Cyclodextrin Catalysis as a Model for Enzyme Action

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Cyclodextrin is the name of a class of cyclic oligomers of glucose whose members are α- (hexamer), β- (heptamer), γ- (octamer) cyclodextrin, etc. Cyclodextrin[1] is a well-constructed miniature of an enzyme in the sense that it has a hydrophobic cavity of appropriate size, sites for introduction of catalytic groups at juxta positions, and satisfactory water solubility. The basic understanding of specific binding and catalysis of enzyme action is one of the most significant targets of cyclodextrin chemistry. Further development of the concept of inclusion catalysis from simple enzyme models into the more artificial, well-organized catalytic system of "superenzyme" activity may be one of the future targets.

Crystallographic studies of cyclodextrins,[2] their derivatives,[3] and inclusion complexes[4] reveal that water molecules occupy the hydrophobic cavity in the absence of a guest molecule. However, a specific "guest" molecule, when added into the cyclodextrin solution, drives water molecules out of the cavity by occupying the cavity by itself (Figure 1). Importantly, the conformational change of cyclodextrin during this process is not too serious, and for this reason, a conformational difference between cyclodextrin hydrate in the crystalline state and cyclodextrin in aqueous solution should be very small. This is even more the case for β-cyclodextrin where the crystalline hydrate has a conformation very close to the expected torus in contrast to the α-hydrate's partly tilted torus. Thus, β-cyclodextrin is free of the rather difficult conformational problems involved in complex formation.

The driving force ($-\Delta G°$) which brings a hydrophobic guest molecule into the cavity of cyclodextrin and drives water molecules out seems to be a complex composite of different "elemental" forces such as activated (less hydrogen-bonded) water,[5] a change in degrees of motional freedoms, conformation energy,[6] etc. But the major driving force binding a nonpolar guest molecule is the so-called "hydrophobic interaction", which dominates when electrostatic or coordination interactions are not important.

A hydrocarbon-like molecule dissolved in water is surrounded by water assemblies along its exposed surface. This process is entropically very unfavorable, as is typically shown for transfer of methane from the gaseous to the aqueous phase. The hydrophobic interaction, most simply expressed, is the tendency of a hydrocarbon-like molecule to reduce these water assemblies by making contact at its surface with the surface of another hydrocarbon-like molecule. This hydrophobic interaction contributes in a most significant way to the hydrophobic recognition by cyclodextrins.

Recognition of the Shape of a Guest Molecule by The Cyclodextrin Cavity

Most enzymes have a hydrophobic pocket or cleft to bind a substrate, inhibitor, activator, or other substance through hydrophobic interaction. Apparently, a cleft or a pocket is a much stronger recognition site than a simple plane because of larger surface area contact to produce enhanced "destruction" of a water assembly. In the cyclodextrin–guest complex formation, we have a situation similar to the enzyme–counterpart complex (more generally, agonist–antagonist complex). Cyclodextrin can strip the unfavorable water assembly around a guest molecule by making an inclusion complex. As apparent from the X-ray studies, no water molecules remain in α-cyclodextrin cavity, and the guest is in van der Waals contact with the cavity wall. Even counterions stay outside the cavity when an ionic guest is bound. Although limited, this information affords a reasonable picture about the nature of the host–guest shape recognition and is relevant to an understanding of enzyme–counterpart complexes.

According to the concept of hydrophobic interaction, the total interaction between α-cyclodextrin and a benzene derivative was calculated on the basis of the following thermodynamic processes (eq 1; see Table I and Figure 2).[7]

$$\Delta H_{\text{inclusion}} = (H^{c}_{\text{vdw}} - H^{w}_{\text{vds}}) + (H^{c}_{\text{conf}} - H^{w}_{\text{conf}}) - \Delta H^{g}_{\text{cluster}} - 2\Delta H^{w}_{\text{vap}} - 2H_{\text{H-bond}} \quad (1a)$$

$$\Delta S_{\text{inclusion}} = (S^{g}_{\text{rot(1-D)}} - S^{g}_{\text{rot(3-D)}} - S^{g}_{\text{trans}}) - 2(S^{w}_{\text{rot(3-D)}} - S^{w}_{\text{trans}}) + 2\Delta S^{w}_{\text{gas}\rightarrow\text{liq}} - \Delta S^{g}_{\text{cluster}} \quad (1b)$$

Quantitative estimation of the destruction of the water

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(1) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry", Springer-Verlag, 1978.
(2) (a) W. Saenger, M. Noltemeyer, P. C. Manor, B. Hingerty, and B. Klar, *Bioorg. Chem.*, **5**, 187 (1976); (b) W. Saenger and K. Lindner, *Angew. Chem.*, **90**, 738 (1978).
(3) K. Hirotsu, et al., to be presented to Annual Meeting of Chemical Society of Japan, Tokyo, April 1981.
(4) S. Harata, *Bull. Chem. Soc. Jpn.*, **48**, 2409 (1975).
(5) R. L. Van Etten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).
(6) P. C. Manor and W. Saenger, *J. Am. Chem. Soc.*, **96**, 3630 (1974).
(7) I. Tabushi, Y. Kiyosuke, and K. Yamamura, *J. Am. Chem. Soc.*, 100, 916 (1978).

