Inclusion complexes of spin-labeled indoles with cyclodextrins in aqueous solutions

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Guest-host complexes of β- and γ-cyclodextrins (CDs) with two spin-labeled indole derivatives having the same molecular weights but different structures were studied by EPR spectroscopy in aqueous solutions and semiempirical quantum-chemical calculations of these systems were carried out. In the presence of CD the polarity of the NO group environment decreases and the rotational correlation time (τ) of guest molecules increases. Both indole derivatives form 1 : 1 complexes with γ-CD, the binding constants of the complexes being different more than twice. Simulation of EPR spectra made it possible to determine the indole ring orientation relative to the plane of the host molecule (at angles in the range 30—60°) and the rotational diffusion coefficients of the complexes, which corresponded to the hydrodynamic volume of one γ-CD molecule. In contrast to the complexes with γ-CD the rotational correlation times, τ, of the complexes with β-CD correspond to a hydrodynamic volume which much exceeds the volume of a single β-CD molecule. The complexes with β-CD are also characterized by more hydrophobic environment for guest molecules and absence of spin exchange with Ni^{2+} ions in the aqueous solution. There results are consistent with a dimeric structure of β-CD in the complex and with the orientation of the long axis of the guest molecule along the dimer axis. The energies and geometric parameters were calculated for all complexes by the PM3 method with a conventional set of parameters. The optimized energetically stable structures of the 1 : 1 complexes with γ-CD and of the 1 : 2 complexes with β-CD are consistent with experimental data.

Key words: EPR spectroscopy, spin probes, cyclodextrins, guest—host complexes, indoles, rotation dynamics, semiempirical quantum-chemical calculations.

Cyclodextrins (CDs) are cyclic glucose oligomers. Due to the presence of a hydrophobic cavity in the molecule they can form guest—host complexes with a variety of organic compounds.[1—6] There are several reasons for recent considerable interest in these complexes. First, for many hydrophobic compounds binding to CDs dramatically increases the solubility in water, thus making possible delivery of various pharmaceuticals[7] or extraction of biologically important hydrophobic molecules, such as cholesterol, from biomembranes.[8] Second, inclusion into the CD cavity causes significant changes in the photophysical properties and reactivities of guest molecules.[1—6,9—12] For instance, the quantum yield of fluorescence of aromatic hydrocarbons and heterocyclic compounds increases[9] and naphthalene in ternary complexes containing two guest molecules (naphthalene and cyclohexane) exhibits room-temperature phosphorescence.[10] In the presence of CDs, catalytic hydrolysis of esters is accelerated[11] and some short-lived radicals are stabilized.[12]

Inclusion complexes of CDs with various classes of compounds have been the subject of numerous studies by various methods including EPR spectroscopy (see reviews[1—7,9] and studies[12—18]). The use of spin-labeled guest molecules is of particular interest, because their EPR spectra allow not only the thermodynamic binding parameters and local environment polarity but also the dynamics of guest molecules inside the cavity to be determined. This is important for understanding of the character of guest—host interaction, the relaxation mechanisms of electronic excitation, and the reactivity of guest molecules.

One of the important classes of guest molecules comprises the derivatives of an aromatic heterocycle indole. Indole, a constituent of tryptophan amino acid, fluoresces, which allows using spin-labeled indole derivatives as double (optical and spin) molecular probes. The aim of the present study was to carry out an EPR study of inclusion complexes formed by two spin-labeled indole derivatives having the same molecular weight but different

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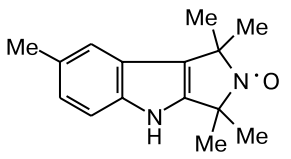
chemical structures with β-CD and γ-CD in aqueous solutions.

Experimental

Compounds used in the experiments were as follows: β-CD and γ-CD (Aldrich); nickel chloride hexahydrate $NiCl_2 \cdot 6H_2O$ (Merck); salts $NiSO_4 \cdot 6H_2O$, $CuSO_4 \cdot 6H_2O$, $MgSO_4 \cdot 7H_2O$ (chemically pure grade); and EtOH, toluene, and glycerol (chemically pure and analytical grades). The synthesis of spin-labeled indoles **1** and **2** was reported earlier;[19,20] samples of these compounds were kindly granted by Dr. A. B. Shapiro (N. M. Emanuel´ Institute of Biochemical Physics, Russian Academy of Sciences).



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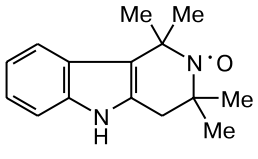

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Complexes of compounds **1** and **2** with β-CD and γ-CD were prepared by adding aqueous solutions containing necessary CD concentrations to aqueous solutions of **1** or **2** and subsequent vortexing for 15 min. The final concentrations of **1** and **2** in the CD-containing solutions were about 10^{-5} mol L^{-1}. The β-CD and γ-CD concentrations were varied in the ranges 0—10 and 0—50 mmol L^{-1}, respectively.

Fluorescence spectra were obtained on a Hitachi spectrofluorimeter in Hellma (USA) quartz cells at 293 K. EPR spectra were recorded on a Bruker ER-200D instrument in a flat quartz cells thermostatted with an accuracy of ±0.5 °C. The magnetic modulation and UHF field amplitudes permitted recording of non-distorted spectra.

The rotational correlation times of the complexes in solutions were determined from EPR spectra using the isotropic rotation model and the relation[21]

$$\tau_{eff} = 6.65 \cdot 10^{-10} \Delta H_{+1} [(I_{+1}/I_{-1})^{1/2} - 1], \quad (1)$$

where ΔH_{+1} is the peak-to-peak linewidth of the low-field hyperfine structure component and $I_{\pm 1}$ are the amplitudes of the low- and high-field hyperfine structure components, respectively.

