Cyclohepta-amylose Inclusion Complexes. A Proton Magnetic Resonance Study

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Summary Proton magnetic resonance provides direct evidence for the inclusion nature of cyclohepta-amylose complex formation with aromatic substrates.

CYCLOAMYLOSES (cyclodextrins) have been used as models for enzymes[1,2] and proteins,[3,4] because their structures are well defined[3,5,6] and because they interact with many substrates in a manner similar to that of enzymes and proteins. It has been suggested[1–4,7,8] that cycloamyloses form inclusion complexes with organic substrates in aqueous solution. Little direct evidence has yet been presented (except from *X*-ray investigation of several crystalline complexes[9,10]) verifying inclusion of a molecular substrate within the cavity of cycloamylose.

We present the first such evidence from proton magnetic resonance (^{1}H n.m.r.) studies of the interaction of cyclohepta-amylose (C7A) (Figure 1a) with a variety of aromatic substrates in aqueous solution. Previous n.m.r.[6] and *X*-ray[5] studies have unequivocally established the C-1 chair conformation for the constituent glucose units in cycloamyloses. Cyclohepta-amylose thus has primary and secondary hydroxy-groups crowning opposite ends of its torus, H-3 and H-5 directed toward its interior and H-1, H-2, and H-4 located on its exterior. It was expected† *a priori*, in light of the screening environment associated with aromatic moieties,[11] that if inclusion does indeed occur, protons located within or near the cavity (*e.g.* H-3, H-5, or H-6) should be strongly shielded.‡ Alternatively, if association takes place at the exterior of the torus, H-1, H-2, and H-4 should be the more strongly affected.

The effect on the high resolution ^{1}H n.m.r. spectrum of C7A in D_2O upon the addition of increasing amounts of the aromatic substrate *p*-hydroxybenzoic acid (III), which is similar in behaviour to many of the substrates examined, is illustrated in Figure 2. Assignments, based on first-order analysis for the various C7A resonances§ are also indicated in this Figure and correspond to proton chemical shift positions shown in Figure 1b. From Figure 2, it is evident that the low-field triplet, assigned to H-3 (δ 4·43), shifts progressively to higher-field value as the molar concentration of (III) is increased relative to C7A. Additionally, the H-5 signal, whose multiplicity is not directly observable, shifts from its initial position (δ *ca.* 4·27) under the C-6 methylene signal to higher field (δ *ca.* 4·08), under the H-2 signal. The observation that spectral regions δ 4·55—4·20 and 4·20—3·80 integrate for 4 and 2 protons respectively when no substrate is present and 3 protons each when the molar ratio of substrate (III) to C7A is one, firmly establishes that one proton, namely H-5, moves significantly upfield. The remaining signals (H-1, H-2, H-4, and H-6) experience marginal upfield shifts.

The above chemical-shift behaviour for the C7A protons definitively establishes that the phenyl ring of substrate (III) is positioned within the cycloamylose cavity. Further, the fact that only one signal is recorded for H-3 irrespective of the relative molar proportions of C7A and (III) in solution, demonstrates (a) that the reversible association between free and associated C7A species in solution is fast on the n.m.r. time scale (otherwise signals arising from free and complexed forms would be observed), and (b) that each time the phenyl ring of (III) enters the cavity of C7A, it assumes a different orientation about the axis parallel to

† According to space-filling molecular models, the diameter of the cavity of C7A is approximately 7·5 Å. The molecular diameter of the benzene nucleus, including van der Waals radii of the aromatic hydrogens, is 6·8 Å. Thus before a substituted aromatic molecule can be included within the C7A cavity, the longest axis of the guest must be alligned parallel to the cavity axis (*i.e.* parallel to the *z*-axis, Figure 1a).

‡ From Johnson–Bovey calculations, (ref. 12) the shielding component normal to the plane of an aromatic nucleus is greater in magnitude than the deshielding component in the plane of the ring for any given distance, *r*, from the centre of the ring. Thus, for the model adopted in this study† r = 7·5 Å/2 (*ca.* 3·8 Å). The magnitude of the shielding and deshielding components normal and parallel to the plane of the included molecule for r = 3·8 Å are +0·965 p.p.m. and −0·502 p.p.m. respectively (ref. 12b). It is obvious therefore that, unless an aromatic substrate has a preferred fixed orientation within the cyclodextrin cavity, all protons within the cavity will experience a net upfield shift upon adduct formation.

