BIOORGANIC CHEMISTRY 5, 437–439 (1976)

The Molecular Disposition of *p*-Nitrophenol and Sodium *p*-Nitrophenolate in the Cyclohexaamylose Cavity: A ^{13}C Probe

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Received July 20, 1976

The direction in which both sodium *p*-nitrophenolate and *p*-nitrophenol penetrate the cyclohexaamylose cavity in aqueous solution has been examined by ^{13}C-nmr. Both sodium *p*-nitrophenolate and *p*-nitrophenol penetrate the cavity asymetrically and quite specifically nitro group first with the phenolic oxanion or hydroxyl group pointing out into solution as evidenced by the nature of the changes in the *meta*-carbon-^{13} shifts. The stoichiometries of the complexes can be defined for various host-to-guest molar ratios. Finally, the potential of these cycloamylose complexes as models for studying the effects of intermolecular interaction of the enzyme substrate type on the ^{13}C-nmr of both host and guest molecules is discussed.

INTRODUCTION

In recent years the cycloamyloses have received a great deal of attention as enzyme active site models (*1*, *2*). Although there has been a concerted and successful effort to improve their catalytic ability (*3*, *4*) and expand their spectrum of catalysis (5), very little emphasis has been placed on an understanding of the nature of the cycloamylose substrate binding forces. There have been three suggestions as to the origins of these forces: release of cycloamylose strain energy (*6*), release of high-energy water from the cavity, or London dispersion forces interactions (*1*, *7*). We feel it is unlikely that any one of these phenomena is solely responsible for clathration, but rather that they all contribute to the complexation driving forces. Although we were recently able to demonstrate that release of strain energy plays at best a small role in complexation (*7*), any further partitioning of these binding forces will require considerable information about the structure of the complexes in aqueous solution. In pursuing this matter, we have successfully employed both the changes in cycloamylose and sodium *p*-nitrophenolate ^1H chemical shifts on complexation and an intermolecular NOE as probes (*8*). In this paper we report on our efforts to employ ^{13}C-nmr as a probe in the determination of cycloamylose substrate conformations in solution and on the potential of the complexes as models for studying the effects of intermolecular interactions of the enzyme substrate type on the ^{13}C-nmr of both the host and guest molecules.

EXPERIMENTAL

Materials. The cyclohexaamylose was obtained from Aldrich Chemical Company and further purified by the method of Cramer and Henglein (*9*). The *p*-nitrophenol, also

obtained from Aldrich, was recrystallized three times from chloroform, (mp 114°C). Deuterium oxide 99.8% was purchased from Merck and Company.

Sample preparation. Stock solutions of cyclohexaamylose were made up in trisodium phosphate buffer, pH 11.00, $I = 0.5$ and in disodium phosphate buffer pH 5.5, $I = 0.5$. The pH was adjusted with phosphoric acid. The pH 5.5 cyclomylose solutions were prepared 1 hr before use. The *p*-nitrophenol and sodium *p*-nitrophenolate solutions were made up in 20% D_2O, 90% H_2O (v/v), pH 11.00 and PH 5.5 phosphate buffers, respectively ($I = 0.5$). The final concentration of D_2O in each sample was 10% (v/v). Sodium formate, 100 mg, was added to each 3-ml sample.

Nuclear magnetic resonance spectra. ^{13}C-pulsed Fourier transform ^1H decoupled nmr spectra, 25.2 MHz, were obtained on a Varian XL-100 spectrometer. The samples were run in 10-mm sample tubes with sodium formate as the internal reference.

In the pH 11.00 samples the sodium *p*-nitrophenolate concentrations varied from 0.025 to 0.097 *M* and the cyclohexaamylose varied from 0.009 to 0.05 *M*. In the pH 5.5 samples the *p*-nitrophenol concentration was held at 0.025 *M* and the cyclohexaamylose concentration varied from 0.005 to 0.05 *M*.

Preparation of 2,6-dideutero p-*nitrophenol.* *p*-Nitrophenol (0.100 g, 7.0×10^{-4} *M*) was dissolved in 2.0 g (2×10^{-2} *M*) of concentrated deuterosulfuric acid and heated to 66°C until the high field *ortho* proton doublet disappeared and the low-field doublet collapsed to a singlet. This required about 1 hr. The sample was poured into D_2O and the resulting precipitate was isolated by centrifugation and washed several more times with D_2O.

