# 12
# Cyclodextrins as Enzyme Models

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## 12.1 INTRODUCTION

Molecular design of artificial enzymes has been widely attempted. The aims of these studies are: (i) to obtain a better understanding of the mechanisms of enzymatic reactions, (ii) to develop useful industrial catalysts showing large activities and selectivities, and (iii) to regulate various bioreactions artificially. Other applications are of course possible. In most of the cases, artificial enzymes are constructed by hybridization of the catalytic residues, which are responsible for the

chemical transformation, and the substrate-recognition moieties which selectively bind the target guest compound(s).

Cyclodextrins (CDs) have been widely employed as the substrate-recognition moieties in artificial enzymes, mainly because (i) the water solubilities are high, (ii) the guest compounds available are versatile, (iii) the molecular structures are well-defined, and (iv) the hydroxyl groups as effective catalysts are regularly arranged around the rims of the cavities. In addition, a variety of functional residues can be introduced to CDs by use of chemical reactions with the hydroxyl groups. All these properties make the molecular design of CD-based artificial enzymes successful and fruitful.

Soon after CDs were discovered, their catalytic activities were recognized.[1,2] Furthermore, Bender and co-workers made detailed and systematic studies on the CD-induced hydrolysis of phenyl acetates, and discovered a significant substrate specificity in the reaction rates.[3,4] The finding clearly showed that CDs are superb models of enzymes, and opened the way to the subsequent marvelous developments in the field. Since then, the number of the reactions catalyzed by CDs has been continuously increasing at an enormous rate. The catalysis mechanisms have been precisely clarified by the use of analytical and physicochemical information. Furthermore, various advanced enzyme models have been envisioned by the chemical modification of CDs according to detailed molecular design. The development of techniques for the precise chemical modification of CDs has undoubtedly made an essential contribution to progress.

All the reactions catalyzed by CDs (and by their derivatives) proceed via the complexes between them and their substrates, in which the chemical transformation takes place. The reaction scheme is exactly identical to that employed by natural enzymes. In most cases, the hydroxyl residues of CDs are responsible for the catalytic functions. Large reaction rates are achieved, since the catalysis occurs intramolecularly in the CD–substrate complexes. Furthermore, the chemical transformation is selective (with respect to the substrates, products, and/or stereochemistry), mainly because CDs provide a chemically and sterically specific reaction field. In the chemically modified CDs as enzyme models, the catalytic residues are located in precise positions so that the catalysis is effective and selective. Multiple molecular recognition by CDs both in the initial state and in the transition state is essential.

This chapter mainly concentrates on the recent developments in the use of CDs as enzyme models. Details of previous work in the field can be found in Refs. 5–11.

## 12.2 HYDROLYSIS

### 12.2.1 Hydrolysis of Amides (Protease Models)

Proteases such as serine proteases and papain have been the most widely studied, and the preparation of their mimics has been one of the most challenging topics for bioorganic and bioinorganic chemists. However, amide bonds are so stable in the absence of natural enzymes that they are only hydrolyzed by the use of metal ions ($Cu^{II}$ and $Co^{III}$). In addition, highly acidic or alkaline conditions are required here. Neither CDs nor their organic derivatives were available for the purpose (this is one of the reasons why most of the previous studies on protease models took advantage of hydrolysis of esters, especially of activated *p*-nitrophenyl esters, rather than the amide hydrolysis itself (see Section 12.2.4)).

However, CDs are effective for the hydrolysis of activated anilides such as 2,2,2-trifluoroacetanilides.[12] The secondary alkoxide ions of CDs as nucleophiles attack the substrates, which are included in their cavities, and the decomposition of the resultant tetrahedral intermediates (release of the aniline derivatives) is promoted by acid catalysis of other hydroxyl group(s) of CDs. Introduction of an aminoethyl group to the secondary hydroxyl side of the CD provides a better catalysis.[13] The highly active amide bonds in acylimidazoles and β-lactams are also hydrolyzed by CDs.[14,15]

Quite recently, peptide bonds in natural (unactivated) dipeptides and tripeptides were efficiently hydrolyzed by a combination of cerium ion ($Ce^{IV}$) and γ-CD.[16] The conversion was around 80% at pH 7 and 60 °C for 24 h. The terminal carboxylate and/or the terminal amino residue coordinate to the $Ce^{IV}$, and the hydroxide ion intramolecularly bound to the $Ce^{IV}$ attacks the carbonyl carbon in the hydrolyzed amide bond (Figure 1). One of the main functions of the γ-CD is to solubilize the $Ce^{IV}$ ion, rather than to directly participate in the catalytic functions.[17] Without γ-CD, $Ce^{IV}$ ions rapidly precipitate due to the formation of hydroxide gel. It has been proposed that hydrogen

bonds are formed between the hydroxyl residues of CDs and the coordination water around the $Ce^{IV}$ ion, and thus the metal ion is located at the top of the cavity.

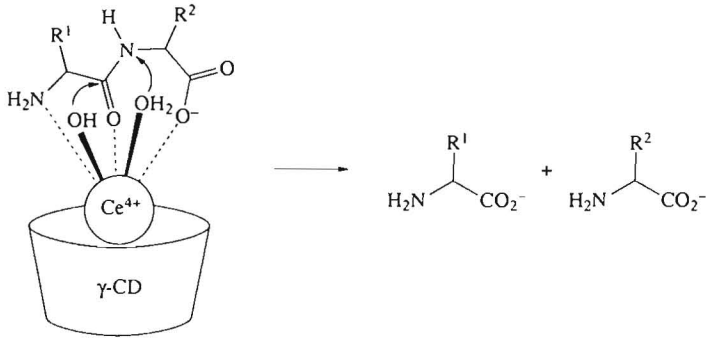



**Figure 1** Amide hydrolysis by the CD–$Ce^{IV}$ complex.

### 12.2.2 Hydrolysis of Phosphates

Phosphate esters play essential roles in processes of life. In RNA and DNA, ribonucleosides are connected by phosphodiester linkages. Phospholipids also have phosphodiester linkages, as expected from their names. In addition, phosphoesters are essential for information transfer, metabolism, and other functions *in vivo*. Thus, artificial enzymes for the hydrolysis of phosphoesters have various potential applications.

#### *12.2.2.1 Hydrolysis of RNAs (ribonuclease models)*

RNA hydrolysis by ribonuclease is highly regioselective in that only the fragments with terminal phosphates at the 3′-positions are selectively formed (Figure 2, pathway A).[18] In nonenzymatic hydrolysis, however, both pathways A and B proceed at almost the same rate. The selectivity in enzymatic reactions comes from the regioselective cleavage of the 2′,3′-cyclic monophosphate intermediate, formed in the first step of the reactions: only the P—O-2′ bond is hydrolyzed while the P—O-3′ bond kept intact.

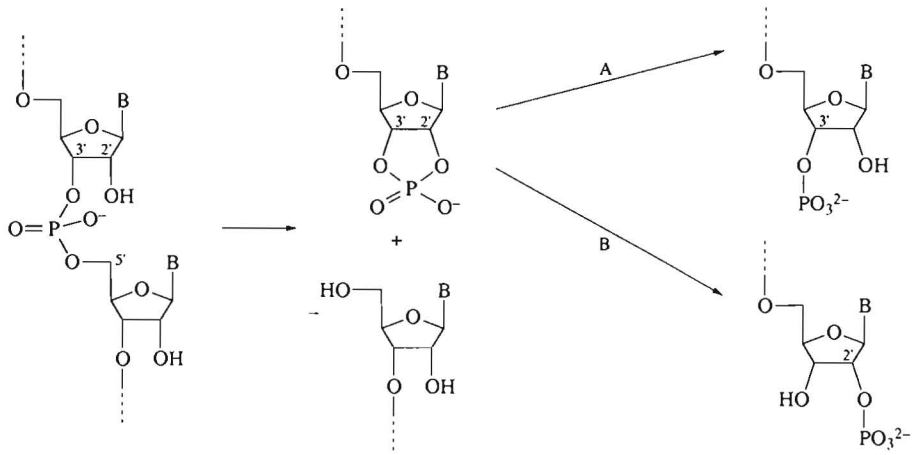



**Figure 2** Scheme for the hydrolysis of RNAs.

CDs successfully mimic the regioselective reaction: either the P—O-2′ or P—O-3′ bond of the intermediate is selectively hydrolyzed, depending on the kind of CD used. With the use of α-CD,[19] the P—O-2′ bonds are dominantly cleaved, giving the corresponding 3′-phosphates in high selectivities as does ribonuclease. The hydroxyl groups of α-CD are essential for the catalyses, since

hexa-2,6-dimethyl-α-CD is ineffective. Figure 3(a) depicts the structure of the complex between α-CD and C > p, determined by NMR spectroscopy. The phosphate residue of C > p forms hydrogen bonds with the secondary hydroxyl group of α-CD, and the oxygen atom at the C-2 carbon of the cytosine forms another hydrogen bond with the secondary hydroxyl group of the farthest glucose. The plane of the cyclic phosphate ring is nearly parallel to the longitudinal axis of the α-CD cavity. As a result, the chemical environments of the P—O-2′ bonds and the P—O-3′ bonds of C > p, which are otherwise almost identical to each other, are largely differentiated. Scission of the P—O-3′ bond, which is located in or near the apolar cavity of the α-CD, is highly suppressed, since the formation of an alkoxide intermediate in the apolar environments is energetically unfavorable. P—O-2′ cleavage, however, is facilitated by effective solvation of the alkoxide intermediate.

