ESR, STESR, DFT, and MD Study of the Dynamical Structure of Cucurbituril[7]–Spin Probe Guest–Host Complexes

Vsevolod A. Livshits,† Boris B. Meshkov, Vitaly G. Avakyan, and Sergei V. Titov

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1. INTRODUCTION

Cucurbit[n]urils (CB[n]), a new class of supramolecular hosts, have attracted significant attention over the past years, owing to their capability to form strong and selective host–guest complexes with various organic molecules and cations in aqueous media.1–3 It has also been demonstrated that the photophysical properties, including fluorescence, of guest molecules often change substantially upon inclusion into the CB[n] cavities.4–7 These properties allow the utilization of CB[n] as artificial molecular receptors in chemos- and biosensors, vehicles for drug transport, light-driven molecular switches, motors, etc.8–14

In addition, the fundamental studies on the structure and dynamics of the cucurbituril host–guest complexes can provide important insights into the nature of noncovalent interactions. Various experimental and computational methods have been employed to study host–guest CB[n] complexes. The use of spin probes as guests is attracting special interest because the analysis of the electron spin resonance (ESR) spectra allows one to investigate the structure, binding thermodynamics, reaction kinetics, and environment polarity of the spin probe guests in the host cavities. Several papers on the spin probe–cucurbituril complexes have been published.15–22 Of principal importance is the information on the dynamic properties of the complexes, including the molecular mobility of the guests within the host cavities and kinetic accessibility to water-soluble reagents. However, these properties have not been sufficiently explored by ESR as far as we know.22–25 Recently, we studied, by ESR, the structure and molecular dynamics of the guest–host complex of the spin probe, TEMPO, with cucurbituril CB[7].23

In this work, we continue the ESR study on the molecular dynamics and structure of the host–guest complexes of CB[7] with several nitroxide spin probes with different chemical structures, both in an aqueous solution and the solid phase. Neutral and positively charged nitroxides of different sizes and shapes were chosen, including TEMPOamine in its protonated form (I); cationic nitroxide, 4,4(N,N-dimethyl N-octyl ammonium-2,2,6,6-tetramethyl-piperidinyl-N-oxy bromide) (II); and spin-labeled indole, 2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-γ-carboline-3-oxyl (III) (see the chemical structures below).

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To study the rotational dynamics of the spin probes within the guest–host complexes and eliminate the effects of both their overall rotation and magnetic dipole/dipole/exchange interactions between the complexes, the diluted solid solutions
of the complexes spin probe@CB[7] in the CB[7] matrix have been prepared. It was shown that the formation of the CB[7] matrix results in the hydrophobic environment for the embedded complexes and a decrease in the environment polarity for spin probes inside the CB[7] cavities, most probably owing to the removal of water on matrix formation. The combination of ESR and the saturation transfer ESR (STESR) spectra is applied to determine the character of the spin probe rotational motion inside the CB[7] hosts embedded in the solid CB[7] matrix.

The information on the spin probe location relative to the CB[7] cavity has been deduced from the polarity parameters of the NO group environment. These parameters have been obtained from the measurements of the isotropic hyperfine interaction (hfi) in aqueous solutions and anisotropic hfi for the immobilized complexes in the CB7 matrix. Additionally, similar information has been deduced from the kinetic accessibilities of the reporter NO groups to the paramagnetic complexes in aqueous solutions using the Heisenberg exchange (HE) broadening of the ESR spectra.

Notably, the additional application of the NMR method is limited in the study of these complexes due to the paramagnetic nature of the guests.

Along with the experimental studies, we perform density functional theory (DFT) and molecular dynamics (MD) computations to determine the spatial structures, formation energies of the complexes, and the changes in the atomic charge and the spin density distributions on the complex formation in the version of the DFT method that considers both the quantum mechanical electrostatic effects and the van der Waals (vdW) interactions. In our opinion, the hydrophobic environment of the complexes in the solid CB[7] matrix, in the first approximation, makes these computations possible, which are technically applicable to the complexes in the gas phase. The effects of surrounding water molecules have been simulated by considering the aqueous clusters that incorporate the spin probes and complexes.

2. EXPERIMENTAL AND COMPUTATIONAL METHODS

2.1. Materials. TEMPOamine and cucurbituril CB[7] were purchased from Sigma-Aldrich and used as received. The protonated form of TEMPOamine (I) was prepared by dissolving its powder in water at a pH of 5.5. The compound 4,4-(N,N-dimethyl-N-octyl ammonium-2,2,6,6-tetramethyl-piperidinyl-N-oxo bromide), (Cat8) (II), was a gift from Dr. G.B. Khomutov (Physics Department of the Moscow State University). The spin-labeled indole, 2,2,4,4-tetramethyl-carboline-3-oxyl (TEMPOamine@CB[7]), was a gift from Dr. A.B. Shapiro from the Institute of Biophysical Chemistry, RAS.26

2.2. Preparation of the Complexes. Aqueous solutions of the spin probes and their complexes with CB[7] were prepared using water purified in a Millipore Model Direct-Q UV purification system. Solutions of I and complex I@CB[7] were prepared in a 2 mM Tris buffer at pH 5.0 or by adding HCl to provide the protonated form of tempamine. The compound II@CB[7] was prepared in water. Due to the very low solubility of spin probe III in water, a 1% ethanol–water mixture, instead of water, was used for the preparation of the solutions of III. For the preparation of the complexes in the solid CB[7] matrix, the solutions of the spin probes and CB[7] were mixed in a molar ratio of 1:60, whereupon the mixture was slowly evaporated and dried at room temperature to a constant weight.

2.3. ESR Measurements. The ESR spectra were recorded at a microwave frequency of 9.2 GHz on a Bruker EPR-200 spectrometer equipped with a nitrogen gas flow temperature unit. Sample capillaries were positioned along the symmetry axis of the standard 4 mm quartz ESR sample tube that contained mineral oil for thermal stability. The root-mean-square microwave magnetic field $\langle B^2 \rangle^{1/2}$ measurement for a “point” sample of aqueous peroxylamine disulfonate with known $T_1$ and $T_2$ values gave a maximum value of 0.51 G. The low-power ESR spectra were recorded at a power of 26 dB ($H_1 \approx 0.0255$ G) and a small modulation amplitude, which rules out spectral distortion. The STESR spectra, i.e., the ESR absorption spectra detected on the second harmonics of modulation in the 90° phase with a modulation field ($V_m^2$), were recorded under partial microwave saturation, essentially as described in ref 27. The microwave amplitude ($H_1$) was 0.25 G; the modulation frequency and amplitude were 50 kHz and 5 G, respectively. The smaller modulation amplitudes, $H_m = 1.5$ and 3 G, were also used to check the weak dependence of $V_m$ line shape on the $H_m$ value. The 90° modulation phase was carefully adjusted by the zero method.27 The temperature dependencies of the ESR and STESR spectra of the complexes were studied in the range of 140−340 K.

