Using Cyclodextrins to Encapsulate Oxygen-Centered and Carbon-Centered Radical Adducts: The Case of DMPO, PBN, and MNP Spin Traps

Mariana Spulber and Shulamith Schlick*

Department of Chemistry and Biochemistry, University of Detroit Mercy, 4001 West McNichols Road, Detroit, Michigan 48221

Received: January 26, 2010; Revised Manuscript Received: April 29, 2010

We present electron spin resonance (ESR) experiments that describe the interaction of β-cyclodextrin (β-CD) with spin adducts of three spin traps: 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), *N-tert*-butyl-α-phenylnitrone (PBN), and 2-methyl-2-nitrosopropane (MNP). The focus was on spin adducts of oxygen-centered radicals trapped by DMPO and PBN and on carbon-centered radical adducts trapped by MNP. The radicals were generated by reaction with hydroxyl radicals and the spin adducts studied were DMPO/OH and PBN/OH, MNP/CH_2COOH generated in CH_3COOH, and MNP/CF_2COOH in CF_2HCOOH. Di-*tert*-butyl nitroxide $((CH_3)_3C)_2NO$ (DTBN) was also detected in experiments with MNP as the spin trap. A *range* of interactions of the spin adducts and DTBN with β-CD was identified. The presence of β-CD led to significant stabilization of DMPO/OH and PBN/OH but to a negligible effect on the ^{14}N hyperfine splitting of the adducts, a_N, indicating that the N−O group is *outside the β-CD cavity*. An increase of a_N was detected for DTBN and MNP/CH_2COOH in CH_3COOH in the presence of β-CD, a result we assigned to bonding at the *rim of the host*. Experiments with methylated β-CD (Me β-CD) provided support for this conclusion. A different type of complexation was detected for DTBN and MNP/CF_2COOH in CF_2HCOOH: for specific host concentrations both *"in"* and *"out" species* were detected. We suggest that the hydrophobicity of the fluorinated adduct leads to insertion of the adduct inside the host cavity. Calculation of the association constant K_a indicated the competition between DTBN and the adduct for inclusion in the host. For MNP as spin trap, the two nitroxide radicals (adduct and DTBN) have the same type of interaction with the host: at the rim in acetic acid, and inside the host cavity in CF_2HCOOH. Experiments with DTBN in the absence of the spin trap and of adducts illuminated the effect of the local polarity and of the pH on the hyperfine splittings and indicated that the presence of acetic acid encourages *rim complexation*.

Introduction

The ability of cyclodextrins (CDs) to form inclusion complexes by noncovalent bonding with a variety of guests has become an exciting field of research, with important applications in chromatography, drug delivery, and catalyst stabilization.[1,2] CDs are cyclic oligosaccharides containing six (α-CD), seven (β-CD), or eight (γ-CD) D-(+) glucopyranose units attached by α-(1,4) glucosidic bonds, with lipophilic inner cavities and hydrophilic outer surfaces.[11] In aqueous solutions the slightly nonpolar cyclodextrin cavity contains water that can be replaced by guests with lower polarity.[3]

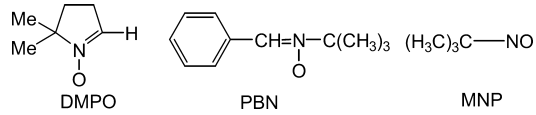
CD inclusion complexes with nitroxide radicals have been studied early on by electron spin resonance (ESR) spectroscopy and take advantage of the ability of this method to detect the effect of the local environment on the guest with great specificity and sensitivity. Atherton and Strach have investigated the association of di-*tert*-butyl nitroxide $((CH_3)_3C)_2NO$ (DTBN) with α-CD and β-CD at 296 K and calculated the equilibrium constant for β-CD, taking into consideration that the solution is not ideal.[4] Martinie et al.[5] have demonstrated the complexation of α-CD and β-CD with several nitroxides by ESR. In both papers[4,5] the nonpolar environment was reflected in lower hyperfine splitting (hfs) from the ^{14}N nucleus, a_N, in guests enclosed in the CDs, compared to aqueous solutions. Lowering of a_N by interaction with the host has become an important indicator of guest−host complexation in numerous ESR studies. Kotake and Janzen have studied the effect of pH on the inclusion process and assigned the lower degree of inclusion at higher pH values to the protons generated by dissociation of the OH groups; the same argument was also used to explain that CDs are poor hosts for ionic species.[6] The same authors detected "multimodal" inclusion: in the case of a nitroxide with several functional groups, different complexes were generated, depending on the group that became the guest.[7] The choice of an interesting nitroxide led to the detection, with excellent resolution due to D-enrichment, of different ESR spectra for the "in" and "out" nitroxides (inside and outside the CD host), with assignment based on the a_N values.[8] More recently, it was demonstrated that pulse ESR methods such as electron spin−echo envelope modulation (ESEEM) and double electron−electron resonance (DEER) can lead to additional structural information compared to continuous wave (CW) ESR.[9]

NMR studies have added important details on the relative location of guests in α-, β-, and γ-CDs.[10] An NMR study of a series of α-phenyl-*N-tert*-butylnitrones (PBNs) in β-CD has determined the stoichiometry of the guest−host complex (1:1) and the corresponding binding constants.[11] These NMR studies, which focus on the host as modified by the guest, offer complementary information to the ESR study of CD−nitroxide complexes, whose focus is on the guest.

* Corresponding author. Telephone: 1-313-993-1012. Fax: 1-313-993-1144. E-mail address: schlicks@udmercy.edu (S. Schlick).

10.1021/jp100777u
Published on Web 05/12/2010

CHART 1: Spin Traps Used in the Present Study



In contrast to the extensive literature on ESR studies of CD−nitroxide complexation, there are only few reports on the interaction between CDs and nitroxide *spin adducts*. These systems are more complicated than the CD−nitroxide systems because they also include the spin trap, the radical source, the attacking species (typically HO• radicals), and in some cases additional paramagnetic species that can also exhibit "in" and "out" ESR spectra. Despite this complexity, study of spin adducts in CDs and the ability of CDs to increase the lifetimes of spin adducts have been reported recently. Tordo and co-workers have described the increased stability of nitrone adducts of the superoxide radical anion and their inclusion complexes with randomly methylated or selectively methylated β-CD.[12,13] Of particular interest for the present study is the inclusion of MNP adducts of alkyl radicals in β-CD: Encapsulation led to broader line widths in the ESR spectra of the adducts, but the higher solubility of MNP in water and the increased lifetimes of the adducts were major advantages.[14] In an interesting recent approach, Han et al. described the interaction of a β-CD−nitrone conjugate with the superoxide radical anion and used molecular modeling to predict the stability of adducts and their longer lifetimes in the presence of the host.[15]

The major motivation in initiating our study of nitroxide complexation by CDs has emerged from spin trapping studies in our laboratory whose goal was to detect and identify oxygen-centered and carbon-centered free radicals generated during the fragmentation of polymeric membranes used in fuel cells and of corresponding model compounds.[16−19] Ex situ experiments in the laboratory have been performed by generating hydroxyl radicals, HO•, via UV irradiation of hydrogen peroxide.[20] Direct ESR detection of radicals was possible only in a limited number of cases, by lowering the temperature in order to increase the lifetimes of unstable intermediates.[16] Most systems required spin traps in order to scavenge the short-lived radicals, a process that leads to the formation of the more stable nitroxide radical adducts.[21,22] The spin traps used in our studies were 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), *N*-*tert*-butyl-α-phenylnitrone (PBN), and 2-methyl-2-nitrosopropane (MNP), Chart 1, and identification of adducts was facilitated in some cases by the spin trapping database.[23] In the study of model compounds[19] and in the in situ study of a fuel cell inserted in the resonator of the ESR spectrometer[24,25] we have described the detection and identification of carbon-centered radicals (CCRs) and oxygen-centered radicals (OCRs) as DMPO and MNP adducts. In some cases the detection of spin adducts was hampered because of their low stability. For these reasons we explore here the effect of CDs on oxygen-centered radical adducts of DMPO and PBN, and on carbon-centered radical adducts of MNP. The focus in the present study was on the effect of CD on the lifetimes of spin adducts, the variation of hfs due to encapsulation, the location of the nitroxide in the complex, the effect of the pH, and the importance of guest hydrophobicity on the interactions CD−guest.

