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NMR STUDY ON THE COMPETITIVE INCLUSION OF SUBSTRATE AND DETERGENT INTO CYCLOHEPTAAMYLOSE CAVITY

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The hydrolyses of 4-nitrophenyl and 2,4-dinitrophenyl sulfates have been accelerated in the presence of a functional detergent such as *N,N*-dimethylhexadecylamine or *N,N*-dimethyl-*N*-hexadecyl-*N*-(4-imidazolium)methylammonium dichloride even at concentrations below cmc, as well as in the presence of cycloheptaamylose (β-CD). Nevertheless, the presence of both β-CD and functional detergent resulted in the diminished rate of hydrolysis of the esters relative to the individual catalysis. Through NMR study on the inclusion behavior of the detergent into the β-CD cavity, the inhibiting effect of β-CD in the hydrolysis of aryl sulfates catalyzed by the functional detergents was ascribed to the competitive inclusion of the substrate and detergent molecules into β-CD.

The hydrolyses of 4-nitrophenyl and 2,4-dinitrophenyl sulfates were accelerated fourfold and eightfold, respectively, by cycloheptaamylose (β-CD) at pH 9.98 and 50.3° C.[1] Congdon and Bender suggested[1] that the acceleration effect was caused by the induced strain within the substrate molecules upon their inclusion into the β-CD cavity, and not by the covalent participation of secondary hydroxyl groups situated in the interior cavity.[2] On the other hand, the bimolecular nucleophilic participation of amines have been well examined[3,4] in nonenzymatic hydrolysis of aryl sulfates. These previous results prompted us to attempt to establish a simple holoenzyme model system which consists of an apoenzyme model providing only the binding site for the sulfates and a coenzyme function acting as the catalysis center. Therefore, we selected β-CD as the former model and functional detergents such as *N,N*-dimethylhexadecylamine (DMCA) and *N,N*-dimethyl-*N*-hexadecyl-*N*-(4-imidazolium)methylammonium dichloride (Im-I) as the latter. Contrary to what would be expected, the rate of nucleophilic decomposition of aryl sulfate esters which was originally catalyzed by these functional detergents have been inhibited upon addition of β-CD. This was attributed to the competitive inclusion of dertergent and substrate molecules into the cycloamylose cavity without forming the effective ternary complex. In this paper we provide the evidence for the favorable incorporation of detergent molecule within cycloheptaamylose cavity through NMR study.

4-Nitrophenyl (PNPS) and 2,4-dinitrophenyl sulfates (DNPS) have been prepared by the method of Fendler and Fendler[5] with some modifications. In order to avoid the

formation of the *N*-substituted anilines upon the C-O cleavage by the nucleophilic attack of amines on the aromatic carbon atom,[3] tertiary amines, DMCA[6] and Im-I,[7] were used. Cycloheptaamylose (β-CD)[8] was a gift of the Research Institute of Teijin Co., Ltd. Concentrations of β-CD and DMCA had to be at least tenfold greater than the initial substrate concentration to ensure the significant rate acceleration under the present reaction conditions. Both DMCA and Im-I were used at the concentration ranges lower than their cmc.[9] A Hitachi-124 recording spectrophotometer with the thermostatted cell compartment (±0.1°C) was used for kinetic assay following the procedures described in literatures.[3,5] Table 1 gives observed first-order rate constants for hydrolyses of both sulfate esters in the presence or absence of β-CD and functional detergents.

Table 1. Inhibition with cycloheptaamylose (β-CD) in the hydrolyses of 4-nitrophenyl (PNPS) and 2,4-dinitrophenyl sulfates (DNPS) catalyzed by functional detergents, DMCA and Im-I

10^5[DMCA] M	10^5[β-CD] M	$10^2 k_{obs}$ min^{-1}	10^5[Im-I] M	10^5[β-CD] M	$10^2 k_{obs}$ min^{-1}
$[PNPS]_0=1.17\times10^{-5}$ M and 1.10N-NaOH in aqueous solution at 90.5°C			$[DNPS]_0=1.011\times10^{-5}$ M and 0.118N-NaOH in 0.73%(v/v) aqueous acetonitrile at 40.0°C		
None	None	2.1	None	None	0.63
10.5	None	3.6	0.92	None	1.24
10.5	25.6	3.4	0.92	37.7	0.94
10.5	205	3.0	None	37.7	0.99
None	20.5	2.8			
None	51.2	5.3			

For both sulfate esters, the rate of hydrolysis was, a little but obviously, accelerated in the presence of β-CD or detergents in cases where these respective catalysts were independently used. In the presence of both catalysts, β-CD and Im-I or β-CD and DMCA, however, the inhibition with β-CD was apparently observed. The result suggests that the functional detergents used may be more favorably incorporated into β-CD cavity[10] than the substrate due to the greater hydrophobic nature of the former relative to the latter. The incorporation may prevent the catalytic detergents from the nucleophilic attack on the substrate. If this is the case, the NMR chemical shift of protons situated in the interior cavity of the host molecule may be affected upon the incorporation of guest molecules.[11a] NMR chemical shifts were measured in D_2O at 100 MHz relative to TMS as the external reference and the accuracy was maintained within ±2 Hz. The measurements were run on a JEOL MH-100 spectrometer equipped with a frequency counter. Signal assignments were made with reference to the data previously reported,[11] and on the basis of chemical shift behaviors and decoupling experiments.

