pH-Dependent Inclusion Complexes

Detection of Paramagnetic pH-Dependent Inclusion Complexes between β-Cyclodextrin Dimers and Nitroxide Radicals**

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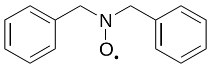
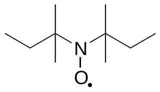
We have recently designed a series of paramagnetic probes based on benzyl *tert*-butyl nitroxide to investigate, in aqueous solution, several types of supramolecular reaction kinetics in the submicrosecond time range by means of EPR spectroscopy.[1–3] The EPR spectra of these probes show a significant difference in the hyperfine splittings both at nitrogen, a(N), induced by the less polar environment of the host component with respect to the solution, and at the benzylic protons, $a(2H_\beta)$, because of conformational changes, so that the signals of both the complexed and uncomplexed species are easily distinguishable.

When benzyl *tert*-butyl nitroxide was investigated in aqueous solution in the presence of β-cyclodextrins (β-CDs) only one inclusion paramagnetic species was detected; this was identified as the 1:1 complex, similar to what was found by NMR spectroscopy for the parent amine compound.[1,4] Since the tendency of CDs to form inclusion complexes with guest molecules and simultaneously to self-associate to give dimers can be exploited to produce supramolecular structures at an higher level of organization,[5] we have been looking for paramagnetic organic guests capable to form CD complexes with a stoichiometry other than 1:1.[6] Herein we report the first EPR detection and characterization of paramagnetic inclusion complexes between symmetric dialkyl nitroxides (**1a**–**2a**) and two molecules of β-cyclodextrin.



1a



2a

The EPR spectrum **1a**, which is produced by oxidation of dibenzyl amine (2.0 mM) with the magnesium salt of monoperoxyphthalic acid (1.0 mM) in water, is shown in Figure 1. The spectrum is easily interpreted on the basis of the coupling of the unpaired electron with nitrogen and four equivalent benzylic protons (see Table 1). In the presence of β-CD (3.0 mM), additional signals assigned to the radical included in the cavity of β-CD (**1b**), in equilibrium with the free nitroxide, were observed (see Figure 1 and Figure 2).

By increasing the absolute concentration of β-CD, the ratio between included and free species varied linearly and, at 6 mM of β-CD, the dominant spectrum was that of the 1:1 inclusion complex **1b**. When β-CD concentrations higher than 9 mM were used, new signals appeared in the EPR spectrum (see Figure 1), which arise from a third radical species, characterized by spectroscopic parameters similar to those of the previous two species. The relative concentration of the third species changed reversibly on changing the β-CD concentration. This third species was identified as a second inclusion complex having a 1:2 stoichiometry (**1c**). Its EPR nitrogen hyperfine splitting (a_N) is significantly smaller than that of both the uncomplexed radical (**1a**) and the 1:1 complex (**1b**; see Table 1), which indicates that the nitroxide group is located in a less polar environment[7] consistent with a 1:2 complex in which the nitroxide group is completely shielded from the aqueous solvent. The molar ratio [**1c**]/[**1b**] of the two complexes, obtained from the EPR spectra, increased linearly with increasing concentration of the dissolved β-CD. When recording the EPR spectra in strongly basic solutions (pH > 12.5) the signals of the 1:2 complex **1c** disappeared while those of the 1:1 complex remained (see Supporting Information), similar to what had been found previously with a pyrene-modified γ-CD[8a] and with the 1:2