Assignment of the ^{13}C*-nmr of* p-*nitrophenol and sodium* p-*nitrophenolate.* Application of the additivity rules (*10*) to both *p*-nitrophenol and sodium *p*-nitrophenolate generated ^{13}C-nmr spectra were not commensurate with our experimental findings; the chemical shift differences between the *ortho* (*o*) and *meta* (*m*) carbons were poorly predicted. The predicted difference for *p*-nitrophenol was 8.3 ppm and the observed was 11.89 ppm. For sodium *p*-nitrophenolate the predicted was 4.0 ppm while the observed difference was 8.93 ppm. Furthermore, owing to the rather peculiar shifts induced in the *o* and *m* carbons of the *p*-nitrophenol and sodium *p*-nitrophenolate ^{13}C spectra by cycloamylose complexation, we felt it was necessary to verify the assignment of the *o* and *m* resonances. This was accomplished by deuterating both the 2 and 6 positions of the *p*-nitrophenol. Because of the strongly deactivating influence of the nitro group, this deuteration required rather severe conditions, concentrated deuterosulfuric acid at 60°C for 1 hr. The deuteration was, however, complete after this time as evidenced by ^1H-nmr. Heating the *p*-nitrophenol in neat deuterotrifluoroacetic acid at 60°C for 25 hr had no effect. Since the ^{13}C spectra are ^1H decoupled and not ^2H decoupled, the *o*-deuterated carbon should now display a triplet pattern, $2N(I) + 1$ where $N = 1$ is the number of equivalent neighbors and $I = 1$ is the spin number of the nuclei. However, owing to the fact that the carbons to which the deuteriums are fixed can no longer experience a complete NOE, their relaxation times T_1 are long and their signals are weak (*11*). Taking advantage of this and using short pulse intervals, the signals of the carbons with the deuteriums fixed should be of low intensity because of partial saturation. Indeed the signal for the *o*-carbons almost disappeared, barely revealing the corresponding triplet in the base line. The high-field signal was definitely the *o*-carbon signal.

Effects of sodium p-*nitrophenolate and* p-*nitrophenol on the* ^{13}C-*nmr of cyclohexaamylose.* Demarco and Thakkar (*12*) clearly demonstrated substrate-induced chemical shifts in the ^{1}H spectra of a variety of cycloheptaamylose aromatic substrate complexes. The H-5 and H-3 protons inside of the cycloamylose cavity were noticeably shifted upfield, i.e., deshielded. Assignment of the cycloamylose ^{13}C spectra by Takeo *et al.* (*13*) has now made it possible to determine the types of changes occurring at each

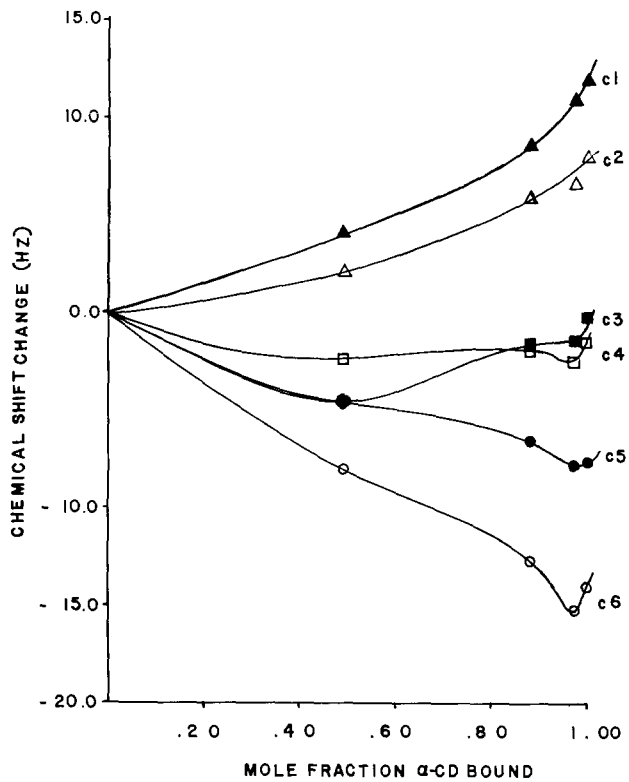

FIG. 1. Effects of sodium *p*-nitrophenolate on the ^{13}C-nmr spectra of cyclohexaamylose. Sample preparation is described in the Experimental section. The cyclohexaamylose and sodium *p*-nitrophenolate concentrations varied between 0.009–0.05 and 0.025–0.097 *M*, respectively. The sodium *p*-nitrophenolate induced shifts in the cyclohexaamylose ^{13}C-nmr are plotted relative to free cyclohexaamylose.