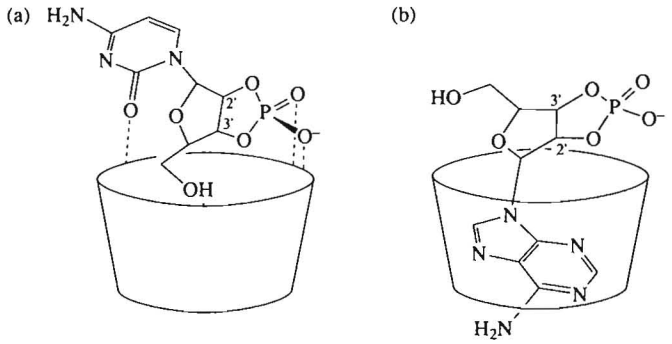


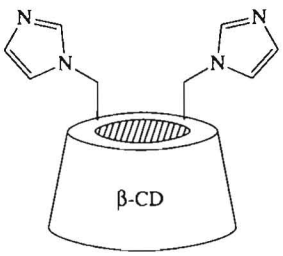

**Figure 3** Structures of (a) the α-CD–C > p complex and (b) the β-CD–A > p complex.

In contrast, β- and γ-CD enhance P—O-3′ cleavage, selectively forming ribonucleoside 2′-phosphates.[20] The hydroxyl groups of CDs are again essential for this purpose. The P—O-3′ bond, which is far from the cavity (see Figure 3(b)), is preferentially cleaved, in the same way as described above for selective P—O-2′ cleavage by α-CD. Remarkable dependence of the regiospecificity on the kind of CD comes from the difference in the structure of the CD–substrate complex (hydrogen bonding type for α-CD, and inclusion type for β- and γ-CDs). The hydrolyses proceed via nucleophilic attack by water or hydroxide ion toward the phosphorus atom, as supported by pressure effect experiments.[21]

The regioselective catalysis of β-CD for P—O-3′ cleavage is greatly promoted by alkali metal halides.[22,23] The selectivity-increasing activity is in the following order: KCl > RbCl > CsCl > NaCl. In contrast, LiCl, $MgCl_2$, and $CaCl_2$ decrease the regioselectivity. The regioselective catalysis of α-CD for P—O-2′ cleavage is also promoted: note that the effect of alkali salts is entirely opposite to that in β-CD catalysis, being consistent with the above arguments.

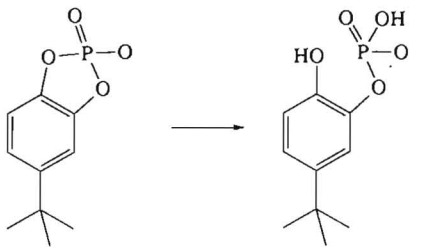
Regioselective catalysis of ribonuclease for RNA hydrolysis (selective formation of the 3′-terminal phosphate fragments) has been mimicked by α-CD.[24] On the other hand, nonnatural fragments (2′-terminal phosphate fragments), which cannot be otherwise obtained, are formed by β- and γ-CDs.

Efficient RNA hydrolysis, although being nonregioselective, is achieved by the combination of a modified β-CD (**1**) and europium(III) ion.[25] The modified β-CD promotes the catalysis of the metal(III) ion[26] by around 1.5-fold. The $Zn^{II}$ complex of the β-CD bearing the diethylenetriamine residue is also effective for RNA hydrolysis.[27]




(1)

Even in the absence of metal ions, the modified β-CD, (**1**), hydrolyzes a cyclic monophosphate of catechol (**2**), a model compound of the cyclic intermediate in RNA hydrolysis (see Figure 1). The manner of cooperation of the two histidines at the active site of ribonuclease has been studied in detail by the use of these mimics.[11,25,28–31] Three isomers, AB, AC, and AD types, which have two imidazolyl residues in the corresponding glucose units of β-CD, were prepared. The pH–rate constant profiles for all the modified β-CDs are bell-shaped, showing that neutral imidazole (a base catalyst) and protonated imidazole (an acid catalyst) function cooperatively (Figure 4). A similar bell-shaped pH–rate constant profile is obtained in catalysis by ribonuclease itself. Interestingly, the AB isomer, in which the two imidazolyl residues are in adjacent glucose units, is much more effective than the AC- and AD-isomers. Based on this geometric information (together with other evidence), a new mechanism in which the imidazolyl cation as an acid catalyst protonates the phosphate oxyanion is proposed (Figure 5). Prior to this experiment, it was believed that, in catalysis by ribonuclease, the imidazolyl cation (of the two imidozoles at the active site) protonates the leaving group (the alkoxide ion) and facilitates its removal from the pentacoordinated intermediate. Whether this mechanism proposed on the model systems is applicable to enzymatic catalysis has not yet been established.




(2)

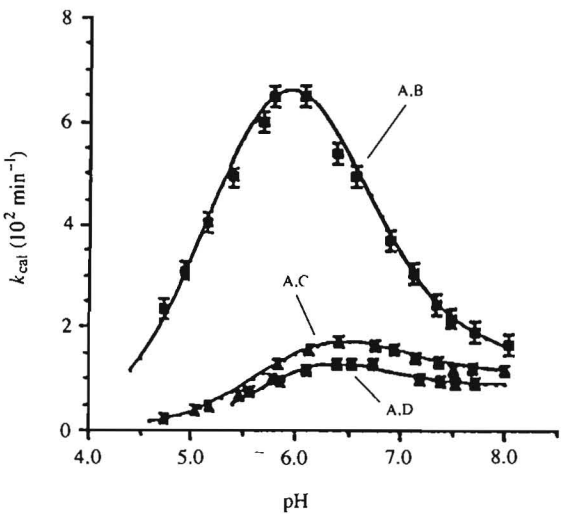



**Figure 4** pH–rate constant profiles for the hydrolysis of cyclic phosphate of catechol (**2**) by β-CDs with two imidazoles.

The product of hydrolysis of (**2**) by modified β-CDs (**1**) is mostly (**3**) not (**4**), showing that the P—O-1 linkage is dominantly cleaved. In the ordinary hydrolysis of (**2**), however, an essentially equal mixture of (**3**) and (**4**) is formed. A study using a molecular model indicates that the delivery of water by the neutral imidazole groups (a general base catalyst) can be in line with the P—O bond to O–1 of (**2**), not O–2. The resultant intermediate goes directly to the product without pseudorotation (if pseudorotation was taking place before release of the leaving group, the regioselectivity should disappear).

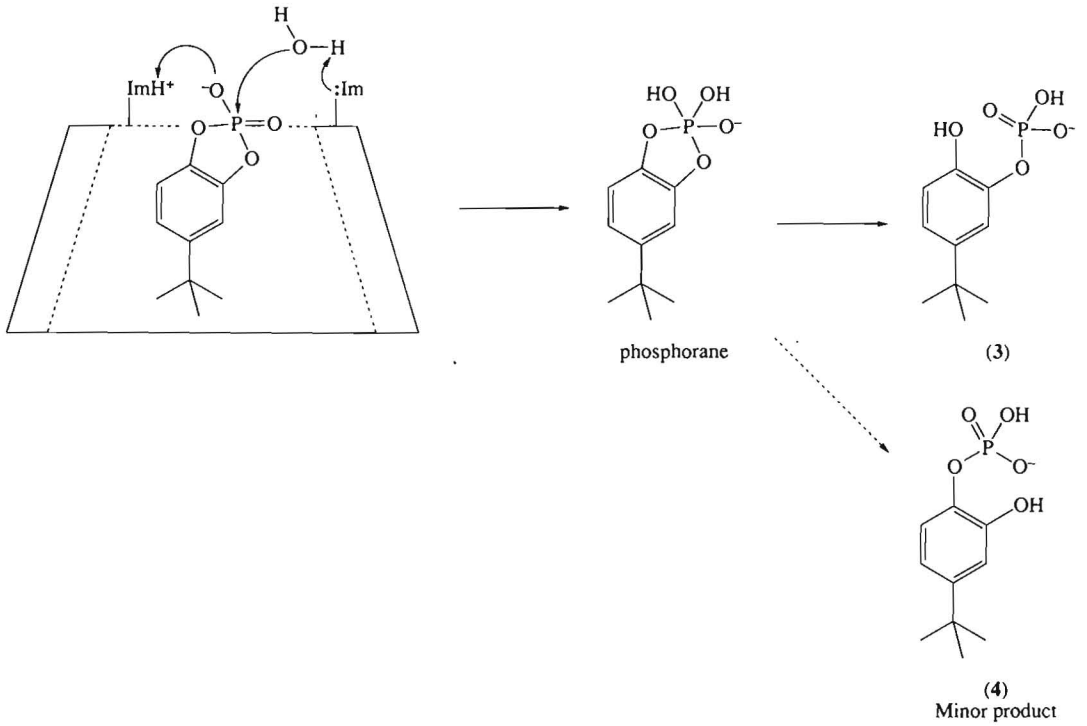



**Figure 5** Proposed mechanism for the hydrolysis of cyclic phosphate of catechol (**4**) by β-CDs with two imidazoles.

### *12.2.2.2 Hydrolysis of DNAs (nuclease models)*

Complexes of $Ce^{IV}$ and CD (α-, β-, and γ-) efficiently hydrolyze DNA at pH 8 and 50 °C.[32] The half-life of a phosphodiester linkage is around 5 h. Since the half-life in the absence of the complexes is 200 million years, acceleration by the complexes is almost $10^{12}$-fold! In the presence of CDs, the reactions proceed totally homogeneously. In their absence, however, the metal ion is precipitated as the metal hydroxide and the system readily becomes heterogeneous. The homogeneous complexes can be used as active sites for the artificial nucleases which selectively hydrolyze DNAs at the target sites.[33]

