Pure Appl. Chem., Vol. 80, No. 7, pp. 1511–1524, 2008.
doi:10.1351/pac200880071511

Advances in physical chemistry and pharmaceutical applications of cyclodextrins*

Noriaki Funasaki[1,‡], Seiji Ishikawa[1], and Saburo Neya[2]

[1]Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan; [2]Graduate School of Pharmaceutical Sciences, Chiba University, Yayoi, Inage, Chiba, 263-8522, Japan

Abstract: Cyclodextrins (CDs) attract much attention for industrial applications and academic research. A few experimental methods for determination of the binding constant between CD and a guest molecule were reviewed critically. A hydrophile–hydrophobe matching model for host–guest docking was proposed for estimation of the binding constant and the solution structure of the complex. Rather detailed solution structures of CD complexes were determined by proton NMR spectroscopy, aided by calculations of molecular mechanics and surface areas, and were used to analyze the binding constants. The binding constants of CDs with multi-site guests were analyzed on the basis of their solution structures. The working mechanisms and physicochemical predictions in a few pharmaceutical applications of CDs were proposed on the basis of detailed solution structures and accurate binding constants.

Keywords: cyclodextrins; solution structures; binding constants; docking model; pharmaceutical applications.

INTRODUCTION

Cyclodextrins (CDs) have homogeneous toroidal structures of different cavity sizes. Three of the most characterized CDs are α-, β-, and γ-CDs, which contain six, seven, and eight glucose units, respectively. The toroidal structure has a hydrophilic surface resulting from the 2-, 3-, and 6-position hydroxyls, making CDs water-soluble. Its cavity consists of the methin groups, giving it a hydrophobic character. As a consequence, CDs can include other hydrophobic molecules of appropriate dimensions inside their cavities. To a first approximation, the magnitude of binding constants correlates with the fit of the guest in the CD cavity.

CDs can give beneficial modifications of guest molecules not otherwise achievable: solubility enhancement, stabilization of labile guests, control of volatility and sublimation, and physical isolation of incompatible compounds. Because they are practically nontoxic, they are added into pharmaceuticals and foods. Modified CDs are synthesized to enhance aqueous solubility, functions, and guest specificity of native CDs. Some of them can exhibit high specificity, remarkable catalysis, and chiral separations [1,2].

In some reviews and books, the data on the crystal and solution structures of CD complexes and the binding constants were summarized and several driving forces of CD complexation were suggested

*Paper based on a presentation at the International Conference on Modern Physical Chemistry for Advanced Materials (MPC '07), 26–30 June 2007, Kharkiv, Ukraine. Other presentations are published in this issue, pp. 1365–1630.
‡Corresponding author

[1–12]. These forces between CD and guest include van der Waals forces, hydrophobic interactions, and hydrogen bonds, and dipole–dipole interactions. Such driving forces of complex formation, despite the many papers dedicated to this problem, have not yet been understood fully. Molecular mechanics and molecular dynamics calculations have been applied to estimate the structures of CD complexes and have been compared with experimental data [7].

CD is one of the best-characterized host molecules and provides a wealth of knowledge for supramolecular chemistry. Supramolecular chemistry has by now become a major field of chemistry. For instance, the development of supramolecular chemistry requires the availability of powerful methods for the investigation of the structural, dynamic, and physicochemical features of supramolecular chemistry [13].

On the basis of our work, recent advances in a few experimental methods for binding constant determination, a novel docking model, the solution structures of CD inclusion complexes, the solution structures and binding constants of multiple complexes, and a few pharmaceutical applications of CDs will be reviewed.

BINDING CONSTANT DETERMINATION

Most of the physicochemical properties of a mixed guest and CD solution will be related with the binding constant between them. This fact suggests that these properties can be used to determine the binding constants. The binding constant is determined by spectroscopic methods, thermodynamic methods (calorimetry, potentiometry, molar volumes, surface tension, and others), measurements of transport properties, measurements of colligative properties [6,8,11,12]. These methods utilize the difference in property between the free and bound species. Any property of a solution containing free guest, free CD, and one or more complexes is related to the sum of contributions weighted by the concentrations of some or all of these species. If a guest molecule forms the dimer and larger multimers, these self-associations should be taken into account. The contribution and the concentration of each species are usually fitted to the observed value of the property by nonlinear least-squares method and are used to determine the binding constant and the property of the complex. To analyze any solution property, one will make some assumptions and approximations. These lead to more or less inaccurate binding constants. It is generally difficult to determine very small and very large binding constants. The reliability of the binding constant will be judged from the agreement among the values determined by several methods and researchers and from comparison with the binding constants of related compounds. Three novel or refined methods, developed by us, will be outlined below.

Surface tension method

No natural CD changes the surface tension of water. As shown in Fig. 1, the complex of a CD with a surface-active substance, such as surfactant, could be assumed not to be absorbed at the air–water interface, so that it would not reduce the surface tension of their aqueous solution. Under these conditions, the surface tension of the aqueous solution depends on the concentration of the free surfactant molecule alone [14–19]. Therefore, we can determine the concentration of free surfactant molecules and the binding constant from the observed surface tension. In the surface tension method, it is a problem whether the complex is completely surface inactive or not. A piece of evidence for the surface inactivity is that the surface tension of surfactant solutions above the critical micelle concentration (cmc) is not influenced by the presence of CDs. The second evidence is that the surface tension of aqueous solutions of a constant surfactant concentration approaches that of pure water with increasing CD concentration [14,15].

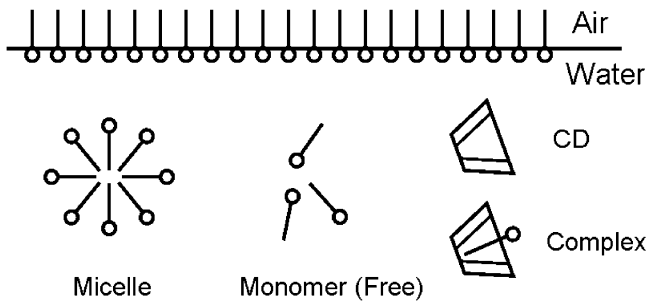

Fig. 1 Equilibria among the surface adsorption, the micelle formation, and the complex formation in a mixed CD and surfactant solution.

