



Dedicated to the memory of
Academician Bogdan C. Simionescu (1948–2024)

AN OVERVIEW ON HOST PROPERTIES OF γ -CYCLODEXTRIN AND HYDROXY-PROPYL- γ -CYCLODEXTRIN FOR NITROXIDES AND β -PHOSPHORILATED NITROXIDES

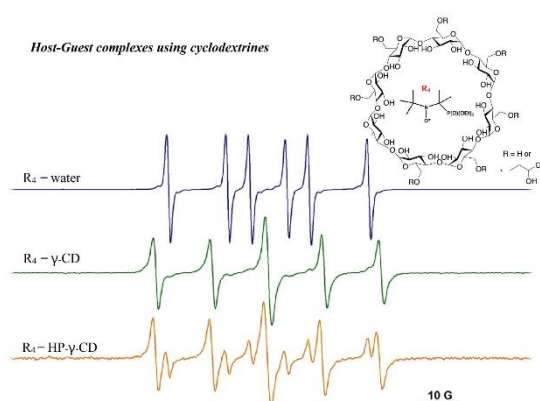
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In this study, electron paramagnetic resonance (EPR) spectroscopy was used to investigate the formation of host-guest complexes of γ -cyclodextrin and 2-hydroxy-propyl- γ -cyclodextrin with nitroxides linked to long aliphatic chains and β -phosphorylated nitroxides. The host-guest complexation of nitroxides is often accompanied by changes in EPR parameters reflecting the change in polarity around the nitroxide group, as well as changes in nitroxide dynamics. The EPR spectra of the solutions of the two series of paramagnetic compounds in the presence of γ -cyclodextrin or 2-hydroxypropyl- γ -cyclodextrin showed significant changes in the hyperfine splitting constants (a_N and a_P) proving the formation of host-guest complexes. Simulation of the experimental EPR spectra allowed to estimate the association constants for each radical/cyclodextrin pair. The values for these constants were lower for the functionalized cyclodextrin.



INTRODUCTION

Electron paramagnetic resonance (EPR) spectroscopy is a technique that can be used to study the physico-chemical properties of species containing one or more unpaired electrons, by analyzing the EPR spectral features (*e.g.* resonance frequencies, splitting, line shapes and line widths) that are sensitive to the electronic distribution,

molecular orientation, molecular motion, and the microenvironment of paramagnetic moieties.¹ The EPR spectroscopy methods have emerged as important tools in studying the supramolecular systems and in particular host-guest complexes of nitroxides, and this has been well documented in some review articles.^{2–6} The specificity and strength of non-covalent interactions between host and guest compounds are determined by the complementary

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matching between host and guest in terms of shape, size, charge distribution and functional groups.⁷

The application of EPR spectroscopy in the investigation of host-guest inclusion complexes is determined by the sensitivity of the hyperfine splitting constant to the polarity of the medium, which decreases as a consequence of paramagnetic moiety inclusion in the hydrophobic host's cavity, and by increases of the rotational correlation time, indicating slower motion as a result of complexation. It is noteworthy that the formation process of nitroxide/cyclodextrin inclusion complexes leads to variations of the hyperfine splitting constant up to 1 G.^{2, 8-10} In the case of large molecules or macromolecules containing nitroxide groups, only partial complexation by the host molecules can occur. The EPR spectra of such complexes can provide precise information on the mode of complexation, in particular whether or not the paramagnetic group is included in the cavity of the host molecule. If a variation in the hyperfine splitting constant, a_N , is observed, it can be concluded that the nitroxide group is entering the cyclodextrin cavity.¹⁰⁻¹²

Starting from the information obtained from literature data on the investigation of inclusion complexes by EPR spectroscopy, in this study the interactions of a series of ten radicals shown in Fig. 1 with 2-hydroxy-propyl- γ -cyclodextrin (HP- γ -CD) or γ -cyclodextrin (γ -CD) were investigated. As can be seen, the series of these radicals consists of six β -phosphorylated nitroxides R1-R6 from which two have a cyclic structure similar with TEMPO radical (R1 and R2) and the others have acyclic structure. Radicals RL1 and RL2 are derivatives of R3, bearing a long alkyl chain. RL3-RL6 are nitroxides, each containing a TEMPO or PROXYL group either at the end or in the middle of the alkyl chain.