2.4. ESR Spectral Simulations. The nonlinear least-squares analysis of the low-power ESR spectra based on the stochastic Liouville equation was performed using a fitting program by Budil et al.28 The hyperfine $A_{\mu
u}$ and $g_{\mu
u}$ tensor components required for the spectral simulations were measured from the rigid-limit ESR spectra at 77 K. The STESR spectra detected on the second modulation harmonic were simulated using an approach based on solving Bloch equations coupled with uncorrelated jump rotational diffusion. This approach was developed in refs 30−34 and is partly presented in S12.

2.5. DFT Computations. The computation of the structures and formation energies of the complexes was carried out by the DFT-D3 method with the full geometry optimization using the Perdue–Burke–Ernzerhof (PBE) functional in the SVP basis with the ORCA program package, version 3.01. The PBE functional opposite to the BLYP (B3LYP) functional has no empirical fitting parameters. We used two program packages adapted for parallel computing: “Prirodina”35,36 and “ORCA”.37 The former was used to preliminarily optimize the geometry of the starting molecules and complexes and estimate the energy minima, which should not lead to imaginary vibrational frequencies. The use of the

Figure 1. Structures of spin probes I−III.
Priroda package, with a three-exponential basis of Gaussian functions, specifically optimized for use with the PBE functional, allowed performing fast analytical calculations of the Hessian and vibrational frequencies. The ORCA package, version 3.01, with an SVP basis set, was used to conduct a full geometry optimization of the complexes, considering the dispersion contribution to the complex formation energy by the Grimme method D3 starting from the structures obtained in the first stage of the calculations.

The contribution of the van der Waals (vdW) interaction to the complex formation energy was considered using Grimme’s dispersion term that was added to the full electrostatic energy. Thus, the total complex formation energy ($\Delta E_{\text{tot}}$) is given as follows:

$$\Delta E_{\text{tot}} = E_{\text{el.stat}} + E_{\text{vdW}},$$

where $E_{\text{el.stat}}$, $E_{\text{vdW}}$, $E_{\text{comp}}$, $E_{\text{SL}}$, and $E_{\text{CB}[7]}$ are the computed energies of the complex, spin probe, and CB[7], respectively. HyperChem 8.0 and Chemcraft packages were utilized to prepare the starting files and visualize the results.

### 3. RESULTS AND DISCUSSION

#### 3.1. Properties of the CB[7]–Guest Complexes in Aqueous Solutions

**3.1.1. Complex Formation.** The dependencies of the ESR spectra on the CB[7] concentration were studied for all of the spin probes. The 0.2 mM solutions of I, II, and III were mixed with equal volumes of CB[7] solutions having different concentrations of CB[7] in the range of 0.2–6.0 mM. After mixing equal volumes of a spin probe and CB[7] solutions, all concentrations decreased by 2 times the final CB[7] concentrations and effects of the concentration change are shown in Figures S1a,b, S2a,b, and S3a,b, SI1. All of the mixtures were incubated for 1 h and stirred at 60°C.

For all of the CB[7] concentrations, the ESR line shapes correspond to a rapid rotation region (Figure 2). The complex formation was monitored by measuring the effective rotational correlation time of the spin probe ($\tau_R$) and the isotropic N14 hyperfine ($a_{\text{N}}$), which is sensitive to the polarity of the spin probe environment. The $\tau_R$ value was estimated in terms of the isotropic rotation using the well-known expression from the second-order perturbation theory:

$$\tau_R = 6.65 \times 10^{-10} \Delta H_{\text{pp}} (\sqrt{I_{\text{pp}}/I_{\text{m}}} - 1)$$

where $\Delta H_{\text{pp}}$ is the pp line width of the hfi component $m = \pm 1$ and $I_{m=\pm 1}$ is the intensity of the hfi components for $m = \pm 1$. For

![Figure 2. Experimental ESR spectra of complexes I@CB7, II@CB[7], and III@CB7 in aqueous solutions. For all of the complexes, the guest concentration was 0.1 mM and the host concentration was 3 mM.](image-url)
all spin probes I–III, an increase in the CB[7] concentration results in an increase in $\tau_0$, owing to the successive binding of the free spin probes to CB[7] (see S11).

Apart from an increase in $\tau_0$, a decrease in $a_{ns}$ was observed for I and III, which signifies a decrease in the local polarity of the NO group environment on the complex formation. For spin probe II, unlike the cases of I and III, an increase in $a_{ns}$ dependencies reached plateau values at the CB[7]/II molar ratio $\cong 1$ (see S11), which suggests the formation of the 1:1 complex. For spin probes I and chiefly III, the concentration dependencies of $\tau_0$ and $a_{ns}$ are indicative of a rather weak binding because the g/h complex is completely formed only at large CB[7] concentrations. The 1:1 stoichiometry for these complexes was deduced from the careful examination of the relationships of the host and guest dimensions (see Section 3.4).

3.1.2. Polarity of the NO Group Environment in Aqueous Solutions. The ESR spectra of the guest–host complexes of CB[7] with spin probes I–III were recorded in aqueous solutions in a large molar excess of CB[7]; the spin probe and CB[7] concentrations were 0.1 and 3 mM, respectively. This CB[7]/spin probe molar ratio corresponded to the complete complexation at 292 K (see the concentration dependencies of $\tau_0$ and $a_{ns}$ in S11).

The dependence of the isotropic hyperfine constant ($a_N$) on the environment polarity is due to the averaged electric fields associated with nearby molecules and groups, which affect the spin density on the nitrogen atom in the NO fragment of a spin probe.41 The main contributions to this dependence are from the sum of two terms: van der Waals interactions of the NO group with the environment and hydrogen bonding with the adjacent proton donor molecules. As a consequence, the $a_{ns}$ values may provide information on the location of the spin probe. Therefore, we measured the isotropic hyperfine constants ($a_N$) for all of the spin probes in the CB[7] complexes ($a_{N,cb}$) and, for reference, in pure solvents as well. For the polar solvents, we used water or a 50/50 v/v glycerol/water mixture ($a_{N,w}$); for the nonpolar solvents, we used toluene ($a_{N,T}$). From these data, we constructed a dimensionless polarity parameter

$$p_{solub} = \frac{a_{N,cb} - a_{N,T}}{a_{N,w} - a_{N,T}},$$

which allows the comparison of the polarities of the NO group environment for different spin probes in the complexes. For all of the spin probes in an aqueous environment, $p_{solub} = 1$; in toluene, $p_{solub} = 0$. The $p_{solub}$ values for all of the CB[7] complexes in water are given in Table 1.

