Differential Pressure Effect on Bimodal Inclusion Complex of β-Cyclodextrin with a Nitroxide Radical Probe as Studied with Electron Paramagnetic Resonance

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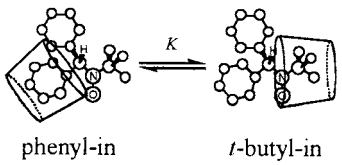
Differential pressure effect was found in the inclusion equilibrium of bimodal inclusion (phenyl-in and *t*-butyl-in) complexes of β-cyclodextrin with the free-radical probe diphenylmethyl *t*-butyl nitroxide. A high-pressure electron paramagnetic resonance (EPR) system was employed to determine the shift of the EPR spectra under high static pressure. With increasing external pressure, the equilibrium between phenyl-in and *t*-butyl-in complexes shifts to the *t*-butyl-in side.

The hydrophobic cavity in a cyclic oligosaccharide, cyclodextrin (CD), has been shown to produce inclusion complexes with a variety of molecules in water.[1] This inclusion phenomenon has been used as a model for molecular recognition that occurs in enzyme active sites.[2] Moreover, various derivatives of CD have been developed as drug delivery tools.

When cyclodextrin includes a molecule that has more than one bulky group to form inclusion complex, each functional group could produce distinct inclusion complex. These isomeric complexes have been shown to be spectroscopically discriminated employing electron paramagnetic resonance (EPR) spectrometry if the probe is paramagnetic.[3–11] A rapid in-and-out inclusion equilibrium exists between these complexes, therefore, EPR has been the only technique that can discriminate these complexes. The formation of diastereomeric complexes was observed when a prochiral probe was included, using electron nuclear double resonance (ENDOR) spectrometry.[11] When two distinct complexes are identified, they are called bimodal inclusion complexes.[3] In this study, the effect of external pressure on bimodal inclusion complexes was investigated using a high-pressure EPR technique. The equilibrium between two distinct inclusion complexes, phenyl-in and *t*-butyl-in complexes, of β -CD with diphenylmethyl *t*-butyl nitroxide (Scheme 1)



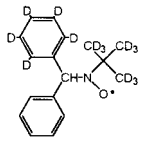
Scheme 1.

was monitored using EPR spectroscopy. When high external pressure such as 637 bar was applied to the sample, EPR spectral pattern was altered due to the shift in the inclusion equilibrium. The difference in inclusion volume for the two complexes was calculated from the shift of the equilibrium constants. This is the first report on the differential pressure effect in group-in inclusion complexes with cyclodextrin.

Temperature dependence studies on these complexes indicated that ΔH and ΔS for the inclusion equilibrium were different for each complex.[7,9] Therefore, it is expected that the application of static pressure would modulate these thermodynamic parameters and shift the equilibrium differentially to each complex. This would result in the alteration of EPR spectral pattern and provide the volumetric information on the included group.

In this study, we synthesized deuterated diphenylmethyl *t*-butyl nitroxide, and determined pressure dependence of EPR spectra of its β-CD bimodal inclusion complexes. The use of the deuterated probe is expected to provide a narrower EPR-spectral line widths which would help in the determination of precise thermodynamic constants.

The nitroxide spin probe DPBN was prepared using the



DPBN

Grignard reaction by mixing equivalent amounts of phenylmagnesium bromide (Aldrich Chemical Co., St, Louis, MO U.S.A.) and perdeuterated phenyl *t*-butylnitrone (PBN-d_{14}, C_6D_5–CH=N(O)–$C(CD_3)_3$) in dry benzene, followed by air oxidation. PBN-d_{14} was previously synthesized in this laboratory. The solvent of the reactant solution was evaporated by nitrogen gas flow and the residue was redissolved in ethanol to make a stock solution for the nitroxide probe. Typically 20 μL of the stock solution was taken into a test tube and ethanol was evaporated off by nitrogen gas. To the residue, 1 mL of 10 mM β-CD aqueous solution was added. This solution was loaded into a high-pressure cell. The procedures for the observation of EPR signals at high pressures were identical with those described elsewhere.[12] Briefly, the solution was contained in a thick wall quartz capillary tubing (i.d. 1 mm, o.d. 6 mm) which was connected to a copper–beryllium high-pressure line using epoxy resin. The pressure was applied with a plunger pump and its magnitude was measured by a Haise–Bourdon gauge. After applying pressure, the stop valve was closed and the tubing was disconnected from the high pressure system and set into the EPR cavity. EPR spectra were obtained using a JEOL FE3XG spectrometer at room temperature and spectrometer settings are listed in the figure legend.

