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Microscopic Medium Effects on a Chemical Reaction. A Theoretical Study of Decarboxylation Catalyzed by Cyclodextrins as an Enzyme Model

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Abstract: The reaction of decarboxylation of phenylcyanoacetate anion catalyzed by cyclodextrins (CDs) was studied theoretically. The reaction field theory developed in our previous study, which makes it possible to investigate the electronic structure of a given molecule in the heterogeneous environment, is applied with the MNDO method. First, the reaction path in vacuo is investigated. The stationary state points and the corresponding molecular structures are found. Subsequently, the activation energies are evaluated for the reaction occurring in some homogeneous dielectrics to simulate normal solvent environments. The calculated values of the activation energy become smaller as the substrate is more deeply penetrated into the apolar cavity of CD, which is consistent with the fact that the reaction of decarboxylation proceeds faster in the β-CD cavity. One of the most interesting findings is that the reaction can be effectively accelerated even if the reaction center, the carboxyl group of the substrate, is not surrounded by the low dielectric medium formed by the CD cavity but exposed to the bulk water. According to Arrhenius' theory, both calculated and observed activation energies in the CD-catalyzed reaction correspond to 10^2–10^6 times acceleration effects in comparison with the aqueous solution case. Its efficiency is comparable to enzymic catalysis. On the basis of these results, the role of active sites of enzymes is discussed.

Introduction

One of the goals in host–guest chemistry is the creation of artificial enzymes that can recognize only a specific type of substrate and accelerate its reaction under mild conditions. Cyclodextrins, hereafter abbreviated as CDs, have served as the mainstays in this field of chemistry since the pioneering work of Bender et al.[1] CDs have a well-constructed molecular cavity composed of at least six glucose residues (Figure 1a) and form inclusion complexes with various guest molecules in aqueous solution.

The decarboxylation of phenylcyanoacetic acid and the hydrolysis of ester substrates are well-known CD-catalyzed reactions. There is some evidence to presume that the decarboxylation is accelerated without any covalent type of interaction between CD and the bound substrate.[2,3] In this case, the lowering of the reaction barrier is thought to be mainly due to microscopic solvent effects of the hydrophobic CD cavity. On the contrary, the hydrolysis is considered to be initiated by attack of the secondary hydroxyl groups of CD to the ester carbonyl of a bound substrate, resulting in the formation of a tetrahedral intermediate and subsequently leading to the formation of the alcoholate anion and acylated CD.[4]

The above rationales for the catalytic mechanism may be reasonable from a view point of standard organic chemistry. However, these alone are insufficient to understand all aspects of the CD-catalyzed reactions even if our attention is confined to a few cases of ester hydrolysis. A typical example is the hydrolysis of ferrocenylacrylate esters in the β-CD cavity. Its *p*-nitrophenyl ester is hydrolyzed 10^7-fold faster than the corresponding ethyl ester when they are complexed with β-CD, despite that their rate difference is only 56 times in favor of *p*-nitrophenyl ester in the free aqueous solution.[5] Recently, the molecular mechanics calculation has been applied to investigate the steric or geometric origin of such a marked difference in reactivity.[6–10] However, there was found no significant difference in geometries between the tetrahedral intermediates of both esters.

In order to obtain a better understanding of CD-based catalysis, more accurate methodologies for determining the reaction coordinate are required. In particular, it is of necessity to consider how the solvent molecules influence the electronic structure of the reaction center of a bound substance. This is one of the important factors that have not been explicitly taken into account by the previous molecular mechanics studies. As pointed in our previous papers,[11,12] the substrates complexed with CD lie in a highly heterogeneous environment. For example, according to NMR studies of the α-CD inclusion complexes with benzene derivatives,[13] a part of the substrate is accommodated into the cavity and the residual part is exposed to the aqueous medium (Figure 1b). A similar situation is also seen in the β-CD inclusion complexes with ferrocenylacrylate esters, with the ferrocenyl moiety lying in the cavity.[5] Thus, in these CD-catalyzed reactions, the reaction center is near the boundary between the highly polarizable aqueous medium and the relatively hydrophobic (lower dielectric) CD cavity. Such a situation is remarkably different from that in the normal liquid condensed state and in the active site of enzymes, where the reaction center is shielded almost completely from the solvent and instead surrounded by the

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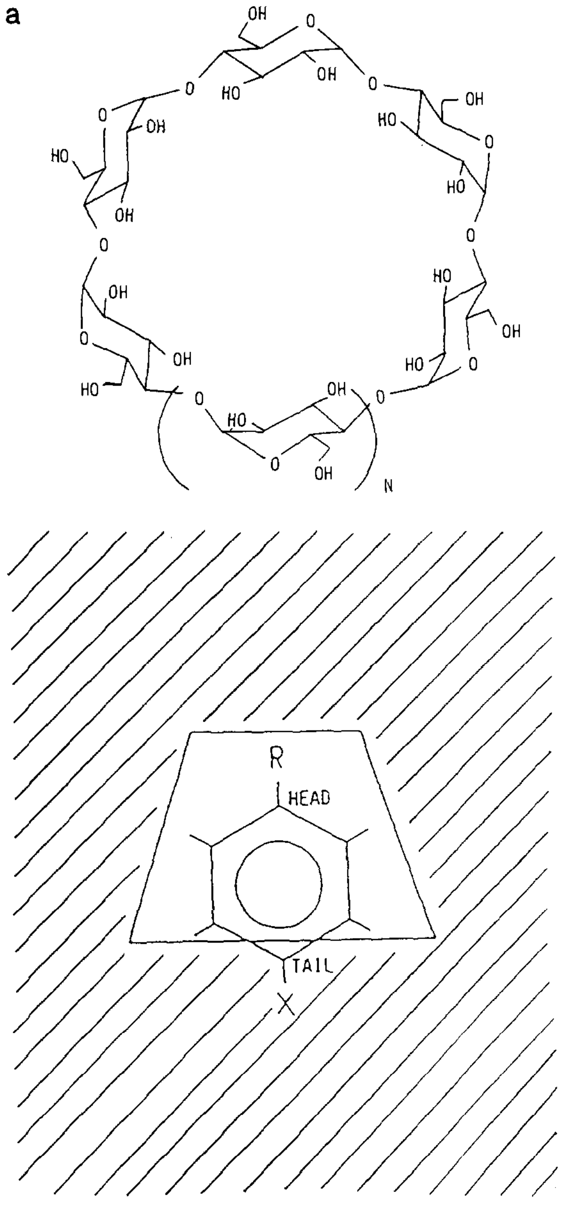

Figure 1. (a) Schematic representation of CD. CDs are cyclic oligosaccharides composed of (1–4)-linked α-D-glycosyl residues. The CDs with 6 (i.e., $N = 1$) and 7 (i.e., $N = 2$) residues are conventionally called as α-CD and β-CD, respectively. (b) Side view of α-CD—aromatic guest complexes in aqueous solution. The trapezoid indicates α-CD. The large part of the guest molecule is inserted into the hydrophobic field (CD cavity), while the tail chemical group is exposed to the aqueous medium.

relatively lower dielectric medium formed by protein. On the other hand, the heterogeneous nature of the environment surrounding a substrate may commonly occur in inclusion complexes using macrocycles other than CDs unless their cavities are large enough to encircle the substrate sufficiently. In this regard, the mechanistic elucidation of CD-catalyzed reaction seems to be useful to obtain a general picture for the catalytic mechanism of macrocycles and consequently to make a molecular design of efficient and specific catalysis for various reactions.