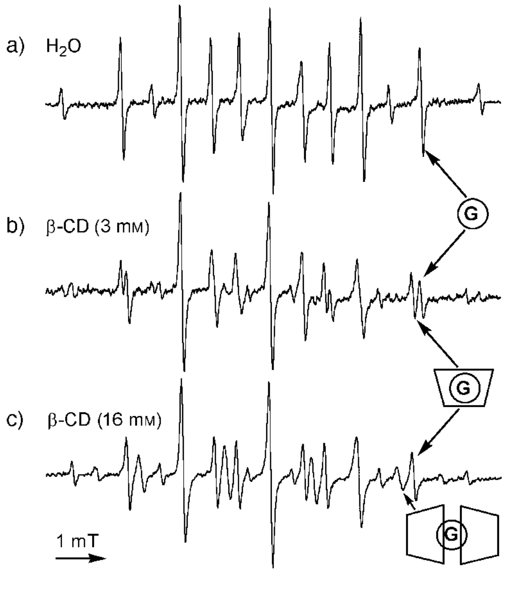


Figure 1. EPR spectra of **1a** in water at 295 K in the absence (a) and in the presence (b), (c) of various amounts of β-CD.

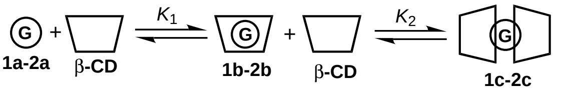


Figure 2. Schematic representation of the equilibria taking place in the presence of β-cyclodextrin.

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Table 1: Spectroscopic and thermodynamic parameters for CD complex formation.

System[a]	$a_{H\beta}$ [mT]	a_N [mT]	ΔH [kJ mol^{-1}]	ΔS [J K^{-1} mol^{-1}]	ΔG^{295} [kJ mol^{-1}]	K_{eq}^{295} [b] [M^{-1}]
1a (H_2O)	1.081	1.649				
1b (β-CD, m)	1.007	1.601	−13.5	5.1	−14.6	385
1c (β-CD, d)	0.796	1.559	−41.0	−108.9	−8.5	32
1d (DM-β-CD)	0.958	1.584			−15.5	539
1e (γ-CD)	0.923	1.595			−10.3	67
2a (H_2O)		1.682				
2b (β-CD, m)		1.620	−18.0	−2.7	−17.3	1106
2c (β-CD, d)		1.565	−37.5	−94.1	−9.9	56
2d (DM-β-CD)		1.602			−18.0	1503
2e (γ-CD)		1.616			−13.6	257

[a] In parenthesis is reported the host species (m = monomer, d = dimer). [b] $K_{eq} = K_1$ or K_2.

complex between 4-(dimethylamino) benzonitrile and β-CD.[8b] The lability of the 1:2 complex at high pH values is attributed to the fact that in basic media dissociation of the secondary CD hydroxy group ($pK_a = 12.5$) takes place to form an anionic species. Since molecular modeling studies have demonstrated that the driving force responsible for the stabilization of the β-CD dimer is the formation of intermolecular hydrogen bonds between the secondary hydroxy groups of the two larger rings,[9] the anionic β-CD unit can not associate to give dimers because of Coulombic charge repulsion. This hypothesis was confirmed by using as host 2,6-dimethylated β-cyclodextrins (DM-β-CDs) in which some of the hydroxylic hydrogen atoms responsible for the intermolecular hydrogen bonding have been replaced with methyl groups. Actually, the EPR spectra of dibenzyl nitroxide in the presence of 0.1 M of DM-β-CD (**1d**) did not show any signals other than those of the 1:1 complex (see Supporting Information).

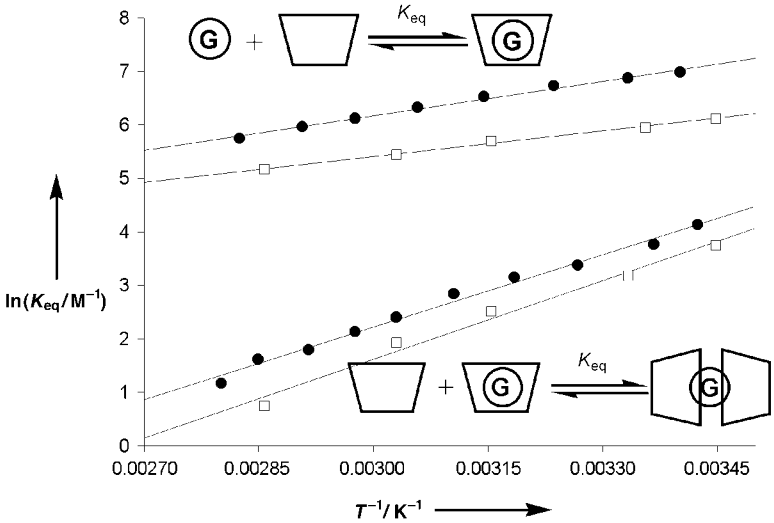

Figure 3. Van't Hoff plot for the temperature dependence of the equilibrium constants, K_1 and K_2; □ = **1a** and ● = **2a**.

