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Importance of Cycloamylose Substrate Geometry and Dynamic Coupling in the Deacylation of 3- and 4-Nitrophenyl Acetates

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Abstract: The dynamic coupling of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate to cyclohexaamylose is determined using ^{13}C NMR. The coupling is evaluated in terms of the geometry of the respective complexes as determined by intermolecular nuclear Overhauser effects. The results suggest that the cyclohexaamylose-catalyzed hydrolyses of 3- and 4-nitrophenyl acetate proceed by different mechanisms.

Introduction

The cycloamyloses are cyclic oligosaccharides containing from 6 to 12 α-1,4-linked D-glucopyranose units.[1,2] They are synthesized by an enzyme induced in *Bacillus macerans* and *Bacillus megaterium* when the bacteria are grown on a culture media rich in amylose.[3] One of the most interesting properties of these oligosaccharides is their ability to complex a variety of different guest molecules in their hydrophobic interiors,[4,5] and, in some cases, catalyze the reaction of the guest molecule.[6,7] Because of these phenomena, the cycloamyloses have received a great deal of attention as enzyme active site models.

Recent efforts focused on the structural modification of the cycloamyloses have improved their catalytic abilities[8] and have expanded the scope of reactions they catalyze.[9] It is clear from these studies that the effectiveness with which the cycloamyloses catalyze a reaction can be related to (1) the position of the substrate in the cavity and (2) the tightness of substrate coupling to the cycloamylose. The importance of these geometric and coupling parameters is best seen in the work of Bender and Breslow.

Bender observed that the cyclohexaamylose-catalyzed hydrolysis of 3-nitrophenyl acetate was more effective than that of the corresponding 4-nitrophenyl acetate.[2,10] His explanation of these relative rates of hydrolysis focused on the differences in the structure of the cycloamylose substrate complexes. He suggested that both substrates bound in the cyclohexaamylose cavity at the 2,3-hydroxyl side nitro group first (Figure 1). This placed the acyl group of 3-nitrophenyl acetate closer to the cycloamylose's catalytic 2-hydroxyls and thus in a better position for transacylation than the acyl group of 4-nitrophenyl acetate.

Breslow, on the other hand, observed that by capping the cycloheptaamylose 6-hydroxyl side (i.e., putting a floor in the cavity) the catalytic hydrolysis of 3-nitrophenyl acetate was substantially more effective.[8] He attributed this improved catalysis to rotational restrictions imposed on the substrate, i.e., to a tighter coupling of the motions of the host and guest molecules.

In this investigation, we evaluate the position and coupling of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate in the cyclohexaamylose cavity. These complexes are used as models for the corresponding 3- and 4-nitrophenyl acetate cyclohexaamylose complexes. The substrate disposition is determined by ^1H homonuclear intermolecular nuclear Overhauser effects while the cycloamylose substrate coupling is determined by $^{13}C[^1H]$ T_1 measurements of the host and guest molecules. The results indicate that, although the geometry and coupling properties of the cyclohexaamylose 3-nitrophenyl acetate complex would be in accord with the suggested deacylation mechanism, the acyl group of 4-nitrophenyl acetate would be too far from the 2-hydroxyl groups to sustain catalytic hydrolysis.

Experimental Section

Materials. The cyclohexaamylose, 4-nitrophenol, 2,6-dimethyl-4-nitrophenol, and deuterium oxide (99.7%) were obtained from Aldrich Chemical Co. The 4-nitrophenol was crystallized from chloroform while the 2,6-dimethyl-4-nitrophenol was purified by high-vacuum sublimation.

Sample Preparation for ^1H Homonuclear Overhauser Enhancement (NOE) Experiments. The cyclohexaamylose hydroxyl protons were exchanged for deuterium by lyophilizing 600 mg of the carbohydrate from 40 mL of D_2O three times. This helps to minimize the HOD in the final sample. The buffer solutions were made up with anhydrous Na_3PO_4 in D_2O and the pD adjusted with deuteriophosphoric acid. The final pD value was 11.00 ± 0.02, $I = 0.5$. The pD value was obtained by adding 0.4 to the pH meter reading,[11] using an electrode

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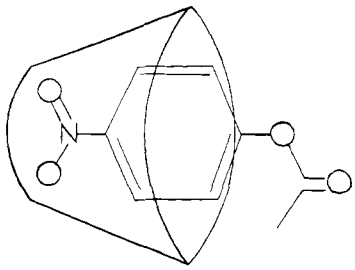


Figure 1. A schematic representation of 4-nitrophenyl acetate bound in the cyclohexaamylose cavity at the "wide" 2,3-hydroxyl side, nitro group first.

which had been standardized with pH 10.00 ± 0.01 and 4.01 ± 0.01 buffers in H_2O and then rinsed with D_2O.