As seen below, we have determined the various effects of β-CD presence: From the much higher stability of DMPO/OH and PBN/OH adducts in the presence of β-CD in aqueous solutions at 300 K, to the different types of complexes of β-CD with the protiated adduct MNP/CH_2COOH and with the fluorinated adduct MNP/CF_2COOH. These latter adducts were generated by attack of HO• radicals on acetic acid (CH_3COOH, AA) and difluoroacetic acid (CF_2HCOOH, DFAA), respectively.[19] DTBN was also generated in experiments with MNP as the spin trap, and its complexation by β-CD was detected. Most experiments were performed with β-CD, but additional clarifications were obtained by using methylated β-CD (Me β-CD). In addition, the presence of both adduct and DTBN in systems with MNP as the spin trap necessitated experiments of DTBN/β-CD (in the absence of MNP); these experiments illuminated the roles of AA and DFAA in complex formation and validated our conclusion that in some systems the guest was located on the *rim* of the host. Some of the aspects studied in this paper, for example, fluorinated spin adducts and the effect of the local polarity, have not been discussed in the existing literature.

Experimental Section

Materials. DMPO, PBN, MNP, β-CD, and Me β-CD were purchased from Sigma-Aldrich and used as received. Hydrogen peroxide, H_2O_2, was obtained from Sigma-Aldrich as a 3% aqueous solution. Deionized "ultrapure" water with low conductivity and <10 ppb total organic carbon was provided by a Millipore Model Direct-Q UV system and was used in all experiments. MNP was supplied by Sigma-Aldrich as a diamagnetic dimer, which is known to dissociate in aqueous solutions[26]

$$(CH_3)_3C{-}\underset{\downarrow}{N}{=}\underset{\downarrow}{N}{-}C(CH_3)_3 \rightarrow 2(CH_3)_3C{-}N{=}O$$
$$\qquad\qquad\quad O \quad O$$

Sample Preparation. The DMPO/OH and PBN/OH spin adducts were obtained by mixing 0.1 mL of aqueous solution of the spin trap (1×10^{-3} M DMPO and 2.5×10^{-3} M PBN, respectively) with 0.01 mL of 3% H_2O_2. The pH of each solution was adjusted to the range 5−7 with 0.1 M NaOH calibrated solution; this procedure was performed because nitroxide radicals are less stable in acidic media.[27] DMPO and PBN adducts were generated at a neutral pH, to enhance their stability. The generation and decay ("time scans") of the DMPO and MNP adducts were followed by in situ UV irradiation of the appropriate solutions (in the presence and in the absence of β-CD) at 300 K in quartz capillary tubes placed inside the ESR resonator, using a 300 W ozone-free Xe arc equipped with a water filter (Oriel).

Attack of hydroxyl radicals on acetic acid leads to the formation of the •CH_2COOH radical; the corresponding MNP adduct has $a_N = 15.7$ G and $a_H = 8.6$ G (2H) at pH = 7.[19] In the case of CF_2HCOOH as the model compound, the •CF_2COOH radical is generated and the corresponding MNP adduct has $a_N = 13.4$ G and $a_F = 22.6$ G (2F) at pH = 7; a_N is significantly lower than for MNP/CH_2COOH because of the higher fluorine electronegativity, and a_F is much higher than that for the ^{1}H hfs in •CH_2COOH, 22.6 vs 8.6 G.[19] The MNP/CCR adducts were prepared by mixing 0.1 mL aqueous solution of MNP (2×10^{-4} M) with 1 mL AA or DFAA (2M) and 0.05 mL of H_2O_2 3%. The pH of the solution was adjusted to 5 with NaOH, in order to facilitate the inclusion process that is enhanced at lower pH values.[6,28] All mixtures were UV irradiated at room temperature in quartz capillary tubes for 10 min with a low-pressure mercury source (Mineralight Model PCQX1). The stability of the MNP adducts prepared as described above is high, typically 3 days at ambient temperature; see next section.

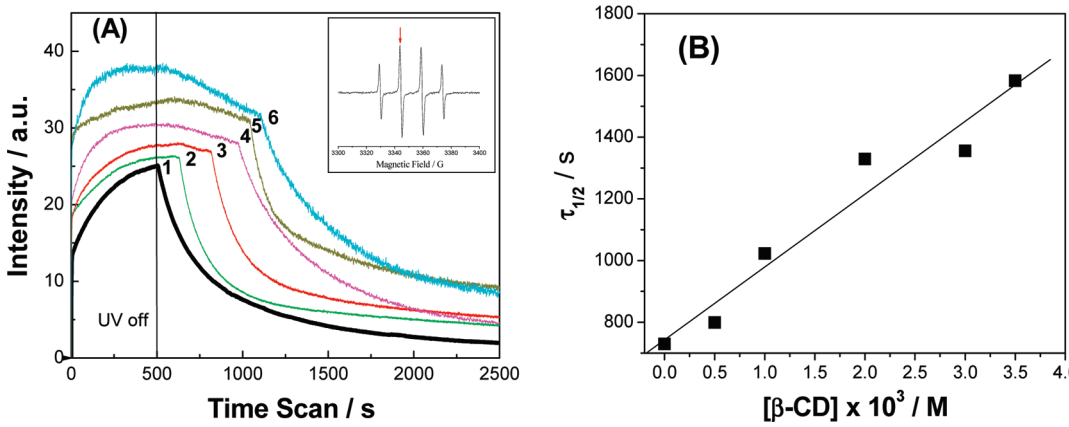


Figure 1. (A) Generation of the DMPO/OH adduct and its decay at 300 K in the absence (line 1) and in the presence of several β-CD concentrations: 0.5×10^{-3} M (line 2), 1×10^{-3} M (line 3), 2×10^{-3} M (line 4), 3×10^{-3} M (line 5), 3.5×10^{-3} M (line 6). The red arrow indicates the signal whose intensity was monitored in the time scan. (B) The half-life of the DMPO/OH adduct as a function of β-CD concentration, determined at the half-maximum intensity of the signal shown by the red arrow after stopping the UV irradiation. The solid line was obtained by linear fit.

The appropriate amounts of β-CD were added to an aliquot of the spin adduct, and the mixture was transferred to capillary tubes for ESR measurements. The MNP adducts are more stable and complexation by β-CD was performed at pH 5; the hyperfine splittings under these conditions are slightly different compared to those published in ref 19; see next section.

ESR Measurements. Spectra were recorded at 300 K using Bruker X-band EMX spectrometers operating at 9.7 GHz and 100 kHz magnetic field modulation, and equipped with the Acquisit 32 Bit WINEPR data system version 3.01 for acquisition and manipulation, and the ER 4111 VT variable temperature units. The microwave frequency was measured with the Hewlett-Packard 5350B microwave frequency counter. The hyperfine splittings of the spin adducts were determined by fitting the spectra using the WinSim (NIEHS/NIH) simulation package;[29] the fitting also determines the relative intensity of each component for spectra that consist of a superposition of contributions from different adducts or radicals. The weak satellites from ^{13}C, indicated by arrows in Figure 11, were not included in the simulations shown in Figures 4A, 11, and 12. Typical acquisition parameters for all ESR spectra were sweep width 100−160 G, microwave power 2 mW, time constant 20.48 ms, conversion time 41.94 ms, 2048 points, modulation amplitude 0.2−0.4 G, receiver gain 5×10^4, and 20−40 scans.