Besides, the EPR spectra of the complexes were simulated using the non-spherical rotation model, which approximates the spin probe or its complex with CD by an ellipsoid of revolution with the diffusion coefficients corresponding to axial ($R_\parallel$) and off-axial ($R_\perp$) rotation and the angle θ between the z axis of the magnetic tensors (A, g) and the symmetry axis of the diffusion tensor. Spectra were fitted using a program,[22] which determines these parameters and the individual linewidths by the nonlinear least squares method and the Levenberg—Marquardt algorithm.

Polarity of the spin probe environment was characterized by the isotropic hyperfine coupling (HFC) constant (a) on the ^{14}N nucleus and a dimensionless parameter[23]

$$h = (a_w - a)/(a_w - a_{HC}), \quad (2)$$

where a_{HC} and a_w are the a values in toluene and water, respectively. The h values in hydrophobic environment (toluene) and in water are 1 and 0, respectively.

Results and Discussion

Fluorescence spectra. In order to exclude the contribution of reduction products of radicals **1** and **2** to fluorescence, aqueous solutions of the radicals were shaken with lead dioxide powder for 1 h and then PbO_2 was removed by centrifugation. The indole fluorescence spectra in solvents of different polarity and in water in the presence of 10 mM of β-CD and γ-CD are shown in Fig. 1, *a*; the corresponding spectra of compound **2** are shown in Fig. 1, *b*. Compounds **1** and **2** exhibit similar fluorescence spectra. Figures 1, *a* and 1, *b* show that the spin-labeled indole derivatives retain the indole fluorescence band and the dependence of the fluorescence maximum (λ_m) on the polarity of the medium, although the absolute values of λ_m for these molecules are different. The λ_m values for indole and its spin-labeled derivatives in both inclusion complexes correspond to an environment polarity, which is higher than the polarity of ethanol and similar to the polarity of the 1 : 1 ethanol—water

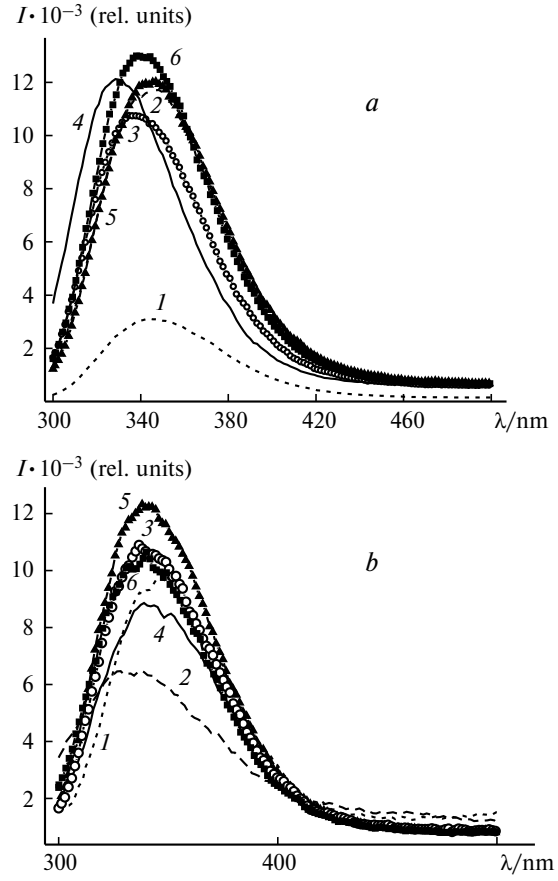

Fig. 1. Fluorescence spectra of indole (*a*) and radical **2** (*b*) in different media and of the complexes with β-CD and γ-CD: water (*1*), ethanol (*2*), toluene (*3*), glycerol—water (1 : 1) (*4*), β-CD (*5*), and γ-CD (*6*); λ_e = 280 nm. The intensities (in relative units) in Fig. 1, *a* are 500 times higher compared to those shown in Fig. 1, *b*.

mixture. However, the fluorescence of radicals **1** and **2** is about three orders of magnitude less intense than that of indole. This decrease is most probably due to an increase in the intersystem crossing to the triplet state in the presence of unpaired electron. Large width and asymmetry of the fluorescence bands of indole and its spin-labeled derivatives in different solvents and in the complexes with CD (see Fig. 1, *a*, *b*) make the determination of λ_m difficult. Finally, even at 10 mmol L^{-1} of β-CD the solution contains not only complexes but also unbound guest molecules. These features make the fluorescence spectra of spin-labeled indoles inconvenient for the investigation of polarity environment and another structural-dynamical parameters of the inclusion complexes. A much larger body of information can be extracted from EPR spectra (see below).

EPR spectroscopy of complexes with γ-cyclodextrin. *Stoichiometry and binding constants*. The EPR spectra of compound **1** at different γ-CD concentrations are shown in Fig. 2. Complex formation manifests itself as an increase in the effective rotational correlation time of spin probes (τ_{eff}) and a decrease in the HFC constant (*a*). These parameters of **1** and **2** are plotted *vs.* γ-CD concentration in Fig. 3. At γ-CD concentrations higher than 10^{-2} mol L^{-1}, the dependences for both spin probes reach a plateau. This means that almost all molecules **1** and **2** are bound in the complexes with γ-CD.