Recently, we have developed a theory of medium effect capable of estimating the electronic structure of a solute molecule surrounded by the heterogeneous medium.[14,15] In a preliminary study using this theory, we have successfully demonstrated that the characteristic changes in ^{13}C NMR chemical shifts of guest molecules, induced on complexation with α-CD, are originated in the electrostatically heterogeneous environment around the bound guests.[12] In the present study, this computational methodology is applied to investigate how a reaction of a bound substrate is influenced on complexation with CDs. The reaction studied here is the decarboxylation of phenylcyanoacetic acid, which is, as mentioned above, expected to be noncovalently catalyzed by CDs and is thereby one of the most appropriate reactions to investigate microscopic solvent effects of CD cavities.

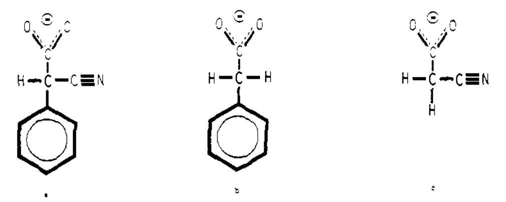

Figure 2. Chemical structure of the substrate studied here: (a) phenylcyanoacetate anion, (b) phenylacetate anion, and (c) cyanoacetate anion.

Model Building. First, we describe the basic grounds required for characterizing the decarboxylation of phenylcyanoacetate anion (shown in Figure 2a, hereafter abbreviated PCAA) in the CD cavity. This type of reaction is known to be accelerated by lowering the polarity of solvent in solution.[2,3] It is thus reasonably said that the interior of the CD cavity is relatively apolar (hydrophobic), consistent with the results from some spectroscopic studies.[4,16,17] As described above, the size of the hydrophobic region formed by the CD cavities is very small although it varies, depending on the number of constituent glucose units. The size is often not large enough to accommodate the whole of bound substrates. From CPK model analysis, the α-CD cavity cannot completely accommodate PCAA. In addition, PCAA has a highly polarized electronic distribution. Its reactive sites are composed of an acetate anion and a highly polar cyano group. In contrast, its residual portion consists of a relatively hydrophobic group. Considering these facts, it is naturally said that the bound substrate should orient in the CD cavities so as to point the reactive site toward the aqueous medium and the residual portion inwards toward the cavity. In the present calculation, the orientation of the substrates, as shown in Figure 1, is assumed. Thus, the reaction occurs in the highly heterogeneous medium.

Next, we consider the reaction mechanism of decarboxylation. Its mechanism is classified into the following two types,[18-20] that is, S_E1 and S_E2 mechanisms:

$$S_E1$$

$$\text{R-COOH} \rightarrow \text{R}^- + \text{COOH}^+ \text{ (slow)}$$

$$\text{R}^- + \text{H}^+ \text{ (from COOH)} \rightarrow \text{R-H (Fast)}$$

$$S_E2$$

$$\text{R-COOH} + \text{H}^+ \rightarrow \text{HRCOOH}^+ \text{ (slow)}$$

$$\text{HRCOOH}^+ \rightarrow \text{R-H} + \text{CO}_2 + \text{H}^+ \text{ (fast)}$$

The S_E1 mechanism involves the heterolytic cleavage of the bond between the carbonyl carbon and its α-carbon, which is the rate determining step. This mechanism proceeds without any acid

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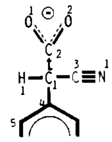

Figure 3. Numbering of the atoms of phenylcyanoacetate. The numbering is distinguished for each kind of atom. For instance, carbon atoms C1, C2, C3, C4, and C5. Oxygen atoms O1 and O2.

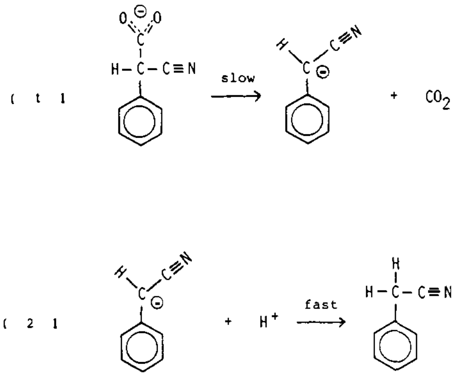

Figure 4. Reaction mechanism of the decarboxylation of phenylcyanoacetate anion.

catalysis. The reaction would be facilitated by introducing an electron-withdrawing group into the chemical group R. In the S_E2 mechanism, the rate determining step is the attack of a proton to the group R. The reaction would be facilitated by introducing an electron-donating group into the chemical group R. According to Straub and Bender,[2] the rate of decarboxylation of PCAA is independent of pH from 4 to 12. It is thus presumed that the decarboxylation of PCAA follows the S_E1 mechanism. Consequently, the heterolytic cleavage of the bond between the carbonyl carbon and its α-carbon, namely, the C1–C2 bond (see Figure 3) is the rate determining step.

The observed values of the decarboxylation cited in ref 2 are at pH = 8.6. Considering that phenylcyanoacetic acid has a pK_a of 1.88,[2] it is presumed that the carboxyl hydrogen is already dissociated before the cleavage of the bond C1–C2 is initiated. Hence, the reaction scheme can be represented as shown in Figure 4.

In our model, the counterion of the carboxylate was omitted, because the reaction rate of decarboxylation of PCAA derivatives is independent of pH and buffer types.[2]

It is of necessity to find the transition state of the reaction. In recent ab initio and AM1 studies on decarboxylation,[20-22] it has been indicated that in the transition state reactants form circular complexes made from their carboxyl groups, α-carbon atoms, and some of the surrounding water molecules. In the present study, such detailed structural information is not available, because our main purpose is to investigate how the energies of both the ground and transition states are influenced on complexation with CD.

In our calculation, the solvent is represented by the dielectric continuum on the basis of a reaction field theory recently developed by us. In the continuum model a solute is embedded in a vacuum cavity formed in a dielectric continuum. The shape of this cavity (Figures 5 and 6a) was selected to be identical with that of the CPK molecular model, namely, that is formed by van der Waals spheres of their constituent atoms.

The intramolecular cavity of CD is approximated by a cylindrically-shaped dielectric. Its dielectric constant ϵ_C is assumed to be 2.0 (Figure 6a), the value of which is based on the observation of complexation-induced UV spectral changes of guest molecules; for example, the spectrum of *tert*-butyl phenol in the complexed state with α-CD is similar to that in dioxane.[4]

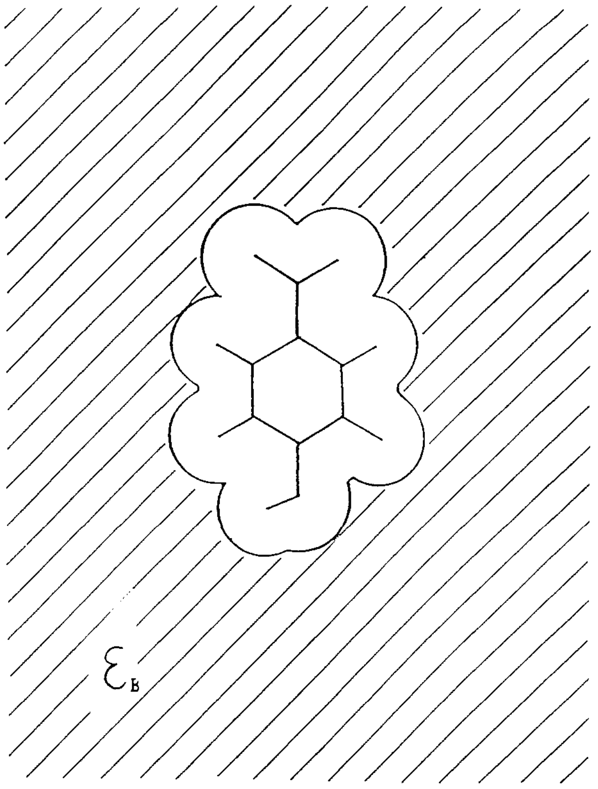

Figure 5. Homogeneous dielectric model. The benzene derivative is surrounded by dielectric with dielectric constant ϵ_B.

