Transient Binding Mode of Phenolphthalein–β-Cyclodextrin Complex: An Example of Induced Geometrical Distortion

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Abstract: Phenolphthalein is transformed into its colorless lactonoid dianion within the cavity of β-cyclodextrin at pH 10.5 with a rate constant of 3.7×10^7 M^{-1} s^{-1}. The structure (including the ionization of the phenolate groups) was demonstrated by ^{13}C NMR spectroscopy, while the rate constant was measured by *T*-jump kinetics. The dissociation constant for the binding at 20 °C is 2.7×10^{-5} M. These findings require that the geometrical distortion of phenolphthalein as it enters the cavity of the cyclodextrin produces a remarkable new ionized form of phenolphthalein, with accompanying enormous rate acceleration; the reaction, in these ways, mimics enzymic processes.

The enormous rate enhancement and specificity of enzymes have variously been ascribed to entropy factors,[2-4] distortion of the substrate[5-7] in the enzyme–substrate complex, and other factors. In this paper, the change in conformation of phenolphthalein as it enters a complex with β-cyclodextrin is presented as a simple chemical model for an enzymic system.

Phenolphthalein (PP), a typical indicator dye, exhibits the equilibria shown in Scheme I.[8] When β-cyclodextrin (β-CD) is added to a solution of the red (alkaline) form, the color instantly disappears.[9] In this paper, we have shown by ^{13}C NMR spectroscopy that, when the red, ionized form of phenolphthalein is enclosed in β-cyclodextrin, it is forced into its lactone structure, without, however, protonating the phenolic groups. That is to say, within the cyclodextrin cavity, the phenolic residues are ionized, but the carboxyl group is bound into a lactone as shown in Scheme II. Furthermore, this lactonization (which destroys the red color) takes place with a rate constant of 3.7×10^7 M^{-1} s^{-1}, a rate only 1 order of magnitude less than the diffusion limit. The cyclodextrin here is acting very much as does an enzyme in increasing the reaction rate.

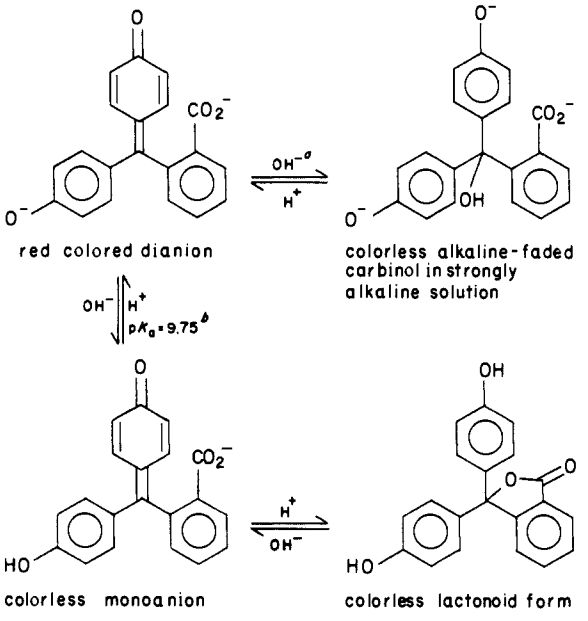
Experimental Section

Materials. β-Cyclodextrin used herein was either prepared at Teijin Ltd. or purchased from Tokyo Kasei Co. Further purification was made by repeated recrystallization from water, and the hydrated β-CD was dried at 80 °C under reduced pressure; purity was assessed by thin-layer chromatography on Eastman cellulose sheets with a fluorescent indicator. The solvent employed for development was 1-butanol–dimethylformamide–water (2:1:1, v/v/v).[10] Phenolphthalein, buffers, and other chemical reagents, purchased from Tokyo Kasei Co., were of analytical grade and were used as received.

General Methods. All solutions of phenolphthalein for the UV–visible spectral measurements were freshly prepared each day. A weighed amount of PP was first dissolved in a small amount of NaOH solution and the resultant mixture then diluted with the appropriate solution. For measurements in unbuffered solution the stock solution was adjusted to a pH slightly lower than pH 10.5, and aliquots were mixed with β-CD solution, thermostated, and diluted to the specified concentration. The pH was finally adjusted to pH 10.5 by addition of a small volume of NaOH solution just prior to the measurements. pH readings were also made after the spectral measurements for the unbuffered solution; the pH remained unchanged within 0.02 unit.