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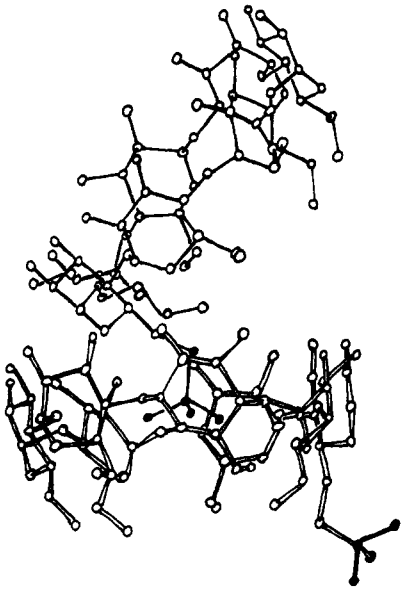


Figure 1. X-ray crystal structure of mono primary *tert*-butylsulfenyl-β-cyclodextrin.

rotation around the *z* axis is assumed to remain in the complex. In this way, the total entropy, enthalpy, and free-energy changes for the inclusion in water were calculated for the possible conformations. The most stable conformation thus obtained is that found by the X-ray crystallography[8] (Figure 3), and the absolute values of thermodynamic quantities calculated are also in good agreement with observed values.[9]

Activity of Hydroxyl Group as Acid and/or Base in the Active Site

Cyclodextrin not only has a specific hydrophobic binding site but also has many hydroxyl groups along its "rim". The hydroxyl group is acidic even though very weak (pK_a = ca. 12) and can act as an acid in a wide range of pH. In a strongly alkaline solution, it is dissociated to the corresponding anion to a reasonable extent and can act as a base. In these circumstances, cyclodextrins themselves satisfy the minimum necessary and sufficient conditions to be models of certain enzymes, which involve acid and/or base catalyses. Many

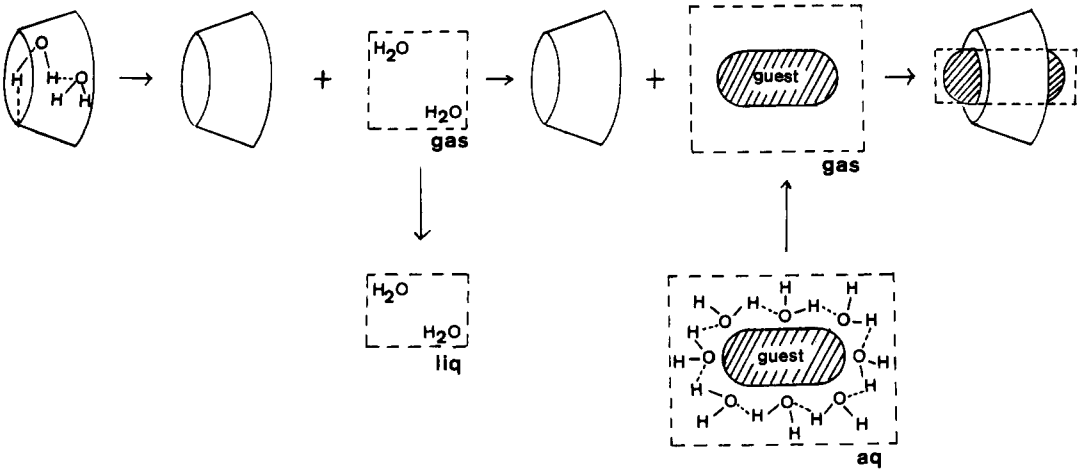


Figure 2. Hypothetical thermodynamic process of formation of the cyclodextrin inclusion complex.

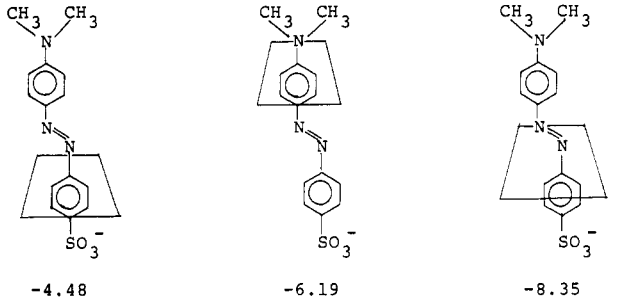
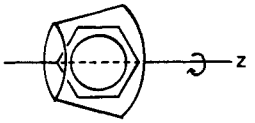


Figure 3. Possible conformation and stabilization energy (kcal/mol).

assembly is made by counting the number of water molecules liberated during the complex formation based



on either CPK models or computer counting. As for the change in rotational freedom of the guest, only the

