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Modification of the hydrogen bonding network at the reversed micelles interface by near infrared radiation

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Abstract. The purpose of this paper is to prove that near infrared radiation (NIR) modifies hydrogen bonds localized in the interface of reversed micelles. The degree of modification of the hydrogen bonds was monitored by TEMPO-palmitate spin probe introduced into the structure of reverse micelles formed by cetyltrimethylammonium bromide (CTAB) (TCAB/phosphate buffer/isooctane/hexanol and TCAB/NaCl/isooctane/hexanol (W = 15)). Electron paramagnetic resonance (EPR) spectra were performed on the argonated samples. The isotropic tumbling correlation time (τ_c) and the hyperfine coupling constant $A_+ = h_0 - h_{+1}$ (h_{+1}, and h_0 correspond to the low-, and centre-field lines, respectively) were determined from the EPR spectra as a quantitative measure for monitoring the action of NIR radiation. A_+ values depend on the composition of the water pool (1.640 mT for phosphate buffer and 1.630 mT for NaCl). NIR irradiation led to decrease in A_+. This parameter reached the same value for both solutions (1.625 ± 0.003 and 1.626 ± 0.003 mT) after exposition to NIR. The tumbling correlation time after exposure to NIR decreased for TCAB/phosphate buffer/isooctane/hexanol reversed micelles from 2.10×10^{-10} s to 1.44×10^{-10} s but did not change for TCAB/NaCl/isooctane/hexanol). The results obtained confirm the possibility of modification of the hydrogen bonds by NIR radiation.

Key words: electron paramagnetic resonance (EPR) • hydrogen bonds • near infrared radiation (NIR) • cetyltrimethylammonium bromide (TCAB) reverse micelles • TEMPO-palmitate spin probe • water structure modification

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Introduction

Near infrared radiation (NIR) is mainly absorbed by water; 749, 880, 980, 1211, 1450, 1787 nm and absorption bands are attributed to the overtones of stretching or combination vibrations [4, 13, 28]. What is known from the literature [17, 18] pulsed single-photon excitation of the overtones of stretching vibrations of pure liquid water produces H_3O^+ and OH^- ions. The study of quantum yield as a function of excitation wave number clearly showed its correlation with the water absorption spectrum.

In the present study, in order to elucidate the role of NIR radiation in modification of hydrogen bonds, we studied EPR spectra of the TEMPO-palmitate spin probe localized in the TCAB (cationic surfactant cetyltrimethylammonium bromide) reversed micelles. The long hydrocarbon chain of TEMPO-palmitate molecule is located in the hydrophobic area, predominantly perpendicular to the micellar interface [7, 12, 16]. The polar, slightly negative TEMPO molecule easily diffuse to the positively charged TCAB interface. The spin probe can effectively describe their surrounding on a molecular level as expressed by the average rotational correlation time (τ_c) and the isotropic nitrogen hyper-

fine coupling constant (A_N). The spin coupling constant (A_N) are affected by interactions between the radical and the surrounding solvent including dipolar aprotic interactions, hydrogen bonding, and complex formation [26]. The hydrogen bonds formed by NO groups are considered as main contributors on the nitrogen hyperfine coupling constant (A_N) [22]. Study of the A_N of TEMPO-palmitate spin probe localized in the TCAB reversed micelles before and after irradiation allowed us to observe modification of hydrogen bonds induced by NIR. Since the lifetime of the formed hydrogen bonds is determined by rotational correlation times of the solvent and spin probe molecules [29], the observed effects should depend strongly on the composition of water pool. Therefore, we investigated the effects of NIR radiation on the hydrogen bonds formed in the water pools with different content: phosphate buffer or NaCl water solution.