Results and Discussion

DMPO and PBN as Spin Traps. The effect of β-CD on the stability and hyperfine splittings of the DMPO/OH adduct was studied at 300 K, and the β-CD concentrations were in the range $(0.5-3.5) \times 10^{-3}$ M. Changes in the values of a_N with increasing β-CD concentration were within experimental error, ≈0.1 G, and only slight increase in the line widths, typically 0.2 G, were detected when the ESR spectra were measured in the temperature range 290−320 K. However, a significant increase in the stability of the adduct was measured: The time scans presented in Figure 1A show the intensity of the adduct during UV irradiation and the decay after the irradiation was stopped, for the indicated β-CD concentrations; the red downward arrow in the inset points to the monitored signal. We note the higher intensity of the adduct during its generation, indicating the formation of the inclusion complex, and its slower decay in the presence of β-CD; both effects are more pronounced for the higher β-CD concentrations. In the absence of β-CD the adduct intensity decays immediately after stopping the UV irradiation; in the presence of the host, however, the signal can be detected for time intervals that depend on β-CD concentration. The decay rate of the DMPO/OH spin adduct is slower with the increase of host content; for example, 2500 s after the initial start of the irradiation, the concentration of the spin adducts is 2−5 times higher in the presence of β-CD than in its absence.

Figure 1B presents the half-life of DMPO/OH, $\tau_{1/2}$, determined at the half-maximum intensity of the signal shown in the inset of Figure 1A; encapsulation of the DMPO/OH adducts leads to an increase of their half-life from 700 s the absence of β-CD to 1600 s for the highest host concentration, [β-CD] = 3.5×10^{-3} M.

The complexation process between PBN/OH spin adducts and β-CD was studied at 300 K for host concentrations in the range $(1-100) \times 10^{-3}$ M. The presence of CD resulted in a 2−10-fold increase in the adduct intensity, but a_N and a_H splittings were unchanged, a_N = 15.1 G and a_H = 3.4 G.[12b] The major effect of the host presence is the increased adduct stability, as clearly seen in the time scans shown in Figure 2A. In the absence of β-CD the decay of the adduct is fast and no signal was detected after 100 s, in agreement with the literature.[30] It is interesting to note that the variation of $\tau_{1/2}$ with host concentration is different for the two spin traps: Figure 1B shows a linear dependence for DMPO/OH, but Figure 2B shows an abrupt increase of $\tau_{1/2}$ for PBN/OH in a narrow range of β-CD concentrations, $(15-30) \times 10^{-3}$ M.

The greater stability of the DMPO/OH and PBN/OH adducts and the negligible effect of the host on a_N and a_H values of the adducts are taken as indicators that the nitroxide group is *outside* the host cavity.[31] In Figure 3 we present the proposed structures for the *partial* inclusion of these adducts in β-CD: "in" for the methyl groups of DMPO and for the benzene ring of PBN,[7] but "out" for the N−O groups, which are exposed to the solvent. To obtain the structure shown in Figure 3, the structures of the adducts were optimized using the Gaussian 03 software followed by insertion in the CD, based on the picture of complexation deduced from the ESR spectra (nitroxide group outside the host).

The MNP/CH_2COOH Adduct and DTBN in Acetic Acid. Attack of hydroxyl radicals on acetic acid (AA) in aqueous solutions at 300 K and pH = 5 leads to the generation of the MNP/CH_2COOH adduct with a_N = 15.4 G and a_H = 8.3 G (12%), and of the DTBN nitroxide radical with a_N = 16.7 G (88%), as seen in Figure 4A. The a_N value for DTBN is lower

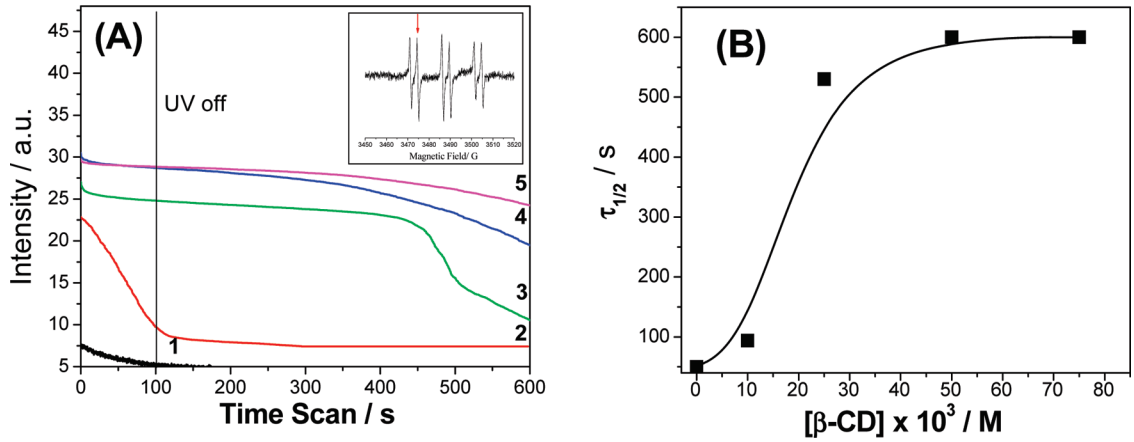


Figure 2. (A) Generation of the PBN/OH adduct and its decay at 300 K in the absence (line 1) and the presence of various β-CD concentrations: 10^{-2} M (line 2), 2.5×10^{-2} M (line 3), 5×10^{-2} M (line 4), 7.5×10^{-2} M (line 5). The red arrow indicates the signal whose intensity was monitored in the time scan. (B) The half-life of the PBN/OH adduct as a function of β-CD concentration, determined at the half-maximum intensity of the signal shown by the red arrow after stopping the UV irradiation. The solid line was obtained by connecting the points by a β spline line.

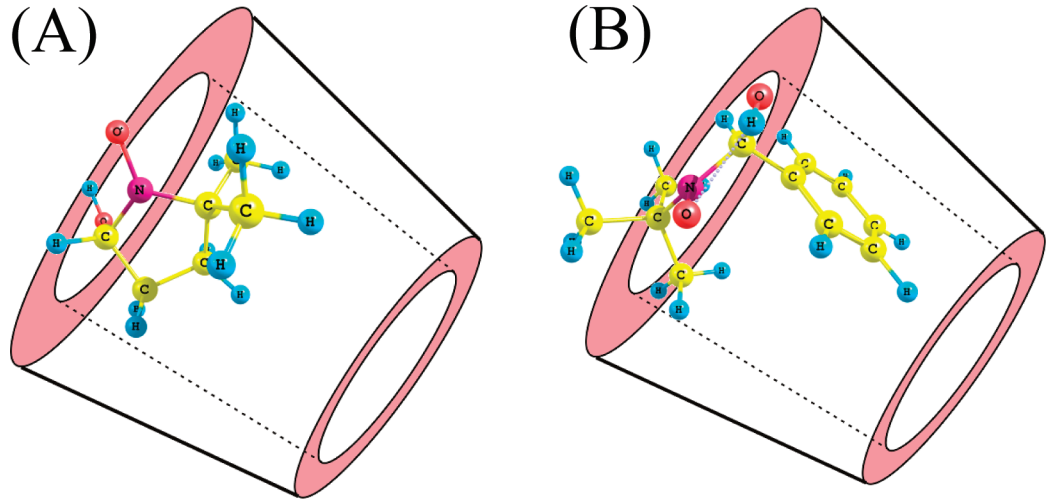


Figure 3. Proposed inclusion of the DMPO/OH adduct (A) and of the PBN/OH adduct (B) in β-CD. Note the position of the N−O group *outside* the host cavity for both adducts. The structures of the adducts were optimized using the Gaussian 03 software, and the adduct was inserted in the CD, based on the picture of complexation deduced from the ESR spectra (nitroxide group outside the host). See text.

than that measured in water, where a value of 17.2 G was reported.[4] This difference will be discussed below, in experiements with DTBN/β-CD in the absence of the adduct.

