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Translocation of alkali metal cations by lipophilic cyclodextrin derivatives through black lipid membranes

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Abstract

Lipophilic cyclodextrin (CD) derivatives, synthetic ionophores, were prepared to transport alkali metal cations across a black lipid membrane (BLM). The purpose of this study is to develop a new class of an artificial transportation system of alkali metal cations via bilayer lipid membranes, by using CD derivatives as a cation carrier. A lipophilic CD derivative incorporated into a BLM forms a complex with an alkali metal cation at one surface of the membrane. This charged complex migrates to the opposite side of the membrane and then releases the cation into the subphase. CD derivatives have various types of acyl groups as a complexing site and formed a 1:1 complex with the alkali metal cation. The complex formation was interpreted by an induced-fit mechanism. It is found that the ability of CD derivative for forming a complex and/or transporting cations across the BLM depends on the bulkiness of acyl groups. The conductivities of heptakis (2,6-di-*O*-propyl-3-*O*-propionyl)-β-CD were higher than those of valinomycin regardless of sizes of cations. The order of the conductivity in all derivatives is $Li^+ < Na^+ < K^+ \approx Rb^+ \approx Cs^+$, regardless of the types of acyl groups in the derivatives. The effects of alkali metal cation concentration in the aqueous phase and CD concentration in the membrane on the translocation are also discussed. © 1998 Elsevier Science B.V.

Keywords: Black lipid membrane; Lipophilic cyclodextrin derivative; Cation transportation

1. Introduction

Biological membranes perform a variety of important functions [1]. Membranes are highly selective permeability barriers rather than impervious walls because they contain specific molecular pumps and gates. These transport systems regulate the molecular and ionic composition of the intracellular medium. The permeability of biological membranes is highly

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selective [2]. The flow of molecules and ions between a cell and its environment is precisely regulated by specific transport systems. Of the important functions of biomembranes, the transportation of cations across the bilayer lipid membrane has attracted considerable attention. There are two possible contrasting ways antibiotics allow the transportation of cations across the bilayer lipid membrane. In the first, a particular antibiotic (e.g., gramicidin A) forms a channel that traverses the membrane [3–7]. Ions enter such a channel at one surface of the membrane and diffuse across it to the opposite side of the membrane. A second group of antibiotics (e.g., valinomycin) enables cation transportation by carrying ions across the hydrophobic region of the membrane [8–11]. This type of transportation antibiotics, called ion-carriers, form a complex with an ion at one surface of the membrane, permeate across the membrane and then finally release the ion at the opposite side of the membrane. A model system for the investigation of the translocation phenomena of ions across a biomembrane is the black lipid membrane (BLM) which only consists of lipids [12–14]. A typical conductivity of BLMs is 10^{-8} S cm^{-2}, a value comparable to that of an insulator. Incorporation of antibiotics increases the conductivity of the BLM by several orders of magnitude.

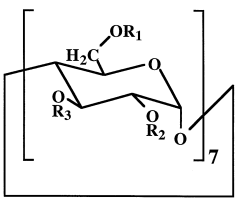
Cyclodextrins (Cds) are well-known as typical examples of organic host compounds. CD molecules are cyclic oligosaccharides containing six (α-CD), seven (β-CD), eight (γ-CD), or more anhydroglucose units joined by α-1,4 glucosidic linkages as shown in Fig. 1 [15–17]. CDs are hydrophilic hosts that can include hydrophobic guest molecules into their hydrophobic cavity. Metal cation binding properties of host compounds, which are determined by solvent extraction method, have been reported previously [18–27]. In general, the selectivity of metal cations depends on the cavity size of the host compounds. In other words, the metal cation which fits best to the host compounds is the most extractable. In the previous papers, we reported on the synthesis method of the lipophilic CD derivatives [27–31] and also reported that lipophilic CD derivatives can extract alkali metal cations from a water phase to an organic phase [27]. The purpose of this study is to develop a new class of lipophilic CD derivatives that have better ion-transportability and/or ion-selectivity than either crown ethers or valinomycin. In this paper, we report on the construction of an artificial carrier system of alkali metal cations using lipophilic CD derivatives which were incorporated into a BLM as ion carriers.