The size of the cylinder (Figure 6b) was determined from the CPK molecular model for CD. For α-CD, the length and radii of the cylinder were 8.0 and 7.0 Å, respectively, and for β-CD, they were 8.0 and 9.0 Å, respectively. In order to examine the effect of the size of the CD cavity, a virtual CD model is defined having the cavity size different from those α- and β-CDs. Its radius and length of the cylinder were taken to be 7.0 and 10.0 Å, respectively.

The environment surrounding the bound substrate is approximated by a multidielectric system. The pseudomolecular complex formed from the substrate and the cylinder is embedded into the aqueous medium with a dielectric constant of 80.

According to the above modelling, the substrate-binding process in aqueous solution is approximated as the transfer of the substrate from the homogeneous dielectric with $\epsilon_B = 80.0$ to the cylindrically shaped dielectric with $\epsilon_C = 2.0$. The subscripts B and C mean bulk and cylinder, respectively. The substrate studied here was oriented so that the molecular axis of the substrate was identical with that of the CD cavity, i.e., cylinder axis. The substrate was inserted from the side of the phenyl ring. We selected three different binding states which are discriminated by the degree of penetration of the substrate into the cylinder (Figure 7). The parameter *d* was introduced in order to indicate three binding states. The *d* value is 0.0 when the carbon C4 of the substrate is placed on the boundary between the top surface of the cylindrically-shaped dielectric and its outer aqueous region. Its negative values indicate deeper penetration of the substrate into the cylinder, namely the CD cavity. The *d* value of 0.35 Å corresponds to the geometry of the most stabilized complexes between α-CD and benzene derivatives like *p*-nitrophenol and benzaldehyde in the crystalline state.[23]

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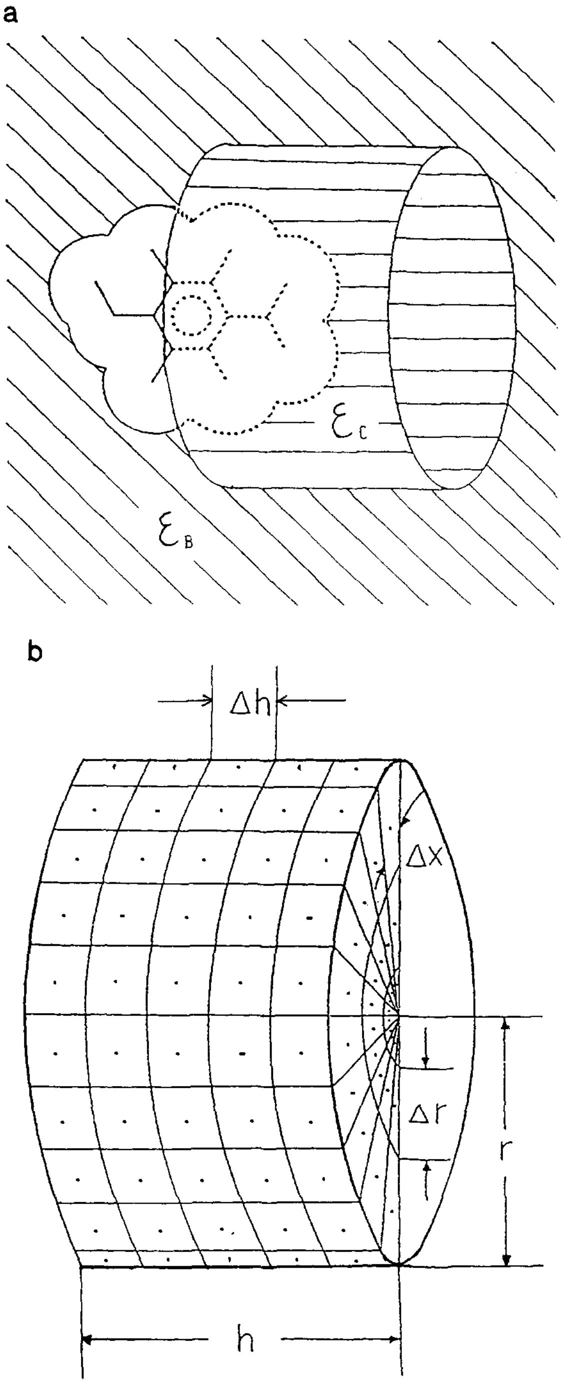

Figure 6. Theoretical model of the CD inclusion complex in aqueous solution. (a) A benzene derivative is situated on the borderline of two dielectrics whose dielectric constants are ϵ_B and ϵ_C. (b) Division of the cylinder surface. The dots indicate the representative points, at which the Laplace equation is solved with the aid of the boundary element method. The values of h and r are described in the text. Δh, Δr, and $\Delta \chi$ are taken to be $h/10$, $r/10$, and 18.0°, respectively.

In order to compare the CD-catalyzed reaction with the normal solution one, some calculations were carried out for the latter case where the solute was embedded in the homogeneous continuum corresponding to a normal solvent environment. We selected three different media corresponding to dioxane, 2-propanol/water (57.7 W/W %), and water, whose dielectric constants were assumed to be 2.0, 12.7, and 80.0, respectively. According to the study of the CD-catalyzed decarboxylation of PCAA, the reaction rate in β-CD cavity is nearly equal to that observed in the above mixed solvent.[2] The dielectric constant of the mixed solvent was obtained by taking the volume fraction weighted-average of dielectric constants of the constituent solvents.

Calculation. The interaction between the solute and the medium is calculated according to the formalism given in refs 12, 14, 15, and 24. The Hamiltonian $\mathcal{H}_0$ of the solute in vacuo is modified by the coulombic interaction V with a reaction field generated by the polarization of the surrounding dielectrics. The total energy of the solute-medium (dielectrics) system is given by

$$H = \langle\psi|\mathcal{H}|\psi\rangle - (^1/_2)\langle\psi|V|\psi\rangle \qquad (1)$$

where $\mathcal{H}$ is $\mathcal{H}_0 + V$ and the second term of the right hand side indicates the energy required to polarize the medium. In the framework of the continuum model, the value of H is a free energy contribution.[14] However, it does not explicitly include an entropic contribution from so-called hydrophobic interaction between the solute and water molecules and from the formation of the complex of the substrate with CD. Hence the calculated data indicate the enthalpic contribution to the energy of a given system rather than the free energy contribution.[24] Since the decarboxylation is an enthalpically-dominated reaction,[2,3,25] the reaction field theory using the continuum model is expected to be useful to analyze the reaction.

The reaction field was determined by solving numerically the Laplace equation with the aid of the boundary element method. The boundary surfaces, formed between the dielectric and the molecular cavity (Figure 5 and 6a) and the boundaries between each dielectric (Figure 6b), are divided into a sufficient number of elements. The details of numerical calculation have been described in refs 12, 15, and 24. The wave function of the solute was obtained under the MNDO approximation.

