A Novel Transmembrane Electron Channel Containing Flavin Units

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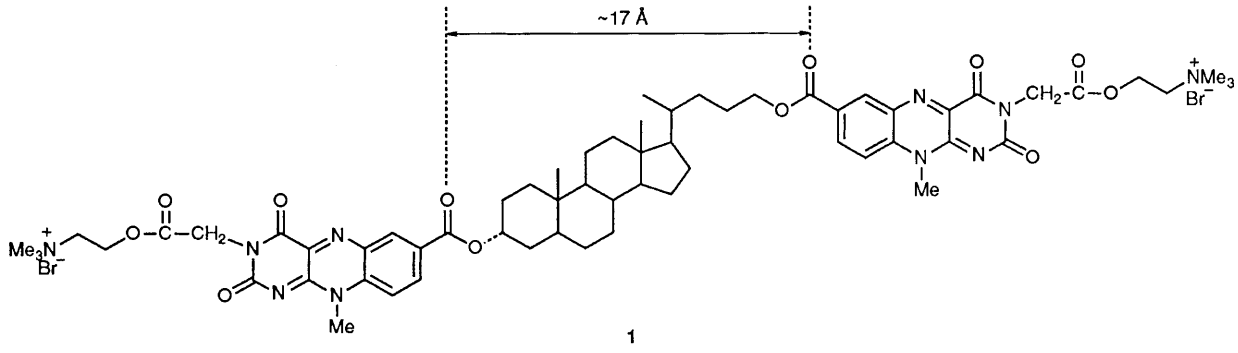
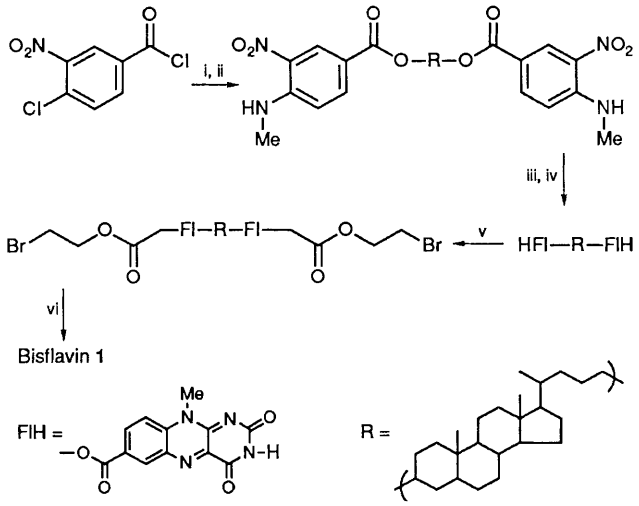
A new amphiphile, consisting of two flavin units bearing quaternary ammonium groups attached to both ends of a rigid steroidal spacer, has been synthesized as a transmembrane electron channel; the electron transfer rate across a liposomal membrane through a separation distance of *ca.* 17 Å was measured as 11.8 s^{-1}.

Electron transfer through biological membranes has attracted interest because photosynthetic and respiratory systems accumulate chemical energy through proton transfer coupled with this process.[1] This was mimicked by membrane-incorporated catalytic units, such as porphyrins[2] and flavins.[3] In these studies, electron transfer occurred through an encounter pair, one participating from the inner bilayer and the other from the outer to make a transient channel.

In this report, a transmembrane electron channel, the bisflavin **1**, with a fixed distance as long as *ca.* 17 Å from one flavin edge to the other was constructed. Studies on such long-range electron transfers, *e.g.* over a separation of more than 15 Å,[4] are interesting in view of oxido-reduction processes between enzymes. Electron transfer between a ground state molecular pair having a small driving force with ΔG° as low as zero is another point of interest in this study.[4c,e,f,5]

The transmembrane electron channel molecule was designed as follows. Two flavin units were attached to both ends of a rigid and hydrophobic steroidal skeleton. To the outer





Scheme 1 *Reagents and conditions*: i, HO–R–OH tetrahydrofuran (THF), Et_3N, 85%; ii, $MeNH_2$, THF, 85%; iii, H_2 PtO_2 THF, not isolated; iv, alloxane, MeOH, HCl, reflux for 1 h, 85% (iii and iv); v, $BrCH_2CH_2Br$ K_2CO_3, dimethylformamide, 40 °C, 25%; vi, Me_3N, THF. 75%

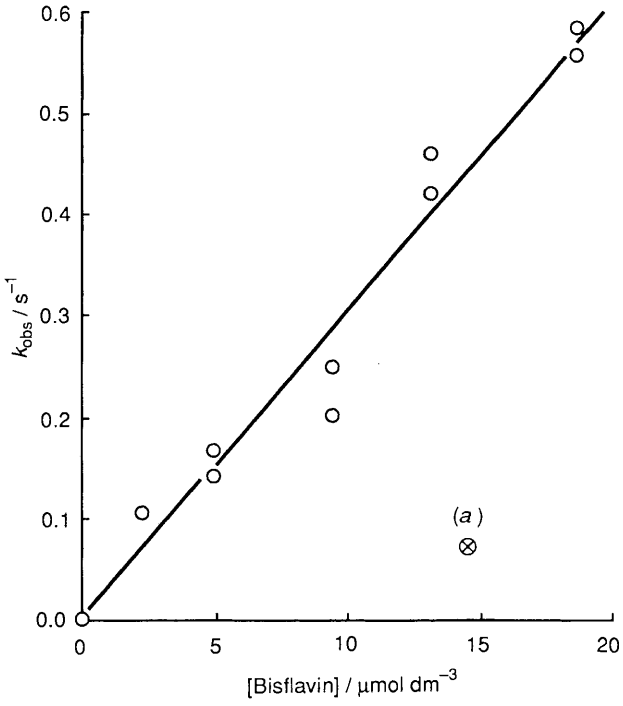


Fig. 1 The dependence of the electron-transfer rate, k_{obs}, on bisflavin concentration. Point (*a*) corresponds to the rate obtained for the previously reported flavolipid.[3d]

