ESR Investigation of the Rotational Mobility of Spin Probes in Ethanol Solvent Adsorbed to β-Cyclodextrin[1)]

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Molecular interactions between spin probe reagents and β-cyclodextrin (β-CyD) in ethanol were investigated on the basis of the rotational correlation time of the spin probes as estimated by the ESR spectrum simulation. The effect of the functional groups of spin probes, possibly hydrogen-bonding interactions, on the hydroxylated surface of the β-CyD molecule were suggested from the thermodynamic data on the rotational correlation times, which could be combined with the rotational mobilities of spin probes confined in a microscopic liquid phase.

Keywords ESR; activation energy; spin probe; adsorption; β-cyclodextrin; molecular mobility

The spin probe method has recently been extensively utilized as a refined method to study the fluidity of biological lipid membranes and so on.[2)] By use of this method, the fluidity of the membranes can be estimated from the rotational correlation time (τ) of spin probes used as one of the basic parameters in ESR spectrum simulation. In this case, the fluidity estimable from the correlation time of spin probes labeled in microscopic liquid spaces should be distinct in general from the ordinary fluidity of macroscopic liquid spaces, because the former is sensitive to miscellaneous short-range intermolecular forces acting on spin probes confined in microscopic liquid spaces.

We may thus expect that, for spin probes labeled in liquid adsorbed to the surface of any adsorbents, this method presents unique information on characteristics of the microscopic adsorbent surfaces which would strongly influence the molecular mobility of spin probes. This information will be helpful for the characterization of the molecular properties of adsorbent materials, some of which have pharmaceutical availability. As far as the above system is concerned, however, it seems that applications of the spin probe method have not been reported in terms of the elaborate calculation of ESR spectrum simulation.

In the present work, the rotational correlation times of spin probes dissolved in ethanol, as well as in ethanol adsorbed to β-cyclodextrin (β-CyD) adsorbent, are investigated on the basis of the ESR spectrum simulation method developed by Freed and Polnaszek.[3)] Thermodynamic data on the correlation times of spin probes will be discussed in terms of the difference in chemical properties of the functional groups of spin probes.

Experimental

Spin probes such as 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), 4-amino-TEMPO, 4-hydroxy-TEMPO and 4-oxo-TEMPO were obtained from Aldrich Chemical Co. and used as accepted. Prior to use, these probes were dissolved in ethanol at a concentration of 1.0×10^{-3} M/l. β-CyD of analytical grade, obtained from Wako Pure Chemical Co., was dried under a vacuum at 80 °C, and was then ground to a fine powder with an agate mortar. When necessary, ethanol solutions of the spin probes were adsorbed to the powdered β-CyD.

ESR measurements were carried out on a JEOL JES-RE3X. All of the spectra were recorded at a microwave power of 5 mW, modulation width of 0.1 mT (100 kHz), time constant of 0.1 s and sweep time of 4 min/10 mT. The temperature was controlled by a JEOL ES-DVT2 variable temperature system. ESR spectrum simulation was performed on a PC-9801RA personal computer.

The ESR simulation program developed by Freed and Polnaszek[3)] was slightly modified in order to compile and install it on the personal computer. The function of the modified program was reconfirmed by comparing the line shape calculated with this program with that calculated by Dr. S. Noji on a HITAC 8700 computer by use of the original program.[4)]

Results and Discussion

As can be seen from Fig. 1, the ESR line shapes of spin probes in ethanol exhibit remarkable temperature dependencies. This indicates that restrictions of the probe rotation in ethanol solvent are caused by lowering the temperature. The above effect was sensitive to a difference in the functional groups of spin probes, and was most prominently observed in the case of amino-TEMPO. This suggests that the amino group of amino-TEMPO may interact with ethanol solvent.

As can be seen from Fig. 2, the temperature effect on the restriction of the probe rotation in ethanol adsorbed to β-CyD is far more profound, particularly in TEMPOs having polar functional groups, compared to the case of spin probes solved in ethanol solvent. This suggests that the functional groups of the spin probes may have a hydrogen-bonding interaction with the hydroxylated surface of the β-CyD molecule surrounded by peripheral hydroxyl groups.