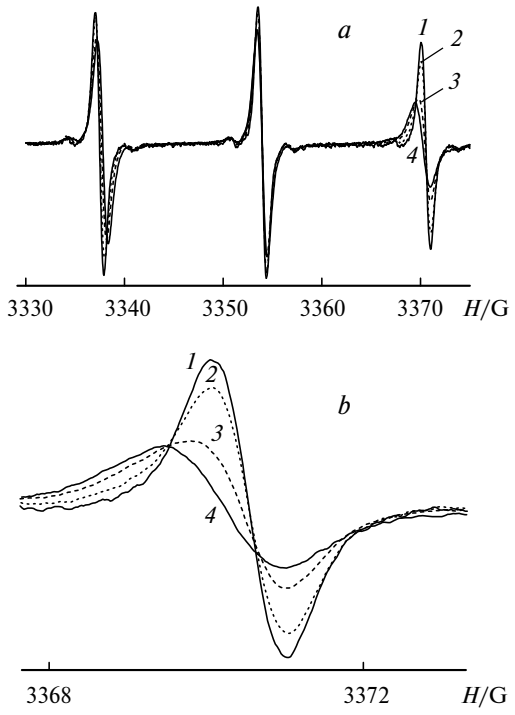

Fig. 2. EPR spectra of radical **1** (*a*) at γ-CD concentrations of 0 (*1*), 4 (*2*), 10 (*3*), and 30 mmol L^{-1} (*4*); the $m = -1$ HFC component of the same spectra (*b*).

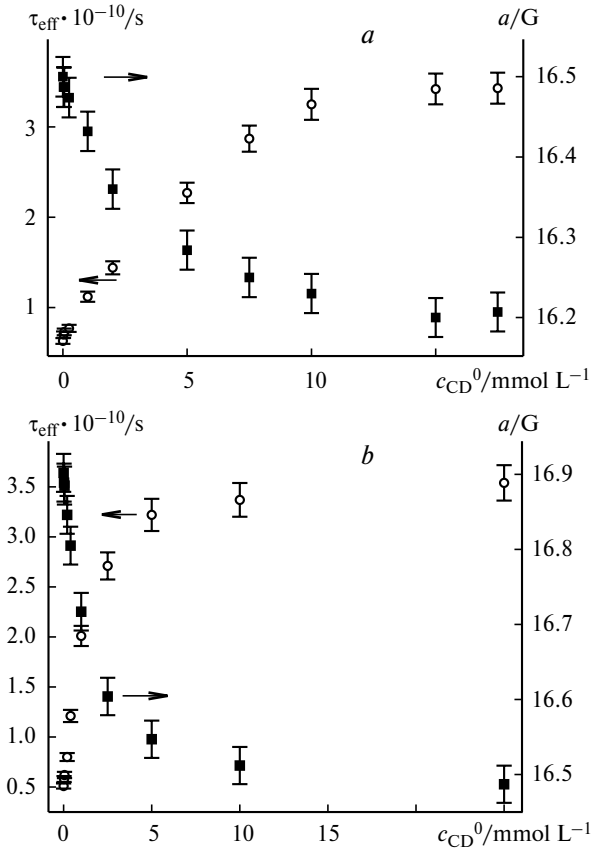

Fig. 3. Polarity parameter (*a*) and effective rotational correlation time (τ_{eff}) plotted *vs.* γ-CD concentration for spin probes **1** (*a*) and **2** (*b*). The probe concentrations were $2 \cdot 10^{-5}$ mol L^{-1} and the temperature was 20 °C.

Assume initially that exchange between free molecules in solution and the molecules bound in the complex with γ-CD is slow on the EPR time scale, namely, the exchange frequency $\omega_e \ll \gamma\Delta a \simeq 4 \cdot 10^6$ s^{-1} (Δa is the difference between the *a* values for the aqueous solution and for the complex with γ-CD). Then, the EPR spectra recorded at intermediate γ-CD concentrations represent superpositions of the signals of free probes and complexes:

$$F_i = \alpha F_b + (1 - \alpha) F_w, \qquad (3)$$

where F_b, F_w, F_i are the spectra respectively recorded in excess, in the absence, and at an intermediate concentration of γ-CD, respectively, and normalized to the double integral; α is the mole fraction of the probes bound in the complex. Meeting condition (3) means the existence of an isosbestic point, which is actually observed in the experiment (see Fig. 2, *b*). Using a computer program, we varied the α value in the linear combination (3) until the best fit of the spectrum at the intermediate γ-CD concentration. Good agreement between the experimental and calculated spectra (see, *e.g.*, Fig. 4, *a*) substantiates the assumption of slow exchange between the free and bound

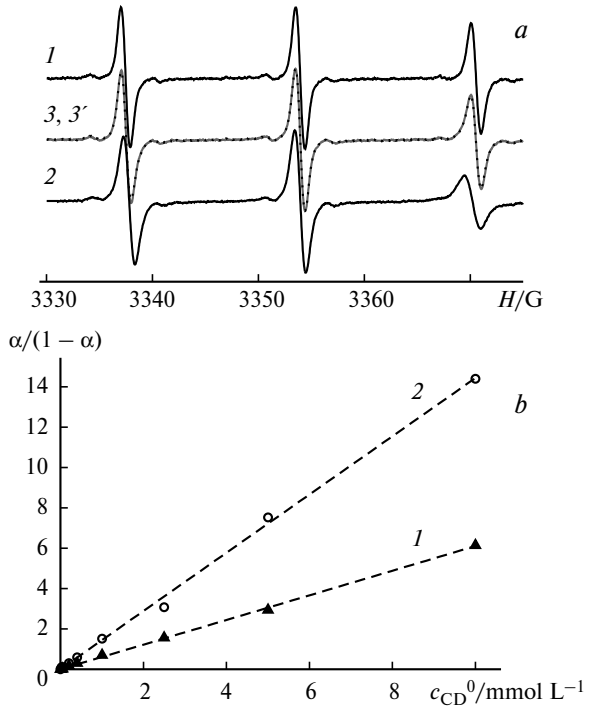

Fig. 4. Binding parameters of spin probe **1** with γ-CD: *a*. The proportion of bound probes (α) is determined by adding the spectra of the free probe in water (*1*) and the probe in the complex in an excess of γ-CD at a molar ratio γ-CD : **1** = 350 (*2*), *3* (solid line) — the experimental spectrum at γ-CD : **1** = 150, *3′* (dashed line) — the calculated spectrum obtained by adding the spectra *1* and *2* with the statistical weights 0.77 and 0.23, respectively; *b*. The binding constants are determined from linear dependence of $\alpha/(1-\alpha)$ on the concentration of γ-CD for **1** (*1*) and **2** (*2*). Dashed line represents the results of simulation of linear dependence by the least squares method.

