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"Induced-Fit"-Type Complex Formation of the Model Enzyme α-Cyclodextrin*

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On the basis of X-ray and neutron data for several α-cyclodextrin·substrate complexes it is shown that basically two different structures for α-cyclodextrin exist, one "tense," the other "relaxed." An "induced-fit"-like mechanism for α-cyclodextrin complex formation is proposed.

INTRODUCTION: THE "INDUCED FIT"

In enzyme-catalyzed processes, the first step is the binding of the substrate to the enzyme active site, which is followed by a sequence of reactions until the product is formed and finally expelled. The high specificity and the catalytic activity of enzymes for their proper substrates was initially explained by the "lock and key" hypothesis (*1*), and later extended by several theories including the "induced fit" (*2, 3*) for conformational changes of the enzyme and "nonproductive binding" as well as "strain and distortion" (*4*) on the part of the substrate.

While in the former hypothesis both enzyme and substrate were considered as rigid entities, they are assumed to be flexible in the latter theories, the conformational changes being triggered by the approach and binding of the substrate to the active site of the enzyme. The conformational change of enzymes was visualized in a number of cases by spectroscopic methods and more elaborately in X-ray diffraction analyses of crystallized enzymes and enzyme-inhibitor complexes (*2, 5*). Since most diffraction studies are limited to about 2 Å resolution, the exact details of the process are still obscure. It is therefore of interest to investigate a suitable model system such as α-cyclodextrin inclusion complexes in order to understand what happens at the atomic level when intermolecular complex formation occurs.

α-CYCLODEXTRIN AS AN ENZYME MODEL

α-Cyclodextrin (α-CD (Fig. 1)) is an enzymatic degradation product obtained from starch and represents the smallest member of a family of cyclic oligosaccharides.

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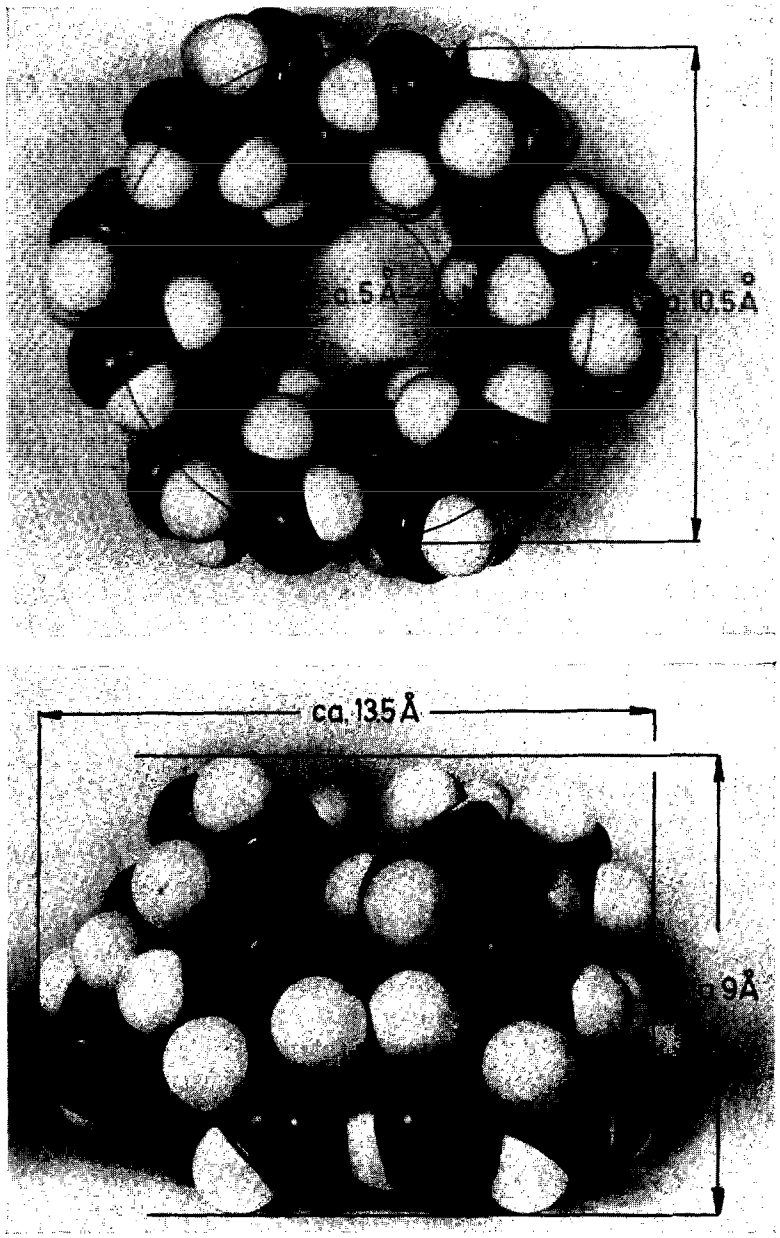

