# Inhibitory Effect of Guest Molecules on Acid-catalysed Ring-opening of β-Cyclodextrin

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The ring-opening reaction of β-cyclodextrin was markedly decelerated by inclusion of guest molecules, as shown by $^1$H NMR and kinetics studies.

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of more than six glucose units linked through α-1,4 glycosidic bonds, and form inclusion complexes with various guest molecules with appropriate size and shape.[1] The chemical reactivity, such as hydrolysis,[2] oxidation and reduction,[3] isomerization[4] and dimerization[5] of guest molecules is markedly changed by the formation of inclusion complexes with CDs; thus CDs have been utilized as models for various enzymes.[6] However, little is known about the changes in reactivity of CDs when guest molecules are included in the

**Table 1** Ring-opening rate constants ($k$/h$^{-1}$)[a] of β-CD ($1.0 \times 10^{-3}$ mol dm$^{-3}$) in the absence and presence of guest molecules ($5.0 \times 10^{-3}$ mol dm$^{-3}$) and association constants ($K$/dm$^3$ mol$^{-1}$)[b] of the complexes in 1.0 mol dm$^{-3}$ HCl

| CD + guest[e] | $k$ ($k_c$, $k_0/k_c$)[c] | $K$ |
|---|---|---|
| β-CD alone | 0.0612 | |
| β-CD + **1** | 0.0562 (0.0354, 1.7) | 50 |
| β-CD + **2** | 0.0382 (0.0184, 3.3) | 260 |
| β-CD + **3** | 0.0357 (0.0120, 5.1) | 240 |
| β-CD + **4** | 0.0258 (0.00644, 9.5) | 420 |
| β-CD + **5** | 0.00623 (0.00266, 23) | 3790 |
| β-CD + **6**[d] | 0.0231 (0.00273, 22) | 5370 |
| Maltoheptaose alone | 0.250 | |
| Maltoheptaose + **2** | 0.248 | |
| Maltoheptaose + **5** | 0.243 | |

[a] Accuracy: <±1%, temperature: 60 °C. [b] Accuracy: <±3%, temperature: 25 °C. [c] $k_c$: rate constant of the complexes determined from the $K$ value, $k_0/k_c$: decelerating ratio. [d] [**6**] = $1.0 \times 10^{-3}$ mol dm$^{-3}$. [e] **1** = aniline; **2** = *p*-nitrophenol; **3** = 1-naphthylamine; **4** = 2-naphthylamine; **5** = adamantane-1-amine; **6** = adamantan-1-ol.

cavity, despite the numerous studies on the change of reactivity of guest molecules in inclusion complexes. CDs are known to be subject to acid-catalysed hydrolysis giving linear oligosaccharides.[7] Therefore, we investigated the effect of guest molecules on the acid-catalysed ring-opening of β-CD and report here that the ring-opening was markedly modified by the inclusion of guest molecules in the cavity.

The hydrolysis of β-CD ($1.0 \times 10^{-3}$ mol dm$^{-3}$) was conducted in the absence and presence of guest molecules (normally $5.0 \times 10^{-3}$ mol dm$^{-3}$) in 1.0 mol dm$^{-3}$ HCl (reagent grade, free from metals and higher oxidised chlorine) at 60 °C. A concentration of adamantan-1-ol of $1.0 \times 10^{-3}$ mol dm$^{-3}$ had to be used because of its low solubility in water. At appropriate times, an aliquot sample was removed, neutralized with 1.0 mol dm$^{-3}$ NaOH, deionized on an ion-exchange column, and analysed by HPLC under the following conditions: Aminex HPX-42A cation-exchange column (BIO-RAD Co, USA) coated with $Ag^{2+}$; mobile phase, water; flow rate, 0.6 ml min$^{-1}$; column temperature, 72 °C; RI detector (SE-61, Shodex, Japan). β-CD, linear oligosaccharides (glucose to maltoheptaose) and an internal standard (sorbitol) were completely separated from each other under these conditions. The reaction was monitored within about one half-life and followed first-order kinetics with respect to concentration of β-CD. The ring-opening rate of β-CD showed a first-order dependence upon hydrogen-ion concentration,[8] indicating the scission of one glycosidic bond of the cyclic CD to acyclic maltoheptaose under the experimental conditions. The association constant of the β-CD complexes was determined from changes in UV or fluorescence spectra of guests by the addition of the host in 1.0 mol dm$^{-3}$ HCl at 25 °C. In the case of adamantanes, the association constant was determined as an inhibition constant using 2-naphthylamine by the fluorescence method.[9] $^1$H NMR spectra were measured on a Jeol GX-400 spectrometer at 400 MHz.