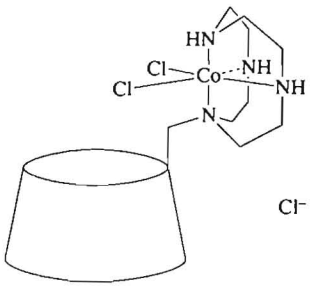
The $Ce^{IV}$–CD complexes are also effective for the hydrolysis of monophosphates of ribonucleosides and deoxyribonucleosides, and thus also mimic phosphomonoesterases.

### *12.2.2.3 Cleavage of other phosphate compounds*

β-CD accelerates the cleavage of bis(4-nitrophenyl) phosphate 92-fold.[34] The secondary alkoxide anion as a nucleophile attacks the phosphorus atom, resulting in phosphoryl transfer to β-CD. For the hydrolysis of phosphodiesters in RNAs, however, nucleophilic catalysis is not effective (see Section 12.2.2.1). The leaving groups (the hydroxyl residues of ribonucleosides) in RNA hydrolyses are much poorer than 4-nitrophenolate in the hydrolysis of bis(4-nitrophenyl) phosphate, which makes nucleophilic catalysis by β-CD (especially the removal of the leaving groups from the intermediate) less efficient.

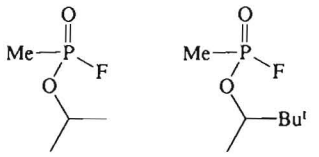
β-CD with a diethylenetriamine residue catalyzes the hydrolysis of bis(2,4-dinitrophenyl) phosphate.[35] Here, a protonated amino residue and a neutral amino residue in diethylenetriamine show cooperative acid–base cooperation, as do the two imidazoles at the active site of ribonuclease. β-CD attached to a $Co^{III}$ complex (**5**) also catalyzes the hydrolysis of bis(4-nitrophenyl) phosphate.[36] In both cases, the β-CD moiety functions as the binding site for the substrates, increasing the local concentrations of the corresponding catalytic residues around the substrates.

Activated phosphate compounds such as (**6**) and (**7**), which are vigorously poisonous to animals due to irreversible deactivation of choline esterase *in vivo*, are cleaved by CDs.[37] Significant enantioselectivities are observed. However, the reactions can not be catalytic (with respect to CD), since phosphoryl CDs are formed as stable intermediates by nucleophilic attack of the secondary




(5)

alkoxide groups (a similar reaction with the serine residue at the active site is responsible for the deactivation of choline esterase).




(6) Sarin (7) Soman

In order to achieve catalytic cleavage of these poisonous compounds, a modified CD (**8**) is prepared by conjugating β-CD with iodosobenzoic acid (Figure 6).[38] Iodosobenzoic acid is an effective catalyst for their cleavage, during which no stable intermediate is formed. When a phosphonofluoridate soman (**7**) is treated with a catalytic amount of (**8**) at pH 7.4 and 37 °C, almost all of (**7**) is rapidly cleaved within 30 min. Successful turnover is taking place. The reaction proceeds via nucleophilic attack by the OH residue on the iodine toward the phosphorus atom. Iodosobenzoic acid without the β-CD residue is only marginally active (three orders of magnitude less), and thus binding of the substrate to the β-CD residue is essential for the effective decomposition of (**7**) (a soman hydrolase mimic).

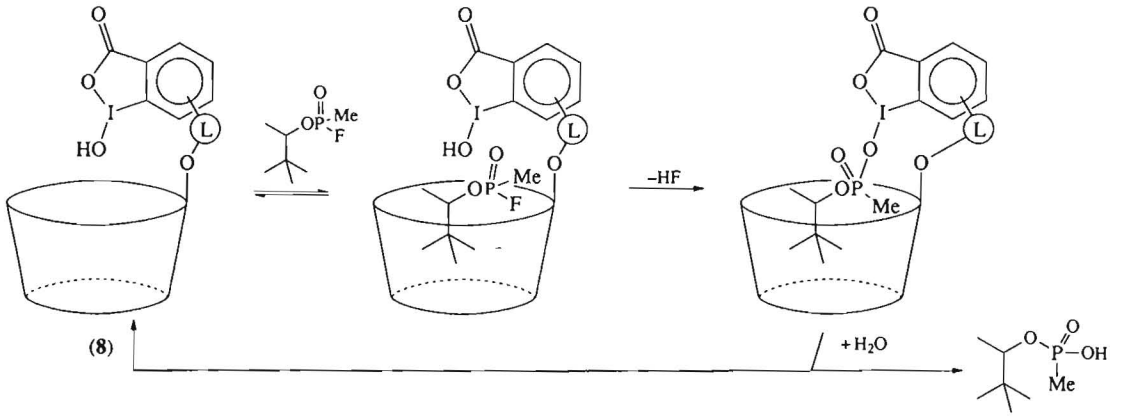
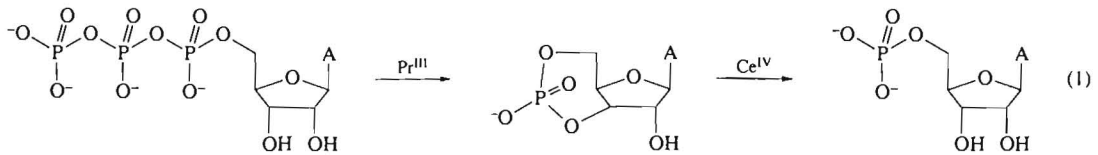



**Figure 6** Catalytic hydrolysis of soman (**7**) by the iodosobenzoic acid-appended β-CD (**8**).

### 12.2.3 Hydrolysis of Second-messenger for Cell-to-cell Communication and its Formation from Adenosine Triphosphate (Models of Phosphodiesterase and Adenylate Cyclase)

The 3′,5′-cyclic monophosphate of adenosine (**9**) is the second messenger for cell-to-cell communication. When a cell is activated by an external stimulus (the first messenger), the cyclic

phosphate is formed in the cell from ATP. An enzyme named adenylate cyclase catalyzes this reaction. The cyclic phosphate (**9**) activates various enzymes in the cell, resulting in the response of the cell against the external stimulus. The response terminates when the cyclic monophosphate is hydrolyzed by another enzyme, phosphodiesterase (Equation (1)).




(9) (1)

The function of adenylate cyclase (formation of (**9**) from ATP) has been successfully mimicked by the combination of β-CD and lanthanide(III) ions.[39] The praseodymium(III) ion (the third metal in lanthanide series) is the most active. This is the first nonenzymatic formation of (**9**) from ATP under physiological conditions. All the previous attempts to cyclize ATP were unsuccessful, since only the hydrolysis of ATP to its monophosphate and diphosphate took place without the required cyclization.

For the cleavage of (**9**), the combination of γ-CD and $Ce^{IV}$ is enormously effective.[40] At pH 7 and 30 °C, (**9**) is hydrolyzed to adenosine monophosphate with a half-life of only 6 s. Without this combination, the half-life of (**9**) is estimated to be 3 000 000 yr. Thus, about $10^{13}$-fold acceleration has been accomplished! This man-made adenylate cyclase and phosphodiesterase should open the way to artificial regulation of cell functions.