Changes in the stability, relative intensity, and hfs were measured for β-CD concentrations in the range $(1-100) \times 10^{-4}$ M. At the lower host concentrations, $[\beta\text{-CD}] < 5 \times 10^{-4}$ M, changes in the relative intensities of the two spectral components were detected: a decrease for DTBN and an increase for MNP/CH_2COOH, accompanied by very slight changes of a_N and a_H values ($\pm$0.1 G). For higher host concentrations, the relative intensity of the adduct continued to increase; even more important were the pronounced increases in a_N values for both nitroxides. The ESR spectra for $[\beta\text{-CD}] = 10^{-3}$ M are shown in Figure 4B: a_N for both DTBN and the MNP/CH_2COOH increased from 16.7 to 17.3 G for DTBN and from 15.4 to 6.1 G for MNP/CH_2COOH. These results are clear indications that both the adduct and DTBN in the presence of the host are in a more polar environment. We propose that these guests are located *at the rim* of the host where the higher polarity is due to the presence of OH groups.[32]

Experiments with Me β-CD as host were performed in order to better understand the local polarities and to validate the proposed location for the spin adduct and DTBN in the inclusion complexes. The concentrations of Me β-CD were in the range $(1-30) \times 10^{-4}$ M, and the ESR spectra at two Me β-CD concentrations (10^{-4} M and 3×10^{-3} M) are presented in Figure S1 (Supporting Information). The a_N value for the MNP/CH_2COOH adduct is the same for the two Me β-CD concentrations, $a_N = 16.1$ G, and equal to that in the presence of β-CD, concentration 10^{-3} M (Figure 4B); this value of a_N is higher than that for the adduct in water, thus confirming the rim location proposed in β-CD. The a_N values for DTBN, however, followed a different pattern: 16.7, 16.9, and 16.3 G for $[\text{Me } \beta\text{-CD}] = 0$, 10^{-4}, and 3×10^{-3} M, respectively. The lower a_N value at the highest Me β-CD concentration suggests inclusion in the host cavity. A possible explanation for this behavior is the higher hydrophobicity of both Me β-CD (compared to β-CD) and of DTBN compared to the MNP/CH_2COOH adduct. The subtle effects of host and guest polarities seem to dictate the complexation site: *at the rim for the adduct*, and *inclusion for DTBN* for a high concentration of the less polar host, Me β-CD.

The stability of the MNP/CH_2COOH adduct increased significantly upon addition of the β-CD host. Figure 5 presents the intensity of the adduct in the absence of β-CD and for $[\beta\text{-CD}] = 1 \times 10^{-3}$ M. The initial concentration of the adduct is larger by a factor of $\approx$2, and the adduct is detectable for $\approx$150 h compared to 70 h in the absence of β-CD. The plots

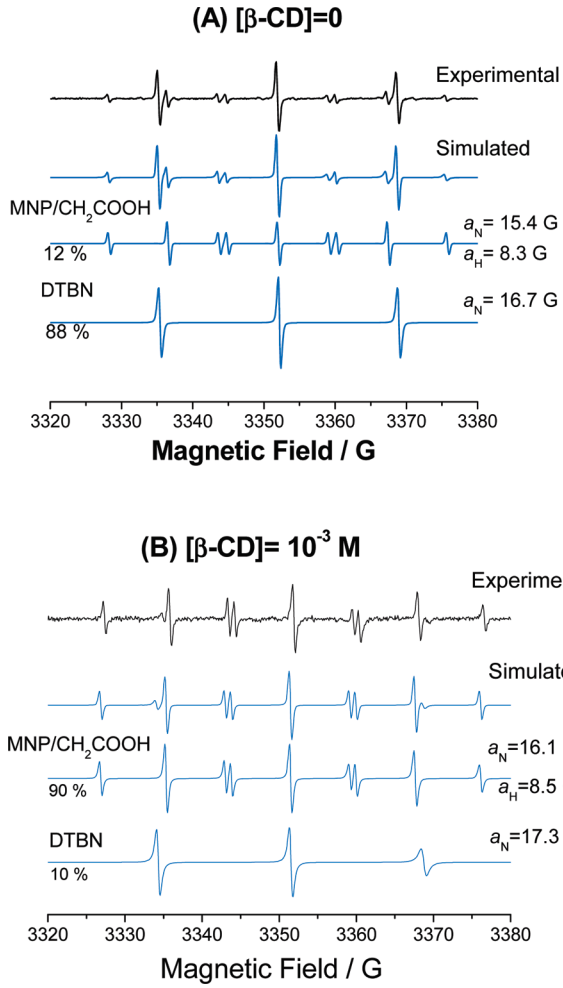


Figure 4. Experimental and simulated ESR spectra of the MNP/CH_2COOH adduct and of DTBN detected at 300 K and pH 5: (A) [β-CD] = 0, (B) [β-CD] = 10^{-3} M. The hyperfine splittings of the adducts are shown on the right and their relative intensity on the left.

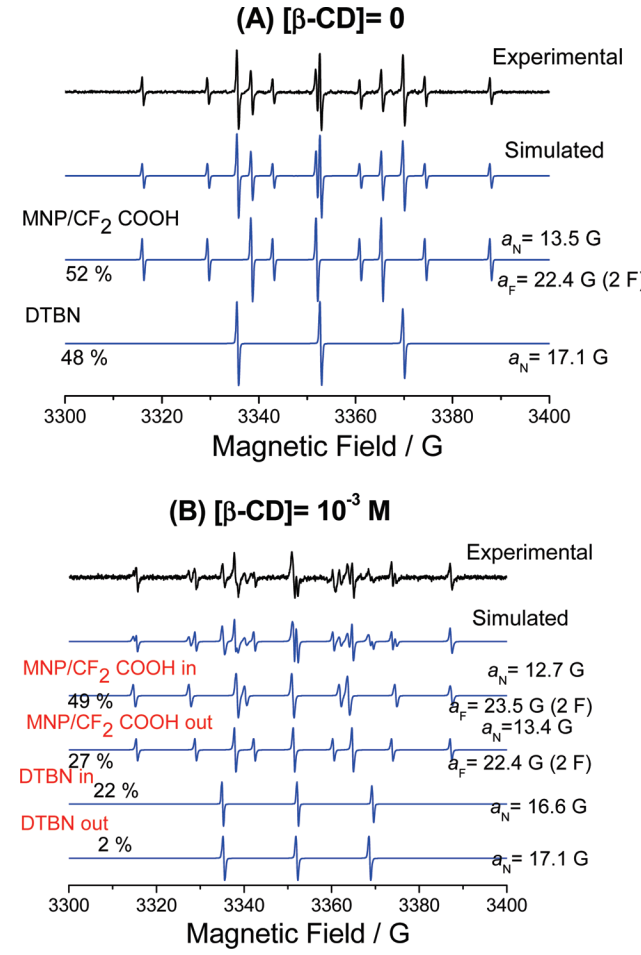


Figure 6. Experimental and simulated ESR spectra of the MNP/CF_2COOH adduct and of DTBN detected at 300 K and pH 5: (A) [β-CD] = 0, (B) [β-CD] = 10^{-3} M. The hyperfine splittings of the adducts are shown on the right and their relative intensity on the left.