The choice of this series of radicals was aimed to highlight the influence that γ -CD functionalization has on the formation of host-guest complexes. The EPR spectra of paramagnetic compounds containing several atoms with non-zero nuclear quantum number exhibit several spectral lines. The EPR spectra of β -phosphorylated nitroxides exhibit several lines, depending on the environmental conditions, the interaction of the unpaired electron with the magnetic moments of the nitrogen and phosphorus nuclei (with values $I_N=1$, $I_P=1/2$) and the dihedral angle formed by the half-occupied molecular orbital with the C-P bond. Dipole-dipole

interactions may also occur between the negatively charged oxygen atom in the nitroxide group and the positively charged phosphorus atom in the phosphoryl group.¹³⁻¹⁶ As such, the EPR spectra of compounds R1-R4, RL1, RL2 shows six lines in aqueous solutions, while the EPR spectra of RL3-RL6 shows only three lines as these radicals possess one N atom.

The interaction of these radicals with cyclodextrins leads to changes in the ratio of the lines or changes in their number. Studies reported in literature evidenced a decrease of a_N values for various nitroxides up to 1 G.¹⁰⁻¹² In a previous study, it was shown that in the case of a β -phosphorylated radical upon complexation with γ -CD, at a sufficiently high cyclodextrin concentration, the spectrum of radical R3 shows five lines instead of the six lines as it presents in water.¹³ The aim of this study is to evidence the influence of γ -CD functionalization on binding properties for this series of radicals, and in the case of nitroxides to evidence the influence of the position of paramagnetic moiety on the aliphatic chain and also its size.

Materials and methods

The β -phosphorylated nitroxides R1-R4, RL1, RL2 and the nitroxides RL3-RL6 were prepared by the group of Prof. S.R.A. Marque at Aix-Marseille University (France). γ -CD and HP- γ -CD were purchased from Sigma Aldrich. Solutions of the cyclodextrins of concentration 10^{-1} M were prepared. In the case of the radicals, stock solutions in ethanol (10^{-2} M) were prepared. After evaporation of a chosen volume of stock solution, spin probes were redissolved in water to ensure a concentration of 2.5×10^{-4} M or in the presence of cyclodextrins (γ -CD and HP- γ -CD 10^{-1} M).

The EPR spectra were recorded using a JEOL FA100 X-band EPR spectrometer EPR JEOL FA100 (Japan) equipped with a TE011 cylindrical resonator. For room temperature measurements the following parameters were used: modulation frequency 100 kHz, microwave power 0.998 mW, modulation amplitude 0.5 G, swept field 150 G, sweep time 4 min, time constant 0,1 s. The EPR spectra were simulated with WinSim software available from NIEHS using the LMB1 optimization algorithm.¹⁷

