Facilitated Ion Transport in a Biomembrane Model. Artificial Ion Channels as Pores in Dihexadecyl Phosphate Vesicles

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An artificial ion channel, composed of stacks of crown ether rings, forms a pore in bilayers of dihexadecyl phosphate vesicles and facilitates the transmembranous transport of cobalt ions.

The membrane mimetic properties of simple surfactant vesicles are currently a focal point of attention.[1] Of special interest are the ion transport processes in these systems as they may give insight into the mechanisms that control the permeability of biological membranes. Generally, two different modes of transmembranous ion transport can be recognized: the carrier and the channel mode.[2] The latter type of transport is believed to be the more important one in biological systems.[3] However, in contrast to the former type it has hardly been studied in synthetic membrane systems.[4]

Recently, we reported the synthesis of a molecular ion channel which could serve as a model of a natural channel type ionophore.[5] The compound is a polymer of an isocyanide, $(R\text{–}N{=}C{<})_n$ (**1**), which contains benzo-18-crown-6 side chains. Polymers of isocyanides are rigid helices with four repeating units per helical turn.[6] Owing to the rigid structure of the polymer backbone, the crown ether rings in (**1**) are placed on top of each other and form four channels which run parallel to the polymer helix axis (Figure 1). Compound (**1**) displays a number of features also found in natural ionophores: (i) pores with a polar interior and an apolar exterior, (ii) a hydrophilic top and bottom which may face the aqueous sides of a membrane, (iii) a chain length (≃4 nm[5]) sufficiently long to bridge the membrane. We present evidence here that channel compound (**1**) enhances the ion permeability of membranes of dihexadecyl phosphate (DHP) vesicles by forming a transmembranous pore.

DHP and compound (**1**) were mixed in water in different molar ratios and dispersed by sonication at 60 °C.† Dynamic light-scattering measurements showed that vesicles were formed whose diameters decreased with increasing sonication time down to a plateau value. This plateau value, which was reached after 25 min, amounted to 45 nm for vesicles both with and without (**1**). Turbidity measurements carried out according to the method of Barrow and Lentz[7] indicated that the vesicles were unilamellar. Incorporation of (**1**) into the bilayers of the vesicles was confirmed by gel permeation chromatography.[8] These experiments showed that the vesicles and channel compound (**1**) were both present in the same peak in the void volume of the column. Vesicle dispersions