Table I
Calculated Free-Energy Change in Inclusion Complex Formation[a]

guest	$\Delta H_{inclusion}$	$-T\Delta S_{inclusion}$	$\Delta G_{inclusion}$ calcd	obsd
benzene	-3.99	-0.51	-4.50	
p-iodoaniline	-7.35	-1.64	-8.99	-5.9
methyl orange	-6.53	+0.33	-6.20	-5.1

[a] kcal mol^{-1}, 25 °C, α-cyclodextrin.

studies have been carried out along the lines after two successful examples discovered independently in early days.[10,11] Thus, cyclodextrin, as a monoanion, accelerates the hydrolytic cleavage of certain pyrophosphates[10] or carboxylates.[11] Two important and interesting enzyme-like characteristics were found: (1) phosphorylated and carboxylated cyclodextrin were

(8) K. Harata, *Bull. Chem. Soc. Jpn.*, **49**, 1493 (1976).
(9) K. Harata, *Bull. Chem. Soc. Jpn.*, **49**, 2066 (1976).
(10) (a) F. Cramer and W. Dietsche, *Chem. Ind (London)*, 892 (1958); (b) R. L. Van Etten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).
(11) F. Carmer, *Angew. Chem.*, **73**, 49 (1961).

Table II
Relative Catalytic Hydrolysis Efficiency of α-Cyclodextrin toward Substituted Phenyl Acetates

substituent	H	*o*-CH_3	*m*-CH_3	*p*-CH_3	*m,m*-$(CH_3)_2$	*m*-Bu-*t*	*m*-Cl	*p*-Cl	*m*-NO_2
k_{cat}/k_0	9.7	7.7	39	3.8	8.6	226	113	3.0	103

Table III
Rate Constants of ω-Bromoethyl-1-naphthalene Solvolysis

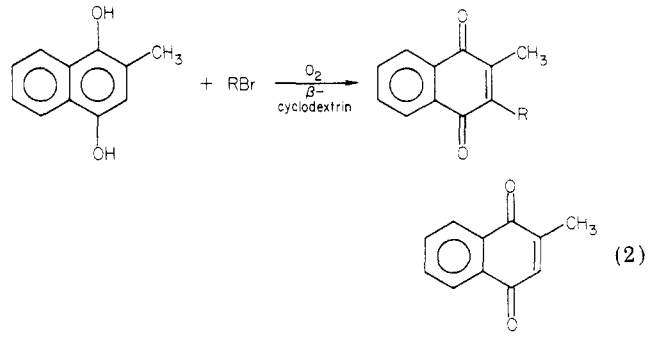
noncatalytic		CD catalytic	
k_{E_1}	1.8×10^{-6} s^{-1}	k_{E_1}	2.6×10^{-6} s^{-1}
k_{E_2}	4.2×10^{-4} s^{-1} M^{-1}	k_{E_2}	160×10^{-4} s^{-1} M^{-1}
k_{SN_1}	1.3×10^{-6} s^{-1}	k_{SN_1}	very small
k_{SN_2}	3.8×10^{-5} s^{-1} M^{-1}	$k_{SN_2(OH^-)}$	very small

obtained as intermediates, although these intermediates were slowly hydrolyzed, leading to very inefficient "turnover"; (2) acceleration was very sensitive to the shape of a guest molecule as apparent from the observed meta acceleration which is almost independent of the electronic nature of the substitutent (Table II). This was the first systematic observations of shape recognition (or pattern recognition) afforded by a simple organic compound.

As discussed above, in an appropriate pH range, cyclodextrin has an acidic group (OH) as well as a basic (O^-) group and can act efficiently as an acid–base multiple catalyst. A typical example of this acid–base multiple catalysis is the reaction-pathway control observed for ω-bromethyl-α-naphthalene hydrolysis. The hydrolysis gives the corresponding alcohol via S_N1 and S_N2 routes and vinyl-α-naphthalene via E1 and E2 routes. In a water-containing solvent at pH 11, E_1, E_2, and S_{N_1} mechanisms are competitively operating (Table III), but in the presence of excess β-cyclodextrin (the specific host for naphthalene derivatives) the E_2 contribution alone is greatly enhanced. This is due to the very effective simultaneous acid and base catalysis of CD–OH and CD–O^-, respectively, leading to discriminating elimination.[12] Several examples of this type of multiple catalysis are known.[13]

As a synthetase model, a one-pot synthesis of a vitamine K analogue was successfully carried out from 2-methylnaphthohydroquinone included in β-cyclodextrin. Addition of an allylic bromide to the inclusion complex in aqueous solution at pH 9 in the presence of a very small amount of dioxygen led to spontaneous allylation followed by controlled oxidation to give the desired product (eq 2).[14] This type of spontaneous substitution–oxidation was extended to other hydroquinones.[15]