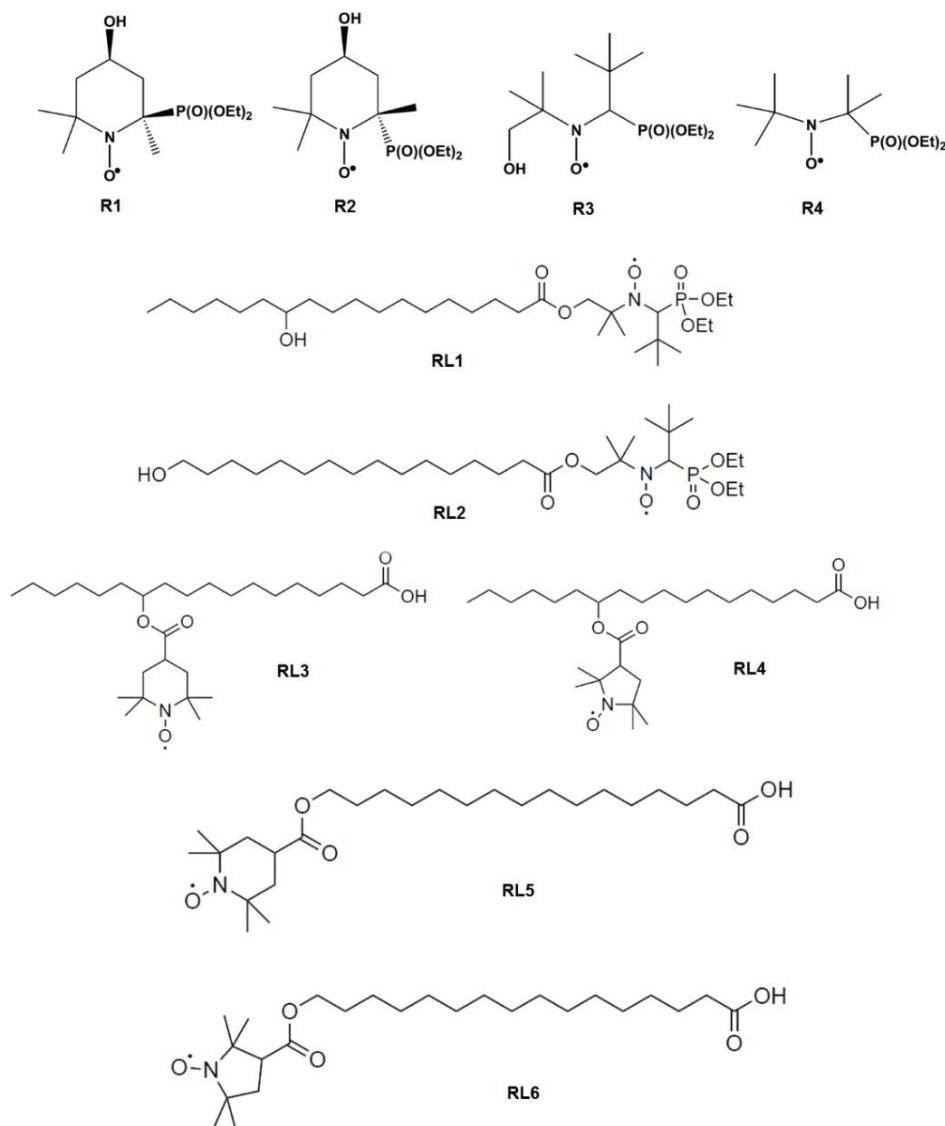


Fig. 1 – The structures of the nitroxides and β -phosphorylated nitroxides used in this study.

RESULTS AND DISCUSSION

β -Phosphorylated nitroxide radicals

The interaction of R1-R4 radicals with γ -CD has been reported previously. On short, in the presence of cyclodextrins, the hyperfine splitting constants a_N and a_P change less for radicals R1-R3. R1 and R2 have cyclic chains, and R3, due to the hydroxyl group, can form hydrogen bonds with water, but also with the external hydroxyl groups of the cyclodextrin. The R4 probe with an increased nonpolar character enters into the cyclodextrin cavity, leading to changes in the dihedral angle θ and in the hyperfine phosphorus splitting parameter a_P from 22.8 G to 30 G in the presence of γ -cyclodextrin. In consequence, in the presence of

γ -CD (10^{-1} M), the EPR spectrum of R4 shows five lines, while in water it shows six lines.¹³

The EPR spectra of probes R1-R4 in hydroxypropyl- γ -CD (10^{-1} M) solution are shown in Fig. 2.

In the case of probe R1 no significant change in a_N or a_P values is observed, indicating that nitroxide and phosphoryl groups are not included in the cavity. The fourth line in the R1 spectrum has lower intensity indicating a slower rotation, proving the formation of a complex.