spin probes. At the 1 : 1 binding stoichiometry, the α values obey the following equation

$$K = \alpha/[(1-\alpha)c_{CD}], \qquad (4)$$

where c_{CD} is the concentration of free CD in the aqueous solution. If the γ-CD concentration (c_{CD}^0) is much higher than the spin probe concentration, one has $c_{CD} \simeq c_{CD}^0$, the ratio $\alpha/(1-\alpha)$ becomes a linear function of c_{CD}^0, and the binding constant K can be determined from the slope of this plot. For both radicals the plots are linear (see Fig. 4, *b*), which confirms that the complex has a 1 : 1 composition.

The binding constants of radicals **1** and **2** at 25 °C, obtained from the slopes of these dependences, are $(6.1\pm0.5)\cdot10^2$ and $(1.4\pm0.1)\cdot10^3$ L mol^{-1}, respectively. Radicals **1** and **2** have the same molecular weights but different structures. The more than twofold difference between the binding constants may result from steric hindrances for molecule **1** in the γ-CD cavity owing to the presence of an additional Me group. It is also possible that structural "adjustment" of molecule **2** in the CD cavity owing to conformational flexibility of the piperidine ring also makes some contribution to the increase in binding for this molecule.

Rotational mobility. Figure 3 shows an increase in the τ_{eff} values to $\simeq 3.5\cdot10^{-10}$ s for both complexes. By simulating EPR spectra one can evaluate the hydrodynamic volume of the complex and determine the orientation of the guest molecule in the cavity. The γ-CD molecule can be treated as an oblate ellipsoid of revolution with the ratio of the average diameter to the height of trapezoid, $\langle d\rangle/L \simeq 2$ ($\langle d\rangle \simeq 15.8$ Å, $L \simeq 8$ Å [3]). Since the γ-CD molecule is much larger than the solvent (water) molecules, rotation of the complex can be described by a Brownian diffusion model. Spectral simulation was carried out using a known program.[22] The components of the HFC tensor were determined using the isotropic HFC constants (*a*) for **1** or **2** in the complexes with γ-CD, linear dependence between *a* and A_{zz} (A_{zz} values were measured at 77 K) obtained for these radicals in solvents of different polarities (see Ref. 24), and assuming $A_{xx} = A_{yy}$. The A_{xx}, A_{yy}, and A_{zz} values thus determined for **1** and **2** are respectively 6.3, 6.3, and 35.45 and 6.5, 6.5, and 35.3 Gs. The *g*-tensor components were taken equal to those measured for structurally similar pyrrolidine and piperidine radicals in the 2-mm UHF band,[25] namely, 2.00805, 2.00602, and 2.00218 for **1** and 2.00856, 2.0059, and 2.00208 for **2**.

Simulation of the spectrum of the **1**—γ-CD complex gave the best fit if the molecular plane of **1** made an angle $\theta \simeq 30$—60° relative to the basal plane of γ-CD molecule; rotation about the symmetry axis of γ-CD is characterized by $R_\parallel = 5.75\cdot10^8$ s^{-1} and rotation about the perpendicular axes is characterized by $R_\perp = 7.9\cdot10^8$ s^{-1}. Using the modified[26] Perrin relations for the ellipsoid of revolution and the diffusion coefficients given above, one can evaluate the effective hydrodynamic volume V_{eff} of a sphere having a volume equal to that of the γ-CD molecule ($V_{eff} \simeq 1100$ Å^3). This value is in reasonable agreement with the estimate of the volume of a single γ-CD molecule (≃1450 Å^3), made based on the structure of γ-CD, being much smaller than the dimer volume. Hence, in accord with the data on the stoichiometry of binding, the **1**—γ-CD complex contains one γ-CD molecule.

Simulation of the spectra of the **2**—γ-CD complex gave similar θ values. However, in this case one has $R_\parallel > R_\perp$ ($R_\parallel \simeq 8.9\cdot10^8$ s^{-1} and $R_\perp = 5.9\cdot10^8$ s^{-1}). A somewhat larger $R_\parallel$ value compared to $R_\perp$ and large absolute values of $R_\parallel$ and $R_\perp$ may be due to the contribution of conformational transitions of the piperidine ring.

The EPR lineshapes of both inclusion complexes show that the γ-CD cavity contains only one spin probe molecule; otherwise, one would observe an additional splitting or broadening due to the strong dipolar interaction between two adjacent spins, modulated by Brownian ro-

Table 1. Hydrophobicity (h) and rotational mobility (τ_{eff}) parameters and constants of spin exchange with paramagnetic ions (k_e) for complexes of compounds **1** and **2** with β-CD and γ-CD in aqueous solutions and reference solvents