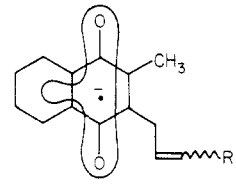
A detailed investigation of this process shows that the dissociation of the hydronaphthoquinone to the corresponding anion is enhanced by a factor of 2 (the anion may be stabilized through hydrogen bonding). A hydrophobic allyl bromide incorporated in the cavity to form the HG_1G_2 ternary complex then reacts with the monoanion of naphthohydroquinone which is activated due to the marked desolvation in the cavity. Allylation is accelerated in the cavity by factor 3 and gives a



(2)

a, R = $-CH_2CH=CH_2$; b, R = $-CH_2CH=CHCH_3$;
c, R = $-CH_2C(CH_3)=CH_2$; d, R = $-CH_2CH=C(CH_3)-CH_3$

specifically allylated product since allylation geminate to the methyl substituent is inhibited by serious steric hindrance in the cavity. A single electron is then abstracted by dioxygen from the 3-allyl-2-methylnaphthohydroquinone monoanion thus formed, and proton dissociation gives the corresponding anion radical.



This is readily observed by ESR spectroscopy at room temperature in aqueous solution. Further electron abstraction by dioxygen gives the corresponding quinone (vitamin K analogue). In contrast to these electron abstractions, which may be taking place at the naphthol oxygen atom outside the cavity, further oxidation of the included quinone with hydrogen peroxide (another product of the electron abstraction) is remarkably retarded, probably due to poor incorporation of HO_2^- in the cavity. This remarkable retardation is ascertained by independent experiments (direct interaction of quinone and H_2O_2 in the presence or absence of β-cyclodextrin) and estimated to be $^1/_{14}$ (deceleration by inclusion).[14]

Anisole was rather specifically chlorinated at the para position with sodium hypochlorite after α-cyclodextrin inclusion. This interesting specificity was interpreted by the formation of the "rim" hypochlorite of cyclodextrin in a close proximity of the para carbon atom of the included anisole.[16]

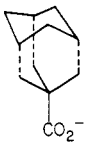
Cyclodextrins Functionalized with Apolar Moiety. Enhanced Hydrophobic Recognition

Despite its unique enzyme-like recognition and catalysis discussed above, β-cyclodextrin is still a poorer binding site than most enzymes partly because of a limited hydrophobic surface area with which it can

(12) I. Tabushi, International Symposium on Solute–Solute–Solvent Interaction, Firenze, 1980.
(13) F. Cramer and W. Kampe, *J. Am. Chem. Soc.*, **87**, 1115 (1965).
(14) I. Tabushi, K. Fujita, and H. Kawakubo, *J. Am. Chem. Soc.*, **99**, 6456 (1977); I. Tabushi, K. Yamamura, K. Fujita, and H. Kawakubo, *ibid.*, **101**, 1019 (1979).
(15) I. Tabushi, Y. Kuroda, K. Fujita, and H. Kawakubo, *Tetrahedron Lett.*, 2083 (1978).
(16) R. Breslow and P. Campbell, *J. Am. Chem. Soc.*, **91**, 3085 (1969).

contact the surface of a given guest molecule. A possible improvement, then, is to introduce certain hydrophobic moieties onto the rim of cyclodextrins on the basis of the concept of the hydrophobic interaction.

By use of this idea flexibly[17] or rigidly[18] capped cyclodextrins have been prepared. The association constant of a flexibly capped cyclodextrin with a round guest molecule like adamantane-1-carboxylate is very much enhanced (80-fold), but with a flat guest such as a substituted benzene, association decreases considerably. However, the situation for the rigidly capped cyclodextrins are much clearer, and association constants of β-cyclodextrin with most hydrophobic guests increase remarkably;[18] e.g., the association constants (M^{-1}) for 1,8-ANS with β-CD is 58, with terephthalate cap is 640, and with diphenylmethanedisulfonate cap is 1300. In this enhanced binding, hydrophobic recognition seems to be integratable over the whole interacting region and precise enough to recognize every carbon (or hetero) atom incorporated. A typical example is the almost complete recognition of the bottom cyclohexane ring of adamantane-1-carboxylate for the *p,p'*-diphenylmethanedisulfonate cap at room temperature, compared with pivalate. For adamantane-1-carboxylate there is very close to the sum of six units (0.6 kcal/CH_2 or CH, at least) of "hydrophobic" interaction energy (see eq 3).[18]