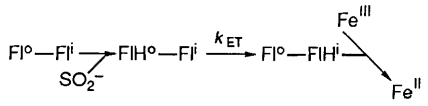
end of the flavin units, an ammonium grouping was introduced to give an amphiphilic nature. The combined interactions, hydrophobic in the membrane and ionic at the interface, with lipid constituents may facilitate the incorporation of the molecule in an extended form without folding in the membrane. The total length of the molecule was designed to fit the bilayer membrane, *i.e.* 36–52 Å.[6] The edge–edge distance between the two flavins was estimated as *ca.* 17 Å from a Corey–Pauling–Koltun (CPK) model. The synthetic route is illustrated in Scheme 1. Litocholic acid was converted into the diol, to which two flavins were attached through ester bridges. The imide nitrogens of the flavin units were used for introducing ammonium groups. The final product, bisflavin **1**, was isolated *via* silica gel column chromatography in a total yield of 3.4% based on the starting diol. After recrystallization, the ^1H NMR, IR, UV and FAB mass spectra as well as elemental analyses were in agreement with the assigned structure.†

† ^1H NMR (CD_3SOCD_3) δ 0.30–2.20 (m, 37H, steroidal CH_3, CH_2, CH), 3.13 (s, 18H, NMe_3), 3.61 (t, 4H, $-CH_2-N$, *J* 7.5 Hz), 4.4 (s, 6H, Me–N), 4.10–4.67 (br. s, 2H, CH_2-O), 4.43–4.73 (br. s, 4H, $-CH_2-O-$), 4.60–5.23 (br. s, 1H, CH–O), 4.76 (s, 4H, $-N-CH_2CO_2$), 8.11 (d, 1H, 9′-H, *J* 9.4 Hz), 8.13 (d, 1H, 9′-H, *J* 9.4Hz), 8.43 (dd, 2H, 8′-H, *J* 1.9, 9.4 Hz), 8.61 (d, 1H, 6′-H, *J* 1.9 Hz) and 8.65 (d, 1H, 6′-H, *J* 1.9 Hz); IR (KBr), ν/cm^{-1}, 1703 and 1649; FAB mass: 1159 ($M-Br_2$ + H)$^+$; Calc. for $C_{62}H_{82}Br_2N_{10}O_{12}$, C, 56.45; H, 6.3; N, 10.6; Br, 12.1. Found, C, 56.35; H, 6.5; N, 9.6; Br, 9.9%; UV(chloroform): λ_{max}/nm 334 (ε 1.3 ×10^4), 420 (1.35 × 10^4), 438 (1.49 × 10^4) and 465 (1.06 × 10^4).

Bisflavin **1** was easily incorporated into the membrane phase of phospholipid liposomes (SUV)‡ of egg yolk lecithin. The decay of internally trapped potassium ferricyanide (3.7 × 10^{-4} mol dm^{-3}) by externally added sodium dithionite (4.8 × 10^{-4} to 3.7 × 10^{-3} mol dm^{-3}) mediated by bisflavin (2 × 10^{-6} to 1.8 × 10^{-5} mol dm^{-3}; 0.5–1.7 wt%[6] relative to total lipid) was traced through the change of the absorbance at 430 nm as described previously.[3d]

The electron-transfer rate showed a half-order dependence at lower concentrations of sodium dithionite and then a saturation, illustrating a change of the rate-determining step from a reduction of the flavin at the outer interphase to a transmembrane electron transfer.[3d] The saturation values, k_{obs}, are plotted as a function of bisflavin concentrations in Fig. 1. An almost linear concentration dependence was observed, which contrasts with the second-order dependence in the case of the flavolipid, where the formation of a transient channel between outer and inner flavolipids was proposed.[3c]

‡ The mean diameter of the liposomes was estimated as 28(±9) nm from dynamic light-scattering measurements.



Scheme 2

The kinetic scheme may be represented in Scheme 2, where Fl and FlH denote oxidized and reduced forms of the flavin, respectively, and the superscripts (°) and (i) the outer and an inner bilayer, respectively. Under conditions of rate-determining transmembrane electron transfer, a steady state concentration of [Fl°–FlH^{i}] may reasonably be assumed. The transmembrane electron-transfer rate k_{ET} may then be expressed by eqn. (1).

$$k_{ET} = k_{obs}\,[Fe^{III}]/[Fl^{o}\text{–}Fl^{i}]_0 = 11.8\ s^{-1} \quad (1)$$

A similar analysis of the electron-transfer rate for the flavolipid reported previously[3d] gave 5.1 s^{-1}. The difference in the electron-transfer rate by a factor of 2 may be ascribed to the difference in the connecting chain and therefore in the distance, since the present electron transfer is through 16 chemical bonds, while that previously reported is through-space in nature.[3c,d] In the present example the electron transfer is occurring from the outer flavin to the inner, FlH°–Fl^{i} to Fl°–FlH^{i}, and the free energy change should intrinsically be zero. The long-range electron-transfer rate with almost zero ΔG^{o} has been reported for ruthenium-modified cytochrome c as 1.7 s^{-1} for an edge–edge separation of 15 Å with $\Delta G^{o} = -0.2$ eV,[4f] and 0.01 s^{-1} for a 17 Å separation with $\Delta G^{o} = -0.05$ eV.[4e] More information is required on various biological as well as artificial systems in order to elucidate the factors controlling the process involved.

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