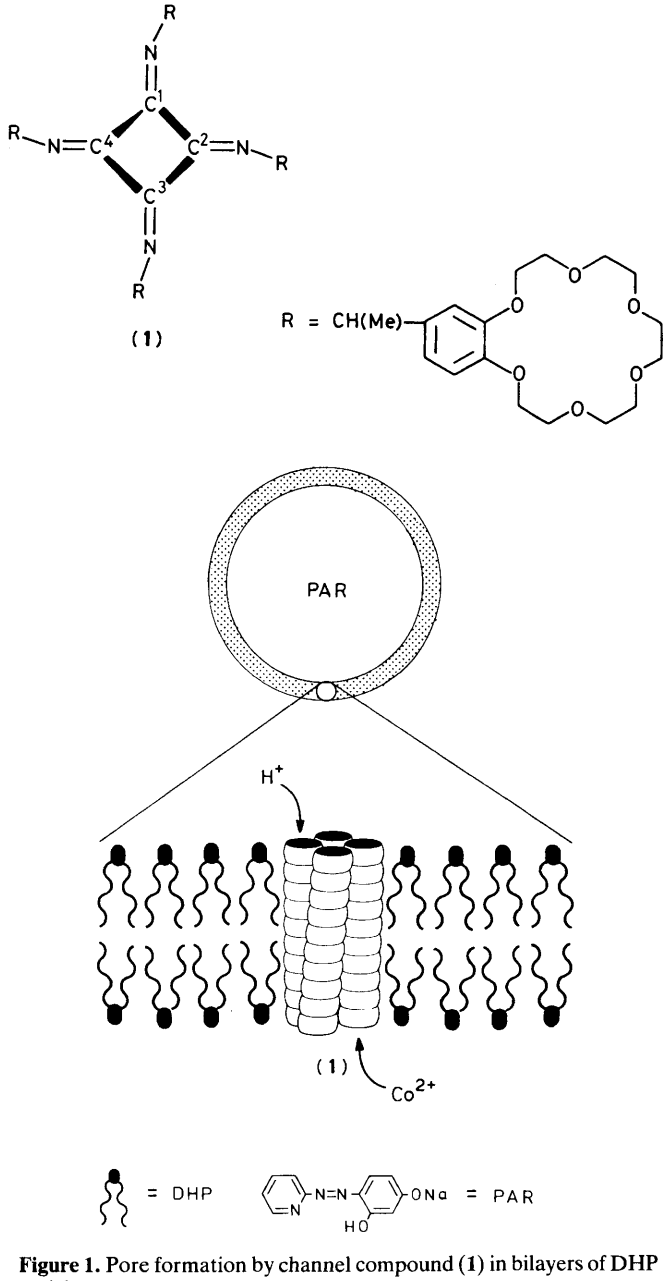

Figure 1. Pore formation by channel compound (**1**) in bilayers of DHP vesicles.

Table 1. Cobalt influx rate as a function of temperature.[a]

T/°C	$v \times 10^9$/mol dm^{-3} s^{-1}
25	1.7 ± 0.1
42	3.1 ± 0.3
58	4.2 ± 0.6

[a] [DHP] 1.55×10^{-3} mol dm^{-3}; molar ratio (**1**)/DHP 0.0018; [Co] 8.8×10^{-5} mol dm^{-3}.

† In a typical experiment DHP (40 mg, 73 μmol), channel compound (**1**) ($\bar{M}_v$ 15000, 1 mg, 0.067 μmol), and an aqueous solution containing PAR (4 cm^3, 24 μmol) were sonicated at 60 °C for 45 min using a Branson B-12 sonifier, power 80 W. The exovesicular PAR was removed by ultrafiltration (Amicon Diaflo XM 100 filter, 4 × 40 cm^3 of doubly distilled water) and the volume of the resulting dispersion was adjusted to 10 cm^3. The latter dispersion (0.5 cm^3) was mixed with an aqueous solution of $Co(NO_3)_2$ (0.04 cm^3, 1.19×10^{-3} mol dm^{-3}) and after osmotic equilibration (3 min) the increase in absorption at 510 nm in the u.v.–visible spectrum was measured. The observed rates generally followed pseudo first order kinetics. Initial rates (v_0) were calculated according to the equation $v_0 = k_1 \times C_0$, in which k_1 is the measured apparent first order rate constant and C_0 is the initial concentration of cobalt ions.

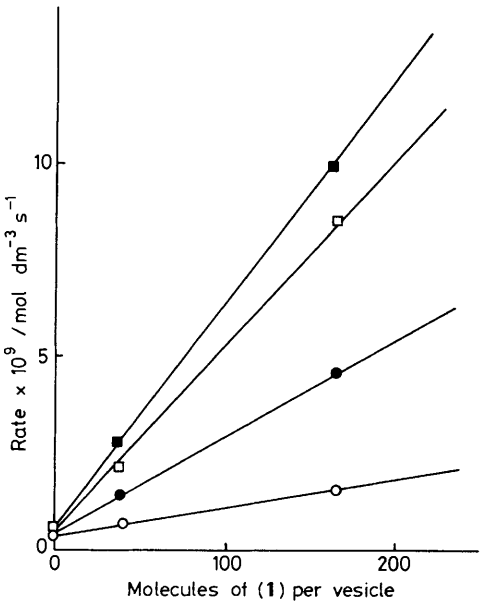

Figure 2. Initial cobalt influx rates at different vesicle concentrations as a function of the number of channels per vesicle; 10^3 DHP/mol dm^{-3}: 4.2 (■), 3.1 (□), 2.0 (●), 1.1 (○); initial [Co] 8.8×10^{-5} mol dm^{-3}; T = 25 °C.

