Characterization of Water Soluble/Bilayer Active β-Cyclodextrin-linked Porphyrin Derivatives

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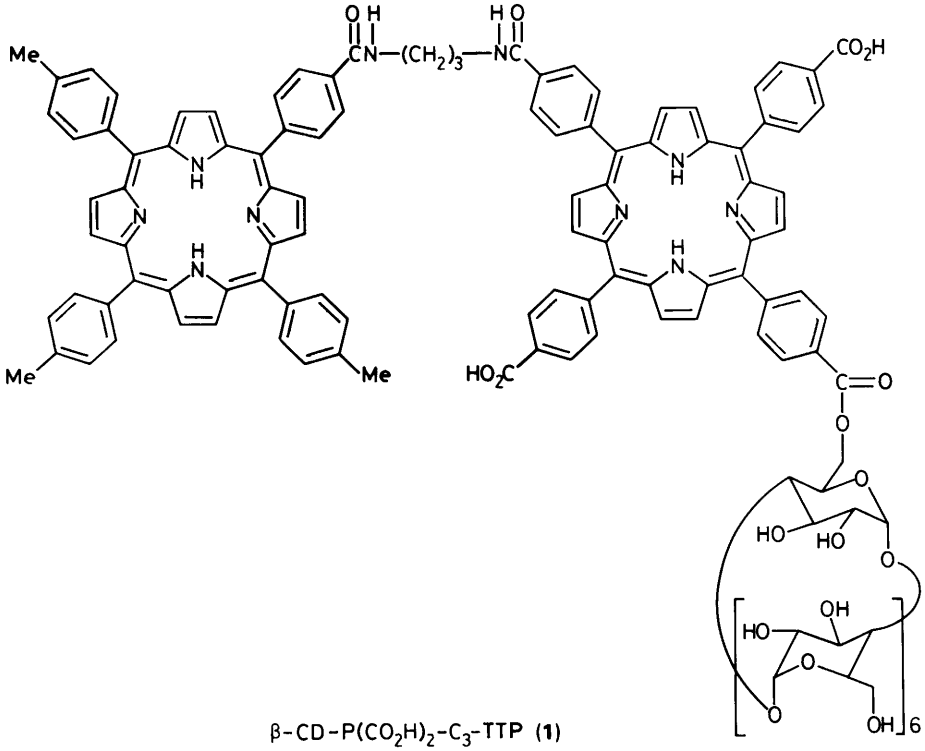
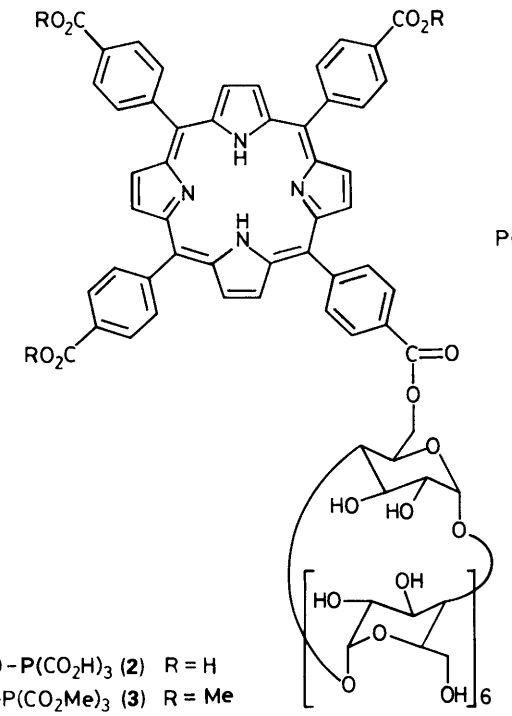
The porphyrin portion of cylodextrin(CD)-linked porphyrin derivatives (**1**)—(**3**) has been anchored onto a lipid bilayer and a light-induced intramolecular electron transfer between the porphyrin on (**1**) and (**2**) and the acceptor, α-naphthoquinone held in the CD cavity in aqueous solution, has been examined.