### 12.2.4 Hydrolysis of Carboxylate Esters (Esterase Models)

#### *12.2.4.1 Catalysis by native CDs*

Of all the catalytic reactions by CDs, carboxylate ester hydrolyses have been the most widely studied, and have provided much important and fundamental information. In their pioneering work, Bender and co-workers showed that *meta*-substituted phenyl acetates are hydrolyzed by CDs much faster than the corresponding *ortho*- and *para*-substituted phenyl acetates.[3,4] The specificity has been ascribed to the mutual position of the nucleophilic center of CD (the secondary hydroxyl group) and the electrophilic center (the carbonyl carbon of the substrate ester). In the inclusion complexes of the *meta*-compounds, the distance between the nucleophilic center and the electrophilic center is small. Thus, only small structural change of the inclusion complex (shift of the guest compound in the cavity) is required to proceed from the initial state to the transition state. For the *para*-compounds, however, the distance is so large that the process accompanies a significant structural change.[41] The energy required for the process increases the activation free energy and decreases the reaction rate. This interpretation has been confirmed by the structures of the inclusion complexes determined by $^1H$ and $^{13}C$ NMR spectroscopy.[42]

The argument is further supported by the fact that *p*-nitrophenyl 3-ferrocenylacrylate (**10a**) is cleaved at a remarkable rate by β-CD ($3.3 \times 10^5$-fold acceleration).[43–5] The acceleration is much larger than the corresponding value for *m-t*-butylphenyl acetate (260-fold acceleration). A molecular model study suggests that the β-CD–(**10a**) complex can go to the tetrahedral intermediate with full retention of the optimum binding geometry in the CD cavity (Figure 7). In β-CD induced hydrolysis of *m-t*-butylphenyl acetate, however, the substrate has to be pulled out of the cavity to form the tetrahedral transition state (although being small compared with the hydrolysis of *o*- and *p*-butylphenyl acetates). Consistently, a still greater acceleration is achieved when the flexible acrylate side chain is incorporated into a fused ring ((**10b**) in Figure 7) so that a better geometry is attained.[46] The rates of β-CD induced hydrolysis of these ferrocenylacrylate esters are nearly comparable with those for enzymatic hydrolysis, clearly evidencing the importance of the "proximity effect" in enzymatic reactions.

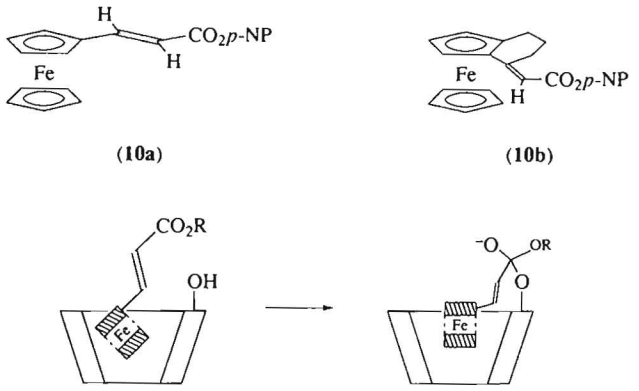
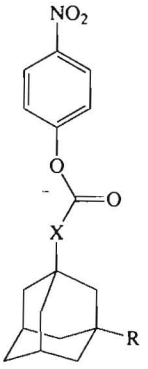



**Figure 7** Structures of *p*-nitrophenyl ferrocenylacrylate (**10a**) and its derivative (**10b**) as well as schematic views for their hydrolysis by β-CD.

However, catalysis by β-CD is remarkable only for phenyl esters of ferrocenylacrylate, and is virtually nil when the leaving groups are replaced by poorer ones. For example, acceleration by β-CD on the cleavage of ethyl, benzyl, and *p*-nitrobenzyl esters of ferrocenylacrylate is less than twofold.[47] The basicity of the leaving group is overwhelmingly important for the effective reactions. These results are interpreted in terms of the instability of the resultant acyl CDs, in which the ferrocenyl residue is incorporated into the cavity of β-CD and the acrylate residue covalently binds to a secondary hydroxyl residue of β-CD. This simultaneous fixation at the two sites causes a significant steric strain in the acyl CD. Thus, the tetrahedral intermediate, formed by nucleophilic attack by the secondary hydroxyl residue of β-CD, can only proceed to the acyl CD (accompanied by the release of the leaving group) when the leaving group is sufficiently good. Otherwise, the intermediate returns to the ferrocenyl ester and β-CD, and no ester cleavage takes place. Molecular mechanics calculations are qualitatively consistent with these ideas, although details of the mechanism are still controversial.[48–50]

The cleavage rates of the relevant substrates (**11a**)–(**11c**) by β-CD are highly dependent on the linker moiety between the adamantyl residue and the 4-nitrophenyl residue. The hydrolysis of (**11a**) is retarded 28-fold by β-CD, which is in contrast with a threefold acceleration by β-CD for the hydrolysis of (**11b**).[51] Furthermore, hydrolysis of (**11c**) is accelerated by 2150-fold. Here the adamantyl portion is located inside the cavity because of its apolar character. Thus the positions of the electrophilic centers (the carbonyl carbon atoms) in the cavity are governed by X in (**11**), resulting in remarkable substrate specificity. In the hydrolysis of trifluoroethyl 4-nitrobenzoate, α-CD exhibits a general base catalysis rather than a nucleophilic catalysis.[52]




(**11**)
(**a**) X = none, R = H
(**b**) X = —$CH_2$—, R = H
(**c**) X = —≡—, R = H

The hydrolysis of nitrophenyl esters of alkanoates by α- and β-CDs has been extensively investigated.[53–9] The structures of the inclusion complexes formed are governed by the length of the

alkyl chains in the alkanoates. When the alkyl chains are short, the nitrophenyl residues of the substrates are accommodated in the cavity of CDs, with the alkyl chains located outside. On the other hand, the alkyl groups are incorporated in the cavity when they are sufficiently long. Furthermore, ternary complexes, in which both alkyl and phenyl groups are individually accommodated by two CD molecules, are also formed if the concentrations of CDs are sufficiently large. The hydrolysis of nitrophenyl esters of aromatic acids involves inclusion of the aromatic acid residue, rather than inclusion of the nitrophenyl moiety.[60]

A significant stereoselectivity is observed in CD-catalyzed hydrolysis of *p*-nitrophenyl esters of dipeptide diastereomers.[61] The hydrolysis of (*Z*)-D-Phe-L-Phe-*p*-NP by γ-CD is 46-fold faster than that of (*Z*)-L-Phe-L-Phe-*p*-NP. The specificity is not due to the difference in the binding activity by γ-CD (the former isomer is only 2.4-fold more favorably included than the latter), and thus is undoubtedly associated with the catalytic process itself. α- and β-CDs also show specificities ($k_{DL}/k_{LL}$ = 22 and 42, respectively). However, the stereoselectivity is smaller for the hydrolysis of (*Z*)-Leu-Phe-*p*-NP ($k_{LL}/k_{DL}$ = 1.7–5.7). Smaller enantioselectivity by CDs has been observed for the hydrolysis of nitrophenyl esters of carboxylates and carbonates.[53–5,62,63]

The hydrolysis of *p*-nitrophenyl esters also has been performed with CD-appended polymers and copolymers.[64–6]

### *12.2.4.2 Catalysis by modified CDs*

The methods of chemical modification of CDs are divided into the following two categories: (i) introduction of catalytic residues for versatile and efficient catalysis, and (ii) improvement of binding properties of the cavities for better geometries of CD–substrate complexes.

#### *(i) Introduction of catalytic residues*

Attachment of an imidazolyl residue to β-CD provides a better chymotrypsin model than β-CD itself, which has only hydroxyl groups as the catalytic residues.[67] In fact, *p*-nitrophenyl acetate is hydrolyzed efficiently at neutral pH by the modified β-CD, although catalysis by native β-CD is effective only in alkaline solutions. In addition, the artificial enzyme has been successfully recycled, since no stable intermediate (such as acyl CD in the CD-promoted reaction) is formed. Noncovalent mixtures of β-CD and imidazole derivatives also efficiently cleave *m*-nitrophenyl acetate.[68] It has been suggested that the imidazoles act as general base catalysts for CD esterolysis.

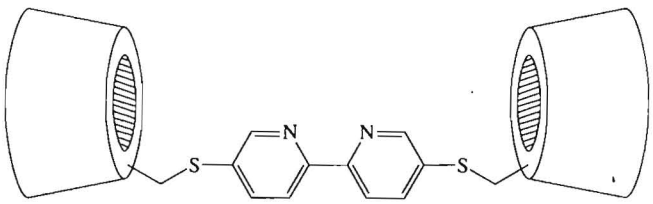
More recently, a β-CD derivative in which an imidazole moiety is directly attached to a C-2 carbon has shown a remarkable accelerating effect on the hydrolysis of *p*-nitrophenyl acetate.[69] The acceleration at pH 7.5 is more than 1000-fold larger than that by native β-CD. When an imidazole is attached to the C-6 position of β-CD, however, the accelerating effect is only marginal. The ratio of catalytic activities of these two modified CDs is more than 70. The large difference in the activity is ascribed to the difference in the mutual orientation between the catalytic residue (imidazole) and the reaction center (the carbonyl carbon of the substrate) in the inclusion complexes. Only the first isomer gives rise to an appropriate orientation for the reaction, since the substrate penetrates into the cavity of β-CD from the secondary hydroxyl side, with the nitro residue first. β-CD bearing a hydroxylamino group, which is an eminent nucleophile due to the so-called α-effect, efficiently cleaves *p*-nitrophenyl acetate even at pH 6.[70]

Imidazole-appended γ-CD, in which the imidazole moiety is attached to the C-6 position, is interesting, since the catalytic activity can be regulated by the third guest compound.[71] Although its intrinsic activity is rather small, the catalytic activity increases when 1-naphthylacetate is added to the system. Here, the third guest compound functions as a spacer and narrows the wide cavity of γ-CD so that the cavity efficiently accommodates the substrate. Consistently, the catalytic activity of the corresponding modified β-CD for the hydrolysis is suppressed by 1-naphthylacetate, since it competitively inhibits the binding of the substrate to the cavity of β-CD which can accommodate only one guest molecule.