carbon in cycloamylose on substrate complexation. A series of ^{13}C-nmr spectra were taken of both cyclohexaamylose *p*-nitrophenol and cyclohexamylose sodium *p*-nitrophenolate complexes at varying ratios of cycloamylose to substrate. The change in chemical shift of each of the cycloamylose carbons was then expressed as a function of the mole fraction of bound cyclohexaamylose. The dissociation constants employed in the plots were (4.0×10^{-4} *M*) (*7*) for the sodium *p*-nitrophenolate complex and (2.6×10^{-3} *M*) (*1*) for the *p*-nitrophenol complex. In the sodium *p*-nitrophenolate system (Fig. 1) C-1 and C-2 are shielded, C-5 and C-6 are deshielded, while C-3 and C-4 experience little change. In the *p*-nitrophenol system (Fig. 2), the perturbations are

somewhat different; C-1 and C-2 are shielded, C-3 and C-6 deshielded, while C-4 and C-5 experience relatively small but discontinuous change.

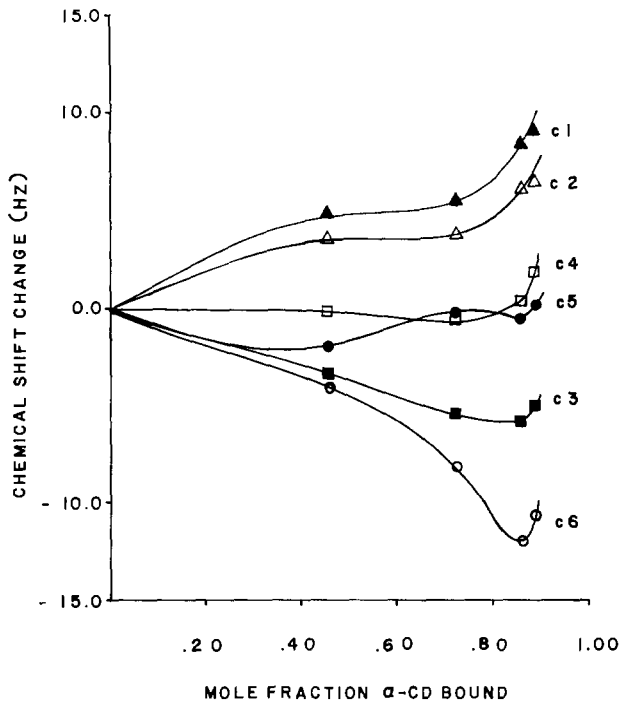

FIG. 2. Effects of *p*-nitrophenol on the ^{13}C-nmr spectra of cyclohexaamylose. Sample preparation is described in the Experimental section. The *p*-nitrophenol concentration was held constant at 0.025*M* and the cyclohexaamylose concentrations varied between 0.005 and 0.05 *M*. The *p*-nitrophenol-induced shifts in the cyclohexaamylose ^{13}C-nmr are plotted relative to free cyclohexaamylose.

Effect of cyclohexaamylose on the ^{13}C-nmr spectra of sodium p-*nitrophenolate and* p-*nitrophenol.* Although workers have shown substrate-induced shifts in the cycloamylose ^{1}H spectra (*12*), it was not until the recent work in these laboratories that cycloamylose-induced shifts in the substrate were analyzed (*9*). We have now shown that rather substantial shifts in the ^{13}C spectra of both sodium *p*-nitrophenolate and *p*-nitrophenol substrates occur on cycloamylose complexation (Fig. 3). Although all of the carbons experienced a change in the chemical shift on complexation, only changes in the *o*- and *m*-protons were followed in each case. Because both the phenolic oxygen and nitro group are unable to effectively relax the carbons to which they are fixed, these carbons have long T_1's and therefore very weak signals; and as a result of this, the time required to measure all of the signals was prohibitive at the substrate concentrations evaluated. The differences in the cycloamylose-induced changes in chemical shift between the *o*- and *m*-carbons of sodium *p*-nitrophenolate at pH 11.00 are substantial. When the sodium *p*-nitrophenolate is 100% bound, the greatest changes are seen for the *m*-carbons which move downfield from the uncomplexed phenolate by 1.43 ppm. Although the absolute shifts for the *o*-carbons are smaller, they move upfield from the free phenolate

by 0.28 ppm in the opposite direction. This is an absolute difference of 1.83 ppm between the two signals. The cyclohexaamylose-induced changes in chemical shifts for the *o*- and *m*-carbons of *p*-nitrophenol at pH 5.5 are unlike the phenolate system. The differences in the induced changes in chemical shift between the *o*- and *m*-carbons are

