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A,B,D,F-TETRASUBSTITUTED β-CYCLODEXTRIN AS ARTIFICIAL CHANNEL COMPOUND

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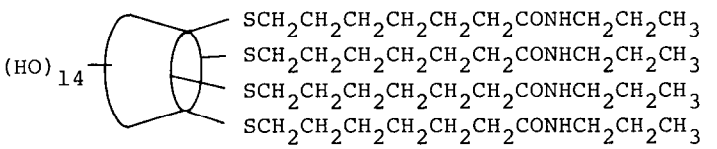
As a channel forming compound, β-cyclodextrin having four hydrophobic tails and three metal binding sites, 1, [A,C,D,F-tetra-6-(6-n-butyrylamino-n-hexylsulfenly)-β-cyclodextrin] was synthesized as a "half channel". Artificial liposome modified with 1 transported Co^{++} efficiently with the rate, $4.5 \times 10^{-4} sec^{-1}$ for 55 μM 1, much faster than the rate for the unmodified liposome.

Two general strategies are adopted in biological systems to achieve specific ion transport--utilization of carrier polypeptides[1] and formation of channels.[2] After the preparation of the first member of the entirely man-made ion carriers, 18-crown-6,[3] a tremendous number of chemists have been concentrated into the preparation and utilization of a variety of artificial carrier molecules,[4] attaining remarkable successes.[5]

However, in order to gain further sophistication, simple carrier molecules are not always satisfactory. Especially, in the case where regulation of ion transport is requested man-made channels must be considered. Unfortunately, the chemistry of artificial channel is far behind. One of possible approaches to an entirely artificial channel is to prepare a molecule of an appropriate hydrophilic part and an appropriate hydrophobic part, of a length comparable to a lipid molecule, and of appropriate numbers of ionophilic sites.

The authors have been currently investigating regiospecific introduction of a variety of functional groups in β-cyclodextrin for the preparation of "artificial proteins".[6] Based on this concept, A,C,D,F-tetra-6-(6-n-butyrylamino-n-hexyl-1-sulfenyl)-β-cyclodextrin (hereafter abbreviated as "half channel", 1) was chosen as a promising candidate for artificial channel compound. Thus 1 was prepared from 600 mg of purified A,C,D,F-tetraiodo-β-cyclodextrin[7] on treatment with 1.11 g (13 molar excess) of sodium 6-n-butyrylamino-n-hexyl-1-mercaptide in carefully dried DMF at 50°C for 5 h. After evaporation of DMF, 10 ml of water was added to the residue and pH was adjusted to 3 with 1N HCl. The precipitate formed was filtered and Soxlet-extracted with acetone for 3 days. This residue (200 mg) was purified by repeated column chromatography (silica gel Wako C 200, $CH_3CN : H_2O = 6.5 : 1$) and 30 mg of pure 1 (6.3% yield) was obtained. ^1H NMR (DMSO d_6), δ 7.6 (4H; NH) 4.8 (7H; β-CD C_1H) 3.2 - 3.7 (59H; others), 2.8 - 3.1 (8H; C_6H), 2.5 (t, 8H; C_1H), 2.2 (t, 8H; C_8H), 1.3 - 1.7 (40H; C_2 - C_5, C_9H) 0.95 (t, 12H; $C_{10}H$). IR, 2920, 1630, 1545, 1140 cm^{-1}. Analysis, calcd. for

$C_{82}H_{146}O_{35}N_4S_4\ 3H_2O$: C 51.02, H 7.94, N 2.90, S 6.64 found C 50.65, H 7.67, N 3.39, S 6.20.

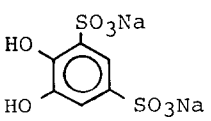


1

"Half channel" 1 is soluble in usual organic solvents ($CHCl_3$, n-BuOH, MeOH) but practically insoluble in water in spite of the strong hydrophilicity of cyclodextrin moiety. It showed a *moderate* association constants toward various metal ions, satisfying the necessary condition to be a channel component, since K_{ass} should be large for the better binding and should be small for the better liberation to the interior solution.[8]

$$K_{ass}(Cu^{++}) = \text{ca } 10^{5.1} \qquad K_{ass}(Co^{++}) = \text{ca } 10^{4.6}$$

An artificial liposome incorporating Tiron, 2, in its interior was prepared from 100 mg of carefully purified egg lecithin[8] and 831 mg of Tiron at pH 7.0 (Tris-HCl buffer, 1 mM) with 3 X 5 min ultrasonic irradiation.[9] This liposome was purified by the procedure reported in our previous paper.[10] The resultant solution (1 ml), containing ca 1 mM of Tiron, Tiron(i)|Lip, was diluted to 6.7 ml with the Tris buffer solution. To 3.8 ml of this solution, desired amount of 1 (in 100 μl MeOH) was added. After ultrasonic irradiation for 10 sec. under Ar with ice cooling, totally functionalized liposome, Tiron(i)|Lip·1 was obtained. The latter solution (3.3 ml) was mixed with 0.1 ml of an aqueous $CoSO_4$ or $CuSO_4$ solution (1 mM Tris buffer), and the rate of metal ion transport from the outside (pH 7.0, Tris-HCl buffer) to the interior aqueous solution across the liposomal membrane "functionalyzed" with "half channel" were measured by following increase of the characteristic absorption (310 nm) of $2{\cdot}M^{++}$.