FIG. 1. Space filling model of α-cyclodextrin.

It has a toroidal shape (Fig. 1) and owing to its 5 Å wide inner aperture it is able to form (inclusion) complexes in aqueous solution with a wide variety of substances ranging from the purely hydrophobic (paraffins, noble gases) to the hydrophilic (water, alcohols, salts), the only requirement being that they fit into the α-CD cavity (*6–8*).

It is surprising to find that the cyclodextrins exert catalytic activity on suitable included substrate molecules; they catalyse the hydrolysis of phenylacetates (*8*), of organic

pyrophosphates (*9*), and of penicillin derivatives (*10*), aromatic chlorinations and diazo coupling (*11*) by means of their primary and/or secondary hydroxyl groups, the rates of hydrolysis being faster by a factor of up to 400 (*8*). In case of phenylacetates, it was found that cyclodextrins mimic the action of α-chymotrypsin with an "acylated enzyme" as intermediate (*8*, *12*).

The complex formation of cyclodextrins has been of interest since their discovery, and many data on these have been accumulated using different methods of investigation. However, the force driving complex formation and the mechanism of inclusion are still unclear and a matter of speculation (*6–8*). We have crystallized several complexes and studied their structures by X-ray diffraction techniques. The neutron diffraction results recently obtained for the α-cyclodextrin · water complex are compared with the structures of the other complexes and a detailed "induced fit"-like mechanism for complex formation is derived.

STRUCTURES OF NONWATER ADDUCTS OF α-CYCLODEXTRIN

α-CD has been cocrystallized from aqueous solution with methanol (*13*), *n*-propanol (*14*), iodine (*15*), polyiodide (*16*), krypton (*17*), *p*-iodoaniline (*18*), and potassium acetate (*19*) and the crystals analyzed by X-ray diffraction. In all these complexes, the α-CD molecule assumes the almost "round" structure displayed in Fig. 2. The glucoses are in a C1 chair conformation and the dihedral angles about the C(4)–O(4)–C(1′) glucosidic bonds, Φ and Ψ, are about +165 and −168° (*20*) which corresponds closely to the global minimum derived from potential energy calculations for the rotation of vicinal glucoses about these bonds (*21*, *22*). In this particular conformation, the O(2)···O(3) distances are so close to the 2.8 to 3.0 Å required for hydrogen bond formation that a ring of six interglucosidic, intermolecular O(2)···O(3) hydrogen bonds is formed, or can be formed by only slight rotation about the glucosidic linkages (*20*) (Fig. 2). The C(6)–O(6) bonds are preferentially directed away from the centre of the α-cyclodextrin molecule (the O(5)–C(5)–C(6)–O(6) angles are in the (−)*gauche* range); but in some complexes where hydrogen bonding to the enclosed substrate is possible, e.g., with the alcohols (Fig. 2), one or two of the C(6)–O(6) bonds point towards the center of the molecule to allow such a bond to form. In these latter cases the angle about the C(5)–C(6) bond is in the (+)*gauche* range.[4] The substrate molecule(s) occupy the centre of the α-CD host; and if they are too small to fill the 5 Å wide cavity, they are statistically disordered to do so. This is true for krypton (*17*), methanol (*13*) (Fig. 2), and *n*-propanol (*14*).

STRUCTURE OF THE α-CYCLODEXTRIN · WATER ADDUCT

Prior to complex formation in aqueous solution, α-CD exists in a "empty" state and can be crystallized as such. The structure of these crystals was first solved from X-ray data (*23*) and further refined in a combined X-ray and neutron study (*24*). In this latter

[4] In the α-CD · potassium acetate complex, all C(6)–O(6) bonds are in a (+)*gauche* orientation, probably caused by the peculiar crystal packing scheme (*20*).

