Dynamic Molecular Motions of *p*-Methylcinnamic Acid Included into β-Cyclodextrin Derivatives: A New Type of Free-energy Relationship in Complex Formation

Yasuhisa Kuroda,* Masahiko Yamada, and Iwao Tabushi
Department of Synthetic Chemistry, Kyoto University, Sakyo-ku, Kyoto, 606 Japan

The deuterium quadrupolar relaxation times of (*E*)-[β,*methyl*-$^2H_2$]-*p*-methylcinnamic acid (**G**) included into various β-cyclodextrin derivatives have been measured by line-shape analysis. The association constants for the complexation of host with (**G**) are largely affected by additional recognition elements substituted into the parent β-cyclodextrin. The correlation times for the molecular motion of G which have been estimated from the corresponding relaxation times are also greatly affected by these additional recognition elements. Thus, the relationship between the association constants and the correlation times of (**G**) may be described simply in the present case, *i.e.*, the host molecule having the larger association constant restricts the molecular motion of (**G**) more significantly. One exception is 6,6′-dideoxy-6,6′-di-iodo-β-cyclodextrin (CDI_2) which has a large association constant but restricts only slightly the molecular motion of (**G**). The observed large association constant and the small correlation time for CDI_2 are reasonably interpreted in terms of the effect of the large polarizability of iodine. The free-energy relationship between the binding process and the molecular motion of (**G**) is discussed on the basis of these new observations.

The concept of molecular recognition is now widely recognized as a most important conceptual basis for the design and construction of mimics of biological molecules such as enzymes and receptors. One objective for the investigation of molecular recognition is to provide a systematic methodology which makes possible the design of a host molecule bearing the desired function and specificity for a given guest molecule.

Cyclodextrins are one of the most suitable host molecules for such purposes because of their versatile recognition ability; various types of enzyme and receptor models have been developed by using unmodified and modified cyclodextrins.[1] Extensive investigations of cyclodextrins in the past two decades, have demonstrated that cyclodextrins recognize the size and the shape of the guest molecule basically by hydrophobic interaction;[2] recognition abilities of cyclodextrins have also been shown to be largely affected by the additional recognition elements introduced by substitution in the parent cyclodextrin. Since these additional recognition interactions between modified cyclodextrins and guest molecules are sometimes observed to operate according to the additivity rule,[3] it is now possible to control the binding ability of a cyclodextrin by selective substitution of the parent structure.

However, there still remain several problems which should be solved in order to understand completely the mimicking of biological molecular recognition. One of these problems is the control of the dynamic molecular motions of the guest molecule in the complex. For example, since the interaction operating in the unmodified cyclodextrin complex is mainly the non-directional van der Waal's force, the guest molecule included into an unmodified cyclodextrin sometimes retains appreciable thermal-motional freedom, especially in the form of rotation along the *z*-molecular axis (see Figure 1).[4] In order to utilize cyclodextrin derivatives bearing additional recognition elements as more effective and sophisticated enzyme or receptor mimics, it will become increasingly important to be able to predict and control the dynamic molecular motion of the guest molecule in the cyclodextrin cavity. There are, however, no systematic data for the dynamic molecular motions in this field which suggest, for example, relationships between the motion of guest molecules and the characteristic indices of the complex formations such as association constants or the types of interactions.

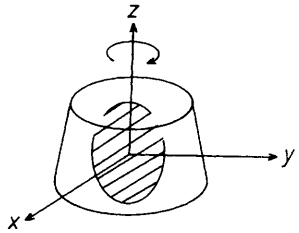


Figure 1. Rotation of guest molecule in cyclodextrin cavity.

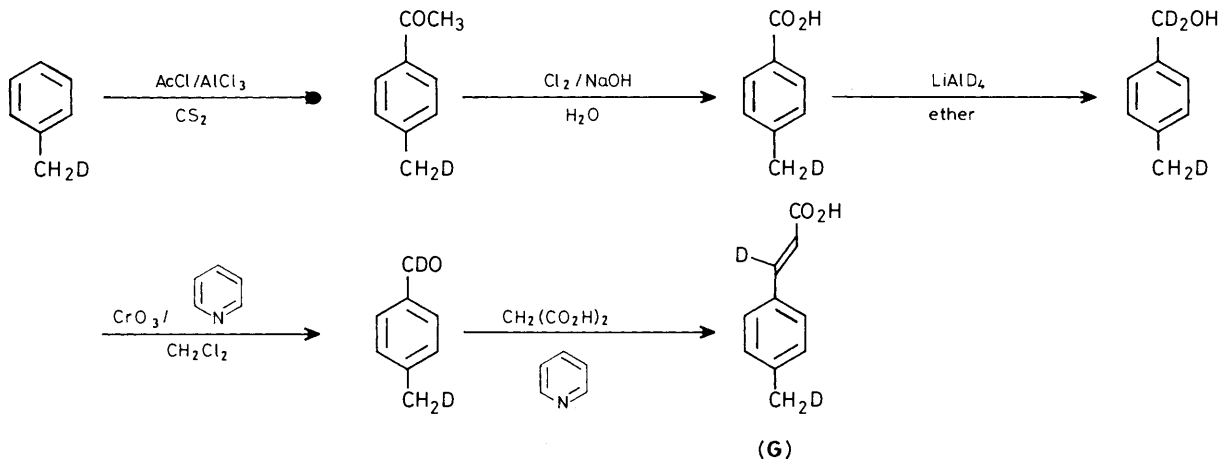
In this paper, we report the first example of such data which suggest a relationship between the free-energy changes for complex formation (ΔG°_{ass}) and the tumbling-motion barrier of the guest molecule ($\Delta G^{\ddagger}_{M}$) included into various β-cyclodextrin derivatives.[5]