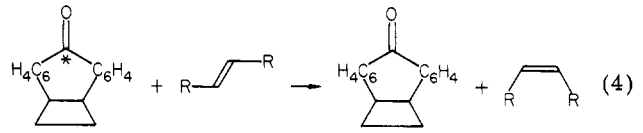
$$K(\mathrm{AdCO_2H}) = 50\,000\ \mathrm{M^{-1}}$$

$$K(\mathrm{Me_3CCO_2H}) = 250\ \mathrm{M^{-1}}$$

$$\text{gives } \Delta\Delta G = -3.2\ \text{kcal/mol} \qquad (3)$$

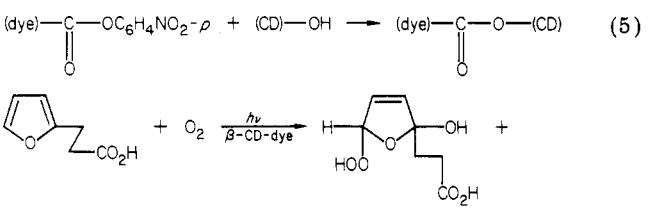
Flexible or rigid capping not only acts as enhanced hydrophobic recognition but also affords some special interaction between the host and guest due to their unusually close proximity. One example of this special interaction may be seen in the very efficient energy transfer between benzophenone-*p,p'*-dicarboxylate-capped cyclodextrin and an appropriate naphthalene derivative. Even at the low concentration of bromonaphthalene (down to 10^{-4} M), irradiation of the benzophenone moiety at 353 nm afforded the complex phosphorescence comming from both the benzophenone moiety and bromonaphthalene. From the observed superposition of the two phosphorescences, the (triplet) energy transfer efficiency was estimated as ca. 60%. Such energy transfer was not observed from the benzophenone cap to very hydrophilic and/or bulky energy acceptors of similar E_T values which were incapable of binding. Similarly no energy transfer was observed from dialkyl benzophenone-*p,p'*-dicarboxylate to bromonaphthalene. These observations strongly demonstrate that the energy transfer took place between the host and the guest.[19]

This energy transfer was successfully applied to the enhanced and specific sensitization of the olefin photoisomerization[20] (eq 4). Similarly, singlet oxygen



(4)

formation by use of an eosin-capped cyclodextrin was observed to enhance $^1O_2$ addition by a factor of 14, probably due to retarded $^1O_2$ quenching in a locally hydrophobic environment (eq 5 and 6).[21]



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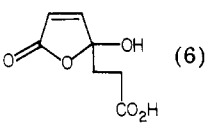


(6)

(trace)

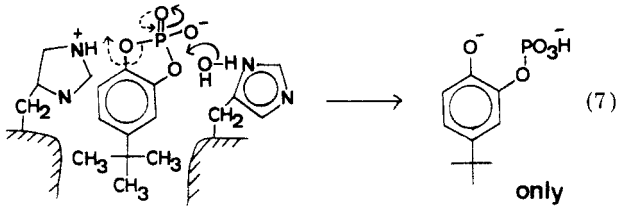
Cyclodextrin Functionalized with Active Catalytic Moiety. Unique and Enhanced Specificity by Multirecognition

The major problem of cyclodextrin models of enzyme catalysis is the fact that cyclodextrin has only limited catalytic activity. This problem may be solved by introduction of potent catalytic functional group(s) onto cyclodextrin, such as imidazole[22] or hydroxamic acid[23] as an effective hydrolytic catalyst, or B_6 as the catalyst for amino acid synthesis.[24]

If the catalytic site has two functional groups, recognition should be much more precise and strict, just like enzyme recognition. Detailed investigation of the hydrolysis activity of bisimidazolyl-β-cyclodextrin prepared from rigidly capped cyclodextrin for a specific

(17) J. Emert and R. Breslow, *J. Am. Chem. Soc.*, **97**, 670 (1975).
(18) I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata, and K. Fujita, *J. Am. Chem. Soc.*, **98**, 7855 (1976).
(19) I. Tabushi, K. Fujita, and L. C. Yuan, *Tetrahedron Lett.*, 2503 (1977).
(20) I. Tabushi, L. C. Yuan, and K. Fujita, "Photosensitized Isomerization of cis-trans Olefins through Host-Guest Energy Transfer", The 36th Annual Meeting of the Chemical Society of Japan, Osaka, April 1977, Abstracts, II, p 791.
(21) I. Tabushi, L. C. Yuan, and K. Yamamura, "Photooxygenation Reaction by Molecular Oxygen Sensitized by dye-Capped Cyclodextrin in Aqueous Solution", The Symposium on Photochemistry, Japan, Kyoto, Nov 1978, Abstract, p 16.
(22) F. Cramer and G. Mackensen, *Angew. Chem.*, **78**, 641 (1966); Y. Iwakura, K. Uno, F. Toda, S. Onozuka, K. Hattori, and M. L. Bender, *J. Am. Chem. Soc.*, **97**, 4432 (1975).
(23) W. B. Gruhn and M. L. Bender, *Bioorg. Chem.*, **3**, 324 (1974).
(24) R. Breslow, M. Hammond, and M. Lauer, *J. Am. Chem. Soc.*, **102**, 421 (1980).

substrate demonstrates that the catalyst recognizes even a small structural change in a specific substrate as shown in eq 7.[25] An elegant way to introduce a cata-