As seen in Table 1, the $a_N$ and $p_{solub}$ values for I in the I@CB[7] complex at pH 5 correspond to the moderately polar environment ($p_{solub} = 0.61$). This result with allowing for the probe dimensions can be preliminarily interpreted in terms of the structure in which the NO group is located inside the CB[7] near carbonyl dipoles, while the protonated charged amine group of I resides nearby the negatively charged portal region of another portal of the same CB7 molecule (we discuss its structure in more detail together with other results in Sections 3.1.3 and 3.3 and S1).

As noted, the $a_{ns}$ values for II in the II@CB[7] complex, unlike that for I@CB[7], increase with the CB[7] concentrations, so at large CB7 concentrations, these values become greater than for the free probe in an aqueous environment (see Figures S1–S3 and Table 1). Besides, as already noted, the rotational correlation time, $\tau_0$, increases with the CB7 concentration. These dependencies suggest that the NO group of II is bound to the highly polar region of CB7 or exposed in an aqueous environment. However, these dependencies do not allow the location of the II relative to CB[7] to be completely specified (see also Sections 3.1.3 and 3.3 and S1).

Notably, the $a_{ns}$ dependencies on the CB[7] concentration were obtained earlier for the structural analogue of II, Cat1.16 II differs from Cat1 by the presence of an octyl substituent bound to the quaternary nitrogen instead of the methyl group in Cat1. Based on the above dependencies and results of the $^1$H NMR titration of the diamagnetic analogue of Cat1, Jayaraj et al.16 proposed a model in which the trimethylammonium group of Cat1 is bound to a portal of CB[7], while the rest of Cat1 including the piperdine-N-oxyl group is exposed to water. The presence of a hydrophobic octyl group in II might alter the spin probe location compared with the case of Cat1. Therefore, to better understand the guest location in II@CB[7] and also in two other complexes, we studied the Heisenberg spin exchange (HE) of these complexes with a water-soluble paramagnetic reagent and performed the DFT and MD computations of their structures (see below).

The $a_N$ and $p_{solub}$ values for spin probe III in the CB[7] complex correspond to the aqueous environment of the NO group, as seen in Table 1. This means that two modes of complex formation, in principle, are possible: external complexation or the location of III inside the CB[7], leaving the NO group bulk exposed.

3.1.3. Accessibility of the Reporter NO Group for the Water-Soluble Paramagnetic Complexes. The measurement of the exchange interaction of the reporter NO group with water-soluble paramagnetic compounds is a well-known approach to determining the kinetic accessibility of the NO group to water-soluble reagents.42 Therefore, to determine the location of this group relative to the surrounding water, we used the chromium trioxalate anion, Cr(Ox)$_3^{2-}$, which is formed in the aqueous solution of potassium chromium(III) oxalate trihydrate (CrOx). Crox is known to produce maximum exchange broadening for free nitroxides.43 The advantage of this broadening reagent is also the existence of a rather strong negative charge; therefore, it is expected that the

| spin probes | complex with CB[7] | glycerol/water 50/50 v/v | water $a_N$ | toluene $a_N$ | $p_{solub}$ |
|------------|------------------|------------------------|-----------|-------------|----------|
| I          | 16.29 ± 0.02 (pH 5) | 16.70 ± 0.03 | 16.85 ± 0.015 (pH 5) | 15.42 ± 0.02 | 0.61 ± 0.04 (pH 5) |
| II         | 16.86 ± 0.02 | 16.57 ± 0.03 | 16.72 ± 0.015 | 15.41 ± 0.02 | 1.107 ± 0.04 |
| III        | 16.88 ± 0.02 | 16.90 ± 0.015 | 15.35 ± 0.02 | 0.987 ± 0.04 |

*Free spin probes in water or a 50/50 v/v glycerol/water mixture ($a_N^{cb}$) and in toluene ($a_N^{T}$) were determined as a distance between the hyperfine components, $m = +1$ and 0, at 292 K. The polarity parameters, $p_{solub}$, in the aqueous solutions of the spin probe@CB[7] complexes were determined from eq 3.
Crox anions would not be bound to the CB[7] host having a negative surface electrostatic potential on the carbonyl rims. The values of the exchange broadening produced by Crox for the complexes of I\(^-\)\(^{\text{III}}\) with CB[7] and free spin probes I\(^-\)\(^{\text{III}}\) are given in Table 2.

As seen in Table 2, free I and II spin probes have similar HE broadening (\(\Delta H_{\text{comp}}^{\text{ex}}\)) values, while the \(\Delta H_{\text{comp}}^{\text{ex}}\) value for bound II is much greater than that for bound I. The accessibility of Crox to the NO group for a particular spin probe in the complex can be estimated by obtaining the ratio of the HE broadening in the complex to that of the free probe (\(\Delta H_{\text{comp}}^{\text{ex}} / \Delta H_{\text{free}}^{\text{ex}}\)). Noticeably, the accessibility parameter of Crox for the NO group in the I@CB[7] complex is significant, although it is rather far from unity, which is indicative of the location of the NO group within the cavity not close to the portal. The accessibility to Crox is much greater for II in the II@CB[7] complex than for I in the I@CB[7] complex. The latter result suggests that the location of the NO group in the II@CB[7] complex is similar to that for Cat1.16 The ratio, \(\Delta H_{\text{comp}}^{\text{ex}} / \Delta H_{\text{free}}^{\text{ex}}\) is less than unity, which can be attributed to the steric hindrance against the interactive contact of Crox with the NO group, owing to the presence of CB[7] and binding II. One can see in Tables 1 and 2 that there is a qualitative correlation between the parameters of polarity and accessibility for both I@CB[7] and II@CB[7] complexes, although the absolute values of these parameters appreciably differ for the two complexes.