Experimental

Materials

The surfactant, cetyltrimethylammonium bromide (CTAB) was purchased from Aldrich and was used without further purification. Predetermined amounts of the aqueous buffer at the desired pH or aqueous solution of NaCl (0.077×10^{-2} mol·dm^{-3} phosphate buffer pH = 7.3 or 0.154×10^{-2} mol·dm^{-3} NaCl equilibrated by NaOH to pH = 7.3) were mixed with TCAB solution in isooctane and 1-hexanol (5:1 v/v) to form the reversed micelles with required $W = 15$ (molar ratio of water to surfactant). Then, the mixture was shaken until it became optically transparent. TEMPO-palmitate (TP) spin label was synthesized at the University of Łódź (Poland), the chemicals buffer employed were of reagent grade purity, deionized and redistilled water was used.

Spin labelling

The ethanolic solution (10^{-3} mol·dm^{-3}) of the spin label TEMPO-palmitate was deposited on the glass wall of the round-bottom flask and was dried in vacuum. The CTAB reversed micelles were added to the spin label dry film. Then, the spin label was equilibrated with TCAB micelles by vortex mixing (1–2 min) and incubating the dispersion for about 30 min in argon atmosphere. The molecular ratio of spin label to TCAB surfactant was always 1:100.

Irradiation and EPR spectra

The nitrogen hyperfine coupling constant A_N, in magnetic field units, is equal to half of the difference in the resonance field of the high- and low-field signals. However, the spacing between hyperfine lines $A_+ = h_0 - h_{+1}$ and $A_- = h_{-1} - h_0$ where h_{+1}, h_0 and h_{-1} correspond to the low-, centre- and high-field lines respectively, differentiate as the rotational correlation times increase due to increasing viscosity of the reversed micelles structure. For purposes of measuring polarity of the reversed micelle surface, A_+ is preferred. See Refs. [1, 2] for discussion of this point. Prior to EPR measurements, all samples were kept in an argon atmosphere for 30 min sealed into capillaries and housed within a quartz EPR tube for the measurement. Conventional EPR spectra of the spin probe in TCAB/phosphate buffer/isooctane/hexanol and TCAB/NaCl/isooctane/hexanol reversed micelles were recorded at 30 ± 1°C on a X-band, SE/X-28 spectrometer made at Technical University of Wrocław. The MX-20/R microwave unit with a frequency counter was applied. Spectra were recorded at 0.1 mT modulation amplitude, time constant 0.03 s, sweep width 5 mT, and sweep time 64 s. A mean value of the sweep width was calculated from 10 measurements.

Irradiation was accomplished *in situ* through a slotted grid in the front of cavity. The light source was a halogen lamp equipped with a 700–2000 nm filter (power density of incident light was 6.9 mW/cm^2).

For evaluation of the free radical mobility, a correlation time τ_c was calculated. The rotational correlation time for the motion in the range $10^{-10} \le \tau \le 3 \times 10^{-9}$ s (motion in the rapid tumbling limit) is defined as a function of the line width of the centre line Δh_0 and amplitude of h_{-1} [15]:

$$\tau_c = k\Delta h_0 \left(\sqrt{\frac{h_0}{h_{-1}} - 1} \right)$$

where h_0, h_{-1} and Δh_0 are parameters taken from the EPR spectrum; h_0 and h_{-1} are mid-field and high-field line amplitudes, respectively; Δh_0 is the line width in gauss of the mid-field signal of TEMPO-palmitate free radical. The constant $k = 6.5 \times 10^{-10}$ s·rad^{-1}·G^{-1}.

Results

Three-line EPR spectra of TEMPO-palmitate spin probe in TCAB reversed micelles typical of nitroxide free radicals undergoing approximately isotropic motion were observed under all conditions in our experiments.

Table 1 gives the data for TEMPO-palmitate spin probe localized in TCAB/phosphate buffer/isooctane/hexanol and TCAB/NaCl/isooctane/hexanol reversed micelles. The central linewidths Δh_0, Δh_{-1}, and Δh_{+1} of all the spectra in this study was found to be stable within empirical error and independent of water pool composition and irradiation.

In Table 1 we also reported values of the hyperfine coupling constant A_+ for TEMPO-palmitate spin probe incorporated into reversed micelles before exposition to NIR and the minimal values of the mentioned parameters after irradiation. Values A_+ before exposition to NIR for TEMPO-palmitate introduced to TCAB/NaCl/isooctane/hexanol suspension were smaller relative to those in TCAB/phosphate buffer/isooctane/hexanol reversed micelles. The decrease in A_+ after irradiation was observed in all our experiments for water pools containing phosphate buffer. The final values of A_+ were equalized for both solutions (1.625 ± 0.003 and 1.626 ± 0.003 mT).