Sample Preparation for Proton Decoupled ^{13}C Spin-Lattice Relaxation Experiments. The buffer solutions were made up using Na_3PO_4 (10% D_2O, 90% H_2O (v/v)) and the pH was adjusted with phosphoric acid to give pH 11.00, $I = 0.1$. The sample solutions of free sodium 4-nitrophenolate (0.10, 0.15, and 0.20 M), free sodium 2,6-dimethyl-4-nitrophenolate (0.10, 0.15, and 0.20 M), free cyclohexaamylose (0.10 M), and the corresponding cyclohexaamylose complexes (0.20 M substrate and 0.10 M cyclohexaamylose) gave final pH readings of 9.64 ± 0.02, $I = 0.5$. The substrate in both the sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate cyclohexaamylose complexes was 50% bound while the cyclohexaamylose was 99% bound (assuming K_D's of $3.7 \pm 0.2 \times 10^{-4}$ and $1.96 \pm 0.53 \times 10^{-4}$ M, respectively[12]).

Determination of Sample Viscosities. The viscosities of the samples used in the $^{13}C[^1H]$ spin-lattice relaxation experiments were determined using a calibrated Cannon-Finske Kinematic viscometer. The viscosities are calculated from the formula $\eta = Bt\rho$ where B is the calibration constant for the viscometer, t is the efflux time between the two marks, and ρ is the density of the solution. The temperature of the viscometer and sample was maintained at 25 ± 1 °C.

^1H Homonuclear Overhauser Enhancements (NOEs). The ^1H homonuclear Overhauser enhancements (NOEs) were determined on a Varian 100.1-MHz FT XL-100 spectrometer at 25 ± 1 °C. The concentration of sodium 2,6-dimethyl-4-nitrophenolate was 0.025 M while the cyclohexaamylose concentration was 0.050 M. Assuming a $K_D = 1.96 \pm 0.53 \times 10^{-4}$ M,[12] the substrate in the resulting solution was 99% bound by cyclohexaamylose. The NOE is reported as the percentage enhancement in the integrated intensity of the resonance being observed when the second radio frequency was first set to a vacant region in the spectrum and then applied at the radio frequency to be irradiated.

Determination of ^{13}C Spin-Lattice Relaxation Times. The proton-decoupled ^{13}C Fourier transform NMR spectra were obtained at 25.2 MHz on a Varian XL-100 spectrometer equipped with a Nicolet NT440 frequency synthesizer using 12-mm sample tubes maintained at 25 ± 1 °C. ^{13}C spin-lattice relaxation times were measured by the fast inversion recovery method (FIRFT)[13] with a pulse sequence of $(180°-\tau-90°-W)_n$ where W, the waiting time between pulses, was $\leq 5T_1$.

The values for K, a measure of imperfections in the rf pulse, M, the equilibrium magnetization, and T, the spin-lattice relaxation time, were determined by a nonlinear least-squares fit of the signal intensities and τ values to the expression[14]

$$M(\tau) = M_\infty(1 - (1 - K(1 - \exp(-w/T_1))\exp(-\tau/T_1)) \quad (1)$$

Results

Intermolecular Homonuclear Overhauser Effects in the Cyclohexaamylose–Sodium 2,6-Dimethyl-4-nitrophenolate Complex. A ^1H homonuclear Overhauser experiment was performed on the cyclohexaamylose–sodium 2,6-dimethyl-4-nitrophenolate complex (0.025 M substrate and 0.050 M cyclohexaamylose, i.e., 99% substrate bound). Irradiation of the C-3 methine protons at 100.1 MHz resulted in a substantial enhancement in the phenolate's meta protons, 22 ± 1%. However, owing to the width of the decoupler's power curve, we were unable to separate potential contributions from the C-5 methines. Although the C-6 methylene protons also fall under the power curve, because of the apparent distance between the methylene[12] and substrate protons as evidenced by the lack of shielding in the C-6 methylenes at high substrate concentrations, any NOE contributions from these are unlikely.

^{13}C Spin–Lattice Relaxation Times. The spin–lattice relaxation times for the ^{13}C nuclei of free cyclohexaamylose (0.10 M), free sodium 4-nitrophenolate (0.10, 0.15, and 0.20 M), free sodium 2,6-dimethyl-4-nitrophenolate (0.10, 0.15, and 0.20 M), and the respective phenolate cyclohexaamylose complexes (0.10 M cyclodextrin and 0.20 M substrate) are given in Table I.