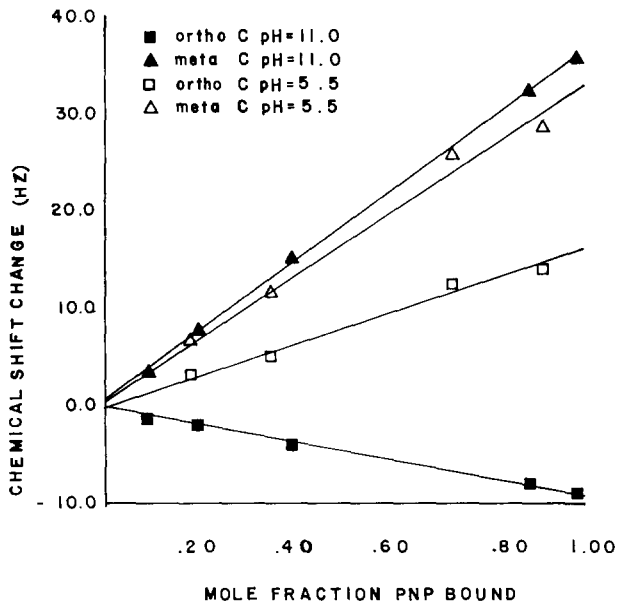

FIG. 3. Effects of cyclohexaamylose on the ^{13}C-nmr spectra of sodium *p*-nitrophenolate and *p*-nitrophenol. The cyclohexaamylose and sodium *p*-nitrophenolate concentrations varied between 0.009–0.05 and 0.025–0.09 *M*, respectively. The cyclohexaamylose concentration varied between 0.005 and 0.05 *M* and the *p*-nitrophenol was held constant as 0.025 *M*. The cyclohexaamylose-induced shifts are plotted relative to free *p*-nitrophenol and sodium *p*-nitrophenolate.

smaller 0.68 ppm and the changes are in the same direction. When the *p*-nitrophenol is 100% bound, the *o*- and *m*-carbons move upfield 1.31 and 0.63 ppm, respectively, from the free phenol. The greatest change then in chemical shift is observed for the *m*-carbon in the sodium *p*-nitrophenolate system.

DISCUSSION

In an earlier study, by observing both intermolecular NOE's and changes in chemical shifts in the ^1H-nmr of both the cyclohexaamylose and sodium *p*-nitrophenolate on complexation, we were able to show with considerable certainty the directions of substrate penetration. The sodium *p*-nitrophenolate enters from the C-2, C-3 side nitro group first. The ensuing discussion will be couched in these findings and focused on offering further proof of asymmetric substrate penetration.

Binding of Sodium p-*Nitrophenolate in the Cyclohexaamylose Cavity*

From the observed changes in both the ^1H and ^{13}C chemical shifts for the sodium *p*-nitrophenolate guest and the cyclohexaamylose host as the host/guest ratio is varied

as well as from the on and off rate constants (5.2×10^8 m^{-1} sec^{-1} and 1.3×10^5 sec^{-1}, respectively) (*2*), it is clear that the system is in the nmr chemical shift fast-exchange limit. That is, the *p*-nitrophenolate resonances appear at the average of the chemical shift of free *p*-nitrophenolate and the chemical shift of *p*-nitrophenolate bound in each possible orientation to cyclohexaamylose, weighted by the fractional population of *p*-nitrophenolate molecules in each environment. The cyclohexaamylose resonances also occur at its fast exchange position, weighted by the fraction of empty cyclohexaamylose molecules and the fraction of cyclohexaamylose molecules which have *p*-nitrophenolate guests.

In principle the sodium *p*-nitrophenolate guest can penetrate the cavity in only two different orientations, either oxygen first or nitro-group first (Fig. 4). The third orientation with penetrating *ortho* and *meta* position is unreasonable, simply because very

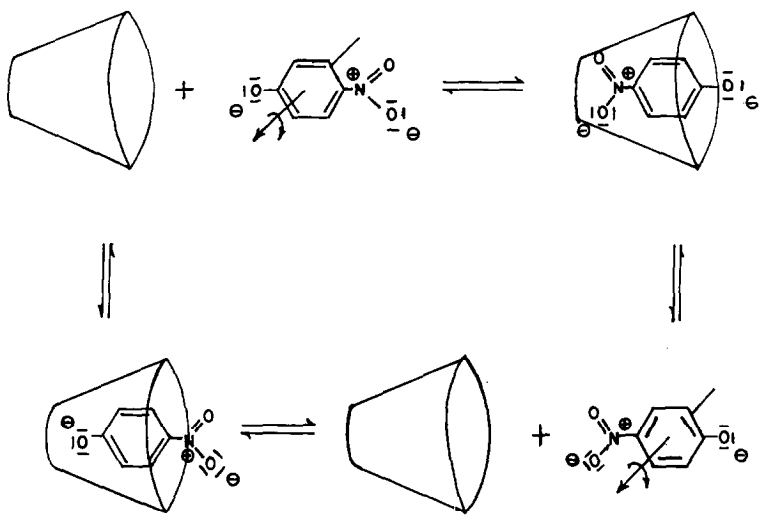

FIG. 4. Possible penetration modes of the cyclohexaamylose cavity by sodium *p*-nitrophenolate.