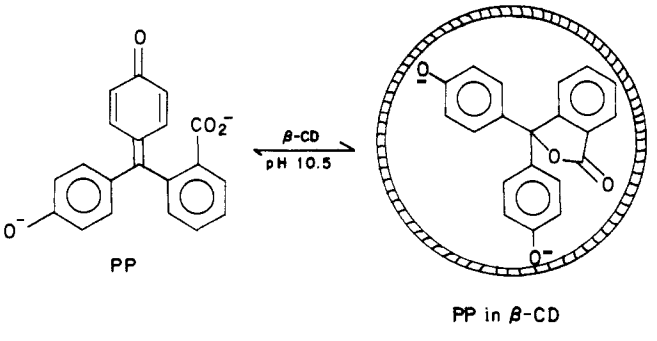
If not specified otherwise, the present studies were conducted at pH 10.5, where about 90% of the total PP is in the red-colored dianion form.[8b,11] Triphenylmethane dyes have been known to aggregate at high

Scheme I



[a] Reference 8b: a slow step, $k = 9.4 \times 10^{-3}$ min^{-1} at pH 12.4. [b] Reference 8b: $pK_a = 9.75 \pm 0.05$.

Scheme II



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(2) Westheimer, F. H. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1962**, *24*, 455.
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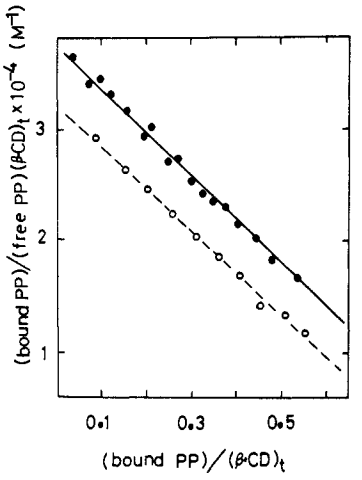


Figure 1. Binding of PP to β-CD at pH 10.5 and 20 °C in 0.1 N NaCl unbuffered solution (●) and in 0.05 N glycine buffer (○). The total concentration of β-CD was 3×10^{-4} M and that of unbound PP was in a range of $(0.1-5) \times 10^{-5}$ M.

concentration. However, under the present condition at pH 10.5 in 0.1 N NaCl solution, a linear relationship of absorbance with concentration was observed at least up to 5×10^{-5} M. The measured extinction coefficient, $\epsilon = 3.06 \times 10^4$ M^{-1} cm^{-1} at 553 nm, was used throughout the present studies.

Physical Measurements. UV-visible absorption spectra for equilibrium measurements in various solutions were recorded on a Hitachi Model 323 recording spectrometer equipped with a thermostated cell holder. The temperature was controlled within 0.1 °C.

Temperature-jump relaxation kinetics was conducted with an electrical discharge temperature-jump device (Type R-105, Union Giken Co., Osaka) equipped with a 200-m coaxial cable as capacitor. The temperature increase, which occurred in less than 4 μs, was achieved by a 25-kV pulse in 0.1 N NaCl solution. The measured relaxation curves were stored on a Tectronics Type-7623A storage oscilloscope, and the traces were then photographed on a Polaroid Pack films. Since the change of intensity is only a small fraction of the final intensity, the absorption changes were assumed proportional to the observed intensity changes. The relaxation reactions, carried out in triplicate, were monitored at 553 nm, the wavelength where the maximum change was observed. The initial temperature in the cell was controlled within 0.1 °C through a thermostated circulating water bath.