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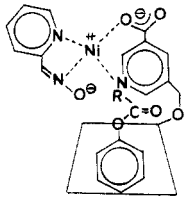
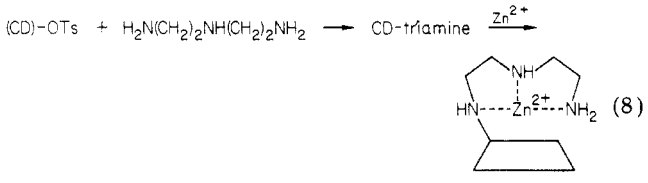
lytic group indirectly into cyclodextrin was devised by Breslow who connected nickel oximate, a most effective catalytic group, and cyclodextrin, the binding site, through metal coordination[26] as shown. We call this



type of recognition "double recognition",[27] i.e., a single enzyme model with two independent recognition sites.

In most metalloenzymes, the metal behaves as an important (coordination) recognition site to enhance binding as well as catalysis. At the same time, enzyme walls recognize the shape of a specific guest molecule mostly through hydrophobic and/or hydrogen bonding interaction. However, details of this double (or multiple) recognition are still uncertain, and the elucidation of detailed mechanisms requires appropriate and much simplified model systems.

We have synthesized compound **12** as one of the simplest hosts for double recognition (eq 8). The



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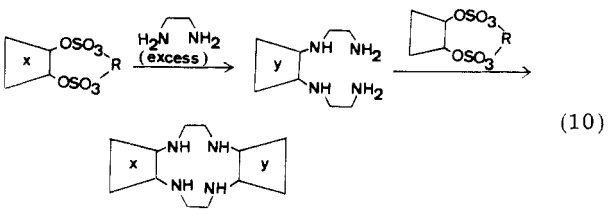
molecule has a hydrophobic recognition site and a metal coordination site in a close proximity. Binding of a series of guest molecules, each of which has both a coordinating group(s) and a hydrophobic surface, were investigated. Analysis of the association constants (Table IV) between these guests and the doubly recognizing host gives simple relationship (eq 9)[26] for the

$$\Delta G^\circ_{\text{overall}} \approx \Delta G^\circ_{\text{hydrophobic}} + \Delta G^\circ_{\text{coordination}} \quad (9)$$

limited examples listed. On the basis of this observation the elemental recognition free energy is additive in principle. Thus one can hope to design and build new host molecules with a given total recognition free energy toward a certain guest molecule (hopefully a bioactive compound of practical significance). An example of a host molecule built with triple recognition is "duplex cyclodextrin", prepared according to eq 10.[27]

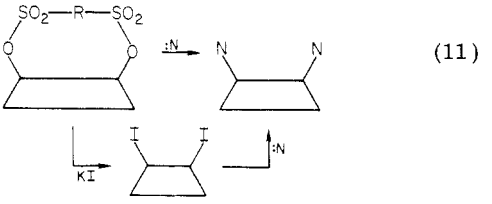


(10)

Preparation of Specifically Multifunctionalized Cyclodextrins. Further Approaches to More Advanced Enzyme Models

In order to approach real enzyme activity by use of cyclodextrins, one should prepare a series of modified cyclodextrins, each of which has appropriate functional groups exactly at the required spatial environment (e.g., distance or angle between functional groups). One possible way to do so is by careful separation of cyclodextrin isomers as, for example, has been accomplished for symmetrically trisubstituted α-cyclodextrin.[28] But we also need simpler and more general approaches to this problem. Recently we have been attempting to modify β-cyclodextrin regiospecifically.

To date, a considerable number of cyclodextrins containing a rigid sulfonate cap are known. These are listed in Table V. These capped molecules are ideal for further nucleophilic substitutions leading to a series of bifunctional cyclodextrins (eq 11).[29] However, this



(11)

direct substitution proceeds satisfactorily only for relatively strong nucleophiles and is accompanied by undesired side reactions for poor nucleophiles. A more facile and complete conversion into bifunctional CD's were attained for double substitution of the sulfonate cap via diiodocyclodextrin. For example, bisimidazolyl-β-CD can be prepared by treating diphenylmethanedisulfonate cap at 85–90 °C with a large excess of imidazole for 96 h, whereas when the diiodide is used at 80 °C for 4 h the product was obtained in 46% yield.[30]

Since the capped cyclodextrins have been gradually recognized as important key intermediates of disubstituted cyclodextrins, the "regiochemistry" of the cap has attracted increasing attention. The first member of the cap family was the mixture of A,C and A,D regioisomers shown in Figure 4.

The present regiochemistry is determined by the degree to which the first substitution controls the site of the second substitution (see I). The nature of R is

(25) (a) R. Breslow, J. Doherty, G. Guillot, and C. Lipsey, *J. Am. Chem. Soc.*, **100**, 3227 (1978); (b) R. Breslow, P. Bovy, and C. L. Hersh, *ibid.*, **102**, 2115 (1980).

