Dynamic Aspects of Cyclodextrin Host–Guest Inclusion as Studied by an EPR Spin-Probe Technique

Marco Lucarini,*[a] Barbara Luppi,[a] Gian Franco Pedulli,[a] and Brian P. Roberts[b]

Abstract: EPR spectroscopy was used to study the inclusion of the nitroxides $PhCH_2N(O^{\bullet})tBu$ (**1a**), $CH_3N(O^{\bullet})tBu$ (**2a**) and $PhCH_2N(O^{\bullet})CH_3$ (**3a**) by α-, β- and γ-cyclodextrins (CDs) and by chemically modified β-CDs in aqueous and in aqueous-methanolic solutions at 294–356 K. The nitrogen and β-proton hyperfine splittings for free and included nitroxides differed significantly, especially for **1a**. Equilibrium measurements of the concentrations of free and included radicals afforded binding constants for the nitroxides. Selective line broadening was also evident in the EPR spectra, and this was attributed to modulation of the spectroscopic parameters by exchange between free and included nitroxides. Computer simulation of these spectra enabled the rate constants for association and dissociation to be determined. These nitroxides are particularly suitable probes for the study of inclusion by cyclodextrins (and probably by other complexing agents), because free and complexed nitroxides have spectroscopic parameters that differ significantly more than those of all other radicals used previously for this purpose. Free and bound nitroxides are readily differentiated by EPR spectroscopy, equilibrium constants for association are easily determined and the dynamics of the inclusion process can be studied as function of temperature, concentration, solvent and other experimental parameters.

Keywords: cyclodextrins · EPR spectroscopy · host–guest chemistry · molecular dynamics · radicals

Introduction

Cyclodextrins (CDs) and chemically modified cyclodextrins can recognise a variety of aliphatic and aromatic organic molecules in aqueous solution, giving rise in most cases to 1:1 inclusion complexes.[1] The CDs can be regarded as practical enzyme models and have found many applications in pharmaceutical science and separation technology.[2–3] One of the most useful tools for the study of CD complexes is NMR spectroscopy, although the spectra obtained from most systems represent concentration-weighted averages since exchange between the free and the complexed guest molecule is usually fast on the NMR timescale. With radicals as guest molecules, it should be possible to detect different signals from the complexed and uncomplexed species because of the much shorter timescale of EPR spectroscopy. There are a few reports of radical inclusion by CDs, all related to very persistent radicals such as sterically protected nitroxides,[4] nitroarene radical anions,[5] *N*-alkylphenothiazine radical cations,[6] semidiones[7] and sterically hindered phenoxyl radicals.[8] They showed that the paramagnetic substrate is inclued in the CD on the basis of the recognition of a particular functional group by the cyclodextrin cavity. Competitive complexation of a radical probe and a diamagnetic compound was used in experiments to determine the association constant between the CD and the diamagnetic guest. For example, the enantioselective inclusion of (+)- and (−)-fenchone was studied by means of the competitive displacement of a nitroxide biradical.[9] Diastereoisomeric inclusion complexes formed between β-CD and a prochiral radical guest were distinguished by ENDOR spectroscopy.[10] Although in most cases the binding constants with the CD were determined, more detailed studies on the dynamics of the inclusion process could not be made because of the similar spectroscopic parameters of the radical in the two sites.

Two of us recently described the characterisation by EPR spectroscopy of CD inclusion complexes of short-lived radicals in aqueous solution.[11] A carboxyalkyl radical and two *para*-substituted phenoxyl radicals were studied, along with the longer lived benzyl *tert*-butyl nitroxide[12] **1a**. Upon complexation, the latter radical showed a marked decrease in the hyperfine coupling constants of the nitrogen nucleus and the β-protons, and this indicates that significant conformational changes accompany the inclusion of the nitroxide. The strong dependence on temperature of the EPR linewidths of

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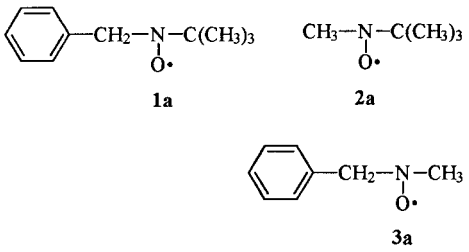
1a indicates that the lifetime of the radical in the associated and free forms is comparable to the EPR timescale. This suggested that the rates of complex formation and dissociation might be measurable by EPR line-shape analysis, which would provide valuable mechanistic information beyond that available from equilibrium measurements alone. Despite the large number of equilibrium binding constants for CD complexes reported in the literature, there are only few determinations of the rates of association and dissociation; these data were obtained by stopped-flow, temperature-jump, ultrasonic absorption and voltammetric techniques. [13]

Here we report the use of the dialkyl nitroxides **1a**–**3a** as probes for studying the effects of structure and the medium on the dynamics of the CD host–guest interaction, and we show that kinetic data for the association/dissociation processes can be obtained by studying the line broadening of the experimental EPR spectra.