Results and Discussion

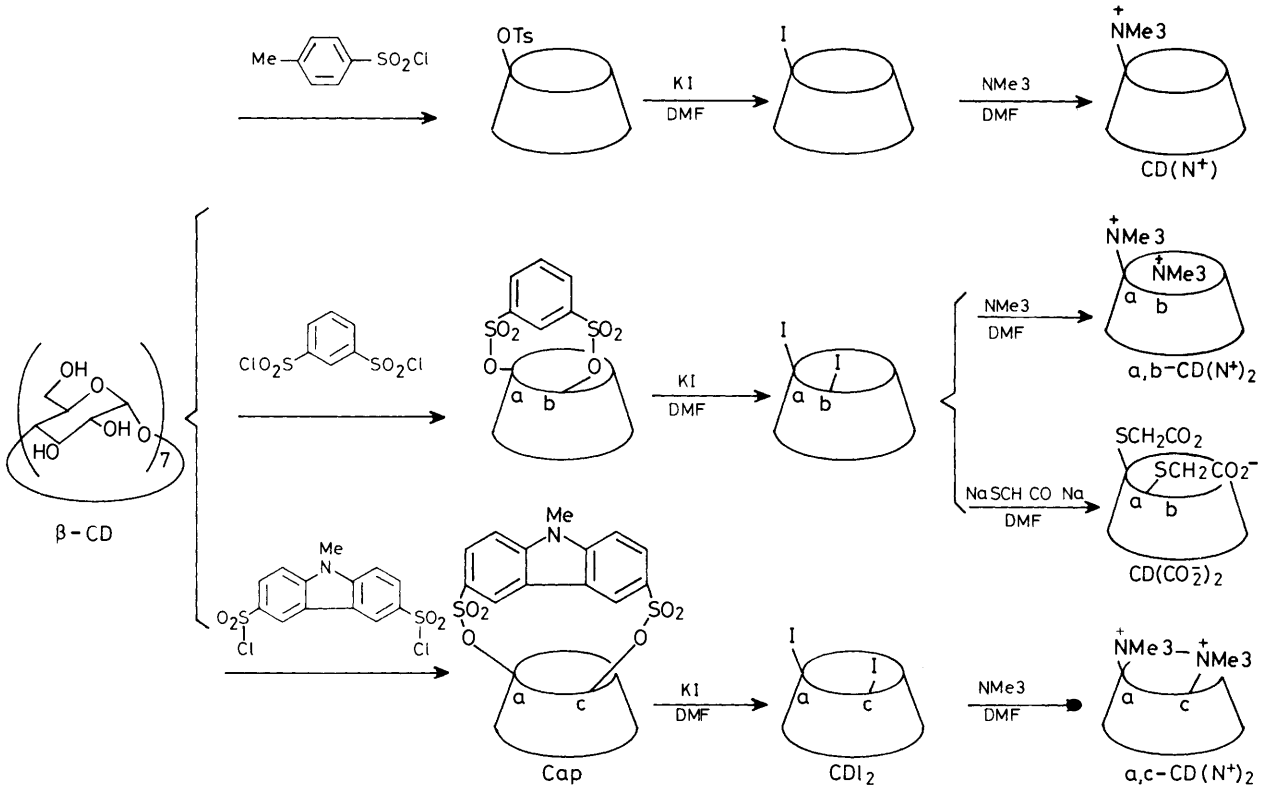
Preparation of Cyclodextrin Hosts and Guest.—The guest molecule employed was (*E*)-[β,*methyl*-$^2H_2$]-*p*-methylcinnamic acid (**G**) which was first applied by Lehn to the estimation of the molecular motion in the α-cyclodextrin inclusion complex. Compound (**G**) was prepared by the method shown in Scheme 1(*a*) a partial modification of Lehn's original method.

The host molecules, monosubstituted and disubstituted β-cyclodextrins, were prepared *via* well established methyl toluene-*p*-sulphonate[3b,6] and capping routes,[7] respectively, as shown in Scheme 1(*b*). The cyclodextrin hosts bearing ammonium substituents, $CD(N^+)$, *a,b*-$CD(N^+)_2$,† and *a,c*-$CD(N^+)_2$, and carboxylate substituents, *a,b*-$CD(CO_2^-)_2$, were

† Lower case letters here represent substituent locants on the cyclodextrins.



Scheme 1(*a*).



Scheme 1(*b*).

successfully purified by CM-Sephadex (C-25) cation-exchange and DEAE-Sephadex (A-25) anion-exchange columns, respectively, using a linear NH_4HCO_3 gradient. All new host molecules synthesized in this work were characterized by ^1H n.m.r. spectroscopy and by elemental analysis.

Determination of Association Constants.—The association constants for (**G**)–host-molecule complexes were determined by the competitive binding of (**G**) with methyl orange as described previously.[8] The data thus obtained spectroscopically supported the 1:1 ratio of host–guest coupling, and was further confirmed by the conductimetric measurements reported by Laufer *et al.*, since they reported the significant 1:2 (**G**):host ratio of complex formation between (*E*)-[α,*methyl*-$^2H_2$]-*p*-methylcinnamic acid and α-cyclodextrin.[9]

The data for the conductimetric measurement for (**G**) and β-cyclodextrin shown in Figure 2, however, did not indicate the existence of the 1:2 complexes, *i.e.*, the data were analysed by using both of the standard 1:1 (model 1) and 1:2 (model 2) complex-formation models and both models gave an almost exact fit for the data as shown in Figure 2 with the corresponding association constants, $K_1 = 470 \pm 40$ dm^3 mol^{-1} for model 1 and $K_1 = 690 \pm 300$ dm^3 mol^{-1}, $K_2 = 10 \pm 10$ dm^3 mol^{-1} for model 2, respectively.

$$\left.\begin{array}{l}(\mathbf{G}) + \text{host} \overset{K_1}{\rightleftharpoons} (\mathbf{G})\cdot\text{host} \text{ - - - - model 1} \\ (\mathbf{G})\cdot\text{host} + \text{host} \overset{K_2}{\rightleftharpoons} (\mathbf{G})\cdot(\text{host})_2\end{array}\right\} \text{model 2}$$

Obviously, the standard deviations for the association constants calculated by the non-linear lest-squares method are small enough for model 1 but are unusually large for model 2.

