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Formation of Cyclodextrin Inclusion Compounds of Stable Nitroxide Radicals as Monitored by Electron-Spin Resonance Spectra

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Hyperfine coupling constants and rotational correlation times, calculated from electron-spin resonance spectra of cyclodextrins incubated with stable nitroxide radicals, indicate inclusion compound formation of β- and γ-cyclodextrin with certain nitroxide radicals. In contrast, α-cyclodextrin exhibits no effect on the spectra of the radicals, probably because its central cavity is too small to form such inclusion compounds. Furthermore, one 1 : 1 molar ratio complex of β-cyclodextrin and a nitroxide radical (isolated as crystalline precipitate and identified both by combustion analysis and ir measurements) is shown by electron-spin resonance data to be an inclusion compound.

INTRODUCTION

Cyclodextrins are cyclic ring-shaped oligosaccharides consisting of 6, 7, or 8 D-glucopyranose units (α-, β- or γ-cyclodextrin) with a central cavity of 6–10 Å diameter in which other molecules can be included (*1–3*). The rather hydrophobic character of the central cavity might be one cause for the existence of such inclusion compounds. Among the methods used to indicate inclusion compound formation (*1*) electron-spin resonance spectroscopy (ESR spectra) (*7–12*) of spin-labeled compounds suitable in size and character for inclusion, in particular, provides telling information, as has been shown in two earlier investigations (*4–6*). Inclusion compounds are valid models for the interaction of a hydrophobic pocket of an enzyme with a low-molecular-weight substrate (*13*, *14*) and might perhaps also be characteristic for one type of association of polymer carbohydrates and low-molecular-weight compounds.

RESULTS

ESR Spectra of Nitroxide Radicals in the Presence of Cyclodextrin in Aqueous Solution

The ESR spectra of the nitroxide radicals **1–5** at room temperature in aqueous solution consist of three equidistant lines of identical intensity, generated by the hyperfine coupling of the unpaired electron with the ^{14}N nucleus ($I = 1$). Upon addition of

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simple carbohydrates, e.g., α-methyl glucoside, these spectra remain unaltered; however, in some cases, upon addition of β-, or γ-cyclodextrin they exhibit considerable alterations (Fig. 1).

Concomitantly, the isotropic hyperfine coupling constants a_N of the radicals **1**–**5** remain unaltered upon addition of either α-methyl glucoside or of α-cyclodextrin. However, radicals **1** and **4** when incubated with β-cyclodextrin, as well as **2** and **3**, when incubated with β- or γ-cyclodextrin, show small but significant decreases of the coupling constants (Table 1). In particular there is a large effect with radical **3** using β- or γ-cyclodextrin. The observed constants lie between those found in pure water and those measured in pure methanol (*15*, *16*). Since the a_N values are critically dependent on the polarity of the surrounding medium, these results indicate a rather hydrophobic environment for these NO groups.

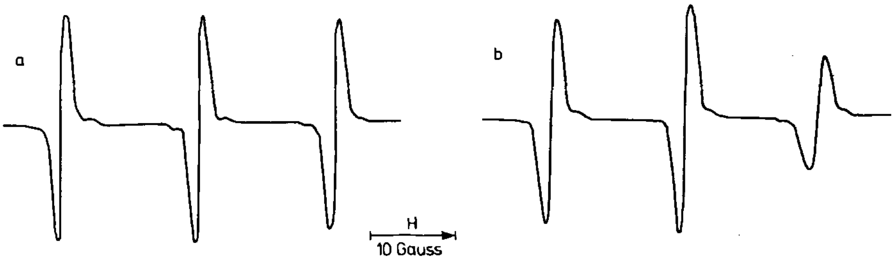


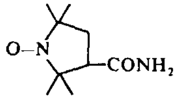
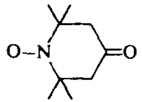
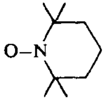
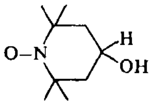
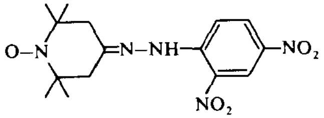
FIG. 1. ESR spectra of (a) 1.06 × 10^{-4} *M* radical **1** and (b) 0.91 × 10^{-2} *M* β-cyclodextrin with 1.06 × 10^{-4} *M* radical **1** in aqueous solution.

