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Note

Deprotonation of β-cyclodextrin in alkaline solutions

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ABSTRACT

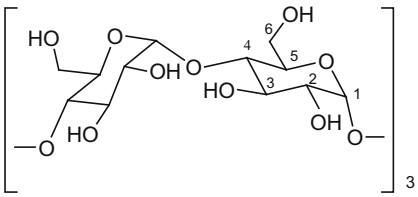
Variable pH ^{13}C NMR and ^{1}H NMR spectroscopic studies of the β-cyclodextrin (β-CD) in alkaline aqueous solutions revealed that β-CD does not deprotonate at pH < 12.0. Further increase in solution pH results in the deprotonation of OH-groups adjacent to C-2 and C-3 carbon atoms of β-CD glucopyranose units, whereas the deprotonation of OH-groups adjacent to C-6 carbon atoms is expressed less markedly. The pK_a values for β-CD OH-groups adjacent to C-2 and C-3 carbon atoms are rather close, $pK_{a1,2}$ being 13.5 ± 0.2 (22.5 °C).

The unique ability of cyclodextrins to form inclusion complexes is widely exploited in many areas, for example, in drug carrier systems, food industry and cosmetics.[1] The principal factors involved in guest–host complex formation with organic molecules are van der Waals, hydrophobic interactions, size effects, and hydrogen bonding.[2–5] Other factors prevail upon complex formation with metal ions, when a covalent bond is formed between metal ion and deprotonated OH-group, i.e., O^--group.[6–18] Therefore, acidity of terminal OH-groups plays a key role in metal–cyclodextrin complex formation.

Deprotonation of β-cyclodextrin (β-CD) in alkaline medium was previously investigated by pH-dependent kinetic study[19] and by pH potentiometry combined with ^{13}C NMR titration,[20] and pK_{a1} values of 12.1[19] and 12.201[20] were reported.

Results obtained in our laboratory on Cu(II)-β-CD[17] and Cu(II)-saccharose[21,22] complex formation suggested that deprotonation of β-CD takes place in more basic solutions than was previously reported. Published pK_{a1} values for β-CD (12.1[19] and 12.201[20]) and saccharose (12.43[23]) are rather similar, suggesting that complex formation in Cu(II)-β-CD and Cu(II)-saccharose systems should be similar, since only deprotonated OH-groups (O^--groups) can serve as donors for Cu(II) ion in highly basic media. This conclusion is supported by complexation results obtained in Cu(II)-tartrate,[24] Cu(II)-glycerol,[22,25] Cu(II)-dextran,[26] Cu(II)-D-mannitol,[27] and Cu(II)-D-sorbitol[28] systems, where formation of soluble complexes is detected polarographically at pH's *ca* 1–3 units lower than pK_a value of corresponding polyhydroxylic ligand. Soluble Cu(II)-saccharose complexes begin to form at pH above 11[21,22] (i.e., at pH ≈ pK_a − 1), whereas Cu(II)-β-CD complexes begin to form only at pH above 12.5[17] (i.e., at pH higher than reported[19,20] pK_a values). Furthermore, metal ion-β-CD complexation data[7–11,17] indicate that at least doubly deprotonated anion β-CD^{2-} is involved in complex formation. Dissociation of two OH-groups adjacent to C-2 and C-3 was already suggested,[20] although no pK_{a2} value was reported. Accurate pK_{a2} value, however, is required in order to study any metal ion-β-CD equilibrium. Consequently, reinvestigation of β-CD deprotonation by means of ^{13}C and ^{1}H NMR titrations was carried out in this work.

NMR spectroscopy is a rather sensitive method to study the deprotonation of hydroxyl groups, since the change in the environment of the latter results in chemical shift change of the adjacent methine and methylene protons and the respective carbon atoms. The necessary information on this equilibrium may be obtained



Scheme 1. Numbering of C-atoms in α-D-glucopyranose unit of β-cyclodextrin.