In previous studies, we have indicated that molecular orbital calculation combined with this theory quantitatively reproduces the hydration enthalpy of various organic molecules, including anion and cations,[24] and that the apolar nature of the CD cavity can be represented by a multidielectric system which replace the CD cavity and aqueous medium by dielectric continuums with the dielectric constants of 2 and 80, respectively.[12]

The QCPE 438 program[26] was used after some modifications including the incorporation of the solvation theory.

Results

Reaction Path in Vacuo. First, we determined the reaction path of decarboxylation of PCAA in vacuo before considering medium effects on its kinetic parameters. Figure 8 shows the plots of the calculated enthalpy ΔH vs Δr, where Δr indicates the increment of the C1–C2 bond length from its equilibrium value (1.613 Å) corresponding to the energy minimized structure of the reactant, and similarly ΔH is the increment of enthalpy from its value in the initial state, i.e., at $\Delta r = 0$ Å. ΔH shows a maximum at $\Delta r = 0.65$ Å. Thus, the reaction of interest attains the transition state at this point, which was also supported by the fact that this structure has only one negative eigenvalue in the Hessian matrix.[27] Activation energy (E_{act}) was evaluated to be 74.8 kJ mol^{-1},[28] by subtracting ΔH in the initial state from that in the transition state. Hereafter the initial and transition states are called the states R and T, respectively.

The value of ΔH is almost kept constant in the range of Δr beyond 2.0 Å. The net charge on the carboxyl group is 0.001 when Δr is 3.0 Å. The negative charge is almost completely

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[28] The MNDO method may give some little larger activation energies of organic reactions than restricted Hartree–Fock ab initio methods using 3-21G, 4-31G, or 6-31G* basis set. However, the MNDO method reproduces the qualitative features of ab initio potential surfaces and transition structures for a variety of reactions. See: Schroder, S.; Thiel, W. *J. Am. Chem. Soc.* **1985**, *107*, 4422–4430.

Table I. The Geometrical Parameters[a] for Each Reaction State Obtained by the MNDO Method

	Δr^b (Å)			
	0.0	0.65	3.00	∞
	Bond Length[c]			
C1–H1	1.120	1.098	1.093	1.093
C1–C3	1.460	1.411	1.397	1.397
C1–C2	1.613	2.263	4.613	∞
C2–O1	1.254	1.205	1.186	1.187
C2–O2	1.252	1.203	1.186	1.187
C1–C4	1.518	1.442	1.404	1.405
	Bond Angle[d]			
C1–C2–O1	115.8	103.0	84.9	
C1–C2–O2	117.8	104.8	97.9	
H1–C1–C4	108.6	116.1	119.1	119.0
C3–C1–C4	112.8	123.3	127.1	127.0
C2–C1–C4	111.2	103.5	75.1	
	Dihedral Angle[d]			
H1–C1–C4–C5	16.1	15.7	0.7	–0.1
C3–C1–C4–C5	132.0	160.9	180.2	180.0
C2–C1–C4–C5	258.9	274.8	272.3	

[a] Numbering of the atoms is given in Figure 3. [b] The increment of the bond C1–C2 from its initial length. For Δr = 0.0, 0.65, and 3.00 Å, corresponding reaction states are defined as the states R, T, and P, respectively, in the text. [c] Given in Å. [d] Given in deg.

Table II. Change in Charge Distribution of Phenylcyanoacetate Anion Along the Reaction Coordinate in Vacuo

	Δr^a (Å)			
group	0.0	0.65	3.00	∞
COO	–0.745	–0.184	0.001	0.000
C≡N	–0.204	–0.238	–0.266	–0.267
Ph[b]	–0.112	–0.261	–0.413	–0.390
C1	0.024	–0.371	–0.378	–0.375
H1	0.030	0.046	0.032	0.031

[a] The increment of the bond C1–C2 from its initial length 1.613 Å. For Δr = 0.0, 0.65, and 3.00 Å, corresponding reaction states are defined as the states R, T, and P, respectively, in the text. [b] Ph represents the phenyl group.

Table III. Dependence of Activation Energy on the Dielectric Constant of Medium in the Decarboxylation of Phenylcyanoacetate Anion[a]

ϵ^b	calcd[c] E_{act}	obsd[d] E_{act}	ΔH_R[g]	ΔH_T[g]
1.0	74.8			
2.0	94.9		–89.3	–69.2
12.7	132.3	107.1[e]	–245.0	–187.4
80.0	150.9	131.0[f]	–290.2	–214.1

[a] E_{act}, ΔH_R, and ΔH_T are given in kJ mol^{-1}. [b] Dielectric constant of the medium. [c] E_{act} has the following relationship: $E_{act}(\epsilon = 80.0) \equiv H_T(\epsilon = 80.0) - H_R(\epsilon = 80.0) = \{\Delta H_T(\epsilon = 80.0) + H_T(\epsilon = 1.0)\} - \{\Delta H_R(\epsilon = 80.0) + H_R(\epsilon = 1.0)\} = \Delta H_T(\epsilon = 80.0) - \Delta H_R(\epsilon = 80.0) + \{H_T(\epsilon = 1.0) - H_R(\epsilon = 1.0)\} = \Delta H_T(\epsilon = 80.0) - \Delta H_R(\epsilon = 80.0) + 74.8$. [d] Cited from ref 2. The data indicate the enthalpic contribution to the activation energy. The substrate is 4-chlorophenylcyanoacetate. [e] Measured in the mixed solvent (2-propanol/water). [f] Measured in water. [g] ΔH_R and ΔH_T are defined as follows: $\Delta H_R(\epsilon = 80.0) \equiv H_R(\epsilon = 80.0) - H_R(\epsilon = 1.0)$, $\Delta H_T(\epsilon = 80.0) \equiv H_T(\epsilon = 80.0) - H_T(\epsilon = 1.0)$.

transferred from the carboxyl group to the carbon C1 side. As mentioned below, the atomic orbitals of the C1 atom exhibits a character of sp^2 hybridization at Δr = 3.0 Å. These findings indicate that the transient species at Δr = 3.0 Å can be approximately regarded as the product of the reaction. Hereafter we call it the state P.

The values of the geometrical parameters are summarized in Table I. As the cleavage of the bond C1–C2 proceeds along the above reaction path, the following geometrical changes are induced: (i) the lengths of the C1–C4 and C1–C3 bonds monotonously decrease about 0.1 Å, (ii) the dihedral angles H1–C1–C4–C5 and C3–C1–C4–C5 approach 0 and 180°, respectively, (iii) the bond angle C2–C1–C4 decreases, finally approaching 78.1° in the state P. The item (ii) indicates that the atoms C1, C3, and C4 are on the same plane in the state P, indicating that the hybrid state of the C1 atom changes from the sp^3 to sp^2 types. Apparent geometrical change was not found in the phenyl group (not shown in Table I) throughout the reaction.

Table II summarizes the charge distribution on PCAA in each state. The negative charge is mainly localized on the carboxyl group in the state R, while it is distributed over the whole molecule in the state T. In the state P, no fractional negative charge is found on the carboxyl group, which is a major difference from the case of the state T. On the basis of these results, it is understood that the significant amount of intramolecular charge transfer is induced as the reaction proceeds. For example, the total amount of negative charge on the carboxyl group changes by +0.561 on going from the states R to T, and by +0.185 from the states T to P. Similarly, the amount of charge change on the atom C1 is –0.395 for the former case and –0.007 for the latter case. As will be described hereafter, this characteristic charge rearrangement is an important factor to interpret how the solvent environment influences the reaction rate.