The linewidths of the high-field hyperfine coupling component $\Delta H_{(m=-1)}$ do not exhibit any alteration either upon addition of α-methyl glucoside or upon addition of α-cyclodextrin (Table 1). In contrast, radicals **1**–**5**, when incubated with β-cyclodextrin, as well as radicals **1**–**3** and **5**, when incubated with γ-cyclodextrin, show considerable line broadening. Large effects can be observed with radical **1** or **2** and β-cyclodextrin. The ESR spectrum of radical **5** already shows some line broadening even without an added cyclodextrin, probably because a somewhat restricted mobility is already inherent in the size of the molecule. Additionally a specific solvation by β- or γ-cyclodextrin might slightly strengthen this effect. In some cases anomalous linewidths observed at high field strengths can be ascribed to the anisotropy of the hyperfine coupling and of the g-factor (*18*, *19*), caused by a hindered tumbling motion of the radical. For the rotational correlation time of the tumbling motion one finds $\tau_c \approx 10^{-10}$ sec for radicals **1**–**5** when incubated with β-cyclodextrin as well as for radicals **2** and **5** when incubated with γ-cyclodextrin, and $\tau_c \approx 10^{-11}$ sec for radicals **1**–**4** in the absence of β- or γ-cyclodextrin (see Appendix).

Space-filling models of the nitroxide radicals **1**–**5** fit best into the cavity of space-filling models of β-cyclodextrin. Because of the decrease of the a_N values and the increase in the τ_c values of the radicals **1**–**4**, complete inclusion of these radicals into β-cyclodextrin can be supposed. In contrast, with radical **5** and β- or γ-cyclodextrin, partial inclusion (perhaps of the dinitrophenyl residue) is more probable, since a small perturbation of the linewidth but no measurable alteration of the a_N values was detected.

TABLE 1

ESR DATA OF INCLUSION COMPOUNDS OF CYCLODEXTRINS WITH STABLE NITROXIDE RADICALS

Radical	Systematic name	Hyperfine coupling constants a_N(G)[a] in aqueous solution						Relation of linewidth $(\Delta H_{(m=-1)}/\Delta H_{(m=+1)})$[b,c]					Summarizing scheme of cyclodextrin inclusion compound formation			
			+ α-Methyl-glucoside	+ Cyclodextrin			in CH_3OH		+ α-Methyl-glucoside	+ Cyclodextrin			In solution			In solid state
				α	β	γ				α	β	γ	α	β	γ	β
	2,2,5,5-Tetramethyl-3-carbamido-pyrrolidine-1-oxyl (1)	16.1	16.1	16.05	15.8	16.0		1.03	1.04	1.04	1.32	1.07	−	+	−	
	2,2,6,6-Tetramethyl-4-piperidone-1-oxyl (2)	15.55	15.55	15.5	15.4	15.4	14.5	1.04	1.05	1.04	1.36	1.21	−	+	+	+
	2,2,6,6-Tetramethyl-4-piperidine-1-oxyl (3)	16.8	16.7	16.65	16.15	16.3	15.75	1.03	1.04	1.03	1.14	1.06	−	+	+	−
	2,2,6,6-Tetramethyl-4-piperidinol-1-oxyl (4)	16.45	16.45	16.4	16.2	16.45	15.5	1.03	1.04	1.04	1.19	1.04	−	+	−	
	2,2,6,6-Tetramethyl-4-piperidone-1-oxyl-4-(2',4'-dinitrophenyl)-hydrazone (5)	15.45	15.5		15.45	15.45		1.12	1.12		1.18	1.17	−	(+)	(+)	

[a] Measured to ±0.05 G.

[b] Measured to a precision of ±0.03.

[c] $\Delta H_{(m=-1)}$ and $\Delta H_{(m=+1)}$ are the linewidths of the *high-field* and *low-field* band, respectively. They have been calculated from the derivative height of the bands according to the equation of Pake (*17*): for bands of identical intensity (derivative height) × (derivative width)^2 = constant.

