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GROUP-RECOGNITION ABILITY OF DERIVATIZED β-CYCLODEXTRIN AS STUDIED BY THE ESR SPIN PROBING TECHNIQUE

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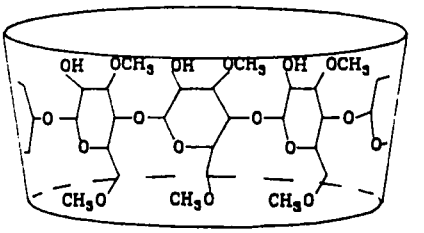
The effect of modifying the entrance face of β-cyclodextrin on the inclusion of various functional groups in aminoxyl spin probes was determined by the use of heptakis (2,6-O-dimethyl)-β-cyclodextrin and heptakis (2,3,6-O-triacetyl)-β-cyclodextrin. The methylation of six of the secondary hydroxyl groups on the wider end of β-cyclodextrin has a variable effect on the association constant of inclusion depending on the nature of the included group. The role of the methoxy group on the rim is discussed on the basis of the thermodynamic data derived from association constants. Contrary to the unmodified β-cyclodextrin the pH of the solution does not influence the inclusion behaviour.

KEY WORDS: Spin probe, cyclodextrin, electron spin resonance.

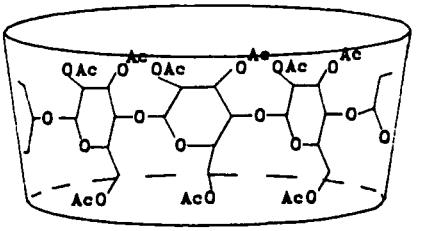
INTRODUCTION

Cyclodextrin, a cyclic oligomer of glucose having a cavity structure exhibits inclusion of organic substrates in aqueous solutions.[1] The role of the hydroxyl groups in the vicinity of the rim of the cyclodextrin cavity upon inclusion of substrate is not well understood. However the fact that the modification or derivatization[2] of these hydroxyl groups greatly influences the equilibrium properties of inclusion complexes indicates that those groups may play a significant role either on the kinetic process or on the equilibrium state.[3-8] Also it has been shown that when hydroxyl groups on the rim of the cyclodextrin are dissociated in highly basic solutions, organic substrates are expelled from the cavity.[9]

Recently developed aminoxyl spin probes having proton and nitrogen hyperfine splittings (hfs's) enable us to study the recognition ability of each *functional group* by the molecular receptor with the use of electron spin resonance spectroscopy.[10-15] The



I

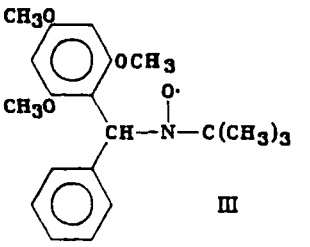


II (Ac=CH_3CO)

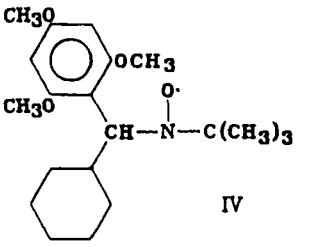
aim of this study is to clarify how "entrance groups" i.e. groups which modify the entrance affect the recognition ability of various functionalities. Thus we describe comparable studies with two derivatized β-cyclodextrins, heptakis (2,6-O-dimethyl)-β-cyclodextrin, $M_{14}CD$ (or β-DMCD)[4-8] **I** and heptakis (2,3,6-O-triacetyl)-β-cyclodextrin, $(AC)_{21}CD$, **II**.

RESULTS AND DISCUSSION

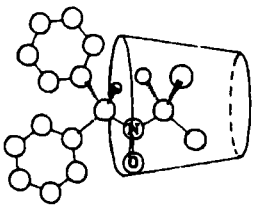
It has been reported that an aminoxyl probe such as **III** is included by cyclodextrin in two different ways as illustrated below, either phenyl-in or *tert*-butyl-in (bimodal inclusion) and the ESR spectrum shows clear separation of these complexes.[12] Only



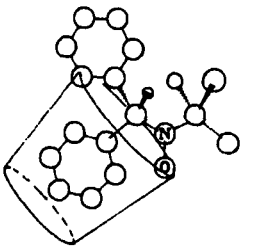
III



IV



t–butyl–in



phenyl–in

two way inclusion of **III** by cyclodextrin occurs because the trimethoxyphenyl group is too bulky to be included. This is the result of the ability of the cyclodextrin cavity to recognize each functional group in a substrate independently.[14] The same phenomena was observed in derivatized cyclodextrin **I** but not in **II**.