To investigate effects of substituents attached to the atom C1 on the activation energy E_{act}, the reaction coordinates were also obtained for phenylacetate and cyanoacetate anions (Figure 2 (parts b and c, respectively)). The values of E_{act} for the former and the latter compounds are 139.9 and 172.1 kJ mol^{-1}, respectively. The value of E_{act} for PCAA is considerably smaller than these values; it is ca. 100 kJ mol^{-1} smaller than the value of E_{act} for cyanoacetate anion. Thus, the phenyl group plays an important role in lowering the activation energy E_{act}, which is consistent with the prediction from the classical theory of organic reaction.

Reaction in Homogeneous Media. Since the decarboxylation is an enthalpically-dominated reaction,[2,3,25] it is of primary importance to calculate the enthalpic contribution to the acceleration of the reaction, induced by lowering the polarity of solvent. In order to make sure of the reliability of the continuum model described above, the activation enthalpies were calculated for the normal solution reactions of PCAA.

Table III summarizes the calculated data for E_{act} in the media of dielectric constants ϵ = 2, 12.7, and 80, and the available experimental data are cited together. The observed E_{act} values for ϵ = 12.7 and 80.0 are measured in the mixed solvent (2-propanol/water) and water, respectively. In both cases, the calculation well reproduces the observed data, although the calculated values are smaller than the corresponding observed ones by ca. 20 kJ mol^{-1}. A more important point to be stressed here is that the calculation better reproduces the amount of lowering of the activation energy, with decreasing dielectric constant. The calculated value for E_{act} is decreased by 18.6 kJ mol^{-1} on going from the medium of ϵ = 80.0 to ϵ = 12.7. The corresponding observed value is 23.9 kJ mol^{-1}. These results indicate that some factors not explicitly considered in the present model, such as a contribution of hydrogen bonding to enthalpy, are canceled. Even if there is such an artificial effect, it is of great surprise that the present simple solvent model provides good prediction on the reaction rate of interest.

Table III also shows the data for the enthalpy differences ΔH_R and ΔH_T, where ΔH_R (ΔH_T) is evaluated by subtracting the H value in the state R (T) at ϵ = 1.0 from that in the state R (T) at ϵ = 2.0, 12.7, or 80.0, respectively. These data indicate that in the states R and T the enthalpy change ΔH becomes larger (more negative) with an increase in dielectric constant. In more detail, the amount of solvent-induced energy change is larger in the state R than in the state T, resulting in the increase of E_{act}

Table IV. Dependence of Charge Distribution on the Dielectric Constant of Medium in the Decarboxylation of Phenylcyanoacetate Anion

	ϵ^a			
group	1.0	2.0	12.7	80.0
	State R			
COO	−0.745	−0.779	−0.850	−0.868
C≡N	−0.204	−0.205	−0.207	−0.206
Ph[b]	−0.112	−0.086	−0.034	−0.023
C1	0.024	0.031	0.045	0.048
H1	0.030	0.034	0.044	0.048
	State T			
COO	−0.184	−0.196	−0.207	−0.212
C≡N	−0.238	−0.247	−0.272	−0.276
Ph[b]	−0.261	−0.233	−0.176	−0.163
C1	−0.371	−0.375	−0.389	−0.393
H1	0.046	0.045	0.044	0.045

[a] Dielectric constant of the medium. [b] Ph represents the phenyl group.

Table V. Thermodynamic Parameters[a] for the Decarboxylation of Phenylcyanoacetate Anion in the β-CD Catalysis and in Free Aqueous Medium

	calculated			observed[c]				
	E_{act}	$\Delta E_{act}{}^b$	$k/k_{\epsilon=80}{}^c$	E_{act}	$\Delta E_{act}{}^b$	$k/k_{\epsilon=80}{}^c$	$\Delta H_R{}^d$	$\Delta H_T{}^d$
				Complex[f]				
				109.2	−21.8	6.6×10^3		
0.35[g]	142.5	−8.4	3.0×10				−277.8	−210.1
−0.75[g]	136.7	−14.2	3.1×10^2				−262.3	−200.4
−1.60[g]	115.3	−35.6	1.7×10^6				−236.8	−196.3
				Free				
1.0[h]	74.8	−76.1	2.2×10^{13}				0.0	0.0
2.0[h]	94.9	−56.0	6.6×10^9				−89.3	−69.2
12.7[h]	132.3	−18.6	1.8×10^3	107.1[i]	−23.9	1.5×10^4	−245.0	−187.4
80.0[h]	150.9	0.0	1.0	131.0[j]	0.0	1.0	−290.2	−214.1

[a] E_{act}, ΔE_{act}, ΔH_R, and ΔH_T are given in kJ mol^{-1}. [b] ΔE_{act} is the activation energy difference evaluated by substracting the E_{act} value at $\epsilon = 80.0$ from that in each medium. [c] The ratio of the kinetic constant in each medium to that in the medium at $\epsilon = 80.0$. [d] ΔH_R (ΔH_T) is evaluated by subtracting the H value in the state R (T) at $\epsilon = 1.0$ from that in the state R (T) in each medium. [e] Cited from ref 2. The data indicate the enthalpic contribution to the activation energy. The substrate is 4-chlorophenylcyanoacetate. [f] β-CD catalysis. [g] The value of parameter d in Å. Details are described in the text and Figure 7. [h] Dielectric constant of the medium. [i] Measured in mixed solvent (2-propanol/water). [j] Measured in water.

in higher dielectric solvent. A similar result has been reported for S_N2 reaction in the gas and liquid phases.[29]

From the analysis of charge distribution, one can find the reason why the energy of the state R is more largely perturbed through the interacting with the dielectric medium than that of the state T. Table IV summarizes the charge distribution on PCAA embedded in the homogeneous dielectric. Here, for the convenience of analysis, we introduce the symbol ρ (X, e) representing the net charge on the chemical group X in the medium of dielectric constant ϵ. In the state R, ρ (COO, 1.0), ρ (COO, 2.0), and ρ (COO, 80.0) for the carboxyl group are −0.745, −0.779, and −0.868, respectively. And in the state T corresponding values are −0.184, −0.196, and −0.212, respectively. The larger the dielectric constant becomes, the more negative charge is induced on the carboxyl group. On the other hand, ρ (Ph, 1.0), ρ (Ph, 2.0), and ρ (Ph, 80.0) for the phenyl group are −0.112, −0.086, and −0.023 in the state R, respectively, and in the state T those values are −0.261, −0.233, and −0.163, respectively. Thus, in both states, intramolecular charge transfer occurs from the phenyl to carboxyl groups with increasing dielectric constant. More importantly, the amount of such charge transfer is larger in the state R than in the state T. This is shown by comparing the values of $\Delta\rho$ (COO, (80.0, 1.0)), defined by ρ (COO, 80.0) − ρ (COO, 1.0), between the states R and T. Those values for the states R and T are −0.123 and −0.028, respectively. These results indicate that the reaction field from the dielectric medium acts so as to localize the negative charge on the carboxyl group and this tendency is remarkably large in the state R. According to the theory of electric polarization in a continuous medium, the values of energy of charged species are generally influenced by the way that the charge is distributed in it.[30] For example, according to Born's theory[31] for a charged sphere, its energy gain, generated by transfer of the sphere from in vacuo to the dielectric medium, is inversely proportional to the radius. This means that an increase in localization of a charge in a molecule results in larger energy gain, which accounts for the fact that the energy of the state R is lowered more largely than the state T (Table III).