ESR Spectra of Solid Materials containing Cyclodextrins and Nitroxide Radicals

From equimolar aqueous solutions of β-cyclodextrin and nitroxide radicals **2** or **3**, pink precipitates can be obtained. Combustion analysis of these materials gives evidence of a 1 : 1 molar ratio of both constituents. Correspondingly, the ir spectrum of the precipitate from β-cyclodextrin and radical **2** reveals the presence of the nitroxide as well as of the carbonyl group of the radical. The ESR spectrum of this material exhibits a broad triplet, whereas an extensively ground pale yellow, 1 : 1 mixture of radical **2** and β-cyclodextrin, or the radical itself in the solid state, exhibit a sharp singlet (Fig. 2a).

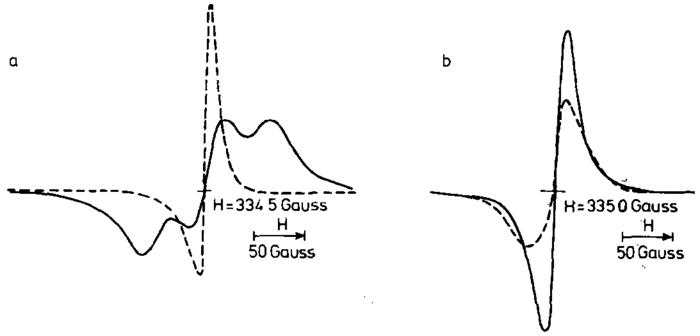


FIG. 2. ESR spectra of (a) solid precipitate of β-cyclodextrin with radical **2** (——) and of a solid 1 : 1 mixture of β-cyclodextrin and radical **2** (– – –) and (b) solid precipitate of β-cyclodextrin with radical **3** (——) and of a solid 1 : 1 mixture of β-cyclodextrin and rdical **3** (– – – –).

Furthermore, the 1 : 1 mixture of radical **2** and α-methyl glucoside, obtained from an aqueous solution of both compounds, also exhibits an ESR spectrum identical to that of the pure radical in the solid state. After redissolving the pink precipitate of radical **2** and β-cyclodextrin it is found that $a_N = 15.65$ G and $\Delta H_{(m=+1)}/\Delta H_{(m=-1)} = 1.04$ in accordance with the values in Table 1, since at a molar ratio of 1 : 1most of the complexes will be dissociated. From this it has to be concluded that in this pink precipitate the radical molecules are separated from each other by carbohydrate layers, the reason for this probably being the inclusion phenomenon.

An extensively ground solid mixture of β-cyclodextrin and radical **3**, on the other hand, is pink as is the precipitate from an aqueous solution of both compounds. The ESR spectra of both preparations exhibit a sharp singlet (Fig. 2b). Hence it is concluded that in this precipitate no inclusion compound is present.

CONCLUDING REMARKS

The present ESR investigation shows for the first time the existence of an inclusion compound of a cyclodextrin with a nitroxide radical in the solid state and confirms earlier ESR investigations (*4–6*) showing inclusion compound formation in solution, using, however, partly different radicals. These results are in good accordance with NMR (*20–23*) and CD measurements (*24*), demonstrating also restricted mobility of the radicals, as well as thermodynamic considerations (*25*).

EXPERIMENTAL

Cyclodextrins were prepared according to Cramer and Henglein (*26*) and purified according to French *et al.* (*27*). The nitroxide radicals were synthesized by oxidation of the corresponding amines (*15*, *28–31*) and purified by recrystallization. Radical **5** was synthesized starting with 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (**2**). The radicals were checked for purity by thin-layer chromatography on silica gel plates F_{254} (Merck, Darmstadt, GFR) in petroleum ether/acetone in varying proportions.

The uv spectra were measured with a Cary 14 spectrometer, ir spectra with a Perkin–Elmer model 137B spectrometer, and ESR spectra with a Varian E-3 (X-band) and a JEOL JES-PE-1x (X-band) spectrometer, each at 16–20°C.