For instance, dodecyl maltoside and α-CD can form the 1:1 and 1:2 complexes. The surface tension of dodecyl maltoside solutions above the cmc remained unchanged with addition of α-, β-, and γ-CDs. This fact clearly indicates that all of their complexes are surface-inactive. As shown in Fig. 1, no CD molecule is bound to the micelle, so that the concentration of free surfactant molecules in mixed dodecyl maltoside and CD solutions above the cmc is equal to the cmc of dodecyl maltoside, regardless of the kind and concentration of CD. Thus, the apparent increase of the cmc with addition of CD is due to the presence of the complex [14].

The surface tension method was applied to the binary systems of natural CDs with surfactants and drugs and allowed us to determine reasonable stoichiometries of complexes and binding constants [14–17]. This method will be applied to solutions below the cmc. The surface tension method is applicable to weakly surface-active modified CDs [18].

Chemical shift method

The NMR chemical shift is referred to an internal or external standard (Fig. 2). The external standard method has the merit of no intermolecular interaction of the standard with all solutes, because they are separated in two tubes. This method, however, has the demerit of the disparity between the volume magnetic susceptibilities of the two solutions. On the other hand, the internal standard method has the merit of an equivalent magnetic susceptibility, though it has the demerit of possible intermolecular interactions of the standard with solutes in a single solution [20–22]. The chemical shift method for binding

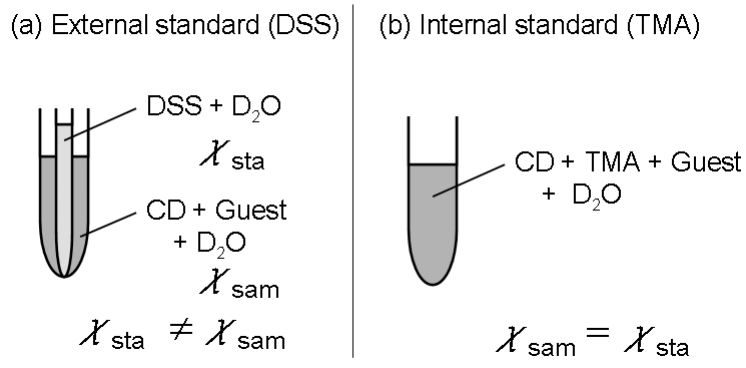

Fig. 2 External and internal standard methods for the chemical shift determination: DSS = sodium 4,4-dimethyl-4-silapentanesulfonate, TMA = tetramethylammonium chloride, and χ = volume magnetic susceptibility.

constant determination has the merit of providing information about the solution structure of the complex [23–33].

The chemical shifts of the free and bound species are generally different from each other, so that they can allow us to determine the binding constant. The chemical shift, δ, of internal tetramethylammonium chloride (TMA), referred to an external standard, changed linearly with increasing CD concentration C_2. This linear change was ascribed to the change in volume magnetic susceptibility of the CD solution

$$\delta = \delta_0 + 4\pi(\chi_2 - \chi_w)V_2C_2/3000 \quad (1)$$

Here, χ_2 and χ_w denote the volume magnetic susceptibilities of CD and water, and V_2 stands for the molar volume of CD. This equation holds true for linear and cyclic oligosaccharides, oligoglycines, organic solvents, and sodium chloride [21]. The chemical shift corrected for this magnetic susceptibility change gives a reasonable binding constant [20,23]. The internal standard method does not require this correction, but needs an inert internal compound.

The internal standard method for chemical shift determination will be better than the external method. However, one should select an internal standard most carefully, because it can interact with solutes present in a solution [20,21]. Electrostatic and hydrophobic interactions between solutes play important roles in aqueous solutions. For instance, sodium 4,4-dimethyl-4-silapentanesulfonate, which is very often used as the internal standard in aqueous solutions, can form complexes with CDs and hydrophobic cations [22]. TMA and sodium methyl sulfate are very good internal standards for cationic and anionic compounds, respectively. Methanol is a very good internal standard for most solutes [22]. Water is a good internal standard, if temperature is kept accurately constant [20,24].

Indirect competitive potentiometry

Recent advances in electrochemistry enable us to determine the concentration of organic ions with an ion-selective electrode. Potentiometry is one of the best methods for binding constant determination of ionic guests [34,35]. For instance, the concentration of the free octyltrimethylammonium (OT) ion can be determined from the electromotive force of an OT ion electrode. This concentration is used to determine the binding constant (K_{OT}) between OT and α-CD [36]

$$\mathrm{CD + OT} \overset{K_{OT}}{\leftrightarrow} \mathrm{CD{\cdot}OT} \quad (2)$$

This method has very recently been extended to nonionic guests. CDs can bind nonionic organic compounds, such as alcohols (AL)

$$\mathrm{CD + AL} \overset{K_{AL}}{\leftrightarrow} \mathrm{CD{\cdot}AL} \quad (3)$$

The addition of alcohol in a mixed CD and OT solution indirectly increases the concentration of the free OT ion, as the result of competitive binding of AL and OT to CD. Quantitative analysis of this increase measured with the OT ion electrode yields the 1:1 binding constant (K_{AL}) between AL and α-CD. This was close to the literature value of the alcohol [36].

However, there are a few difficulties in indirect competitive potentiometry. For instance, an appropriate ionic compound must be chosen to construct an electrode. Nonionic compounds may dissolve into the polyvinyl chloride membrane and may modify the response of the electrode. The reproducibility of electromotive forces determined by indirect competitive potentiometry is not very high at the present stage [36]. Nevertheless, this will become a promising technique for determination of nonionic compounds by potentiometry. This method is similar to the dye method that utilizes competitive binding of a dye and a guest to CDs [37].

NOVEL HOST–GUEST DOCKING MODEL

Water-accessible molecular surface areas of solutes are calculated by analytical and numerical methods and are very often used to estimate the hydrophobic properties of the solutes, such as oil–water partition coefficients, aqueous solubilities, chromatographic retention times, cmc's, and binding constants [6,12,38,39].