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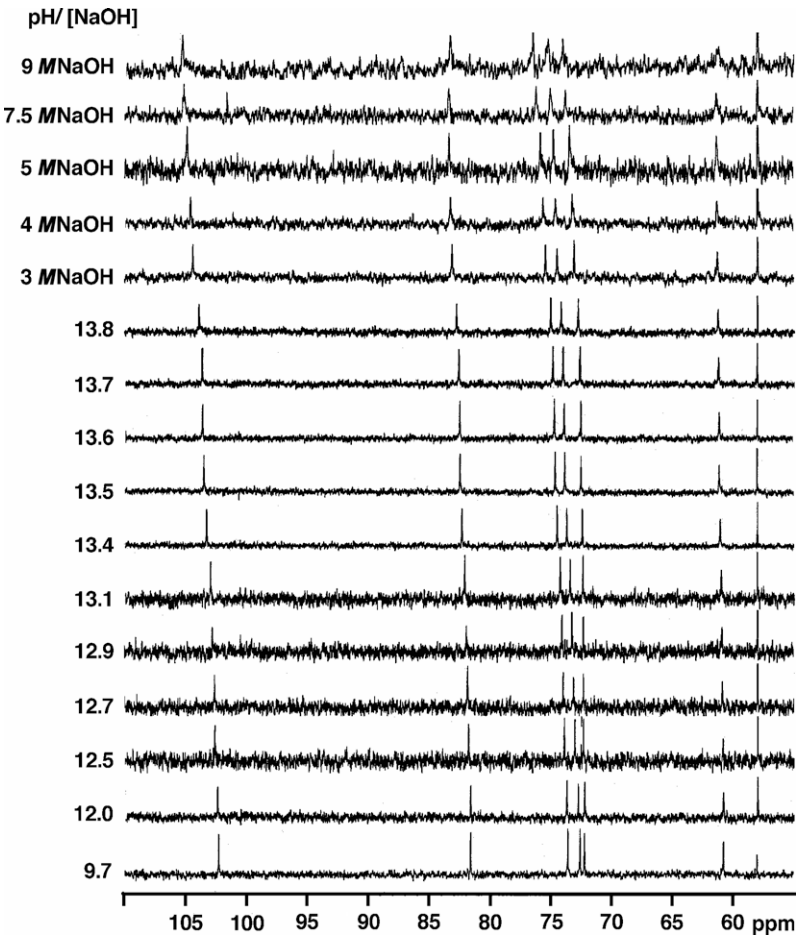

Figure 1. Variable pH ^{13}C NMR spectra of the aqueous β-CD (15.5 mM) solution. For highly basic solutions (pH > 14), total molar concentrations of NaOH are given (from 9 M to 3 M), otherwise pH values of final solutions are shown (from 13.8 to 9.7). Signal at 58.05 ppm is due to ethanol used as internal reference. Signal-to-noise ratio is different because various scan numbers were used to acquire some spectra.

due to the chemical shift change during the titration experiment by varying the pH of the solution. Variable pH ^{13}C and ^{1}H NMR spectroscopy was used to obtain the number of deprotonating OH-sites and to determine accurate pK_a values.

The ^{13}C spectrum of β-CD obtained at neutral pH (~7) exhibit six resonances at 102.31 (C-1), 81.58 (C-4), 73.65 (C-3), 72.65 (C-2), 72.28 (C-5), and 60.83 ppm (C-6) (the numbering of C-atoms is given in Scheme 1), and is in a good agreement with previously published results.[20,29]

Due to the symmetry of β-CD molecule, a single set of signals is observed. Chemical shifts for all six resonances do not change significantly up to pH 12.0, showing that no deprotonation occurs at pH < 12.0 (Fig. 1). Upon increasing pH, all resonances shift in the downfield direction indicating deshielding of the deprotonated hydroxyl groups in glucopyranose residues. ^{13}C-resonance displacements $\Delta\delta$ (ppm) = $\delta_2 - \delta_1$, where δ_2 is the chemical shift observed in 5 M NaOH solution, and δ_1 is observed in pH 9.7 solution, decrease in the following order: C-1(2.3) > C-3(1.96) > C-2(1.92) > C-4(1.51) > C-5(0.97) > C-6(0.56). Significant displacement of C-1 resonance may be ascribed to the acidity and electric field sensitivity of this anomeric carbon. The hydroxyl group at the primary hydroxyl group at C-6 is shifted least as expected. In this work and previously published $\Delta\delta$ values for β-CD are summarized in Table 1. Apparently, published values are significantly lower than those obtained in this work (except for C-5), suggesting that β-CD in 5 M NaOH is ionized more than at pH 14 (Ref. 30) and especially more than in 0.1 M NaOH solution containing 0.02–0.05 M of β-CD.[20]

