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# A TWO-STEP MECHANISM FOR THE INCLUSION REACTIONS OF $\alpha$-CYCLODEXTRIN WITH MONO ALKYL-SUBSTITUTED HYDROXYPHENYLAZO DERIVATIVES OF SULFANILIC ACID

Akitoshi SEIYAMA, Noboru YOSHIDA, and Masatoshi FUJIMOTO*
Department of Chemistry II, Faculty of Science,
Hokkaido University, Sapporo 060

Kinetic studies on the inclusion reactions of the title azo compounds with $\alpha$-cyclodextrin ($\alpha$-CD$_x$) in aqueous solution by means of the stopped-flow method reveal a two-step inclusion mechanism involving a fast binding process of the guest compounds to $\alpha$-CD$_x$ followed by the slow intramolecular transformation of the intermediate inclusion complex. Activation parameters for each step were determined.

$\alpha$-Cyclodextrin forms inclusion complexes with a variety of guest compounds in aqueous solution usually in 1 : 1 stoichiometry.[1] However, the static parameters such as the strength and the selectivity of the host-guest interaction in the inclusion complexes, as expressed by the values of the binding constants and their ratios respectively, are not sufficient to clarify the mechanism of the inclusion phenomena and the discriminating ability of the $\alpha$-CD$_x$ for the guest molecules. The dynamic feature for the inclusion phenomena is required.

In the present paper, we propose a two-step mechanism for the inclusion reactions of the title azo compounds with $\alpha$-CD$_x$ on the basis of the stopped-flow measurement.

The dissociation constants, $K_d$, of 1 : 1 inclusion complexes of $\alpha$-CD$_x$ with a series of guest azo compounds, p-(3-alkyl-4-hydroxyphenylazo)benzenesulfonate ion (abbr. 3-R-HAB; Fig. 1), formed in aqueous solution[4] were spectrophotometrically determined to be in the order of magnitude $10^{-4}$ mol dm$^{-3}$ (Table 1). The values of $K_d$ are almost in the same order of magnitude and do not depend on the substituent ($R_3$) in the guest molecule and the pH values of the solution. This indicates that the guest molecule interacts with $\alpha$-CD$_x$ at the same binding site in these inclusion complexes.[3]

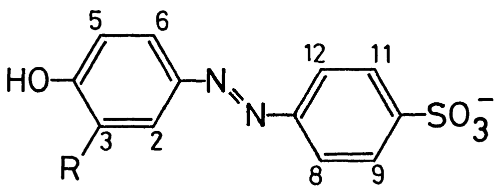

Fig. 1. Structural formula of the guest molecule, 3-R-HAB(HA$^-$).

**1**. $R_3 = -CH_2CH_2CH_3$
**2**. $R_3 = -CH(CH_3)_2$
**3**. $R_3 = -CH(CH_3)CH_2CH_3$

Figures 2(a) and (b) show the changes in absorbance at 390 nm during the course of the inclusion reaction of HA$^-$ species of the guest compound **1** with $\alpha$-CD$_x$. These changes clearly indicate that two steps of relaxation, a fast (a) and a

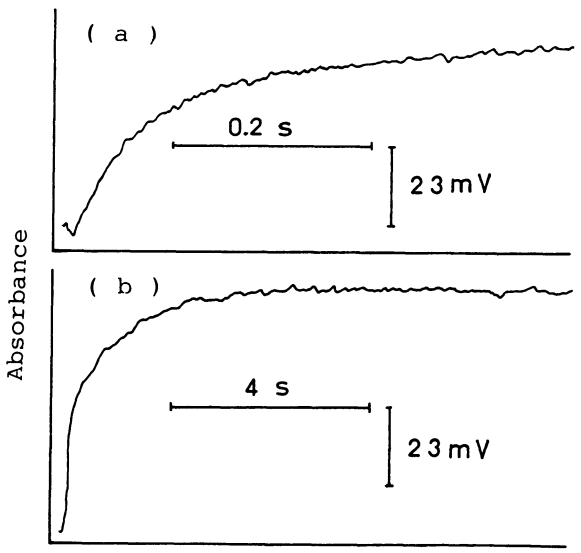

Fig. 2. Typical stopped-flow signals for the change in absorbance of the fast (a) and the slow (b) processes at 390 nm. [3-Pr-HAB] = $3.2 \times 10^{-5}$ and [$\alpha$-CD$_x$] = $3.0 \times 10^{-4}$ mol dm$^{-3}$. pH = 4.7.