The spin–lattice relaxation times, T_1's, for the ^{13}C nuclei of free phenolate or free cyclohexaamylose are dependent on the rate of reorientation of the molecules in solution, τ_c. Anything which influences this rate of reorientation, e.g., viscosity and/or molecular association, will affect the spin–lattice relaxation time.[14] Therefore, the T_1's given in Table I for the sodium phenolates, cyclohexaamylose, and their corresponding complexes have been viscosity corrected. These corrected values are given as $(T_1)^v_{free}$ and $(T_1)^v_{obsd}$ in Table II. The $(T_1)^v_{free}$ values refer to the viscosity corrected T_1's for the free substrate and cyclohexaamylose. The $(T_1)^v_{obsd}$ values refer to the viscosity-corrected T_1's for the corresponding complexes prior to 100% bound corrections.

Because the rates of reorientation between the free and bound states of the host and guest molecules differ, the T_1's observed for each ^{13}C nuclei are dependent upon the mole fraction substrate bound, α. Since the rate of dissociation is small compared to the rate of reorientation, the T_1's for 100% bound can be obtained from the relationship[15,16]

$$(T_1)^{-1}_{obsd} = \alpha(T_1)^{-1}_{complex} + (1 - \alpha)(T_1)^{-1}_{free} \quad (2)$$

The viscosity-corrected $(T_1)^v_{free}$ values obtained for cyclohexaamylose, sodium 4-nitrophenolate, and sodium 2,6-dimethyl-4-nitrophenolate, as well as the viscosity-corrected values, $(T_1)^v_{obsd}$, for cyclohexaamylose and the sodium nitrophenolates in their respective complexes were used to calculate values of $(T_1)^v_{complex}$, the spin–lattice relaxation times for 100% bound.

Correlation Times. Measurements of the NOEs of protonated carbons indicate that dipole–dipole interactions are of major importance in the relaxation of ^{13}C nuclei.[15,17] However, in the special case of CH_3 groups, spin rotation as well as dipole–dipole relaxation contributes to the T_1 values of ^{13}C nuclei.[15,17] The intermolecular dipole–dipole relaxation of a nucleus I_1, separated a distance r from a nucleus I_2, can be expressed as

$$(T_1)^{-1}_{D\text{-}D} = 3\gamma_1{}^2\gamma_2{}^2h^2I_2(I_2 + 1)r_{1,2}{}^{-6}\tau_c \quad (3)$$

where γ_1 and γ_2 are the gyromagnetic ratios for the respective nuclei and τ_c is the reorientation rate or correlation time.[18,19] The viscosity-corrected $(T_1)^v_{free}$ values for cyclohexaamylose (0.10 M), sodium 4-nitrophenolate (0.10, 0.15, and 0.20 M), and sodium 2,6-dimethyl-4-nitrophenolate (0.10, 0.15, and 0.20 M), as well as the viscosity-corrected $(T_1)^v_{complex}$ values generated from eq 2 for the sodium phenolates and cyclohexaamylose in their corresponding complexes, were used to calculate the respective τ_c's from eq 3. The resulting τ_c values are given in Table II.

Internal Rates of Rotation of the Sodium Phenolates Bound in the Cyclohexaamylose Cavity. Component analysis of local molecular motions has been accomplished by the use of correlation times obtained from NMR line shape analysis of –CHD–proton resonances.[20] Similarily, the analysis of local molecular motions can be accomplished using correlation times determined from ^{13}C spin–lattice relaxation times. In general, when the rotation is occurring among a large number of

Table I. The ^{13}C Proton Decoupled Spin-Lattice Relaxation Times for Both Free and Bound Cyclohexaamylose (αCD), Sodium 4-Nitrophenolate (I), and Sodium 2,6-Dimethyl-4-nitrophenolate (II), ± 8%

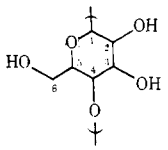
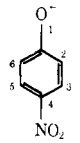
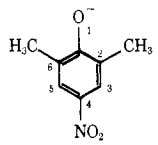
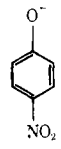
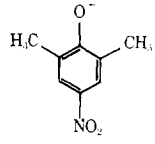
compd	concn	^{13}C spin-lattice relaxation times, s						
		1	4	3	5	2	6	$\langle T_1 \rangle_{1\text{-}5}$
	0.1	0.123	0.133	0.137	0.142	0.119	0.067	0.131
[αCD, I]	0.1, 0.2	0.108	0.118	0.120	0.127	0.127	0.073	0.120
[αCD, II]	0.1, 0.2	0.116	0.119	0.115	0.119	0.116	0.074	0.117
		2,6	3,5					$\langle T_1 \rangle_{2\text{-}6,3\text{-}5}$
 I	0.10	4.67	4.93					4.80
	0.15	3.62	3.59					3.61
	0.20	3.42	3.28					3.35
[I, αCD]	0.2, 0.1	1.38	1.38					1.38
		3,5	2,6-CH_3					
 II	0.10	1.93	4.19					
	0.15	1.36	3.38					
	0.20	1.41	3.52					
[II, αCD]	0.2, 0.1	0.434	1.59					