The values for the K_1 and K_2 binding constants were obtained by plotting the ratio between the concentrations of the 1:1 complex and the free species ([β-CD] 0–8 mM) and the ratio between the concentrations of the 1:2 and 1:1 complex ([β-CD] 8–15 mM) as a function of the cyclodextrin concentration in water. The temperature dependence of these equilibrium constants, reported in van't Hoff plots (Figure 3), shows that K_1 changes only slightly with temperature, while K_2 significantly decreases with increasing temperature.

The first step, that is, the formation of the 1:1 complex, is associated with a small negative $\Delta H°$ and an entropy change, $\Delta S°$, close to zero (see Table 1). Although the entropy of complexation of a guest by an empty host in the gas phase would be negative by 120–200 J mol^{-1} K^{-1} the complexation in solution is expected to have entropies of complexation much closer to zero, because of the desolvation of the guest from water molecules is entropically favorable.[10]

The formation of the 1:2 complex, however, is characterized by largely negative enthalpy and entropy changes. The negative ΔH value is the result of the formation of a large number of intermolecular hydrogen bonds between the two wider rings of the cyclodextrins. This process also implies a loss of entropy because of the reduced freedom of molecular motion of the complex which, in contrast to the 1:1 complexation, is not compensated by the desolvation of the guest as this is already included in the cavity of one CD molecule. Thus, the measured thermodynamic parameters are consistent with the formation of a 1:2 complex.[8b]

To gain a more detailed picture of the 1:2 complex formation, stochastic dynamics (SD) simulations were performed in water at constant temperature by using AMBER* force field.[11,12] After the sample had been heated to 295 K, the different complexes were equilibrated for 200 ps with time step of 1 fs and the total simulation time was set to 15000 ps to achieve full convergence (see Table 2).[13]

Consistent with the experimental evidence, the SD simulations show that on average, the formations of the 1:1 and 1:2 complexes are energetically favorable by 50 and 101 kJ mol^{-1}, respectively. While in the 1:1 complex only one phenyl ring is located inside the torus, in the 1:2 complex the nitroxide is totally embedded in the macrocylic rings (see

Table 2: Summary of data obtained by SD simulation at 295 K in water.

Equilibrium	Energy of reactants [kJ mol^{-1}]	Energy of complex [kJ mol^{-1}]	ΔE [kJ mol^{-1}]
1a + β-CD ⇌ **1b**	767 (116 + 651)	717	−50
2β-CD ⇌ dimer	1302 (2 × 651)	1238	−64
1b + β-CD ⇌ **1c**	1368 (717 + 651)	1267	−101
1a + dimer ⇌ **1c**	1354 (116 + 1238)	1267	−87

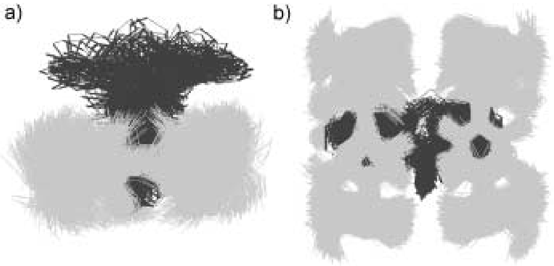

Figure 4. Clustered molecular display. Dynamics of the 1:1 (a) and 1:2 (b) complexes of β-CD (gray) with dibenzyl nitroxide (black). The drawings include only 500 structures that refer to the 15 000 ps simulation. Hydrogen atoms have been omitted for clarity.