Results

The EPR spectrum at 298 K of benzyl *tert*-butyl nitroxide **1a**, produced by reaction of the magnesium salt of monoperoxyphthalic acid (5.0×10^{-4} M) with benzyl *tert*-butyl amine (5.0×10^{-4} M) in water, is shown in Figure 1a. The spectrum was straightforwardly interpreted on the basis of the coupling of the unpaired electron with the nitrogen nucleus and with the two equivalent benzylic protons; the spectroscopic parameters are reported in Table 1. The additional signals observed in the presence of 1.3×10^{-3} M β-CD (Figure 1b) were assigned to the cyclodextrin-included radical **1b** in equilibrium with the free nitroxide [Eq. (1)]. In a control experiment, the use of methyl-α-D-glucopyranoside in place of the CD did not lead to any observable change in the EPR spectrum of the free nitroxide.

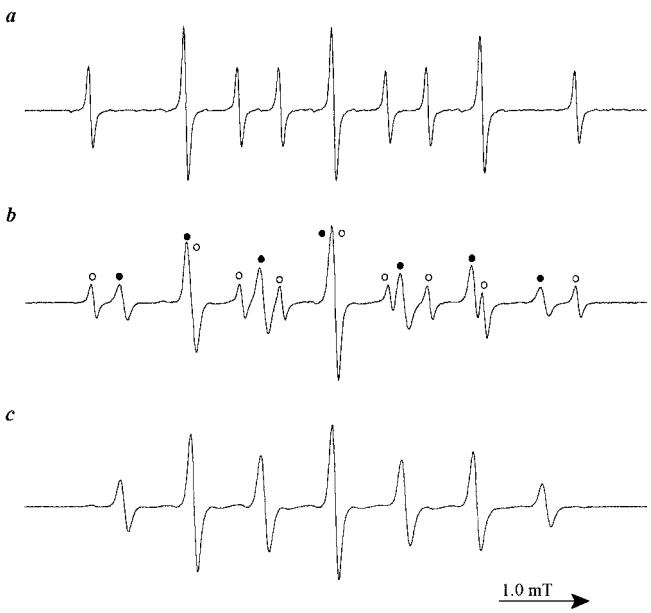


Figure 1. EPR spectra of nitroxide **1a** in water (a), in the presence of 1.3×10^{-3} M β-CD (b) and 1.3×10^{-2} M β-CD (c) at 298 K. Empty (○) and full symbols (●) refer to the free and included radicals, respectively.

$$\underset{\text{1a-3a}}{\text{R–N(O•)–R'}} + \text{CD} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\text{1b-3b}}{\left(\text{R–N(O•)–R'}\ \text{CD}\right)} \qquad K_1 = \frac{k_1}{k_{-1}} \qquad (1)$$

Since, under our conditions, the parent amine might compete with the nitroxide radical for inclusion by β-CD, a series of experiments were performed with different initial concentrations of amine and the salt of the peroxy acid. For concentrations of the amine and oxidising agent up to 1×10^{-3} M, the ratio of free to included radicals remained constant; this suggests that the affinity of the amine and the peroxyphthalate for β-CD is much lower than that of the nitroxide.[14]

When the concentration of β-CD was increased to 1.3×10^{-2} M, the spectrum of the complexed radical **1b** became dominant (Figure 1c), and the spectroscopic parameters for the included nitroxide could be measured. The a_N value of **1a** decreases only slightly on inclusion in the less polar environment of the β-CD host cavity, while the $a(2H_\beta)$ value, which is sensitive to conformational changes, decreases considerably on inclusion and gives rise to significant differences in the resonance fields for the $M_I(2H_\beta) = \pm 1$ lines of the included

Abstract in Italian: *L'inclusione dei nitrossidi $PhCH_2N(O^\bullet)tBu$ (**1a**), $CH_3N(O^\bullet)tBu$ (**2a**) e $PhCH_2N(O^\bullet)CH_3$ (**3a**) da parte di ciclodestrine naturali (CDs) e modificate chimicamente è stata studiata mediante la spettroscopia di risonanza di spin elettronico (EPR) sia in acqua sia in miscele acqua metanolo a 294–356 K. Le costanti di accoppiamento iperfine all'azoto e a i protoni in posizione β, particolarmente per il radicale **1a**, differiscono in modo significativo nella specie inclusa e in quella libera. Misure all'equilibrio delle concentrazioni del radicale nitrossido in soluzione e di quello incluso all'interno della cavità della ciclodestrina hanno permesso di determinare la costante di affinità dei vari nitrossidi. Si è anche osservato che gli spettri EPR mostrano allargamenti selettivi di alcune righe spettrali dovuti alla modulazione dei parametri spettroscopici in seguito allo scambio tra la specie libera e quella inclusa. Poiché questo processo avviene in tempi paragonabili a quelli che caratterizzano le transizioni EPR è stato possibile, tramite simulazioni al calcolatore, determinare le costanti di velocità di associazione tra nitrossido e CD e di dissociazione del complesso. I nitrossidi esaminati si sono rivelati particolarmente utili per studiare la dinamica del processo di inclusione con ciclodestrine (e probabilmente con altre sistemi in grado di alloggiare una specie ospite).*

Table 1. EPR spectral parameters for free and included dialkyl nitroxides.