The difference in electronic distribution between the states R and T can be attributed to the change in hybridization of the C1 carbon. In the state T, this carbon is approximately assigned to the sp^2 hybrid state as described above. As a result, the π-conjugated system is generated in the region including the carboxyl, phenyl, and cyano groups, allowing the more extended delocalization of the negative charge in a high dielectric medium in the state T than in the state R. This is exemplified by comparing the values of $\Delta\rho$ (CN, (80.0, 1.0)) between the states R and T. Those values are −0.002 and −0.038 for the states R and T, respectively.

Reaction in Heterogeneous Media. As described above, the calculation gives the enthalpic contribution to the energy of a CD complex system rather than the free energy. This causes no problem for analysis of CD catalyzed decarboxylation of PCAA. Because the CD catalysis for the reaction of PCAA derivatives is an enthalpically dominated reaction.[2,25]

Some reports have been published about the effect of α- and β-CDs on the rate constant of decarboxylation of PCAA derivatives.[2,25] In those reports, a series of para-substituted compounds, such as 4-methyl- and 4-chlorophenylcyanoacetates, were used as substrates. However, the data for the activation energy are available only for the β-CD inclusion complex with 4-chlorophenylcyanoacetate. In Table V are summarized experimental data and the calculated activation energy for the β-CD catalyzed reduction and, for comparison, the data for the cases of homogeneous media cited from Table III. The observed data for β-CD catalysis cited in Table V indicate the enthalpic contribution. As can be seen from the experimental data, E_{act} of decarboxylation is lowered by 21.8 kJ mol^{-1} on going from the aqueous medium to the CD-catalyzed state (see ΔE_{act} in Table V). The value of E_{act} in the catalyzed state is nearly equal to that in the mixed solvent composed of 2-propanol and water. These results for β-CD catalysis are well reproducible by the present model calculation as shown in Table V. The calculated values of E_{act} become smaller as the substrate penetrates more deeply into the CD cavity. When the value of the parameter d is −0.75 Å, the value of E_{act} is nearly equal to that in the medium of ϵ = 12.7. Then, the value of ΔE_{act} is 14.2 kJ mol^{-1}, which is 6–7 kJ mol^{-1} smaller than the experimental value (21.8 kJ mol^{-1}). From the results of the calculated activation energy, it is predicted that the value of ΔE_{act} would be nearly equal to the corresponding observed value when the value of d is a little smaller than −0.75 Å. As can be understood from Figure 7, such a structure of the inclusion complex is energetically reasonable, because the polar groups of the substrate are wholly surrounded by the aqueous medium, with the phenyl ring embedded in the apolar domain (low dielectric medium) of the CD cavity. As a result, the activation energy of the reaction is very sensitive to the property of the environment surrounding the phenyl group. This is

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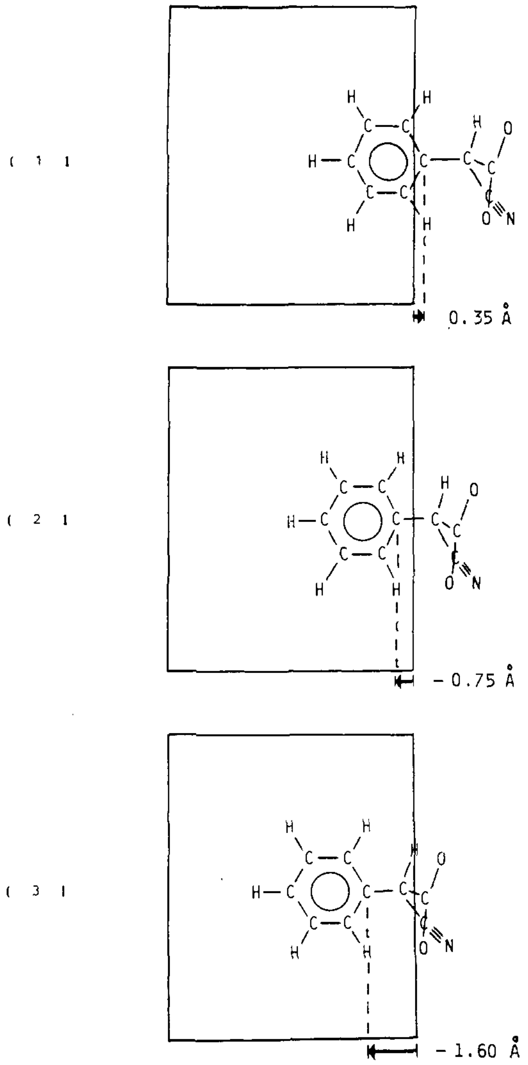

Figure 7. Schematic representation of three different binding states of the substrate into the CD cavity. The definition on the depth of inclusion is described in the text.

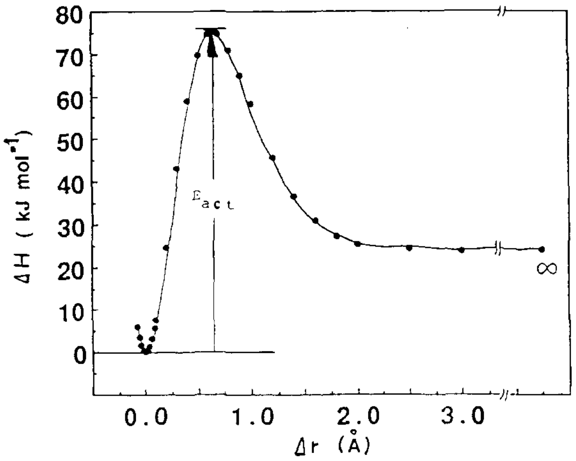

Figure 8. The reaction path in the decarboxylation of phenylcyanoacetate anion in vacuo. The distance between the carbons C1 and C2 (the numbering shown in Figure 3) is used as the reaction coordinate. The ordinate represents the energy difference from the initial state.

consistent with the result for the reaction path in vacuo showing that the activation energy is drastically lowered by an addition of the phenyl group. Therefore, the present calculation can consistently account for the experimental results for both homogeneous and heterogeneous media.

Table VI. Dependence of Activation Energy on the Structure of Inclusion Complexes in the Decarboxylation of Phenylcyanoacetate Anion[a]

d[b]	E_{act}	ΔE_{act}[c]	ΔH_R[d]	ΔH_T[d]
		α-CD		
0.35	143.8	-7.1	-278.1	-209.2
-0.75	137.7	-13.2	-263.3	-200.4
-1.60	129.4	-21.5	-251.2	-196.7
		β-CD		
0.35	142.5	-8.4	-277.8	-210.1
-0.75	136.7	-14.2	-262.3	-200.4
-1.60	115.3	-35.6	-236.8	-196.3
		V-CD[e]		
0.35	143.8	-7.1	-278.1	-209.1
-0.75	137.9	-13.0	-263.3	-200.2
-1.60	129.6	-21.3	-251.2	-196.4

[a] E_{act}, ΔE_{act}, ΔH_R, and ΔH_T are given in kJ mol^{-1}. [b] The value of parameter d in Å. Details are described in the text and Figure 7. [c] ΔE_{act} is the activation energy difference evaluated by subtracting the E_{act} value at $\epsilon = 80.0$ from that in each inclusion complex. [d] ΔH_R (ΔH_T) is evaluated by subtracting the H value in the state R (T) at $\epsilon = 1.0$ from that in the state R (T) in each inclusion complex. [e] V-CD indicates virtual CD. Details are described in the text.