In the case of the R2 radical, the spectrum is the sum of two components, one corresponding to the uncomplexed probe and the second to the probe included in the cyclodextrin cavity. The spectrum of the R3 probe still shows six lines but there is a change in a_N as the nitroxide group enters the cyclodextrin cavity and a noticeable decrease in the

intensity of the high-field line. The spectrum of the R4 radical is the result of the superposition of two

(six lines) and the γ -CD complexed radical (five lines). Unlike γ -CD, the HP- γ -CD derivative does not have the ability to fully complex the R4 radical.

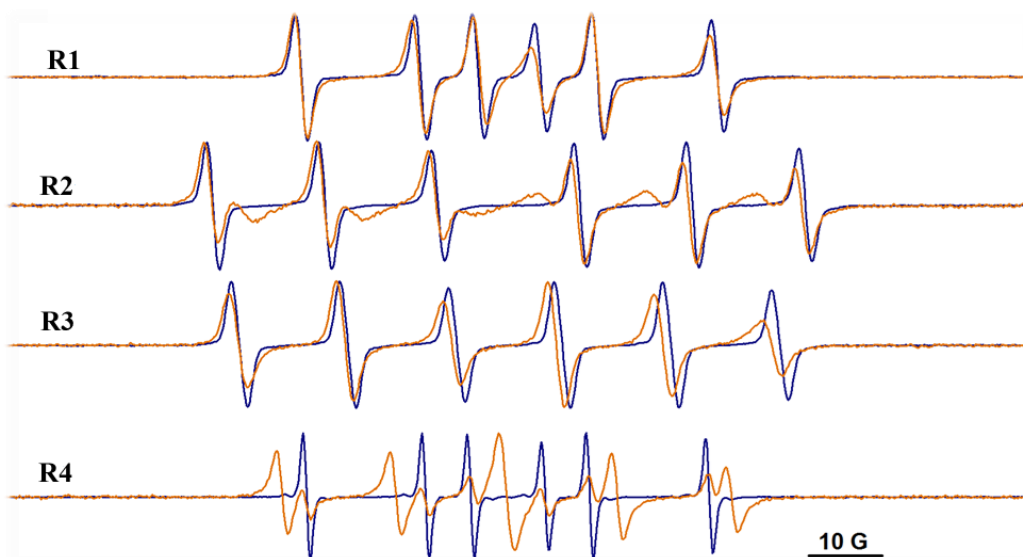


Fig. 2 – EPR spectra of probes R1-R4 in water (blue) and in HP- γ -CD (orange).

The paramagnetic compounds RL1 and RL2 are derivatives of the R3 radical. The hydrophobic chain and the existence of intramolecular dipolar interactions in the paramagnetic fragment determine the insolubility of these two probes in

water. In solution of HP- γ -CD (10^{-1} M), the probes RL1 and RL2 are solubilized due to the complexation process. Small decreases in the hyperfine splitting parameters a_N and a_P are observed (Fig. 3).

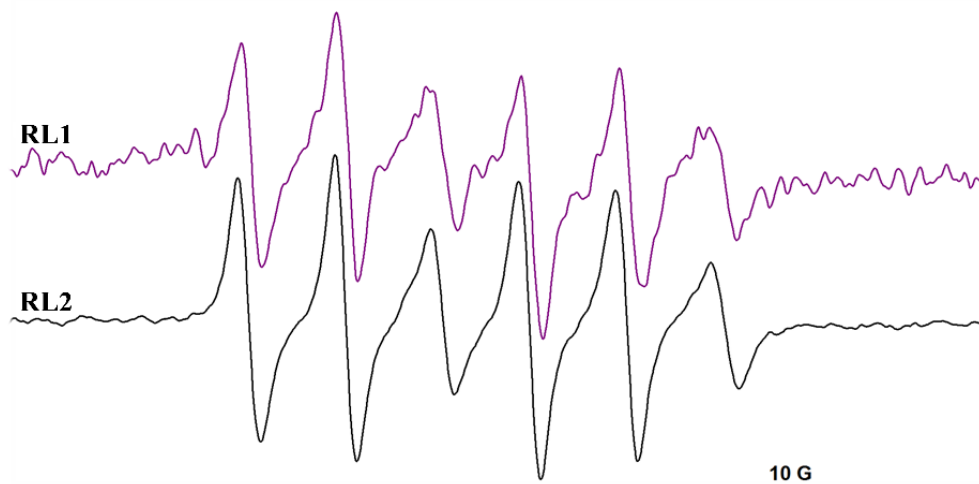


Fig. 3 – EPR spectra of RL1 and RL2 probes in HP- γ -CD.