Modified β-CD bearing an L or D-histidine residue at the secondary hydroxyl side shows enantioselective cleavage of *p*-nitrophenyl esters of amino acids.[72] The ratio of the hydrolysis rates of the L and D derivatives of phenylalanine by β-CD-L-His is 2.3. Here the L-His residue is located in a favorable position for catalysis of the hydrolysis of the L-phenylalanine derivative, but is not favorable for the hydrolysis of the D-counterpart. Native β-CD slightly prefers hydrolysis of the D-isomer (L:D = 0.72).

Introduction of an imidazole to the secondary hydroxyl side of heptakis-2,6-dimethyl-β-CD provides a useful α-chymotrypsin model.[73–5] For the hydrolysis of *p*-nitrophenyl acetate, the catalytic rate constant is almost comparable to the value of chymotrypsin, although the binding is weaker. Catalytic turnover is also achieved. Interestingly, the catalytic rate constant is nine times as large as that of native β-CD bearing a histamine residue, indicating that the apolar character in the dimethyl-β-CD residue promotes the catalysis. Catalysis is less effective for the hydrolysis of *m*- and *o*-nitrophenyl acetates.

The combination of $Cu^{II}$ ion with a β-CD dimer having a bipyridine ligand (**12**) shows both large substrate-binding activity and large catalytic activity.[76] For example, the hydrolysis of (**11c**) is accelerated by $2.2 \times 10^5$-fold. In complex formation with (**12**), both the adamantyl and 4-nitrophenyl residues are incorporated into each of the two β-CD cavities, and thus the ester moiety is located in the vicinity of the catalytically active $Cu^{II}$ ion. A similar type composite was formed from two molecules of the modified β-CD having an ethylenediamine residue and a $Cu^{II}$ ion.[77] Here the ethylenediamine residues of two of the modified β-CDs coordinate to a $Cu^{II}$ ion, forming a noncovalently bound β-CD dimer.




(12)

At the active site in serine proteases, carboxylate (aspartate), imidazole (histidine), and hydroxyl residue (serine) form triad charge-relay system. It was proposed that a proton relay from the serine to the histidine is accompanied by proton transfer from the histidine to the aspartate, which is a key for the enormous catalytic activities of these enzymes. Whether this proposal is valid or not is still a subject of argument.

As a mimic of the charge-relay system, the effect of 2-benzimidazoleacetic acid on the α-CD-catalyzed hydrolysis of *m*-*t*-butylphenyl acetate was examined.[78] About 10-fold acceleration, which is ascribable to cooperation of the carboxylate, the imidazole, and the hydroxyl residues, was observed.

An even better mimic for the charge-relay system (**13**) has been reported.[79,80] Both carboxylate and imidazole were attached to α, β-, and γ-CDs. The kinetic parameters for ester hydrolysis by the β-CD-based artificial enzyme are almost comparable with the values of chymotrypsin. The specificities are dependent on the kind of CD. Whereas α- and β-CD-based artificial enzymes are better than the γ-CD-based artificial enzyme in phenyl ester hydrolysis, γ-CD-based artificial enzyme hydrolyzes tryptophan ethyl ester faster than the other two. In the same way as chymotrypsin, the artificial enzymes show presteady-state acylation and steady-state deacylation and turnover (Figure 8). Thus, a superb artificial enzyme is now in hand.

Alternatively, the serine–histidine–aspartate catalytic triad has been introduced as a tripeptide (Ser-His-Asp) to the primary hydroxyl side of β-CD.[81] This compound hydrolyzes *p*-nitrophenyl esters with various alkyl group lengths. *p*-Nitrophenyl hexanoate is hydrolyzed 3.4-fold faster than by the tripeptide itself.

*(ii) Modulation of binding process*

In order to strengthen the hydrophobic interactions between CD and guest compounds, a capped β-CD (**14a**) was synthesized.[82] As expected, they show stronger binding towards *m*- and *p*-nitrophenyl acetates. However, hydrolysis by this modified β-CD is slower than that by native β-CD. Here, the substrate penetrates into the cavity so deeply that the secondary hydroxyl group of the β-CD cannot attack the carbonyl carbon atom efficiently. However, a flexibly capped β-CD (**14b**), in which all the primary hydroxyl groups are converted into *N*-formal amino groups, show larger acceleration than native β-CD.[83] Here, penetration of the substrate into the cavity is rather

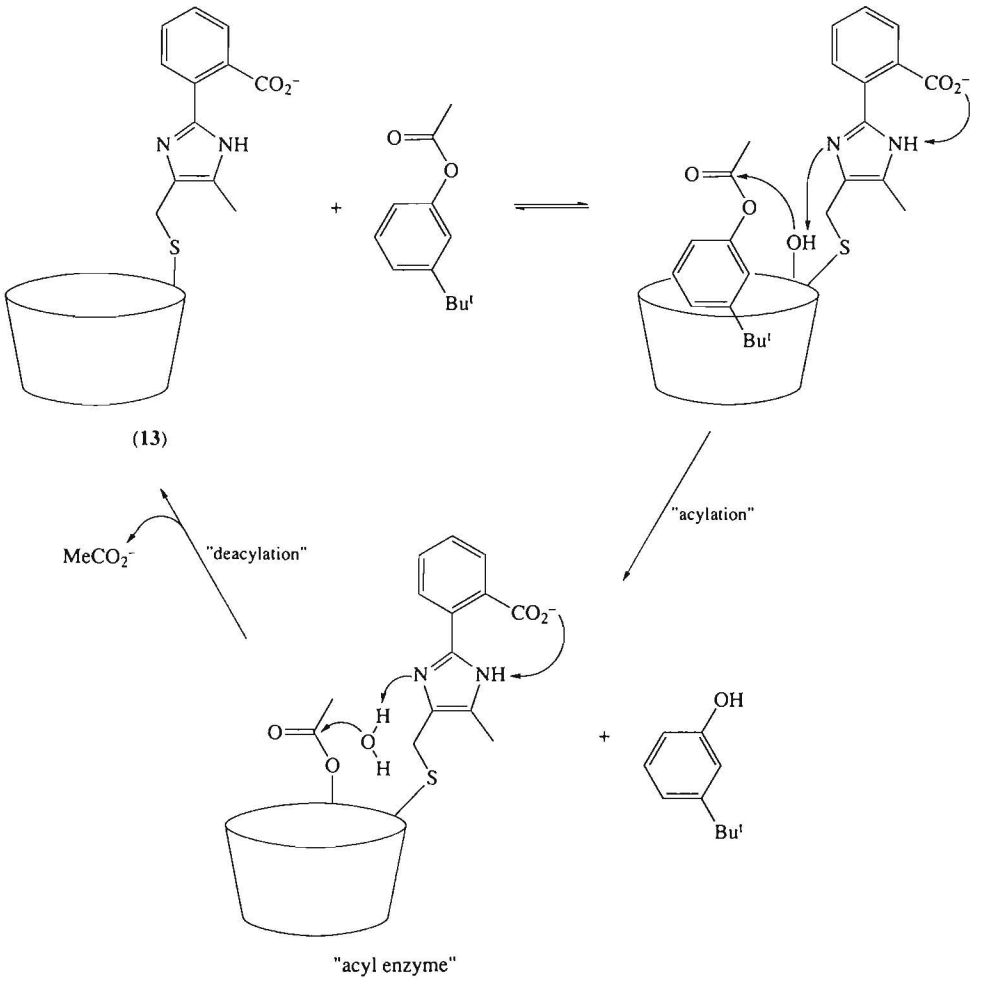
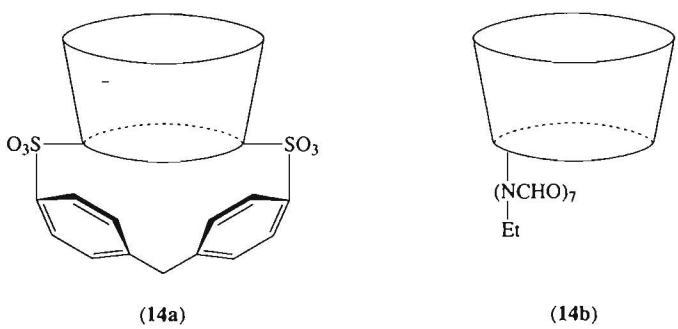



**Figure 8** Mechanism of ester hydrolysis by artificial chymotrypsin (a modified CD involving the charge-relay system composed of hydroxyl, imidazolyl, and carboxylate residues).

shallow so that the attack by the secondary hydroxyl group can proceed smoothly. Importance of the proximity of the reaction centers, evidenced above for the hydrolysis of ferrocenyl esters, is again substantiated. Introduction of positively or negative charged residues to β-CD has been also investigated, and the results are satisfactorily interpreted in terms of the electrostatic interactions of the charges with the substrates.[84,85]




**(14a)** **(14b)**

The hydrolysis of *m*- and *p*-nitrophenyl acetates by γ-CD bearing a naphthyl residue is more than 10-fold faster than that by native γ-CD.[86] Here, the naphthyl residue functions as a spacer to

narrow the large cavity of γ-CD to fit the substrate. Consistently, the pseudo-first-order rate constants for cleavage by the modified γ-CD are only three times as large as the values by native γ-CD. Thus the larger activities of the modified γ-CDs are, at least partially, ascribed to the effect in the binding process. When the modified γ-CD binds *p*-nitrophenyl acetate, the naphthyl moiety makes its carbonyl group stand at the correct position for attack of the secondary hydroxyl side of the modified γ-CD (Figure 9). This mimics "induced-fit" observed in natural enzymes.