This result clearly indicates that model 1 is adequate for the analysis of the present complex formation and the value of K_2 is safely estimated to be <20 dm^3 mol^{-1} if it exists at all. Therefore, only the 1:1 complex-formation model was considered in the analysis of the present β-cyclodextrin host series.

The typical example of competitive binding based on the measurement of methyl orange absorption intensities is shown in Figure 3 where the spectra are recorded as the difference spectra. In all cases, the difference spectra showed clear isospecific points (Figure 3). Thus, the stoicheiometry of the complex formation was confirmed again to be 1:1 under the present conditions. The association constants obtained are summarized in Table 1 together with those for the host–methyl orange complexes.

Table 1. Association constants between cyclodextrin hosts and guests.[a]

Host	Association constant K_{ass}/dm^3 mol^{-1}	
	p-Methylcinnamate	Methyl orange
$CD(CO_2^-)_2$	280 ± 30	1 700 ± 150
CD	440 ± 50	2 300 ± 300
$CD(N^+)$	720 ± 60	3 500 ± 250
a,c-$CD(N^+)_2$	790 ± 80	2 600 ± 300
a,b-$CD(N^+)_2$	800 ± 100	2 700 ± 300
Cap	3 200 ± 500	22 000 ± 3 000
CDI_2	7 400 ± 700	2 700 ± 400

[a] At 35 ± 0.1 °C, pH 7.0 ± 0.1 in 0.1 mol dm^{-3} phosphate buffer.

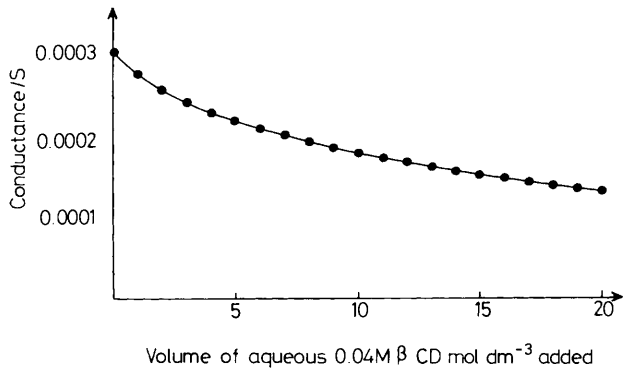


Figure 2. Conductimetric titration curve of 0.05 mol dm^{-3} lithium *p*-methylcinnamate with aqueous 0.04 mol dm^{-3} β-cyclodextrin at 35 °C: observed (· · · ·) and simulated (– – – –) calculated by the method of Laufer using 22.4 of $\lambda^{\circ}_{complex}$ and 34.3 cm^2 S equiv.$^{-1}$ of λ°_{free} (λ°; equivalent ionic coductance, for details, see ref. 9) for model 1. The calculation for model 2 shown in the text gave almost the same simulated curve.

As was expected, the association constants of this β-cyclodextrin series with (**G**) are largely dependent on the additional recognition elements. Thus, the association constants for $CD(N^+)$·(**G**) and $CD(N^+)_2$·(**G**) are 1.6 and 1.8 times larger than that for CD·(**G**) due to the attractive coulombic interaction between ammonium and carboxylate ions in these complexes, although the positional isomers of $CD(N^+)_2$, *a,b*- and *a,c*-isomers, gave the same values of K_{ass} within experimental error. Interestingly, the negatively charged recognition element also follows the additivity rule as shown in the case of $CD(CO_2^-)_2$·(**G**), where the repulsive coulombic interaction results in K_{ass} being 1.6 times smaller than that for CD·(**G**). In contrast with the relatively small alterations of K_{ass} induced by the coulombic interaction, the addition or expansion of the hydrophobic interaction region results in the much larger enhancement of K_{ass}, *i.e.*, the association constants of Cap·(**G**)* and CDI_2·(**G**) are 7.3 and 17 times larger than that for CD·(**G**). In spite of the apparent smaller hydrophobic area of CDI_2 than that for Cap, the former host showed a much larger association constant than the latter. This large enhancement of K_{ass} for CDI_2 may be attributed to a strong London dispersion force due to the large polarizability of the iodine atom.[10]

Dynamic Molecular Motions of (**G**) *Included into Modified* β-*Cyclodextrin.*—The molecular motions of (**G**) included into the cyclodextrin cavity were measured by the deuterium quadrupolar-relaxation method.[11] The method of analysis employed in this work was the line-shape analysis originally suggested by Pople[12] and developed later by Lehn.[13]

The most striking advantage of the deuterium relaxation method is that the deuterium relaxation may be directly related to the molecular motions and independent of other relaxation mechanisms such as dipole–dipole and spin–rotational interactions because the relaxation of the deuterium nucleus is overwhelmingly dominated by the single quadrupolar mechanism.[11,14] As a consequence, relationship between the measured quadrupolar-relaxation time (t_q) and the correlation time (τ_q) is straightforward when a pseudoisotropic motion is assumed [see equation (1).[14] Furthermore, the line-shape

$$t_q^1 = 0.43 \times 1012\tau_q \quad (1)$$

analysis makes it possible to measure the relaxation time with a

* Cap·(**G**) represents a capped cyclodextrin–guest complex.

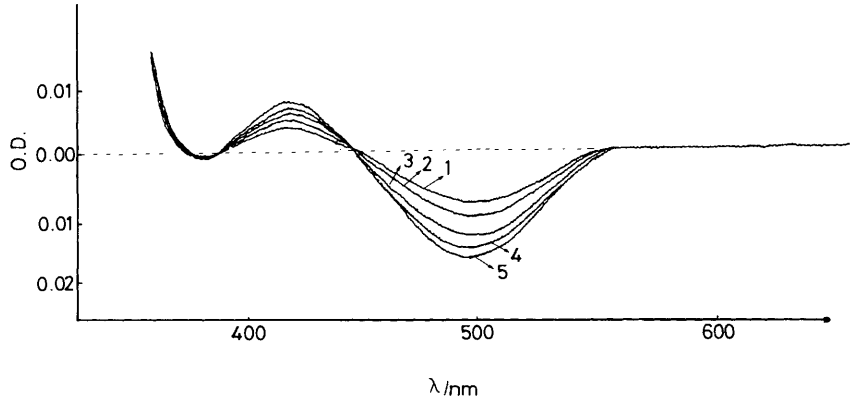


Figure 3. Difference electronic spectra of methyl orange in the presence of Cap. At 35 °C, pH 7.0 in the 0.1 mol dm^{-3} phosphate buffer. [Methyl orange] = 2.1 × 10^{-6} mol dm^{-3}, [Cap] = 1.53 × 10^{-4} mol dm^{-3} and [G] × 10^3: 1, 0.952; 2, 2.17; 3, 1.69; 4, 2.26; and 5, 3.80 mol dm^{-3}.