In this case, the a_N and a_P values of the complexes have been plotted against the values of hyperfine splitting constants characterizing the EPR spectrum of the R3 in water, assuming that the aliphatic chain does not have a significant influence on these values. The fact that the lines of the spectra have different intensities is an indication for the

formation of γ -CD inclusion complexes. However, the RL1 and RL2 radicals are also not completely dissolved in the presence of HP- γ -CD.

Table 1 summarises the hyperfine splitting constants a_N and a_P and the spin probe ratio in water/CD obtained by simulating the experimental spectra using the WinSim program.

Table 1

Values of a_N and a_P corresponding to the complexed and uncomplexed species of R1-R4 and RL1 and RL2 radicals in the presence of HP- γ -CD, and their contributions to each EPR spectrum, obtained by simulations

Sample	Water (free)		% uncomplexed species in HP- γ -CD	HP- γ -CD (complexed)		% complexed species in HP- γ -CD
	a_N [G]	a_P [G]		a_N [G]	a_P [G]	
R ₁	16.20	24.59	44	15.91	24.08	56
R ₂	15.26	49.79	28	14.87	43.49	72
R ₃	14.74	43.77	34	14.13	42.25	66
R ₄	16.15	22.82	21	15.44	30.00	79
RL ₁	–	–	–	14.50	41.92	–
RL ₂	–	–	–	14.49	41.97	–

Knowing the ratios of complexed to uncomplexed species determined by simulation for each R1-R4

radical and assuming a 1:1 stoichiometry, the association constants evaluated with the formula:

$$K = \frac{[\text{complexed species}]}{[\text{free species}] \times [\text{free cyclodextrin}]} = \frac{\% \text{complexed nitroxide} \times [\text{nitroxide}]}{\% \text{free nitroxide} \times [\text{nitroxide}] \times ([\text{cyclodextrin}] - \% \text{complexed nitroxide} \times [\text{nitroxide}])}$$

have the values K (R1) = 12.75 M^{-1} , K (R2) = 25.76 M^{-1} , K (R3) = 19.44 M^{-1} , K (R4) = 37.69 M^{-1} .

The EPR spectra of R1-R4 radicals in the presence of the two cyclodextrins highlight the effect of functionalization of the host molecules on complexation. R1-R4 interactions with γ -CD were analyzed in the previous study.¹³ The EPR spectra of R1-R3 do not change significantly in the presence of γ -CD or HP- γ -CD. Table 2 shows the proportions of the corresponding uncomplexed and complexed probe components for each case. As a general trend,

a slight increase in the proportion of the corresponding complex is observed in γ -CD and less in HP- γ -CD. In contrast, in the case of the R4 probe in γ -CD only one component is observed (Fig. 4), which means that it is fully incorporated. In solution of the same concentration of HP- γ -CD, the EPR spectrum shows two components. This leads to the conclusion that the hydroxypropyl groups decrease the complexing ability of functionalized cyclodextrin.