little of the substrate would fit into the cavity. If the penetration were random, both the *ortho*- and *meta*-carbon would experience similar environments and complexation-induced changes in chemical shift should be similar. If the sodium *p*-nitrophenolate penetrates the cyclohexaamylose cavity asymmetrically and selectively the *o*-carbon will experience a different environment than the *m*-carbons, a difference which should be reflected in their change in chemical shifts and should be more apparent as more of the substrate becomes bound. The carbon most intimately associated with the cavity should demonstrate the largest change in chemical shift. These ideas were well borne out in the ^{1}H-nmr experiments: the *m*-proton was deshielded by 0.30 ppm and the *o*-proton only by 0.16 ppm relative to the uncomplexed sodium *p*-nitrophenolate. In the ^{13}C-nmr spectra the *m*-carbons are deshielded by 1.43 ppm while the *o*-carbons are shielded by 0.28 ppm, again relative to the uncomplexed phenolate. The magnitude of these shifts is clearly copacetic with the asymmetric penetration concept. Furthermore, the *o* and *m* ^{13}C shifts are 1.75 and 4.76, respectively, as great as the corresponding ^{1}H shifts.

However, the direction of the ^{13}C shifts is somewhat surprising and cannot be explained on the basis of simple steric interaction (*14*). If the shielding were a case of simple steric polarization, the fact that both the *o*- and *m*-protons of the substrate are deshielded would lead one to believe the corresponding carbon resonances should be shielded. Since this sterically induced movement of the carbon resonances in the opposite directions to the corresponding hydrogen resonances has been demonstrated in a large number of systems (*15*), either the idea of opposite shifts of ^{13}C and ^{1}H is really not a general phenomenon or other shift mechanisms are operating more effectively. There are, however, any number of phenomena which could be contributing to the direction of the *meta*-carbons shift: microsolvent effects, substrate hydrogen bonding, selective stripping of substrate solvent, etc. Clearly, when the substrate penetrates the cavity, the included portion of the substrate experiences an environment entirely different from the environment of the bulk solvent. Furthermore, simple calculations reveal that on cavity penetration any solvent shell around the *p*-nitrophenol or sodium *p*-nitrophenolate would have to be stripped in order for the aromatic ring to fit into the cavity. This means that there is a type of specific solvent removal from the substrate; parts of the aromatic ring experience the bulk solvent and parts experience the cavity's environment. Concomitant with solvent stripping is a decrease in hydrogen bonding to the acceptor on the substrate which is inside the cavity.

On complexation by cycloamylose we observe that the *para*-carbon to which the hydrogen bond accepting nitro group is fixed is shielded. This would of course be in keeping with a decrease in hydrogen bonding of the bulk solvent to the nitro groups. The deshielding of the *m*-carbon could then be a type of β effect because of the *m*-carbon's position relative to the nitro group. Regardless of the mechanism responsible for the cycloamylose-induced changes in chemical shifts in the substrate, one fact is undeniable. If the substrate cyclohexaamylose penetration were equivalent, the *o*- and *m*-carbon would experience the same average environment and their shifts should be of similar magnitude. The shifts of the *o*- and *m*-carbons are clearly of different magnitudes.

Binding of p-*Nitrophenol in the Cyclohexaamylose Cavity*

The most noticeable features of the changes in the *p*-nitrophenol spectra (pH 5.5) are the similarity in magnitude of the changes in chemical shifts, of both the *o*- and *m*-carbons, the direction of the shifts, and the fact that the differences between the *o*- and *m*-carbon shifts are much smaller than in the sodium *p*-nitrophenolate system. If changes in hydrogen bonding to the substrate are responsible for the perturbation of the substrate's ^{13}C shifts, clearly *p*-nitrophenol would be expected to experience different effects because of its neutral charge. Although it is possible that the changes in the chemical shifts of the *p*-nitrophenol carbons reflect a more random substrate penetration, we have no evidence to suggest this as yet. It seems that if the penetration were random, the induced shifts would be more alike.

Sodium p-*Nitrophenolate- and* p-*nitrophenol-Induced Changes in the Cyclohexaamylose* ^{13}C-*nmr*

Owing to the simple symmetry of cyclohexaamylose, belonging to the C6 point group all of the constituent glucopyranose residues are chemically and physically indistinguishable. The ^{13}C-nmr is a simple six line spectra with the C-1 carbon at lowest field

followed by the C-4, C-2, C-3, C-5, and the C-6 carbon (*13*). There are at least four parameters which could contribute to changes in the ^{13}C chemical shifts experienced by the cycloamylose on complexation of the sodium *p*-nitrophenolate or *p*-nitrophenol substrates. These parameters are removal of water from the cavity, shielding by the guest's aromatic π cloud, conformational changes, and steric interactions.