2. Experimental

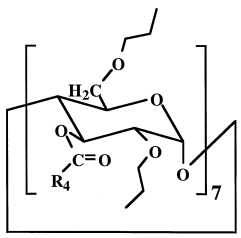
2.1. Materials

Diphytanoyl phosphatidylcholine (Avanti), valinomycin (Sigma), chloroform (Merck) and *n*-decane (Fluka) were used without further purification. LiCl, NaCl, KCl, RbCl, and CsCl (Merck) were of analytical grade and used as received. Water was purified by using a Millipore Milli-Q system.

The synthetic method of CD derivatives used in this work was reported previously [27]. The chemical structures are shown in Fig. 1. DPCD, DPACCD, DPPRCD, DPBUCD, DPISCD together with valinomycin were used for the BLM experiments.



1 R_1=R_2=R_3=H; β-CD
2 R_1=R_2=n-propyl, R_3=H; DPCD



3 R_4=CH_3; DPACCD
4 R_4=CH_3CH_2; DPPRCD
5 R_4=$CH_3CH_2CH_2$; DPBUCD
6 R_4=$(CH_3)_2CH$; DPISCD

Fig. 1. Molecular structures of β-CD and its derivatives.

2.2. BLM measurement

The lipid solution was prepared as follows: Diphytanoyl phosphatidylcholine was dissolved in chloroform and then mixed with the various amounts of valinomycin or CD derivatives. After the evaporation of chloroform, *n*-decane was added as solvent. The electrolyte solution contained one of the alkali metal chloride salts in various concentrations in Milli-Q water, the pH of which was adjusted to 6.0 by adding HCl or NaOH solution just before use. BLMs were formed by smearing the BLM-forming solution (1% (w/v) lipid in *n*-decane solution) across a 8.8×10^{-4} m diameter hole in the septum of a thermostated Teflon cell according to the way described by Müller et al. [32,33]. Two Ag/AgCl electrodes were introduced into the two separate compartments containing subphase. After smearing the BLM-forming solution the membrane became black within 10 min [34] and then ion current flowing through the membrane was measured with an electrometer-amplifier by applying a typical d.c. voltage by the two electrodes at 22°C. The conductivity per unit area of the membrane ($S\,cm^{-2}$) was calculated from the current–voltage data and the membrane area. All data are mean values from about 60 single measurements of different individual membranes and voltages.

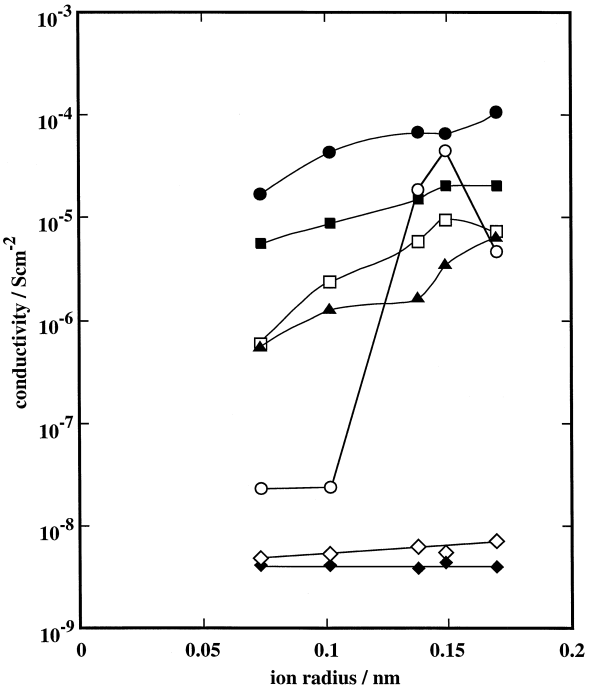


Fig. 2. Plots of conductivities of BLMs in the absence and presence of 10^{-3} M of valinomycin or CD derivatives against the radii of the alkali metal cations (Li^+: 0.074 nm, Na^+: 0.102 nm, K^+: 0.138 nm, Rb^+: 0.149 nm, Cs^+: 0.170 nm) at 22°C. The pH of aqueous phase was adjusted to 6.0. ◇: bare membrane, ◆: DPCD, □: DPACCD, ■: DPBUCD, ●: DPPRCD, ▲: DPISCD, ○: valinomycin.

