EPR STUDY OF THE DYNAMICS OF SOME SPIN LABELS INCLUSION IN CYCLODEXTRINS

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Abstract. In this paper we present the results obtained by using the EPR spin label technique for testing the inclusion of monoradical nitroxide compounds in β-cyclodextrin, in liquid phase. Different concentrations of TEMPO (2, 2, 6, 6-tetramethyl-piperidine-1-oxyl), and TEMPONE (4-Oxo-2,2,6,6-tetramethyl piperidine-1-oxyl) solutions in distilled water were mixed with a water solution of β-cyclodextrin in various ratios and the EPR spectra of the mixtures were recorded on an X-band EPR spectrometer. The mixture spectra were compared to the pure spin label spectra in water. The double integration of the spectra did not revealed significant difference between the two types of samples. However, the analysis of the spectra in terms of the rotational correlation time revealed a strong difference between the samples. For TEMPO solutions, the presence of cyclodextrin leads to a fivefold increase in the rotational correlation time, in the case of TEMPONE the rotational correlation time being around three times higher in the presence of β-cyclodextrin. These results were interpreted as an indication of the complex formation and they correlate with precipitate formation when mixing high concentrations of spin labels with cyclodextrin solutions. In the presence of the ascorbic acid the intensity of the EPR signal is strongly reduced. The remaining signal is anisotropic and can be attributed to the fraction of the spin label molecules having the nitroxide group inside the cyclodextrin cavity. These results strongly support that EPR signals of monoradical nitroxides can also bring valuable informations about host-guest interactions.

Key words: EPR, spin labels, cyclodextrin.

INTRODUCTION

Cyclodextrins (CD) and their inclusion complexes with various substances have been extensively studied in the last decade due to their potential use in pharmacy, biochemistry, analytical chemistry, food industry and cosmetology. For studying the interaction between the CD and inclusion molecules different physical techniques, including NMR, X-ray diffraction, IR spectroscopy, circular dichroism

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and differential scanning calorimetry were applied, in order to characterize the complex formation and its stability in diverse media [3].

Spin labels and especially nitroxides exhibit an EPR spectrum which is very sensitive to slight changes in the environment and therefore have been used to characterize the interactions of labeled molecules. Earlier attempts made to use nitroxides in the study of CD complex formation did not reveal significant changes in the EPR spectra of nitroxides in the presence of CD and that only the change in the kinetics of the nitroxides reduction by ascorbic acid can be used for characterizing the host-guest interactions [1]. More recently it was demonstrated that biradicals are particularly suitable probes for studying inclusion phenomena, because the EPR signals of free and included species are well separated [4].

The aim of this study was to check the possibility of using the EPR technique of common, commercially available, monoradical nitroxides in order to characterize inclusion of spin labeled molecules in CD.

MATERIALS AND METHODS

Different concentration of TEMPO (2,2,6,6-tetramethyl-1-piperidinyl-N-oxide and TEMPONE 2,2,6,6-tetramethyl-4-piperidine-oxyl (Aldrich) solutions in double distilled water were mixed with a water solution of β-cyclodextrin (Frères Roquette) in various ratios. When mixing solutions of 10 mM β-CD with the same concentration of the nitroxide solutions, precipitate formation can be observed. This is an indication of the complex formation between the two types of molecules. Because the aim of this work was to check the possibility of using the EPR technique for studying complex formation in liquid phase, we reduced the concentration of both, nitroxide and CD, until we obtained clear, mixtures.

The clear mixtures were aspirated in a capillary tube and introduced in the cavity of an X-band EPR spectrometer (ART-6-IFIN Bucharest) which was coupled to a PC for data acquisition. In order to compare spectra for different samples, a set of measurement was performed on the same capillary tube, placed in the spectrometer cavity in the same position.

RESULTS AND DISCUSSIONS

The EPR spectra of the nitroxide-CD mixture were compared to the pure spin label spectra in water. The mixture spectra seemed to be similar to those of the free spin labels in water (Fig. 1). The double integration of the spectra did not revealed significant differences between the two types of samples. Plotting de double integral of the absorption spectra for both, free spin label and CD-spin label

solutions, we did not obtained significant differences between the two sets of data (Figs. 2 and 3).