If the magnetic fields of the aromatic ring and nitro π clouds were of significance in perturbing the carbon resonances, the direction and magnitude of these perturbations should be similar for given sets of carbons. In particular, carbons C-2 and C-3 should experience approximately similar fields as well as carbons C-1 and C-4, while C-5 and C-6 should experience different fields. The changes in chemical shift of carbons C-2 and C-3 are both different and in opposite directions and the same is true of carbons C-1 and C-4. Although this is not true of carbons C-5 and C-6, the fact that their shifts are so different from the shifts of the other carbons suggests that π cloud deshielding is unlikely. Again, because of the differences in magnitude and direction of the shifts, it seems unlikely that stripping of cavity-solvent water is a major consideration. Furthermore, although it is interesting that both carbons C-3 and C-5 move in the direction predicted for steric polarization, i.e., opposite to that observed for the protons, steric arguments do not explain the differences between the magnitudes and directions of the C-1, C-2, C-4, and C-6 shifts.

A number of workers have clearly demonstrated that the conformation of the cycloamylose cavity changes on substrate complexation. This phenomenon has been demonstrated in both solution studies and in the solid phase. The differences in magnitude and direction of the various carbon shifts would be in keeping with the differences in various bond distortions generated during the complexation process. The same arguments would certainly apply to the *p*-nitrophenol system as well.

The most notable difference between the pH 11.00 studies and the pH 5.5 work with respect to perturbations in the cycloamylose ^{13}C spectra is the difference in which carbons are now shielded or deshielded. In the sodium *p*-nitrophenolate system, the order of shielding is C-1 > C-2 > C-3 $\approx$ C-4 > C_5 > C_6 while with *p*-nitrophenol as a substrate the shielding order is $C_1 > C_2 > C_4 \approx C_5 > C_3 > C_6$. The C-3, C-4, and C-5 carbons change positions while C-1, C-2, C-5, and C-6 maintain the same relative order. Although less obvious, there is also a corresponding change in the magnitude of shifts with the *p*-nitrophenol inducing smaller shifts in the host molecule. If indeed the *p*-nitrophenol penetrates the cavity in a different fashion than sodium *p*-nitrophenolate, this would be reflected in the magnitude and direction of shifts.

Stoichiometry

In an earlier ^1H-nmr study by Konrad Bloch (Machida *et al.*, *16*), it was clearly demonstrated that several cycloamyloses could fit over a single substrate, e.g., palmitate. Although the palmitate chain is a great deal longer than *p*-nitrophenol, the aromatic substrate, because of its "width," presents an opportunity for stronger (tighter fitting) interaction with the cycloamylose cavity. Furthermore, our NOE studies implied that sodium *p*-nitrophenolate was not completely in the cavity and, therefore, it is possible that two cycloamyloses could be involved with the same *p*-nitrophenolate.

Depending on the ratio of *p*-nitrophenol or sodium *p*-nitrophenolate (M) to cyclohexaamylose (L) two host–guest stoichiometries are possible ML_2 or M_2L. At high

ratios of M to L, an M_2L complex could exist while at low ratios, i.e., high cycloamylose concentrations, an ML_2 complex is possible. Plots of the molar ratio of αCD/PNP versus the $\Delta\delta$ (change in chemical shifts of the *o* and *m* carbons); Fig. 5, clearly suggest ML_2 is an unlikely species. However, plots of the molar ratio of PNP/αCD versus $\Delta\delta$ (changes in the chemical shifts of the cycloamyloses carbons) are not so revealing. At a molar ratio of one for the cyclohexaamylose/sodium *p*-nitrophenolate system the sodium *p*-nitrophenolate is 88% bound and the *o*- and *m*-carbons have shifted 91 and 87%, respectively, of the shift they experience when the cyclohexaamylose/sodium