Table VI summarizes the data for the activation energy E_{act} and the enthalpy differences ΔH_R and ΔH_T in the three different structures of the inclusion complexes as defined in Figure 7, where ΔH_R (ΔH_T) is evaluated by subtracting the H value in the state R (T) at $\epsilon = 1.0$ from that in the state R (T) in each complex. For both cases of α- and β-CDs, as the value of d changes from 0.35 to -1.60 Å, the value of ΔH_R is increased by 30–40 kJ mol^{-1}, while the corresponding energy change of ΔH_T is ca. 15 kJ mol^{-1}. Thus, the energy of the state R is more largely perturbed through the interaction with the surrounding dielectric than that of the state T, which is similar to the case in the homogeneous medium.

In contrast to the intuitively acceptable results for E_{act}, ΔH_R, and ΔH_T, the charge distribution on the substrate exhibits some characteristic features on complexation with CD, especially in the state T. Table VII shows the data for the charge distribution on the substrate embedded in the cylinder dielectrics corresponding to α- and β-CDs. For a comparison of the charge distribution between the CD-complexed and free aqueous states, we introduce the charge difference $\Delta\rho$ evaluated by subtracting the net charge in the aqueous medium from those in the complexed states descriminated by the d value. In the state R, with a decrease in the d value, the negative charges on the polar groups, COO and CN, decrease, while that on the phenyl group tend to increase. Thus, the intramolecular charge transfer occurs in the same direction as observed with decreasing dielectric constant in the free state. In other words, the electronic polarization of the substrate decreases with increasing penetration depth of the substrate into the cavity. These are consistent with the behavior of ΔE_{act} and ΔH_R mentioned above. In the state T, the net charge change is less remarkable than that in the state R, which is also consistent with the results for the enthalpy changes ΔH_T in both states. However, it should be stressed that the charge distribution in the state T is quite characteristic. Irrespective of the value of d, the values of $\Delta\rho$ of the polar groups are negative, namely, the net charge on them are more negative than those in the aqueous medium of $\epsilon = 80.0$. On the other hand, the net negative charge on the phenyl group decreases on complexation with CD. The magnitudes of these changes are very small (<0.019), but may be nonnegligible on consideration that the energy change in the free state is accompanied by almost equal amounts of charge change as shown in Table VII. In particular, it is noticable that such an amount of charge change is nearly equal to that induced by the transfer of the substrate from the medium of $\epsilon = 80.0$ to that of $\epsilon = 12.7$ (Table IV). As a result, in the state T, the charge distribution is more polarized in the complexed state than in the

Table VII. Dependence of Charge Distribution on the Phenylcyanoacetate Anion in CD Catalysis[a]

	α-CD catalysis			β-CD catalysis			V-CD catalysis[d]		
group	0.35[b]	−0.75[b]	−1.60[b]	0.35[b]	−0.75[b]	−1.60[b]	0.35[b]	−0.75[b]	−1.60[b]
				State R					
COO	0.001	0.013	0.012	0.001	0.010	0.031	0.001	0.012	0.012
C≡N	0.000	−0.003	0.023	0.000	−0.002	−0.005	0.000	−0.004	0.023
Ph[c]	−0.003	−0.008	−0.012	−0.003	−0.007	−0.008	−0.003	−0.007	−0.012
C1	0.001	−0.002	0.002	0.001	−0.002	−0.007	0.001	−0.002	0.002
H1	0.000	−0.003	−0.007	0.000	−0.002	−0.013	0.000	−0.003	−0.007
				State T					
COO	−0.006	−0.005	−0.003	−0.006	−0.006	−0.005	−0.006	−0.005	−0.003
C≡N	−0.005	−0.011	−0.011	−0.004	−0.012	−0.011	−0.005	−0.012	−0.012
Ph[c]	0.018	0.018	0.013	0.017	0.016	0.014	0.018	0.019	0.013
C1	−0.002	0.002	0.004	−0.002	0.003	0.004	−0.002	0.002	0.004
H1	−0.005	−0.004	−0.008	−0.005	−0.004	−0.009	−0.005	−0.008	−0.004

[a] Charge values are evaluated by subtracting the charge value at $\epsilon = 80.0$ from that in each complexed state. [b] d are given in Å. This parameter indicates the position of the substrate in the CD cavity. Details are described in the text and Figure 7. [c] Ph represents the phenyl group. [d] V-CD indicates virtual CD.

aqueous medium. It will be again discussed whether this unexpected result is physically realistic or due to the artificial property of the theoretical model used here.

In order to examine the effect of the size of CD cavity (hydrophobic pocket) on the electronic structure of the substrate, similar calculation was carried out for a virtual model having the cavity sizes different from those of α- and β-CDs: namely, the radius and length of the cylinder are taken to be 7.0 and 10.0 Å, respectively. The calculated data for this model are listed as V-CD in Tables VI and VII. If the data for the three kinds of CD are compared for each d value, no apparent difference is found except for the case of β-CD with the d value of −1.60. Thus, electronic property of the substrate is insensitive to the dimensions of the hydrophobic pocket surrounding it.

Discussion

CD is one of the most important compounds providing chemists the possibility of realizing artificial enzymes. However, many aspects of its functional mechanism have not been fully understood in molecular level. Although molecular orbital calculation is effective for solving various problems of molecules, its direct application to the CD catalysis is difficult because one must treat a large system composed of CD, substrate, and their surrounding water molecules. Here, we attempted to treat approximately the effects of the environment surrounding the substrate. The results described above indicate that essential aspects of CD-catalyzed decarboxylation are reproducible by the simple dielectric model approximating the CD cavity as a lower dielectric constant medium of microscopic dimensions. This successful consequence seems to be basically ascribed to the high ability of the solvation theory used, confirmed by our previous studies showing that it can quantitatively evaluate the solvation enthalpies of organic molecules in the aqueous medium[24] and depict the heterogeneous character of the environment surrounding a molecule.[12] However, some important factors about the CD substrate interaction may be lost owing to the exaggerated simplification. Next, we discuss this point.

The following factors are not explicitly considered in the present calculation: (1) temperature dependence of the dielectric constant of the medium, (2) orientational freedom of the substrate phenylcyanoacetate anion in the CD cavity, and (3) steric hindrance between the substrate and CD. With regard to the factor (1), in the calculation we adopted the value of dielectric constant at 20 °C, while the experiment for the CD-catalyzed decarboxylation has been performed at 60 °C.[2] The value of dielectric constant ϵ of water is 80.0 at 20 °C and 66.8 at 60 °C,[32] indicative of an apparent difference between the experimental and computational conditions. However, this factor is almost negligible, because the reaction field generated from the dielectric is not a linear function of ϵ but a hyperbolic type of function expressed by $(\epsilon - 1)/(\epsilon + 1)$. In the case of water, the value of this function is 0.975 and 0.971 for ϵ at 20 and 60 °C, respectively. The factors (2) and (3) are related to each other. According to the CPK molecular modeling for the CD–substrate complexes, the orientation of the substrate in the α-CD cavity is confined to the case that the molecular axes of both molecules have a parallel orientation. Thus, the orientation assumed in the present calculation (Figure 7) is reasonable for the α-CD inclusion complex studied. However, the substrate can almost freely rotate in the β-CD cavity if we consider only the steric factor between CD and the substrate. The assumed orientation is thus a special case for the β-CD inclusion complex but a representative of orientations stabilized by the electrostatic interaction between the substrate and its surrounding environment.