3. Results and discussion

In Fig. 2, the conductivities of BLMs, each with 10^{-3} M of the various types of CD derivatives incorporated, are plotted against the radii of the translocated alkali metal cations, together with the data of a bare membrane and one which was doped by 10^{-3} M valinomycin. The conductivity of the BLM in which DPCD was incorporated was less than $10^{-8}\,S\,cm^{-2}$ and almost the same as that of bare membrane, regardless of the radii of alkali metal cations. The conductivities of BLMs in which DPACCD, DPPRCD, DPBUCD, DPISCD were incorporated ranges from 6×10^{-7} to $1 \times 10^{-4}\,S\,cm^{-2}$ which were much higher than those of a bare membrane and DPCD. The difference between DPCD and other derivatives is the acyl group in the 3-position of the CD derivatives. Therefore, acyl groups in CD derivatives are obviously needed for the interaction with inclusion of alkali metal cations. The order of the conductivity values in all cases is $Li^+ < Na^+ < K^+ \approx Rb^+ \approx Cs^+$. The order of the conductivity values in CD derivatives is DPISCD < DPACCD < DPBUCD < DPPRCD. The difference in these CD derivatives is the bulkiness. DPPRCD might have the best matching cavity towards the alkali metal cations. Although the bulkiness of acyl groups changes the conductivity, these CD derivatives did not have particular ion-selectivity for alkali metal cations as valinomycin has. The ion radius difference from Li^+ to Cs^+ is about 0.1 nm. CD derivatives have less ability to recognize this ion radius difference. Previously [27], we reported the results of solvent extraction experiments for the same samples as the ones used in this work. DPACCD, DPPRCD, and DPBUCD could extract the alkali metal cations from a water phase to an organic solvent, but not DPISCD, although the inclusion ability towards the alkali metal cations is almost the same. The cation complex of a CD derivative is required to interact with a co-anion and extract at the

same time because of the charge neutralization in the case of solvent extraction. Since DPISCD could not form a complex with the alkali metal cation and the co-anion due to the bulkiness of isobutyryl group, it could not extract alkali metal cations from a water phase into an organic phase. On the other hand, as DPISCD does not need to interact with a co-anion in the case of a BLM experiment, it could transport alkali metal cations across a membrane.

The increase in membrane conductivity is strictly proportional to the CD derivative concentration in the membrane over several orders of magnitude. This is shown in Fig. 3 for DPPRCD in the presence of 1 M KCl, pH 6.0. In the concentration region from 10^{-5} to 10^{-2} M examined in this work, the conductivity is proportional to the concentration of CD derivatives with a slope of nearly 1.

The conductivity of a BLM, in which 10^{-3} M of DPPRCD was incorporated, is plotted in Fig. 4 as a function of the KCl electrolyte concentration. From 10^{-3} to 10^{-1} M, the conductivity is proportional to the concentration of KCl in the subphase with a slope of 1. At higher concentrations of KCl in the electrolyte, a gradual saturation is found.

From the results of Figs. 3 and 4, it is concluded that the translocation of metal cations by CD derivatives through the BLMs depends in a linear way on the concentrations of CD derivatives in the bilayer membrane and of the metal ion at the water–lipid interface. The higher the concentrations of metal cations at the interface and CD derivatives in the membrane, the better is the transportation efficiency of alkali metal cations owing to the probability of the complexation or inclusion reaction at the membrane surface.

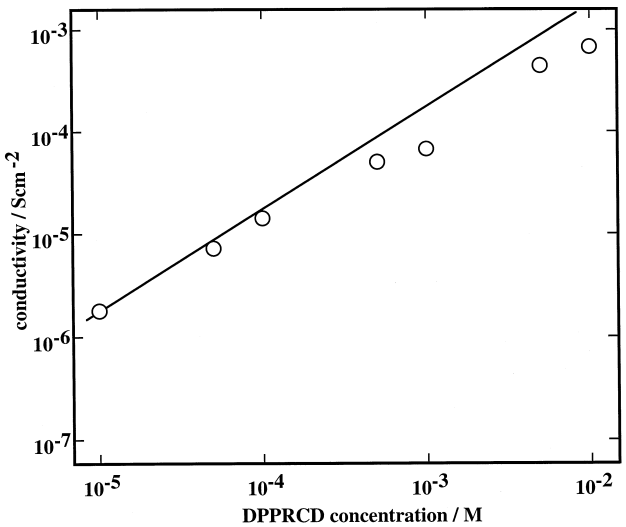


Fig. 3. Plots of conductivities of BLMs against the DPPRCD concentration in the membrane at 22°C. Aqueous phase was 1 M KCl, pH 6.0. The line with a slope of 1 in this figure is shown to compare with the obtained data.