Figure 4), in agreement with the decrease of a_N observed experimentally when passing from **1b** to **1c**.

Moreover, the SD calculated $\langle \cos^2\theta \rangle$ values [14] for **1a** and **1c**, where θ is the dihedral angle between the symmetry axis of the $2p_\pi$ orbital of nitrogen atom and the N-C-H_β plane in the nitroxide, were 0.41 and 0.37, respectively. These values are in agreement with the large reduction of $a_{H\beta}$ found experimentally when passing from **1a** to **1c**. The computations indicate that in the 1:2 complex the only populated conformers are those in which θ is $-30°$ and $90°$, while in water the energy minimum in which the phenyl group is eclipsed by the oxygen atom is also significantly populated (see Supporting Information) and contributes to the value of $\langle \cos^2\theta \rangle$.

To increase the life time of the paramagnetic 1:2 complex we investigated the behavior of symmetric di-*tert*-alkyl nitroxides, because substitution of the β-hydrogen atoms with alkyl groups increases considerably the life time of the nitrogen containing species.[15] In the absence of β-hydrogen atoms, however, the characterization of the complexed species by EPR spectroscopy is not as straightforward as for **1a**, since the changes with complexation of the only measurable splitting constant, that is a_N, are not sufficiently large to clearly differentiate the spectra of the various species. However, when using di-*tert* amyl nitroxide (**2a**) partial resolution of the high-field EPR lines of the free and complexed nitroxides is observed.[16] Actually, we were able to distinguish two different signals, attributed to the radical in water (**2a**) and to the 1:1 complex (**2b**) in the presence of 3.7 mM β-CD, and to the 1:1 and 1:2 complex (**2c**) at 16 mM β-CD concentration (see Supporting Information). The assignment of the signals to the different species was on the basis of considerations similar to those reported above for dibenzyl nitroxide. In this case, however, the increased radical life time (several days) made it possible to switch reversibly from the 1:1 to 1:2 complex many times by successive acid–base treatments without any EPR evidence of side reactions.

Summarizing, we have been able to construct the first three-component organic free-radical complex which can be reversibly formed by changing the pH value or temperature. Simple chemical modifications of the radical guest can give species able to self associate. The combination of this property with the tendency of cyclodextrins to form inclusion complexes and simultaneously to self-associate and form dimers can be exploited to produce paramagnetic supramolecular structures at an higher level of organization. The formation of such supramolecular ordered polyradical assemblies can be useful for the preparation of functional molecular magnetic materials.

Experimental Section

Radicals **1a** and **2a** were generated by mixing a methanol solution (1 μL) containing the corresponding amine (0.2 M) and a water solution (1 μL) containing the Mg salt of monoperoxyphthalic acid (0.1 M) with a water solution (100 μL) containing variable amounts of β-CD. The pH value of the solution was adjusted by adding NaOH. for pH > 12 Oxone was used as the oxidant. Samples were transferred in capillary tubes (1 mm i.d.), heated for 30–60 s at 80 °C and then placed inside the thermostatted cavity of EPR spectrometer (Bruker ESP300). The instrument settings were: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. The computed spectra were best-fitted to the experimental ones by using a Monte Carlo minimization procedure.[1b,17]

SD simulations were carried out using the MacroModel 7.0 program. Extended nonbonded cutoff distances were set to 8 Å and 20 Å for the van der Waals and electrostatic interactions. The generalized Born/surface area (GB/SA) solvation model was used when modeling the solvent effect under MacroModel. All C–H and O–H bond lengths were held fixed using the SHAKE algorithm. Translational and rotational momentum were removed every 0.1 ps. β-Cyclodextrin with a C_7 symmetry was used as the starting host. All the complexes were generated by docking the guest to the host in a suitable orientation. In all cases the origin of a Cartesian reference frame was placed on the center of mass of the CD and the z axis aligned with the C_7 symmetry axis of CD. No significant differences were obtained by using different starting orientations.

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