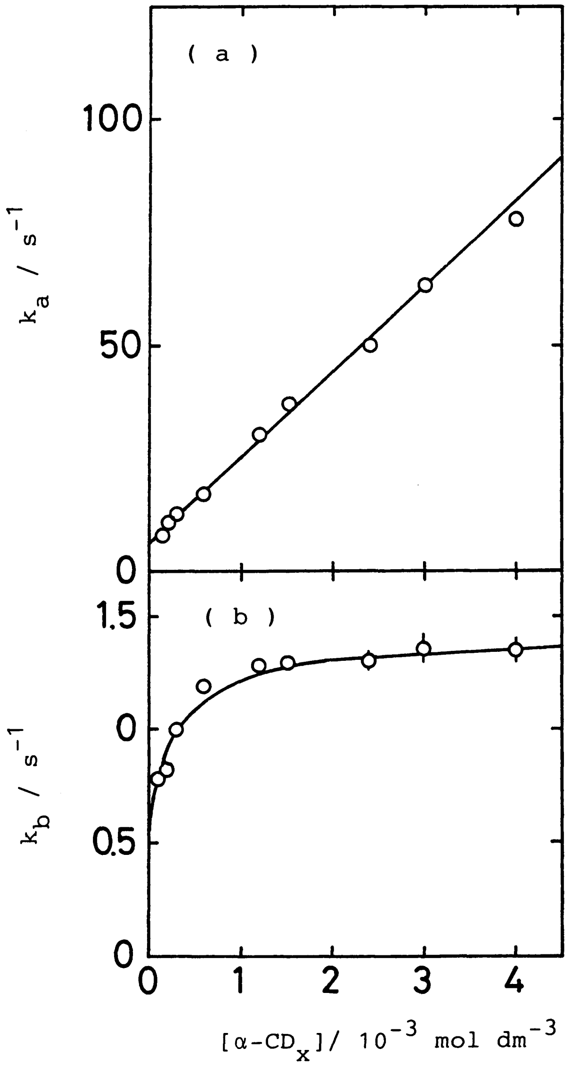

Fig. 3. Plots of the observed rate constants, $k_a$ and $k_b$, vs. the concentration of $\alpha$-CD$_x$ at pH = 4.7 (phosphate buffer) and I = 0.1 mol dm$^{-3}$ (NaCl). [3-Pr-HAB] = $3.2 \times 10^{-5}$ mol dm$^{-3}$.

slow relaxation process (b), are involved. Kinetic data were obtained under pseudo-first-order conditions in the presence of a large excess of $\alpha$-CD$_x$. The rate constants, $k_a$ and $k_b$, for the fast and the slow relaxation processes are determined from the respective plots of log $\Delta$Abs. vs. time. The plot of $k_a$ vs. [$\alpha$-CD$_x$] gave a straight line and that of $k_b$ a saturation curve (See Fig. 3). These results suggest that this inclusion reaction is a consecutive reaction with an intermediate as shown in Scheme 1

$$\mathrm{G} + \alpha\text{-CD}_x \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{G}\text{-}\alpha\mathrm{CD}^{*}_{x} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} \mathrm{G}\text{-}\alpha\mathrm{CD}_{x},$$

Scheme 1.