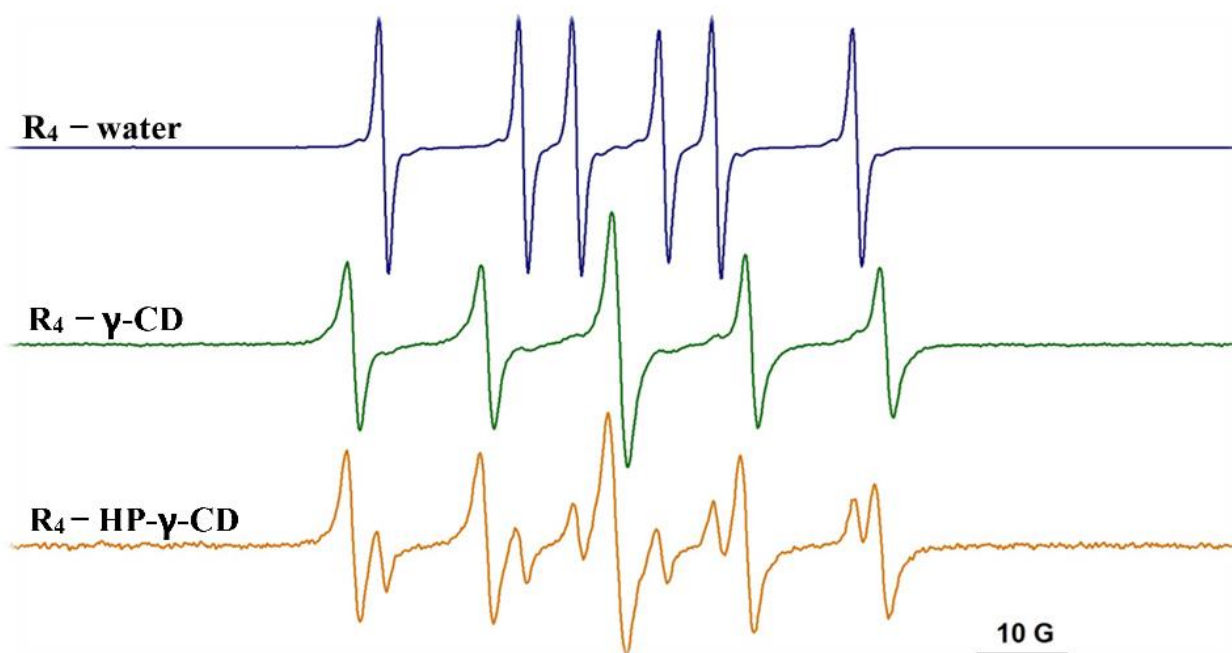


Fig. 4 – EPR spectra of probe R4 in water (blue), in γ -CD (green) and in HP- γ -CD (orange).

Table 2 presents the hyperfine splitting parameters of nitrogen, a_N , phosphorus, a_P , and the spin probe ratio in water/CD for the HP- γ -CD and γ -CD complexes. It can be observed that the

proportion of complex species increases in γ -CD compared with HP- γ -CD, leading to the conclusion that binding properties of the functionalized cyclodextrin are limited compared with γ -CD.

Table 2

Hyperfine splitting constants a_N and a_P of R1-R4 probes in solutions of HP- γ -CD and γ -CD and their contributions to each EPR spectrum, obtained by simulations

Sample	HP- γ -CD			γ -CD		
	a_N [G]	a_P [G]	% complexed species	a_N [G]	a_P [G]	% complexed species
R1	15.91	24.08	56	15.81	24.74	61
R2	14.87	43.49	72	14.91	42.13	86
R3	14.13	42.25	66	13.78	42.36	73
R4	15.44	30.00	79	15.59	30.44	100

Nitroxide radicals

The EPR spectra of the RL3-RL6 nitroxides show three lines due to the hyperfine coupling of the free

electron and the nitrogen atom (Fig. 5). In contrast to the β -phosphorylated nitroxides RL1 and RL2, the nitroxides RL3-RL6 are soluble in water because in their structures is present the carboxylic fragment.