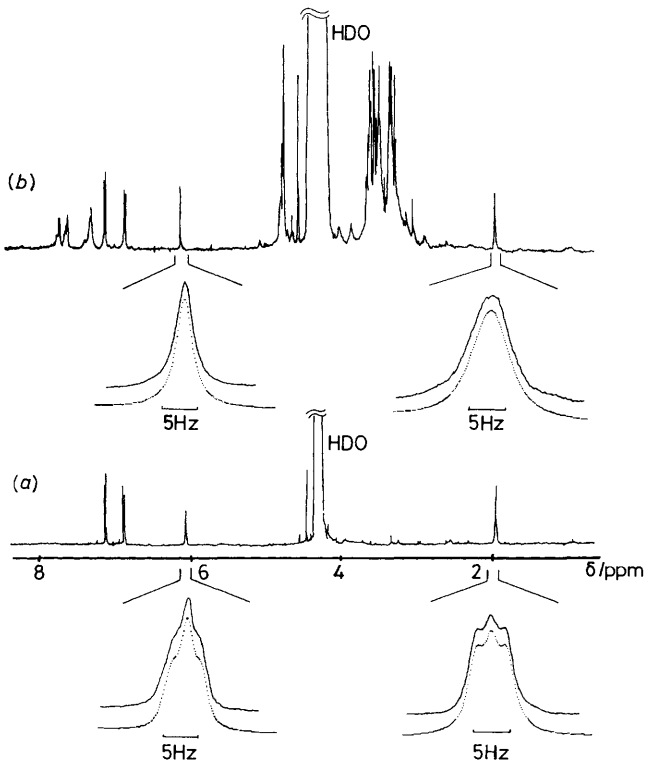


Figure 4. ^{1}H N.m.r. spectra of (**G**). At 35 °C, pD 7.0 in D_2O, [**G**] = 3×10^{-3} mol dm^{-3}, (*a*) free, (*b*) [Cap] = 5×10^{-3} mol dm^{-3}. In each expanded spectrum; observed (——), simulated (– – – –) line shape obtained from equation (5) using the following T_q and T_2 values: (*a*) alkenic, 0.110, 0.329; methyl, 0.910, 0.127; (*b*) alkenic, 0.010, 0.144; methyl, 0.087, 0.079 s, respectively.

Table 2. Values for τ_q^M, τ_q^{Me}, and τ_q^i for deuterium labelled *p*-methylcinnamic acid.[a]

Host	τ_q^M/ps	τ_q^{Me}/ps	τ_q^i/ps
b	20 ± 1	2.6 ± 0.3	<1[c]
$CD(CO_2^-)_2$	51 ± 4	*d*	*d*
CD	73 ± 2	21 ± 5	19 ± 5
$CD(N^+)$	106 ± 10	48 ± 4	53 ± 10
a,c-$CD(N^+)_2$	150 ± 8	17 ± 5	<1[c]
a,b-$CD(N^+)_2$	160 ± 5	22 ± 2	5.1 ± 0.2
Cap	270 ± 13	30 ± 2	<1[c]
CDI_2	122 ± 22	14 ± 2	<1[c]

[a] At 35 ± 1 °C, pD 7.0 ± 0.1 in D_2O. [b] Without the host molecule. [c] The values are smaller than the limit of experimental error. [d] Not estimated since the methyl signal was too broad.

relatively low concentration of the target molecule since only a proton and not a deuterium nucleus, couples with the adjacent deuterium which is required to be measured.*

The line-shape analysis of the two ^{1}H n.m.r. signals for the methyl (*ca.* δ 1.8 ppm) and alkenic (*ca.* δ 6.0 ppm) protons in (**G**) which couple with corresponding deuterium atoms gave their deuterium quadrupolar-relaxation times, t_q^{alkene} and t_q^{Me} (for details, see the Experimental). The typical results of the analysis are shown in Figure 4 where the original spectra and the simulated line shapes calculated from the corresponding best fit values of t_q are represented. Usually, each simulated line shape fits the observed one within the error due to experimental noise; the standard deviation for t_q obtained by the non-linear least-squares calculation was less than 10% of t_q.

Since it is well established that, at the reaction temperature used, the rotational barrier about the bond between the double bond and the benzene ring is sufficiently high (*ca.* 10 kcal mol^{-1});[15] τ_q^{alkene} obtained from t_q^{alkene} by using equation (1) is equal to the overall reorientational correlation time (τ_q^M) of (**G**).[14] Alternatively, τ_q^{Me} similarly obtained from t_q^{Me} contains the terms derived from both τ_q^M and τ_q^i which is the correlation time for the internal rotation of the methyl group. Since the relationship between τ_q^M, τ_q^i, and τ_q^{Me} is given by equation (2) as

$$\tau_q^{Me} = 0.11\tau_q^M + 0.89(1/\tau_q^M + 1/\tau_q^i)^{-1} \quad (2)$$

a good approximation in the present case,[14,16] τ_q^i can be estimated using observed τ_q^{Me} and $\tau_q^M (= \tau_q^{alkene})$. The results are summarized in Table 2.