It has often been suggested that steric complementarity in size and shape of host and guest plays an essential role in docking between them [13]. This model had often been suggested for qualitative interpretations of the structure and function of protein. We have recently proposed that the complementarity could be estimated from the magnitude of the contact area (ΔA) between host and guest, as shown in Fig. 3. The second factor for better docking is related to the nature of the contact area. The molecular surface area of each molecule consists of the hydrophobic area (A_o) and the hydrophilic area (A_w). As the contact area (ΔA_{oo}) between the hydrophobic surfaces of host and guest and that (ΔA_{ww}) between their hydrophilic surfaces increase, respectively, their complex will be stabilized better. On the other hand, as the contact area (ΔA_{ow}) between the hydrophobic surface and the hydrophilic surface of host and guest decreases, their complex will be stabilized better. Namely, better hydrophile–hydrophobe matching of the contact surface causes stronger binding [40].

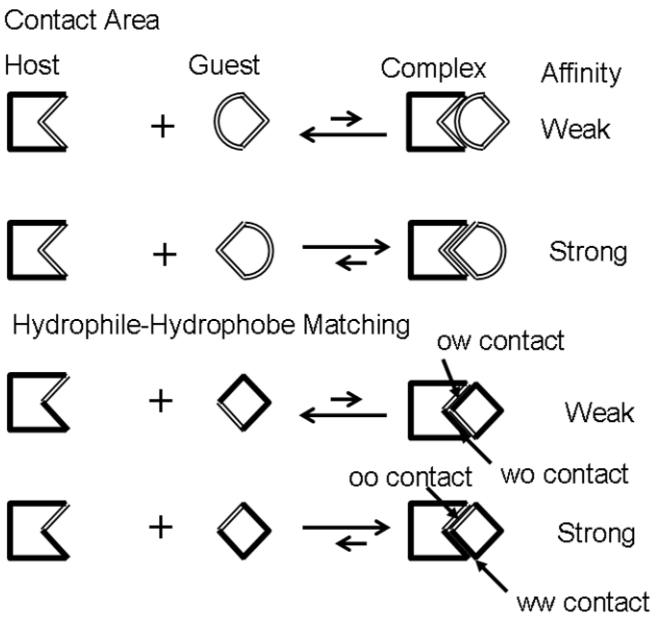

Fig. 3 Effects of sizes, shapes, and hydrophile–hydrophobe matching upon host–guest docking. Thick lines and double thin lines denote the hydrophilic and -phobic surfaces, respectively.

To verify this model, we developed molecular modeling software named Mihochan (Molecular Information Handling Option for Chemical Analysis). A Mihochan's view on a display is shown in Fig. 4, where a cross-section of the crystal structure of the 1:1 complex between β-CD and 4-*tert*-butylbenzyl alcohol is depicted. Mihochan has several useful functions to investigate the structure of the complex; independent movements of host and guest, calculations of molecular structural parameters (atomic coordinates, dihedral angles, interatomic distances, molecular surface areas, and others), visualization of the structure of the complex, and theoretical calculations of proton chemical shifts induced by benzene. These calculations can be performed by pushing in some buttons on the display [40].

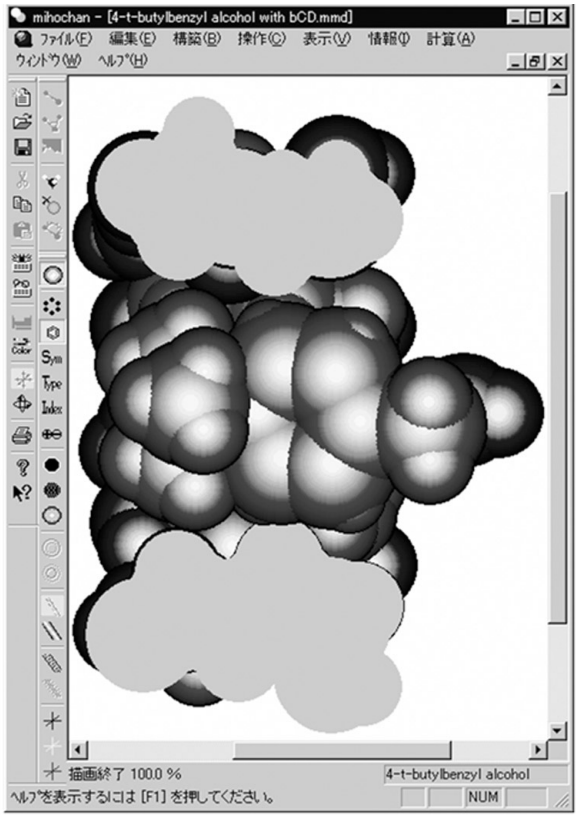

Fig. 4 A Mihochan's view of the crystal structure of the complex between β-CD and 4-*tert*-butylbenzyl alcohol on Windows. These molecules can be moved independently to change their structures. Atomic coordinates, dihedral angles, inter-atomic distances, molecular surface areas, and other structural parameters can be obtained by pushing in some buttons on the display.

We moved the 4-*tert*-butylbenzyl alcohol molecule along the symmetry axis of β-CD and calculated the ΔA_{oo} value at each translational movement. The ΔA_{oo} value exhibits the maximum around the crystal structure: this structure is stabilized by hydrophobic interactions. Therefore, from the maximal ΔA_{oo} value, we can predict a stable solution structure of the CD complex. Furthermore, for 11 binary complexes of aliphatic and aromatic guests with α-, β-, and γ-CDs, the observed 1:1 binding constants were correlated with the maximal ΔA_{oo} values as follows [40]:

$$\text{Log } K = 1.803\Delta A_{oo} - 2.023 \text{ (correlation coefficient = 0.9087)} \quad (4)$$

Applications of this equation to other complexes will be described below.

SOLUTION STRUCTURES AND BINDING CONSTANTS OF CD COMPLEXES

To verify the hydrophile–hydrophobe matching model, we need the coordinates of all atoms of the complex. We determined rather detailed solution structures of CD complexes by NMR techniques aided with molecular mechanical calculations. As two protons approach more closely, the cross-peak on the rotating frame Overhauser effect spectroscopy (ROESY) spectrum becomes larger. This relation between the ROESY cross-peak intensity and the inter-proton distance was quantitatively analyzed to determine a detailed solution structure of the complex. The chemical shift change with complex formation provides important clues to image the solution structure of the complex. The vicinal spin–spin

coupling constants were used to estimate the conformations of guests and CDs. When the crystal structure of the complex is available, it was used as the starting structure. Molecular dynamics simulations provide detailed information about molecular motions of CD complexes. However, we do not have experimental data to test most of the predicted information. The prediction of flexibility of the CD cavity needs calculations of molecular mechanics or molecular dynamics. Molecular surface area calculations were used to predict the position of guest in the CD cavity [25,27,40]. Finally, it is noted that the major solution structure is estimated by NMR and molecular mechanical calculations, whereas minor structures are usually neglected.