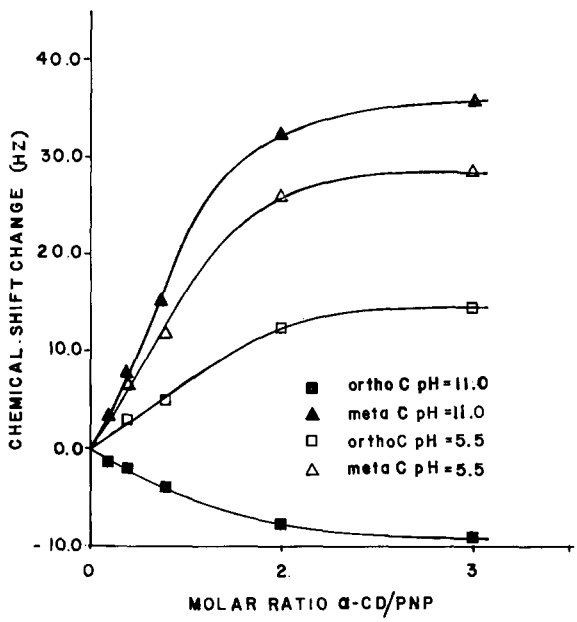

FIG. 5. Effects of cyclohexaamylose on the sodium *p*-nitrophenolate and on the *p*-nitrophenol ^{13}C-nmr. The cyclohexaamylose-induced shifts are plotted as a function of the molar ratio of cyclohexaamylose to the phenol or phenolate substrate.

p-nitrophenolate ratio is 2 (99% PNP bound) (Table 1.) This relationship between the percentage of substrate bound and the percentage change in the chemical shift is roughly maintained throughout the various αCD/PNP ratios for both the sodium *p*-nitrophenolate system and the *p*-nitrophenol system. The important feature is that after a molar ratio of one for αCD/PNP, no further substantial changes in chemical shift occur. These results are similar for *p*-nitrophenol and strongly suggest that after a one-to-one complex between sodium *p*-nitrophenolate or *p*-nitrophenol and cyclohexaamylose is formed further addition of cyclohexaamylose does not result in formation of an ML_2 system. More accurately, if an equilibrium exists of the type $ML + L \rightleftharpoons ML_2$, the constant must be very small if one assumes perturbations of similar magnitude are generated in the aromatic ring on ML_2 formation.

Similar considerations of the data for the cyclohexaamylose carbon shifts (Fig. 4) are difficult to interpret because of the apparent discontinuities in some of the curves.

TABLE 1

pH	Percentage of PNP bound	Percentage of *m* (shift)	Percentage of *o* (shift)	αCD/PNP
11.0	98	≈100.00	≈100.0	2.0
11.0	88	90	87	1.0
11.0	39	43	43	0.4
11.0	19	22	20	0.2
11.0	10	10	16	0.1
5.5	91	91	87	2.0
5.5	73	83	81	1.0
5.5	35	38	32	0.4
5.5	18	22	19	0.2

Note: The percentage of cycloamylose-induced shift in the ^{13}C signals of the *o* (*o* shift) and *m* (*m* shift) carbons of sodium *p*-nitrophenolate relative to the total possible shift approximated from the plots in Fig. 5 are tabulated against the percentage of bound phenolate (PNP bound) and the molar ratio of cyclohexaamylose to sodium *p*-nitrophenolate, (αCD/PNP).

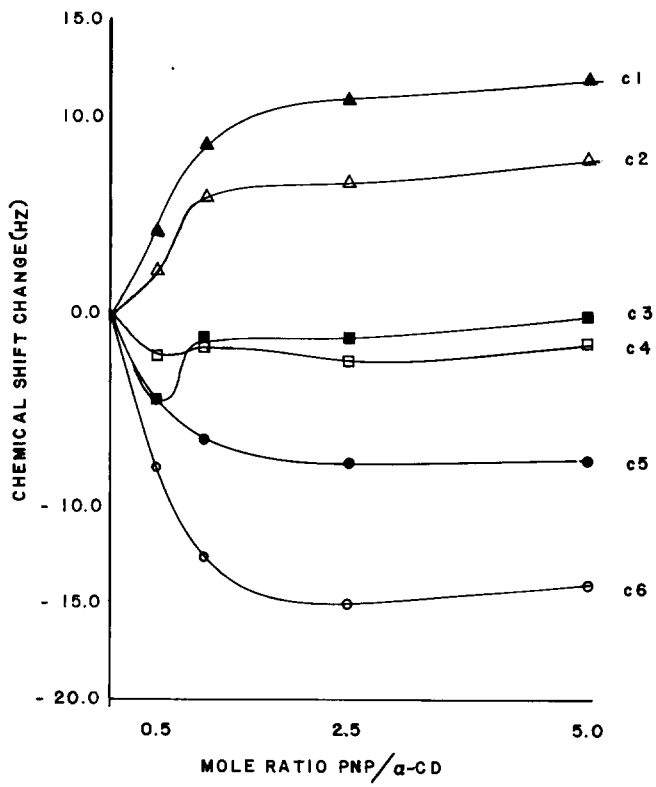

FIG. 6. Effects of sodium *p*-nitrophenolate on the ^{13}C-nmr spectra of cyclohexaamylose. The sodium *p*-nitrophenolate induced shifts are plotted as a function of the molar ratio of sodium *p*-nitrophenolate to cyclohexaamylose.