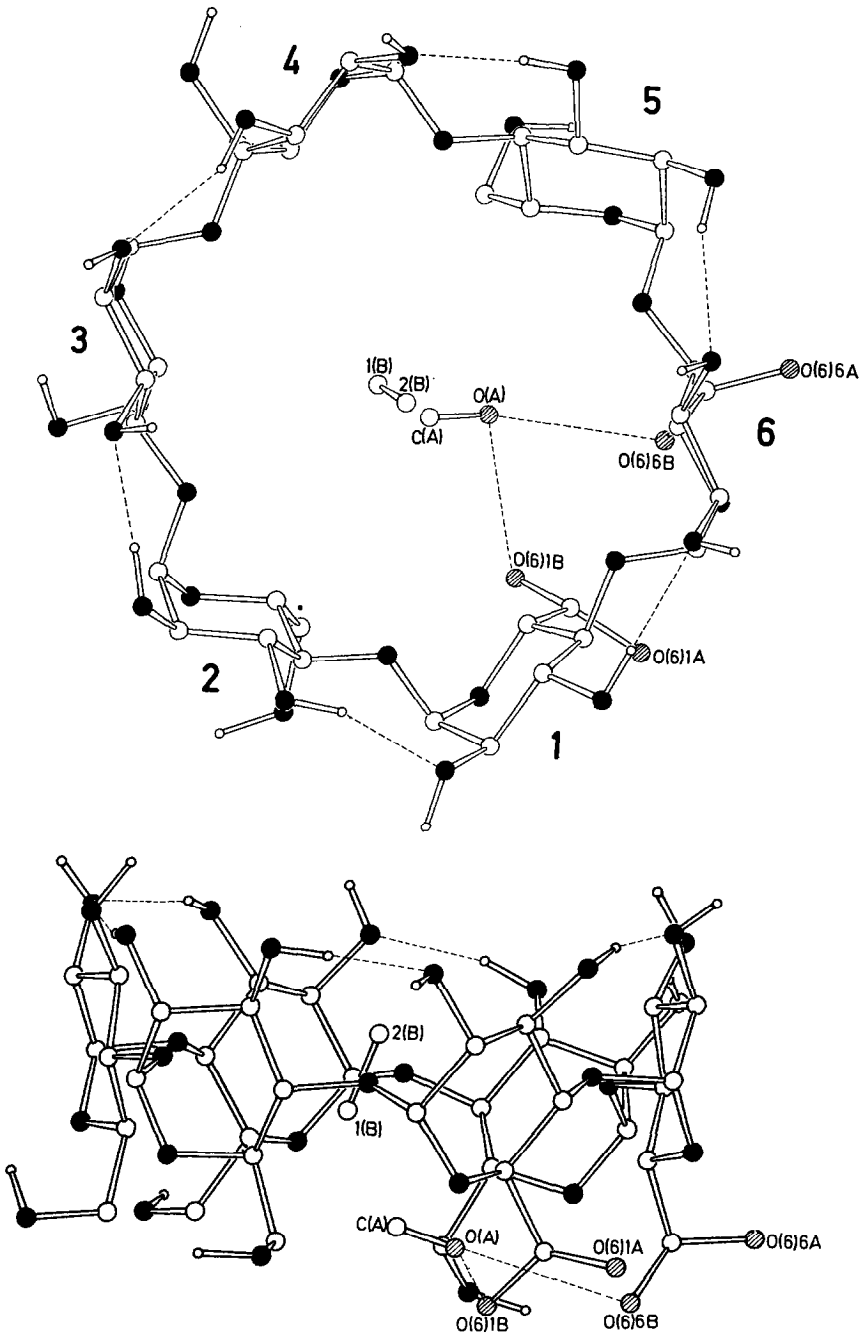

FIG. 2. The α-CD·methanol complex as a representation for nonwater adducts. ○ = C, ● = O, ◉ = disordered O, ○ = H. Hydrogen bonds indicated as dashed lines. The enclosed methanol molecule is twofold disordered, at positions (A) and (B). The molecule in the (A) site is hydrogen bonded to two O(6) hydroxyl groups which are themselves disordered (*13*). All H atoms were located from X-ray difference Fourier syntheses.