Spin probe (Environment)	h	τ_{eff}/ns	k_e/L mol^{-1} s^{-1}		
			$NiCl_2 \cdot 6H_2O$	$NiSO_4 \cdot 6H_2O$	$CuSO_4 \cdot 6H_2O$
1 (H_2O)	0	0.06	$1.2 \cdot 10^9$	$(8.7 \pm 0.2) \cdot 10^8$	$(1.2 \pm 0.12) \cdot 10^9$
2 (H_2O)	0	0.05	$1.3 \cdot 10^9$	—	—
1 (γ-CD)	0.18	0.34	$1.3 \cdot 10^8$	$(0.5 \pm 0.02) \cdot 10^8$	$(1.7 \pm 0.2) \cdot 10^8$
2 (γ-CD)	0.21	0.37	$1.52 \cdot 10^8$	—	—
1 (β-CD)	0.52	0.57	$<1.5 \cdot 10^7$	—	—
2 (β-CD)	0.65	0.54	$4.0 \cdot 10^6$	—	—
1, **2** (EtOH)	0.48	—	—	—	—
1, **2** (EtOH—H_2O, 1 : 1)	0.25	—	—	—	—

tation. This conclusion is also quite consistent with the data on the stoichiometry of the complexes.

Environment polarity and accessibility to ions from aqueous solution. The hydrophobicity parameters h of both spin probes in the complexes with γ-CD and in some reference solvents are listed in Table 1. As can be seen, the environment of both spin probes in the complexes with γ-CD is less hydrophobic than ethanol ($h = 0.48$) but more hydrophobic than water ($h = 0$), being similar in hydrophobicity to 1 : 1 ethanol—water mixture ($h = 0.25$). This suggests that the oxygen atom of NO is involved in a hydrogen bond with water molecules or with a peripheric OH group of γ-CD molecule. As mentioned above, the h parameter characterizes the environment hydrophobicity of the NO group, whereas the fluorescence maximum (λ_m) characterizes the environment hydrophobicity of the chromophore indole group. Comparison of Fig. 1, *b* and the data in Table 1 shows that these parameters in the complexes of **2** with γ-CD are similar but not the same; the chromophore has a more polar environment than the environment of the NO group of the spin probe.

In addition to environment polarity, information on location of the spin probe in the CD cavity can be obtained by studying the accessibility of NO group to spin exchange with paramagnetic aquaions. Spin exchange was studied using broadening of the EPR spectra in the presence of a paramagnetic probe (here, a paramagnetic salt $NiCl_2 \cdot 6H_2O$). Besides, for radical **1** we carried out additional measurements with two salts ($NiSO_4 \cdot 6H_2O$ and $CuSO_4 \cdot 6H_2O$) and with $MgSO_4 \cdot 7H_2O$ for diamagnetic control. The spin exchange rate constants (k_e) of the complexes and free probes in aqueous solutions were obtained from the slopes of the dependences of linewidths on the concentration of paramagnetic ion[27]:

$$k_e = (3/4)^{1/2} \gamma_e d(\Delta H_{pp})/d[P], \quad (5)$$

where ΔH_{pp} is the peak-to-peak width of the $m = 0$ HFC component, [P] is the paramagnetic ion concentration (mol L^{-1}), and γ_e is the magnetogyric ratio for an electron.

For complexes, measurements were carried out at a γ-CD concentration of 5 mmol L^{-1} corresponding to binding of almost all spin probes to γ-CD. The k_e values are listed in Table 1. For both spin probes the absolute values of k_e with Ni^{2+} ions ($NiCl_2 \cdot 6H_2O$) in water in the absence of γ-CD (k_e^w) correspond to the case of strong exchange (collision controlled exchange rate). As to the complexes with γ-CD, the k_e^{CD} values for both probes decrease by about nine times compared to k_e^w.

For radical **2**, the k_e values in water and in the complex are somewhat larger than the corresponding characteristics of radical **1** (see Table 1). Such a correlation between k_e values suggests that the mechanism of spin exchange in the complexes involves escape of the guest molecule from the cavity, interaction with the Ni^{2+} ion in the free state, and re-binding in the complex. Would this mechanism occur, the observed spin exchange rate must be proportional to the concentration of the free probe, *i.e.*, be inversely proportional to the binding constant in accord with Eqn. (4). However, the binding constants of compounds **1** and **2** with γ-CD differ by more than two times and in such a fashion that contradicts the prediction made using this model. Therefore, this exchange mechanism is not active. The decrease in the exchange rate upon incorporation of the probe into the cavity of the γ-CD molecule is probably due to steric shielding, *i.e.*, spin exchange involves the entry of Ni^{2+} ions into the γ-CD cavity. This mechanism is consistent with the fact that the spin exchange rate of compounds **1** and **2** in the complexes are similar to each other, because in this case the exchange is controlled by the probability of the entry of ions into the cavity. Such a probability can be characterized by the k_e^{CD}/k_e^w ratio. This exchange mechanism implies that this ratio depends on the nature of the paramagnetic ion and the anion, which is actually observed in the experiments (see Table 1). Indeed, if the spin ex-

change rate is controlled by the exposure of the NO group probe into the aqueous phase, these ratios must be equal for different paramagnetic salts. Note that information on the incorporation of transition metal complexes in the CD cavity is available.[28]

The spin exchange mechanism considered above also shows that the high environment polarity of the NO group (see Table 1) is due to the formation of hydrogen bonds with water molecules inside the cavity rather than localization of this group on the surface of γ-CD. X-Ray analysis data revealed the presence of three water molecules at three out of eight binding sites inside the γ-CD cavity.[4,5]

Note that a slight decrease in the HFC constant a by 0.25 ($NiSO_4 \cdot 6H_2O$), 0.2 ($NiCl_2 \cdot 6H_2O$), and 0.15 Gs ($CuSO_4 \cdot 6H_2O$) is also observed along with the exchange broadening of spectral lines upon increase in the concentrations of the Ni^{2+} and Cu^{2+} ions. The a parameter reaches a plateau at salt concentrations of 50—100 mmol L^{-1}. This change in the environment polarity apparently can not be attributed to the stronger binding in the presence of ions, because the a values in the presence of paramagnetic ions were smaller than the limiting a values in excess of γ-CD in the absence of ions. Besides, a blank experiment with a diamagnetic salt $MgSO_4 \cdot 7H_2O$ revealed a decrease in a by 0.07 G only.