Table 2. HE Broadening of the ESR Spectra Produced by Crox in an Aqueous Solution for Complexes I@CB[7], II@CB[7], and III@CB[7] (\(\Delta H_{\text{comp}}^{\text{ex}}, \Delta \omega_{\text{comp}}^{\text{ex}}(\text{s}^{-1})\)) and Free Spin Probes I–III\(^a\)

| Crox (mM) | complex      | \(\Delta H_{\text{comp}}^{\text{ex}}\) (G), \(\Delta \omega_{\text{comp}}^{\text{ex}}(\text{s}^{-1})\) | \(\Delta H_{\text{comp}}^{\text{ex}}\) (G), \(\Delta \omega_{\text{comp}}^{\text{ex}}(\text{s}^{-1})\) | \(\Delta H_{\text{comp}}^{\text{ex}} / \Delta H_{\text{free}}^{\text{ex}}\) |
|----------|--------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 5        | I–CB[7]      | 1.58 ± 0.02, 2.41 \(\times 10^7\)             | 0.34 ± 0.02, 0.52 \(\times 10^7\)             | 0.215 ± 0.04                                  |
| 5        | II–CB[7]     | 1.41 ± 0.02, 2.15 \(\times 10^7\)             | 1.09 ± 0.02, 1.66 \(\times 10^7\)             | 0.77 ± 0.04                                  |
| 5        | III–CB[7]    | 0.56 ± 0.02, 0.99 \(\times 10^7\)             | 0.57 ± 0.02, 1.00 \(\times 10^7\)             | 1.02 ± 0.04                                  |

\(\Delta H_{\text{free}}^{\text{ex}}(\text{G})\) and \(\Delta \omega_{\text{free}}^{\text{ex}}(\text{s}^{-1})\) are in Gauss and \(\text{s}^{-1}\), respectively. \(\Delta \omega^{\text{ex}} = \gamma \cdot \Delta H^{\text{ex}}\); \(\gamma\) is the electron gyromagnetic ratio, and \(\gamma = 1.76 \times 10^7 \text{ s}^{-1} \text{ G}^{-1}\). \(T = 292\) K.

Figure 3. Experimental (red lines) and best simulated (black lines) ESR spectra of complexes I@CB[7], II@CB[7], and III@CB[7] in the solid solution in the CB[7] matrix. The concentrations of the spin probes are 0.1 mM; the molar ratio of CB[7] to spin probes is 60.
The exchange broadening for the III@CB[7] complexes is practically the same as for free probe III. Thus, the exchange broadening data for the III@CB[7] complexes also qualitatively agrees with the polarity data. These data indicate the high degree of accessibility to water molecules by the NO groups in the III@CB[7] complexes. The rather small decrease in $a_N$ and an increase in $r_8$ with an increase in CB7 concentration together with very low solubility of III in water support the formation of the “endo” complex. Also, as opposed to I and II, spin probe III has no positive charge to be bound to CB7. However, in principle, these results cannot exclude the possibility of the formation of the exo-complex where spin probe III is bound outside the CB7 cavity. (The locations of I–III in the CB7 complexes are finally discussed in Section 3.4.)

3.2. Properties of the CB[7]–Spin Probe Complexes in a Solid Phase. Figure 3 shows that the ESR line shapes for all of the spin-labeled guests in the temperature range of 142–345 K correspond to the slow rotation regime, where the most prominent change in the ESR spectra is a decrease in the splitting between the outer extrema, $2A_{zz}'$, with an increase in temperature. Correspondingly, a decrease in the motion-sensitive parameter, $S = A_{zz}'/A_{zz}$, also occurs (where $A_{zz}$ is the rigid-limit value measured at 77 K).

3.2.1. Polarity of the NO Group Environment for the Complexes in the Solid CB[7] Matrix. We determined the polarity parameters for spin probes I–III in complexes with CB[7] by measuring the distance between the outer extrema in their ESR spectra at 77 K, which is equal to the doubled z-components of the hyperfine tensors, $A_{zz}$, with the assumption of no motion. The polarity parameters ($p_{solid}$) were defined similarly to those for the aqueous solution (see Tables 1 and 3)

$$p_{solid} = \frac{A_{zz}^{ch} - A_{zz}^{T}}{A_{zz}^{W} - A_{zz}^{T}}$$

As seen in Tables 1 and 3, the polarity parameters in the solid CB[7] matrix, $p_{solid}$, are considerably smaller than those in the aqueous solutions of the complexes, $p_{solat}$. These differences most probably are due to the decrease in the water content around the NO groups inside the CB[7] matrix that results from the preparation of the solid samples. It is noteworthy that a similar decrease in $A_{zz}$ values was observed for lipid spin labels embedded in hydrated phospholipid multilayers on their dehydration and subsequent measurement at 77 K.

We also found out that in the solid samples, a new ESR signal appears with an increase in temperature, the line shape of which corresponds to a rapid rotation regime and the $A_{zz}$ values (15.3–15.5 G) correspond to the hydrophobic environment (see S14). This signal most probably belongs to the nonbonded spin probes dissociated out of the complexes, which should be located in the interstitial sites in the solid CB[7] matrix. Thus, these ESR signals report on the hydrophobic environment inside the solid CB[7] matrix.

The decrease in the water content around the NO groups may result in some changes in the location of the bound spin probe inside the CB[7] cavity compared with the case in the aqueous solution. To determine the locations of the NO groups and the whole spin probe molecules, DFT computations were also performed for the complexes without and in the presence of water, which were modeled using water clusters (see Sections 3.4 and S13).

3.2.2. Rotational Mobility of Spin Probes I–III in the CB[7] Complexes Incorporated into the Solid CB[7] Matrix. The decrease in the water content around the NO groups may not only immobilize the CB[7] hosts and shift the spin probe location but also cause some change in the molecular dynamics of a spin probe relative to the CB[7] host. It is of great interest to investigate the character of the spin probe motion in such a system.

First, we carried out the spectral simulations in terms of the Brownian rotational diffusion model over the full solid angle for the whole indicated temperature range. The input parameters for the simulations, the $A_{xx}$ and $g_{xx}$ tensor components, were determined from the ESR spectra recorded at 77 K (see $A_{xx}$ values in Table 3).

The experimental and best simulated ESR spectra of the I@CB[7], II@CB7, and III@CB7 complexes at different temperatures are given in Figure 3.

From these figures, it can be observed that, generally, there is a satisfactory agreement between the experimental and simulated ESR spectra. However, as was emphasized earlier,24,25,34 the parameters of the molecular rotation of the spin probes, rotational frequencies, and angular amplitudes cannot be determined unambiguously using the $A_{xx}$ (or $S$) values, since the same $A_{xx}$ or $S$ values can be obtained by varying the slow rotation frequency in the full solid angle (4$\pi$) or by varying the angular amplitude in the fast librations model. In particular, it is difficult to correctly and unambiguously choose between these two limiting models of molecular motion for the often-occurring case of high $S$ values ($S \geq 0.9$) from the simulations of the conventional first-harmonic ESR spectra because of the large inhomogeneous broadening. However, this choice, as shown below, can be made by STESR.34

Previously, STESR was successfully applied to the study of the “super slow” molecular rotations with correlation times in the range of $10^{-6}–10^{-3}$ s, to which the conventional ESR spectra are not sensitive.77 The approach used in our study is based on the fact that the STESR line shapes, i.e., the second harmonics absorption signals detected in the 90° phase (out of phase) relative to the phase of the modulation field under a moderate microwave saturation ($V_2$), differ substantially for the two limiting motion models: (1) fast librations with $r_8 \leq 1$
ns and limited angular amplitude and (2) unrestricted slow diffusional rotations in the whole solid angle or of axial diffusional rotation with $T_R \approx 10^{-8} \sim 10^{-6} \text{s}$. For the first model, the $V_2'$ line shape is very similar to that for the no-motion absorption line shape, whereas for the second model, the $V_2'$ line shape is essentially different, being similar to the second derivative of the absorption signal. The $V_2'$ line shapes simulated for the cone angles of 25 and 90° and different rotational frequencies ($f = \tau_R^{-1}$) in the range of $10^5 \sim 10^{10} \text{s}^{-1}$ are illustrated in Figure 4.