work, all the hydrogen atoms of the complex could be located with certainty (Fig. 3). In the "empty" α-CD molecule, two water molecules of van der Waals diameter 3.8 Å occupy the void. In contrast to complexes with krypton atoms as well as methanol and *n*-propanol molecules, they are in *fixed* positions because the 5 Å wide α-CD void of Fig. 2 is diminished through rotation of one glucose out of the toroidal arrangement

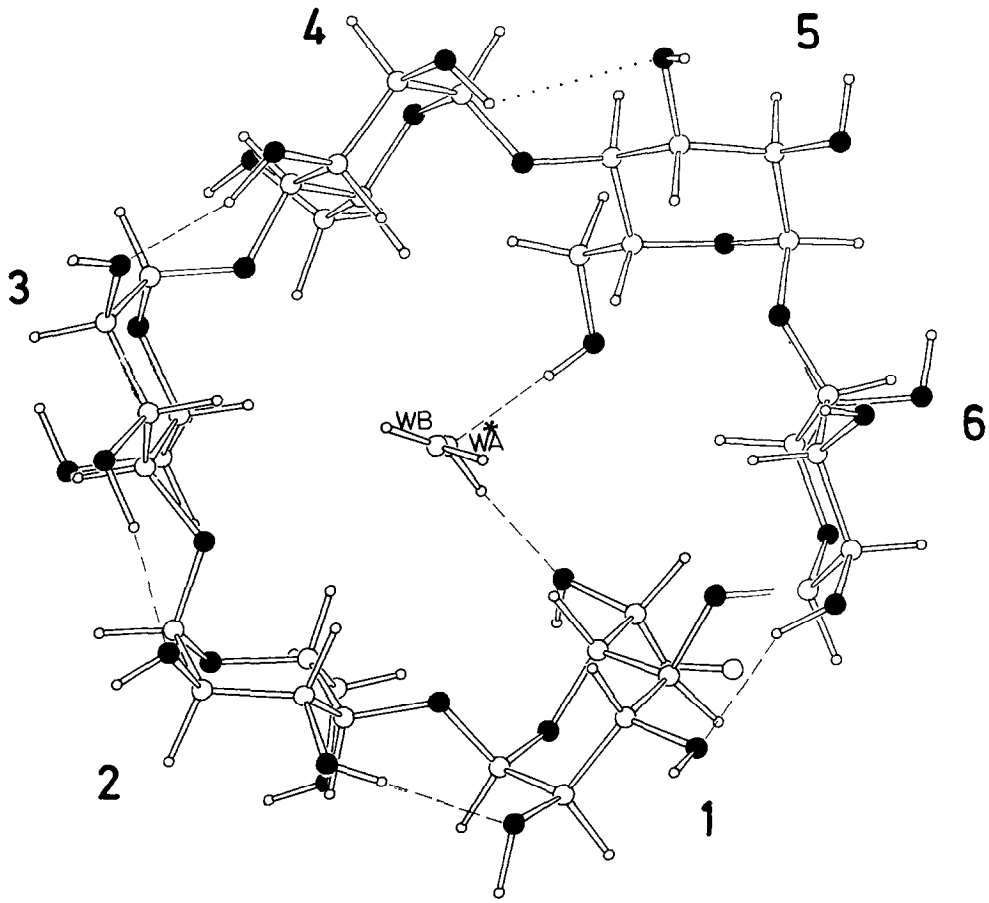

FIG. 3. The "empty" α-CD·2 H_2O complex as obtained from neutron diffraction (*23*, *24*). Numbering scheme of glucoses and atom designation as in Fig. 2. The dotted line between the most rotated glucose 5 mentioned in the text and glucose 4 represents a questionable H bond of 3.36 Å O···O distance. Water molecule WB is above WA and hydrogen bonded to WA and to two O(6) hydroxyl groups of α-CD. The * marks the center of the α-CD molecule; the water molecules are shifted by 0.6 Å out of this center. All H atoms were located from X-ray and from neutron difference Fourier syntheses.