Aromatic guests

When sodium benzenesulfonate (BS) is incorporated in an α-CD cavity, the chemical shifts of the CD protons depend on the geometry of the complex. The ring current effect of benzene on the chemical shift is well established theoretically and allows us to estimate the solution structure of the BS-α-CD complex from the observed chemical shifts of the α-CD protons. Four solution structures of the benzenesulfonate (BS)-α-CD complex were estimated from analysis of the observed proton chemical shifts and molecular surface area calculations. The crystal structure of this complex is available. Further, the binding constants for these five structures were predicted from eq. 4 using the calculated ΔA_{oo} values. Although these structures are similar to one another at first glance, the theoretical chemical shifts for the NMR30 structure are the best fitting to the observed values and the calculated binding constant (11.8 M^{-1}) for this structure is close to the observed value of 9.8 M^{-1} [25]. The NMR30 structure is consistent with the ROESY spectrum of the BS-α-CD complex.

The ring current shift was also employed to determine the solution structure of the propanetheline bromide (PB)-α-CD complex. Because PB has two phenyl groups, the theoretical chemical shifts of the CD protons are the sum of these contributions. PB can form the dimer, which dissociates into a bivalent cation and two bromide ions. The chemical shift changes with this dimerization must be taken into consideration [24]. Molecular mechanical calculations (e.g., with CVFF force fields) predict energetically stable structures in the presence of water. The solution structure of the PB-α-CD complex, estimated by molecular mechanics calculations, is in a good agreement with the observed chemical shift data [41].

Aliphatic guests

In the crystal structures of the 1:1 complexes with α-CD, the sulfonate group of propanesulfonate is at the secondary alcohol side, whereas the hydroxyl group of propanol is at the primary alcohol side. The solution structures of these α-CD complexes were determined from the best correlation between the ROESY cross-peak intensity and the inter-proton distance. The hydrophilic groups of these two guest molecules are both at the secondary alcohol side. Thus, the solution structure of the propanol-α-CD complex is different from the crystal structure [27].

Furthermore, we investigated the intermolecular interactions between α-CD and propanol on the basis of molecular surface area calculations. The solution structure of this complex has the maximal values of ΔAoo and ΔAww and the minimal ΔAow value in all translated structures. These results clearly demonstrate the validity of our hydrophile–hydrophobe matching model of the contact area [27,40].

The solution structures of the 1:1 complexes of α-CD with hexyl (HTAB), octyl (OTAB), and dodecyltrimethylammonum bromide (DTAB) were determined by ROESY [28,30]. As shown in Fig. 5, the position of the alkyl chain in the α-CD cavity regularly changes with increasing length of the alkyl chain. The chemical shift change with complex formation depends on the position of the proton of the guest molecule in the α-CD cavity. The proton of the guest molecule between H3 and H5 of α-CD exhibits the largest change. This largest change decreases with increasing length of the alkyl chain. This

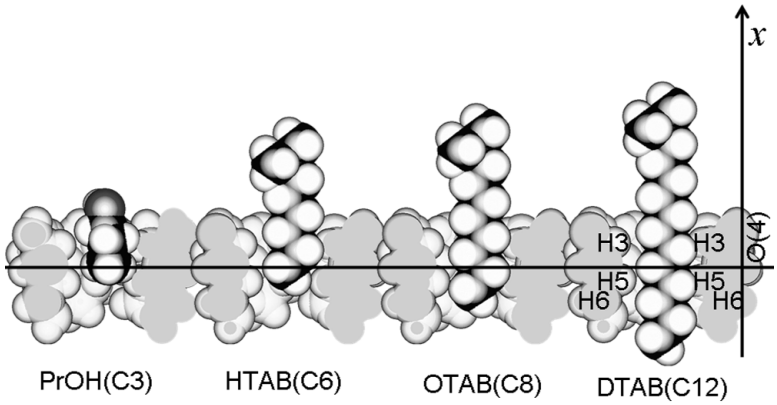

Fig. 5 Solution structures of the 1:1 complexes of α-CD with propanol and HTABs, OTABs, and DTABs.

result suggests the translational motion of the alkyl chain: the translational motion seems to become wider with increasing length of the alkyl chain [30].

The translational and other motions of DTAB in the α-CD cavity were investigated theoretically by molecular dynamics simulations. The most probable structure predicted by these simulations is close to the NMR structure [42].

The analysis of the chemical shift change with the concentration of DTAB reveals that DTAB and α-CD form the 1:2 complex. The solution structure of this complex was determined by ROESY spectroscopy: the secondary alcohols of two α-CD molecules are in close contact [30].

STRUCTURES AND BINDING CONSTANTS OF MULTIPLE COMPLEXES

The dissociation constants of acids and bases are investigated most extensively in the multiple equilibria. These results serve to analyze the multiple equilibria of CD complexations. The literature values of macroscopic 1:2 and 2:1 binding constants are much less reliable than macroscopic 1:1 binding constants. There is no rigorous method to determine these microscopic binding constants, though the chemical shift method will generally provide better values than any others. To estimate two microscopic 1:1 binding constants of a bivalent guest, we would use the macroscopic binding constants of two related univalent guests [31]. If we estimate the microscopic 1:1 binding constants, we can analyze the molecular interactions between the two binding sites. In general, when they are distant from each other, they can bind independently.

Diheptanoylphosphatidylcholine

Diheptanoylphosphatidylcholine (DHPC) has two heptanoyl chains at positions 1 and 2 of the glycerol moiety. DHPC has three conformers (gauche$^+$, gauche$^-$, and trans) different in dihedral angles of the glycerol moiety, and their populations can be determined from the vicinal spin–spin coupling constants of the HXC(2)-C(1)HAHB spin system (Fig. 6). DHPC forms the 1:1 and 1:2 complexes with α-CD, whereas it forms mainly the 1:1 complexes with β- and γ-CDs [23,26]. The α-methylene protons of 1- and 2-heptanoyl chains have slightly different chemical shifts, which allowed us to estimate preferential binding of these chains to CD: there was no preference to α-CD [23].