Table 1
$\Delta\delta = \delta_2 - \delta_1$ values for β-CD

	This work[a]	Ref. 30[b]	Ref. 20[c]
C-1	2.30	1.1	0.52
C-2	1.92	0.0[d]	0.65
C-3	1.96	1.0	0.45
C-4	1.51	0.8	0.33
C-5	0.97	1.4[d]	0.07
C-6	0.56	0.3	0.17

[a] Difference between chemical shifts in 5 M NaOH and pH 7.
[b] Difference between chemical shifts at pH 14 and pH 7.
[c] Difference between chemical shifts in 0.1 M NaOH and 0 M NaOH.
[d] In Ref. 30 C-2 and C-5 were assigned in reversed order.

The ^{1}H NMR spectrum is a complex one with distinct signals at 5.07 ppm (d, H-1), 3.97 (t, H-3), 3.64 (dd, H-2), 3.58 (t, H-4) and less distinct group from 3.85 to 3.91 (m, H-5, H-6) (Fig. 2, pH 10.9), which is in agreement with previously published results.[29] All resonances do not shift at pH below 12. Only upon further increase in solution pH, all resonances shift upfield, indicating that substantial deprotonation of β-CD takes place at pH > 12. No plateaus were observed for all ^{1}H chemical shifts, even though spectra were taken in 5 M and 8 M NaOH solutions. Qualitatively, ^{1}H NMR titration results are in perfect agreement with ^{13}C NMR titration results, although pK_a values in D_2O usually are slightly different from those obtained in deuterium-free media.[31]

^{13}C and ^{1}H chemical shifts as a function of pH for these atoms are shown in Figure 3. Since only hydroxyl groups adjacent to