Radical	Solvent	CD	T [K]	a_N [mT][a]	$a(2H_\beta)$ [mT]	a_H(other) [mT]
1a	H_2O		298	1.669	1.057	
	H_2O	α-CD	298	1.668	1.056	
				1.656	**0.944**	
	H_2O	β-CD	298	1.666	1.056	
				1.574	**0.788**	
	H_2O	γ-CD	294	1.671	1.063	
				1.597	**0.802**	
	H_2O	DM-β-CD	298	1.664	1.058	
				1.560	**0.780**	
	H_2O/MeOH (70/30)	DM-β-CD	299	1.651	1.005	
				1.562	**0.793**	
	H_2O/MeOH (50/50)	DM-β-CD	301	1.625	0.943	
				1.551	**0.808**	
	H_2O	TM-β-CD	294	1.668	1.054	
				1.570	**0.827**	
2a	H_2O		294	1.680	–	1.433 (CH_3)
	H_2O	DM-β-CD	294	1.683	–	1.432 (CH_3)
				1.625	–	**1.325** (CH_3)
3a	H_2O		294	1.737	1.044	1.448 (CH_3)
	H_2O	DM-β-CD	294	1.737	1.044	1.448 (CH_3)
				1.666[b]	**0.912**[b]	**1.344**[b] **(CH_3)**

[a] In the presence of cyclodextrin; the values given in bold type refer to the included radical. [b] Values obtained by computer simulation.

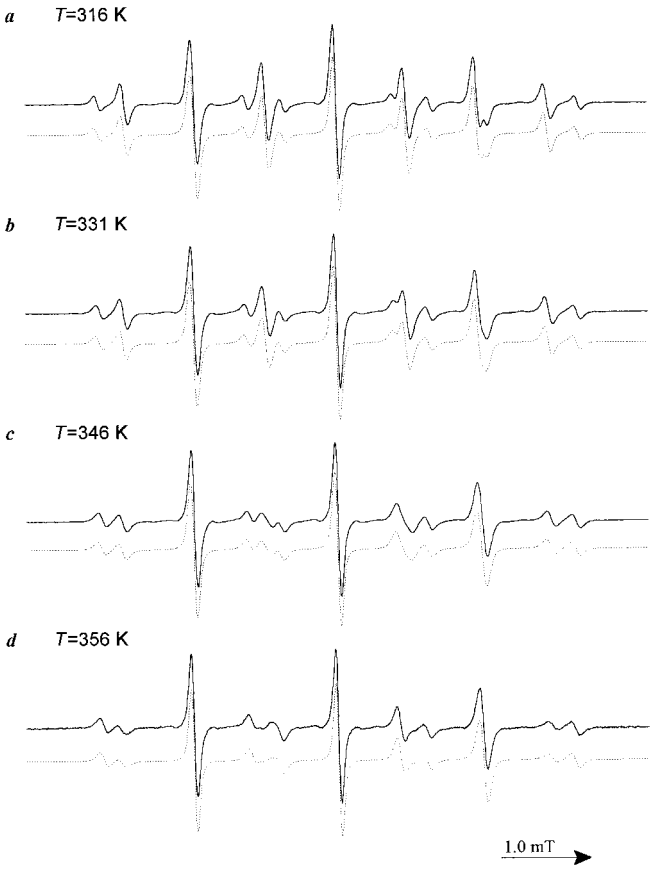


Figure 2. EPR spectra of nitroxide **1a** recorded in water in the presence of 3.25×10^{-3} M β-CD at different temperatures. The dotted lines show the corresponding simulations. a) $k_1=1.0\times10^9\,M^{-1}s^{-1}$, $k_{-1}=1.6\times10^6\,s^{-1}$; b) $k_1=1.5\times10^9\,M^{-1}s^{-1}$, $k_{-1}=2.9\times10^6\,s^{-1}$; c) $k_1=2.0\times10^9\,M^{-1}s^{-1}$, $k_{-1}=5.1\times10^6\,s^{-1}$; d) $k_1=2.3\times10^9\,M^{-1}s^{-1}$, $k_{-1}=8.7\times10^6\,s^{-1}$. As an example, spectrum (b) was simulated by assuming the presence of the free species (37%) in the bulk aqueous phase (g factor 2.0056, linewidth 0.029 mT, a(9H) 0.018 mT, a(N) 1.659 mT, a(2H) 1.031 mT), and the included radical (63%) (g factor 2.0058, linewidth 0.031 mT, a(9H) 0.018 mT, a(N) 1.584 mT, a(2H) 0.802 mT).