To confirm the above, the rotational correlation times of spin probes in ethanol, as well as in ethanol adsorbed to β-CyD, were estimated by means of the aforementioned ESR spectrum simulation method.[3)] Typical results of the simulated ESR spectra are illustrated in Fig. 3, together with the spectra observed for amino-TEMPO in ethanol.

The rotational correlation time (τ) of spin probes was usually estimated as $1/6D_R$ from the rotational diffusion constant (D_R), used as one of the basic parameters for the ESR spectrum simulation.[3)] According to the well-known potential barrier model, the rate constant ($\tilde{k}$) of activation for the molecular rotation is expressible as follows:[5)]

$$\tilde{k} = (kT/h)\exp(\Delta S^{\ddagger}/R)\exp(-\Delta H^{\ddagger}/RT) \quad (1)$$

where $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the activation entropy and activation enthalpy respectively, k the Boltzmann constant,

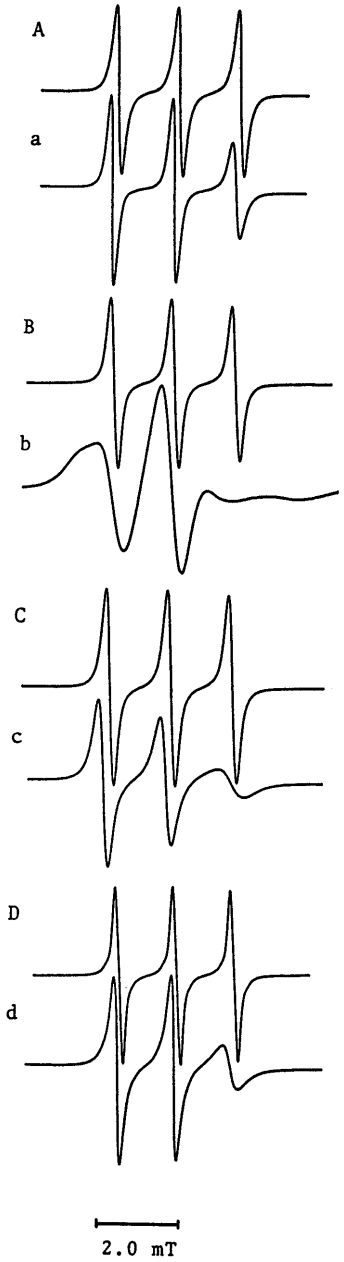

Fig. 1. Temperature Effects of the ESR Spectra of TEMPO Probes in Ethanol

The spectra are, A) TEMPO: 20 °C, a) TEMPO: −100 °C, B) amino-TEMPO: 20 °C, b) amino-TEMPO: −100 °C, C) hydroxy-TEMPO: 20 °C, c) hydroxy-TEMPO: −100 °C, D) oxo-TEMPO: 20 °C, d) oxo-TEMPO: −100 °C, and the recorded gains were A) 5×1, a) 2×1, B) 6.3×1, b) 3.2×10, C) 6.3×1, c) 5×1, D) 4×1, d) 7.9×1, respectively.