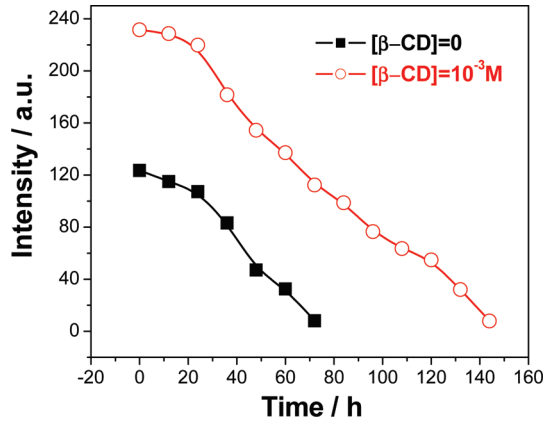


Figure 5. The stability of the MNP/CH_2COOH adducts at 300 K in the absence (black solid square) and in the presence (red open circle) of β-CD, concentration 10^{-3} M. The solid line was obtained by a spline line through the experimental points.

also show that the rate of decay of the adduct intensity in the presence and in the absence of the host are similar.[33]

The relative intensity of MNP/CH_2COOH increased significantly in the presence of β-CD, for example to 90% for [β-CD] = 10^{-3} M. A similar behavior was also observed in the case of Me β-CD, up to a host concentration of 10^{-3} M. At higher concentrations of Me β-CD, the DTBN radical becomes dominant in the system; this increase of the DTBN relative intensity is accompanied by its inclusion in the Me β-CD cavity. We note that at pH = 7 the addition of β-CD had no effect on the hfs of both adduct and DTBN: no evidence for complexation was detected.

MNP/CF_2COOH and DTBN in CF_2HCOOH (DFAA). The results for the MNP/CF_2COOH adduct generated when aqueous solutions of CF_2HCOOH are exposed to hydroxyl radicals are shown in Figures 6−9. Figure 6A presents experimental ESR spectra, corresponding simulated ESR spectra, and their deconvolution into contributions from the fluorinated adduct (a_N = 13.5 G and a_F = 22.4 G (2F), 52%), and from DTBN (a_N = 17.1 G, 48%). The a_N value for DTBN is higher in the DFAA system compared to that in AA (a_N = 16.7 G, Figure 4A). At this stage we tentatively assign this difference to the higher polarity in aqueous DFAA compared to aqueous AA; this conclusion will be validated by experiments with DTBN in the absence of the adduct; see below.

Figure 6B shows the results for a host concentration of 10^{-3} M. Addition of the host leads to the detection of two types of MNP/CF_2COOH adducts and DTBN radicals, with different a_N values. For the adduct we assign the two species to the "in" and "out" adducts on the basis of their a_N values, 12.7 G for "in" and 13.4 G for "out". We notice that one type of adduct is identical to that detected in the absence of β-CD (a_N = 13.5 G,

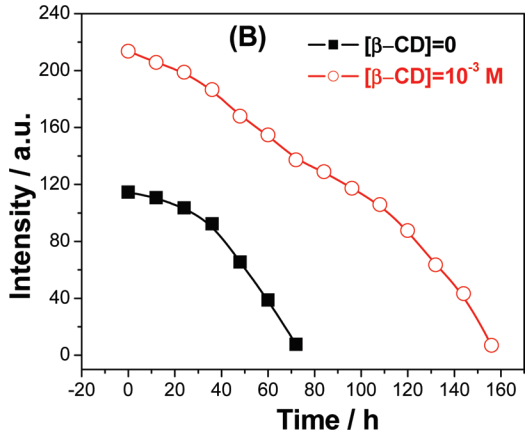


Figure 7. The stability of the MNP/CF_2COOH adducts at 300 K in the absence (black solid square) and (2) in the presence (red open circle) of β-CD, concentration 10^{-3} M. The solid line was obtained by a spline line through the experimental points.

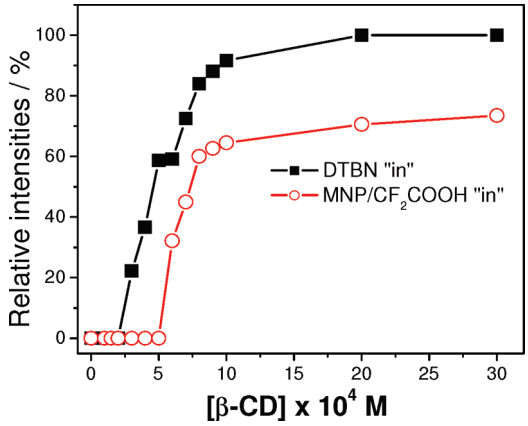


Figure 8. Variation of the % relative intensities for MNP/CF_2COOH "in" and for DTBN "in" as a function of β-CD concentration. The relative intensities were calculated separately in % total intensity for the each nitroxide. See Table 1.

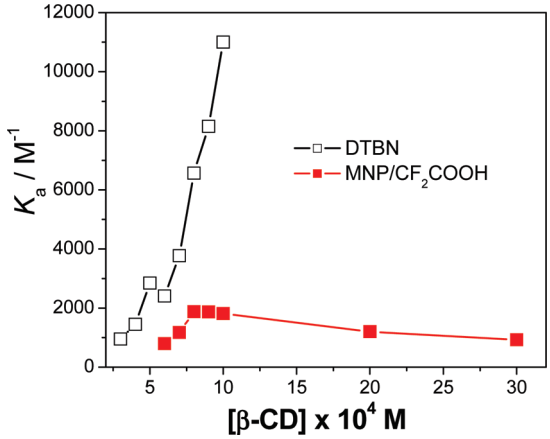


Figure 9. The association constant K_a at 300 K of DTBN and of the MNP/CF_2COOH adduct as a function of β-CD concentration. See text.

a_F = 22.4 G), the "out" adduct. Similar arguments apply for the DTBN radicals: "in" with a_N = 16.6 G, "out" with a_N = 17.1 G. The stability of the MNP/CF_2COOH adduct increases significantly upon addition of the host. Figure 7 presents the intensity of the spin adduct in the absence of β-CD and in the presence of host concentration of 10^{-3} M. The effect of host addition is similar to that detected for the MNP/CH_2COOH adduct (Figure 5): The initial concentration of the adduct is larger by a factor of ≈2, and the adduct is detectable for >150 h compared to 70 h in the absence of β-CD, and as in the case of MNP/CH_2COOH, the plots also show that the rate of change of the adduct intensity in the presence and in the absence of the host are similar.[33]

Table 1 shows the variation of the "in" and "out" species for MNP/CF_2COOH and DTBN as a function of β-CD content. It is interesting to note that at the highest β-CD concentration all the DTBN is "in" (and 17% of the total ESR signal intensity), while only 73% of the total adduct is "in" (and 61% of the total ESR signal intensity). Additional important details on the effect of β-CD were observed: First, DTBN "in" was detected at a lower β-CD concentration compared to the spin adduct. Second, the large increase of both "in" species in a narrow range of β-CD concentration was detected, $(2.5-10) \times 10^{-4}$ M for DTBN and $(5-8) \times 10^{-4}$ M for the spin adduct, as shown in Figure 8.