The overall correlation times, τ_q^M, of (**G**) included into Cap, *a,b*-$CD(N^+)_2$, *a,c*-$CD(N^+)_2$, CDI_2, $CD(N^+)$, CD, and $CD(CO_2^-)_2$ are *ca.* 14, 8, 7.5, 6.1, 5.3, 3.7, and 2.6 times larger, respectively, than those in the free state. Comparison of these τ_q^M values with the corresponding association constants shown in Table 1 clearly indicated a general trend that the host having the larger binding capacity restricts the overall reorientational motion of (**G**) more significantly, except for the case of CDI_2. Since the overall correlation times of β-cyclodextrin host molecules usually lie in the range 400–700 ps,[4] the dynamic coupling coefficient for the benzene ring of (**G**) and Cap may be estimated to be larger than *ca.* 0.4 which indicates the existence of the appreciable coupling between the molecular motion of (**G**) and Cap.† In contrast, the complexations of (**G**) with the hosts having much smaller association constants such as CD and $CD(CO_2^-)_2$ do not result in an appreciable restriction of the molecular motion of (**G**) as estimated from their small dynamic coupling coefficient (*ca.* 0.1).

In spite of the clear relationship between τ_q^M and K_{ass}, there seem to be no correlations between τ_q^i and K_{ass}, *i.e.*, the complexations of (**G**) with CD and $CD(N^+)$ result in significant restriction of the methyl rotation but those with Cap and $CD(N^+)_2$ do not.[17] One of the possible explanations for the rapid methyl rotation in Cap and $CD(N^+)_2$ is the steric-compression effect operating in these complexes which does not allow the existence of the special stable rotameric conformer.[18]

The Energetic Relationship between Complexations and Molecular Motions of (**G**).—The relationship between τ_q^M and K_{ass} discussed above becomes clearer when the energetic terms for the corresponding processes are considered. The free-energy change associated with the complexation process (ΔG°_{ass}) and the activation energy for the molecular motion ($\Delta G^\ddagger_M$) are given by equations (3) and (4), respectively, where *k* is Boltzmann's

$$\Delta G^\circ_{ass} = -RT \ln (K_{ass}) \quad (3)$$

$$\Delta G^\ddagger_M = RT \ln \left(\frac{kT}{h} \times \tau_q^M \right) \quad (4)$$

constant and *h* is Planck's constant. The plot of ΔG°_{ass} *vs.* $\Delta G^\ddagger_M$ thus obtained from these equations gives a straight line (slope = 1.30 and correlation coefficient = 0.95) with one deviating point for CDI_2 (see Figure 5).

The relationship observed indicated that the energy gain

* The n.m.r. sensitivity of deuterium is only 0.009 65 relative to hydrogen due to its low magnetogyric ratio.

† The dynamic coupling coefficient in the present case is defined as τ_q^M(G in host)/τ_q^M(host) and varies from 1 (complete coupling) to 0.11 (complete decoupling) in the simplest case (ref. 14).

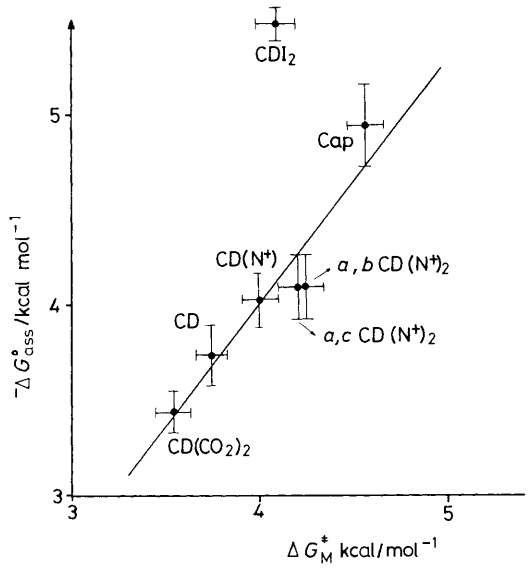


Figure 5. Plot of ΔG°_{ass} *vs.* ΔG_{M}. The values of ΔG°_{ass} and ΔG_{M} were calculated from K_{ass} and τ_{q}^{M} by using equation (3) and equation (4), respectively. Error ranges are given by accompaning bar lines.

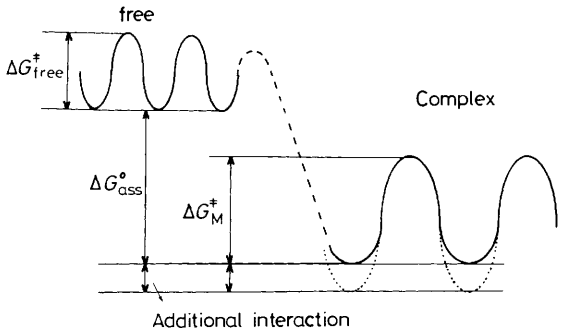


Figure 6. Hypothetical energy diagram for free and complexed (**G**).

resulting from the complexation is almost negated in the activated state of the molecular motion process of (**G**) included in the host cavity. Although it is difficult to draw a general scheme for the present observation because of the lack of information on the geometric configurations of (**G**) in host cavities,* the most probable scheme may be represented as in Figure 6 which shows the energy diagram for the present host–guest complexation and the molecular motions, *i.e.*, if one can reasonably assume the common energy level for activated states of molecular motions of (**G**) where the interactions between (**G**) and hosts are almost lost, the present linear relationship between ΔG°_{ass} and $\Delta G^{\ddagger}_{M}$ the slope of which is nearly equal to unity, is explainable as the natural result of this.