![Figure 4](image-url)

**Figure 4.** Simulated $V_2'$ ESR spectra for different rotation frequencies ($f$) in terms of the uncorrelated jump model for the reorientations of the magnetic $Z$-axis inside the cone angles of (A) 25° and (B) 90°.

For the quantitative estimates of the effects of the rotational frequency and angular amplitude on the $V_2'$ line shape, we used the dimensionless ratio, $L''/L$ (see Figure 4) introduced earlier to characterize the sensitivity of the $V_2'$ spectra to very slow rotational motions. $L''$ is the amplitude in the low-field region corresponding to the maximum rate of variation of the resonance field vs the angle ($\Omega$) between the magnetic field direction and the principal $z$-axis of the $A$ and $g$ tensors ($dH_{res}/d\Omega$). $dH_{res}/d\Omega$ is maximum at $\Omega \approx 45^\circ$, and $L''$ is the amplitude corresponding to $dH_{res}/d(cos \Omega) = 0$ and $\Omega \approx 0$. It was shown, for the model of the slow rotation in the whole cone angle ($\pi/2$), that the $L''/L$ ratio decreases with a decrease in $\tau_R$ and for $\tau_R \lesssim 10^{-7} \text{s}$, it becomes negative, while the $V_2'$ line shape itself approaches that of the second derivative of the absorption signal. The dependencies of the $L''/L$ ratio on $f = \tau_R^{-1}$ for different cone angles including $\Delta \theta = \pi/2$ are presented in Figure 5.

![Figure 5](image-url)

**Figure 5.** Dependencies of the diagnostic parameter, $L''/L$, on the rotational frequency ($f$) for different cone angles.

The $V_2'$ line shape for the model of small amplitude librations is observed to be similar to that for the absence of motion, i.e., it is very close to the absorption line shape for the powder sample for which the ratio $L''/L \gtrsim 2$. An increase in the libration amplitude results in an increase in $L''/L$ for high rotation frequencies in the range of $10^6 \sim 10^{10} \text{s}^{-1}$ (see Figure 5).

The qualitative explanation of the decrease in the ratio, $L''/L$, with increasing rotational frequency is as follows. The out-of-phase absorption signal (the imaginary component of the absorption), $V_2''$, records the delay effects of the response of the spin system on the modulated magnetic field. The amplitude of the $V_2''$ response depends on the product, $\omega_mT_{rel} = 2\pi \cdot \nu$, where $\nu$ is the modulation frequency. $\omega_mT_{rel} = \omega_m/T_{rel}$, where $T_{rel}$ includes all of the relaxation processes, particularly the rotational diffusion that results in the spectral diffusion out of the resonance position and a decrease in the signal amplitude. The effect of the spectral diffusion should be maximum at point $L''$ and minimum at point $L_0$ where $dH_{res}/d(cos \Omega) = 0$. An increase in the rotational frequency results in an increase in the spectral diffusion, which, accordingly, results in a decrease in the $L''/L$ ratio. A further increase in the rotational frequency at $f \gg \omega_m$ leads first to the averaging of the signal over the rotational motion and subsequently to the response of the averaged signal to the magnetic field modulation, which results in an increase in the $L''/L$ ratio. The effect of the spectral diffusion also increases with an increase in the cone angle, resulting in a decrease in parameter $L''/L$.

The $V_2'$ spectra of the inclusion complexes of I–III with CB[7] were recorded at different temperatures in the range of 245–345 K. The values of the microwave field ($H_m$) and modulation field (H$_{mod}$) were 0.25 and 5 G, respectively. The temperature dependencies of the $V_2'$ spectra for all three spin probes are presented in Figure 6.

As noted above, the $R_2$ values obtained from the simulations of the conventional first-harmonic ESR spectra in terms of the Brownian rotation model are in the range of $10^6 \sim 10^7 \text{s}^{-1}$. Simultaneously, it follows from the $V_2''$ simulations (see Figure 5) that the diffusional rotation in the full cone angle ($\Delta \theta = \pi/2$) with frequencies in the range of $10^6 \sim 10^7 \text{s}^{-1}$ should lead to approximately zero or negative $L''/L$ values. However, the experimental $L''/L$ values for the complexes of I–III with CB7 in the temperature ranges studied are much greater than the above values: 1.06–0.79 for I@CB7, 0.97–0.65 for II@CB7,
and 1.41–0.775 for III@CB7 (see Figure 6). These experimental $L''/L$ values correspond to the frequencies in the range of $10^5−3 \times 10^5$ s$^{-1}$ for the overall Brownian rotation in the solid CB[7] matrix. The spin probe concentrations are 0.1 mM; the molar ratio of CB[7] to spin probes I, II, and III is 60:1. The magnetic field modulation frequency and amplitude are 50 kHz and 5 G, respectively; the microwave field amplitude is 0.25 G.

Figure 6. Temperature dependencies of the $V''_1$ STESR spectra of complexes I@CB[7], II@CB[7], and III@CB[7] in the solid CB[7] matrix. The spin probe concentrations are 0.1 mM; the molar ratio of CB[7] to spin probes I, II, and III is 60:1. The magnetic field modulation frequency and amplitude are 50 kHz and 5 G, respectively; the microwave field amplitude is 0.25 G.

where $A_{1}A_{1}$ is the averaged value of the perpendicular component of the $A$ tensor, $A_{1} = (A_{xx} + A_{yy})/2$, in the absence of rotational motion (at 77 K). It is difficult to precisely determine the $A_{1}$ value from our experimental ESR spectra. The typical $A_{1}$ values for the six-membered piperidine oxide radicals vary in a rather narrow range of $6−7$ G; moreover, the $\Delta \theta$ values determined in eq 5 are weakly dependent on the $A_{1}$ values in the above range. Therefore, we can insert $A_{1} = 6$ G. The $A'_{xx}$ and $A'_{zz}$ values were determined as a half separation between the outer extrema of the ESR spectra. The $\Delta \theta$ values calculated in eq 5 using the experimental $A_{ii}$ components for the complexes of I−III with CB[7] are given in Figure 7.