^{13}C NMR spectra were recorded on a JLM PFT-100 Fourier transform NMR system (JEOL, Tokyo) interfaced with a JEC-6 spectrum computer. T_1 measurements were performed by using the inversion-recovery method with a ($>5T_1$–180°–τ–90°) pulse sequence for the carbons that are directly bonded to hydrogen atoms. The T_1 values were calculated internally for each value of the experimental sequence and calculated from a least-squares fit to the best line on a semilogarithmic form based on the equation $M(\tau) = [M(0)][1 - 2\exp(-\tau/T_1)]$, where $M(0)$ is the equilibrium value of magnetization and $M(\tau)$ the value at time τ. The room temperature was kept at 25 °C. The chemical shifts were expressed in ppm downfield from Me_4Si contained in a coaxial tube. The molar concentrations were 0.2 M for free PP, 0.02 M for free α- and β-CD, and 0.07 M for each component for the measurement of the PP–β-CD complex. With these concentrations for PP and β-CD, about 98% of both components is in the bound state.

Results

Dissociation Constant of the Complex of PP and β-CD. The dissociation constant of the complex of PP and β-CD at pH 10.5 at 20 °C was determined by the visible absorption changes at 553 nm. Figure 1 shows the data in the form of Scatchard plots[11] based on the equation for binding to n independent sites on each molecule of β-CD

$$\frac{[\text{bound PP}]}{[\text{free PP}][\beta\text{-CD}]_t} = \frac{n}{K_d} - \frac{[\text{bound PP}]}{[K_d][\beta\text{-CD}]_t}$$

where K_d is the intrinsic dissociation constant and $[\beta\text{-CD}]_t$ is the total concentration of β-CD. Since n was found to be 1.0 within the experimental error in unbuffered solution, 1 to 1 complex formation of PP and β-CD was verified at 20 °C and pH 10.5 in 0.1 N NaCl solution. The dissociation constant (K_d) was determined to be 2.7×10^{-5} M.

In 0.05 N glycine buffer solution at 20 °C, the value of n was found to be 0.9, with an apparent K_d of 3.1×10^{-5} M (20 °C). This is slightly higher than that obtained in unbuffered solution. Therefore, the subsequent measurements were made in unbuffered solution.

The dissociation constants of the PP–β-CD complex were also determined at various temperatures between 10 and 30 °C. The logarithmic values of K_d against the reciprocal of the absolute temperature were plotted to evaluate the thermodynamic changes for the complex formation (Figure 2; Table I). The enthalpy change of the complex formation, $\Delta H°$, is −5.8 kcal/mol. The entropy change, +1 eu, is almost null within the experimental error.

Table I

Temperature Dependence of the Rates and the Dissociation Constant in Unbuffered Solution at pH 10.5

temp, °C	$10^{-7}k_1$,[a] M^{-1} s^{-1}	$10^{-3}k_{-1}$,[a] s^{-1}	10^5K_d,[b] M
10.0	2.1	0.45	2.0
15.0	2.8	0.69	2.4
20.0	3.7	1.0	2.7
30.0	5.4	2.2	3.9

Thermodynamic[b] and Activation Parameters in Unbuffered Solution at pH 10.5 and 20 °C
$\Delta F°(293) = -6.1$ kcal/mol, $\Delta H° = -5.8$ kcal/mol, $\Delta S° = +1$ eu

	$\Delta F^{\ddagger}(293)$, kcal/mol	$\Delta H^{\ddagger}$,[c] kcal/mol	$\Delta S^{\ddagger}$,[c] eu
association	+7.0	+7.5	+2
dissociation	+13.0	+13.0	0

[a] Uncertainty: k_1, k_{-1}, 8%. [b] The values directly obtained by the equilibrium binding measurements. Estimated uncertainties: K_d, ±5%; $\Delta H°$, ±0.5 kcal/mol; $\Delta S°$, ±2 eu. [c] Estimated uncertainties: $\Delta H^{\ddagger}$, ±2 kcal/mol; $\Delta S^{\ddagger}$, ±5 eu.

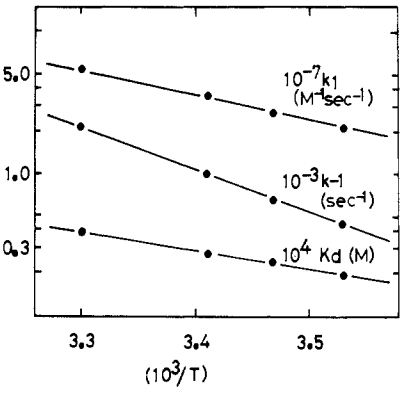


Figure 2. Logarithmic plots of the dissociation constants (K_d) and the rate constants (k_1 and k_{-1}). All measurements were performed at pH 10.5 in 0.1 N NaCl unbuffered solution.