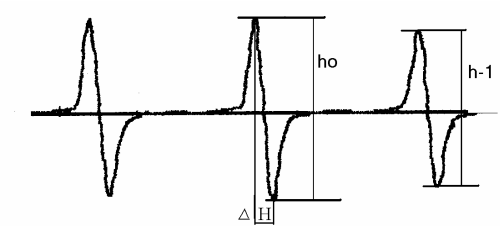

Fig. 1. EPR first derivative absorption spectrum of a mixture of TEMPO (0.3 mM) and β-CD (4 mM). The spectrum parameters used to calculate the rotational correlation times are also depicted.

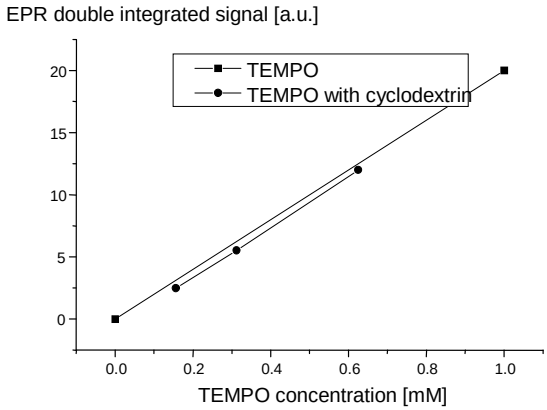

Fig. 2. Double integrated EPR signal values of TEMPO and TEMPO-CD mixtures.

The same behavior was observed for both spin labels TEMPO and TEMPONE. However, the analysis of the spectra in terms of the rotational correlation time revealed a strong difference between the samples.

For calculating the rotational correlation times for the free spin labels and for their mixtures with CD we used the well known formula [2]:

$$\tau_R = 6.5 \cdot 10^{-10} \Delta H \left(\sqrt{\frac{h_0}{h_{-1}}} - 1 \right) \quad (1)$$

where the meaning of each term is given in Figure 1.

This type of analysis can be applied for isotropic spectra, as they are in our case. In the absence of spin-spin interactions, an increase in τ_R indicates a decrease in the molecule mobility. In our case the spin labels bound to CD should increase the values of τ_R (Tables 1 and 2). For TEMPO solutions, the presence of CD leads to a fivefold increase in the rotational correlation time, in the case of TEMPONE the rotational correlation time being around 3 times higher in the presence of β-CD.

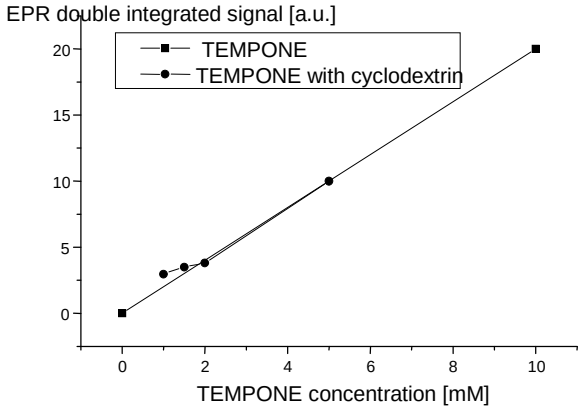

Fig. 3. Double integrated EPR signal values of TEMPONE and TEMPONE-CD mixtures.

Table 1.

Rotational correlation times for TEMPO and TEMPO-cyclodextrin mixtures.

h_0(a. u.)	h_{-1}(a. u.)	[TEMPO] (mM)	Correlation times (s)	Sample
0.4	0.3	0.16	$2.8 \cdot 10^{-10}$	5mM CD
0.7	0.5	0.31	$2.1 \cdot 10^{-10}$	5mM CD
1.2	1.0	0.63	$2.0 \cdot 10^{-10}$	5mM CD
1.1	1.0	0.40	$4.0 \cdot 10^{-11}$	free
12.0	11.3	10	$4.7 \cdot 10^{-11}$	free

Table 2.

Rotational correlation times for TEMPONE and TEMPONE-cyclodextrin mixtures.

h_0(a.u.)	h_{-1}(a.u.)	[TEMPONE] (mM)	Correlation times (s)	Sample
5.6	4.5	1	$1.7 \cdot 10^{-10}$	5mM CD
8.1	6.5	1.5	$1.7 \cdot 10^{-10}$	5mM CD
8.8	7.0	2	$1.7 \cdot 10^{-10}$	5mM CD
4.4	3.7	2.5	$1.3 \cdot 10^{-10}$	5mM CD
13.6	12.6	10	$5.5 \cdot 10^{-11}$	free

To obtain information about the site of the spin label binding to CD, we followed the kinetics of the EPR spectra of the spin label CD solutions in the presence of ascorbic acid. It is well known that ascorbic acid reduces drastically the EPR signal of the spin labels.