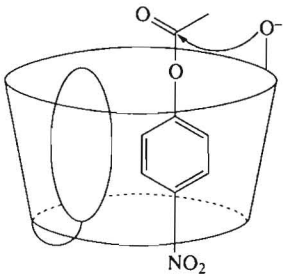



**Figure 9** Mechanism of nitrophenyl acetate hydrolysis by the γ-CD bearing a naphthyl residue: the ellipse shows the naphthyl residue which functions as a spacer.

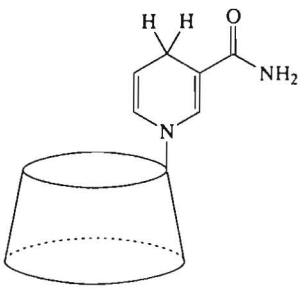
Enantioselective hydrolytic cleavage of nitrophenyl esters is performed by heptakis-[6-deoxy-6-(*N*-methylacetoamide)]-β-CD, heptakis-2,6-dimethyl-β-CD, and heptakis-2,3,6-trimethyl-β-CD.[87] The hydrolysis of 2,4-dinitrophenyl sulfate is accelerated by methylated β-CD derivatives in mixed solvent systems of reduced water content.[88]

## 12.3 OXIDATION AND REDUCTION

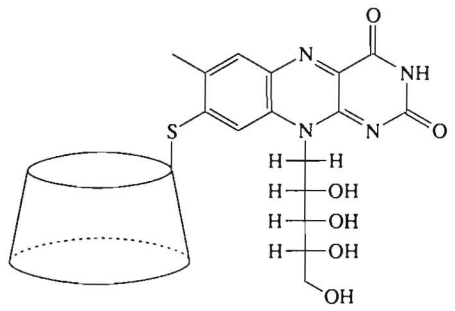
Attachment of a nicotinamide residue (NAD), a well-known cofactor for redox reactions, to β-CD provides a superb NADH coenzyme model (**15a**).[89–94] Reduction of ninhydrin by this mimic (in a reduced form) is 400-fold faster than that by dihydronicotinamide without β-CD. Here, ninhydrin and the β-CD residue form an inclusion complex ($K_d = 2.1 \times 10^{-5}$ M), which increases the effective concentration of the cofactor moiety at the ninhydrin. In addition, the dihydronicotinamide residue in the modified β-CD, which is otherwise rather unstable, is stabilized in water, because of encapsulation in the hydrophobic cavity of β-CD. Both effects give rise to a prompt reaction.

Compound (**15a**) is also effective for the asymmetric reduction of benzoylformic acid.[95] When benzoylformic acid is reduced in water by (**15a**) in the presence of $Cu^{II}$, D-mandelic acid of 46% *ee* is formed as the major product. The use of $Mg^{II}$ and $Zn^{II}$ in place of $Cu^{II}$, however, results in the predominant formation of benzoic acid (rather than mandelic acid). The chemical yield is low and, in addition, the enantiomeric excess in mandelic acid is decreased.

There have been several attempts to connect a flavin adenine dinucleotide (FAD), another cofactor for redox reactions, to CDs. Tabushi and Kodera constructed the first flavoenzyme mimic by the attachment of a riboflavin onto the primary face of α-CD (**15b**).[96] In the oxidation of an NADH analogue, *n*-hexyldihydronicotinamide, the binding constant and the reaction rate are $2500\,M^{-1}$ and $0.5\,s^{-1}$, respectively. A modified β-CD bearing a flavin moiety at the secondary hydroxyl side has also been prepared.[97]



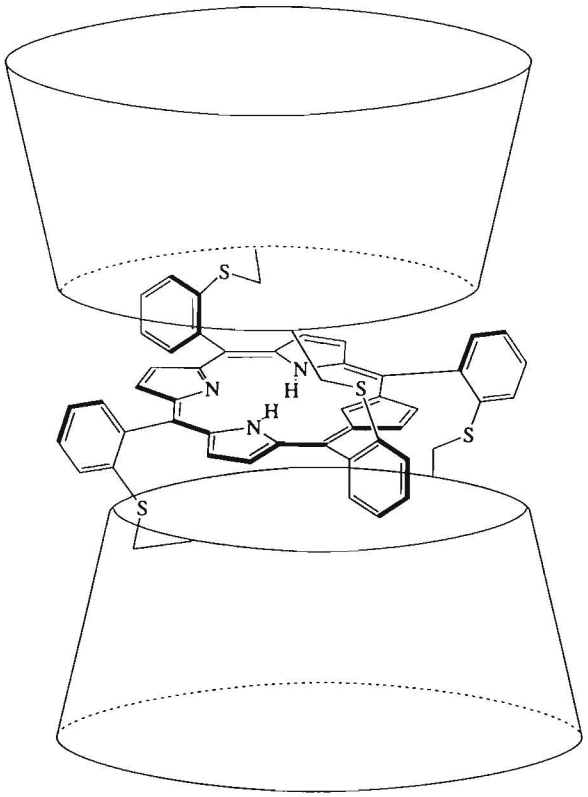

(15a)



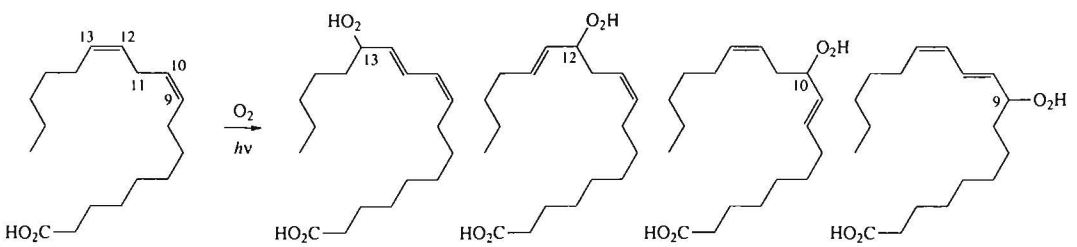

(15b)

2-[(7α-*O*-10-Methyl-7-isoalloxazino)methyl]-β-CD shows significant catalysis for the photooxidation of substituted benzyl alcohols.[98] The modified β-CD is rather stable under the photochemical conditions employed and exhibits more than 100 turnovers, although riboflavin itself readily decomposes and shows up to seven turnovers. It has been proposed that the geometry of the artificial enzyme–substrate complex is favorable for the photoreaction. β-CD, which is substituted at the 6-position with an electrophore (2-benzoylbenzoate), forms complexes with benzyl esters and promotes their cleavage at relatively modest reduction potentials.[99]

A porphyrin molecule sandwiched by two β-CD residues (**16**) regio- and stereoselectively oxidizes linoleic acid on photoirradiation (Equation (2)).[100] When the mixture is photoirradiated in the presence of this porphyrin derivative as catalyst, singlet oxygen is generated from the chromophore. The active species selectively attacks the $\Delta^{12,13}$ double bond, resulting in hydroperoxidation of the corresponding bond (the first two products in Equation (2): the selectivity is 82%). However, when a water-soluble porphyrin without a β-CD residue is used as the catalyst, the reaction takes place at both the $\Delta^{12,13}$ and $\Delta^{9,10}$ bonds almost nonselectively. Probably both linoleic acid and singlet oxygen are simultaneously accommodated in the hydrophobic pocket of the β-CD(s) throughout the hydroperoxidation reaction. Consistently, some enantiomeric excess (12–20%) is perceived in the products, confirming that the reaction is taking place in the asymmetric cavity. This is a mimic of lipoxygenase.