Table II. Viscosity-Corrected Spin-Lattice Relaxation Times and Rotational Correlation Times for Both Free and Bound Cyclohexaamylose (αCD) and the Sodium Nitrophenolates (I and II)

compd	concn	η, cP	$(T_1)_{free}$	$(T_1)^v_{free}$	$(T_1)_{obsd}$	$(T_1)^v_{obsd}$	$(T_1)^v_{complex}$	τ_c^v, ps
				α-CD Overall				
[αCD]	0.1	1.265	0.131	0.166				296
[αCD, I]	0.1, 0.2	1.357	0.131	0.166	0.120	0.163	0.163	302
[αCD, II]	0.1, 0.2	1.394	0.131	0.166	0.117	0.163	0.163	302
				Primary Hydroxyl				
[αCD]	0.1	1.265	0.067	0.085				289
[αCD, I]	0.1, 0.2	1.357	0.067	0.085	0.073	0.099	0.099	249
[αCD, II]	0.1, 0.2	1.394	0.067	0.085	0.074	0.103	0.103	240
				Substrate Overall				
[I]	0.10	0.974	4.80	4.68				10.5
[I]	0.15	0.985	3.61	3.55				13.9
[I]	0.20	1.012	3.35	3.39				14.5
[I, αCD]	0.2, 0.1	1.357	4.80	4.68	1.38	1.88	1.17	42.0
[I, αCD]	0.2, 0.1	1.357	3.61	3.55	1.38	1.88	1.27	38.7
[I, αCD]	0.2, 0.1	1.357	3.35	3.39	1.38	1.88	1.28	37.9
				Substrate Overall				
[II]	0.10	0.958	1.93	1.84				26.7
[II]	0.15	0.969	1.36	1.32				37.3
[II]	0.20	1.030	1.41	1.45				33.9
[II, αCD]	0.2, 0.1	1.394	1.93	1.84	0.434	0.606	0.362	136
[II, αCD]	0.2, 0.1	1.394	1.36	1.32	0.434	0.606	0.393	125
[II, αCD]	0.2, 0.1	1.394	1.41	1.45	0.434	0.606	0.382	129
				Methyls				
[II]	0.10	0.958	4.19	4.01				4.09
[II]	0.15	0.969	3.38	3.27				5.02
[II]	0.20	1.030	3.52	3.62				4.53
[II, αCD]	0.2, 0.1	1.394	4.19	4.01	1.59	2.21	1.52	10.8
[II, αCD]	0.2, 0.1	1.394	3.38	3.27	1.59	2.21	1.67	9.83
[II, αCD]	0.2, 0.1	1.394	3.52	3.62	1.59	2.21	1.59	10.3

equilibrium positions, the rate of internal rotation, τ_i^{-1}, around a bond which is in turn rotating at a overall rate, τ_m^{-1}, results in an effective correlation time, τ_c, given by the relationship[20]

Table III. Coupling Coefficients and Internal Rates of Rotation for the Sodium 4-Nitrophenolate- and Sodium 2,6-Dimethyl-4-nitrophenolate-Cyclohexaamylose Complexes

compd	ξ	τ_i, s	τ_i^{-1}, s^{-1}
	0.14	6.68×10^{-11}	1.50×10^{10}
	0.13	5.99×10^{-11}	1.67×10^{10}
	0.13	5.83×10^{-11}	1.72×10^{10}
	0.45	4.15×10^{-10}	2.41×10^{9}
	0.41	3.52×10^{-10}	2.84×10^{9}
	0.43	3.75×10^{-10}	2.67×10^{9}

$$\tau_c = A\tau_m + B\left(\frac{1}{\tau_m} + \frac{1}{\tau_i}\right)^{-1} + C\left(\frac{1}{\tau_m} + \frac{4}{\tau_i}\right)^{-1} \quad (4)$$

In this equation A, B, and C are the geometric parameters:

$$A = {}^1/_4(3\cos^2\theta - 1)^2$$

$$B = {}^3/_4\sin^2 2\theta$$

$$C = {}^3/_4\sin^4\theta$$

where θ is the angle between the relaxation vector or the main field gradient and the principal axis of rotation. The principal axis of rotation for the sodium nitrophenolate-cyclohexaamylose complexes passes through the C_6 symmetry axis of the cyclodextrin cavity and the C_2 symmetry axis of the cyclodextrin cavity and the C_2 symmetry axis of the substrate, i.e., through the C-1 and C-4 carbons of the sodium phenolate molecules. The relaxation vector for the sodium phenolates lies along the C–H bonds and make an angle of 60 or 120° with the rotational axis of these substrates inside the cyclohexaamylose molecule.