Relaxation Kinetics. The temperature-jump measurements were performed in the equilibrium concentration range of $\bar{C}_{pp} = \bar{C}_{\beta\text{-cd}} = (1-10) \times 10^{-5}$ M at pH 10.5 in 0.1 N NaCl solution by recording the increase in the absorption at 553 nm (Figure 3). The rate constants were evaluated from the following equation:

$$\text{PP} + \beta\text{-CD} \rightleftharpoons \text{PP–}\beta\text{-CD}$$

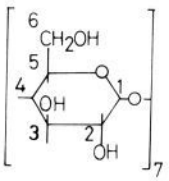
$$1/\tau = k_1(\bar{C}_{pp} + \bar{C}_{\beta\text{-cd}}) + k_{-1}$$

The results are summarized in Table I, and the concentration dependence of the reciprocal relaxation times ($1/\tau$) is also presented in Figure 4.

The observed rate constant for the association obtained in 0.05 N glycine buffer at pH 10.5 was slightly less than that in unbuffered solution, whereas the dissociation rate constant remained the same.

The temperature dependence of the rate constants of the association and dissociation was also determined at various tem-

Table II. Carbon-13 Chemical Shift Changes and Spin–Lattice Relaxation Times (T_1) of Cyclodextrin



	β-CD in D_2O		β-CD–PP complex, pD 10.5		α-CD in D_2O	
assgnt[a]	δ	NT_1,[b] s	δ	NT_1, s	δ	NT_1, s
C-1	102.51 (d)	0.11	102.66 (d)	0.07	102.54 (d)	0.15
C-4	81.93 (d)	0.12	82.05 (d)	0.07	82.37 (d)	0.14
C-3	73.87 (d)	0.10	74.15 (d)	0.07	74.47 (d)	0.16
C-5	72.90 (d)	0.12	73.06 (d)	0.08	73.20 (d)	0.15
C-2	72.71 (d)	0.10	72.57 (d)	0.08	72.86 (d)	0.15
C-6	61.16 (t)	0.10	61.06 (t)	0.08	61.62 (t)	0.22

[a] Assignment following ref 12. [b] N is the number of hydrogen atoms bonded to a given carbon atom, (d) and (t) refer to doublet and triplet in off-resonance spectra, respectively, and chemical shifts were expressed in ppm downfield from Me_4Si as standard. The estimated uncertainties for T_1 values are about ±10%.

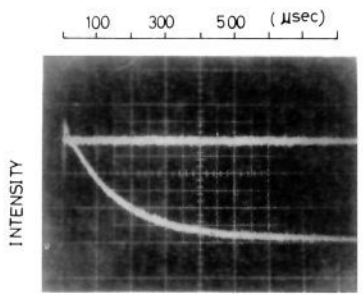


Figure 3. Typical oscilloscope trace of the change in transmittance at 553 nm due to the temperature perturbation on the lactonization at 30 °C and pH 10.5 in 0.1 N NaCl unbuffered solution. The horizontal scale is 100 μs/larger division, and the vertical scale is an arbitrary unit in transmittance. The concentration in the T-jump cell was 8×10^{-5} M for PP and β-CD.

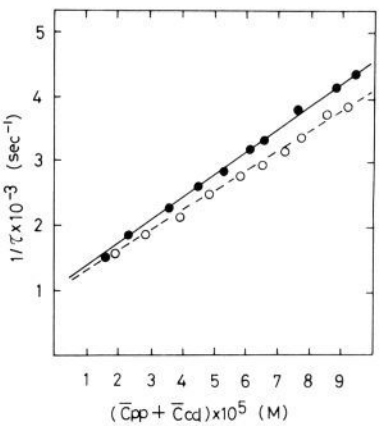


Figure 4. Concentration dependence of the reciprocal relaxation times ($1/\tau$) for the 1 to 1 PP–β-CD complex system at pH 10.5 and 20 °C: (●) in unbuffered 0.1 N NaCl solution; (○) in 0.05 N glycine buffer solution. The equilibrium concentrations, $\bar{C}_{pp}$ and $\bar{C}_{\beta\text{-cd}}$, were determined by the absorbance at 553 nm.

peratures between 10 and 30 °C. The results are presented in Table I and Figure 2.