(**16**)

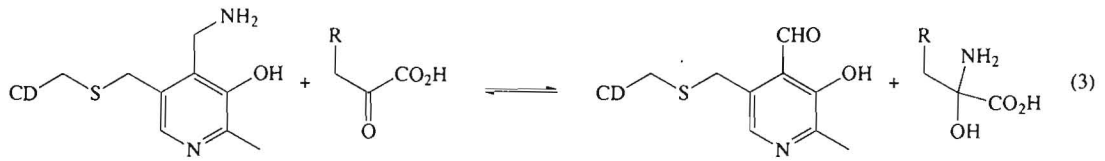



(2)

A manganese(III)–β-CD complex was prepared as a model for the photosynthetic water oxidizing enzyme.[101] In a proposed structure, two $Mn^{3+}$ ions, which are in the bis(μ-hydroxo)-bridged form, are placed near the primary hydroxyl side. Four primary hydroxyl groups are coordinated to the ions.

## 12.4 TRANSAMINATION (TRANSAMINASE MODELS)

A modified β-CD (**17a**), which contains a pyridoxamine moiety in the primary hydroxyl side, catalyzes the transamination of amino acids as shown in Equation (3).[102] The forward reaction between (**17a**) and indolepyruvic acid (**18**) (R = indole), which produces the intermediate (**19**) and tryptophane, is 200 times faster than the reaction between a pyridoxamine (without β-CD) and (**18**). This is because the first reaction is intracomplex and the second intermolecular. The transamination is completed by the back reaction of (**19**) with another amino acid, where another α-keto acid is formed and (**17a**) is regenerated. This is a model of the enzyme transaminase.




(17a) (18) (19) (3)

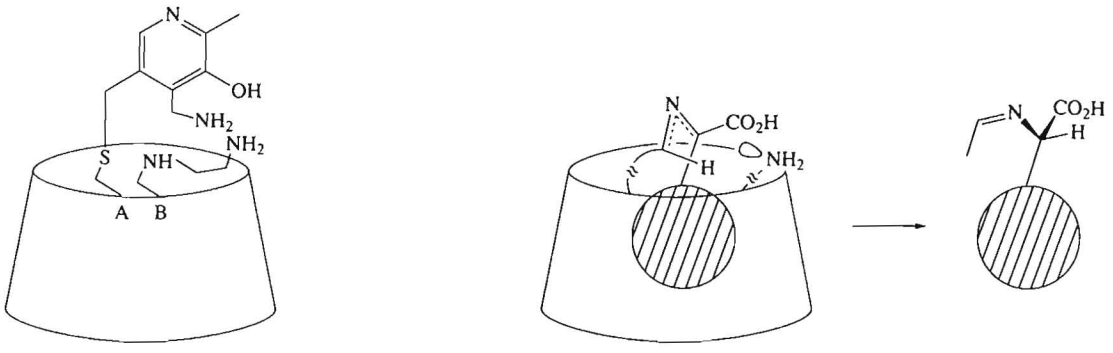
In addition to the large acceleration, the reactions by the artificial enzyme (**17a**) proceed enantioselectively, because of the chirality of the β-CD cavity. The tryptophane obtained from (**17a**) and indolepyruvic acid has 12% *ee*, whereas phenylalanine from phenylpyruvic acid has 67% *ee*. Both are rich in the L-forms.

The modified β-CD (**17b**), which has a pyridoxamine residue in the secondary hydroxyl side, also catalyzes the transamination.[103] Quite interestingly, the tryptophane prepared from indolepyruvic acid by the use of (**17b**) is rich in the D-form (33% *ee*), which is in great contrast to the preferable formation of the L-form by (**17a**). Thus, the position of the pyridoxamine residue (either in the primary hydroxyl side of the cavity or in the secondary side) governs the direction of the enantioselectivity.

A still more advanced transaminase mimic (**20**) showing a larger enantioselectivity has been prepared by the introduction of an additional catalytic moiety to (**17a**).[104] In (**20**), a pyridoxamine is attached to a glucose residue A, and an ethylenediamine residue is attached to the adjacent glucose unit B. The AB type isomer was separated by column chromatography from the BA type isomer, in which two functional groups changed positions with each other. The tryptophane obtained from (**20**) and indolepyruvic acid is rich in the L-form and has 95% *ee*. Both phenylalanine and alanine, obtained in a similar way from the corresponding α-keto acids, show 98% *ee*. Quite high enantioselectivities have been obtained. The reactions involve a planar C···N···C intermediate, and it is the direction of the attack by a proton (with respect to the plane of the intermediate) that determines the enantiomeric property of the product. In this modified β-CD, an ethylenediamine residue as a catalyst for proton transfer is fixed in one side of the plane so that the proton attack occurs dominantly from only one side of the plane, giving rise to a high enantioselectivity (Figure 10).

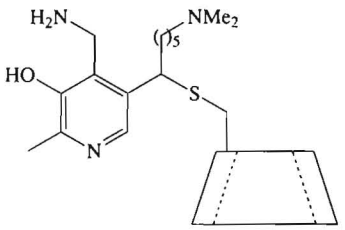
Enantioselective transamination has also been achieved by the modified β-CD, which has an amino residue in the linker between a pyridoxamine and β-CD (**21**). When (**18**) is reacted with (**21**), the maximum optical ratio (L:D) in the phenylalanine product is 6.8.[105] Increase of the length of the moiety between the linker and the amino residue decreases the enantioselectivity, probably due to an increased flexibility.

A modified β-CD, in which a pyridoxamine is connected to β-CD by two linkers, was also prepared.[106] Here, the geometry of the pyridoxamine, with respect to the β-CD residue, is strictly regulated, although remarkable selectivity has not yet been obtained.



(20)

**Figure 10** Mechanism of transamination by β-CD having a pyridoxamine moiety (**20**).

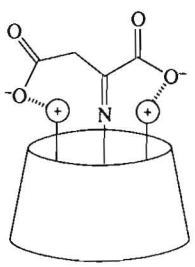


(21)

## 12.5 DECARBOXYLATION

CDs catalyze decarboxylation either by covalent catalysis of the hydroxyl groups or by microsolvent effect of the apolar cavities, as evidenced by Bender and co-workers.[107–9] However, the activities for the reactions are greatly promoted by the chemical modification of CDs.

β-CD attached with seven amino residues, heptakis(2,3-di-*O*-methyl-6-amino-6-deoxy)-β-CD (**22**), is highly active for the decarboxylation of oxalacetate.[110] At a catalyst concentration of $2 \times 10^{-3}$ M (at 25 °C, pH 5), the half-life is only around 6 s. In contrast, a modified β-CD with only one amino residue has virtually no catalytic activity. Apparently, two or three amino residues participate in the catalysis. The pH–rate constant profile is bell-shaped and shows a maximum around pH 4.5. The Michaelis constant is unusually small ($8.68 \times 10^{-4}$ M) for an anionic substrate, corresponding to quite strong binding of the substrate to (**22**). An imine intermediate is probably formed, which is stabilized by electrostatic interactions with the two neighboring protonated amino groups (Figure 11). To confirm the proposed mechanism, isomers of diamino-β-CDs, which have two amino residues at various glucose units, were isolated.[111] As expected, the AB isomer is the most active, and the AD isomer is inefficient for catalysis.



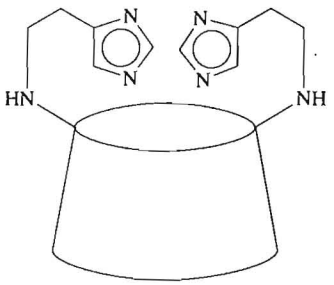
(22)

**Figure 11** Mechanism of decarboxylation of oxalacetate by the modified β-CD (**22**).

Attachment of a cyclic peptide (cyclo-(L-histidyl-l-histidyl) to β-CD is reported.[112] This host can bind various metal ions (e.g., $Cu^{II}$), and in addition has a number of molecular recognition sites towards substrates. Potentiality as an interesting catalyst has been indicated.

## 12.6 HYDRATION OF CARBON DIOXIDE (CARBONIC ANHYDRASE MODELS)

Carbonic anhydrase, which is responsible for the metabolism of carbon dioxide in animals and plants, has a zinc(II) ion surrounded by three imidazoles at its active site. Carbon dioxide is bound to the active site with the assistance of hydrophobic interactions, and is fixed in a close proximity to the zinc(II) ion. In the hydration, water is activated by coordination to the zinc(II), and attacks the carbon dioxide. It is assumed that a base catalysis by an imidazole is involved here. The zinc(II) complex of the modified β-CD (**23**) as a model for this enzyme efficiently catalyzes the reaction.[113] A zinc(II) ion is bound by the two imidazolyl residues, and the β-CD cavity mimics the apolar character of the enzyme active site. In fact, catalysis by the 1:2 zinc–imidazole complex without β-CD is much less efficient. The aliphatic amino residue attached to the β-CD is a substitute of the imidazole which functions as a base catalyst in the enzymatic reactions.




(23)

## 12.7 CONDENSATION

Benzoin condensation catalyzed by cyanide ion is about twofold accelerated by γ-CD.[114] The acceleration is ascribed to accommodation of two benzaldehyde molecules in the large cavity of γ-CD. The two molecules, located in a mutual conformation favorable for the condensation, are smoothly reacted. In contrast, β-CD decelerates the reaction, since the cavity is small and only one benzaldehyde molecule is included therein. Here the benzaldehyde is protected from condensation by the wall of β-CD.