and free species. Because of this favourable feature, the determination of the association constants K_1 was straightforward. When the CD was present in large excess with respect to the radical species, the concentration of which was in the range $(2-6)\times10^{-5}$ M in all experiments, the equilibrium constant for radical inclusion was obtained from Equation (2), where $[CD]_0$ denotes the initial concentration of cyclodextrin, and x_{CD} and x_{water} are the molar fractions of the included and free radical species, respectively, which were determined by simulating the EPR spectra.

$$K_1=\frac{x_{CD}}{x_{water}[CD]_0} \qquad (2)$$

Good agreement with the experimental spectra was obtained at room temperature, while the EPR spectra recorded in the presence of β-CD at higher temperatures could not be correctly simulated. Above about 316 K, the spectrum of **1a** showed marked selective line broadening, which was especially evident on the lines corresponding to $M_I(2H_\beta)=\pm1$ (see Figure 2a–d). Such linewidth effects[15] are indicative of rapid exchange between the nitroxide included in β-CD **1b** and the corresponding unbound species **1a**, which modulates the nitrogen and proton hyperfine splittings and, to a smaller extent, the Zeeman interaction. The rate constants k_1 and k_{-1} for association and dissociation, respectively, were determined as a function of temperature by simulating the exchange-broadened EPR spectra with well-established procedures[15] based on the density matrix theory[16] and by assuming a two-jump model as illustrated in Equation (1). Fitting to the experimental spectra was carried out with a Monte Carlo minimisation procedure.[17]

Figure 2 shows the excellent agreement between simulated and experimental spectra recorded at different temperatures in the presence of 3.25×10^{-3} M β-CD. The rate constant of association of the nitroxide with the CD and of dissociation of the complex are collected in Table 2, while the results of such experiments are summarised graphically in the Eyring plot shown in Figure 3, in which $\ln(k/T)$ was plotted against $1/T$ to derive the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ (Table 2).

The effect of changing the size of the CD on the inclusion process was also investigated with **1a** as radical probe. There was no detectable association of **1a** with α-CD at a concentration of up to 10^{-3} M, while at higher α-CD concentrations ($>2\times10^{-2}$ M) new lines arising from the complexed radical were observed. The spectra of free and bound species were sufficiently different to allow the determination of the spectroscopic parameters of the included radical. With these parameters, simulation of the experimental spectrum obtained at 294 K yielded the rate constants $k_1=1.1\times10^8\,M^{-1}s^{-1}$ and $k_{-1}=7.7\times10^6\,s^{-1}$ for the exchange process. With a γ-CD concentration of 10^{-2} M, an EPR spectrum showing signals due to both free and included nitroxide was obtained, and simulation of this provided the rate and equilibrium constants for complexation reported in Tables 2 and 3, respectively.

The effect on inclusion of modifying the entrance face of β-cyclodextrin was investigated with two derivatives, heptakis-

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Table 2. Rate constants[a] at 294 K and activation parameters[b] for the inclusion of nitroxides **1a**–**3a** by cyclodextrins.

Radical	Solvent	CD	k_1 [M^{-1}s^{-1}]	k_{-1} [s^{-1}]	$\Delta H_1^{\neq}$ [kcal mol^{-1}]	$\Delta S_1^{\neq}$ [cal mol^{-1}K^{-1}]	$\Delta H_{-1}^{\neq}$ [kcal mol^{-1}]	$\Delta S_{-1}^{\neq}$ [cal mol^{-1}K^{-1}]
1a	H_2O	α-CD	1.1×10^8	7.7×10^6				
	H_2O	β-CD	6.8×10^8	5.3×10^5	3.55	−6.0	8.38	−3.8
	H_2O	γ-CD	3.8×10^8	7.6×10^6				
	H_2O	DM-β-CD	6.5×10^8	6.1×10^5	4.55	−2.7	8.98	−1.5
	H_2O/MeOH (70/30)	DM-β-CD	6.6×10^8	1.9×10^6	4.57	−2.6	9.51	2.6
	H_2O/MeOH (50/50)	DM-β-CD	5.8×10^8	4.9×10^6				
	H_2O	TM-β-CD	1.2×10^8	5.7×10^6				
2a	H_2O	DM-β-CD	2.8×10^8	3.3×10^6				
3a	H_2O	DM-β-CD	2.5×10^9	3.7×10^7				

[a] Estimated uncertainty ≈ 10 %. [b] Estimated uncertainties: $\Delta H^{\neq}$, ±0.5 kcal mol^{-1}; $\Delta S^{\neq}$, ±2 cal mol^{-1} K^{-1}.