^{13}C NMR Spectral Assignments. The NMR spectra show that β-CD exhibits completely C-7 symmetry on the NMR time scale. The observed chemical shifts for free β-CD, together with the values of β-CD for comparison, are all in accord with the values previously reported (Table II).[12] Upon the formation of the PP–β-CD complex, only small chemical shift differences of less than 10 Hz for free β-CD were observed. These changes of each of the carbons of β-CD may be attributed to the substrate-induced perturbation,[13] but this feature of the shift changes was not further explored in the present studies.

The ^{13}C NMR spectra of a series of solutions of PP in the various ionic forms were recorded to elucidate the structure of the transient binding form of PP in β-CD, and the assignments are summarized in Table III.

The chemical shift assignments of the alkaline-faded form of PP were made by direct comparison with those reported for a series of triaryl carbinols.[14] The resonance of the carbon (C-1) and that of the carboxylate carbon (C-8′) fall well outside the aromatic region; the resonance at 82.57 ppm is unambiguously assigned to the central carbinol carbon (C-1), and that at 179.08 ppm is due to the carboxylate carbon (C-8′). The resonances at 118.07, 129.92, and 165.77 ppm are attributed to C-4, C-3, and C-5, in this order, and are all in good agreement with the corresponding resonances reported for the phenolate anion.[15] The resonance at 133.39 ppm is due to C-2, which is directly linked to the central carbinol carbon. The resonances of relatively low intensity at 127.97, 129.29, 130.61, 139.97, and 146.75 pm all arise from the aryl residue that bears the carboxylate group, of which the two resonances at 139.97 and 146.75 ppm are due to C-7′ and C-2′, respectively.

The assignments for the resonances of the lactonoid form of PP were made principally by comparison with those for the alkaline-faded form. The central carbon, C-1, is observed at 92.83 ppm, which appears downfield by about 10 ppm from the corresponding resonance for the carbinol carbon of the alkaline-faded form. Furthermore, the chemical shifts for the carbons of the phenolic moiety, C-3, C-4, and C-5, are in good agreement with those for un-ionized phenol previously reported.[15] The resonance at 132.61 ppm is undoubtedly due to C-2, and the rest of the resonances are all from the aryl residue that bears the lactone ring.

The resonances for the carbons, C-2, C-3, C-4, and C-5, of the phenolic moiety of the red-colored dianion form could be assigned on the basis of the resonance intensity with the off-resonance ^{1}H-decoupled ^{13}C spectrum in comparison with the chemical shifts of the alkaline-faded form. The resonances for the aryl residue that bears the carboxylate group coincide with the corresponding chemical shifts for the alkaline-faded form, reflecting that the carboxyl group is indeed ionized. However, the resonances for C-1 and C-2′ could not be located in the spectrum.

The ^{13}C NMR spectrum for the transient binding form of PP in β-CD appears rather complicated due to the close proximity

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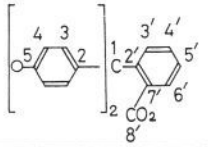
Table III. Carbon-13 Chemical Shift Changes and Spin–Lattice Relaxation Times of Phenolphthalein under Various Conditions