EPR spectroscopy of complexes with β-cyclodextrin. In contrast to γ-CD, the dependences of τ_{eff} and a on the concentration of β-CD for both guest molecules do not reach a plateau even in nearly saturated β-CD solutions where $c_{CD}^0 = 10^{-2}$ mol L^{-1} (data are not shown). The EPR lineshapes for both spin probes indicate superposition of signals of free radicals and the radicals bound in the complex. For radical **2** this manifests itself in splitting of the HFC component $m = -1$ (Fig. 5), for radical **1** in asymmetry of the $m = \pm 1$ components relative to the base line. To separate these signals, we used two methods: (i) subtraction of the signal of radical **1** in water from the its spectrum in the β-CD solution and (ii) spin exchange with Ni^{2+} ions. In the former case we used spin probes in nearly saturated β-CD solution; the symmetrical shape of the components $m = \pm 1$ in the difference spectrum was used as an indication of complete subtraction. Changes in the lineshape of radical **2** in the complex with β-CD upon increase in the $NiCl_2 \cdot 6H_2O$ concentration are shown in Fig. 5. The signal amplitude of free probe in water decreases and the signal is no longer observed because of strong exchange broadening at $NiCl_2 \cdot 6H_2O$ concentrations ≥ 200 mmol L^{-1}. Based on the k_e^w values, the width of the signal of free probe in water must be ~20 Gs at a $NiCl_2 \cdot 6H_2O$ concentration of 200 mmol L^{-1}, which corresponds to a decrease in the signal amplitude by more than two orders of magnitude. Thus, the EPR spectrum recorded at a $NiCl_2 \cdot 6H_2O$ concentration of 200 mmol L^{-1} (see Fig. 5) corresponds to the complex of radical **2** with β-CD.

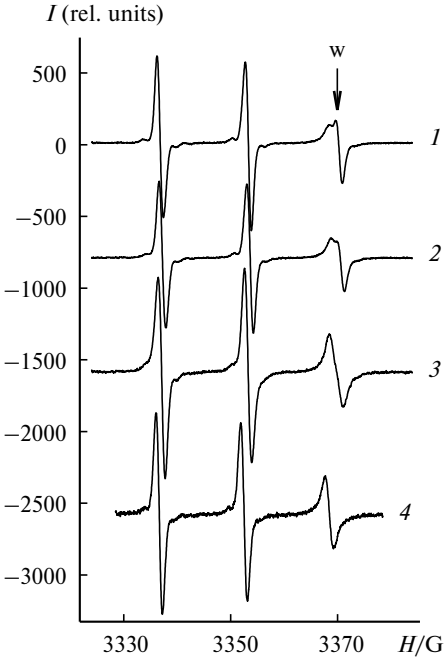

Fig. 5. Effect of the $NiCl_2 \cdot 6H_2O$ concentration on the shape of the EPR spectrum of spin probe **2** in the presence of 5 mM β-CD in aqueous solution: $[Ni^{2+}] = 0$ (*1*), 2 (*2*), 8 (*3*), and 200 mmol L^{-1} (*4*); w — signal of free probe in water.

The EPR lineshapes of the complex, obtained as a result of exchange broadening of the signal of free probe in water or subtraction are similar and exhibit a number of remarkable features. First, the HFC constants correspond to a much more hydrophobic environment of the NO group than in the complexes with γ-CD (see Table 1). Moreover, the environment hydrophobicity somewhat increases in the presence of $NiCl_2 \cdot 6H_2O$ (200 nmol L^{-1}). Second, the $m = 0$ HFC components of the EPR spectra recorded in the presence of 200 and 400 mM of $NiCl_2 \cdot 6H_2O$ have nearly equal widths (1.24 and 1.29 Gs, respectively), which makes it possible to estimate an upper bound of the k_e^{CD} value for the **2**—β-CD complex, namely, a value of $4.0 \cdot 10^6$ L s^{-1} mol^{-1} (see Table 1), which is more than 300 times smaller than the k_e^w value for the free probe and nearly 40 times smaller than the k_e^{CD} value for the **2**—γ-CD complex. Third, the complex **2**—β-CD in the presence of 200 mM $NiCl_2 \cdot 6H_2O$ is characterized by $\tau_{eff} = 0.54$ ns. This is much larger than for the complexes of **1** and **2** with γ-CD ($\tau_{eff} \approx 0.34$ ns, see Table 1), although the γ-CD molecule is much larger than the β-CD molecule: at equal heights the ratio of the average outer diameters of the β-CD and γ-CD molecules is ~0.916, the ratio of the squared diameters and, correspondingly, the hydrodynamic volumes is $V_{eff}^{\beta}/V_{eff}^{\gamma} \simeq 0.84$. Therefore, larger τ_{eff} values may be either due to the formation of 2 : 1 complexes along with the 1 : 1 complexes (two β-CD molecules per probe molecule) or to an incomplete immersion of the probe molecules into the β-CD

cavity. As a result, in both cases, the effective hydrodynamic volume of rotation increases. Hydrophobic environment and a very low rate of spin exchange for radical **2** in the complex with β-CD rule out partial incorporation of radical **2** into the cavity of the β-CD molecule as an explanation for large τ_{eff} vales, *i.e.*, the complex of **2** with β-CD should include two β-CD molecules. Fourth, the intensity ratios of the HFC components, $I_{+1} > I_0 > I_{-1}$, in the EPR spectra of the complex (see Fig. 5, curve *4*) are characteristic of anisotropic rotation, in particular, faster rotation about the axis coinciding with the direction of the N—O bond than the rotation about the perpendicular axes in the molecular frame.