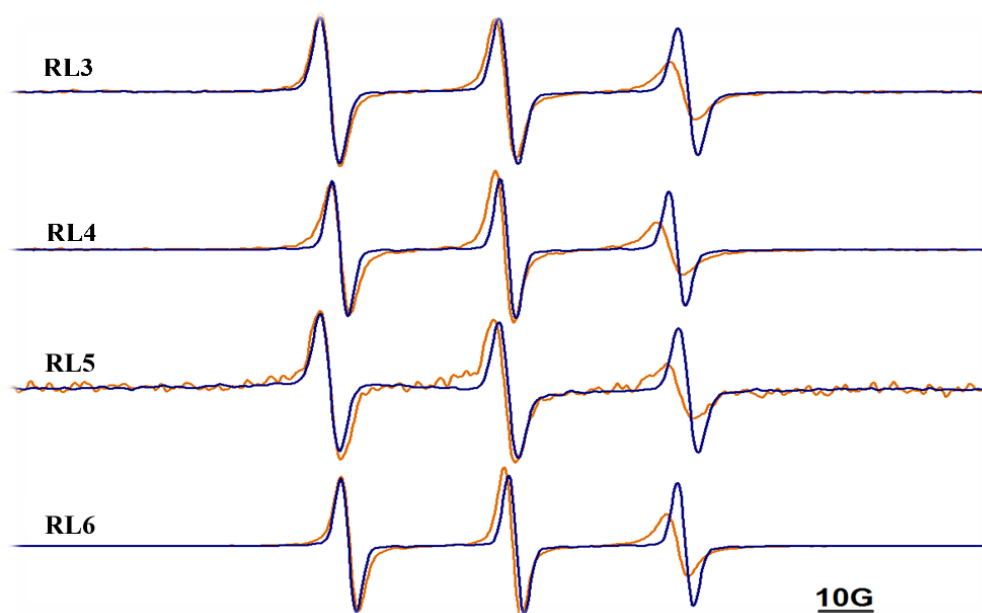


Fig. 5 – EPR spectra of RL3-RL6 probes in water (blue) and in HP- γ -CD (orange).

The parameter that indicates the dynamics of the radical, the rotational correlation time, is determined for isotropic motion using eq. (1), where ΔH_0 is the peak-to-peak width of the center line (in Gauss) and h_{-1} , h_0 and h_{+1} are the height of the bands for the low, center and high field respectively.¹⁸

$$\tau_c = 6.51 \times 10^{-10} \Delta H_0 \left[\left(\frac{h_0}{h_{-1}} \right)^{1/2} + \left(\frac{h_0}{h_{+1}} \right)^{1/2} - 2 \right] \quad (1)$$

It can be seen that the spin probes RL5 and RL6 having the paramagnetic group located at the end of the aliphatic chain have a faster motion than RL3 and RL4 with the paramagnetic group located in the interior of the chain (the values of τ are larger for the compounds having the paramagnetic groups in marginal positions). An even more drastic change occurs following complexation, when τ increases by an order of magnitude due to the lower mobility of the spin probe inside the cyclodextrin.

Table 3

Rotational correlation time values for RL3-RL6 spin probes in water and in HP- γ -CD solution (10^{-1} M)

Sample	$\tau \times 10^{10}$ s	
	Water	HP- γ -CD
RL3	0.9	12.1
RL4	1.2	12.2
RL5	0.6	11.6
RL6	0.7	11.5

In contrast, the value of hyperfine splitting constant of nitrogen is not influenced by the position of the paramagnetic group in the probe chain. The

a_N values and the proportions of the RL3-RL6 radicals in water and in the complexes are shown in Table 4.

Table 4

Values of hyperfine splitting constants for RL3-RL6 probes in water and in HP- γ -CD solution (10^{-1} M).

Sample	Water		HP- γ -CD	
	a_N [G]	%	a_N [G]	%
RL3	17.07	21	16.44	79
RL4	16.02	13	15.44	87
RL5	17.07	7	16.42	93
RL6	16.07	23	15.31	77

From the information reported above it follows that the exposure of the paramagnetic moieties to the aqueous medium is the same irrespective of the position in the alkyl chain. Moreover, at the radical concentration of 10^{-4} M used in the experiments, there is no self-association process or different conformation of the alkyl chain that would bring a polarity change of the paramagnetic group's environment (*i.e.*, the probes do not form micelles, and the aliphatic chain does not surround the paramagnetic group).