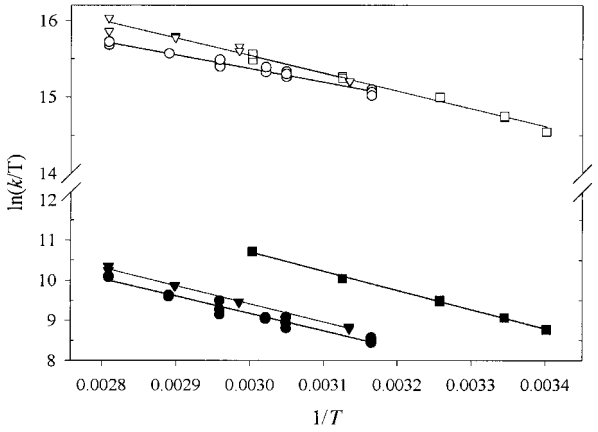

Figure 3. Plots of ln(k/T) *versus* 1/T for the association (empty symbols) and dissociation (full symbols) rate constants for inclusion of radical **1a** by β-CD (○, ●) and by DM-β-CD (▽, ▼) in water. Squares refer to rate constants measured for **1a** in the presence of DM-β-CD in methanol/water (30/70).

(2,6-*O*-dimethyl)-CD (DM-β-CD) and heptakis-(2,3,6-*O*-trimethyl)-CD (TM-β-CD). The former has a molecular conformation which, although it retains many of the features of the parent β-CD, contains an extended internal toroidal region in which larger hydrophobic guest molecules can be accommodated. The experimental data (see Figure 3 and Table 3) show that the association constants of **1a** with DM-β-CD and with unmodified β-CD are similar, while that with TM-β-CD is much lower since no inclusion of the probe was observed for host concentrations up to 10^{-2} M. However, in the presence of 0.1M TM-β-CD, a second spectrum, assigned to the included radical, was detectable alongside that of free **1a**. In addition to the changes in the binding constant, the hyperfine splitting from the two benzylic protons of the included radical are substantially different from the value observed with unmodified β-CD (see Table 1).

Despite the large number of investigations on cyclodextrin-complex formation in binary aqueous/organic solvent mixtures,[18] to the best of our knowledge, the effects of the addition of a co-solvent on the rates of association/dissociation have not yet been studied in detail. We determined the rates of these processes in different water/methanol mixtures with DM-β-CD as host, since this derivative is much more soluble in organic solvents than the unmodified CD. With increasing the molar fraction of methanol in the methanol/water mixture, a decrease in the hyperfine splittings from both nitrogen and the protons in the free nitroxide was observed with a consequent decrease in the separation of the signals of the free and included species. For this reason, solvent mixtures containing more than 50 vol% of methanol could not be studied. From the experimental data reported in Tables 2 and 3, it is evident that the binding constant for **1a** with DM-β-CD decreases when water is replaced by a water/methanol mixture.

We also explored the possibility of using other nitroxides, such as *tert*-butyl methyl nitroxide (**2a**) and benzyl methyl nitroxide (**3a**), to measure the rate of exchange between free and included radicals. Because of the shorter lifetimes of these radicals compared with **1a**, large amounts of the amine precursor and oxidising agent ($> 1 \times 10^{-3}$ M) were required to obtain sufficiently high steady-state radical concentrations in static samples. Since, under these conditions, it is likely that the amine will compete with the radical for inclusion in the CD, the nitroxides were generated by a flow-mixing technique, in which the aqueous solution passes through the microwave cavity immediately after mixing of the reagents. By using a flow rate of about 0.1 mL min^{-1}, spectra with good signal-to-noise ratios could be obtained with only 10^{-3} M

Table 3. Thermodynamic parameters for the inclusion of nitroxides **1a**–**3a** by cyclodextrins.

Radical	Solvent	CD	K_1 [M^{-1}][a] (T = 294 K)	ΔG^0_{294}[a] [kcal mol^{-1}]	ΔH^{0}[a] [kcal mol^{-1}]	ΔS^{0}[a] [cal mol^{-1}K^{-1}]
1a	H_2O	α-CD	14.3	−1.55		
	H_2O	β-CD	1281	−4.18	−4.83	−2.2
	H_2O	γ-CD	50.7	−2.29		
	H_2O	DM-β-CD	1079	−4.08	−4.43	−1.2
	H_2O/MeOH (70/30)	DM-β-CD	342.8	−3.41	−4.94	−5.2
	H_2O/MeOH (50/50)	DM-β-CD	121.0	−2.80		
	H_2O	TM-β-CD	21.5	−1.79		
2a	H_2O	DM-β-CD	84.8	−2.59		
3a	H_2O	DM-β-CD	70.0	−2.48		

[a] Values derived from the measured rate constants, except for **3a** (see text).

solutions of the amine and of the peroxy acid salt. The EPR spectrum of radical **2a** in water at room temperature was readily analysed in terms of the coupling constants listed in Table 1, and the spectrum was unaffected by the presence of methyl-α-D-glucopyranoside (6.0×10^{-2} M). In contrast, when DM-β-CD (8.2×10^{-3} M) was added to the solution, additional signals assigned to the cyclodextrin-included radical **2b** were observed. The values of a_N and a_H(Me) decreased only slightly upon inclusion in the less polar environment of the DM-β-CD cavity; nevertheless, significant differences in the line positions of the two species were clearly evident (see Supporting Information) and allowed the concentration ratio [**2a**]/[**2b**] to be determined. The spectra also exhibited selective line broadening, and good simulations were obtained by assuming that this arises from exchange between free and included nitroxide.