The rate of internal rotation for the phenyl ring of the sodium nitrophenolates was calculated from the overall correlation time, τ_m, of the cyclohexaamylose and the effective correlation time, τ_c, of the sodium phenolates at 100% substrate bound. From the values of the effective local correlation time, τ_c, and the overall correlation time, τ_m, it is possible to determine the degree of coupling between these two rotations. The coupling coefficient, ξ, is the ratio τ_c/τ_m and varies from 0.11 to 1.[20] When the rate of overall rotation is approximated by the rate of internal rotation, the value of ξ approaches 1, its maximum coupling value. However, if the rate of overall rotation is slow compared to that of internal rotation, ξ approaches its minimum coupling value. The values of ξ as well as τ_i^{-1} are given in Table III.

The Effects of Binding on the Spin–Lattice Relaxation and Correlation Times of Cyclohexaamylose. Although there has been some disagreement in the past as to the assignments of the ^{13}C NMR spectrum of cyclohexaamylose, in this investigation we use those of Jennings and Smith.[21] On this basis, the carbon signals in going from the least to the most shielded are C-1 < C-4 < C-3 < C-2 < C-5 < C-6.

The ^{13}C nuclei spin–lattice relaxation times, T_1's, for C-1 through C-5 as determined in a 0.10 M solution in phosphate buffer (pH 9.64; I = 0.5 at 25 ± 1 °C) are all within experimental error of one another and thus are combined as the average $\langle T_1\rangle_{1\text{-}5}$, 0.131 s (Table I). The T_1 for the C-6 primary hydroxyl carbon, determined under identical conditions, is somewhat shorter, 0.067 s. The T_1's for the free cyclohexaamylose were previously determined at 33 ± 2 °C to be 0.144 s for C-1 through C-5 and 0.105 s for C-6; however, the pH was not given nor was the viscosity taken into account. Consequently, no comparison can be made between these values and those reported.[15] The overall viscosity-corrected correlation time, τ_c^v (Table II), for free cyclohexaamylose (0.10 M; pH 9.64; I = 0.5 at 25 ± 1 °C) is 296 ps, while the viscosity-corrected effective local correlation time, τ_c^v, for the C-6 primary hydroxyls is 289 ps. The coupling coefficient, ξ, the ratio of the C-6 effective local correlation time to the overall correlation time, is 0.98. This clearly indicates strong coupling between the two rotations.

The overall spin–lattice relaxation times for cyclohexaamylose in both the sodium 4-nitrophenolate and the sodium 2,6-dimethyl-4-nitrophenolate complexes are 0.163 s (0.10 M cyclohexaamylose, 0.20 M sodium phenolate; pH 9.64; I = 0.5 at 25 ± 1 °C). These values are within experimental error of $(T_1)^v_{free}$ for cyclohexaamylose. The spin–lattice relaxation times for the primary hydroxyls of cyclohexaamylose in the sodium 4-nitrophenolate and the sodium 2,6-dimethyl-4-nitrophenolate complexes are 0.099 and 0.103 s, respectively (0.10 M cyclodextrin, 0.20 M sodium phenolate; pH 9.64; I = 0.5 at 25 ± 1 °C). Similarly, these values are within experimental error of their corresponding $(T_1)^v_{free}$ values.

The Effects of Binding on the Spin–Lattice Relaxation and Correlation Times of the Sodium Phenolates. The ^{13}C NMR spectrum of sodium 4-nitrophenolate was assigned in an earlier paper.[22] From lower to higher field, the ^{13}C resonances are C-3 = C-5 < C-2 = C-6.

The absence of NOE enhancements in the ^1H decoupled spectrum of the ipso carbons of sodium 2,6-dimethyl-4-nitrophenolate as well as the large chemical shift differences between the aromatic and methyl ^{13}C nuclei made the assignment of the C-3, C-5, and methyl carbons fairly simple. The ^{13}C resonances from lower to higher field are C-3 = C-5 ≪ methyl carbons.

The overall T_1's for the free sodium 4-nitrophenolate (0.10, 0.15, and 0.20 M; pH 9.64; I = 0.5 at 25 ± 1 °C) were determined from the average of the T_1's observed for the C-2, C-3, C-5, and C-6 carbons since all were within experimental error of one another ($\langle T_1\rangle_{2\text{-}6,3\text{-}5}$ = 4.80 s at 0.10 M, 3.61 s at 0.15 M, and 3.35 s at 0.20 M). The T_1 for free sodium 2,6-dimethyl-4-nitrophenolate (0.10, 0.15, and 0.20 M; pH 9.64; I = 0.5 and 25 ± 1 °C) was determined from the T_1's observed for the C-3 and C-5 carbons ($\langle T_1\rangle_{3\text{-}5}$ = 1.93 s at 0.10 M, 1.36 s at 0.15 M, and 1.41 s at 0.20 M).