The EPR spectrum of DPBN in β-CD solution under atmospheric pressure (1 bar) exhibits two sets of six lines, each

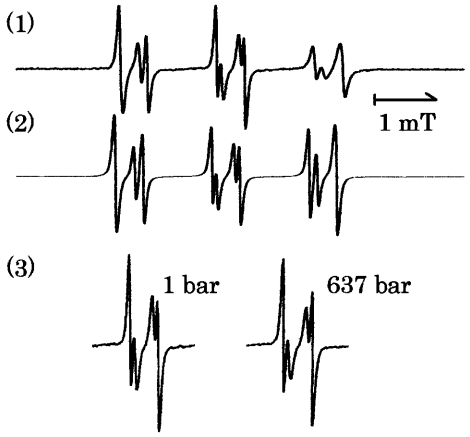


Figure 1. (1) Atmospheric pressure EPR spectrum of DPBN (2×10^{-4} mol dm^{-3}) in the presence of 1.0×10^{-2} mol dm^{-3} β-CD at 298 K (phenyl-in complex A_N=1.61 mT, A_H=0.285 mT; *t*-butyl-in complex A_N=1.56 mT, A_H=0.454 mT). (2) Computer-simulated spectrum for the spectrum (1). (3) The center-field lines at 1 bar and 637 bar.

contains three sets of double lines (Figure 1). This spectrum pattern was analyzed based on hyperfine interaction with one nitrogen nucleus and one hydrogen nucleus. The spectrum was reproduced using computer EPR-spectral simulation, and each species was assigned to "*t*-butyl-in" and "phenyl-in" complex (Figure 1). When a static pressure equivalent to 637 bar was applied to the sample, the EPR spectrum was altered. This EPR spectrum was reproduced with computer spectral simulation by adjusting the relative abundance of the two complexes but keeping the hfs constants the same.

Because the EPR spectrum from uncomplexed (free) probe was not observed, a direct equilibrium between *t*-butyl-in complex (binding constant: 1.8×10^3 mol^{-1} dm^3)[7] and phenyl-in complex (binding constant: 1.2×10^3 mol^{-1} dm^3)[7] was assumed (Scheme 1). The equilibrium constant K was calculated from the concentration ratio of the two complexes (K=[*t*-butyl-in]/[phenyl-in]). The K values increased as a function of increasing external pressure (Table 1).

Table 1. The equilibrium constants and reaction volume for the bimodal inclusion complexes in water at 298 K

K							ΔV
1	98.1	196	343	490	637	(P/bar)	cm^3 mol^{-1}
1.60	1.70	1.80	1.96	2.10	2.30		-14.9

The change in reaction volume ΔV at 1 bar was estimated according to the following equations.

$$\ln K = aP^2 + bP + c \quad (1)$$

$$\Delta V = -RT(\partial \ln K/\partial P)_T \quad (2)$$

Using these equations, the reaction volume for the bimodal inclusion is estimated to be −14.9 cm^3 mol^{-1}.

The reaction volume corresponds to the difference between the inclusion volume between *t*-butyl-in and phenyl-in complexes, i.e.,

$$\Delta V = \Delta V_{t\text{-bu}} - \Delta V_{\text{ph}} \quad (3)$$

When we assume that all water molecules in the CD cavity are repelled out upon inclusion of the phenyl or *t*-butyl group,[13] ΔV_{ph} following inclusion may be indentical to that of the benzene molecule, i.e., ΔV_{ph}= −90 cm^3 mol^{-1},[14] then, the $\Delta V_{t\text{-bu}}$ value can be calculated to be −105 cm^3 mol^{-1}. This is the molar volume of this molecule when it is included from the *t*-butyl side. Because the framework of the *t*-butyl group is more flexible than the phenyl group, it is reasonable to speculate that *t*-butyl group penetrates more than the phenyl group. Previously, the nitrogen hfs in the EPR spectrum of *t*-butyl-in complex showed that the depth of inclusion of *t*-butyl-in complex was more than that of phenyl-in complex,[6] which is in agreement with the present observation.

In conclusion, the present high-pressure EPR study on group inclusion complex of CD made it possible to evaluate the volume difference between functional group in the included molecule.

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