A strong EPR spectrum of benzyl methyl nitroxide (**3a**) was obtained from the corresponding amine in water at 293 K, and the measured hyperfine splitting constants are given in Table 1. In the presence of DM-β-CD, significant variations were observed in a_H(Me) and $a_H(CH_2)$ upon inclusion, although it was not possible to detect separate signals from the free and bound nitroxide. In particular, a_H(Me) and $a_H(CH_2)$ decreased by 0.104 and 0.132 mT, respectively, on changing the solvent from water to an aqueous solution of DM-β-CD (9.16×10^{-2} M). In addition, the slight decrease in a_N indicated a reduction in the polarity of the spin-probe environment, consistent with the inclusion of **3a** in the hydrophobic cavity of the cyclodextrin. In contrast, essentially no change in the coupling constants was observed when the CD was replaced by a corresponding concentration of methyl-α-D-glucopyranoside. Under the above conditions, the experimental EPR spectrum represents the concentration-weighted average of the spectra of the nitroxide in water and included in the CD; the binding constant of **3a** with DM-β-CD can be estimated by means of Equations (2) and (3). In Equation (3), a_H^{CD} and a_H^{water} are the methyl or CH_2 proton splittings for included and free nitroxide, respectively.

$$\langle a_H \rangle = [a_H^{CD} - a_H^{water}]x_{CD} + a_H^{water} \quad (3)$$

By using the value of a_H^{CD} measured at high CD concentrations, for which the coupling constants tend to reach a plateau (Figure 4), K_1 was estimated to be 70.0 M^{-1} at 294 K. At this temperature, the EPR spectrum of **3a** (Figure 5a) in the presence of DM-β-CD (7×10^{-3} M) exhibited selective line broadening, and computer simulation gave the rate constants for exchange as $k_1 = 2.5 \times 10^9\ M^{-1}s^{-1}$ and $k_{-1} = 3.7 \times 10^7\ s^{-1}$.

Discussion

The EPR data are consistent with the formation of 1:1 inclusion complexes between the cyclodextrins and the nitroxides **1a**–**3a**. For *tert*-butyl diphenylmethyl nitroxide (**4a**), Kotake and Jansen[4i] found evidence that this radical is only partially included by β-CD either from the *tert*-butyl or from the phenyl side of the molecule. We believe that this behaviour is related to the presence of the two aromatic rings

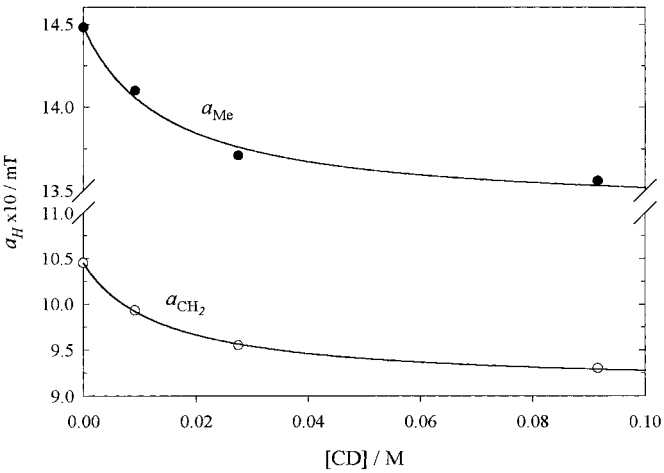

Figure 4. Plot of the mean proton hyperfine splitting constants for **1a** versus the concentration of DM-β-CD in water. Solid lines correspond to the values predicted by Equations (2) and (3).

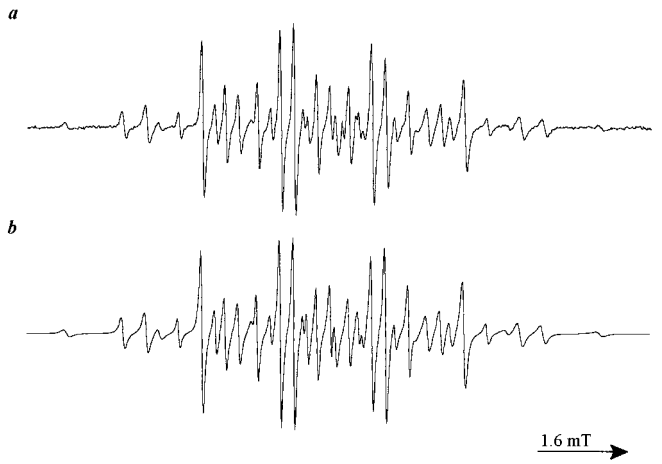

Figure 5. a) EPR spectrum of radical **3a** in water at 294 K in the presence of DM-β-CD (7.0×10^{-3} M). b) Computer simulation of spectrum (a) obtained by using the spectroscopic parameters reported in Table 1, $k_1 = 2.5 \times 10^9\ M^{-1}s^{-1}$ and $k_{-1} = 3.7 \times 10^7\ s^{-1}$.

in **4a**, the bulk of which precludes its full inclusion in the CD cavity. In contrast, the smaller radicals **1a**–**3a** can be completely included in the β-CD cavity. The significant reductions in a_N relative to the values in water that accompany complexation of **1a**–**3a** by β- or γ-CD indicate that the N–O groups of these nitroxides are quite deeply included in the host cavities, consistent with the relatively large internal diameters of β- and γ-CDs (ca. 6.6 and ca. 8.4 Å, respectively).[1] However, with α-CD (internal diameter ca. 5.2 Å), the similar values of a_N for free and included **1a** indicate that the N–O group of the complexed radical is exposed to bulk water.