The obtained $\Delta \theta$ values should agree with both the conventional ESR and STESR spectra. Next, using the above $\Delta \theta$ values and the calibration dependencies in Figure 5, we can, in principle, estimate the rotational frequencies for librational motion at different temperatures. Unfortunately, the calibration curves in Figure 5 are not exactly quantitative dependencies because they were obtained using several approximations: Bloch equations, axially symmetric $A$ and $g$ tensors, small modulation amplitude compared with the homogeneous line width, and the adiabatic approximation for the spin Hamiltonian. Therefore, the $\Delta \theta$ values could be estimated adequately from the $A_{ii}$ components, while the $f$ (or $\tau_R^{-1}$) values are correct within 1 order of magnitude, depending on the $\Delta \theta$ value.

Thus, the comparison of the conventional ESR and STESR spectra enables the selection of the spin probe rotational motion in the CB[7] complexes between two possible models and also the estimation of the angular amplitudes of the librations.

We attribute the obtained libration amplitudes and their temperature dependencies presented in Figure 7 to the motions of the spin probes within the host cavities rather than to the librations of the complexes as a whole in the CB[7] matrix on the following grounds. First, the above temperature dependencies appreciably differ from the data on the angular restricted molecular motions for the doped molecular probes in different disordered media, including supercooled liquids and organic glasses.44−49 The common characteristic feature of the motions in all of these systems is the existence of a temperature region, in which the linear temperature dependency of the mean-squared libration amplitude considerably

Figure 7. Temperature dependencies of the cone angles (amplitudes) of the libration motion for spin probes I–III in the guest–host complexes with CB[7] embedded into the CB[7] matrix. The spin probe/CB[7] molar ratio is 1:60.

and 1.41–0.775 for III@CB7 (see Figure 6). These experimental $L''/L$ values correspond to the frequencies in the range of $10^5−3 \times 10^5$ s$^{-1}$ for the overall Brownian rotation in the solid CB[7] matrix. The spin probe concentrations are 0.1 mM; the molar ratio of CB[7] to spin probes I, II, and III is 60:1. The magnetic field modulation frequency and amplitude are 50 kHz and 5 G, respectively; the microwave field amplitude is 0.25 G.
increases in sharpness and increases nonlinearly with the temperature. Similar phenomena concerning the translational local motion were observed for the neutron scattering on the protons, for Mössbauer absorption of the dissolved iron ions, and for the motions in proteins and lipid membranes.\textsuperscript{50–53} These general phenomena were treated as dynamical transitions from harmonic librations at low temperatures to anharmonic librations for diffusive motions. Some experimental results indicate that the dynamical transitions in molecular glasses and biological systems involve cooperative motion in a cluster of neighboring molecules (see ref 49).

Our ESR data for quite different objects, including the spin probes in the guest–host complexes with the CB[7] host, which form a dilute solid solution in the CB[7] matrix, are also indicative of the rotational motion of limited angular amplitude, which increases with temperature. For better comparison of both kinds of dependencies, we expressed the angular amplitudes in Figure 7 in radians and plotted the temperature dependencies for the mean-squared amplitudes in Figure 8, as was carried out in previous studies.

![Figure 8. Temperature dependencies of the mean-squared libration amplitudes for spin probes I–III inside the CB[7] cavities embedded into the solid CB[7] matrix.](image)

As seen in Figure 8, the mean-squared libration amplitudes for I have a parabolic temperature dependence on the broad temperature range without a visible sharp transition and tend to be linear at relatively high temperatures. A similar dependency without a sharp transition is observed for the II@CB[7] complex. The temperature dependence for the III@CB[7] complex was measured in a minor temperature range due to the partial dissociation of the spin probe out of the CB[7] cavity at elevated temperatures (see SI4). However, in this range, a sharp increase in the libration amplitude was also not observed.

Second, the libration amplitudes are different for spin probes I–III and have different temperature dependencies, whereas the mechanism of the overall rotation predicts approximately equal temperature dependencies for all of the complexes. Furthermore, the libration amplitudes for the different spin probes correlate with the probe dimensions, i.e., they are the greatest for probe I having the minimum size and are minimal for probe III having the maximum size. Next, the temperature dependencies in Figure 8 differ from the similar dependencies for the amorphous solids discussed above, which have a rather long linear region and sharp transition to nonlinear behavior. Moreover, it was shown that the molecules embedded into the solid matrix execute librations of considerably smaller amplitude if they are structurally similar to the matrix molecules compared to the case with other probes.\textsuperscript{49} This means that for the guest–host complexes embedded into the matrix of the host molecules, the librations of the spin probes in the host cavities are predominantly manifested in the ESR spectra rather than the librations of the guest–host complexes as a whole. Finally, the librations of the spin probes within the CB[7] cavities in the dilute solutions are certainly not cooperative as opposed to the case of molecular glasses.\textsuperscript{49}

As noted in Section 1, there are only a few studies on the rotational mobility of the spin probes in the g/h complexes with cucurbiturils.\textsuperscript{22,23} In the previous work,\textsuperscript{52} bis-spiropeperidinium nitroxide derived from Tempone was included in CB[7] to form a strong g/h complex having increased resistance to ascorbate reduction. The unusual ESR line shape of this complex was explained by an anisotropic rotational diffusion, which was more rapid than the Brownian rotational diffusion of the free spin probe in water, i.e., in this complex, a very high mobility of the spin probe is realized inside the CB[7] cavity. Surprisingly, such superhigh mobility is possible for the spin probe having two positive charges on the opposite sides of the molecule, which are capable of interacting with the carbonyl oxygen groups on the walls of CB[7]. The comparison of the work of Casano et al.\textsuperscript{22} with the results from ref 23 and from the present study, where, on the contrary, rather constrained motions were discovered, shows that there is a great potential in researching and applying such different dynamics in different chemical and physical processes occurring in nanosystems.

3.3. Computational Results and Their Comparison with Experimental Data. The hydrophobic environment for the CB[7] complexes in the solid CB7 matrix means low dielectric permittivity of the local environment, which is close to the permittivity of a gas. This fact allows us to suppose that, because of the absence of molecular motion in the solid matrix, the effect of the surrounding results mainly in the immobilization of the embedded inclusion complex and formation of the hydrophobic environment. This conclusion in turn allows us to assume that the inner structural features of the CB[7] inclusion complexes embedded in the solid CB7 matrix in a first approximation might be computed by the DFT method, which is technically applicable to the gas phase.

The formation energies for the complexes of I–III with CB[7] and their structures are given in Table 4 and Figures 9–11. The influence of the aqueous environment on the formation energy and the spatial structure of the g/h complexes with CB[7] are considered in more detail in SI3.