From two vicinal spin-spin coupling constants, we can estimate microscopic binding constants for the three conformers of DHPC with CD. The trans conformer has the largest 1:1 and 1:2 binding constants to α-CD among the three conformers, and has the smallest binding constants to β- and γ-CDs

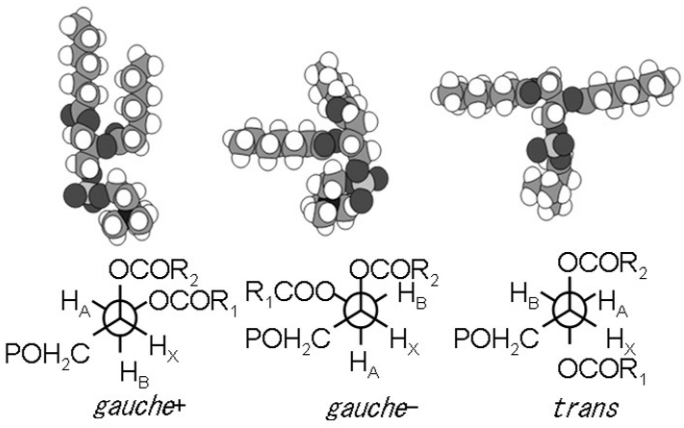

Fig. 6 Rotational isomers of diheptanoyllecithin around the C1–C2 bond axis of the glycerol moiety [23].

among the three conformers. Two heptanoyl chains of the gauche$^+$ conformer are incorporated simultaneously in a cavity for β- and γ-CDs to form 1:1 complexes [23,26].

The proton chemical shift, molecular mechanics calculations, and the ROESY spectra were used to image the solution structures of the complexes of DHPC with α-, β-, and γ-CDs [23,26]. For instance, the macrocycle of β-CD is elliptically deformed by simultaneous incorporation of two heptanoyl chains in the β-CD cavity [26].

Dialkyldimethylammonium bromides

CD and guest can form complexes of 1:1, 1:2, 2:1, 2:2, and other ratios [14]. For simplicity, we deal with multiple equilibria for a system forming two 1:1 complexes and a single 1:2 complex. For instance, hexyldimethyloctylammonium bromide (HDOAB) can form the 1:1 and 1:2 complexes with α-CD, as shown in Fig. 7. The 1:1 complex consists of the hexyl-in and octyl-in complexes. Generally, we write these three complexes of a bivalent guest XY with CD as DXY, XYD, and DXYD. Then we can define four microscopic equilibrium constants as follows: k_{1X} = [DXY]/[XY][D], k_{1Y} = [XYD]/[XY][D], k_{2X} = [DXYD]/[XYD][D], and k_{2Y} = [DXYD]/[DXY][D]. The macroscopic 1:1 and 1:2 binding constants (K_1 and K_2) are connected with the microscopic constants as follows [31,33,35]:

$$K_1 = \{[DXY] + [XYD]\}/[XY][D] = k_{1X} + k_{1Y} \quad (5)$$

$$K_2 = [DXYD]/\{[XYD] + [DXY]\}[D] = k_{2X}k_{2Y}/(k_{2X} + k_{2Y}) \quad (6)$$

If the two binding sites bind CD independently, these microscopic binding constants can be substituted by the macroscopic 1:1 binding constants of CD with two corresponding univalent guests. As univalent guests for HDOAB, HTAB and OTABs were used. Thus, the mole fraction of the hexyl-in complex in the two 1:1 complexes was estimated to be 0.108 from eq. 5 using the binding constants of α-CD with HTAB and OTAB [31].

The hexyl ω-methyl group of HDOAB is distinguishable from the octyl ω-methyl group on a proton NMR spectrum, so that we can independently determine these chemical shift changes upon complex formation with α-CD. For instance, the chemical shift change ($\Delta\delta_1$) of the hexyl ω-methyl group with the 1:1 complex formation is smaller than that ($\Delta\delta_2$) with the 1:2 complex formation. The ratio $\Delta\delta_1/\Delta\delta_2$ gives an estimation of the mole fraction of the hexyl-in complex of 0.102. Similarly, the mole fraction of the octyl-in complex was estimated to be 0.916. The sum of these mole fractions is close to 1. This agreement demonstrates the validity of this second estimation. Thus, the mole fraction of the hexyl-in complex is 0.1 from both of the binding constants and the chemical shift changes [31].

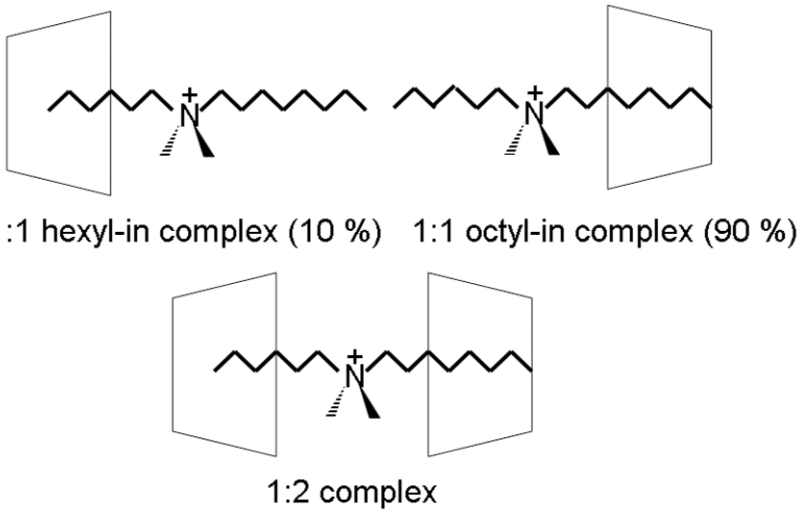

Fig. 7 Structures of 1:1 and 1:2 complexes between α-CD and HDOAB [31].