Dissociation Constants

The ^{13}C-nmr now provides an additional way to measure the dissociation constant for the complexes by observing the changes in chemical shift of the substrate (S) carbons as a function of added cycloamylose (C). This allows verification of other measurements. Consider first the equilibrium expression:

$$CS \overset{K_d}{\rightleftharpoons} C + S,$$

$$K_d = \frac{[S]\,[C]}{[CS]},$$

$$C_0 = C + CS, \tag{1}$$

$$S_0 = S + CS, \tag{2}$$

$$K_d = \frac{[S_0\text{-}CS]\,[C_0\text{-}CS]}{[CS]}. \tag{3}$$

The observed chemical shift will be given by

$$\delta_{obs} = \frac{S}{S_0}\,\delta_s + \frac{CS}{S_0}\,\delta_{CS}. \tag{4}$$

Combining Eqs. (2) and (4) produces

$$\delta_{obs} = \frac{(S_0\text{-}CS)\delta_s}{S_0} + \frac{CS}{S_0}\,\delta_{CS}. \tag{5}$$

The change in chemical shift is given by

$$\Delta\delta = \delta_{obs} - \delta_s. \tag{6}$$

Combining Eqs. (5) and (6) gives

$$\Delta\delta = \left(\delta_s - \frac{CS}{S_0}\,\delta_s + \frac{CS}{S_0}\,\delta_{CS}\right) - \delta_s,$$

which on rearrangement transforms to

$$\Delta\delta = \frac{CS}{S_o}\,(\delta_{CS} - \delta_s), \qquad \text{let } \delta_{CS} - \delta_s = Q.$$

Finally, CS is expressed as

$$\frac{\Delta\delta\, S_o}{Q} = CS. \tag{7}$$

Equation (3) is now rearranged to

$$K_d\,[CS] = (CS)^2 - C_0CS - S_0CS + C_0S_0 \tag{8}$$

and it is possible to solve for K_d two different ways. Equation (8) can be combined with Eq. (7) directly and the K_d solved by computer analysis or Eq. (8) can put in a form to allow for simple graphing. Experimentally, this is limiting because $[CS]^2 \ll C_0S_0$, which (8) transforms to

$$\therefore K_d[CS] = C_0S_0 - C_0CS - S_0CS. \tag{9}$$

Substituting Eq. (7) into Eq. (9) and rearranging generates

$$\frac{K_d}{Q} + \frac{C_0 + S_0}{Q} = \frac{C_0 S_0}{\Delta\delta}.$$

Our experimental conditions did not conform in every case to the $[CS]^2 \ll C_0S_0$ requirement for a linear plot. Equations (7) and (8) were combined and expressed in the below form:

$$f_i(QK_d) = C_o^i S_o^i - \frac{(C_o^i S_o^i \Delta\delta^i - S_o^{i2}\Delta\delta - K_d S_o^i \Delta\delta^i)}{Q} + \left(\frac{+S_o^i \Delta\delta^i}{Q}\right)^2,$$

where $i = 1, 2, 3, \ldots, n$.

The Q values were estimated from Fig. 3 and the K_d values were taken from the literature. A computer analysis was effected on this expression for S_0^i, C_0^i, and ΔS_0^i to minimize the absolute value of $\sum |f_i|$. The K_d for the sodium *p*-nitro-phenolate system is 2×10^{-4} and 1.3×10^{-3} M for the *p*-nitrophenol system. These values are in reasonable agreement with our reported values.

CONCLUSION

It is clear that ^{13}C-nmr can serve as a useful tool in determining the structure of cycloamylose substrate complexes in aqueous solution in addition to providing valuable information about their stoichiometries. More than this we have shown that these complexes offer an excellent opportunity to study some of the fundamental relationships between the intermolecular interactions and changes in the ^{13}C-nmr of both host and guest molecules. The cycloamylose systems offer several advantages in this respect. The position of a variety of substrates in the cavity has been defined by both X ray and nmr, the binding constants can be measured accurately by several methods, both the ^{13}C and ^{1}H spectra have been clearly assigned for these compounds and selective chemical modification of the systems can be effected with facility (*17*). Although this is only a preliminary report we are currently investigating in detail the reasons for these complexation-induced changes in chemical shifts.

ACKNOWLEDGMENTS

Acknowledgment is made to the Donors of The Petroleum Research Fund, administered by the American Chemical Society, for support (PRF 3127) G12 of this research.

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