The EPR spectrum of radical **1** recorded in the presence of β-CD exhibits no splitting on the third HFC component; however, changes in the EPR lineshape in the presence of $NiCl_2 \cdot 6H_2O$ are similar to those observed for **2**. At $NiCl_2 \cdot 6H_2O$ concentrations of at least 200 mmol L^{-1}, the EPR spectrum of this radical in water is not observed because of exchange broadening, and the detected EPR spectrum corresponds to the **1**—β-CD complex. The parameter *a* corresponding to this spectrum, similarly to **2**, corresponds to a sufficiently hydrophobic environment, while the τ_{eff} value corresponds to the presence of more than one β-CD molecule in the complex (see Table 1).

Quantum-chemical calculations of complexes. The structures and energy characteristics of the four 1 : 1 complexes of radicals **1** and **2** with β-CD and γ-CD and the complexes of **1** and **2** with β-CD dimer were calculated by the semiempirical method PM3 with a conventional set of parameters[29] using the GAMESS program.[30] The calculations were carried out in order to establish the most favorable structure of the complexes.

Calculations were performed as follows. The input structure files were prepared manually using a HyperChem graphic package by "incorporating" the substrate into the cavity of the CD molecule. As free CD molecules we used the structures of the β-CD and β-CD dimer molecules, which were optimized by this method earlier.[29] It was shown[31] that the energetically most favorable conformation of the β-CD molecule has a C_7 symmetry, being stabilized by inter-residue hydrogen bonds between the 2-OH group of one glucose residue and the 3′-OH group of a neighboring residue. These hydrogen bonds form a clockwise-twisted structure. According to PM3 calculations, this conformation corresponds to a global energy minimum, the solution being (mathematically) stable. In the present work this was proved by re-optimization of the β-CD parameters after removal of the substrate from the β-CD cavity. The converged structure was similar to the initial one.

The structures of γ-CD and free radicals **1** and **2** were optimized in this work. The heats of formation, ΔH_f, of these compounds and the energies of formation, ΔE, of the complexes are listed in Table 2. The ΔE values were calculated as the differences between the ΔH_f values of the optimized complex and the sum of the heats of formation of the fully optimized components. We evaluated different fashions of incorporation of substrates into the CD cavity. The ΔH_f and ΔE values of the most energetically favorable orientations of the guest molecules in the 1 : 1 complexes with γ-CD are listed in Table 2 and the structures of these complexes are shown in Fig. 6.

Table 2. Heats of formation (ΔH_f) of free components and complexes and the complexation energies (ΔE) calculated for the complexes of spin-labeled indoles **1** and **2** with β-CD and γ-CD

Compound*	ΔH_f	$-\Delta E$
	kcal mol^{-1}	
1	24.82	—
2	25.17	—
γ-CD—**1** (1)	−1668.0	14.1
γ-CD—**2** (1)	−1666.56	13.03
γ-CD—**1** (2)	−1668.0	10.5
γ-CD—**2** (2)	−1666.32	12.79
β-CD—**1**	−1454.36	10.78
β-CD—**2**	−1455.05	11. 8
2β-CD—**1**	−2943.51	19.23
2β-CD—**2**	−2940.89	16.96

* The type of configuration of the complex (see text) is given in parentheses.

According to calculations, the highest energies of formation of the complexes of γ-CD with both spin probes correspond to the configurations in which the NO group of the probes are strongly exposed to the aqueous phase (see Table 2, items labeled with "1" in parentheses). However, these configurations are inconsistent with experimental data, in particular, with the ninefold decrease in the k_e values upon the formation of the complexes with γ-CD and with the values of the hydrophobicity parameters *h*, which are significantly different from zero ($h = 0$ for water). The relative complexation energies, ΔE, correspond to a more stable complex with radical **1** rather than **2**, being also inconsistent with the experimental data. According to calculations, somewhat less stable configurations (see Table 2, items labeled with "2" in parentheses) correspond to a more hydrophobic environment of the NO group and to lesser accessibility of this group to aquaions (see Fig. 6, *a*). In this case the ΔE for the **2**—γ-CD complex is larger than for the **1**—γ-CD complex, which is consistent with the experimental binding constants (see above). Finally, the configuration in which the indole group is rather strongly exposed to the aqueous phase is in better agreement with the fluorescence data, according to which the environment polarity of the indole chromophore is similar to that of water (see Fig. 1, *a*, *b*). Probably, it is these configurations of the complexes that

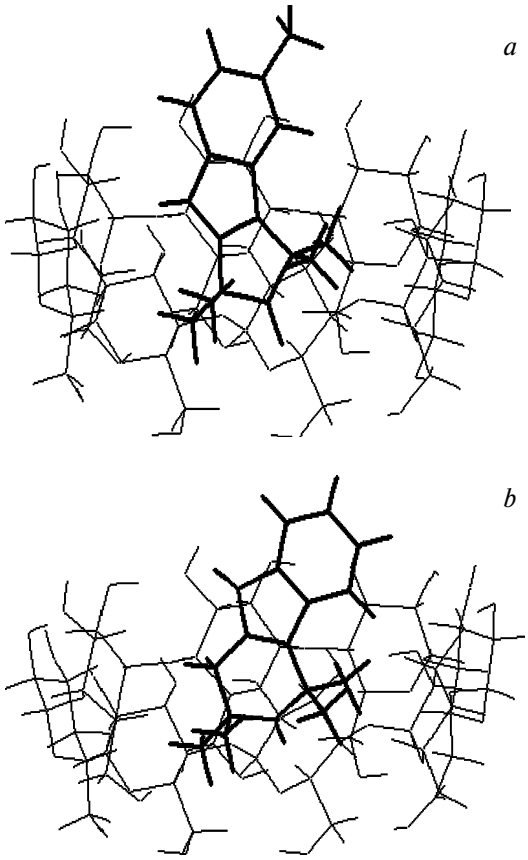

Fig. 6. Structures of complexes of spin-labeled indoles **1** (*a*) and **2** (*b*) with γ-CD obtained from PM3 calculations.

actually exist; additional stabilization of these configurations is provided by the hydrogen bonds between the NO group and water molecules present in the CD cavity, which were ignored in the calculations.

