Carbohydrate Research, 142 (1985) 329–332
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A ^{1}H-n.m.r. study of the formation and structure of cyclomalto-hexaose- and heptaose and inclusion-complexes with aromatic amino acids in aqueous solution

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(Received March 1st, 1985; accepted for publication, April 18th, 1985)

The cyclomaltopolyoses (cycloamyloses, cyclodextrins, CD) are cyclic oligosaccharides that contain at least six (1→4)-linked α-D-glucosyl residues. The most important property of a CD is its ability to admit a variety of guest molecules into its hydrophobic cavity without any covalent bonds being formed[1–4]. Because of this property, CDs serve as models for studying topochemical aspects and catalytic reactions of enzymes. The kinetics and mechanisms of numerous CD-catalysed stereospecific reactions in solution have been studied[1,2,4], and investigations of the molecular geometry of CD inclusion-complexes in solution are therefore important.

The molecular dynamics of the inclusion complexes of cyclomalto-hexaose, -heptaose, and -octaose (α-CD, β-CD, and γ-CD) with aromatic amino acids in aqueous solution have been studied by ^{13}C-n.m.r. spectroscopy[5–7]. The strongest dynamic coupling was observed for the β-CD complexes with L-phenylalanine (Phe) and L-tyrosine (Tyr) at p^{2}H 11, in which the aromatic rings are assumed to be deeply and tightly contained in the CD cavity. The deeper and tighter inclusion of aromatic rings in the β-CD complexes than in α-CD complexes was also suggested by the observation of conformational changes of Phe and Tyr on complexation with α-CD and β-CD by analysis of ^{1}H–^{1}H spin–spin coupling[8]. The geometry in aqueous solution of inclusion complexes of α-CD and β-CD with several *p*-substituted phenols has been studied[9] by ^{1}H- and ^{13}C-n.m.r. spectroscopy. The ^{1}H data for H-3 and H-5 of the host, which are located inside the cavity, show that the guests are more deeply inserted into the cavity of β-CD than into that of α-CD.

We now report on the formation and the molecular geometry of inclusion complexes of α-CD and β-CD with Phe and Tyr in aqueous solution as investigated

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0008-6215/85/$ 03.30

by ^{1}H-n.m.r. spectroscopy. The influence of the hydroxyl group of Tyr on the formation and geometry of the complex may be assessed by comparing the results with those for Phe. Determination of the geometry of the CD–guest inclusion-complexes, as well as the strength of the dynamic coupling between the host and the guest, is essential for an understanding of the mechanism of enzyme-like catalysis by CD.

Formation of the CD–guest complex by insertion of the aromatic ring of the guest into the CD cavity was confirmed by observing the shifts induced in the H-3 and H-5 resonances of CD due to the ring-current effects of the aromatic guest. As shown in Table I, α-CD and β-CD form complexes of the inclusion type with Phe and Tyr, with the exception of the α-CD–Tyr system at p^{2}H 11. Dissociation constants (K_d) for the host–guest complexes were determined from u.v. spectral changes or ^{1}H-n.m.r. chemical shift displacements induced by complexation. The K_d values obtained by assuming the formation of 1:1 complexes and by applying the modified Hildebrand–Benesi equations[9–11] were of the order of 10^{-3}M (from 9.3×10^{-3}M for β-CD–Tyr at p^{2}H 11 to 2.2×10^{-3}M for β-CD–Phe at p^{2}H 11). In the case of the α-CD–Tyr system at p^{2}H 11, the K_d value could not be estimated because of the smallness of the spectral changes. Thus, Tyr may not form a stable inclusion complex with α-CD in alkaline solution. Since the formation of inclusion complexes was found in the other systems, the ionisation[12] of the hydroxyl group of Tyr (pK_a 10.9) is unfavourable for complexation with α-CD, but not for that with β-CD. These results can be explained on the basis of the molecular geometry of the complex.