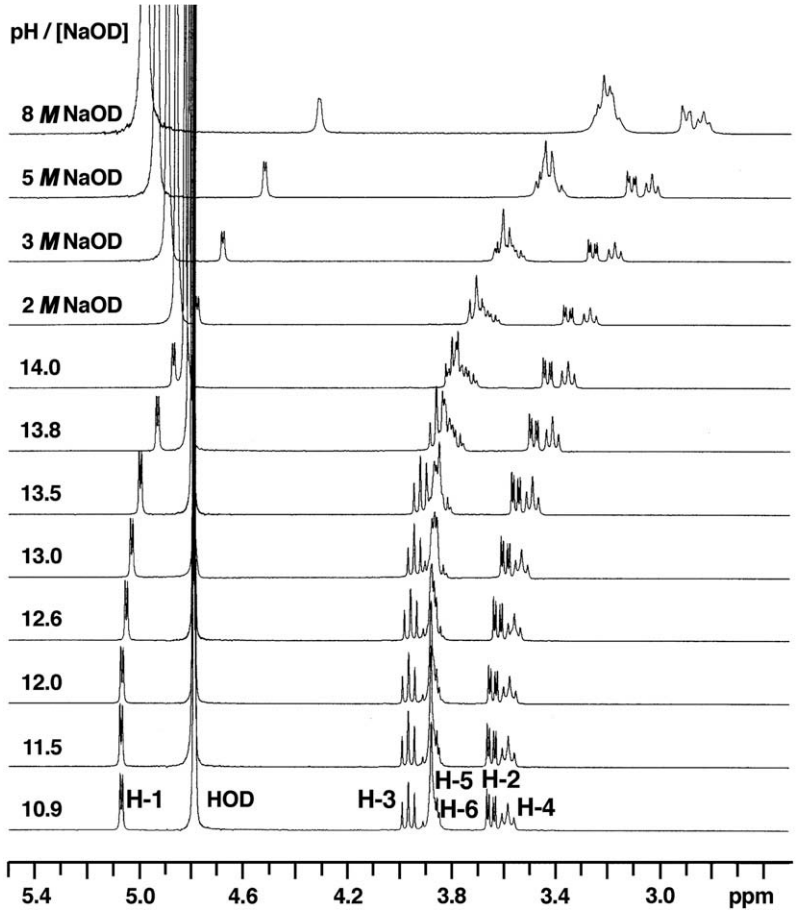

Figure 2. Variable pH ^{1}H NMR spectra of the aqueous β-CD (10 mM) solution. For highly basic solutions (pH > 14) total molar concentrations of NaOD are given (from 8 M to 2 M), otherwise pH values of final solutions are shown (from 14.0 to 10.9). No corrections were made for pH values due to the presence of D_2O.

C-3, C-2, and C-6 can deprotonate, displacements of these carbon atoms were used to determine pK_a values. A plateau of the chemical shift at high pH was not observed even in very concentrated NaOH solutions, thus, K_a values were obtained from a slope of the δ versus $10^{-pH}(\delta_1 - \delta)10$[14] graph (Fig. 4).

NMR spectroscopic study of β-CD showed a significant change of the chemical shifts depending on the pH of the solution. Both ^{13}C and ^{1}H signals shifted downfield indicating the deprotonation of the adjacent hydroxyl groups (Figs. 1 and 2). The most significant downfield shifts were observed for C-5, C-2, C-4, and C-3 of the β-CD ring and for the corresponding hydrogen atoms attached to the respective carbon atoms. The plot of the chemical shifts against the variation of the pH of the solution in a broad range indicated that the C-2 and C-3 carbon atoms of the β-CD cavity are affected most sensibly to the variation of the pH of solution (Fig. 3a and b). Similar slopes of the curves (Fig. 3) indicate that the deprotonation occurs within close range of pH values for all two hydroxyl groups. Only a small change of δ was observed for primary hydroxyl group at C-6. The process and extrapolation of the chemical shift results enable to establish the deprotonation value 13.5 with the accuracy of ±0.2 (Fig. 4).

In conclusion, the results obtained in this work indicate that at high pH values the deprotonation of three hydroxyl groups of β-CD takes place, located at C-2, C-3, and C-6 carbon atoms. It should be noted, however, that the primary hydroxyl groups at C-6 form intermolecular hydrogen bonds and the deprotonation should proceed more readily compared to the secondary hydroxyl groups at C-2 and C-3 forming intramolecular hydrogen bonds. Since it is known that twofold or fourfold deprotonated anion of β-CD forms complexes with some metal ions forming bonds with O^--groups, adjacent to C-2 and C-3 carbon atoms, deprotonation equilibria were calculated namely for corresponding OH-groups. The pK_a values for C-2 and C-3 obtained in this work as from ^{13}C, as well as from ^{1}H NMR measurements, are rather similar and are equal to 13.5 ± 0.2.

1. Experimental

1.1. Materials and sample preparation

β-CD was purchased from TCI (Japan), NaOH from Fisher Scientific. Sodium hydroxide-d (98 atom % D) as a 40% (w/w) solution in D_2O was from Merck, deuterium oxide and deuterium chloride (99.5 atom % D) as 35% (w/w) solution from Cambridge Isotope Laboratories. Samples for ^{13}C NMR spectroscopy were prepared by dissolving β-CD in H_2O (90 vol %)/D_2O (10 vol %) mixture. An appropriate amount of NaOH stock solution (0.01 M, 0.1 M, 1.16 M 2.03 M, 4.92 M, or 10.22 M; all prepared from 50% NaOH) was added to the sample. Final solution contained 15.5 mM β-CD and 10 mM ethanol as an internal reference. Samples for ^{1}H NMR spectroscopy were prepared from 50 mM β-CD stock solution in NaOD/D_2O. The pH was adjusted with diluted DCl or NaOD solutions. Final solution contained 10 mM of β-CD. The pH readings were taken with an Orion 420 A+ pH-meter equipped with a Corning Semi-Micro Combo glass electrode. The electrode was calibrated with standard aqueous buffers at pH 7.00 and pH 10.00.