2 ; 292nm(ε,3300) , Co^{++} complex , 310nm

The observed rates satisfied pseudo-first order kinetics with respect to 2 (see Fig 1). The pseudo-first order rate constants thus obtained for a given content of 1 were then plotted against content of 1 in the artificial liposome (see Fig 2). Interestingly, the rate of the Co^{++} transport followed clearly second order kinetics with respect to "the concentration" of 1 in the membrane in a rage of 0 - 55 μM, while rates of Cu^{++} transport followed satisfactory 1st order kinetics and enhancement of the transport rate was much smaller than Co^{++}.

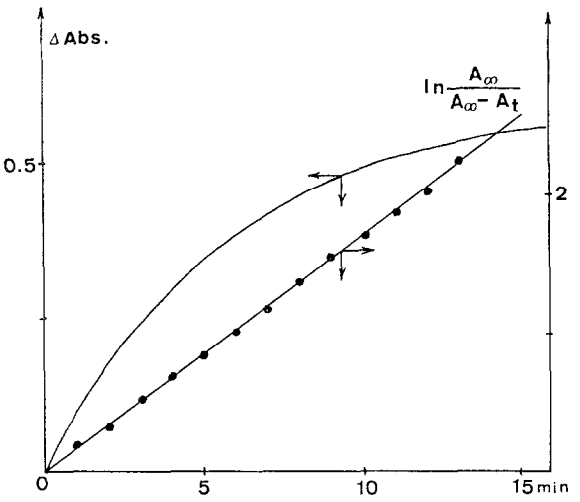


Fig. 1. Change in the absorbance at 310 nm., [Lip] = 0.3 μM, [1] = 24 μM, [2] = 150 μM, $[Cu^{++}]$ = 600 μM, at pH 7.0.

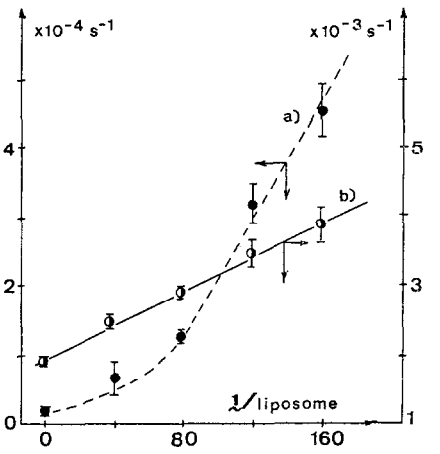


Fig. 2. Dependence of k on the concentration of 1, (a) Co^{++}, (b) Cu^{++}. All of the measurements were carried out at pH 7.0, 25.0°C.

The channel transport rate ($4.5 \times 10^{-4} sec^{-1}$ for 55 μM 1) was much faster than the specific carriers; eg., $5.4 \times 10^{-5} sec^{-1}$ under the corresponding conditions for 18-azacrown-N6. The transport rates were also sensitive to the types of potential gradients applied across the membrane, details of which will be discussed in our forthcoming article.

The present channel is so designed as to allign along lecithin molecules comfortably and to bind the metal ion moderately by several approximately equally-spaced binding sites (see Fig 3).

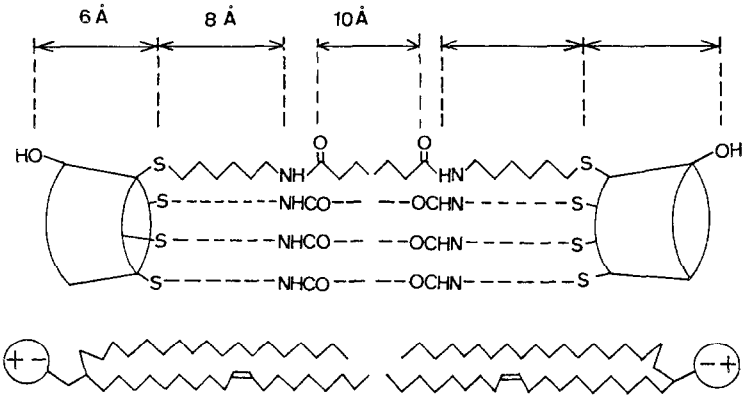
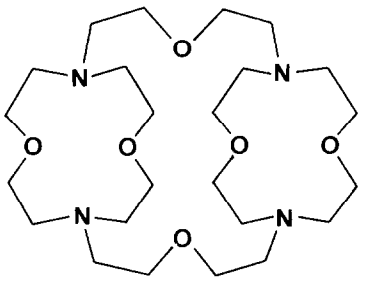


Fig. 3. The schematic representation of the alignment of the complete channel along lecithin.

The presently observed rapid metal ion "jumping" from one to another binding site within a channel is readily interpreted by the rapid metal ion "jumping" (171 sec^{-1} for Sr^{++}, 155 sec^{-1} for Ba^{++}) observed for cryptate 3.[11]



3

M — M distance ; 5.5 ~ 9 Å

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