Hyperfine splitting constants of the complex

The ESR spectrum of the aqueous solution of aminoxyl **III** changes upon the addition of $M_{14}CD$ from Figure 1a to 1b in water at room temperature. Figure 1c shows the ESR spectrum of the same probe in the presence of β-cyclodextrin. The new spectrum can be analyzed in terms of three components with the hfs's shown in Table 1. Also the new species shows weaker intensities in the high field wing due to the restriction

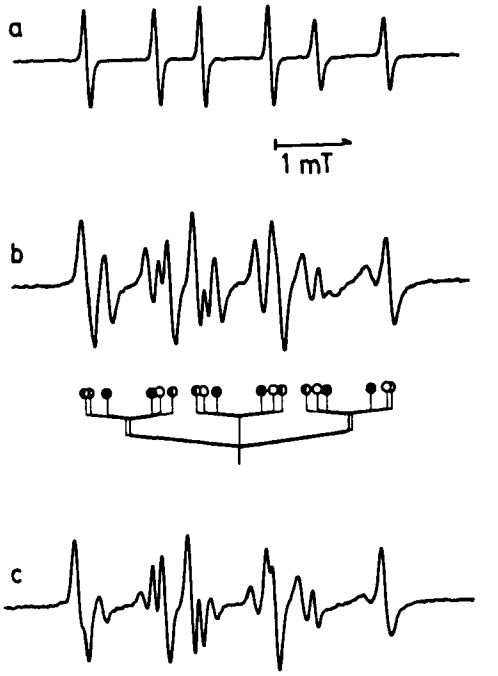


FIGURE 1 a. ESR spectrum of aminoxyl spin probe **III** in water. b. ESR spectrum of aminoxyl spin probe **III** in water in the presence of 3.75×10^{-3}M $M_{14}CD$ at 300 K. c. ESR spectrum of aminoxyl spin probe **IV** in water in the presence of 7.5×10^{-3}M β-cyclodextrin. Stick spectrum shows the line positions of phenyl-in complex (●), *tert*-butyl-in complex (◐), and free probe (○). Field modulation amplitude was 0.0125 mT and incident microwave power was 6 mw.

of motion by the complexation.[16] The assignments of the included species are based on the principle that the insertion of the *tert*-butyl end always produces similar hfs irrespective of the kind of substituents on the other side of the NO function in the probe while the insertion from the other end gives smaller βH-hfs compared to the free species. The small change in hfs by *tert*-butyl inclusion indicates that the inclusion occurs at the remote site from the β-carbon where β-hydrogen is attached. The difference in the hfs of the complex of **I** from that of unmodified β-cyclodextrin is

TABLE 1
Hfs constants of cyclodextrin complexes of aminoxyl probes

probe		β-Cyclodextrin		$M_{14}CD$	
		A_H/mT	A_N/mT	A_H/mT	A_N/mT
III	free	9.78	16.42	9.78	16.42
	Phenyl-in	6.12	15.74	5.96	15.75
	t-Butyl-in	12.54	15.94	11.80	15.81
probe					
IV	free	6.76	16.33	6.76	16.33
	Cyclohexyl-in	5.39	15.75	4.66	15.72
	t-Butyl-in	9.58	15.53	9.65	15.43

Error is ±0.07 mT.

TABLE 2
Association constants and thermodynamic constants of the formation of cyclodextrin complex of aminoxyl probe at 300 K.

	β-CD			M_{14}CD		
	K/M^{-1}	$-\Delta H°$ kcal/M^{-1}	$T\Delta S°$	K/M^{-1}	$-\Delta H°$ kcal/M^{-1}	$T\Delta S°$
probe **III**						
Phenyl-in	93	6.2	-3.4	590	6.1	-2.3
t-Butyl-in	435	3.6	-0.1	1100	4.0	0.2
probe **IV**						
Cyclohexyl-in	2600	3.8	0.9	11200	1.4	4.2
t-Butyl-in	580	3.2	0.6	1300	3.2	1.1

Error for K is ±10%. Error for $\Delta H\cdot$ and $\Delta S\cdot$ is ±20% and ±40%, respectively.

small. The N-hfs which can be a indicator of the hydrophobicity of the surroundings of the NO group shows a similar decrease in magnitude upon inclusion indicating that the environment in the cavity is similar to an alcoholic solvent.[17] This fact means that the structure and the environment of the aminoxyl probe in the inclusion complex does not change by partial methylation of the entrance hydroxyl groups.