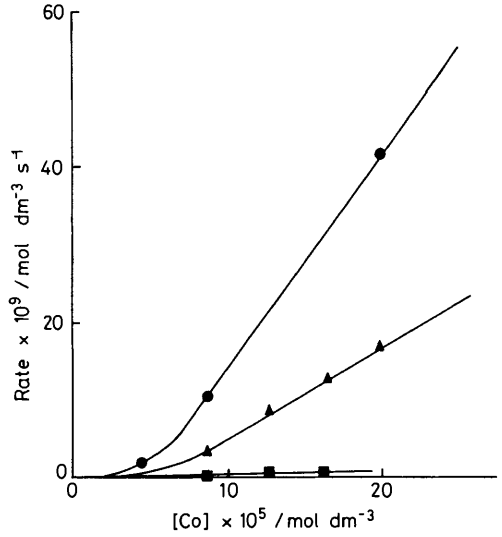

Figure 3. Initial influx rates as a function of cobalt concentration; molar ratio (**1**)/DHP: 3.6×10^{-3} (●), 0.91×10^{-3} (▲), 0 (■); T = 25 °C.

incorporating the dye, 4-(2-pyridylazo)resorcinol monosodium salt (PAR) in their inner aqueous volume were obtained when DHP [with or without (**1**)] was sonicated in the presence of this dye in the solution.† The exovesicular dye was then removed by ultrafiltration.

The ion permeability of the bilayers of the vesicles was measured by adding an aqueous solution of $Co(NO_3)_2$ to the vesicle dispersions and recording either the decrease in absorption of the entrapped PAR at ~400 nm or the increase in absorption of the cobalt–PAR complex at 510 nm. Separately we checked that cobalt ions do not disrupt the vesicle membrane. Even when using a cobalt ion concentration of 2×10^{-4} mol dm^{-3}, dialysis experiments indicated that no leakage of entrapped PAR had occurred. The results of experiments performed at various concentrations of vesicles, containing different amounts of channels, and at various concentrations of cobalt ions are given in Figures 2 and 3. These experiments reveal that channel compound (**1**) has a pronounced effect on the ion permeability of the DHP vesicle bilayer. At several concentrations of vesicles the overall ion transport rate increases with increasing number of channels per vesicle. A rate enhancing effect is also observed when the concentration of cobalt ions in the outer aqueous phase is increased. Determination of the ion transport rate as a function of temperature (Table 1) yields an Arrhenius activation energy E_a = 24 kJ mol^{-1}. This number is consistent with a pore mechanism for the ion translocation process (Figure 1). A very similar value of E_a has been found for the natural pore-forming compound Gramicidin A (E_a = 20.5—22.5 kJ mol^{-1}).[9] A carrier transport mechanism would need a higher energy of activation (E_a = 90—120 kJ mol^{-1}).[10]

Cobalt ions do not easily lose their hydration shell and as a result their complexation to a crown ether ring is relatively weak. Translocation of these ions in our system, therefore, is only likely to occur *via* water molecules present in the interior of the channels. A migration process in which the ion hops from a water molecule bound at a crown ether ring to a water molecule bound at another ring along the exterior of the channels, is less probable. Such a process would require a higher energy of activation (probably E_a = 60—120 kJ mol^{-1})[11] owing to a less effective screening of the ion charge in the lipid environment. Ion transport *via* defective pores caused by disarrangement of the bilayers as a result of incorporation of (**1**) can be ruled out. If this were the case, the vesicle dispersions containing (**1**) would also show a leakage of encapsulated PAR. Repeated dialysis of the dispersions indicated that such a leakage did not occur.

For the conservation of electroneutrality it is required that translocation of cobalt ions is coupled to either a co-transport of nitrate ions or a counter-transport of protons and/or sodium ions. An exchange of cobalt ions for protons seems to be the most likely process. We found that during our experiments the pH inside the vesicle increases. This increase in pH is deduced from the shift in $\lambda_{max.}$ value that occurs in the u.v.–visible spectrum of entrapped, uncomplexed PAR. For instance, at an initial pH of 5.5, the $\lambda_{max.}$ value of entrapped PAR is at 385 nm. In the course of the experiment this maximum value shifts to 410 nm, which corresponds to a pH >7, as was checked separately.

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