assgnt[a]	δ for lactonoid form in Me_2SO-d_6	transient form in the complex, pD 10.5 δ	transient form in the complex, pD 10.5 T_1,[e] s	δ for alkaline–faded form, 0.1 N NaOD	red-colored form, pD 10.5 δ	red-colored form, pD 10.5 T_1, s	δ triaryl carbinols[c] (p-X-$C_6H_4)_3OH$	δ phenol[d] $C_6H_5OH/C_6H_5O^-$
C-1	92.83 (s)	95.59 (s)	1.84	82.57 (s)			79.4–80.2 (79.5)	
C-2	132.61 (s)	130.08 (s)	1.25	133.39 (s)	126.49 (s)	12.9	137.3–152.2 (140.4)	121.2/114.9
		129.19 (s)	1.74					
C-3	129.56 (d)	129.33 (d)	0.16	129.92 (d)	136.38 (d)	0.52	127.6–129.1 (128.6)	129.9/130.4
		128.71 (d)	0.13					
C-4	116.63 (d)	118.6 (d)	0.15[b]	118.07 (d)	120.86 (d)	0.52	110.8–129.7 (112.0)	115.8/120.3
		117.9 (d)						
C-5	158.92 (s)	163.78 (s)	1.46	165.77 (s)	145.56 (s)	10.9	125.7–161.0 (158.1)	154.4/168.1
		162.90 (s)	2.17					
C-2′	153.97 (s)	154.07 (s)	1.66	146.75 (s)				
C-7′	125.90 (s)	125.12 (s)	1.68	139.97 (s)	135.45 (s)	10.8		
C-3′	136.09 (d)	136.67 (d)	0.09		131.68	0.44		
C-4′	130.87 (d)	130.86 (d)	0.07	130.61	130.66 (d)	0.34		
C-5′	126.70	126.78	0.07	129.29	129.88	0.47		
C-6′	125.80	124.61	0.08	127.97	127.07	0.51		
C-8′	170.48 (s)	173.85 (s)	2.17	179.08 (s)	175.26 (s)	12.6		

[a] The numbering of the carbons. [b] The average T_1 value. [c] Reference 14. Substituent X: H, D, F, Cl, Me, MeO, N(Me). The values in parentheses are the shifts of X = MeO. The shifts in ppm downfield from Me_4Si were calculated by substracting the reported values with respect to CS_2 as standard from 192.8 ppm. [d] Reference 15. The shifts in ppm downfield from Me_4Si were calculated by substracting the reported values with respect to benzene as standard from 152.8 ppm. [e] Estimated uncertainties for T_1 values for the carbons with directly bonded hydrogen atoms are about 10%, and those for longer T_1 values for the carbons without bonded hydrogen atom are substantially shorter than the real values.

of several resonances in the region of 125–130 ppm as shown in Figure 5. However, this problem was greatly clarified by examining the PRFT spectra at appropriate τ intervals, because a resonance intensity becomes null at $\tau = T_1 \ln 2$. As seen in Figure 5, the resonances for the carbon atoms without bonded hydrogen ($T_1 \sim 1.5$ s) disappear at the τ value of 0.9 s, whereas those of the carbons directly bonded to hydrogen ($T_1 \sim 0.1$ s) become null at 0.09 s. Further chemical shift assignments were made by direct comparison with those for the lactonoid form and the alkaline-faded form of PP. The observed resonance for the central carbon C-1 at 95.59 ppm clearly indicates that the bound PP is in a lactonoid form, undoubtedly not in a form of carbinol type for which the chemical shift at C-1 of the alkaline-faded form is recorded at 82.57 ppm and those of a series of triaryl carbinols were reported within the range of 79–80 ppm.[14] Furthermore, the chemical shifts for the aryl residue (C-2′–C-7′) of the transient binding form are all in accord with those for the lactonoid form of PP observed in Me_2SO solution (Table III) but considerably different from those for the alkaline-faded form and the red-colored dianion form of PP, for which the carboxyl group is ionized without forming a lactonoid ring with the central carbon atom (C-1) of PP. Likewise, the possibility of a covalent binding reaction between a hydroxyl group on β-CD and PP could be excluded, especially on the basis of the finding that, even upon formation of the PP–β-CD complex, the chemical shifts for all of the carbon atoms of β-CD were observed as essentially unchanged from those for the corresponding carbon atoms of free β-CD (Table II). The chemical shift changes of the phenolic moiety of the transient binding form of PP, especially the chemical shift of C-5 directly linked to the phenolic oxygen atom, are all comparable to those for the corresponding carbons of the alkaline-faded form; these facts support the claim that the phenolic moiety is indeed ionized. The transient binding form of PP is therefore a lactonoid form having two ionized phenolate moieties.

It should be noted that all of the resonances for the carbons of the two phenolate moieties are split as shown in Table III. This evidence clearly indicates that the two phenolate moieties are in different environments.