Based on experimental data, it was concluded that at high β-CD concentrations stable complexes of **1** and **2** with β-CD have a 1 : 2 compositions (two β-CD molecules) (see above). Therefore, we calculated the structural and energy characteristics of the complexes for both spin probes with β-CD monomers and dimers. According to our calculations, the complexes 2β-CD—**2** or 2β-CD—**1** are more stable than the 1 : 1 complexes, the complex 2β-CD—**1** being somewhat more stable than complex 2β-CD—**2** (see Table 2). It should be noted that in the complexes with β-CD dimers, in contrast to the 1 : 1 complexes of β-CD and γ-CD, the nitroxyl fragment is placed in the cavity of the second β-CD molecule (Fig. 7), which must lead to higher hydrophobicity of its environment and lesser accessibility to water-soluble reactants. These results are in good agreement with our experimental data, according to which the environment hydrophobicity of the NO group in the complexes of β-CD dimers is much higher than in the complexes with γ-CD

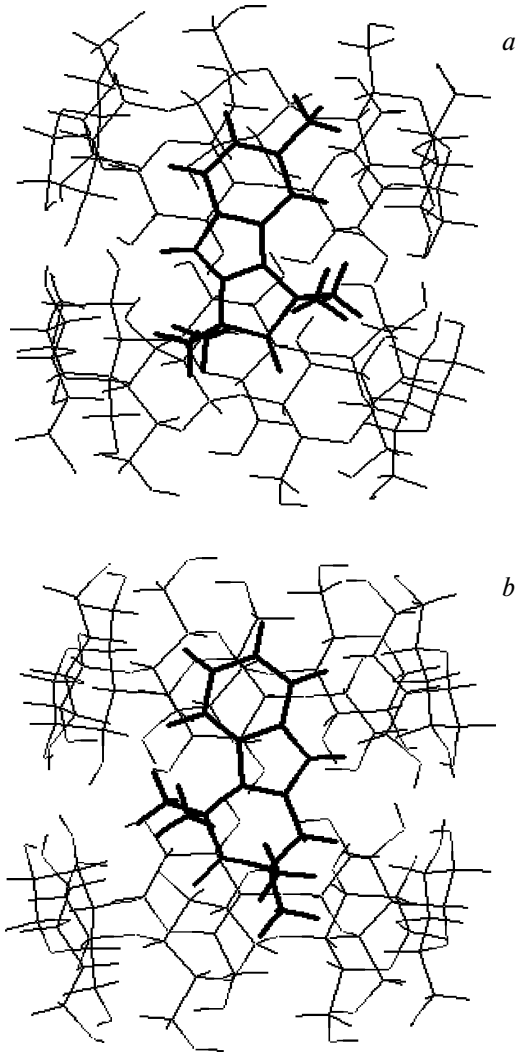

Fig. 7. Structures of complexes of spin-labeled indoles **1** (*a*) and **2** (*b*) with β-CD dimers obtained from PM3 calculations.

monomers and the NO group is much less accessible to Ni^{2+} aquaions than in the monomeric complexes.

* * *

Thus, the results obtained indicate strong structural distinctions between the complexes of both spin-labeled indoles with β-CD and γ-CD. Both spin probes form 1 : 1 complexes with γ-CD in which the long axes of the molecules **1** and **2** are nearly parallel to the γ-CD molecular planes (see Fig. 6). This orientation is consistent with the relative size of the spin probes and cavity; the absence of free volume probably makes free rotation of the guest molecules in the cavity impossible. The NO group is inside the cavity and, as seen from the value of the hydrophobicity parameter *h*, forms a hydrogen bond with one water molecule in the cavity. Spin exchange with Ni^{2+}

ions occurs by diffusion of these ions toward the nitroxyl group.

Compared with the complexes with γ-CD, the complexes with β-CD are characterized by much longer rotational correlation times, much higher hydrophobicity of the environment, much smaller spin exchange constants (compared to the complexes with γ-CD), and anisotropic rotation of molecule **2** (faster rotation about the N—O bond). According to our calculations, both spin-labeled indoles can form the 1 : 1 and 1 : 2 complexes (Fig. 7) with β-CD. Two β-CD molecules form a "head-to-head" dimer and the long axis of the spin probe and the direction of the N—O bond are nearly perpendicular to the molecular planes of β-CD. Note that β-CD is prone to dimerization due to formation of intermolecular hydrogen bonds even in the absence of guest molecules.[4,5]

Thus, in this work we studied the interaction of the spin-labeled indole derivatives **1** and **2** with β-CD and γ-CD in aqueous solutions. The presence of a radical group, NO, reduces the quantum yield of fluorescence of compounds **1** and **2** by 2.5—3 orders of magnitude compared to the quantum yield of indole fluorescence. Analysis of the EPR spectra of these probes provided information on the compositions and binding constants of the complexes, orientation of the guest molecules inside the cavity, polarity of their environment, and accessibility to water-soluble paramagnetic ions. Based on these data, structural models of inclusion complexes were proposed. The structures are consistent with the results of our quantum-chemical calculations.

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