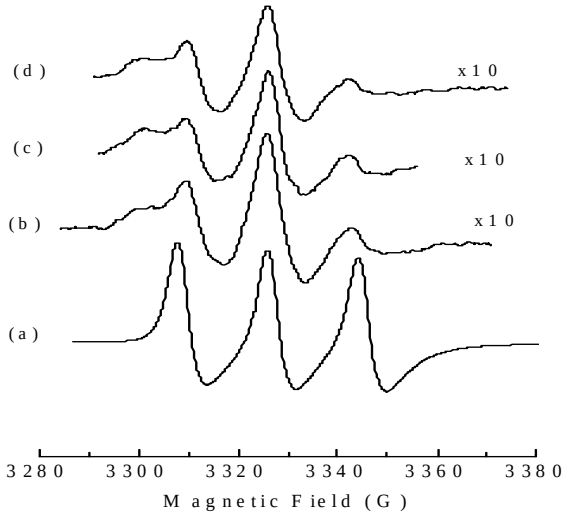

Fig. 4. The EPR spectra of TEMPONE CD a and TEMPONE-CD plus ascorbic acid b, c and d. The gain for the curves b, c and d is 10 times higher as compared to the gain corresponding to curve a.

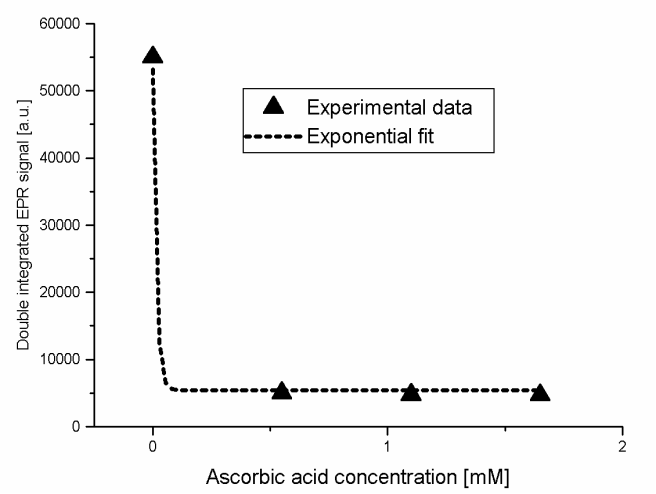

Fig. 5. EPR spectra reduction of TEMPONE-CD mixtures in the presence of ascorbic acid.

In the presence of the ascorbic acid the intensity of the EPR signal is strongly reduced. The remaining signal is strongly anisotropic and can be attributed to the fraction of the spin label molecules having the nitroxide group inside the CD cavity. The reduction in the signal intensity is time dependent. These results were interpreted as an indication of the association between the spin labels and CD molecules. On the other hand, from the data presented in the two tables, one can

see a slight increase in the rotational correlation time as the concentration of spin label increases (the concentration of CD was kept constant). We supposed that only a small fraction of the spin label were bound to the CD molecules. Therefore, the rotational correlation times obtained represent in fact an average for both bound and unbound molecules. As the spin label concentration increases the number of unbound molecules increases and the values of the rotational correlation times are closer to those of free labels.

One can observe also the differences between the two spin labels used in the study. We attributed these differences to the differences in the hydrophobicity of the two molecules, the TEMPONE molecule being more hydrophobic. The same differences were observed for the solubility of the two nitroxides in the presence of CD. From the data presented in the two tables and in Figs. 2 and 3 one can see that the limit concentration at which TEMPO can be mixed with CD to form clear solutions is 8 times lower as compared to the TEMPONE case, for both nitroxides the final CD concentration used being 5 mM.

CONCLUSIONS

Our results strongly support that the tested monoradical nitroxide compounds form inclusion complexes with CD. The rotational correlation times of the nitroxides in the presence of CD are strongly influenced by the association of the two molecules. We believe that developing o theoretical model for calculating rotational correlation times for a mixture of bound and unbound nitroxides can lead to quantitatively calculate the fraction of nitroxide bound to CD. The use of reducing agents, like ascorbic acid, can also improve the significance of the results, as it can indicate the fraction of the bound molecules having the paramagnetic group inside the cavity of CD.

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