The large deviation of the CDI_2 point from the present linear free-energy relationship may be due to the large polarizability of the electron cloud surrounding the iodine atom, which has been shown to reduce the activation energy of the internal rotation around the C–CI bond.[19]

The relationship between ΔG°_{ass} and $\Delta G^{\ddagger}_{M}$ may not necessarily hold for different series of host–guest complexes. For example, τ_{q}^{M} of (**G**) in α-cyclodextrin reported by Lehr[4] is larger than that in β-cyclodextrin but the former association constant is only one third of the latter, which results in a serious deviation of α-cyclodextrin from the present relationship. Another example is the tryptophan–β-cyclodextrin couples.[20] Since the indole ring of tryptophan fits the cavity of β-cyclodextrin more tightly than the simple benzene ring, the molecular motion is almost completely restricted even in the unmodified β-cyclodextrin cavity and the additional recognition elements such as ammonium and carboxylate ions on β-cyclodextrin do not in fact affect the molecular motion of tryptophan, although the association constant is enhanced by addition of these recognition elements.[2e]

Despite these limitations, the relationship is expected to provide fundamental data for the design of more sophisticated host molecules which not only recognize the size and shape of the guest but also control its molecular motion.

Experimental

Apparatus and Materials.—Measurements of proton nuclear magnetic resonance spectra were performed on a JEOL JMN-GX 400 spectrometer. Infrared spectra were measured with a Hitachi model 215 infrared spectrometer. U.v.–visible spectra were recorded on a Union Giken high sensitivity spectrometer SM 401. Thin-layer chromatography was carried out on the 0.25 mm Merck precoated silica-gel plate (60F-254). The spot detection for the cyclodextrin derivatives was carried out by staining with 0.45% anisaldehyde in MeOH–AcOH–H_2SO_4 (860/90/45 v/v).[21] *N,N*-Dimethylformamide (DMF) was dried over P_2O_5 for several days at room temperature, decanted, shaken with KOH pellets and then distilled under reduced pressure. Sodium ethoxide was freshly prepared by dissolving sodium in ethanol and evaporating the solution to dryness. Capped cyclodextrin (Cap), and *a,c*- and *a,b*-di-iodocyclodextrins were prepared by the previously reported method.[7] CM-Sephadex and DEAE-Sephadex were used as the NH_4^+ and HCO_3^- forms, respectively.

(E)-[α,methyl-$^2H_2$]-p-*methylcinnamic acid* (**G**).—This compound was prepared according to Scheme 1(*a*) where each step for the corresponding non-deuteriated compound was well established. Each procedure was performed according to the literature.† The deuterium content in the final product was confirmed to be >99% for the methyl and alkenic groups by ^1H n.m.r. spectroscopy.

6-*Trimethylammonio*-6-*deoxy*-β-*cyclodextrin Chloride* [*CD*(N^+)].—To a stirred liquid trimethylamine (20 cm^3) was added 6-deoxy-6-iodo-β-cyclodextrin[6] (3.0 g, 2.4 mmol) in dry DMF (10 cm^3) at −75 °C. The resultant mixture was autoclaved at 80 °C overnight. After the removal of the solvent by evaporation, the product was chromatographed on CM-Sephadex C-25 eluting with a linear gradient of 0–1.0 mol dm^{-3} ammonium hydrogen carbonate. The appropriate fractions were combined and the ammonium hydrogen carbonate was decomposed by repeated evaporation under reduced pressure. The residue was dissolved in water (20 cm^3), acidified to pH 3.0 with concentrated hydrochloric acid and evaporated to afford

* Since the process indicated by τ_{q}^{M} is the superimposed motion of the overall molecular motion for the whole complex, host (**G**), and the internal motion of (**G**) in the host cavity, the estimated values of $\Delta G^{\ddagger}_{M}$ may be the lower limit for the activation energy of the latter process in the majority of cases.

† [*Methyl*-^{2}H]-*p*-methylacetophenone: C. R. Noller and R. Adams, *J. Am. Chem. Soc.*, 1924, **46**, 1889; [*methyl*-^{2}H]-*p*-benzoic acid: A. M. van Arendoc and A. E. Cupery, *ibid.*, 1931, **53**, 3184; [α,α,*methyl*-$^2H_3$]-*p*-methylbenzylalcohol: R. F. Nystrom and W. G. Brown, *ibid.*, 1947, **69**, 2548 ($LiAlD_4$ was used instead of $LiAlH_4$·); [α,*methyl*-$^2H_2$]-*p*-methylbenzaldehyde: J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Lett.*, 1968, 3363; (*E*)-[β-*methyl*-$^2H_2$]-*p*-methylcinnamic acid: J. A. Elvidge, R. J. Jones, R. B. Mane, and M. Suljoighian, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1191, G. Jones, *Org. React.*, 1967, **15**, 204.

the final product (yield 1.1 g, 38%). δ(D_2O) 4.95 (m, 7 H, 1-H), 3.2–3.9 (m, 42 H, other H), and 3.03 (s, 9 H, NCH_3); $\nu_{max.}$(KBr) 3 600–3 000, 2 920, 1 630, 1 360, 1 150, and 1 010 cm^{-1} (Found: C, 41.0; H, 7.05; and N, 1.15. Calc. for $C_{45}H_{78}O_{34}NCl{\cdot}6H_2O$: C, 40.93; H, 6.92; N, 1.06%).

6a,6b-*Dideoxy*-6a,6b-*bis*-(*trimethylammonio*)-β-*cyclodextrin Dichloride* [a,b-*CD*(N^+)$_2$].—This compound was prepared by the same method for CD(N^+) by using 6*a*,6*b*-dideoxy-6*a*,6*b*-di-iodo-β-cyclodextrin as the starting material (yield 41%): δ(D_2O) 5.26 (d, 1 H, C1-H), 4.93 (m, 6 H, 1-H), 4.0–3.2 (m, 42 H, other H), 3.14 (s, 9 H, NCH_3), and 3.04 (s, 9 H, NCH_3); $\nu_{max.}$(KBr) 3 600–3 000, 2 900, 1 640, 1 400, 1 150, and 1 000 cm^{-1} (Found: C, 41.0; H, 6.7; N, 1.55. Calc. for $C_{48}H_{86}N_2O_{33}Cl_2{\cdot}6H_2O$: C, 41.23; H, 7.12; N, 2.00%).