The correlation time calculated from the EPR spectra for TCAB/phosphate buffer/isooctane/hexanol suspen-

Table 1. Values of tumbling correlation time calculated according to formula 1, hyperfine splitting constant A_+ and the EPR line widths for TEMPO-palmitate spin label incorporated into TCAB reversed micelles for control and irradiated samples. Water pools contained 0.077×10^{-2} mol·dm^{-3} phosphate buffer pH = 7.3 or 0.154×10^{-2} mol·dm^{-3} NaCl equilibrated by NaOH to pH = 7.3. The data are mean values calculated from 10 measurements

Solutions in water pool	Parameters	Dark sample	Irradiated sample
Phosphate buffer pH = 7.3	τ_c (s) rad^{-1} $\times 10^{-10}$	2.100 ± 0.060	1.440 ± 0.060
	A_+ (mT)	1.640 ± 0.004	1.626 ± 0.003
	Δh_{+1} (mT)	0.199 ± 0.004	0.193 ± 0.004
	Δh_0 (mT)	0.186 ± 0.004	0.187 ± 0.004
	Δh_{-1} (mT)	0.210 ± 0.004	0.203 ± 0.004
NaCl pH = 7.3	τ_c (s) rad^{-1} $\times 10^{-10}$	1.880 ± 0.060	1.850 ± 0.060
	A_+ (mT)	1.630 ± 0.003	1.625 ± 0.003
	Δh_{+1} (mT)	0.199 ± 0.004	0.192 ± 0.004
	Δh_0 (mT)	0.189 ± 0.004	0.191 ± 0.004
	Δh_{-1} (mT)	0.210 ± 0.004	0.206 ± 0.004

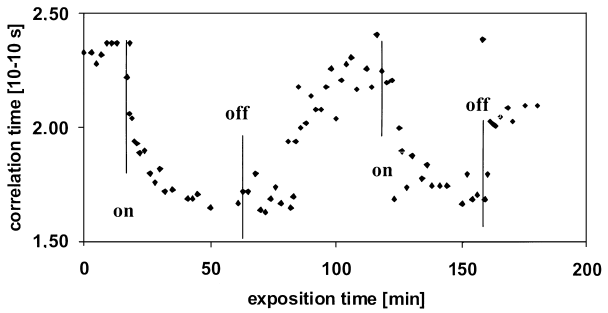

Fig. 1. Changes of correlation time calculated according to formula 1 for the TEMPO-palmitate spin probe incorporated into TCAB reversed micelles during exposition to continuous radiation. Water pool contained 0.077×10^{-2} mol·dm^{-3} phosphate buffer at pH = 7.3. Lines indicate the moment when radiation is switched on or switched off.

sion is plotted against the irradiation time (Fig. 1). The rotational correlation time decreased after irradiation for CTAB/phosphate buffer/isooctane/hexanol while it was independent of the irradiation time for TCAB/NaCl/isooctane/hexanol reversed micelles (Table 1). TEMPO--palmitate spin probe showed a longer correlation time in TCAB/phosphate buffer/isooctane/hexanol than in TCAB/NaCl/isooctane/hexanol reversed micelles (see Table 1).