CONCLUSIONS

Analysis of EPR spectra for four β -phosphorylated nitroxides and a series of six radicals with paramagnetic moieties linked to an alkyl chain, in water or in cyclodextrin solutions, revealed the different ability to form complexes of functionalized and non-functionalized cyclodextrins.

The simulation of the EPR spectra corresponding to the HP- γ -CD solutions allowed the evaluation of the association constants for the inclusion complexes formed by β -phosphorylated nitroxides. These constants have low values, indicating that cyclodextrin functionalization does not lead to the formation of complexes characterized by high stability.

Since a decrease in a_N values is observed for the RL3-RL6 radicals in the presence of HP- γ -CD,

complex formation involves the inclusion of the nitroxide group into the cyclodextrin cavity, the radicals being sensitive to polarity changes.

The results obtained by analyzing the EPR spectra may find applicability in the search for host-guest molecular systems with applications in magnetic resonance imaging. Nitroxide-type paramagnetic groups are included in the cyclodextrin cavity, potentially providing protection against bioreducing agents.

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REFERENCES

1. M. M. Roessler and E. Salvadori, *Chem Soc. Rev.*, **2018**, 47, 2534–2553.
2. M. Lucarini and E. Mezzina, *Electron Paramag. Reson.*, **2011**, 22, 41–70.
3. P. Franchi, M. Lucarini and G. F. Pedulli, *Curr. Org. Chem.*, **2004**, 8, 1831–1849.
4. E. G. Bagryanskaya and S. R. A. Marque, *Electron Paramag. Reson.*, **2017**, 25, 180–235.
5. M. Lucarini, *Eur. J. Org. Chem.*, **2020**, 20, 2995–3008.
6. I. Matei, E. I. Popescu, S. Mocanu, E. N. Hristea, F. Savonea, R. Baratoiu and G. Ionita, *Rev. Roum. Chim.*, **2021**, 66, 9–23.

7. M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, **1998**, *98*, 1875–1917.
8. G. Ionita, A. Caragheorgheopol, H. Caldararu, L. Jones and V. Chechik, *Org. Biomol. Chem.*, **2009**, *7*, 598–602.
9. G. Ionita and V. Chechik, *Phys. Chem. Chem. Phys.*, **2010**, *12*, 6956–6960.
10. G. Ionita, S. Mocanu and I. Matei, *Phys. Chem. Chem. Phys.*, **2020**, *22*, 12154–12165
11. G. Ionita, A. Caragheorgheopol, H. Caldararu, L. Jones and V. Chechik, *Org. Biomol. Chem.*, **2009**, *7*, 598–602.
12. G. Ionita and V. Chechik, *Phys. Chem. Chem. Phys.*, **2010**, *12*, 6956–6960.
13. A. V. F. Neculae, G. Audran, S. Bourdillon, G. Ionita, J. P. Joly, S. R. A. Marque, I. Matei and S. Mocanu, *J. Mol. Liq.*, **2022**, *364*, 119983.
14. G. Audran, L. Bosco, P. Bremond, T. Butscher, J. M. Franconi, K. Kabitaev, S. R. A. Marque, P. Mellet, E. Parzy, M. Santelli, E. Thiaudiere and S. Viel, *RSC Adv.*, **2016**, *6*, 5653–5670.
15. G. Audran, L. Bosco, P. Bremond, T. Butscher and S. R. A. Marque, *Org. Biomol. Chem.*, **2016**, *14*, 1288–1292.
16. G. Audran, L. Bosco, P. Nkolo, R. Bikanga, P. Bremond, T. Butscher and S. R. A. Marque, *Org. Biomol. Chem.*, **2016**, *14*, 3729–3743.
17. D. R. Duling, **1996**. *PEST Winsim, version 0.96*, National Institute of Environmental Health Sciences, Triangle Park, NC.
18. T. J. Stones, T. Buckman, P. L. Nordio and H. M. McConnell, *Proc. Natl. Acad. Sci. U.S.A.*, **1965**, *54*, 1010.