6a,6c-*Dideoxy*-6a,6c-*bis*(*trimethylammonio*)-β-*cyclodextrin Dichloride* [a,c-*CD*(N^+)$_2$].—This compound was also prepared by the same method for CD(N^+) by using 6*a*,6*c*-dideoxy-6*a*,6*c*-di-iodo-β-cyclodextrin as the starting material (yield 40%): δ(D_2O) 4.95 (m, 7 H, 1-H), 4.0–3.2 (m, 42 H, other H), 3.05 (s, 18 H, NCH_3), $\nu_{max.}$(KBr) 3 700–3 000, 2 930, 1 640, 1 400, 1 400, 1 150, 1 020, and 940 cm^{-1} (Found: C, 39.55; H, 7.25; N, 2.0. Calc. for $C_{48}H_{86}N_2O_{33}Cl_2{\cdot}10H_2O$: C, 39.21; H, 7.23; N, 1.91%).

6a,6b-*Bis*-(*carboxymethylthio*)-6a,6b-*dideoxy*-β-*cyclodextrin Diammonium Salt* [*CD*(CO_2^-)$_2$].—To a solution of 6*a*,6*b*-dideoxy-6*a*,6*b*-di-iodo-β-cyclodextrin (5 g, 3.7 mmol) in dry DMF (10 cm^3) stirred under an argon atmosphere was added thioglycolic acid (6.8 g, 74 mmol), followed by sodium ethoxide (10.1 g, 148 mmol) as a finely crushed powder. The suspension was stirred at 60 °C for 10 h. The solution was evaporated to dryness under reduced pressure and the residue was dissolved in water (5 cm^3). After acidification of the solution (pH 3.0) with concentrated hydrochloric acid, ethanol (50 cm^3) was added and the resultant white precipitate was collected by filtration. The solid material was dissolved in water (5 cm^3) and tetrachloroethylene (3 cm^3) was added at O °C with vigorous stirring. The precipitate formed was collected and washed with ethanol (10 cm^3). The white precipitate thus obtained was dissolved in water (20 cm^3), treated with concentrated NH_4OH solution and evaporated to dryness. The pale-yellow powder was dissolved in water (2 cm^3) and applied to a DEAE-Sephadex column. Elution with a linear gradient of 0–1.0 mol dm^{-3} ammonium hydrogen carbonate yielded the desired compound (yield 900 mg, 20%). δ(D_2O) 4.96 (m, 2 H, 1-H), 4.91 (m, 5 H, 1-H), 3.7–3.3 (m, 38 H, other H), 3.15–3.25 (m, 4 H, SCH_2CO_2H), 3.07 (d, *J* 14 Hz, 1 H, 6-H), 3.00 (d, *J* 14 Hz, 1 H, 6-H), 2.82 (dd, *J* 14 and 8 Hz, 1 H, 6-H), 2.70 (dd, *J* 14 and 8 Hz, 1 H, 6-H), $\nu_{max.}$(KBr) 3 600–3 000, 2 900, 1 700, 1 580, 1 270, 1 150, 1 010, and 580 cm^{-1}; R_F = 0.40 (Pr^nOH/AcOEt/H_2O/NH_4aq = 5/2.5/2.5/1) (Found: C, 39.55; H, 6.2; N, 1.8; S, 5.24. Calc. for $C_{46}H_{80}N_2O_{37}S_4{\cdot}4H_2O$: C, 39.77; H, 6.44; N, 2.02; S, 4.62%).

Measurements of Deuterium Relaxation Times.—The samples used for n.m.r. spectroscopic measurements were prepared by dissolving (**G**) as the sodium salt and the host in D_2O and the solution was adjusted to pD 7.0 ± 0.1 using 40% NaOD and 20% DCl. All the ^1H n.m.r. spectra were obtained on a JEOL JMN-GX 400 spectrometer. [2,2,3,3-$^2H_4$]-3-(Trimethylsilyl)-propionic acid sodium salt was used as external reference. The deuterium relaxation times were measured by the line-shape analysis of the ^1H resonances of methyl (*ca.* 1.8 ppm) and alkenic (*ca.* 6.0 ppm) protons coupled to corresponding deuterium atoms of (**G**). The line-shape analysis is performed by using equation (5), which had been originally derived by Lehn

$$I(\omega) = \mathrm{re}\left\{(1,1,1)\begin{pmatrix} D_{11} & 1/5T_q & 2/5T_q \\ 2/5T_q & D_{22} & 1/5T_q \\ 2/5T_q & 1/5T_q & D_{33}\end{pmatrix}^{-1}\begin{matrix}1\\1\\1\end{matrix}\right\} \quad (5)$$

$$D_{11} = i(\Delta\omega + 2\pi J) - 3/5T_q - 1/T_2$$
$$D_{22} = i\Delta\omega - 2/5T_q - 1/T_2$$
$$D_{33} = i(\Delta\omega - 2\pi J) - 3/5T_q - 1/T_2$$

for the ^{14}N quadrupolar-relaxation measurement,[13a] where $I(\omega)$ is the line shape as the function of frequences (ω), re denotes 'real part of' and $\Delta\omega$ ($=\omega_0 - \omega$), *J*, T_q, and T_2 are the shift from the centre of the multiplet, D–H coupling constant, the quadrupolar relaxation time and the relaxation time of the protons. For the calculation, the values of *J* for the methyl and alkenic D–H coupling were fixed as 2.2 and 2.45 Hz, respectively, which were theoretically obtained from the corresponding H–H coupling constants and the magnetogyric ratio of the deuterium nucleus, and T_q and T_2 were employed as the variables for the computer-aided non-linear least-squares calculation using the damping Gauss–Newton[22] and Marquardt method.[23,27,*] The computation was performed on a NEC PC-901VM personal computer.

In order to obtain the T_q value of (**G**) in the pure complex state ($T_q^{complex}$), T_q^{obs} thus determined was corrected using equation (6),[4] where α is the fraction of the complex calculated

$$1/T_q^{obs} = \alpha/T_q^{complex} + (1 - \alpha)/T_q^{free} \quad (6)$$

from K_{ass} and concentrations of (**G**) and the host. Since the concentration of the host (2×10^{-3}–1×10^{-2} mol dm^{-3}) and (**G**) (1.05×10^{-3}–3.15×10^{-3} mol dm^{-3}) were chosen so that $\alpha > 9$, the difference between $T_q^{complex}$ and T_q^{obs} usually lay in the range of 10% of T_q^{obs}. The values shown in Table 2 are those of τ_q values calculated from $T_q^{complex}$ thus obtained.