where G, G-$\alpha$CD$^{*}_{x}$, and G-$\alpha$CD$_x$ denote the guest compound (HA$^-$ or A$^{2-}$ species), an intermediate, and the final product, respectively. Provided that the first step proceeds rapidly compared with the second step, the dependence of $k_a$ and $k_b$ on [$\alpha$-CD$_x$] under the experimental conditions5) can be expressed by the following equations,6)

Table 1. The Dissociation and the Rate Constants for the Inclusion Reactions of **1**, **2**, and **3** with $\alpha$-$CD_x$ at 25 °C

| Guest Molecule | | pH | $K_d$ a) $\text{mol dm}^{-3}$ | $k_{+1}$ $\text{mol}^{-1}\text{dm}^{3}\ \text{s}^{-1}$ | $k_{-1}$ $\text{s}^{-1}$ | $K_{-1}$ $\text{mol dm}^{-3}$ | $k_{+2}$ $\text{s}^{-1}$ | $k_{-2}$ $\text{s}^{-1}$ | $K_{+2}$ |
|---|---|---|---|---|---|---|---|---|---|
| **1** | $HA^-$ | 4.7 | $1.2 \times 10^{-4}$ | $2.0 \times 10^{4}$ | 6.0 | $3.0 \times 10^{-4}$ | 0.87 | 0.55 | 1.58 |
| | $A^{2-}$ | 11.4 | $1.4 \times 10^{-4}$ | $6.9 \times 10^{3}$<br>(±2.0) | 3.6<br>(+1.0) | $5.0 \times 10^{-4}$<br>(±2.0) | 0.25<br>(±0.02) | 0.08<br>(±0.02) | 4.2<br>(±0.8) |
| **2** | $HA^-$ | 4.7 | $2.2 \times 10^{-4}$ | $1.2 \times 10^{4}$ | 9.4 | $7.8 \times 10^{-4}$ | 0.58 | 0.26 | 2.2 |
| | $A^{2-}$ | 11.4 | $2.0 \times 10^{-4}$<br>(±0.4) | $9.0 \times 10^{3}$<br>(±2.0) | 15.0 | $2.0 \times 10^{-3}$<br>(±0.5) | 1.47<br>(±0.20) | 0.10<br>(±0.02) | 14.0 |
| **3** | $HA^-$ | 4.7 | $1.8 \times 10^{-4}$ | $1.1 \times 10^{4}$ | 14.0 | $1.3 \times 10^{-3}$ | 0.8 | 0.16 | 5.0 |
| | $A^{2-}$ | 11.5 | $1.7 \times 10^{-4}$<br>(±0.2) | $7.0 \times 10^{3}$ | 20.0 | $3.0 \times 10^{-3}$ | 1.67 | 0.08 | 21.0 |

a) Determined from the equilibrium measurements at $I = 0.1\ \text{mol dm}^{-3}$ (NaCl).

$$k_a = k_{+1}[\alpha\text{-CD}_x] + k_{-1}$$

$$k_b = k_{-2} + k_{+2}[\alpha\text{-CD}_x]/(K_{-1} + [\alpha\text{-CD}_x]),$$

where $K_{-1} = k_{-1}/k_{+1}$. Plots of $1/(k_b - k_{-2})$ vs. $1/[\alpha\text{-CD}_x]$ gave a straight line with a slope $K_{-1}/k_{+2}$ and an intercept $1/k_{+2}$. The value of $K_{-1}$ thus obtained from the slope and the intercept is in fair agreement with the values of $K_{-1}$ determined independently from the plot in Fig. 3(a). The dissociation constant ($K_d$) is given by the following equation

$$K_d = K_{-1}/(1 + K_{+2})$$

where $K_{+2} = k_{+2}/k_{-2}$. The values of $K_d$ thus obtained agree with those of $K_d$ determined by the equilibrium measurement (Table 1). In the systems investigated here, all values of $k_{+1}$ determined for $A^{2-}$ are only slightly smaller than those of $k_{+1}$ for $HA^-$. These kinetic results support the preferential inclusion at the sulfanilate moiety of the guest molecules into $\alpha$-$CD_x$ cavity in the fast process to form an intermediate.3) In a subsequent slower change, the intermediate inclusion complex is converted into the final stable inclusion complex.