The geometry of the complexes, namely, the probable position of the guests

TABLE I

^{1}H CHEMICAL SHIFT DISPLACEMENTS ($\Delta\delta$) OF THE H-3 AND H-5 RESONANCES OF α-CD AND β-CD INDUCED BY COMPLEXATION WITH Phe AND Tyr IN AQUEOUS SOLUTION

Solvent	*Host*	*Guest*	*Concentration (M)*		*$\Delta\delta$ (p.p.m.)*[a]	
			Host	*Guest*	*H-3*	*H-5*
M ^{2}HCl	α-CD	Phe	0.02	0.10	−0.08	0.00_7
		Tyr	0.02	0.10	−0.07	-0.00_5
	β-CD	Phe	0.01	0.10	−0.04	−0.17
		Tyr	0.01	0.10	−0.07	−0.16
p^{2}H 11	α-CD	Phe	0.03	0.12	−0.08	0.00_1
		Tyr	[b]	[b]	[b]	[b]
	β-CD	Phe	0.01	0.04	−0.07	−0.19
		Tyr	0.001	0.005	−0.05	−0.09
					(−0.16)[c]	(−0.26)[c]

[a]Negative values indicate upfield displacements. [b]No binding. [c]$\Delta\delta$ values for 100% binding calculated using dissociation constant K_d.

in the CD cavity, is deducible by using the complexation-induced chemical shift displacements ($\Delta\delta$) of H-3 and H-5 of the CD with the aid of the calculated ring-current shifts arising from the included aromatic ring[9]. Here, $\Delta\delta$ is defined as a chemical-shift difference between the fully complexed and uncomplexed states. The values of $\Delta\delta$ were estimated from the chemical shifts at known concentrations of CD and guests, in conjunction with the K_d values. The values of chemical-shift displacements shwn in Table I (for β-CD–Tyr in alkaline solution, the values are shown in parentheses) are practically those of $\Delta\delta$ under the conditions investigated here.

For α-CD complexes, the relative magnitude and sign of $\Delta\delta$ for H-3 and H-5 clearly demonstrate that the aromatic rings of Phe and Tyr (except Tyr in alkaline solution) are inserted up to approximately the same position in the CD cavity from the secondary-hydroxyl side of the CD. The centre of the aromatic ring in the most probable geometry of α-CD complexes lies approximately in the plane of the six H-3 atoms.

For β-CD complexes, the relatively large shifts also observed for H-5 indicate that the aromatic rings penetrate more deeply into the cavity. The position of the centre of the aromatic rings in the β-CD complexes is between the H-3 and H-5 planes, and is independent of pH and the presence of the hydroxyl group in the guest molecule. Of course, these results do not indicate from which side of the β-CD cavity the aromatic rings of the guest are inserted.

From these geometries, the exceptional results for the α-CD–Tyr system in alkaline solution can be explained. The cavity size of α-CD is too small for deeper inclusion of the phenyl ring[9]. If the aromatic ring of Tyr having an ionised hydroxyl group is included into the cavity of α-CD in the same fashion as for Phe and Tyr having an unionised hydroxyl group, the ionised hydroxyl group must reside within the non-polar CD cavity. This situation is disadvantageous as compared to the free state. For the β-CD–Tyr system, the hydroxyl anion on the included aromatic ring is situated at the one end of the CD cavity and is exposed to the medium or the hydroxyl groups of β-CD. The results presented here correspond well with the conclusions drawn from the measurements of ^{13}C spin–lattice relaxation times[7]. The most probable position of the aromatic rings in the CD complexes, at least with Phe, is independent of the ionisation states[12] of α-amino (pK_a ~9–11) and α-carboxyl groups (pK_a ~2) of the guests. These groups are not included in the CD cavity[7], but they can interact with the hydroxyl groups on the cavity rim of the CD. This interaction induces significant conformational changes of Phe and Tyr on complexation with β-CD[8].

EXPERIMENTAL

^1H-N.m.r. spectra were recorded with a JEOL JNM FX-270 spectrometer (270 MHz), using a sweep width of 1350 Hz (8192 points, resolution 0.3 Hz). The ^1H chemical shifts were measured in p.p.m. downfield from the signal of external

Me_4Si. U.v. spectra were recorded with a Beckman-25 spectrometer. All spectroscopic measurements were made at 31 ±1°.

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