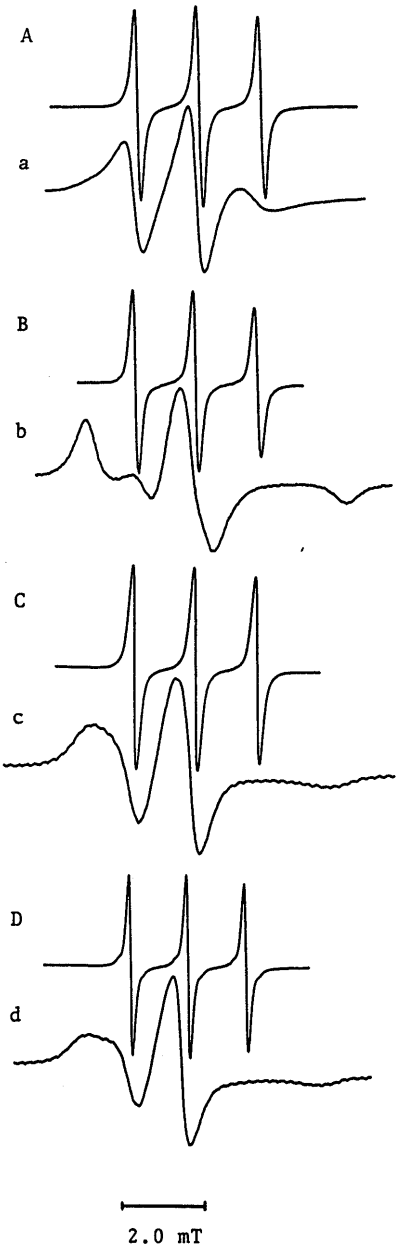

Fig. 2. Temperature Effects of the ESR Spectra of TEMPO Probes in Ethanol Adsorbed to β-CyD

The spectra are, A) TEMPO: 20 °C, a) TEMPO: −100 °C. B) amino-TEMPO: 20 °C, b) amino-TEMPO: −100 °C, C) hydroxy-TEMPO: 20 °C, c) hydroxy-TEMPO: −100 °C, D) oxo-TEMPO: 20 °C, d) oxo-TEMPO: −100 °C, and the recorded gains are A) 3.2×10, a) 7.9×10, B) 5×10, b) 1.6×100, C) 2.5×10, c) 7.9×10, D) 2.5×10, d) 6.3×10, respectively.

T the absolute temperature, $\boldsymbol{h}$ the Planck constant and $\boldsymbol{R}$ the gas constant. For the present purpose, it may be convenient to use Arrhenius-type activation energy (E_a) rather than the activation enthalpy ($\Delta H^{\ddagger}$), because the activation energy (E_a) for the liquid state is obtainable from $\Delta H^{\ddagger}$ by use of the thermodynamic relationship[6]:

$$E_a = \Delta H^{\ddagger} + \boldsymbol{R}T \tag{2}$$

The above equation can then be converted to

$$\tilde{k} = (\boldsymbol{k}T/\boldsymbol{h})\exp(\Delta S^{\ddagger}/\boldsymbol{R})\exp(-E_a/\boldsymbol{R}T+1)$$
$$= (\boldsymbol{e}\boldsymbol{k}T/\boldsymbol{h})\exp(\Delta S^{\ddagger}/\boldsymbol{R})\exp(-E_a/\boldsymbol{R}T) \tag{3}$$

The rotational correlation time (τ) corresponding to the reciprocal of $\tilde{k}$ is thus expressed as

$$\tau = (\boldsymbol{h}/\boldsymbol{e}\boldsymbol{k}T)\exp(-\Delta S^{\ddagger}/\boldsymbol{R})\exp(E_a/\boldsymbol{R}T) \tag{4}$$

This type of equation has been widely applied to investigate the rotational relaxation rate of polar molecules in liquids. The plot of $\boldsymbol{R}\cdot\ln(\boldsymbol{e}\boldsymbol{k}T\tau/\boldsymbol{h})$ *vs.* $1/T$ is useful to estimate the activation energy (E_a) and activation entropy ($\Delta S^{\ddagger}$) for the rotational mobility of spin probes. These plots are shown in Fig. 4, and the E_a and $\Delta S^{\ddagger}$ values estimated from the plots are listed in Table I. The $\Delta S^{\ddagger}$ values were excluded from later discussion because these values were sensitively affected by changing the half-width pa-