^{13}C NMR Spin–Lattice Relaxation Times. Upon the binding of PP, β-CD displays a considerable decrease in the NT_1 values,

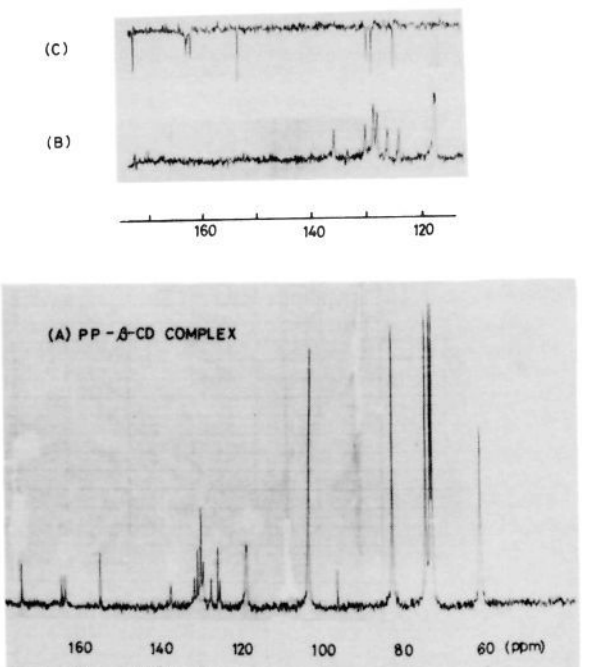


Figure 5. (A) ^{13}C NMR spectrum of the transient binding form of PP. (B) and (C) PRFT spectra with τ intervals of 0.9 s for (B) and 0.09 s for (C).

which are as low as about two-thirds of the corresponding values for the carbons for free β-CD. Although viscosity change upon forming the complex[24] is not considered in this study, the large decrease in mobility cannot be accounted for by an increase of the structural dimension of the bare PP incorporated in β-CD, but this aspect can be attributed to structured water molecules clothing the complex system.[16]

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On the whole, all data on relaxation times shown in Table III demonstrate the considerable restriction of the motion of PP when incorporated in β-CD.

Discussion

Upon the binding to β-CD in aqueous solution at pH 10.5, the red-colored dianion of a trigonal sp^2-conjugated system of PP is rapidly transformed into a colorless lactonoid dianion form having a tetrahedral sp^3 central carbon atom. This is a new and hitherto unforeseen form of PP, which resembles in part the structure of PP predominantly found in acidic medium; the two phenolic moieties of this transient binding form of PP are, however, ionized as is phenol in alkaline solution. This unexpected feature shows that the site of β-CD where PP undergoes this chemical transformation does not merely provide an apolar environment so as to derive the carboxylate group of PP into the lactonoid ring; instead, the intrinsic binding energy is primarily responsible for the distortion of a trigonal sp^2-conjugated PP toward the formation of a tetrahedral sp^3 central carbon atom, even though the two phenolic moieties remain ionized.

The structural aspect of the whole complex is indicated by examining the ^{13}C NMR spectrum, which displays the split resonances with almost equal intensities for each of the corresponding carbons of the two ionized phenolate moieties that are located in different environments; that is, these two phenolate moieties are enantiotopic.[25] This is possible when one of the phenolate moieties is in the cavity of β-CD and the other, together with the aryl residue that bears the lactone ring, is on the surface of β-CD. Alternatively, the aryl residue with the lactone ring is in the cavity, whereas the two phenolate moieities rest on the surface of β-CD in a different environment. At the present stage of our knowledge, it is difficult to determine which of these possibilities is correct. This point requires further study with the PP–β-CD complex.

The dissociation constant for the complex is 2.7×10^{-5} M at 20 °C and pH 10.5, corresponding to a free energy change of −6.1 kcal/mol, and the observed enthalpy of complex formation (ΔH° = −5.8 kcal/mol, ΔS° = 1 eu) indicates that the binding is characterized by what might be termed as atypical[17] or nonclassical[18] hydrophobic interaction between relatively polar substrates. The values of the binding constants generally observed for mononuclear aromatic substrates lie in the small range from 10^{-2} to 10^{-3} M, and the enthalpy–entropy compensation effect caused by solvation change has previously been reported.[19] However, such a low dissociation constant as that found for the PP–β-CD complex (~10^{-5} M) is rarely observed for β-cyclodextrin complexes.