Information on the preferred conformations adopted by radical **1a** in the various environments can in principle be obtained from the magnitude of $a(H_\beta)$. The value of the hyperfine splitting constant to β-protons in alkyl nitroxides depends on the spin population in the $2_{p\pi}$ orbital of nitrogen ρ_N^π and on the dihedral angle ϑ between the symmetry axis of this orbital and the N-C-H_β plane [Eq. (4)].[19]

$$a(H_\beta) = \rho_N^\pi(A_N + B_N\langle\cos^2\vartheta\rangle) \quad (4)$$

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The constant A_N is small and is usually neglected, while $\rho_N^{\pi}B_N$ can be obtained from the splitting of the methyl protons in **3a**, where $\langle\cos^2\vartheta\rangle = 0.5$ for symmetry reasons. Thus, for radical **1a** in water at 298 K when $a(H_\beta)$ is 10.57 G, $\arccos\langle\cos^2\vartheta\rangle^{1/2}$ is calculated to be 52.8°, which can be compared with the value of 45° if rotation about the N–C_β bond were unhindered. For the α-, β- and γ-CD inclusion complexes the corresponding calculated values are 55.0, 57.5 and 57.5°, respectively, assuming that ρ_N^{π} decreases linearly with the decrease of a_N that accompanies inclusion. These values indicate that the minimum energy conformation of **1a** is close to that shown in Figure 6, although the *t*Bu-N-C_β-Ph dihedral angle is somewhat greater than 90° and thus relieves repulsion between the bulky phenyl and *tert*-butyl groups. (The instantaneously nonequivalent β-protons would be rendered magnetically equivalent on the EPR timescale by rapid twofold rotation about the N–C_β bond.)

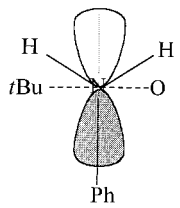

Figure 6. Minimum energy conformation of **1a**

It appears that inclusion into the CD cavities leads to an increased tendency for the phenyl group to eclipse the $2_{p\pi}$ orbital of nitrogen, probably because the nitroxide is thereby rendered more compact, even though steric repulsion between the phenyl and *tert*-butyl groups is increased. Of course, predictions based on Equation (4) are only semi-quantitative, and the conformations indicated are averages over the populated torsional states.

Inspection of the binding constants K_1 given in Table 3 shows that β-CD has a higher affinity for **1a** than either α- or γ-CD, and this indicates that the latter hosts have cavities too small and too large, respectively, to bind **1a** effectively. Consideration of the rate constants for the inclusion process (Table 2) shows that the difference in the binding constants for β-CD and γ-CD appears to result mainly from an increase in the rate of dissociation for the γ-CD complex, since the rates of association are comparable for the two CDs. With α-CD, both a smaller association rate constant and a larger dissociation rate constant contribute to the smaller affinity for **1a**, consistent with a relatively weak interaction between radical and the small α-CD cavity.

The activation parameters for formation and dissociation of the inclusion complexes of **1a** with β-CD and with DM-β-CD in water show some interesting features. At room temperature, the enthalpic contributions ($\Delta H_1^{\neq}$, $\Delta H_{-1}^{\neq}$) to the Gibbs energy term $\Delta G^{\neq}$ are larger than the entropic contributions. The significant enthalpies of activation for the association process (3.55 and 4.55 kcal mol^{-1} for β-CD and DM-β-CD, respectively) indicate that the corresponding rate constants are still far from the diffusion-controlled limit. The various factors governing the magnitude of the entropy of activation are more difficult to visualise, since $\Delta S_1^{\neq}$ and $\Delta S_{-1}^{\neq}$ are related to the change in randomness on passing from reactants or complex, respectively, to the transition state.[20] In general, the following four terms should contribute to the activation entropy: 1) the freezing of motional degrees of freedom for the guest molecule, 2) the desolvation around the guest molecule and the reorganisation of solvent water, 3) the release of water molecules from the CD cavity[21] and 4) any conformational changes of the CD torus. While the first term will be negative, the others are expected to contribute positively to the activation entropy. For association of **1a**, the experimental activation entropy ($\Delta S_1^{\neq}$) is only slightly negative, and this suggests that the positive and negative contributions nearly cancel each other. The entropy of activation for the dissociation process $\Delta S_{-1}^{\neq}$ is also negative but of smaller magnitude than $\Delta S_1^{\neq}$, so that the enthalpy term $\Delta H_{-1}^{\neq}$ provides the dominant contribution to the free energy of activation for the dissociation process.