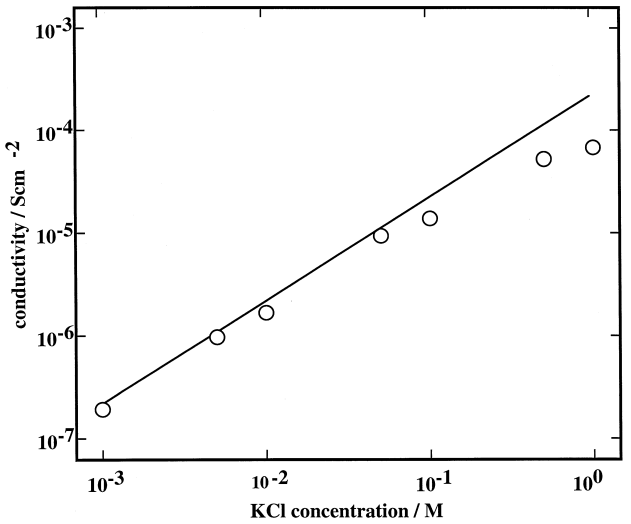


Fig. 4. Plots of conductivities of BLMs, in which 10^{-3} M of DPPRCD was incorporated, against KCl concentration at 22°C. The pH of aqueous phase was adjusted to 6.0. The line with a slope of 1 in this figure is shown to compare with the obtained data.

As noted previously [27], CD derivatives form a 1:1 complex with alkali metal cations as shown in Fig. 5(a). Various types of acyl groups are the complexing sites with the alkali metal cations. These are coordinated to the oxygen atoms of acyl groups. On comparing this with the results from Figs. 3 and 4, we propose a mechanism of cation translocation as shown in Fig. 5(b) in analogy to the valinomycin carrier mechanism [35]. A CD derivative incorporated into a BLM forms a complex with a metal cation at one surface of the membrane. In this adsorbed state, some of the carbonyl groups of the CD derivatives may point towards the aqueous phase whereas the apolar side chains may be oriented towards the hydrocarbon interior of the membrane. This charged complex permeates across the membrane due to the applied voltage and then releases the cation at the opposite side of the membrane.

When substrate and enzyme interact and bind to each other, the substrate generally has a matching