It is clear that the viscosity-corrected $(T_1)^v_{free}$ values for the sodium 4-nitrophenolates show a decrease in going from 0.20 to 0.10 M solutions. However, because of experimental error a clear monotonic relationship is difficult to establish at all three concentrations.

As a result of complexation with cyclohexaamylose the values obtained for the sodium nitrophenolates $(T_1)^v_{complex}$ calculated from eq 2 are significantly shorter than their $(T_1)^v_{free}$ values. In the sodium 4-nitrophenolate-cyclohexaamylose complex the T_1's decrease by a factor of 2.6 while in the sodium 2,6-dimethyl-4-nitrophenolate-cyclohexaamylose complex the T_1's decrease by a factor of 3.4.

The τ_c^v values for both free sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate were determined at three concentrations (0.10, 0.15, and 0.20 M) from the corresponding $(T_1)^v_{free}$ values. Using eq 2, $(T_1)^v_{complex}$ was calculated at 100% bound for each substrate at each concentration. These τ_c^v values for the substrates (effective local correlation time of substrate at 100% bound, τ_c) and the τ_c^v values obtained for cyclohexaamylose (overall correlation time of cycloamylose, τ_m) were then used to calculate τ_i^{-1} from eq 4 (see Table III).

The coupling coefficient, ξ, the ratio of effective local correlation time, τ_c, for sodium 4-nitrophenolate (37.9 ps) to that of the overall correlation time, τ_M, for cyclodextrin (302 ps) in the sodium 4-nitrophenolate cyclohexaamylose complex is

0.13. This indicates weak coupling between substrate and cavity. However, the coupling coefficient, ξ, of the effective local correlation time, τ_c, for sodium 2,6-dimethyl-4-nitrophenolate (125 ps) to that of the overall correlation time for cyclodextrin, τ_M (302 ps), in the sodium 2,6-dimethyl-4-nitrophenolate–cyclohexaamylose complex is at least 0.41. Thus in this case fairly strong coupling between the substrate and cavity is indicated. Finally, the internal rate of rotation, τ_i^{-1}, for sodium 4-nitrophenolate is at least 6.2 times faster than that obtained for sodium 2,6-dimethyl-4-nitrophenolate.

Only an upper limit may be calculated for the correlation times of the methyl groups of sodium 2,6-dimethyl-4-nitrophenolate since spin-rotation relaxation contributes to the relaxation of these ^{13}C nuclei.[15,17] However, it is clear that the effective correlation times of the methyl groups of free sodium 2,6-dimethyl-4-nitrophenolate increase by a factor of 2.4 on complexation with cyclohexaamylose.

Discussion

Structure of Sodium 4-Nitrophenolate– and Sodium 2,6-Dimethyl-4-nitrophenolate–Cyclohexaamylose Complexes in Aqueous Solution. In an earlier investigation we were able to determine the dissociation constants and the stoichiometries of the sodium 4-nitrophenolate– and sodium 2,6-dimethyl-4-nitrophenolate–cyclohexaamylose complexes by observing the complexation-induced changes in the ^1H NMR spectra of the host and guest molecules. Both are 1:1 complexes[12] with dissociation constants of $3.7 \pm 0.2 \times 10^{-4}$ and $1.96 \pm 0.5 \times 10^{-4}$ M, respectively. In these same studies, we determined that the substrates bound at the 2,3-hydroxyl side of the cycloamylose cavity.

Intermolecular homonuclear Overhauser enhancement experiments definitively showed that the nitro group of the 4-nitrophenolate penetrated the cavity to the extent that the meta protons were in contact with the cyclohexaamylose's C-3 methines and no deeper.[23] Complexation-induced chemical shift changes in the host and guest molecules suggested a similar structure for the sodium 2,6-dimethyl-4-nitrophenolate complex; however, definitive NOE evidence for this complex was not available at the time. The present study provides this additional evidence and confirms the structure of this complex. The 22% enhancement of the sodium 2,6-dimethyl-4-nitrophenolate's meta proton signal on irradiation of the cyclohexaamylose cavity protons confirms the substrate disposition to be very similar to that of the cyclohexaamylose–sodium 4-nitrophenolate complex. The sodium 2,6-dimethyl-4-nitrophenolate also binds at the 2,3-hydroxyl side of the cyclohexaamylose cavity penetrating nitro group first. Having established both the stoichiometry and geometry of these cycloamylose substrate complexes in aqueous solution, the dynamic coupling properties could now be evaluated.