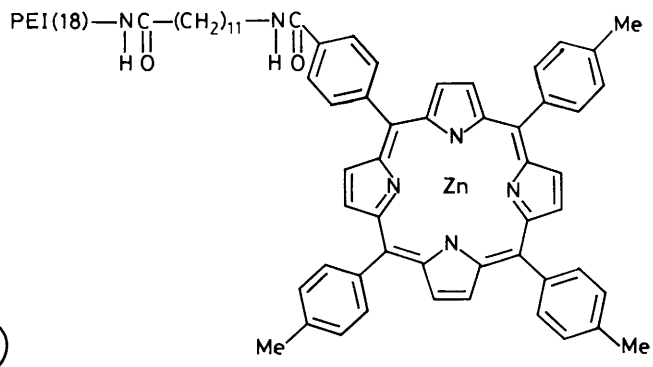
Chemical models have been used to provide insight into the possible reactions of cyclodextrin(CD)–porphyrin complexes, such as photosynthetic reaction and secondary electron transfer.[1—3] Porphyrin derivatives play a crucial role in these electron transfer systems.[4] We now report the preparation of CD-linked porphyrin derivatives (**1**)—(**3**) and the characterization of their interactions with a lipid bilayer and α-naphthoquinone held in the CD cavities in aqueous media. We reasoned that the CD moiety would confer water solubility, while the hydrophobic porphyrin portion should insert into the lipid bilayer. That is, the porphyrin group on the compounds can be anchored onto the lipid bilayer so that a



β-CD-P(CO$_2$H)$_2$-C$_3$-TTP (**1**)



β-CD-P(CO$_2$H)$_3$ (**2**) R = H
β-CD-P(CO$_2$Me)$_3$ (**3**) R = Me



PEI(18)-C$_{11}$-ZnTTP (**4**)

vectorial electron transfer between porphyrin centres may be constructed as a model for electron transfer across a biological membrane.[1] Furthermore, the CD of compounds (**1**)—(**3**) has an inclusion effect for a hydrophobic compound in an aqueous environment. These compounds take advantage of the ability of the CD to complex hydrophobic species into its central cavity and allow the examination of light induced electron transfer between the porphyrin portion on the compounds and quinone acceptors of varying reduction potential held in the CD cavity.[8] We used fluorescence quenching to monitor the electron transfer.

Compounds (**1**)—(**3**) were prepared as follows.† The porphyrins were prepared as described in previous papers.[1,3,6,7] The porphyrin derivatives were treated with *N*,*N*′-carbonyldi-imidazole in refluxing chloroform for 1 h and then

† Compound (**1**) contains the isomer. More detailed synthetic and analytical data will be reported elsewhere.

Figure 1. Plots of I_0/I of PEI(18)–C_{11}–ZnTTP (**4**) *vs.* the concentration of β-CD-linked free base porphyrins (**1**)—(**3**) [free base porphyrins], in egg yolk phosphatidylcholine vesicle, 0.01 M (Tris), pH 8.0 at 25 °C. The *x*-axis is represented by [free base porphyrins (**1**)—(**3**)]/[PEI (18)–C_{11}–ZnTTP (**4**)]. (○): β-CD–P $(CO_2H)_2$–C_3–TTP (**1**), (□): β-CD–P $(CO_2H)_3$ (**2**), (△): β-CD–P $(CO_2Me)_3$ (**3**).

reacted with a large excess of commercially available β-CD in pyridine at 70 °C overnight. The ^{1}H n.m.r. (400 MHz) spectra of compounds, (**1**)—(**3**) isolated by either preparative silica gel t.l.c. or gel filtration, followed by ultrafiltration, support unambiguously the assigned structure. The visible and fluorescence spectra were measured for porphyrin complexes (**1**)—(**3**) in H_2O or 10% tetrahydrofuran (THF)–H_2O, dimethyl sulphoxide (DMSO), and egg yolk phosphatidylcholine(PC) vesicle.‡ A large decrease in the absorptivity of the band near 420 nm (the Soret band) for (**1**) in aqueous solution can be seen in comparison to that in the PC vesicle. By contrast, the spectrum in DMSO is more like that in the PC vesicle. Thus, when (**1**) is added to a vesicle suspension, the porphyrin environment is apolar, consistent with the lipid interior rather than an aqueous environment.

Similar results are observed for (**2**) and (**3**). The relative intensities of the fluorescence emission bands for (**1**)—(**3**) in various media show that higher fluorescence yields are observed in the PC vesicle for (**1**)—(**3**), 56, 83, and 75%,

‡ Experimentally determined absorption band maxima [λ_{max} (nm)] and extinction coefficients [ε (mmol^{-1} dm^3 cm^{-1})] of β-CD-P $(CO_2H)_2$-C_3 TTP (**1**) in several media at 25 °C: 10% THF–H_2O 421 (194), 517 (22.2), 551 (12.7), 590 (8.25), 647 (6.62); DMSO 418 (532), 514 (26.0), 549 (14.2), 589 (8.52), 644 (8.25); PC vesicle (0.01 M Bistris, pH 7.0) 418 (372), 512 (24.2), 549 (13.8), 589 (8.40), 644 (7.98).