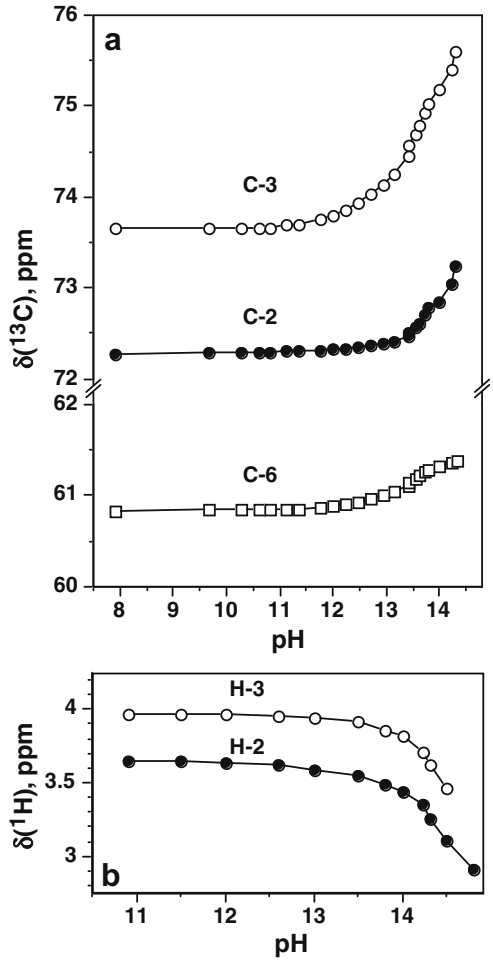

Figure 3. β-CD chemical shift titration curves obtained from spectra shown in Figure 1 for carbon resonances C-3, C-2 ,and C-6 (a) and titration curves obtained from spectra shown in Figure 2 for proton resonances H-3 and H-2 adjacent to the C-3 and C-2 carbon atoms.

Measurements were taken before and after each NMR experiment, and the difference between these values was less than 0.05 pH units. The pH values obtained after spectra acquisition were used for all further calculations. No corrections were made for pH values measured in the presence of the deuterated solvent.

1.2. NMR spectroscopy

$^{13}C\{^1H\}$ and ^1H NMR spectra were recorded at 22.5 ± 0.2 °C on Varian Inova-300 spectrometer operating at 75.472 MHz (carbon) and on Varian Inova-400 spectrometer operating at 400.107 MHz (proton), using standard acquisition parameters. The ^{13}C chemical shifts were referenced to the internal ethanol (δ = 58.05 and 14.47 ppm), and the ^1H chemical shifts were referenced to the external DSS (3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt; δ = 0.015 ppm). The estimated accuracy of the chemical shift is 0.01 ppm for ^{13}C and 0.001 ppm for ^1H.

1.3. pK_a Determination

Since a plateau at high alkaline pH was not observed, K_a values were obtained from the slope of the graph plotted using the equation:[32]

$$\delta = \delta_2 + 10^{-pH}(\delta_1 - \delta)/K_a \quad (1)$$

where δ is the observed chemical shift, δ_1 is the chemical shift of protonated group, and δ_2 is the chemical shift of deprotonated group.

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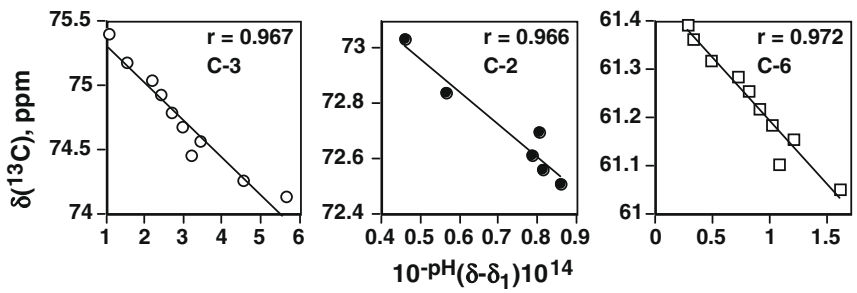

Figure 4. The plots of ^{13}C chemical shift for C-3, C-2, and C-6 as a function of $10^{-pH}(\delta_1 - \delta)10^{14}$ at pH values from the slope region of the titration curves. Correlation coefficients (r) are shown.

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