We emphasize that the complex formation energies in the solid CB[7] matrix presented in the upper rows of Table 4 relate to the gas-phase formation energies. Moreover, these energies should not be directly related to the corresponding binding constants of the spin probes because our DFT computations did not consider the entropic component of the free energy of formation.

It is seen that in the I@CB7 complex there are contributions from both electrostatic and van der Waals interactions. The designations I@CB7perp and I@CB7par relate to the complexes with the orientations of the NO group almost perpendicular or parallel relative to the portal CB[7] plane, respectively. It is seen that in the hydrophobic environment the complex structure with the perpendicular orientation of the
Table 4. Complex Formation Energies, ΔE_{comp.form} and the Contributions to ΔE_{comp.form} from Electrostatic (ΔE_{el.stat}) and Van der Waals (ΔE_{vdW}) Interactions (kcal/mol) for the CB[7] Inclusion Complexes with Spin Probes I–III in the Hydrophobic and Aqueous Environments

| complexes and molecules | ΔE_{comp.form} | ΔE_{el.stat} | ΔE_{vdW} |
|-------------------------|----------------|--------------|----------|
| I@CB[7]perp           | −94.0          | −39.3        | −54.7    |
| I@CB[7]par           | −83.0          | −36.7        | −46.3    |
| I@[36H2O]              | −70.9/−70.2     | −23.2        | −47.7    |
| II@CB[7]-NO-in        | −91.3          | −43.4        | −47.9    |
| II@CB[7]-NO-out       | −90.6          | −24.5        | −66.1    |
| II@[30H2O]:CB[7]-in   | −89.0          | −37.6        | −51.4    |
| II@[30H2O]:CB[7]-out  | −100.3         | −30.7        | −69.6    |
| III@CB[7]             | −55.2          | −55.8        | 0.6      |

“Hydration energy calculated using the PCM approximation.

Hydration energy, calculated by the cluster approximation/hydration energy calculated using PCM. NO group of II is located inside the CB[7] cavity. NO group of II is located outside (out) the CB[7] cavity.

NO group is more stable because of greater contributions of both electrostatic and van der Waals interactions. The spatial structure of the latter I@CB[7] complex is given in Figure 9. I is almost entirely embedded into the CB[7] cavity, and the NO group is located close to the portal inside the cavity.

The rigid computation of the complex formation energy and the spatial structure of the I@CB[7] complex in an aqueous solution is a complicated problem within the framework of the DFT method. According to the present concept, the main driving force for the guest–host complex formation with the cucurbiturils in an aqueous solution is the hydrophobic interaction. This interaction results in the transition of the guest molecule from an aqueous solution to the host cavity and the displacement of the "high-energy" water from the cavity to the solvent bulk. The complex formation in the aqueous phase can be formally divided into the following steps: (i) dehydration of the spin probe, (ii) release of high-energy water from CB[7] and its merger with bulk water, and (iii) the inclusion of the spin probe into the CB[7] cavity.

We applied an approximate approach based on the building up of a solvation shell (cluster) around the solute consisting of a large number of water molecules. The DFT-D3 method was used for computing the interaction energy of the cluster with the solute (I), which is the hydration energy (E_{hydr}) of the solute. Earlier, a similar approach was successfully applied to simulate the solvent influence on the ground and excited states of the various organic solutes: guanine, thymine, and pyrene. The first hydration layer can be built as a closed cluster enveloping the solute with all of the H-bonded water molecules. Further, the water layers can be sequentially added until the new layer does not lead to a change in E_{hydr}. The computation details and spatial structures of hydrated I, the CB[7] molecule, and the hydrated I@CB[7] complex are presented in SI3.

The examination of the I@CB[7] complex in an aqueous solution shows that there are three possible (basic) variants of the complex structures for further consideration. In two of them, the NO group is located inside the CB7 with different orientations of the NO bond relative to the portal, i.e., perpendicular and parallel ones, and the third one represents the so-called exo-complex in which I is bound to CB7 through the positively charged ammonium group, while the other part of I is in the aqueous phase out of the CB7 cavity. The experimental data on the polarity of the NO group environment, P_{water} and the NO group accessibility to Crox, ΔH_{hydr}/ΔH_{free} allow us to reject straightforward the third variant of the complex for which the NO group is open for bulk water and therefore both parameters should be equal to unity. In fact, both the decreased polarity and accessibility parameters correspond to the NO group not easily accessible to water, i.e., located in the cavity (see Sections 3.1.2. and 3.1.3).

The formation energy of the I@CB7 complex for the more stable structure with the perpendicular orientation of the NO bond relative to the portal was found to be −18.6 kcal/mol. Accounting for the free energy changes attributed to an increase in the entropy, owing to the transition of the high-energy water molecules into the bulk water, would afford even more negative values. Thus, the inclusion of water into the energy balance in the complex formation predicts the formation of the I@CB[7] complex in agreement with the experiment.

The DFT-D3 version of the DFT method was also used to compute the structure of the II@CB7 complex in the solid environment, computed by the DFT-D3 method. The red, dark blue, blue, and gray indicate oxygen, nitrogen, carbon, and hydrogen atoms, respectively.
CB7 matrix. The complex formation energies for the locations of the NO group inside and outside the CB7 cavity are given in Table 4, while the corresponding II@CB7 spatial structures are shown in Figure 10. As seen in Table 4, the “in” structure with the NO group inside CB7 is slightly more stable than the “out” structure because of a larger contribution of the vdW interaction in the former one.

First, in Table 4, as for I@CB7, the vdW contribution to the complexation energy of II@CB7 is quite comparable to the electrostatic one.

Figure 10 shows two possible structures of II@CB7, which could simulate the spatial structure of this complex in the hydrophobic environment, i.e., in the CB7 matrix. In II@CB7-NO-in, the NO bond is oriented approximately parallel to the symmetry axis, C7, of CB7 and located close to the portal inside the CB7 cavity. This structure is energetically more favorable by 0.7 kcal/mol than the II@CB7-NO-out structure, in which the NO group is located outside the CB7 cavity. The location of the NO group out of the cavity is also at variance with the slow-motional ESR spectra of this complex in the CB7 matrix because, as shown in SI4, the ESR spectra of the spin probes with the NO group in the hydrophobic CB7 matrix correspond to the rapid molecular rotation. Therefore, the structure of II@CB7-NO-out should be rejected, whereas the structure of II@CB7-NO-in is realized in the hydrophobic environment of the complex in the CB7 matrix.