Discussion

The properties of the interface of reversed micelles at different distances from the head group position had been monitored employing a series of doxylstearic spin probes [16, 25]. Analysis of the isotropic hyperfine splitting constant illustrated that with increasing water content the polarity increases also this is related to the increasing hydration of the head group region. Water molecules in reversed micelles structures are located mainly in the polar core and classified inside the water pool into two categories: near polar heads and interacting with them, or located far from the interface and whose properties are close to bulk water [3, 6, 14, 24, 25, 27]. The selective water pool solvent properties were established in dependence on the hydration shell of resolved ions; the bulk water accumulates mainly univalent anions; $H_2PO_4^-$, Cl^-, Br^- in decreasing order. Subsequently, the small highly hydrated cations (H^+, Na^+), highly hydrated anions – HPO_4^{2-}, OH^- and hexanol are localized in the interfacial region [19]. Thus, interfacial region for TCAB/phosphate buffer/isooctane/hexanol reversed micelles was enriched in HPO_4^{2-}, Br^-, OH^- ions, while TCAB/NaCl/isooctane/hexanol in Cl^-, Br^-, OH^-, due to positive charged surface. Large singly charged ions, with low charge density ($H_2PO_4^-$, Cl^-, Br^-) are chaotropes. They exhibit weaker interactions with water than water with itself and thus interfering little in the hydrogen bonding of the surrounding water. The small or multiply-charged ions, with high charge density, are kosmotropes (HPO_4^{2-}, Na^+, H^+, OH^-). They exhibit stronger interactions with water molecules than water with itself and, therefore, are capable of breaking water-water hydrogen bonds. Thus, the hydrogen bonds between water molecules are more strongly modified in the vicinity of ionic kosmotropes than ionic chaotropes. Optimum stabilization of macromolecules by salt requires a mixture of a kosmotropic anion (HPO_4^{2-}) with a chaotropic cation (Na^+) what is realized in the case of TCAB/phosphate buffer/isooctane/hexanol structure [19].

Computational investigations and experimental data [23] for nitroxyl spin probes in aqueous solution showed that more than half of the spin probe molecules population have two genuine water-nitoxide H-bonds; configuration with just one or with three H-bonds are also represented, while the fraction of lacking any H-bonds is small. The environment of TEMPO-palmitate spin probe localized in TCAB/phosphate buffer or TCAB/NaCl reversed micelles must be different. Highly hydrated anions – HPO_4^{2-} form probably, relatively strong hydrogen bond network associated with the water molecules from solvation shell of nitroxyl groups [24]. The higher value of correlation time before exposition to NIR for TCAB/phosphate buffer reversed micelles compared with TCAB/NaCl may illustrate that phenomenon. Weakening of the hydrogen bonds by NIR leads to decrease τ_c what we indeed observed for TCAB/phosphate buffer micelles (from 2.10×10^{-10} s to 1.44×10^{-10} s). NaCl in water pool is unable to form such hydrogen bonded structures and both parameters were unchanged after exposition to NIR.

The nitrogen isotropic hyperfine coupling constant A_+ is sensitive to electrostatic interactions and hydrogen bonding. Its value is a perturbation measure of the spin density distribution of the radicals in solution

caused by electrostatic interactions and hydrogen bonding with the solvent [22, 26]. Therefore, A_+ should be higher for solvents undergoing hydrogen bonding with the probe than for solvents that are not able to create them. The variation of A_+ dependent on the water pool composition was about 0.010 mT (1.640 mT for phosphate buffer and 1.630 mT for NaCl). The A_+ value for phosphate solvent confirms the suggestion that the phosphates form a network of strong hydrogen bonds which are decomposed by radiation.

Similar values of A_+ for both structures, and decreasing correlation time only for TCAB/phosphate buffer/isooctane/hexanol reversed micelles after exposition to NIR suggest that NIR modifies rather the hydrogen bond network formed by solutes than the hydration shell of TEMPO-palmitate spin probe. The spin labelling method applied in this work provides merely a tool for the observation of the effect of the hydrogen bond breaking. The studied effect of NIR on the hydrogen bonds system in water environment is not limited to the presence of radical molecule what we reported in our earlier papers [5, 8–11, 20, 21].

Conclusions

1. EPR spectra of TEMPO-palmitate spin probe incorporated to TCAB/phosphate buffer/isooctane/hexanol and TCAB/NaCl/isooctane/hexanol reversed micelles under NIR exposure were presented. They were compared with those of non-irradiated samples.
2. Analysis of correlation time and the spacing between hyperfine lines $A_+ = h_0 - h_{+1}$ showed the modification of the network of hydrogen bonds induced by NIR radiation.

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