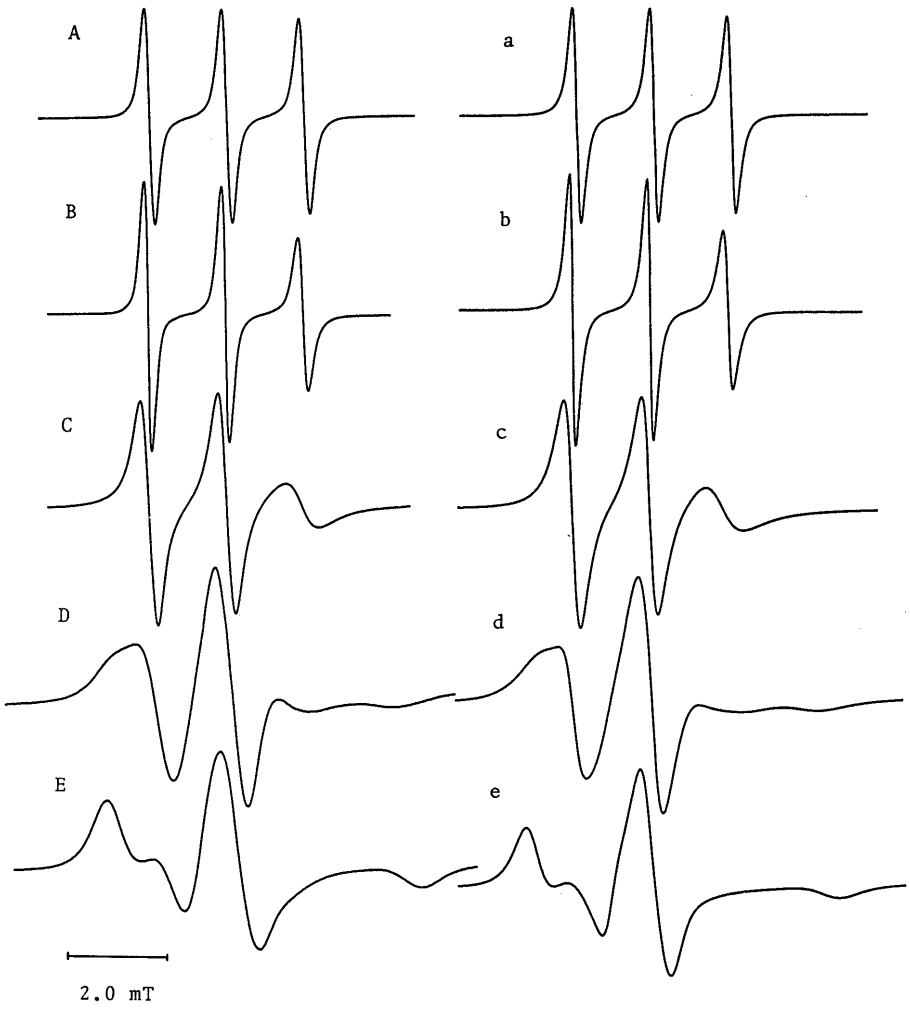

Fig. 3. Comparisons between the Observed and Simulated ESR Spectra of Amino-TEMPO in Ethanol

The spectra A—E) were observed at 20 °C, −40 °C, −80 °C, −100 °C, −115 °C, respectively. The spectra a—e) were simulated with of ESR parameters, $A_{xx}=0.65$ mT, $A_{yy}=0.6$ mT, $A_{zz}=3.4$ mT, $g_{xx}=2.0100$, $g_{yy}=2.0075$, $g_{zz}=2.0025$, half-width = 0.133 mT and rotational correlation times, 3.0×10^{-11}, 2.5×10^{-10}, 2.2×10^{-9}, 3.5×10^{-9} and 6.0×10^{-9} s, respectively.

rameter from the best fit value (0.133 mT) used here to calculate the simulation spectra, although such was not the case with the E_a values.