The association constants, K_a, as a function of β-CD concentration (Table 2) may be estimated based on the reasonable assumption that the concentration of β-CD is higher than those of the nitroxides.[34] For these conditions:

$$K_a = r_1/(r_o[CD]) \quad (1)$$

In eq 1 r_1 is the concentration of the "in" species and r_o is the concentration of the "out" species, as shown in Table 1. The values are plotted in Figure 9 for both MNP/CF_2COOH and DTBN. We note that DTBN is a strong competitor for the adduct in terms of inclusion in the host. For [β-CD] ≈ 9×10^{-4} M, K_a for DTBN increases dramatically, while K_a for the adduct starts to decrease. These results reinforce the idea that the two nitroxide radicals are competitors for inclusion in the host.

The attachments of the two spin adducts, MNP/CH_2COOH in acetic acid and MNP/CF_2COOH in fluoroacetic acid, to the β-CD host are different. The ESR spectra presented in Figures 4 and 6, together with the variations of a_N values determined by the simulations of the spectra, suggest *rim* attachment for the MNP/CH_2COOH adduct and *inclusion* of the fluorinated adduct MNP/CF_2COOH inside the host cavity. It seems reasonable to suggest that the hydrophobicity of the fluorinated adduct drags the adduct into the host cavity. The contrasting complexation for the two adducts is shown in Figure 10A for the MNP/CH_2COOH adduct and in Figure 10B for the MNP/CF_2COOH adduct; Figure 10 was generated as described for the structures shown in Figure 3. It is appropriate to look at these different interactions with β-CD in light of the recent paper that distinguishes between "*inclusion complexation*" (in the host cavity) and "*encapsulation interaction*", when the guest is between the wider rims of the cavity of two CD molecules or on the rim of one host molecule.[35] The first is illustrated by the adduct and DTBN in CF_2HCOOH and the second by the adduct and DTBN in CH_3COOH.

The DTBN Radical in CH_3COOH and in CF_2HCOOH. A surprising result of the present study was that for a given model compound (AA or DFAA), the two nitroxide radicals (the spin adduct and DTBN) have the same type of interaction with the host: at the rim in AA, and inside the host cavity in DFAA. Moreover, in the absence of the host, the a_N value for DTBN is higher in the DFAA system (17.1 G) compared to that in AA (16.7 G). This result emphasizes the complexity of a system that includes the radical source, the spin trap, the adduct, hydroxyl radicals, and DTBN, in addition to the

TABLE 1: Hyperfine Splittings and Relative Intensities of the "out" and "in" DTBN Radical and MNP/CF_2COOH for the Indicated β-CD Concentrations

	DTBN "out"		DTBN "in"		MNP/CF_2COOH "out"			MNP/CF_2COOH "in"		
[β-CD] $\times$ 10^4/M	a_N/G	%	a_N/G	%	a_N/G	a_F/G	%	a_N/G	a_F/G	%
0	17.1	48			13.4	22.4	52			
1	17.1	58			13.4	22.4	42			
1.5	17.1	56			13.4	22.4	44			
0.2	17.1	54			13.4	22.4	46			
0.3	17.1	42	16.7	12	13.4	22.4	46			
0.4	17.1	38	16.7	22	13.4	22.4	40			
0.5	17.1	26	16.7	37	13.4	22.4	37			
0.6	17.1	18	16.6	26	13.4	22.4	38	12.7	23.5	18
0.7	17.1	11	16.6	29	13.4	22.4	33	12.7	23.5	27
0.8	17.1	4	16.6	21	13.4	22.4	30	12.7	23.4	45
0.9	17.1	3	16.6	22	13.4	22.4	28	12.6	23.4	47
10	17.1	2	16.6	22	13.4	22.4	27	12.6	23.4	49
20			16.6	15	13.4	22.4	25	12.7	23.4	60
30			16.6	17	13.2	22.5	22	12.7	23.5	61

TABLE 2: Association Constants, K_a, of DTBN and MNP/CF_2COOH for the Indicated β-CD Concentrations

	K_a/M^{-1}	
[β-CD] $\times$ 10^4/M	DTBN	adduct
2		
3	952	
4	1447	
5	2846	
6	2407	789
7	3766	1169
8	6562	1875
9	8148	1865
10	11000	1815
20		1200
30		924

cyclodextrin, and encouraged us to explore the behavior of DTBN in the absence of other competitors but in the presence of the cyclodextrin host. Ref 4 reported the insertion of DTBN in β-CD and the detection of "in" and "out" ESR signals; as expected, the "in" signal had a smaller value of a_N. We are aware of one study of DTBN in α-CD, which suggested that the N−O group is outside the host cavity.[31] At this stage it seemed reasonable to expect that the γ-CD cavity is too large for the DTBN radical. Therefore the additional experiments were performed with β-CD as the host.

The results are shown in Figures 11 and 12. Figure 11 presents the ESR spectra of aqueous DTBN, at pH = 7.2. In the absence of β-CD, Figure 11A shows a_N = 17.2 G; the same a_N value was measured for low β-CD concentration, 10^{-4} M. The "in" and "out" DTBN species with a_N values of 16.6 and 17.2 G, respectively, were measured in the presence of high host concentration, Figure 11B. These results are in agreement with the data cited in ref 4. Moreover, the association constants given in Table 3 (second column) are similar to those presented in ref 4.

Contrasting results were detected in the presence of acetic acid, Figure 12. In the absence of the host, Figure 12A indicates a_N = 16.8 G, as in the presence of the adduct (Figure 4A). For a low β-CD concentration, a_N becomes 17.1 G, a sign for a higher local polarity; this value of a_N is identical to that presented in Figure 4B and is similarly taken to describe a *rim* location for the nitroxide. In the presence of high host concentrations, 10^{-3} M, Figure 12B, two DTBN species with a_N values of 16.6 and 17.2 G, respectively, were measured. We propose that the a_N values represent "in" and "rim" locations of DTBN. The association constants given in Table 3 (third column) are higher than those for the simpler system in the absence of acetic acid, possibly because of the lower pH value. All results presented in this section reinforce our assumption mentioned above for data in the absence of host: the presence of the acids AA and DFAA, with pK_a values of 4.81[36] and 1.34,[37] modify the local polarity and lead to a lower a_N value in the AA solutions. We conclude that for the system host/DTBN two effects are at play: the local polarity (lower for AA than for DFAA) in the absence of host, and the insertion of DTBN in the host cavity in the absence of the adduct.

An important extrapolation can be made for rationalizing the results for both the adduct and DTBN shown in Figures 4 and 6: In the presence of the host, the more hydrophobic adduct (MNP/CF_2COOH) is inserted in the host, while the less hydrophobic adduct (MNP/CH_2COOH) is attracted to the polar rim.

Conclusions

We have presented experiments that describe the potential of β cyclodextrin (β-CD) to interact with spin adducts of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), *N*-*tert*-butyl-α-phenylnitrone (PBN), and 2-methyl-2-nitrosopropane (MNP). The focus was on spin adducts of oxygen-centered radicals trapped by DMPO and PBN, and on carbon-centered radicals trapped by MNP. We have chosen to study the DMPO/OH and PBN/OH adducts, the MNP/CH_2COOH adduct in CH_3COOH, and the MNP/CF_2COOH adduct in CF_2HCOOH. The radical di-*tert*-butyl nitroxide $((CH_3)_3C)_2NO$ (DTBN) was also generated in experiments with MNP as the spin trap, and its presence provided the opportunity to explore the effect of β-CD in the presence of two nitroxides. The interactions of the nitroxides (adducts and DTBN) with β-CD were examined in terms of their stability and of the variation of the ^{14}N hyperfine splitting of the nitroxides, a_N, which is known to decrease at a location of lower polarity and increase when the N−O group is H-bonded.