Didecyldimethylammonium bromide has two equivalent binding sites to α-CD. If two binding sites are equivalent and independent, we can expect $K_1 = 2k_1$ and $K_2 = k_2/2 = K_1/4$ from eqs. 5 and 6. Using these equations, we analyzed the 1:1 and 1:2 binding constants determined by potentiometry with a didecyldimethylammonium ion electrode. Didecyldimethylammonium bromide forms the 1:1 complex with γ-CD, where two dodecyl chains are simultaneously incorporated in a single γ-CD cavity [35]. Complex formation of α-CD with dioctyldimethylammonium and didecyldimethylammonium bromides was investigated by measurements of proton chemical shifts. From the 1:1 and 1:2 binding constants and the chemical shift changes, it was concluded that the two equivalent chains bind α-CD independently [33]. Recently, complex formation between CD and gemini surfactants was reported [43].

Oxyphenonium bromide

Oxyphenonium bromide (OB) has the phenyl and cyclohexyl groups to bind α-CD. Measurements of electromotive forces and chemical shifts independently established that they form the 1:1 complex alone [29,34]. The proximity of the phenyl and cyclohexyl groups, which both are chemically bound to the asymmetric carbon atom, will prevent OB from forming the 1:2 complex with α-CD. This binary system simultaneously forms two 1:1 complexes, the phenyl-in and cyclohexyl-in complexes, and their ratio has been estimated from binding constants, chemical shift changes, and ROESY data. The conformational change of OB induced by α-CD inclusion was estimated from the chemical shifts of the cyclohexyl protons on the basis of the ring current effect [29]. OB forms only 1:1 complexes with either β- or γ-CD. The cyclohexyl group of OB is incorporated in a β-CD cavity, whereas the phenyl group remains outside the secondary hydroxyl group. These two binding sites of OB are incorporated both in a γ-CD cavity, whereas the ammonium group remains outside the primary hydroxyl group [32].

The binding constants of OB with α-, β-, and γ-CDs were determined by UV spectroscopy, NMR spectroscopy, and potentiometry and they are almost independent from the methods [29,32,34,44]. The binding constant of γ-CD is slightly larger than that of α-CD, and is 90-fold smaller than that of β-CD. Most of the chemical modifications of β-CD decrease the binding constant, though negative ionization increases it by electrostatic attraction of the positive OB ion. Thus, the size of the CD cavity and chemical modifications of β-CD affect the binding constant and the solution structure of the complex with OB [32,34] and other guests [45,46].

PHARMACEUTICAL APPLICATIONS OF CDs

As outlined in the Introduction, CDs have many industrial applications. It is noted that CDs have some toxicities [2,10,47]. Here we will focus on three pharmaceutical applications of CDs.

Suppression of bitter taste

Propantheline bromide (PB) is a bitter anticholinergic drug. As the PB concentration is increased, the aqueous PB solution tastes more bitter. The bitter taste intensity was evaluated as one of the six bitterness scores ranging from 0 (no bitter taste) to 5 (extremely bitter taste). For instance, the 1.5 mM PB solution tastes very bitter (bitterness score of 4). Addition of α-, β- or γ-CD into this solution reduces the bitter score. The reason for this reduction is the formation of PB-CD complexes, which taste less bitter. CDs having a larger binding constant to PB can suppress the bitter taste intensity more strongly. Generally, bitter compounds are hydrophobic. The hydrophobic xanthene ring of PB will be the cause for the bitter taste. This ring is more or less incorporated in a CD cavity [24,41], so that the PB-CD complex exhibits little bitter taste. Therefore, we can assume that the bitter taste intensity of a mixed PB and CD solution is determined by the concentration ([PB]) of free PB [15]:

$$\text{Bitter taste intensity} = f([\text{PB}]) \tag{7}$$

The concentration of free PB molecules can be estimated from the binding constant. The surface tension of this solution depends on the concentration of free PB alone. Therefore, the bitter taste of a mixed PB and CD solution is a function of surface tension alone, regardless of the concentrations of PB and CD and the kind of CD. This relationship enables us to predict the bitter taste intensity of a mixed PB and CD solution from the observed surface tension, without any sensory test [15].

The observed electromotive force, selectively responsible to drugs, is similarly used to predict the bitter taste intensity of a mixed drug and CD solution [34]. For this purpose, potentiometry can be applied more widely than surface tensiometry. CDs can suppress the bitter tastes of PB and OB more strongly than polymer, surfactant, and other nontoxic compounds. The suppressing mechanism of some of them is different from that of CD: eq. 7 does not hold for all of these compounds [48].

Suppression of drug-induced hemolysis

CDs at high concentrations cause hemolysis because they extract lipids from the erythrocyte membrane. However, they can suppress drug-induced hemolysis at low concentrations [16–18]. Complexes between CD and drug are expected to be nonhemolytic because they are hydrophilic. Then, one can assume that the hemolytic activity of a mixed drug and CD solution is determined by the concentration of free drug alone. For such cases, the suppression of hemolysis by CD can be predicted from the observed surface tension. This prediction holds true for drug and surfactants [16–18], though it does not hold for highly hemolytic CDs [17] and weakly binding CDs [18]. CDs can bind intrinsic substances in the body. Phospholipids, cholesterol, and proteins in blood can bind CDs competitively with drug [16]. To analyze the binding equilibrium in this complicated system, we need to determine the binding constant between CDs and each of these intrinsic compounds. The studies on complex formation between DHPC and CDs afford useful information upon the extraction of lecithin by CDs [23,26].

Catalysis and inhibition of hydrolysis of drugs

CDs can accelerate and inhibit chemical reactions [1]. Generally, when the reactive site is located near the catalytic group of CDs, the reaction will be accelerated. On the other hand, when it is deeply incorporated in a CD cavity, the reaction will be inhibited. To understand the effect of CDs on the reac-

tion rate, we need to estimate the solution structure, binding constant, and stoichiometry of the CD-substrate complex.