The E_a values for the spin probes estimated in ethanol adsorbed to β-CyD are clearly higher than those estimated in ethanol. It is especially notable that the above differences are markedly sensitive to the functional groups of spin probes. That is, the differences in the E_a values are 6, 6 and 5 kJ/mol for amino-, hydroxy-, and oxo-TEMPOs respectively, while the difference is only 1 kJ/mol for TEMPO itself. These results suggest that the restriction of the molecular mobility of spin probes is greater, and particularly so for chemically modified TEMPO, in ethanol adsorbed to β-CyD than in ethanol, and also that the restriction is released in the activated state, possibly due to a break in the hydrogen bonding between the spin probes and β-CyD. This may be understood in terms of the fact that TEMPO has no functional groups capable of a hydrogen-bonding interaction with β-CyD, which would cause restrictions of its molecular mobility. The finding obtained here is in accord with a series of ESR studies[7-11] indicating the restricted molecular rotation of TEMPO spin probes complexed with β-CyD.

Table I. Rotational Activation Energy (kJ/mol) and Rotational Activation Entropy (J/mol·K) of the Spin Probes

Media \ Probe		TEMPO	Amino-TEMPO	Hydroxy-TEMPO	Oxo-TEMPO
Ethanol (A)	E_a	14	18	16	16
	$\Delta S^{\neq}$	16	9	14	14
Ethanol adsorbed to β-CyD (B)	E_a	15	24	22	21
	$\Delta S^{\neq}$	−2	23	26	19
Difference in the E_a (B−A)		1	6	6	5

In conclusion, we can recognize that restrictions of the probe rotation in the microscopic adsorbent surface are largely dependent on hydrogen-bonding interactions between the functional groups of spin probes and the hydroxylated surface of the β-CyD molecule.

This work presents interesting new information about the adsorbent effects of β-CyD on the rotational mobility of spin probes, which could be conceived in terms of the microscopic viscosity of ethanol as adsorbed to β-CyD.

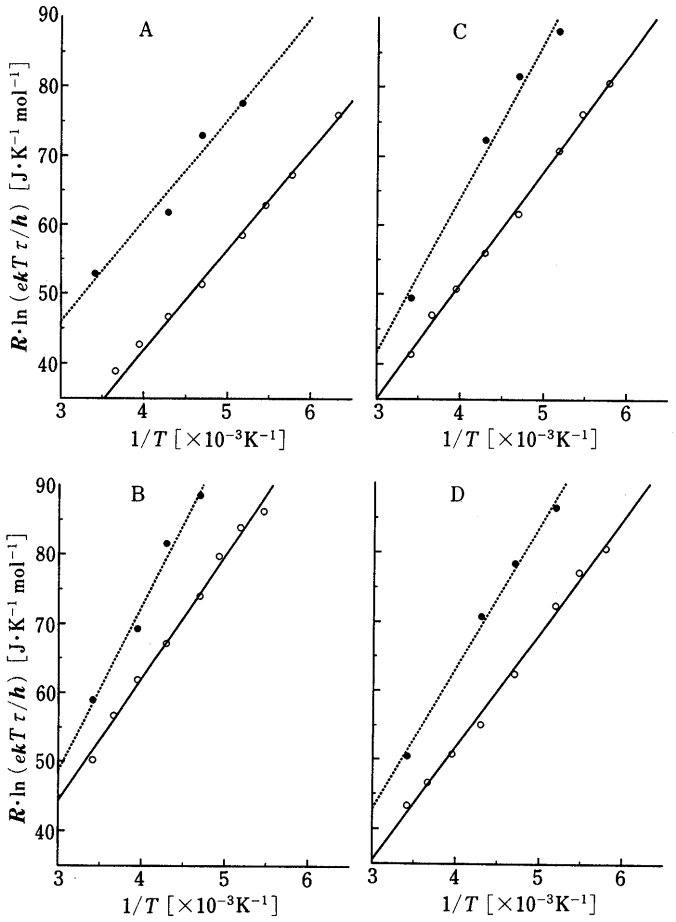

Fig. 4. The Plots of $R \cdot \ln(ekT\tau/h)$ *vs.* $1/T$, in Ethanol (Solid Line) and in Ethanol Adsorbed to β-CyD (Broken Line)

A) TEMPO, B) amino-TEMPO, C) hydroxy-TEMPO, D) oxo-TEMPO.

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References and Notes

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