Earlier cycloamylose–substrate dynamic coupling studies on the sodium 4-methylcinnamate– and sodium 4-*tert*-butylphenolate–cyclohexaamylose complexes were carried out without such information. The workers assumed 1:1 cycloamylose–substrate stoichiometries and, further, that the aromatic ring and not the carboxylate end of the substrate was bound in the cavity. However, Laufer[24] has recently shown that two molecules of cyclohexaamylose complex one molecule of sodium 4-methylcinnamate. In addition, we have demonstrated that the carboxylate end of sodium 3,5-dimethyl-4-hydroxycinnamate binds in the cyclohexaamylose cavity at the 2,3-hydroxyl side.[25] These findings make the initial dynamic coupling experiments somewhat difficult to interpret.

Dynamic Coupling of Sodium 4-Nitrophenolate and Sodium 2,6-Dimethyl-4-nitrophenolate to Cyclohexaamylose. The spin–lattice relaxation times for sodium 4-nitrophenolate, sodium 2,6-dimethyl-4-nitrophenolate, and cyclohexaamylose as well as those for the nitrophenolates in their respective cycloamylose complexes were all viscosity corrected. Therefore, the differences between the τ_c^v values of the free and bound states represent actual differences in rates of rotation due to complexation and not changes in the bulk viscosity. The viscosity-corrected $(T_1)^v_{free}$ values for both substrates are shortened by ~25% on increasing the concentration of free substrate from 0.10 to 0.20 M. This is probably due to self-association of the substrates. Therefore, the reported τ_c^v values are likely to be somewhat smaller than the actual values. This means that the actual coupling between the sodium nitrophenolates and cyclohexaamylose is likely to be somewhat greater than the apparent coupling.

The correlation times for free sodium 2,6-dimethyl-4-nitrophenolate are longer than those for sodium 4-nitrophenolate. This suggests that the added bulk of the methyl groups tends to slow down the rotation of this molecule in aqueous solution. In addition, comparison of the effective local correlation times reveals that on cyclohexaamylose complexation the rate of rotation of sodium 4-nitrophenolate decreases by a factor of 3 while that of sodium 2,6-dimethyl-4-nitrophenolate is reduced by a factor of 4. A comparison of the internal rates of rotation, τ_i^{-1}, of sodium 4-nitrophenolate and sodium 2,6-dimethyl-4-nitrophenolate suggests that the methyl groups of sodium 2,6-dimethyl-4-nitrophenolate are in intimate contact with the cyclohexaamylose's 2- and 3-hydroxyl groups. The sodium 4-nitrophenolate rotates 6.2 times faster in the cyclohexaamylose cavity than sodium 2,6-dimethyl-4-nitrophenolate (1.5×10^{10} vs. 2.41×10^9 s^{-1}, respectively).

In addition, it is clear that the methyl groups of 2,6-dimethyl-4-nitrophenolate rotate more slowly when the substrate is bound. This further suggests intimate contact between the substrate's methyl groups and the cyclohexaamylose's 2,3-hydroxyls. However, because of spin rotation contributions, the apparent τ_c^v values calculated for the methyl groups of sodium 2,6-dimethyl-4-nitrophenolate are likely to be somewhat longer than the actual values.

The Position of Sodium 4-Nitrophenolate in the Cyclohexaamylose Cavity as Related to Catalysis. It was not possible to carry out these experiments on the nitrophenyl acetates cyclohexaamylose complexes because of the low solubility of the acetates. Because it is likely that the aromatic rings of the nitrophenyl acetates and sodium nitrophenolates adopt the same position in the cyclohexaamylose cavity we have extrapolated our present findings with the nitrophenolates complexes to the nitrophenyl acetate complexes.

The NOE data clearly demonstrates that sodium 4-nitrophenolate binds in the cyclohexaamylose cavity at the 2,3-hydroxyl side nitro group first. The sodium 4-nitrophenolate penetrates the cavity to the extent that the meta protons are in contact with the cyclohexaamylose's C-3 methine protons and no deeper. This solution binding geometry is similar to that observed in the solid phase as evidenced by a recent X-ray study of the cyclohexaamylose–4-nitrophenol complex.[26] Therefore, in the case of the cyclohexaamylose–4-nitrophenyl acetate complex, if the substrate were binding in the cyclohexaamylose cavity nitro group first at the 2,3-hydroxyl side deacylation could not occur. The acyl group of the substrate and the cycloamylose's active hydroxyls are too far from each other. Furthermore, the poor coupling between the sodium 4-nitrophenolate and the cyclohexaamylose suggests that the 4-nitrophenyl acetate would also be poorly coupled to the cyclohexaamylose.