A *range* of interactions of the spin adducts and DTBN with β-CD was detected. The presence of β-CD led to significant stabilization of the DMPO/OH and PBN/OH adducts, but to negligible variations of the hyperfine splittings and line widths. The half-life of the DMPO/OH adduct depends linearly on the host concentration and increases from 700 s in the absence of the host to 1600 s for [β-CD] = 3.5×10^{-3} M. The half-life of the PBN/OH adduct shows a major increase in a narrow range of β-CD concentrations, $(1.5-3.0) \times 10^{-2}$ M. The increased stability suggests complexation with the host; the negligible

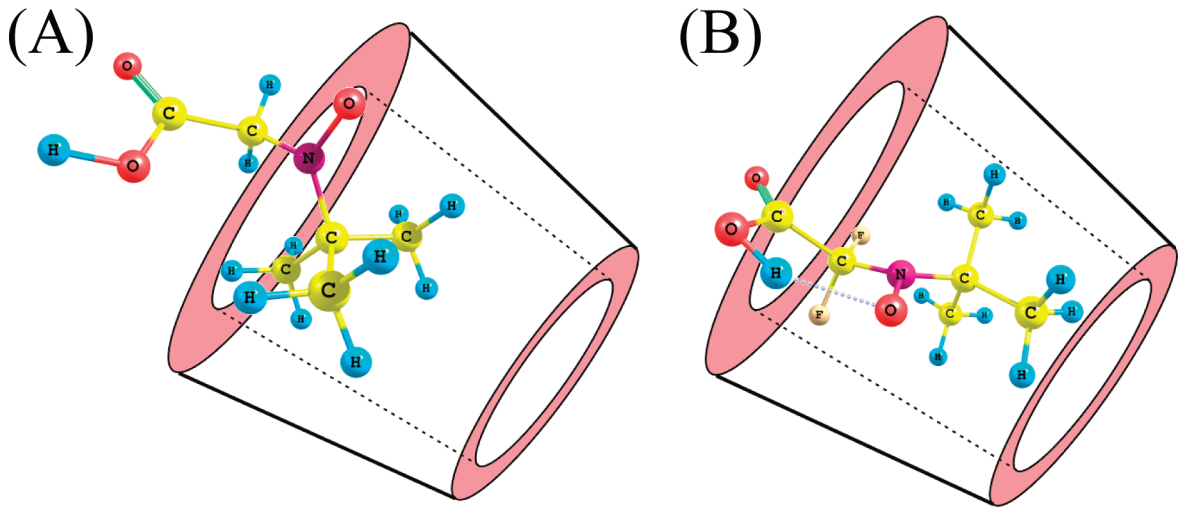


Figure 10. Proposed locations of the MNP/CH_2COOH adduct (A) and of the MNP/CF_2COOH adduct (B) in β-CD. Note the position of the N−O group *close to the rim* of the host for MNP/CH_2COOH and *inside the host cavity* for MNP/CF_2COOH. As for Figure 3, the structures of the adducts were optimized using the Gaussian 03 software, and the adduct was inserted in the β-CD, based on the picture of complexation deduced from the ESR spectra (rim or "in"). See text.

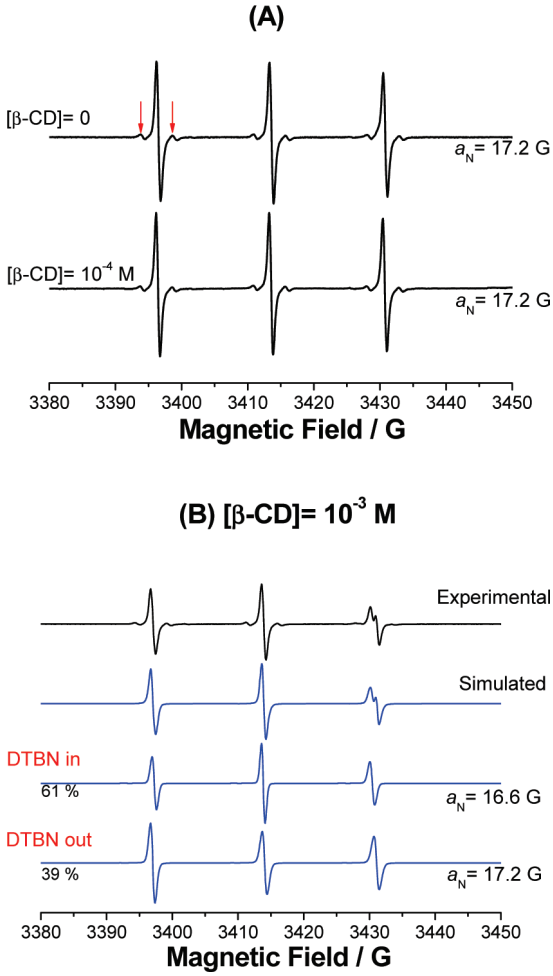


Figure 11. ESR spectra of DTBN at 300 K at pH 7.2: (A) in the absence of β-CD (top spectrum), and for [β-CD] = 10^{-4} M; (B) for [β-CD] = 10^{-3} M. Downward arrows point to ^{13}C satellites in (A), which were not considered in the simulated spectra.

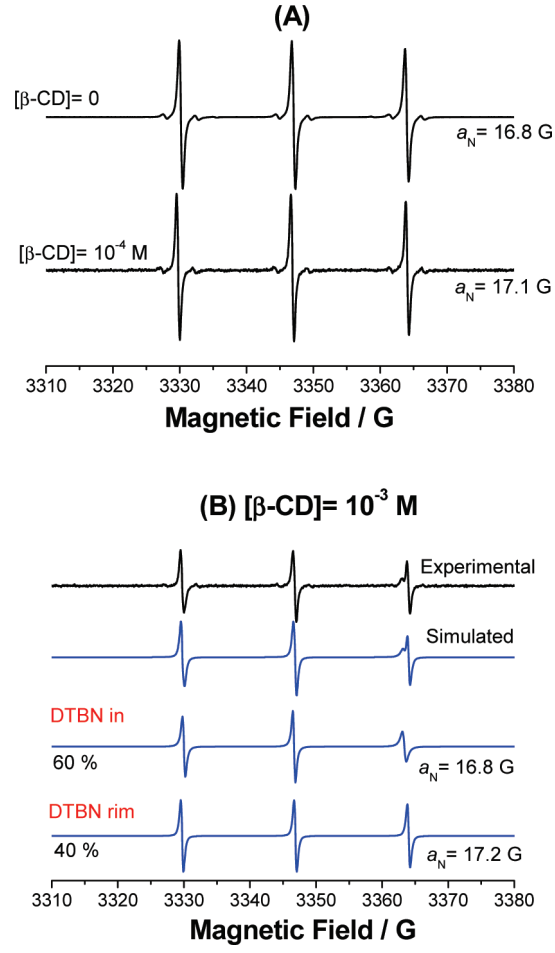


Figure 12. ESR spectra of DTBN at 300 K at pH 5 in the presence of acetic acid (2 M): (A) in the absence of β-CD (top spectrum), and for [β-CD] = 10^{-4} M; (B) for [β-CD] = 10^{-3} M.

effect on a_N indicates that the N−O group is outside the β-CD cavity. This effect is assigned to *partial enclosure of the adducts*.