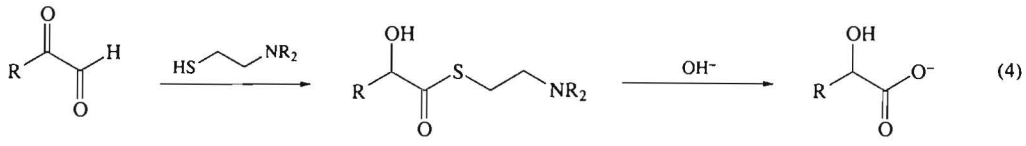
Based on this finding, various *N*-alkylated thiazolium salts, derivatives of a coenzyme thiamine pyrophosphate, were introduced to the C-6 position of γ-CD.[115] These modified γ-CDs efficiently catalyze the benzoin condensation, and the rates are more than 100-fold faster than the values for the corresponding thiazoliums without γ-CD. This is a mimic of the holoenzyme composed of thiamine pyrophosphate and apoenzyme.

Compound (**22**) is active for aldol condensation of pyruvic acid with pyridine aldehyde (aldolase model).[116] The activity is much larger than that of aminoacetonitrile. Here a Shiff base intermediate is formed between the amino residue on the β-CD and pyruvic acid. The pyridine aldehyde is included in the cavity, which increases its local concentration around the pyruvate residue fixed on the β-CD. Shiff base formation and host–guest complexation operate cooperatively to enhance the bimolecular condensation reaction.

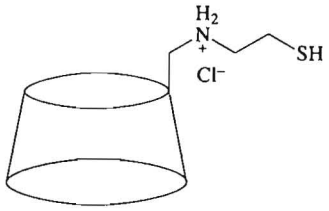
## 12.8 GLYOXALASE MODELS

Glyoxalases (I and II) and glutathione constitute a set of glyoxalase enzymes, which are of interest because of their importance in catalyzing the transformation of α-keto aldehyde into the corresponding α-hydroxycarboxylic acids (Equation (4)). As a mimic of the system, a trifunctional mono[6-(2-mercaptoethylamino)-6-deoxy-β-CD (**24**) was prepared.[117] The reaction with this

artificial enzyme is faster than the value for the reference compound 2-(dimethylamino)ethanethiol, transforming phenylglyoxal into L-mandelic acid in 47% *ee*.



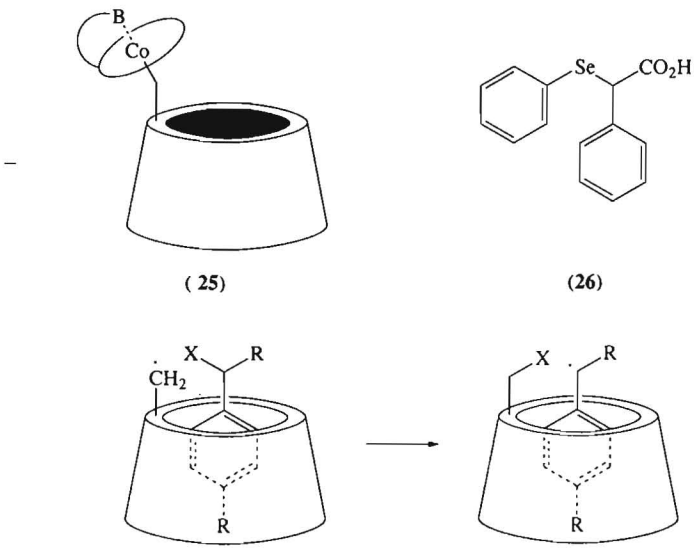
(4)



(24)

## 12.9 MIMICS OF COENZYME $B_{12}$

Homolysis of the carbon–cobalt bond of adenosylcobalamin coenzyme (coenzyme $B_{12}$) is partially mimicked by the β-CD bearing cob(I)alamin (**25**).[118] The standard mechanism for rearrangements catalyzed by this coenzyme is as follows: (i) homolysis to form $B_{12(r)}$ and the enzyme-bound deoxyadenosyl radical, followed by (ii) hydrogen atom transfer from the bound substrate to this radical to form a substrate radical. Then (iii) the derived substrate radical rearranges, followed by (iv) hydrogen atom transfer back to reform the deoxyadenosyl radical, which then couples with $B_{12(r)}$ to reform the coenzyme. The homolysis of (**25**) generates $B_{12(r)}$ and a primary CD radical of a glucose unit which is strongly related to the primary ribose radical formed from the true coenzyme. When (**25**) is treated with a selenide (**26**) in deoxygenated neutral 0.05 M phosphate buffer, the Ph—Se— group is transferred from the selenide substrate to the primary hydroxyl residue of β-CD, as in the enzymatic reaction (Equation (5)). Apparently, the primary CD radical is formed by the homolytic cleavage of the bond between cobalamin and β-CD. This is certainly an interesting approach toward coenzyme $B_{12}$ mimic, although the ultimate function of coenzyme $B_{12}$ has not yet been accomplished.

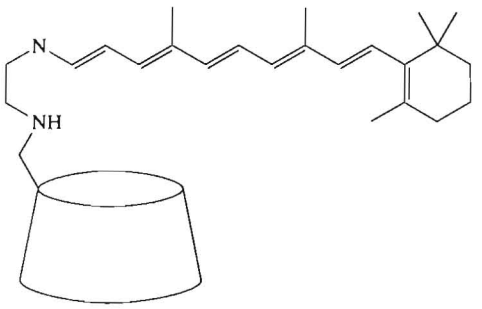


(25) (26)

(5)

## 12.10 MIMICS OF SOME PROTEINS

A modified β-CD (**27**) was prepared as a mimic of a visual pigment, rhodopsin.[119] This has an absorption maximum at 375 nm in its neutral form. When both the nitrogen atoms are protonated at low pH, however, the maximum shows a red shift of 100–120 nm, and the absorption spectrum of the protonated form is almost identical to that of native rhodopsin. Probably the retinal moiety of (**27**) is included in the cavity of the β-CD at low pH, resulting in a red shift due to the combination of an electrostatic effect and the microsolvent effect of the cavity.




(27)

Some of the spectroscopic properties of heme-containing proteins are mimicked by complexation (molar ratio 1:2) with heptakis(2,6-di-*O*-methyl)-β-CD.[120] The porphyrin residue is encapsulated in the cavities of two modified β-CDs via the secondary face first. Both an $Fe_4S_4$ cluster[121] and a porphyrin[122] were sandwiched by two CD residues, mimicking the corresponding proteins.

## 12.11 THE PREPARATION OF MORE ADVANCED ENZYME MODELS

Site-selective introduction of functional residues to CDs has in most cases been achieved via the corresponding tosylates. Capped CDs are available for the introduction of two functional groups to CDs (details are described in Chapter 4). Furthermore, various useful techniques for the preparation of modified CDs as enzyme models have been reported.

Each of ($6^A$-*O*-(4-nitrobenzenesulfonyl)-$6^X$-(β-naphthalenesulfonyl)-β-CD) (X = B–G), which is useful for the preparation of unsymmetrically bifunctionalized CDs, has been synthesized.[123] The preparation of β-CD with pertosylated secondary hydroxyl groups at the 2-positions has also been reported.[124] CD derivatives with a β-*N*-glucosyl residue as the recognition site for cells has also been prepared, which may be useful for the vectorized transport of drugs *in vivo*.[125]

An improved route for the introduction of a functional residue to the secondary hydroxyl side has been presented.[126] First, all the primary hydroxyl groups are silylated by *t*-butyldimethylsilyl groups. This makes β-CD much less polar and soluble in organic solvents. Thus, the products can be purified by chromatography on silica gel on a preparative scale. Subsequently, the compounds can be converted to the desired modified CDs by the usual methods.

Visual observation of CDs and their inclusion complexes by scanning tunneling microscopy[127–31] has been carried out to obtain detailed information for the molecular design of further advanced enzyme models. The specimens are prepared by depositing aqueous solutions of CDs or their complexes on a graphite substrate (or other substrates) and drying the liquid phase. Molecular images of the cyclic oligomers and their complexes have been obtained for the first time.

## 12.12 CONCLUSION

The molecular design and synthesis of CD-based enzyme models have shown remarkable progress in the 1990s, and a variety of bioreactions have been successfully mimicked. The trend should undoubtedly continue, possibly even more dramatically. The development of methods for the site-selective and regioselective modification of CDs have made valuable and essential

contributions to this. In the near future, these artificial enzymes will be widely used *in vivo* and *in vitro* to selectively accelerate, decelerate, and/or regulate the target reactions at will.

## ACKNOWLEDGMENTS

This chapter is dedicated to the memory of Professor Myron L. Bender, one of the most important pioneers in cyclodextrin chemistry, and his wife Mrs. Muriel S. Bender.

Financial support by a Grant-in-Aid for Scientific Research on Priority Areas "New Development of Rare Earth Complexes"(Nos. 06241103 and 06241107) from the Ministry of Education, Science, and Culture is highly acknowledged.

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