Measurements of Association Constants.—The equilibrium constants were determined by the competitive binding of (**G**) with methyl orange. The procedure was the same as that described previously.[8]

The conductimetric measurement for the determination of the association constants of β-cyclodextrin with (**G**) was performed by using a Yanagimoto Model My-8 the cell constant of which was 0.492. The method for the analysis was the same as that reported by Laufer *et al.*[9] (for data, see the text).

* The program for the personal computer was developed in this laboratory.

References

1 (*a*) M. L. Bender and M. Komiyama, 'Cyclodextrin Chemistry,' Springer Verlag, New York, 1978; (*b*) I. Tabushi, *Acc. Chem. Res.*, 1982, **15**, 66; (*c*) I. Tabushi and Y. Kuroda, *Adv. Catal.*, 1983, **32**, 417; (*d*) R. B. Breslow, *Science*, 1982, **218**, 532.

2 (*a*) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, 1962, **36**, 3401, 3382; (*b*) J. Emert and R. Breslow, *J. Am. Chem. Soc.*, 1975, **97**, 670; (*c*) B. Siegel and R. Breslow, *ibid.*, 1975, **97**, 6869; (*d*) I. Tabushi, Y. Kiyosuke, T. Sugimoto, and K. Yamamura, *ibid.*, 1978, **100**, 916; (*e*) I. Tabushi, Y. Kuroda, and T. Mizutani, *ibid.*, 1986, **108**, 4514.

3 (*a*) I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata, and K. Fujita, *J. Am. Chem. Soc.*, 1976, **98**, 7855; (*b*) I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, and K. Yamamura, *ibid.*, 1977, **98**, 1743.

4 (*a*) J. P. Behr and J. M. Lehn, *J. Am. Chem. Soc.*, 1976, **98**, 1743; (*b*) K. Flohr, R. M. Paton, and E. T. Kaiser, *ibid.*, 1975. **97**, 1209; (*c*) K. Uekama, F. Hirayama, and F. Koinuma, *Chem. Lett.*, 1977, 1393; (*d*) Y. Inoue, T. Okada, and Y. Miyashita, *Carbohydr. Res.*, 1982, **101**, 187; (*e*) M. Okazaki and K. Kuwata, *J. Phys. Chem.*, 1984, **88**, 4181.

5 The preliminary part of this work has been reported previously, see I. Tabushi, Y. Kuroda, and M. Yamada, *Tetrahedron Lett.*, 1988, **29**, 1413.
6 (*a*) L. D. Melton and K. N. Slessor, *Carbohydr. Res.*, 1971, **18**, 29; (*b*) I. Tabushi, L. C. Yuan, K. Shimokawa, K. Yokota, T. Mizutani, and Y. Kuroda, *Tetrahedron Lett.*, 1981, **22**, 2273.
7 (*a*) I. Tabushi and Y. Kuroda, *J. Am. Chem. Soc.*, 1984, **106**, 4580; (*b*) I. Tabushi, K. Yamamura, and T. Nabeshima, *ibid.*, 1984, **106**, 5267; (*c*) I. Tabushi, T. Nabeshima, K. Fujita, A. Matsunaga, and T. Imoto, *J. Org. Chem.*, 1985, **50**, 2638.
8 I. Tabushi, Y. Kuroda, and T. Mizutani, *Tetrahedron*, 1984, **40**, 545.
9 R. I. Gelb, L. M. Schwarz, and F. A. Laufer, *J. Am. Chem. Soc.*, 1978, **100**, 5875.
10 E. L. Elliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, 'Conformational Analysis,' Wiley, New York, 1965, p. 449.
11 H. H. Mantsch, H. Saito, and I. C. P. Smith, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1977, **11**, 211.
12 J. A. Pople, *Mol. Phys.*, 1958, **1**, 168.
13 (*a*) J. P. Kinzinger, J. M. Lehn, and R. L. Williams, *Mol. Phys.*, 1969, **17**, 135; (*b*) C. Brevard and J. M. Lehr, *J. Am. Chem. Soc.*, 1970, **92**, 4987.
14 C. Brevard and J. M. Lehr, *Tetrahedron*, 1972, **28**, 2429, 2447.
15 L. M. Jackman and F. A. Cotton, 'Dynamic Nuclear Magnetic Resonance Spectroscopy,' Academic Press, New York, 1975.
16 (*a*) J. N. Noggle, *J. Phys. Chem.*, 1968, **72**, 1324; (*b*) D. E. Woessner, B. S. Snowden, and G. H. Meyer, *J. Chem. Phys.*, 1969, **50**, 719.
17 In the case of the enzyme, the similar lack of the correlation between substrate dynamics and binding energy was observed, see C. R. Sanders II and M.-D. Tsai, *J. Am. Chem. Soc.*, 1988, **110**, 3323.
18 G. C. Levy, *Acc. Chem. Res.*, 1973, **6**, 161.
19 (*a*) F. R. Jensen, C. H. Bushweller, and B. H. Beck, *J. Am. Chem. Soc.*, 1969, **91**, 344; (*b*) C. H. Bushweller, S. Hoogasian, W. G. Anderson, and L. J. Letendre, *J. Chem. Soc., Chem. Commun.*, 1975, 152.
20 I. Tabushi, Y. Kuroda, M. Yamada, and T. Sera, *J. Inclusion Phenomena*, 1988, **6**, 599.
21 N. Wiedenhof, *J. Chromatogr.*, 1964, **15**, 100.
22 K. Levenberg, *Q. Appl. Math.*, 1944, **2**, 164.
23 D. W. Marquardt, *J. Soc. Ind. Appl. Math.*, 1963, **11**, 431.

Received 24*th August* 1988; *Paper* 8/03430C