Table 1 shows the ring-opening rate constants of β-CD and the hydrolysis rate constants of acyclic maltoheptaose in the presence and absence of guest molecules in 1.0 mol dm$^{-3}$ HCl at 60 °C. The guest molecules **1**–**6** used were chosen from consideration of their solubility and chemical stability under the high acidic and temperature conditions, and are known to form inclusion complexes with β-CD with a 1:1 stoichiometry.[10]

The ring-opening of β-CD was decelerated by the addition of guest molecules, the deceleration being marked for guests with a close fit of the β-CD cavity. For example, **5** and **6** slowed the ring-opening rate of β-CD by a factor of about 20, whereas small guests such as **1** and **2** showed only slight deceleration ($k_0/k_c$ = 1.7 and 3.3, respectively). The deceleration effect of **4** was greater than that of **3**; the former guest is known to be more deeply included axially (the long axis of the naphthalene ring is parallel to the axis of the CD cavity) than the latter guest which is included equatorially (the long axis of the naphthalene ring is perpendicular to the axis of the CD cavity).[11] The hydrolysis rate of acyclic, linear maltoheptaose was not affected by the addition of **2** and **5**, as shown in Table 1. Furthermore, plots of the rate of ring-opening of β-CD as a function of concentration of **2** or **5** showed typical saturation kinetics.[6] The minimum rate ($k_c$) and associated constant ($K$) of the complexes were calculated from Lineweaver–Burk plots,[6] and were 0.00318 h$^{-1}$ (rate decelerating ratio, $k_0/k_c$ = 19) and 4200 dm$^3$ mol$^{-1}$ for the complex with **5** and 0.0226 h$^{-1}$ ($k_0/k_c$ = 2.7) and 340 dm$^3$ mol$^{-1}$ for the complex with **2**, respectively. These kinetically determined association constants were in good agreement with those determined spectrophotometrically. The above results indicate that the deceleration of the ring-opening is attributable to the formation of inclusion complexes with guest molecules.

Fig. 1 shows the relationship between $k_0/k_c$ and $K$ values of

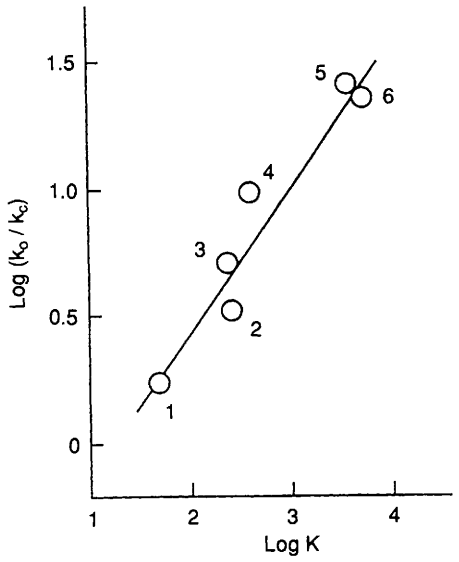


**Fig. 1** Log ($k_0/k_c$) *vs.* log $K$. The numbers stand for the guests employed.

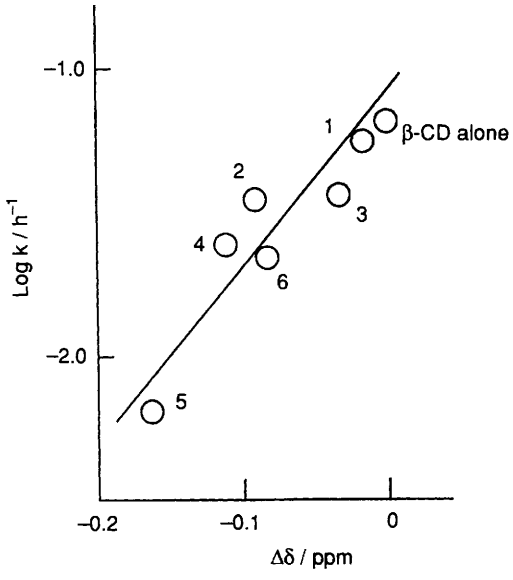


**Fig. 2** Log $k$ *vs.* $^1$H chemical shift changes (Δδ) of 3-H of β-CD. The numbers stand for the guests employed. Δδ: negative sign indicates upfield shift induced by the addition of guest in pH 2.0 (pH meter reading) at 25 °C. The concentration of the host and guests was the same as those used in the kinetic run (Table 1). Accuracy of NMR measurements = ±0.003 ppm.