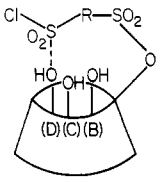
(26) I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, and K. Yamamura, *J. Am. Chem. Soc.*, **99**, 7100 (1977).

(27) I. Tabushi, Y. Kuroda, and K. Shimokawa, *J. Am. Chem. Soc.*, **101**, 1614 (1979).

(28) (a) J. Boger, P. G. Brenner, and J. R. Knowles, *J. Am. Chem. Soc.*, **101**, 7630 (1979); (b) J. Boger and J. R. Knowles, ibid., **101**, 7631 (1979).

(29) I. Tabushi, K. Shimokawa, and K. Fujita, *Tetrahedron Lett.*, 1527 (1977).

(30) I. Tabushi, Y. Kuroda, and A. Mochizuki, *J. Am. Chem. Soc.*, **102**, 1152 (1980).



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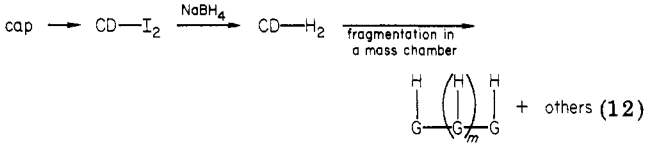
important in the sense that the more rigid R is stricter this restriction becomes. For satisfactorily rigid R's the site of the second attachment, i.e., "looper's walk", is mainly determined by the average distance between the two S atoms. The range of the distances estimated by CPK models is shown in Table V.

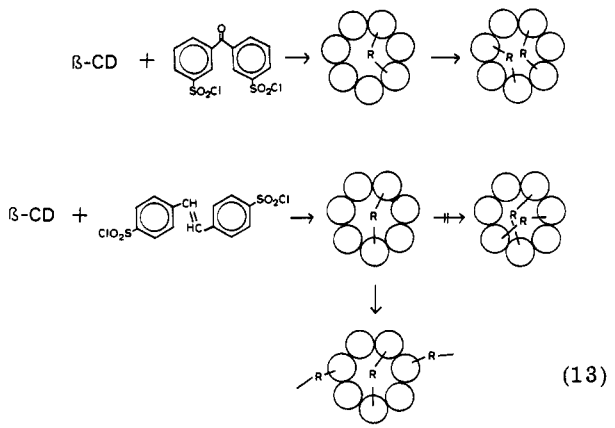
One of the most serious problems encountered in obtaining this type of regiospecificity is the uselessness of spectroscopic structural determination procedures. The effect of a remote substitution on the NMR chemical shift of a ring A proton or ^{13}C, for example, is only observable for a substitutent on the B ring atom,[30] but is not clear for substituent on the C or D rings, although a slight broadening due to a very small shift is readily recognizable. Mass spectroscopy is helpful in demonstrating the sites of the substitution using the pattern of fragments, as shown in eq 12.



(12)

Unfortunately, mass spectroscopy cannot give satisfactory information on the relative ratio of regioisomers. Breslow pointed out that high-pressure liquid chromatography (HPLC) could differentiate the regioisomers, but often the separation is not satisfactory enough to give the regioisomer ratio quantitatively; more seriously, HPLC cannot tell which structure is which.

Very recently we have tried to doubly "cap" β-cyclodextrin with the best candidates for A,C or A,D regiospecific capping[31] and have obtained some interesting results. A 2.5 M excess of *m,m'*-benzophenonedisulfonyl chloride gave the A,C doubly capped β-CD in excellent yield, while a 2.5 M excess of *p,p'*-stilbenedisulfonyl chloride gave a trace amount of the double cap but mostly a polymeric material (eq 13). Fortunately, two



(13)

(31) I. Tabushi, Y. Kuroda, K. Yokota, and L. C. Yuan, *J. Am. Chem. Soc.*, **103**, 711 (1981); *Tetrahedron Lett.*, 2273 (1981).

Table IV
Association Constants (M^{-1}) of Coordinated Hydrophobic Guests

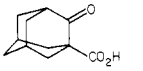
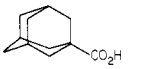
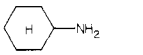
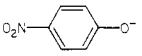
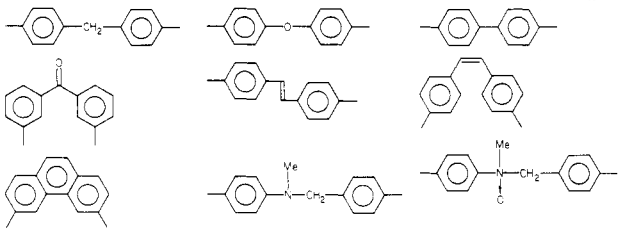
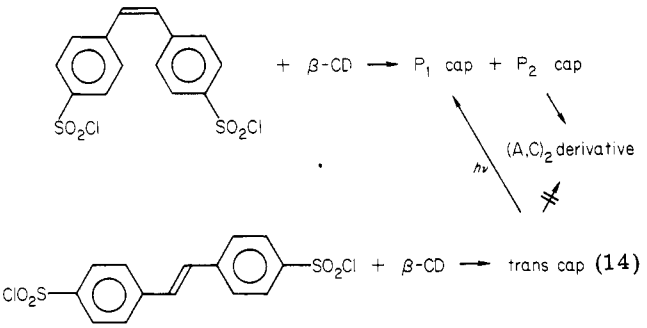
	host		
guest	β-CD	β-CD·N_3·Zn^{2+}	Zn^{2+} enhancement
	830	280000	330
	230	5300	23
	45	210	4.7
	480	1200	2.5