§ Signal assignments were firmly established on the following basis: (1) analysis of individual splitting patterns and coupling constants at 220 MHz, (2) decoupling experiments at 100 MHz and (3) expected chemical shift behaviour, *i.e.* H-3 and H-5, being 1,3-diaxial to the C-1 axial oxygen, should resonate at lower field[13] than H-2 and H-4.

the cyclohepta-amylose cavity and/or is rapidly spinning about this axis within the C7A cavity.

That inclusion complex formation is not a unique phenomenon of substrate (III) is demonstrated by the data given in the Table which summarizes the chemical shift changes, $\Delta\delta$ ($\Delta\delta = \delta_{free} - \delta_{sat.}$), which occur for the different C7A protons upon saturation of a 2% aqueous solution of C7A with a variety of structurally different substrates. It is evident from these $\Delta\delta$-values that all substrates, with the exception of (X), (XI), (XVI), and (XVII), form inclusion complexes with C7A in D_2O solution. The apparent failure of (XVI) and (XVII) to form such complexes can be readily explained in terms of the greater steric requirements associated with both the t-butyl and aromatic substituents in these substrates respectively. An explanation for the failure of (X) and (XI), in contrast to (XII), to form inclusion complexes probably lies in the greater solubility of these substrates in D_2O (45 and 229 g/100 ml. respectively) relative to (XII) (5·9 g/100 ml.). It seems reasonable that the relatively hydrophobic environment of the C7A cavity would be considerably less attractive to highly water-soluble molecules.

Preliminary variable temperature and substrate concentration studies confirm[1,3,8] that the stoicheiometry of

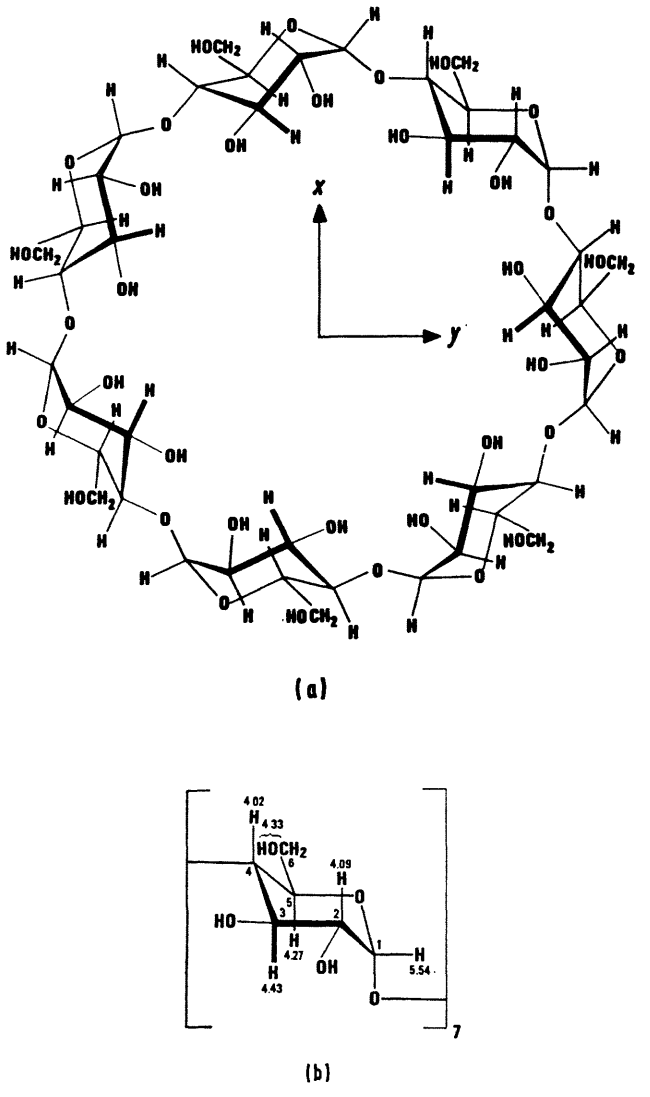

FIGURE 1. (a) *Cyclohepta-amylose molecule. z-Axis is parallel to the cyclohepta-amylose cavity*, i.e. *normal to plane of the page*; (b) *recorded chemical shifts in p.p.m. for various cyclohepta-amylose protons.*