The present relaxation kinetics has revealed that the observed second-order rate constant for the lactonization reaction (k_1 = 3.6×10^7 M^{-1} s^{-1}, 20 °C) is only 1 order of magnitude smaller than a diffusion-controlled rate. The large enthalpies of activation for both the association ($\Delta H^\ddagger$ = +7.5 kcal/mol) and the dissociation ($\Delta H^\ddagger$ = +13 kcal/mol) support the view that breaking the water structure associating with PP and β-CD or the complex is a prerequisite and essential part of the activation stages, followed by rapid reconstitution of the water structure around the complex as suggested for α-CD complex formation.[20] The role of tightly bound water molecules with the solute molecules is indicated by a considerable decrease in the ^{13}C NMR relaxation times (Tables II and III).

These results are consistent with that of a close-fitting tetrahedral form of PP, with the three-site contact with β-CD: the three arms, consisting of the two phenolate and the one aryl group that bears the lactone ring hold β-CD so as to prevent the dissociation of the residue located in the cavity. These three sites must all be broken at the time of dissociation. Therefore, the observed rate of dissociation of PP (k_{-1} = 1×10^3 s^{-1}, 20 °C) is at least 1 order of magnitude slower than those reported for mononuclear aryl substrates incorporated in the cavity,[17] which in turn makes the dissociation constant for PP significantly lower. Chemically modified β-CD, with a hydrophobic floor or cap on one end of the cavity, exhibits dissociation constants only $^1/_{20}$, or less, of those for unmodified β-CD.[21]

The data for the ^{13}C NMR relaxation times have shown that the motion of PP, when incorporated in β-CD, has been considerably reduced; in particular, the observed T_1 values for the carbons of the aryl residue that bears the lactone ring are identical with those of β-CD; i.e., almost no decoupling internal motion of this residue with respect to β-CD is observed. The average dynamic coupling coefficient[16] of about 0.5 for the phenolate moieties of PP in β-CD is obtained from the data in Tables II and III. Therefore, the PP–β-CD complex is relatively tightly bound not only in terms of the thermodynamic dissociation constant but also in the sense of internal residual motion of the bound PP in the β-CD molecule.

The tight-binding mode of the complex further indicates that, as the geometrical distortion starts to develop, the carboxylate anion of PP becomes directed toward and proceeds toward the reactive central carbon of PP so as to stabilize the distorted carbon atom. Once PP is incorporated into the β-CD molecule with simultaneous desolvation around the reactive sites, the lactonization reaction can proceed without further significant loss of entropy.

It is this step in which the lactonization reaction is greatly facilitated, with a second-order rate constant of 3.7×10^7 M^{-1} s^{-1} (20 °C). Since the rates were measured by following the absorption changes at 553 nm, since this change is directly connected with the distortion at the central carbon atom of PP (i.e., directly connected with the lactonization reaction), and since the lactone ring formation is only possible in a 1 to 1 β-CD complex, it can be said that the first-order rate constant for the lactonization is at least 3.7×10^7 s^{-1} *within the field of the* β-CD. This is consistent with the generalization that the efficiency of intramolecular nucleophilic catalysis depends critically on the geometry of approach to a nucleophilic center; in favorable cases, the acceleration reaches 10^6–10^8 M[22] or in some cases even more,[23] whereas that of intramolecular general base catalysis in loose transition states corresponds to a concentration of only about 100 M, accompanied by higher residual entropy.

In summary, the lactonization reaction of PP proceeds extremely fast, following the complex formation at pH 10.5. The specific binding per se provides the driving force to reduce the free energy of activation for this lactonization reaction of PP within the field of a β-CD molecule.

Over the years, considerable interest has been generated in cyclodextrins as enzyme models.[17] The example of induced geometrical distortion presented herein shows a remarkable analogy to those so far found in enzyme–substrate or enzyme–inhibitor complexes.

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