of the other five (glucose 5 in Fig. 3). The dihedral angles Φ, Ψ for these five glucoses have values close to the local minimum of the calculated potential energy (*21*, *22*), while for the angles belonging to the rotated glucose considerable steric strain is indicated. The ring of interglucosidic, intramolecular hydrogen bonds is broken at this glucose with O(2)···O(3) distances of 4.66 and 3.35 Å, and the C(6)–O(6) bonds for this and

for another glucose (numbers 5 and 1 in Fig. 3) are in (+)*gauche* orientation to allow hydrogen bonding to one of the enclosed water molecules. The second water molecule is hydrogen bonded to this first one and then to an adjacent α-CD molecule. It can be inferred from Fig. 3a that the two enclosed water molecules cannot satisfy their hydrogen bonding potentials. Molecule WA is lacking one acceptor bond, and molecule WB one acceptor and one donor bond. The two water molecules are not located on the toro-

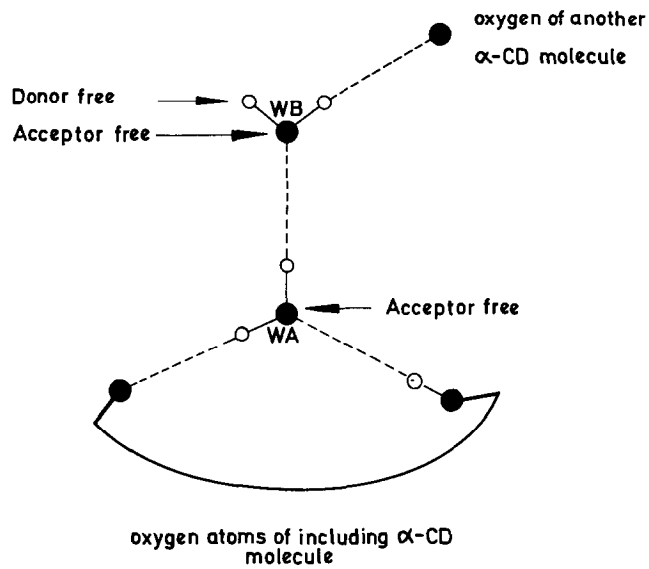

FIG. 3a. A schematic close-up view of the two water molecules WA and WB enclosed in the cavity of the α-CD·water complex. Free donor and acceptor sites are marked by arrows; ○ = hydrogen; ● = oxygen, hydrogen bonds indicated by dashed lines.

idal axis of the including α-CD molecule but displaced by 0.6 Å in order to achieve van der Waals contact with its wall, the contact surface being opposite the most rotated glucose.

THE MECHANISM OF ADDUCT FORMATION

The two types of structures which α-CD is able to assume are summarized in Fig. 4. The "empty" water complex found in the α-CD·water adduct in the crystalline state will also be present in aqueous solution. In this complex, the α-CD molecule is not "round" owing to the distortion of one of its six glucoses and occurs in a high energy, "tense" conformation, as discussed above, because one of the Φ,Ψ angles indicates increased strain energy and the ring of interglucosidic O(2)···O(3) hydrogen bonds is interrupted. This particular "tense" α-CD molecule could undergo complex formation with a substrate (*S*) directly (Scheme A in Fig. 4) to yield an unstrained, "relaxed" α-CD molecule with all Φ,Ψ angles near the global potential energy minimum

and the ring of O(2)···O(3) hydrogen bonds fully established. Two other schemes (B and C) are feasible, depending upon whether the tense complex is in equilibrium with a complex consisting of a relaxed α-CD molecule and included "activated" water molecules H_2O^* or whether the substrate aggregates at the outside of the tense α-CD molecule with inclusion occurring subsequently.

The term "activated water" needs a few words of explanation. The two included water molecules in the α-CD·water complex are not fully coordinated (Fig. 3a) and will therefore be at higher energy potential compared to "bulk" water. Activated water