Thermodynamic parameters, $\Delta H°$ and $\Delta S°$, and activation parameters, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the inclusion reaction of the guest molecule **1** were determined from the temperature-dependency of $K_d$ and the rate constants, respectively (Table 2). The values of the standard enthalpy and the entropy of the total reaction obtained from the kinetic data are in fair agreement with those determined from the equilibrium data. The value of the enthalpy change in the fast process, $\Delta H^{\ddagger}_{+1} - \Delta H^{\ddagger}_{-1}$, is larger than that in the slow process, $\Delta H^{\ddagger}_{+2} - \Delta H^{\ddagger}_{-2}$, and the entropy in the fast process, $\Delta S^{\ddagger}_{+1} - \Delta S^{\ddagger}_{-1}$, is larger than that in the slow process, $\Delta S^{\ddagger}_{+2} - \Delta S^{\ddagger}_{-2}$. These results

Table 2. The Activation Parameters for the Inclusion Reactions of **1**, 3-Pr-HAB, with α-CD$_x$ at 25 °C

| Species | pH | $\Delta H^\circ$ a) | $\Delta S^\circ$ a) | $\Delta H^{\ddagger}_{+1}$ | $\Delta S^{\ddagger}_{+1}$ | $\Delta H^{\ddagger}_{-1}$ | $\Delta S^{\ddagger}_{-1}$ | $\Delta H^{\ddagger}_{+2}$ | $\Delta S^{\ddagger}_{+2}$ | $\Delta H^{\ddagger}_{-2}$ | $\Delta S^{\ddagger}_{-2}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $HA^-$ | 4.7 | -33.3 | -36.0 | 22.5 | -87.0 | 49.6 | -65.3 | 54.5<br>(± 3.0) | -63.4<br>(±11.6) | 63.3<br>(± 2.6) | -37.3<br>(± 9.0) |
| $A^{2-}$ | 11.4 | -31.8 | -32.0 | 29.8 | -68.7 | 56.0 | -48.4 | 65.0<br>(± 1.0) | -38.5<br>(± 3.2) | 76.0<br>(± 1.0) | -12.8<br>(± 3.0) |

a) $\Delta H^\circ$ and $\Delta S^\circ$ were determined from equilibrium measurement. The signs of $\Delta H^\circ$, $\Delta S^\circ$ refer to the formation reaction of the inclusion complex. $\Delta H^\circ$, $\Delta H^{\ddagger}_{+1}$, $\Delta H^{\ddagger}_{-1}$, $\Delta H^{\ddagger}_{+2}$, $\Delta H^{\ddagger}_{-2}$ in kJ mol$^{-1}$. $\Delta S^\circ$, $\Delta S^{\ddagger}_{+1}$, $\Delta S^{\ddagger}_{-1}$, $\Delta S^{\ddagger}_{+2}$, $\Delta S^{\ddagger}_{-2}$ in J K$^{-1}$ mol$^{-1}$.

suggest the formation of a reasonably strong intermediate complex in the first step of the inclusion reaction.

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3) N. Yoshida, A. Seiyama, and M. Fujimoto, Chem. Lett., 1984, 703.

4) The formation of 1 : 1 inclusion complexes was confirmed by the presence of isosbestic points in the spectral change upon varying α-CD$_x$ concentrations and by the Hildebrand-Benesi plot.

5) The first decay in absorbance corresponds to a decrease of G, and the second one a decrease of G and G-αCD$_x^*$.

6) M. Fujimoto, "Kanwa-gensho no Kagaku (The Chemistry of the Relaxation Phenomena)," ed by S. Nagakura and K. Higashi, Iwanami, Tokyo (1973), Chap. 6, p. 313.

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