The addition of MeOH as co-solvent caused almost no change in the association rate constant for inclusion of **1a** by DM-β-CD. In marked contrast, the dissociation rate constant increased appreciably from $6.1 \times 10^5\,\text{M}^{-1}\text{s}^{-1}$ in water to $4.9 \times 10^6\,\text{M}^{-1}\text{s}^{-1}$ in water/MeOH (50/50) at 294 K. The decrease in binding constant with increasing methanol concentration is hence almost wholely a result of an increase in the rate constant for dissociation. Inspection of the activation parameters for the dissociation process in water/MeOH (70/30) reveals that the increase in this rate constant is attributable to an increase in the entropy of activation, which more than compensates for the larger enthalpy of activation. This enthalpy/entropy compensation behaviour in the formation of CD complexes has been reported frequently in the literature.[22]

It is interesting to compare the relative stabilities of the three complexes **1b**–**3b** formed between DM-β-CD and the nitroxides **1a**–**3a**. The binding constants for **2a** and **3a** are quite similar and lower than that of **1a**. Since the hydrophobic interaction is believed to play an important role in controlling the stabilities of CD complexes,[1] we interpret these results in terms of the relative hydrophobicities of the radical species. The nitroxide **1a** bears two strongly hydrophobic substituents and should have a stronger affinity for the CD than either **2a** or **3a**, in which the benzyl group or the *tert*-butyl group of **1a** has been replaced with a less hydrophobic methyl group. Differences in the rate constants that depend on the nature of the guest molecule are also clearly evident. The data of Table 2 suggest that steric hindrance due to the *tert*-butyl group has a marked effect on the association and dissociation rate constants. In particular, the rate constants for the inclusion of **1a** or **2a**, which both possess a bulky *N-tert*-butyl group capable of interacting sterically with the rim of the CD, are appreciably smaller than the rate constant for the inclusion of benzyl methyl nitroxide **3a**.

Conclusion

We have demonstrated that natural and modified cyclodextrin hosts form inclusion complexes with the nitroxide radicals **1a**–**3a**. In particular, benzyl *tert*-butyl nitroxide **1a** exhibits high binding constants for complex formation with β-CD and with modified β-CDs and shows a marked difference in the hyperfine splitting constants for the free and bound forms. This enabled the rate constants for the association and dissociation processes to be measured for the first time by

EPR spectroscopy with a radical probe. In our view, this method for investigating the dynamics of CD host–guest interactions has considerable potential, and experiments are underway to extend this technique to the study of other types of host–guest complexes.

Experimental Section

Commercially available cyclodextrins (Sigma or Aldrich) were used throughout; β-CD was recrystallised from distilled water and dried under vacuum at 80 °C; the α- and γ-CDs and the modified CDs were used as received. The amines (Aldrich) were also used as received. Freshly distilled water and methanol (Aldrich HPLC grade) were used as solvents.

EPR spectra were recorded on a Bruker ESP300 spectrometer equipped with an NMR gaussmeter for field calibration and a Hewlett Packard 5350B microwave frequency counter for the determination of the g factors, which were referenced to that of the perylene radical cation in concentrated H_2SO_4 ($g = 2.00258$). The sample temperature was controlled with a standard variable-temperature accessory and was monitored before and after each run with a copper/constantan thermocouple. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. Digitised EPR spectra were transferred to a personal computer for analysis, and digital simulations were carried out with a program developed in our laboratory and based on a Monte Carlo procedure.[17] The input data for the program are the number of nonequivalent nuclei, the hyperfine splitting constants of the free and included radical at each temperature, the intrinsic linewidth in the absence of exchange (a Gaussian lineshape resulting from the unresolved splittings from the *tert*-butyl protons was employed) and the rate constants for the exchange process.

Radical **1a** was generated by mixing a solution of the corresponding amine (5.0×10^{-4} M) with a solution of the magnesium salt of monoperoxyphthalic acid (Aldrich, technical grade, 5.0×10^{-4} M). To achieve a sufficiently high radical concentration, the mixed solution was sometimes heated at 60 °C for 1–2 min. Aliquots from a concentrated CD stock solution were added to the solution of nitroxide to yield the required concentrations. Samples were then transferred in capillary tubes (1 mm i.d.), and EPR spectra were recorded. Radicals **2a** and **3a** were generated by continuous-flow experiments in a mixing chamber which allowed the simultaneous mixing of two reagent streams (amine 1.0×10^{-3} M, magnesium monoperoxyphthalate 1.0×10^{-3} M) before passing through a flattened cell in the cavity of the EPR spectrometer. Since the oxidation processes were quite slow at room temperature, optimum nitroxide concentrations were obtained by maintaining the flow rate at about 0.1–0.2 mL min^{-1} with a syringe-pump apparatus (model SP200i, Henry Fein Word Precision Instrument).

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