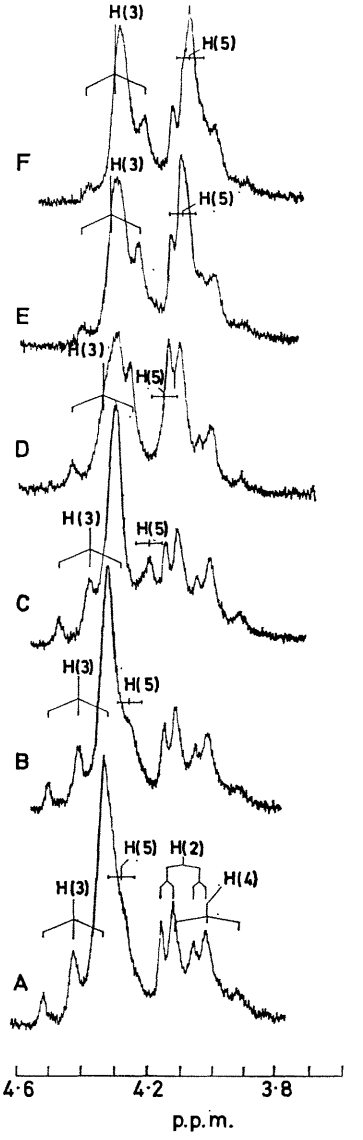

FIGURE 2. 100MHz ^1H *N.m.r. spectra of cyclohepta-amylose in* D_2O ($1·79 \times 10^{-2}$ M) *at* $30 \pm 1°$ *containing various amounts of* p-*hydroxybenzoic acid*: *molar ratio, substrate*: *cyclohepta-amylose*: (A) 0·00, (B) 0·19, (C) 0·40, (D) 0·77, (E) 1·16, (F) 3·09.

Substrate induced shifts[a] (Δδ) *for cyclohepta-amylose protons*

	Substrate	Δδ (p.p.m.)[b]					
		H-1	H-2	H-3	H-4	H-5	H-6
(I)	Benzoic acid	+0·04	+0·04	+0·16	+0·03	+0·19	+0·05
(II)	*m*-Hydroxybenzoic acid	+0·04	+0·04	+0·11	+0·04	+0·19	+0·09
(III)	*p*-Hydroxybenzoic acid	+0·04	+0·04	+0·14	+0·04	+0·21	+0·06
(IV)	*p*-Aminobenzoic acid	+0·04	+0·04	+0·14	+0·03	+0·19	+0·06
(V)	4-Amino-2-hydroxybenzoic acid	+0·02	+0·02	+0·08	+0·01	+0·11	+0·06
(VI)	Methyl *p*-hydroxybenzoate	+0·04	+0·03	+0·14	+0·03	+0·21	+0·05
(VII)	Acetylsalicylic acid	+0·03	+0·03	+0·12	+0·03	+0·20	+0·06
(VIII)	Ethyl *p*-aminobenzoate	+0·03	+0·03	+0·14	+0·03	+0·20	+0·06
(IX)	Phenol	+0·06	+0·08	+0·09	—	+0·26	+0·17
(X)	*o*-Dihydroxybenzene	+0·02	+0·01	+0·03	+0·01	+0·06	+0·02
(XI)	*m*-Dihydroxybenzene	+0·01	+0·01	+0·03	+0·01	+0·06	+0·03
(XII)	*p*-Dihydroxybenzene	+0·10	+0·10	+0·12	+0·10	+0·28	+0·10
(XIII)	D-Phenylalanine	+0·02	+0·02	+0·09	+0·03	+0·16	+0·06
(XIV)	*m*-t-Butylphenol	+0·05	+0·04	+0·20	+0·03	+0·20	+0·13
(XV)	*p*-t-Butylphenol	+0·04	+0·04	+0·21	+0·02	+0·03	+0·11
(XVI)	2,6-di-t-Butylphenol	+0·01	+0·02	+0·01	+0·01	0·00	+0·02
(XVII)	Tetracycline	0·00	0·00	+0·01	0·00	0·00	−0·01

[a] Determined from chemical shifts measured at 100 MHz relative to Me_4Si as external reference in D_2O solution. [b] Accuracy ±0·02 p.p.m.

C7A–substrate interaction is 1:1 and that the energy barrier to complex formation is low.

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