There is a more important structural difference between the α- and β-CD inclusion complexes which may be directly related to the difference in catalytic ability between α- and β-CDs. X-ray[23] and NMR studies[13] of α-CD inclusion complexes and the CPK molecular modeling have indicated that the aromatic ring of benzene derivatives, such as *p*-nitrophenol and *p*-hydroxyacetic acid, is not completely accommodated in the α-CD cavity. This was confirmed by more recent NMR and molecular mechanics study.[33] Deep inclusion is impossible due to some steric hindrance. This experimentally-determined structure can be compared with that of our complex assumed in the calculation if we regard the top surface of the cylinder (see the caption of Figure 7) as the plane constructed from the secondary hydroxyl groups of CD. If we also consider the results of the previous study on NMR spectra of inclusion complexes,[13] the d value of −0.35 is appropriate for the pseudoinclusion complex corresponding to the α-CD inclusion complex. On the other hand, in the case of β-CD, the same substrates could more deeply penetrate into the cavity without undergoing explicit steric hindrance. As described previously, the value of d for the β-CD inclusion complex is estimated to be a little smaller than −0.75 Å. The difference in the attainable values of the parameter d seems to cause the difference in catalytic ability between α- and β-CDs. According to the experimental results for the decarboxylation of phenylcyanoacetic acid, α-CD exhibits no appreciable catalytic ability compared with β-CD. For example, the half-life time of the decarboxylation of *p*-methylphenylcyanoacetate anion is 32.0 min in the absence of CD, while it is 30.5 and 16.0 min in the presence of α- and β-CDs, respectively.[25] Using Arrhenius' theory and the calculated activation energies given in Table V, the k values can be evaluated

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as a function of the parameter d. The acceleration ratio was obtained by calculating the ratio of the kinetic constant k in the presence of CD to that in the absence of CD. The results indicate that the reaction at 298 K is accelerated about 30 and 300 times at the d values of 0.35 and –0.75 Å (Table V), respectively, which is consistent with the tendency of the above experimental data.

One of the most interesting findings in this study is that the reaction center, the carboxyl group, is not necessarily required to be surrounded by the low dielectric medium formed by the CD cavity, in order for the reaction to be effectively accelerated. The reaction can be significantly accelerated if the phenyl ring is partially or wholly embedded in the microscopic domain with lower dielectric constant. In this sense, it seems to be reasonable that we given a term of "microsolvent" to such a solvent-like function of the CD cavity, as described in ref 2.

As remarked in the Introduction, there remain unresolved problems in the CD-catalyzed hydrolysis of some ester compounds. The hydrolysis of ester is known to be accelerated in a lower polar medium.[34,35] Thus, the microscopic solvent effect of the CD cavity may also effectively contribute to the acceleration of this type of reaction. It is of interest to apply the present method to the CD-catalyzed ester hydrolysis, which is now under investigation in our laboratory.

As well-known, enzymes accelerate the reactions of their bound substrates 10^2–10^6 times as much as in the aqueous medium.[36] Early studies have indicated that the dehydration of substrates, resulting from its binding to enzyme, is an important factor accounting for the origin of enzymatic catalysis. For example, the decarboxylation of thiaminepyrophosphate is accelerated 10^4–10^5 times by pyruvate decarboxylase.[37] The main origin of this catalysis was ascribed to the fact that the active site of this enzyme is formed by relatively apolar amino acids similar to dioxane in their dielectric nature.[37] Furthermore, according to a study on the catalytic mechanism of α-chymotripsin using methyl esters as substrates, the hydrolysis is thought to be accelerated more than 10^3 times as a net effect of dehydration of the substrates.[35] It has been reported that the active sites of various enzymes act on their bound substrates like lower dielectric media with dielectric constants of 2–4.[38,39] However, there is some controversy about the dielectric properties of enzymes. In a series of study on electron-transfer reactions in protein, the dielectric constants of their active sites were estimated to be about 50 on the basis of the observation of oxidation–reduction potential.[40] In other cases, a number of water molecules were found near the reaction center of a bound substrate,[41,42] indicating that the active site has higher dielectric constant. These different results suggest that the dielectric property of the reaction center may be considerably different for each enzyme. An alternative interpretation is that only a unique value of dielectric constant is difficult to be attributed to each active site having a complexed structure formed by various amino acid. If so, it may be appropriate to decompose the active site into some microscopic domains with different dielectric constants. From a theoretical view point, the present results assure that such an argument is possible.

A typical example for such a case is the reaction center of α-chymotrypsin, where there is a clearly-defined hydrophobic pocket capable of accommodating the aromatic ring of a bound substrate. According to the X-ray analysis, this pocket is formed by relatively apolar amino acid.[43] Thus, as similar to the CD cavity, it may play a key role in the catalytic reaction of this enzyme.

The electrostatic interaction studied here is an indispensable component of the driving force for binding the substrate to CD. It generates a repulsive force for substrate-binding, because the dehydration of the substrate, which occurs when it penetrates into the CD cavity, is a thermodynamically endothermic process. This can be seen from the data for the energies of the states R and T. As can be seen in Table VI, their energies are increased as the substrate penetrates more deeply into the CD cavity. In order to study the driving force, attractive forces for complexation must be evaluated. In this regard, the so-called cavity formation energy may be the most important contributor. The formation of the inclusion complex leads to a decrease in volume of the in vacuo cavity which accommodates the substrate and CD molecules. This means that the cavity formation energy is decreased on complexation. In fact, it has been experimentally evidenced that such an energy contribution is important for complexation.[44] In a previous study, we have developed an empirical method for calculating this type of energy contribution.[24] However, this energy does not contribute to perturbing the electronic structure of the substrate and thereby to its reaction rate at least in the framework of the continuum model used. Therefore, we did not calculate it in the present study.

In the state T, the electronic distribution on the substrate exhibits the unique change on complexation with CD. The validity of this should be judged through a comparison with appropriate experimental data reflecting the electronic distribution of the substrrate. ^{13}C NMR chemical shifts are one of the most powerful methods to study the electronic distribution on π-conjugated molecules, because there is a good correlation between the electron densities on carbon atoms and their chemical shifts. Although we have no chemical shift data for the substrate PCAA in the state T, the present calculated results may be supported by our previous study[12] showing that the cylinder model used here well reproduces the characteristic chemical shift changes of some benzene derivatives, induced on complexation with α-CD. Thus, the over-polarized electronic distribution in the state T should have a physical reality.

Concluding Remarks

This study theoretically confirmed the experimental observation that the decarboxylation of PCAA is accelerated in the CD cavity. The cavity space was treated as a solvent-like medium with microscopic dimensions. In the framework of the present theory, it is concluded that the reaction field from the cavity space plays an important role in the enzymatic functions of CD. Thus one may call such a function of CD the microsolvent effect.[2] The term microsolvent effect has been often used to explain the fact that a substrate included in CD shows properties similar to those in relatively apolar solvent. However, its physicochemical concept has remained ambiguous. The primary significance of a series of our theoretical studies,[11,12,14,15,24] including the present one, is to give a theoretical background to it. As clearly understood from the present theoretical treatment based on the continuum model, the application of the method is not confined to CD inclusion complexes. Microsolvent effects are also expected to account for the catalytic mechanism of other macrocyclic compounds as well as enzymes.

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