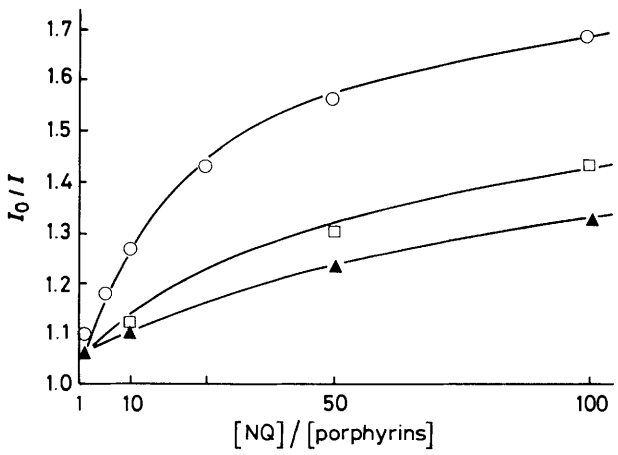


Figure 2. Plots of I_0/I of β-CD-linked porphyrins *vs.* the concentration of α-naphthoquinone (NQ), [NQ], in 0.01 M Tris, pH 8.0 at 25 °C. The *x*-axis is represented by [NQ]/[β-CD-linked porphyrins]. (○): β-CD–$P(CO_2H)_2$–C_3–TTP (**1**) and NQ, (□): β-CD–$P(CO_2H)_3$ (**2**) and NQ, (▲): control [$P(CO_2H)_4$ + β-CD] and NQ.

respectively, and lower fluorescence yields are observed in aqueous solution for (**1**)—(**2**), 10 and 71, respectively (values are normalized to yields for these samples in DMSO). The data imply that in the PC vesicle, all the porphyrin moiety is not at the surface in the aqueous environment but rather is immersed within the hydrophobic interior of the membrane. Furthermore, energy transfer between porphyrins was measured to gain more information on the environment of the porphyrin molecules of (**1**)—(**3**) in the lipid bilayer. Energy transfer from a previously incorporated zinc porphyrin complex on polyethylenimine (PEI, *M* 1800)-linked zinc porphyrin [PEI (18)-C_{11}-ZnTTP (**4**)], in the vesicle to externally added free base porphyrin on compounds (**1**)—(**3**) in the same vesicle was measured. Complex (**4**) was prepared as described previously.[1] With addition of free base porphyrins (**1**)—(**3**), the fluorescence emission of the zinc porphyrin of (**4**) at 613 nm decreased, while the emission of the free base porphyrins at 658 nm dramatically increased, indicating that energy transfer from the zinc porphyrin to the free base porphyrins of compounds (**1**)—(**3**) had taken place.[5] The efficiency of the energy transfer is expressed by the intensity change of the fluorescence emission of the zinc porphyrin at 613 nm, represented by I_0/I. Figure 1 illustrates the change of (I_0/I) with addition of (**1**)—(**3**); (**1**) shows a large increase in I_0/I, indicating a large energy transfer between (**4**) and the porphyrin dimer on (**1**). In contrast, the respective porphyrin monomers (**2**) and (**3**) show a lower increase in I_0/I, a higher value being observed for (**3**) than for (**2**). No change in I_0/I is observed with addition of $P(CO_2H)_4$ (the data are not shown). These results strongly support the conclusion that all the porphyrin portions of (**1**)—(**3**) are embedded in the hydrocarbon region of the bilayer, in the following order: (**1**) > (**3**) > (**2**). This characteristic energy transfer between porphyrins was more accentuated in the PC vesicle than in CH_2Cl_2–10% EtOH (the data are not shown).

The dependence of the fluorescence intensity upon quinone concentration in an aqueous solution of either (**1**) or (**2**), represented by Stern–Volmer plots (I_0/I), is shown in Figure 2. The I_0/I plots for (**1**) show a large increase with addition of α-naphthoquinone (NQ), compared with the control; the value increased rapidly initially, and then more slowly, at the same rate as the control, as the NQ concentration was increased. The initial rapid increase in the quenching efficiency may be attributed to the complexation of NQ by the CD

of (**1**),§ which, on freezing, adopts a conformation in which intramolecular electron transfer between porphyrin and NQ may occur. The lower rate of increase may correspond to intermolecular quenching of the excited state of complexes with NQ in which the molecule exists in unfavourable conformations such that the intramolecular quenching cannot occur.[8,9] Similar quenching behaviour was also obtained between (**2**) and NQ where the initial rapid increase of I_0/I was less characteristic than that of (**1**). In contrast, (**1**) showed no significantly large value of I_0/I, with the increase of NQ concentration in DMSO, in which the complexation of NQ by the CD was inefficient.[7]

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§ The experimentally determined binding constant is 6929 mol^{-1} dm^3 according to the Benesi–Hildebrand equation: H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.

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