For both DTBN and MNP/CH_2COOH, the presence of β-CD leads to an increase of a_N, a result that was interpreted as due to the location of the guests *at the rim of the host, where the polarity is higher*. Experiments with methylated β-CD (Me β-CD) provided support for this conclusion.

A different type of complexation was detected for both DTBN and MNP/CF_2COOH in CF_2HCOOH: for specific host concentrations both "in" and "out" species were detected. It seems reasonable to suggest that the hydrophobicity of the fluorinated

TABLE 3: Association Constants, K_a, of DTBN in β-CD in the Absence (Middle Column) and in the Presence (Last Column) of Acetic Acid

	K_a/M^{-1}	
$[\beta\text{-CD}] \times 10^4/M$	DTBN/β-CD	DTBN/AA/β-CD
7		
8	561	4624
10	612	4493
20	636	2812
30	619	2131
40	583	1886
50		1571
60		2606
70		

adduct drags the adduct inside the host cavity. Calculation of the association constant K_a indicated the competition between DTBN and the adduct for inclusion in the host cavity: For $[\beta\text{-CD}] \approx 9 \times 10^{-4}$ M the K_a for DTBN increases dramatically, while the K_a for the adduct plateaus and starts to decrease. These results reinforce the idea that the two nitroxide radicals are competitors for inclusion in the host.

In a given system containing MNP as spin trap, the two nitroxide radicals (adduct and DTBN) have the same type of interaction with the host: *at the rim* in acetic acid and *inside the host cavity in CF_2HCOOH*.

Experiments with DTBN in the absence of the spin trap and of adducts illuminated the effect of the pH and of the local polarity on the hfs and indicated that the presence of acetic acid encourages rim complexation.

Acknowledgment. This research was supported by grants from the Polymers Program of the National Science Foundation.

Supporting Information Available: Experimental and simulated ESR spectra of the MNP/CH_2COOH adduct and of DTBN detected at 300 K and pH 5 in the presence of methylated β-CD (Me β-CD) as the host. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743–1753.
(2) Hedges, A. R. *Chem. Rev.* **1998**, *98*, 2035–2044.
(3) (a) Spulber, M.; Pinteala, M.; Fifere, A.; Moldoveanu, C.; Mangalagiu, I.; Harabagiu, V.; Simionescu, B. C. *J. Inclusion Phenom. Macrocyclic Chem.* **2008**, *62*, 135–142. (b) Miron, L.; Mares, M.; Nastasa, V.; Spulber, M.; Pinteala, M.; Fifere, A.; Harabagiu, V.; Simionescu, B. C. *J. Inclusion Phenom. Macrocyclic Chem* **2009**, *63*, 159–162, and references therein.
(4) Atherton, N. M.; Strach, S. J. *J. Chem. Soc., Faraday Trans. 2* **1974**, 357–360.
(5) Martinie, J.; Michon, J.; Rassat, A. *J. Am. Chem. Soc.* **1975**, *97*, 1818–1823.
(6) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1989**, *111*, 7319–7323.
(7) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1992**, *114*, 2872–2874.
(8) Franchi, P.; Pedulli, G.-F.; Lucarini, M. *J. Phys. Chem. B* **2008**, *112*, 8706–8714.
(9) Ionita, G.; Florent, M.; Goldfarb, D.; Chechik, V. *J. Phys. Chem. B* **2009**, *113*, 5781–5787.
(10) Eastman, M. P.; Brainard, J. R.; Stewart, D.; Anderson, G.; Lloyd, W. D. *Macromolecules* **1989**, *22*, 3888–3892.
(11) Bardelang, D.; Clement, J.-L.; Finet, J.-P.; Karoui, H.; Tordo, P. *J. Phys. Chem. B* **2004**, *108*, 8054–8061.
(12) (a) Karoui, H.; Rockenbauer, A.; Pietri, S.; Tordo, P. *Chem. Commun.* **2002**, 3030–3031. (b) Bardelang, D.; Rockenbauer, A.; Karoui, H.; Finet, J.-P.; Tordo, P. *J. Phys. Chem. B* **2005**, *109*, 10521–10530.
(13) Clément, J.-L.; Tordo, P. Advances in Spin Trapping. In *Electron Paramagnetic Resonance*; Gilbert, B. C., Davies, M. J., Murphy, D. M., Eds.; A Specialist Periodical Report; Royal Society of Chemistry: Cambridge, 2007; Vol. 20, pp 29−49 and references therein.
(14) (a) Luo, L. B.; Han, D. Y.; Wu, Y.; Song, X. Y.; Chen, H. L. *J. Chem. Soc., Perkin Trans.* **1998**, *2*, 1709–1714. (b) Song, X.; Chen, Y.; Chen, H. *New J. Chem.* **2001**, *25*, 985–988.
(15) Han, Y.; Tuccio, B.; Lauricella, R.; Villamena, F. A. *J. Org. Chem.* **2008**, *73*, 7108–7117.
(16) Kadirov, M. K.; Bosnjakovic, A.; Schlick, S. *J. Phys. Chem. B* **2005**, *109*, 7664–7670.
(17) Bosnjakovic, A.; Schlick, S. *J. Phys. Chem. B* **2006**, *111*, 10720–10728.
(18) Lund, A.; Macomber, L.; Danilczuk, M.; Stevens, J.; Schlick, S. *J. Phys. Chem. B* **2007**, *111*, 9484–9491.
(19) Danilczuk, M.; Coms, F. D.; Schlick, S. *Fuel Cells* **2008**, *8* (6), 436–452.
(20) Bednarek, J.; Schlick, S. *J. Phys. Chem.* **1991**, *95*, 9940.
(21) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31–40.
(22) Lagercrantz, C. *J. Phys. Chem.* **1971**, *75*, 3466–3475.
(23) Spin Trapping Database, http://EPR.niehs.nih.gov.
(24) Danilczuk, M.; Schlick, S.; Coms, F. D. *J. Phys. Chem. B* **2009**, *113*, 8031–8042.
(25) Danilczuk, M.; Coms, F. D.; Schlick, S. *Macromolecules* **2009**, *42*, 8943–8949.
(26) Madden, K. P.; Taniguchi, H. *J. Am. Chem. Soc.* **1991**, *113*, 5541.
(27) Gaffney, B. J. In *Spin Labeling, Theory and Applications*; Berliner, L. J. Ed.; Academic Press: New York, 1976; Chapter 5, pp 1830−238.
(28) Stella, V. J.; Rao, V. M.; Zannou, E. A.; Zia, V. *Adv. Drug Delivery Rev.* **1999**, *36*, 3–16.
(29) The software WinSim can be accessed at: http://www.niehs.nih.gov/research/resources/software/tools/index.cfm.
(30) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1991**, *113*, 9503–9506.
(31) Eastman, M. P.; Freiha, B.; Hsu, C. C.; Lum, K. C.; Chang, C. A. *J. Phys. Chem.* **1987**, *91*, 1953–195.
(32) Vujosevic, D.; Dilger, H.; McKenzie, I.; Martyniak, A.; Scheuermann, R.; Roduner, E. *J. Phys. Chem. B* **2007**, *111*, 199–208.
(33) We are grateful to a reviewer for this observation.
(34) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1989**, *111*, 5138–5140.
(35) Song, L. X.; Bai, L.; Xu, X. M.; He, J.; Pan, S. Z. *Coord. Chem. Rev.* **2009**, *253*, 1276–1284.
(36) Kim, H.-S.; Chung, T. D.; Kim, H. *J. Electroanal. Chem.* **2001**, *498*, 209–215.
(37) Ye, Y. K.; Stringham, R. W. *J. Chromatogr., Sect. A* **2001**, *927*, 47–52.

JP100777U