In contrast, the geometry and coupling properties of the cyclohexaamylose–sodium 2,6-dimethyl-4-nitrophenolate system are much more favorable for deacylation. The NOE data indicates that the sodium 2,6-dimethyl-4-nitrophenolate sits in the cyclohexaamylose cavity in the same orientation as the sodium 4-nitrophenolate. Furthermore, the ^{13}C dynamic coupling experiment places the methyl groups of sodium

2,6-dimethyl-4-nitrophenolate in intimate contact with the 2,3-hydroxyl groups. The ^{13}C T_1 data shows that the methylated sodium nitrophenolate is more tightly coupled to the cavity than parent sodium nitrophenolate, $\xi = 0.41$ vs. $\xi = 0.13$. This coupling is likely due to an interaction between the substrate's methyl groups and the cavity's 2,3-hydroxyl groups. This interaction is further evidenced by a decrease in the spin rate of the substrate's methyl groups on cycloamylose complexation. This evidence implies that the acyl group of 3-nitrophenyl acetate would be very close to the active 2-hydroxyl group if the substrate bound in the cavity nitro group first at the 2,3-hydroxyl side. Furthermore, if methyl groups are so effective in increasing cycloamylose substrate coupling the somewhat larger acyl groups should be even more effective in assisting cycloamylose substrate coupling.

Consequently, the observation that 3-nitrophenyl acetate is deacylated by cyclohexaamylose faster than the 4-nitrophenyl acetate can be attributed to both the steric relationship of the reactive groups and at least in part to the reduced motion of the substrate. The origins of the small catalytic effect observed in the deacylation of the 4-nitrophenyl acetate by cyclohexaamylose must be attributed to some mechanism other than that suggested in the literature.[2,10] The 4-nitrophenyl acetate must either penetrate the cavity more deeply than the corresponding sodium 4-nitrophenolate on cyclohexaamylose complexation, or the aromatic ring of 4-nitrophenyl acetate must improve on the leaving ability of the 4-nitrophenolate. This stabilization of the leaving group has some basis in the observation that the pK_a of 4-nitrophenol is 1 pK_a unit lower when complexed by cyclohexaamylose.[27]

Conclusion

The results suggest that the cyclohexaamylose deacylations of 4-nitrophenyl and 3-nitrophenyl acetate proceed by different mechanisms. The present evidence as well as recent X-ray studies shows that, unless the 4-nitrophenyl acetate penetrates the cyclohexaamylose cavity substantially further than the corresponding nitrophenol or nitrophenolate, its acyl group is simply too far from the cyclohexaamylose's catalytic 2-hydroxyls to react. An alternative explanation of the observed catalytic effect suggested by pK_a studies is that the complexation of the nitrophenol segment of 4-nitrophenyl acetate increases the stability of the leaving group.[27]

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Electron Spin Resonance Studies on Radiolysis of Crystalline Methanol at 4.2 K

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Abstract: Radiolysis of crystalline methanol and the structure of radicals produced have been studied at 4.2 K using electron spin resonance spectroscopy. It has been observed for the first time that $CH_3\dot{O}$ and $\dot{C}H_3$ are formed as major products together with conventional $\dot{C}H_2OH$ in pure CH_3OH irradiated at 4.2 K. The direct evidence was obtained that both $CH_3\dot{O}$ and $\dot{C}H_3$ convert into $\dot{C}H_2OH$ below 77 K. Intramolecular hydrogen atom transfer is suggested for the conversion from $CH_3\dot{O}$ into $\dot{C}H_2OH$. Besides these isolated radicals, considerable amounts of radical pairs between $\dot{C}H_3$ and $\dot{C}H_2OH$ were formed. $\dot{C}H_3$ in this pair also converts into $\dot{C}H_2OH$ forming radical pairs between $\dot{C}H_2OH$ below 77 K. The change in the radical pair separation indicates that CH_3 abstracts a hydrogen atom from the neighboring CH_3 group forming $\dot{C}H_2OH$. It is suggested that the radical pairs are formed from recombination of an electron and a cationic species. The electronic and geometrical structure of $CH_3\dot{O}$ has also been discussed. The radical is the oxygen-centered π radical and the CH_3 group undergoes tunneling rotation at 4.2 K. From the A- and E-line splittings, B_0 and B_2 in the $\cos^2\theta$ rule for the β proton coupling in the oxygen-centered π radicals have been estimated to be 5 and 94 G, respectively.

In recent years, we have been studying the solid-state radiolysis of organic compounds at cryogenic temperatures (4.2 K) with electron spin resonance (ESR) spectroscopy.[1-5] It becomes gradually clear that the solid-state radiolysis at temperatures lower than 77 K involves two important phases: the one is that migration of hydrogen atoms produced by ra-

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