the complexes. The deceleration was greater for the guests with larger association constants (correlation coefficient of the straight line 0.97), suggesting that the guests which fit snugly in the cavity suppress the ring-opening. This was further supported by $^1$H NMR spectroscopic studies. 3-H and 5-H of the glucose units of CDs are known to be subject to magnetic shielding effects due to the included guest molecules because they are directed inside the cavity, whereas the 1-H, 2-H and 4-H on the outer surface are little affected.[12,13] Furthermore, the magnitude of the shielding of 3-H and 5-H may reflect the degree of contact of the guest molecules with the glycosidic bonds (reactive site of the ring-opening), because these protons are located in the neighbourhood of the glycosidic oxygen atoms. In the present system, the $^1$H NMR signals of 3-H and 5-H were shifted upfield (0.01–0.15 ppm) by the addition of guest molecules, whereas those of 1-H, 2-H and 4-H were little changed. As shown in Fig. 2, the ring-opening rates of β-CD could be correlated with the upfield shift of the 3-H signal (correlation coefficient of the straight line = 0.81). Unfortunately, the shift-change for 5-H could not be accurately determined because of overlap with other signals. The above results indicate clearly that the larger the volume of the cavity that is occupied, the greater is the deceleration. A possible mechanism for deceleration caused by inclusion of guest molecules is that water molecules or oxonium ions capable of catalysing the ring-opening are excluded from the cavity, and access to the glycosidic oxygen atoms from the inside of the cavity is difficult.

The present system is of interest from the viewpoint of controlling the ring-opening rate of CDs, and is applicable to the stabilization of CDs from acid- or enzyme-hydrolysis in the stomach and in saliva and to the selective production of CDs, by choosing guest molecules that fit a particular CD.

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## References


1 W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 344; J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982; F. Hirayama and K. Uekama in *Cyclodextrins and Their Industrial Uses*, ed. D. Duchêne, Editions de Santé, Paris, 1987, p. 131.

2 V. T. D'Souza and M. L. Bender, *Acc. Chem. Res.*, 1987, **20**, 146.

3 H. Ye, D. Rong, W. Tong and V. T. D'Souza, *J. Chem. Soc., Perkin Trans. 2*, 1992, 2071, 163; T. Eiki and W. Tagaki, *Chem. Lett.*, 1980, 1063.

4 P. Bortolus and S. Monti, *J. Phys. Chem.*, 1987, **91**, 5046; M. S. Syamala, S. Devanathan and V. Ramamurthy, *J. Photochem.*, 1986, **34**, 219.

5 N. K. Sangwan and H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1223; T. Utsuki, F. Hirayama and K. Uekama, *J. Chem. Soc., Perkin Trans. 2*, 1993, 106.

6 R. Breslow, *Acc. Chem. Res.*, 1991, **24**, 317.

7 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, Berlin, 1977.

8 F. Hirayama, M. Yamamoto and K. Uekama, *J. Pharm. Sci.*, 1992, **81**, 913.

9 I. Tabushi, N. Shimizu, T. Sugimoto, M. Shinozuka and K. Yamamura, *J. Am. Chem. Soc.*, 1977, **99**, 7100; J. W. Park and H. J. Song, *J. Phys. Chem.*, 1989, **93**, 6454.

10 K. A. Connors and J. M. Lipari, *J. Pharm. Sci.*, 1976, **65**, 379; K. Harata, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1807; M. Hoshino, M. Imamura, K. Ikehara and Y. Hama, *J. Phys. Chem.*, 1981, **85**, 1820; J. C. Harrison and M. R. Eftink, *Biopolymers*, 1982, **21**, 1153; S. Hamai, *J. Phys. Chem.*, 1989, **93**, 2074; R. I. Gelb and L. M. Schwartz, *J. Incl. Phenom. Mol. Recognition Chem.*, 1989, **7**, 537; R. Palepu and V. C. Reinsborough, *Aust. J. Chem.*, 1990, **43**, 2119.

11 K. Harata and H. Uedaira, *Bull. Chem. Soc. Jpn*, 1975, **48**, 375.

12 P. V. Demarco and A. L. Thakkar, *Chem. Commun.*, 1970, 2.

13 D. J. Wood, F. E. Hruska and W. Saenger, *J. Am. Chem. Soc.*, 1977, **99**, 1735; M. Komiyama and H. Hirai, *Chem. Lett.*, 1980, 1467.