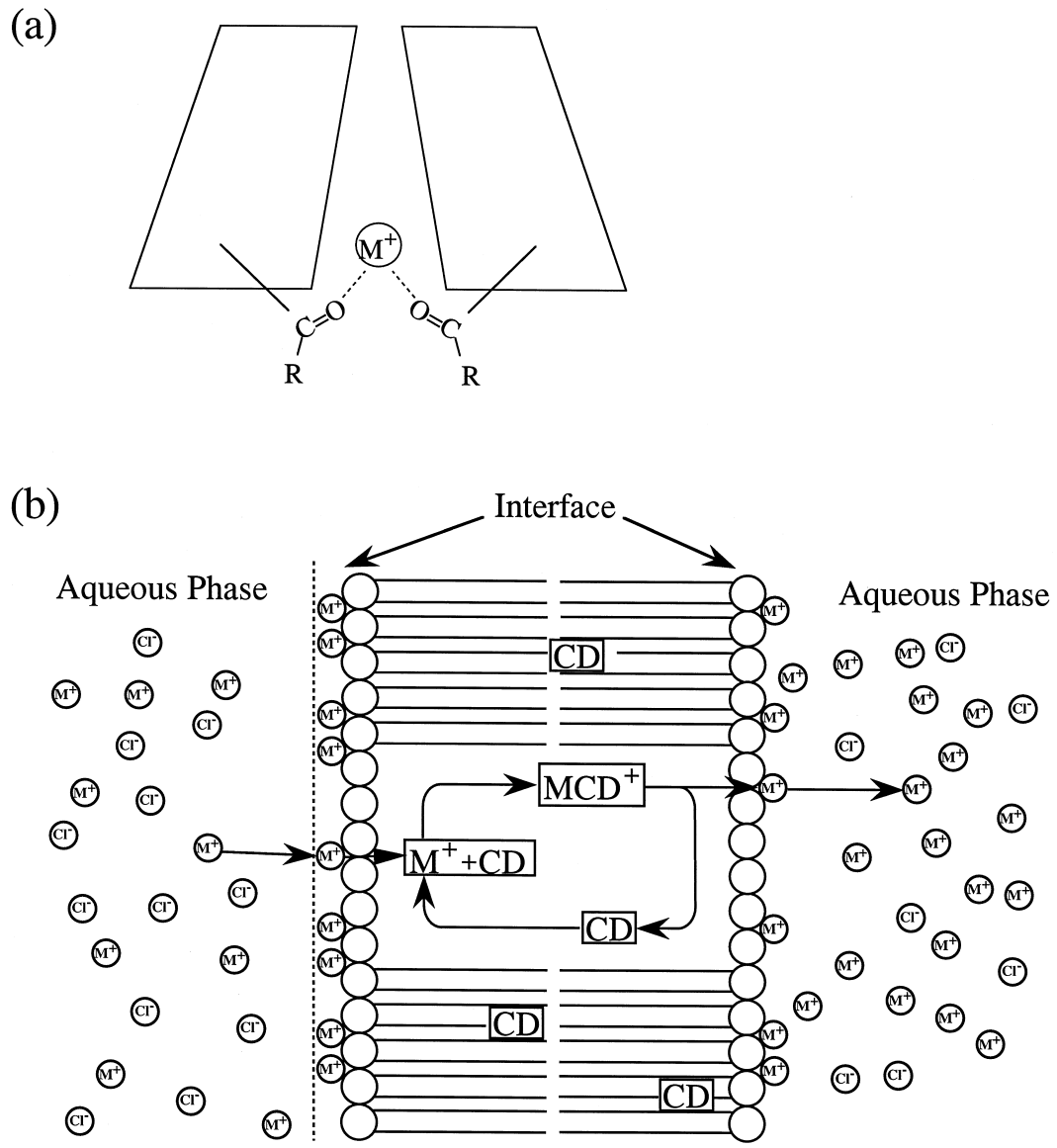


Fig. 5. (a) Schematic diagram of the complex structure between CD derivative and an alkali metal cation. (b) Schematic diagram of the cation translocation mechanism through the BLM by CD derivatives.

shape so as to fit into the site of enzyme (lock and key model). However, the shapes of the active sites of some enzymes (e.g. carboxypeptidase A, yeast hexokinase, citrate synthase, mitochondrial aspartate aminotransferase, etc.) are remarkably modified by the binding of their substrate [36]. The enzymes undergo relatively large structural changes when the substrate is bound. The active sites of these enzymes have shapes that are complementary to that of the substrate only after the substrate is bound. This process of recognition is called induced-fit. Ueno et al. reported that the attachment of a naphthalene moiety to γ-CD enables this modified γ-CD to become a good host for small molecules, due to an induced-fit type of complexation, i.e., space-regulating effect of the appending naphthalene moiety [37]. If the ion binding of the host compound is restricted by only the pore size fitness with the guest cation like in the case of valinomycin or crown ether derivatives, the results obtained by BLM experiments show the well-known ion-selectivity. On the other hand, the translocation behaviors of CD derivatives as shown in Fig. 2

could be interpreted by the above mentioned induced-fit mechanism, provided the CD derivatives undergo structural changes due to the flexibility of the complexing site when the alkali metal cation is included into the cavity. Translocation of alkali metal cations by CD derivatives used in this study depends not only on the size fitness, but also on the ion–dipole interaction between the acyl groups and the cations, steric (depth) effect of the cavity, space-regulating effect (induced-fit), etc.

We have demonstrated the first stage of an artificial membrane system which can transport the univalent metal cation by using lipophilic CD derivatives, although these CD derivatives have no special ion-selectivity for alkali metal cations. CD derivatives catalyze the translocation of cations through membranes by making them soluble into lipid bilayer. On the other hand, the cation transportation mechanism of this experiment could be interpreted by an induced-fit mechanism, so that the complex formation of CD derivatives could be considered as one model of the formation of an enzyme–substrate complex in enzymatic catalysis.

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