Most drugs lose bioactivity by hydrolysis. For instance, penicillin G is a labile antibiotic and reduces its antibiotic activity by hydrolysis. Penicillin G is stabilized by β- and γ-CDs, whereas it is slightly catalyzed by α-CDs [49]. The hydrolysis of PB is accelerated by α-CDs because its ester group is located near hydroxyl groups of α-CDs. The effects of α-, β-, and γ-CDs on the hydrolysis of PB and OB are analyzed on the basis of the stoichiometry and the solution structures of complexes in some detail [41]. The polarity of OB and PB bound to CDs may be estimated from the UV absorption maximum of the guest and has some correlation with the reactivity [42].

SCOPE AND LIMITATIONS

In pharmaceutical and other industrial applications of CDs, the binding constant and the solution structure of the complex are of primary importance. To analyze and predict the binding constant, we need the solution structure of the complex. To apply the hydrophile–hydrophobe matching model for docking, we need the atomic coordinates of the solution structure of the complex.

The chemical shift is the most fundamental quantity in NMR and is used in all branches of chemistry. Sodium 4,4-dimethyl-4-silapentanesulfonate is very often used as the internal standard in aqueous solutions. However, it is a rather hydrophobic compound and has negative charge. It is not suitable for aqueous solutions containing CDs, surfactant, and cations. For binding-constant determination, the internal standard method is better than the external standard [20]. Potentiometry is a good method for binding-constant determination of ionic substances, if an appropriate ion-selective electrode can be constructed. Indirect competitive potentiometry is used to determine the binding constants of alcohols with α-CD [36]. This method will be applicable to other nonionic guest molecules and CDs. Widespread binding constants for a single CD–guest system have been reported in the literature: the methodology and data analysis must be improved [14].

The utility of our hydrophile–hydrophobe matching model has been demonstrated for predictions of the solution structures and the binding constants of several CD complexes. For instance, the solution structures of complexes of α-CD with BS [25], propanol, propanesulfonate [27], and OB [29] and those of β-CD with 4-*tert*-butylbenzyl alcohol [40], predicted by the model are close to the NMR and crystal structures. Furthermore, our model predicted reasonable binding constants [40] and revealed the importance of hydrophile–hydrophobe matching of the contact area in CD complexes [25,27,40]. The binding constants of γ-CDs with guest molecules are smaller than those of β-CD. This result can be explicable on the basis of the finding that the calculated ΔA_{oo} values of the former complexes are smaller than those of the latter. Namely, the contact areas of the former have larger spaces than those of the latter [40]. The weak points of this model at the present stage are that it requires a precise solution structure of a complex and that the contributions of the ΔA_{ww} and ΔA_{ow} values to the binding constant have not yet been estimated. A reasonable solution structure of the complex would be predicted by molecular mechanical calculations. The contributions of the ΔA_{ww} and ΔA_{ow} values in eq. 4 may be estimated from the relations between the structure and binding constant for more complexes. These improvements could allow us to apply our model to more host–guest complexes.

We determined rather detailed solution structures of CD complexes by NMR techniques aided with molecular mechanical calculations: in particular, we used a quantitative relation between the ROE intensity and the inter-proton distance. The number of the structures of complexes reported in the literature decreases in the order α-CD > β-CD > γ-CD. This is related to the number of possible structures of the complexes. In fact, we spent more time to estimate the solution structures of OB with α-, β-, and γ-CDs in the same order. The solution structures of these complexes are remarkably different among the CDs [29,32]. γ-CD has the largest space to entrap a guest molecule and has the most to fit it. The prediction of flexibility of the CD cavity needs calculations of molecular mechanics or molecular dynam-

ics. Finally, it is noted that the major solution structure is estimated by NMR and molecular mechanical calculations, whereas minor structures are neglected.

The reliability of the literature values of binding constants for multiple complexes is still low, and their theoretical analysis and prediction remain almost unsolved. The problem of the multiple equilibria is related to predictions of the macroscopic 1:1 binding constant. For instance, DTAB can bind two α-CD molecules [30]. Namely, it has two binding sites in the dodecyl chain. Therefore, we need to analyze its macroscopic 1:1 binding constant from the microscopic viewpoint. This is the reason for an increase in 1:1 binding constant with increasing alkyl chain beyond the hexyl group. In other words, the solution structure of the DTAB-α-CD complex depicted in Fig. 6 is the most probable over the time. It is expected that the ΔA_{oo} value for this structure is close to that for the OTAB-α-CD complex and that these complexes have close binding constants. However, the observed 1:1 binding constant (18 200 M^{-1}) of the DTAB-α-CD complex is larger than that of the OTAB-α-CD complex (3610 M^{-1}) [28,30]. This difference will be ascribed to more binding sites of DTAB than those of OTAB, as the result of translational motions of the alkyl chains in the α-CD cavity. The observed 1:2 binding constant (K_2) of DTAB with α-CD is 350 M^{-1} [30]. According to the equivalent independent binding model ($K_2 = K_1/4$), a K_2 value of 4550 M^{-1} is expected. This is larger than the observed value by one order. This discrepancy would be explicable in terms of nonequivalent sites and/or inhibitory binding. This case of the DTAB-α-CD system demonstrates our insufficient stage in quantitative analysis of multiple equilibria.

It is not easy to estimate the solution structure of the 1:2 complex in the presence of the 1:1 complex, even under the condition where the former is the major complex. More studies on the equilibria and solution structures of multiple complexes are required.

In pharmaceutical applications, the mechanisms of the suppression of bitter taste and hemolysis and the stabilization of drugs by CD are considered on the basis of quantitative analyses and their physicochemical predictions are proposed. These applications are explained on the basis of the binding constants between CD and drug and the solution structures of their complexes. Furthermore, if we can predict the binding constant from the chemical structures of CD and drug, we can quantitatively predict the suppression of bitter taste intensity and hemolysis of the solution. The present approach will be used for other applications of CDs.

CD is one of the most useful host molecules. Its interactions with guests, such as binding constants, three-dimensional structures of complexes, and intermolecular forces provide a wealth of knowledge for other supramolecular as well as industrial applications [13].