The preparation of the materials for the ESR measurements was as follows: Materials dissolved in water or methanol were saturated for 30 min in the cold with nitrogen and then transferred into the cuvettes (quartz, 0.5 mm). Solid materials were degassed for 2 hr at room temperature under high vacuum (10^{-4}–10^{-5} Torr) in glass tubes (diameter 4 mm) and then sealed.

Isolation of the solid material containing β-cyclodextrin and 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (**2**): Aqueous solutions of β-cyclodextrin (0.2 g/30 ml) and of the radical (0.2 g/30 ml) were mixed at room temperature. The precipitate formed after 3 hr was isolated by centrifugation, washed with a little water and methanol, and dried in vacuum over P_2O_5. The yield was 82 mg of an amorphous pink product of the mp 288–291°C.

Anal. Calcd for $(C_6H_{10}O_5)_7 \cdot C_9H_{16}O_2N$ (1:1, M_r 1304): C, 46.9; H, 6.6; N, 1.1. Found: C, 44.87; H, 6.77; N, 1.05.

Isolation of the solid material containing β-cyclodextrin and 2,2,6,6-tetramethylpiperidine-1-oxyl (**3**): Aqueous solutions of β-cyclodextrin (0.1 g/15 ml) and of the radical (0.1 g/15 ml) were mixed at room temperature. A precipitate was formed after a short period, spun down by centrifugation, washed with a little water and methanol, and dried in vacuum over P_2O_5. The yield was 70 mg of an amorphous pink product of the mp 288–291°C.

Anal. Calcd for $(C_6H_{10}O_5)_7 \cdot C_9H_{18}ON$ (1:1, M_r 1290): C, 47.4; H, 6.8; N, 1.1. Found: C, 46.39; H, 7.25; N, 1.11.

APPENDIX

Calculation of the Rotational Correlation Times of the Nitroxide Radicals in Aqueous Solution at Room Temperature

The anisotropy of the linewidths of the triplet of the nitroxide radicals is given by the theory of ESR bands of McConnell, 1956 (*18*), Freed and Fraenkel, 1963 (*32*), and Kivelson, 1960 (*19*). On the assumption that an ESR absorption curve is described by a Lorentzian function, the linewidths of the three components of the triplet are:

$$\frac{1}{T_2(M)} = \frac{1}{T_2(0)} - \frac{4}{15}\tau_c\, b\, \Delta\gamma\, H_0 M + \frac{\tau_c}{8} b^2 M^2, \qquad \text{(I)}$$

$$\frac{1}{T_2(M)} = \pi 3^{1/2} \Delta H_m. \qquad \text{(II)}$$

$T_2(M)$ is the transverse relaxation time which is inversely related to the linewidth ΔH_m described by the peak-to-peak separation of the first derivative curve ($M = +1, 0, -1$ being the orientations of the ^{14}N nuclear spin), H_0 is the magnetic field strength (G), $\Delta\gamma$ is the anisotropy of the g factor, and τ_c is rotational correlation time (sec). In Eq. (I)

$$b = \frac{4\pi}{3}(A - B). \tag{III}$$

A and B describe the hyperfine coupling of the coupling tensor in the directions of the main axes z and x, respectively. In our case b equals 107 G and $\Delta\gamma$ equals 4.22×10^4 $G^{-1} \cdot sec^{-1}$ (*33*). The coefficients of Eq. (I) are found experimentally:

$$\frac{1}{T_2(M)} = a_0 + a_1 M + a_2 M^2. \tag{IV}$$

According to Eq. (I):

$$a_1 = -\frac{4}{15}\tau_c b \,\Delta\gamma\, H_0. \tag{V}$$

From Eq. (V) together with Eqs. (I) and (II) τ_c can be found:

$$\tau_c = K\,\Delta H_{(m=+1)}\left(\frac{\Delta H_{(m=-1)}}{\Delta H_{(m=+1)}} - 1\right), \tag{VI}$$

$$K = \frac{15\pi\, 3^{1/2}}{8b\,\Delta\gamma\, H_0} = 6.6 \times 10^{-10}\ (G^{-1} \cdot sec)\ (6). \tag{VII}$$

In case of very fast tumbling motion (isotropic spectrum) the anisotropy is averaged out (Fig. 1); this is the case when $\tau_c \leqq 10^{-11}$ sec.

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