The effect of the water environment on the structure of the II@CB7 complex was simulated in a way different from that for the I@CB7 complex. As already mentioned, the complex of CB7 with the spin probe, Cat1, which differs from II by having a methyl instead of an octyl group in II, was concluded in ref 16 to have different spatial structures in crystal and aqueous solutions. Specifically, both in the gas and crystal, Cat1 is entirely located in the CB7 cavity. In the aqueous solution, the trimethylammonium group in Cat1 is bound to the CB7 portal from the outside, while the NO group and the rest of the Cat1 molecule are exposed to water.

The bulky octyl group in II changes the hydrophobic/electrostatic balance of this spin probe, which might affect the mode of its binding to CB7 compared to the case with Cat1. Our experimental data on the environment polarity and accessibility of the NO group to Crox (Tables 1 and 2) demonstrate the almost aqueous environment of the NO group. However, these data are not sufficient to deduce the exact location of the whole radical of II and estimate the complex formation energy. Therefore, it appeared important to simulate the complex formation energy and structure of the II@CB7 complex in an aqueous solution using molecular dynamics simulations. The great quality of this method is the capability to determine the global minimum of the potential energy and free energy corresponding to the equilibrium structure of the supramolecular complexes. The stability of g/h complexes is determined by the dependence of the energy of a complex on a small number of variables responsible for complexation. In most cases, this variable can be considered the distance R between the centers of mass of the components. In the MD method, to determine the global minimum of potential energy, a mean force potential (PMF) is constructed, which is obtained as a result of averaging and is affected not only by the intermolecular interactions of the components but also by the presence of an aqueous solvent.

The most stable structure in the aqueous phase is shown to correspond to the location of the NO group in the aqueous phase out of the CB7 cavity. The outside location of the NO group of II in an aqueous solution agrees well with the experimental data on the environment polarity and accessibility to the Crox of the NO group (see Tables 1 and 2). It is also shown that the octyl group of II takes the bent form of a helix with a maximum inclusion into the cavity. Such a change in conformation compared with the all-trans conformation in the hydrophobic environment or vacuum (see Figure 10) is due to the hydrophobic interaction. The results of the application of this method to the g/h complexes of II and III with CB7 in the aqueous phase are presented in more detail in SI3.

The DFT computation of the spatial structure of III@CB7 in the CB7 matrix showed that the indole ring of III is oriented perpendicular to the portal plane, as shown in Figure 11, while the inclination of the N–O bond to the vertical CB7 axis is about 47°. The NO group itself is at a rather close proximity to the portal plane. Interestingly, however, that this location corresponds to the considerably hydrophobic environment of the NO group, as seen from the $P_{	ext{ehab}}$ value (Table 3).

Along with the computations of the spatial structures of the g/h complexes, we determined, by DFT, some important physical parameters of the spin probes and CB7 and the effect of the complexation on their changes:

1. The total electric charges on the spin probes, partial electric charges on N and O atoms, and the spin densities on the N and O atoms of the NO group for the free spin probes and their complexes with CB7 in the solid CB7 matrix are presented in SI5.
4. CONCLUSIONS

The evidence was obtained for the librational character of the rotational motion of the spin probes in the guest–host complexes inside the CB7 cavities that were embedded in the solid CB7 matrix. This motion is opposed to the diffusional rotation over the whole solid angle. The stated conclusion is based on the comparison of the results obtained by conventional ESR with those of the STESR methods for the above motion models.

The determined libration amplitudes and their temperature dependencies relate to the motions of spin probes I–III within the host cavities rather than to the motions of the complexes as a whole in the solid CB7 matrix. These librations essentially differ from the constrained motions observed for various molecular probes in supercooled liquids, organic molecular glasses, and other disordered media. As opposed to these objects, the temperature dependencies of the libration amplitudes in the g/h complexes have a parabolic shape and turn into linear dependencies at relatively high temperatures.

The integrated experimental ESR- and DFT-based computational approaches were used to characterize the structure of the complexes, particularly, to trace the location of guest molecules in the host structure. For the I@CB7 complex, the observed $\Delta q_x$ and $P_{solid}$ values as well as dynamic accessibility parameters correspond to the NO group location deduced from the DFT structure. A certain correlation also exists for the II@CB7 and III@CB7 complexes. ESR data on polarity and dynamic accessibility in aqueous solutions are indicative of the NO group location in the aqueous phase, in agreement with DFT computations that take into account aqueous clusters around the complex. In the CB7 matrix, the $A_{2\theta}$ and $P_{solid}$ values correspond to the location of the NO group in the nonpolar environment. This again agrees with the computed structures in which the guest molecules and their NO groups are inside the CB7 cavity.

The environment polarities of the NO groups for all of the spin probes in the inclusion complexes are substantially different for the complexes in an aqueous solution and in the solid CB7 matrix. For I@CB7 in an aqueous solution, the polarity is probably due to the partial penetration of the outside water molecules; for II@CB7, to the location of the NO group in the aqueous phase; and for III@CB7, to the location of the NO group at the water/CB7 interface.

The hydrophobic environment for the guest–host complexes in the solid CB7 matrix and, accordingly, low dielectric constant in the first approximation make the DFT computations compatible, which are technically applicable to the gas phase.

The use of the concept of aqueous clusters surrounding the spin probes and CB7 molecules has allowed the estimation of the effects of an aqueous environment on the complexation energy and spatial structure of the guest–host complexes.

The use of the molecular dynamics simulations has allowed the determination of global minima and binding energies for the II@CB7 and III@CB7 complexes in the aqueous solutions.

The spatial structures, formation energies of the complexes, and the changes in the atomic charge and spin density distributions on the complex formation are determined from the computations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03772.

Dependences of the rotational correlation time and polarity parameter, $\Delta q_x$, on the CB7 concentration for spin probes I–III in an aqueous solution (SI1); derivation of the equations for the second-harmonic adsorption ESR line shape for the nitroxide spin probe undergoing strong jump diffusion of limited angular amplitude and unrestricted frequency (SI2); influence of the aqueous environment on the formation energy and spatial structure of the g/h complexes with CB7 (SI3); dissociation of the spin probes out of the guest–host complexes in the solid phase (SI4); electric charges and spin densities of the spin probes in the free-state and in the g/h complexes (SI5); and principal values of the mass moments of inertia and ratios of the long ($d_{l}$) and short ($d_{s}$) ellipse diameters of the portals arising from the complex formation (SI6) (PDF)

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ABBREVIATIONS

CB7, cucurbit[7]uril; ESR, electron spin resonance; STESR, saturation transfer electron spin resonance; DFT, density function theory; Crox, potassium chromium(III) oxalate trihydrate; TEMPOamine (I), 2,2,6,6-tetramethylpiperidinyl-N-oxy-4-amine bromide; (Cat8, II), 4-(N,N-dimethyl-N-octyl
ammonium)-2,2,6,6-tetramethylpiperidinyl-N-oxyl bromide; III, 2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-γ-carboline-3-oxyl

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