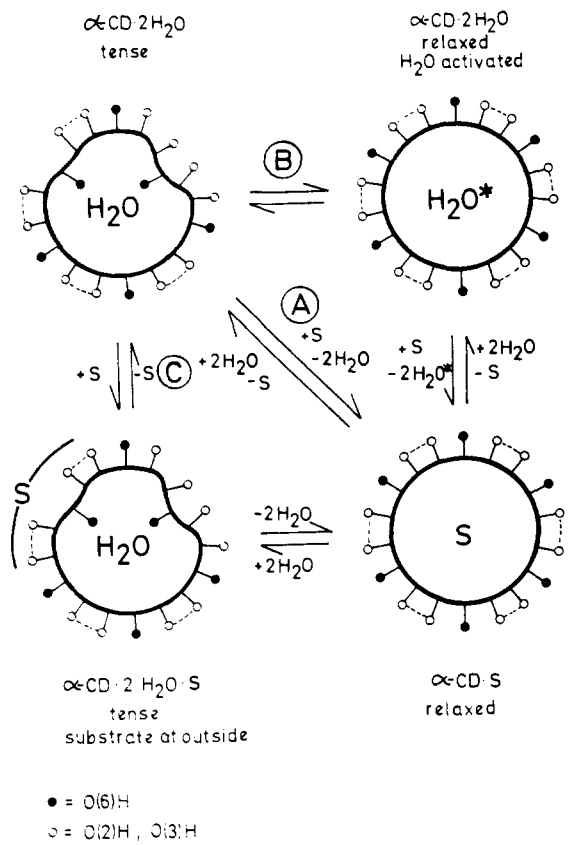

FIG. 4. A schematic illustration of the α-CD inclusion formation. S = substrate, H_2O^* = "activated water", –● = O(6) hydroxyl group, –○ = O(2) and O(3) hydroxyl group. H-bonds marked by dashed lines. For details see text.

(H_2O^* in Fig. 4) is taken to mean such incompletely coordinated water molecules which are located in a "round" α-CD cavity of about 5 Å diameter and not in the diminished cavity of the α-CD·water complex. From anology with the α-CD·krypton complex we assume that the water molecules in the "round" cavity will be disordered in a similar way to the krypton atoms because the van der Waals diameters of krypton and H_2O are comparable (4.0 and 3.8 Å, respectively).

A decision favouring one or the other reaction schemes of Fig. 4 cannot yet be made. It should be noted that this description is strictly valid only for the solid state. In

solution, the α-CD · water and α-CD · substrate complexes will undergo dynamic change, i.e., the hydrogen bonds from the glucoses in the included water molecules will not be stationary but will be broken and reformed (they "rotate"), but the overall picture representing minimum potential energy will be similar to that found in the crystal structures.

Several independent observations support the proposed mechanism of α-CD · substrate complex formation. Circular dichroism spectroscopic data suggest that α-CD undergoes a conformational change when adduct formation occurs (*25*). Thermodynamic data of a number of adducts between α-CD and azo dyes demonstrate that the constants for complex dissociation, $K \sim 10^{-3}$ mole/liter and the reaction enthalpies, $\Delta H \sim 6$–8 kcal/mole, are comparable (*26*). However, kinetic data for these adducts reveal that the rates of complex formation and dissociation may cover seven orders of magnitude, depending on the size, shape, and charge distribution of the substrate molecules.

On the basis of these results, we conclude that α-CD · substrate complex formation is mainly due to a change in conformation and hydrogen bonding energy of the α-CD molecule itself; it goes from a "tense" to a "relaxed" state. The gain in entropy resulting from expulsion of the two water molecules from the α-CD cavity into the bulk water should also add to the complex stabilization, but we assume that this is not so significant as postulated previously (*8*).[5] The proposed mechanism also explains why α-CD forms complexes with such a variety of substrate molecules: individual α-CD · substrate interactions are only of minor importance although they may contribute to the stabilization of the inclusion complexes.

Because a conformational change of the α-CD molecule is involved in complex formation, some relation to the "induced fit" observed for enzyme · substrate interactions is apparent. Assuming that α-CD can really be regarded as an enzyme model (*8*), we conclude that hydrogen bonding energy and conformational strain energy are also released in the much more complicated enzyme molecules when complex formation occurs with the proper substrate. The enzyme is transformed from the empty, tense state to a relaxed enzyme · substrate complex (with the substrate activated) and returns to the initial tense state when the reaction and expulsion of the substrate are accomplished.

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[5] The presence of water or the occurrence of the "tense" α-CD species should be a prerequisite for the mechanism described in Fig. 4. It is known that isopropanol suppresses the complex formation of β-cyclodextrin (*27*) but dimethylsulfoxide does not (*28*). The influence of organic solvents on complex formation of α-CD is not known. The cavity diameters of α- and β-CD are too different (*26*) to permit extrapolation.

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