REFERENCES

1. M. L. Bender, M. Komiyama. *Cyclodextrin Chemistry*, Springer Verlag, Berlin (1978).
2. J. Szejtli. *Cyclodextrin Technology*, Chaps. 2 and 3, Kluwer Academic, Dordrecht (1988).
3. W. Saenger, J. Jacob, K. Gesseler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S. Smith, T. Takaha. *Chem. Rev*. **98**, 1787 (1998).
4. K. Harata. *Chem. Rev*. **98**, 1803 (1998).
5. H.-J. Schneider, F. Hacket, V. Rüdiger, H. Ikeda. *Chem. Rev*. **98**, 1755 (1998).
6. K. A. Connors. *Chem. Rev*. **97**, 1325 (1997).
7. K. B. Lipkowitz. *Chem. Rev*. **98**, 1829 (1998).
8. K. A. Connors. *Binding Constants*, John Wiley, New York (1987).
9. J. Szejtli, T. Osa. *Cyclodextrins*, *Comprehensive Supramolecular Chemistry*, Vol. 3, Elsevier, Oxford (1996).
10. J. Szejtli. *Chem. Rev.* **98**, 1743 (1998).
11. M. V. Rekharsky, Y. Inoue. *Chem. Rev*. **98**, 1875 (1998).
12. H.-J. Schneider, A. K. Yatsimirksi. *Principles and Methods in Supramolecular Chemistry*, John Wiley, New York (2000).

13. J.-M. Lehn. *Supramolecular Chemistry*, Chap. 2, VCH, Weinheim (1995).
14. N. Funasaki, H. Yodo, S. Hada, S. Neya. *Bull. Chem. Soc. Jpn.* **65**, 1323 (1992).
15. N. Funasaki, Y. Uemura, S. Hada, S. Neya. *J. Phys. Chem.* **100**, 16298 (1996).
16. N. Funasaki, M. Ohigashi, S. Hada, S. Neya. *Langmuir* **15**, 594 (1999).
17. N. Funasaki, M. Ohigashi, S. Hada, S. Neya. *Langmuir* **16**, 383 (2000).
18. N. Funasaki, T. Okuda, S. Neya. *J. Pharm. Sci.* **90**, 1056 (2001).
19. U. R. Dharmawardana, S. D. Christian, E. E. Tucker, J. F. Scamehom. *Langmuir* **9**, 2258 (1993).
20. N. Funasaki, M. Nomura, H. Yamaguchi, S. Ishikawa, S. Neya. *Bull. Chem. Soc. Jpn.* **73**, 2727 (2000).
21. N. Funasaki, M. Nomura, S. Ishikawa, S. Neya. *J. Phys. Chem. B* **105**, 7361 (2001).
22. N. Funasaki, S. Ishikawa, S. Neya. *Bull. Chem. Soc. Jpn.* **75**, 719 (2002).
23. S. Ishikawa, N. Funasaki, S. Neya. *J. Phys. Chem. B* **102**, 2502 (1998).
24. S. Hada, S. Neya, N. Funasaki. *J. Phys. Chem. B* **103**, 2574 (1999).
25. N. Funasaki, H. Yamaguchi, S. Ishikawa, S. Neya. *J. Phys. Chem. B* **105**, 760 (2001).
26. N. Funasaki, S. Ishikawa, S. Neya. *Langmuir* **18**, 1786 (2002).
27. N. Funasaki, S. Ishikawa, S. Neya. *J. Phys. Chem. B* **106**, 6431 (2002).
28. N. Funasaki, S. Ishikawa, S. Neya. *J. Phys. Chem. B* **107**, 10094 (2003).
29. N. Funasaki, H. Yamaguchi, S. Ishikawa, S. Neya. *Bull. Chem. Soc. Jpn.* **76**, 903 (2003).
30. N. Funasaki, S. Ishikawa, S. Neya. *J. Phys. Chem. B* **108**, 9593 (2004).
31. N. Funasaki, K. Yamauchi, S. Ishikawa, S. Neya. *Bull. Chem. Soc. Jpn.* **77**, 2165 (2004).
32. N. Funasaki, T. Sumiyoshi, S. Ishikawa, S. Neya. *Mol. Pharm.* **1**, 166 (2004).
33. N. Funasaki, S. Ishikawa, S. Hirota. *Anal. Chim. Acta* **555**, 278 (2006).
34. N. Funasaki, K. Kawaguchi, S. Ishikawa, S. Hada, S. Neya, T. Katsu. *Anal. Chem.* **71**, 1733 (1999).
35. N. Funasaki, S. Neya. *Langmuir* **16**, 5343 (2000).
36. N. Funasaki, M. Nagaoka, S. Hirota. *Anal. Chim. Acta* **531**, 147 (2005).
37. W. Hirsch, C. K. Choy, K. W. Ng, V. Fried. *Anal. Lett.* **22**, 2861 (1989).
38. R. B. Herman. *J. Phys. Chem.* **76**, 2754 (1972).
39. S. Ishikawa, S. Hada, N. Funasaki. *J. Phys. Chem.* **99**, 11508 (1995).
40. S. Ishikawa, S. Hada, S. Neya, N. Funasaki. *J. Phys. Chem. B* **103**, 1208 (1999).
41. N. Funasaki, S. Ishikawa, S. Neya. *J. Pharm. Sci.* **90**, 740 (2001).
42. S. Ishikawa, S. Hirota, S. Neya, N. Funasaki. *Chem. Pharm. Bull.* **54**, 528 (2006).
43. A. Guerrero-Martínez, G. González-Gaitano, M. H. Vinas, G. Tarajos. *J. Phys. Chem. B* **110**, 13819 (2006).
44. N. Funasaki, K. Kawaguchi, S. Hada, S. Neya. *J. Pharm. Sci.* **88**, 759 (1999).
45. T. Kuwabara, M. Takamura, A. Matsushita, H. Ikeda, A. Nakamura, A. Ueno, F. Toda. *J. Org. Chem.* **63**, 8729 (1998).
46. H. Aki, T. Niiya, Y. Iwase, M. Yamamoto. *J. Pharm. Sci.* **90**, 1186 (2001).
47. R. U. Agu, M. Jorissen, T. Willems, G.. van den Mooter, R. Kinget, N. Verbeke, P. Augustijns. *Int. J. Pharm.* **193**, 219 (2000).
48. N. Funasaki, I. Uratsuji, T. Okuno, S. Hirota, S. Neya. *Chem. Pharm. Bull.* **54**, 1155 (2006).
49. S. Hada, S. Neya, N. Funasaki. *Chem. Pharm. Bull.* **45**, 577 (1997).