Table V
List of Sulfonate Capped Cyclodextrins



regioisomers (P_1 + P_2) of the *cis*-stilbene-*p,p'*-disulfonate cap were clearly differentiated by HPLC, one of which, P_1, was identical with the cis cap derived from the trans cap by irradiation. The other cis cap, P_2, was converted to A,C: A',C'-double cap cyclodextrins (eq 14). Therefore trans-stilbenedisulfonyl chloride is



(14)

concluded to be a specific A,D looper.

At present we are studying a candidate for specific A,B capping. In the future, by appropriate use of this set of regiospecific capping reagents, any specifically polysubstituted β-CD may be prepared.

Concluding Remarks

For increase in the sophistication of these enzyme models a special technique to functionalize cyclodextrin with two different functional groups (X and Y) seems necessary. Since the reactivity of the two bridgehead carbons of the sulfonate cap is approximately the same, even an optimized two-step functionalization would afford at most 50% CDXY. Considering the probable similarity of physical properties of CDXY, CDX_2, and CDY_2, isolation of pure CDXY is likely to be very difficult.

Very recently we have discovered a new technique to

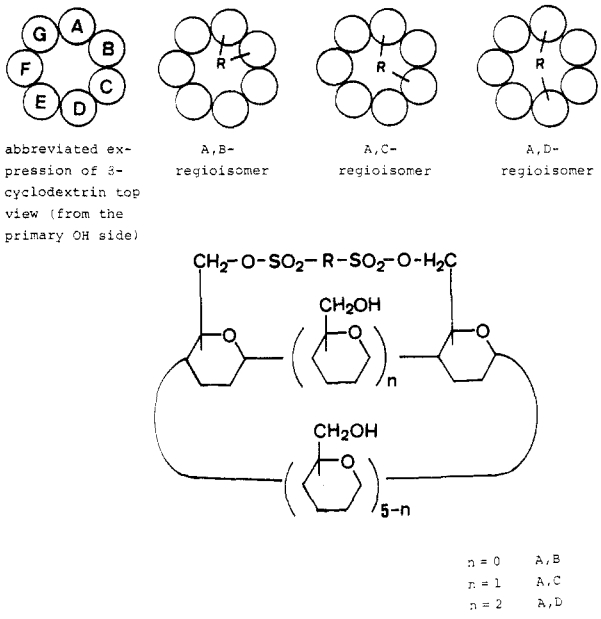
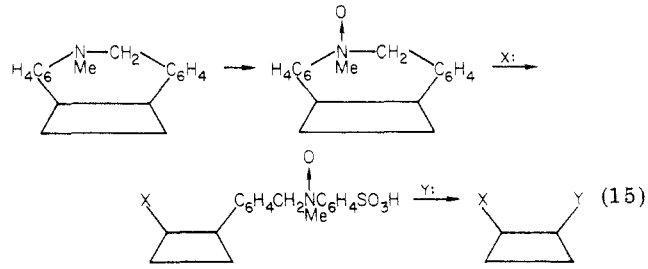


Figure 4. Schematic representation of possible regioisomers of disubstituted β-cyclodextrins.

generate this "combination specificity". By the use of a unsymmetrically activated cap in which the activity



toward the first nucleophile is 1 order of magnitude higher than that for the second, CDXY can be obtained in more than 80% yield (eq 15).[32]

By the future application of these new procedures, we hope to build up many cyclodextrin derivatives, with functions close or even superior to those of native enzymes. Other important biological phenomena in which hydrophobic recognition takes a significant role are also appropriately modeled by modified cyclodextrins,[33] giving promises of future developments of new areas.

(32) I. Tabushi, K. Yamamura, and H. Kitaguchi, "Capped Cyclodextrin Having a Unsymmetrical Reactivity", presented at the Annual Meeting of the Chemical Society of Japan, Tokyo, April 1981. See also *J. Am. Chem. Soc.*, in press.

(33) I. Tabushi, Y. Kuroda, and K. Shimokawa, *J. Am. Chem. Soc.*, **101**, 4759 (1979); I. Tabushi and K. Shimokawa, *ibid.*, **102**, 5400 (1980).