidation of a monoreduced molecule should occur before a second electron is transfered to the same species. The risk of *cis–trans* isomerisation in the monoreduced state should be limited by the transmembrane anchorage of the molecule. A more direct electrical study of electron transfer by $\mathbf{1}^{2+}$ incorporated into black lipid membranes, has yielded positive preliminary results.[23] It would also be of interest to investigate $CV^{2\pm}$ molecules of different length so as to determine its influence on electron transfer efficiency. The processes studied here might have some relevance to the possible electron transfer function of natural polyenes contained in biological membranes.

Various developments can be envisaged. Push–pull carotenoids have been shown to present marked charge-transfer[24] and non-linear optical properties.[25] When oriented in molecular films,[26] these polarised molecular wires might display preferential one-way electron transfer and act as rectifying components.[2] The attachment of redox- or photo-active metal complexes at the ends of the polyenic chain opens entries into electro- or photo-switchable molecular wires.[2,27,28] Finally, addressing by means of scanning tunneling microscopy might allow the direct study of electron flow through a single oriented molecular wire.[29]

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