Demarco and Thakkar have stated[11a] that H-3 and H-5 ptorons of β-CD directed toward its interior are strongly shielded because of the anisotropy of aromatic nucleus upon incorporation of aromatic substrates. NMR spectra (Fig. 1) are closely similar to those presented by Demarco and Thakkar.[11a] It is evident that signals of H-3 (437 Hz) and H-5 (424 Hz) shifts progressively to higher field as the molar concentration

Table 2. Chemical shift changes for β-CD protons upon incorporation of detergents, DMCA and Im-I

Molar ratio of detergent to β-CD	β-CD protons, Hz H-3	H-5
β-CD alone[a)]	437	424
[DMCA]/[β-CD][b)]		
0.48	431	411
0.99	422	408
5.02	421	408
[Im-I]/[β-CD][c)]		
0.49	433	417
1.21	430	412
5.55	421	409

a) Chemical shifts for other protons scarcely changed upon the formation of inclusion complex: H-1, 542; H-2, 404; H-4, 398; and H-6, 428 Hz, respectively. $J_{2-3}=J_{4-5}=J_{3-4}=8.5$ Hz and $J_{1-2}=3.5$ Hz.

b) At 40.0°C, [β-CD]=0.8×10^{-2} M in D_2O.

c) At 34.0°C, [β-CD]=1.0×10^{-2} M in D_2O.

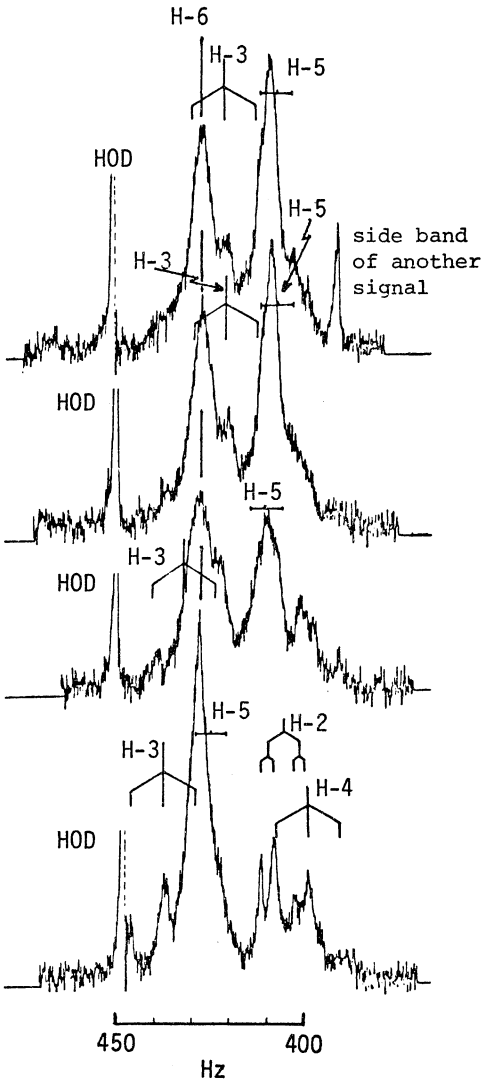

Fig. 1. 100 MHz ^1H NMR spectra of β-CD in D_2O (0.8×10^{-2} M) at 40.0°C containing various amounts of DMCA.

of DMCA is increased relative to β-CD. Im-I also gave out a similar effect. The extent of the induced shifts of these proton signals (Table 2), in addition, are large ($\Delta\delta \simeq +16$ Hz) and comparable to those observed for the aromatic guest molecules.[11a)] In aqueous solution since one or more water molecules are placed within the cavity,[12)] the interior of β-CD may be a little polar at first. Through penetration of a hydrophobic guest molecule into the cavity, however, the interior may become more apolar upon extrusion of water molecules. The above chemical-shift behavior for β-CD protons, therefore, definitely suggests that the induced up-field shifts should be ascribed not to the shielding effect due to guest molecules but to the change of atmosphere of the interior, the well-recognized hydrophobic effect. Since both DMCA and Im-I had to be used at the concentration higher than their cmc's due to experimental reasons in NMR studies, a question arises as to whether or not β-CD is entrapped into the detergent micellar phase. If this is the case, however, NMR signals for protons located at the exterior of the torus (H-1, H-2, and H-4) must also be strongly affected due

to the micellar effect. The present results may preclude such behavior.

It is also important to point out that the induced up-field shifts of interior protons of β-CD upon incorporation of aromatic guest molecules[11a] may be not only due to the anisotropy of aromatic nucleus but primarily to the micro-solvent effect.[12]

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