Association constants of the complex

The association constants for encapsulation of the phenyl or *tert*-butyl groups can be compared for β-cyclodextrin and M_{14}CD (Table II). In spite of the minor change in hfs of the complex the association is greater by 2 to 5 fold for phenyl, cyclohexyl and *tert*-butyl in the case of the cyclodextrin derivative **I**. However, no inclusion of the probe was observed in the case of **II**. The association constant of the *tert*-butyl-in complex of both probe **III** and **IV** with M_{14}CD are similar in magnitude (1100 M^{-1} and 1300 M^{-1}). This means that M_{14}CD produces an inclusion complex on the basis of group recognition just as in the case of β- and γ-cyclodextrins.

Group recognition ability which is estimated by the absolute value of the association constant is increased by the introduction of the methoxy groups to the rim. However, the ability of differentiating functional groups is not always enhanced. The ratio of the association constants of the phenyl group and the *tert*-butyl group ($K_{t\text{-butyl}}/K_{phenyl}$) decreases from 4.7 to 1.8 by methoxy substitution of β-cyclodextrin while $K_{cyclohexy}/K_{t\text{-butyl}}$ is increased from 4.5 to 8.4. This is the result of the alteration in group-recognition preference by introducing methoxy groups onto the rim.

Standard enthalpy and entropy of inclusion

Association constants may not be the best indicator of recognition ability because of a temperature dependence. Standard enthalpy and entropy of association, $\Delta H°$ and $\Delta S°$ are a more accurate measure of recognition ability. Thus $\Delta H°$ and $\Delta S°$ were calculated based on the temperature dependence of the association constant using a Van't Hoff plot. The results are listed in Table III. In spite of considerable experimental error comparative inspection of the data shows that the complexes are formed mostly by enthalpic driving force ($-\Delta H° > T\Delta S°$).[18] As is realized from the Van't

Hoff plot the small change in $\Delta H°$ and $\Delta S°$ causes a large deviation in the association constant. The increase of association constant in cyclodextrin **I** from that of unmodified β-cyclodextrin is the result of an increase in $\Delta S°$. The increase of entropy contribution is accounted for by the defolding of many methoxy groups out of the cavity upon inclusion of the substrate into the cavity. The cyclohexyl-in complex of probe **IV** with $M_{14}CD$ shows a large entropy increase compared to β-cyclodextrin which is enough to compensate for the decrease in $\Delta H°$. The high hydrophobicity of the cyclohexyl group and the steric flexibility to fill the cavity efficiently could contribute in repelling all methoxy groups out of the cavity. The reason for the loss of inclusion ability in cyclodextrin **II** is either steric crowding or occupation of the cavity by the acetyl group.

Finally cyclodextrin **I** does not reject the substrate at pH values as high as 13. In β-cyclodextrin the dissociation of hydroxyl groups at pH 12 causes the rejection of inclusion.[9] Thus it is confirmed that the lack of the hydrogen bond network among hydroxyl groups helps raise the pKa of the hydroxyl groups.

MATERIALS AND METHODS

β-Cyclodextrin and derivatized cyclodextrins **I** and **II** were purchased from Aldrich Chemical Co. and were used as received. Aminoxyl spin probes **III** and **IV** were prepared by the reaction of β-2,4,6-trimethoxyphenyl-N-*tert*-butylnitrone (MO_3PBN) with phenyl lithium and cyclohexyl magnesium bromide followed by air oxidation. MO_3PBN is available in these laboratories and phenyl lithium and cyclohexyl magnesium bromide were obtained from Aldrich Chemical Co. Water was treated by a Millipore Mili Q system. The reactant solution in benzene was washed with sodium bicarbonate saturated solution twice and the organic portion was purged with nitrogen gas. The dry residue was dissolved in water and was used as a probe solution. The concentration of the probe was about 10^{-4}M. ESR spectra were obtained using a Bruker ER 200D spectrometer with 100 kHz field modulation and the temperature was controlled by a Bruker ER4111 VT variable temperature unit. Association constants were determined based on the formation of a one to one complex. The relative concentrations of the free probe and the complexed probe were calculated by computer spectrum simulation.

Acknowledgement

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