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ESR spectroscopic characterization of spin labeled procaine in homogeneous solutions and membrane mimetic systems

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ABSTRACT

Procaine labeled at the aromatic amino group with the paramagnetic 2,2,5,5-tetramethylpyrrolin-1-oxyl moiety (sl-PRC) has been used as a model compound in several studies on interactions of local anesthetics with lipid membranes, but these works were not preceded with a detailed spectral characterization of the compound in simple, well defined systems. To fill the gap we examine here ESR spectra of sl-PRC solutions in solvents encompassing a large range of dielectric constants, as well as in aqueous solutions containing sodium dodecylsulfate (SDS) micelles, sodium bis(2-ethylhexyl)sulfosuccinate (AOT) vesicles or small unilamellar liposomes of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (DPPG, sodium salt). Results obtained for the homogeneous systems are discussed in terms of the effect of solvent properties (polarity, electrophilicity, H-bonding ability, and viscosity) on the ^{14}N hyperfine splitting parameters and on the rotational mobility of the spin label. For the microheterogeneous systems amphiphile/water partition coefficients of the drug have been evaluated from ESR spectra. Their values indicate that binding of procaine (protonated at biological pH) to the anionic aggregates is determined by both electrostatic and hydrophobic interactions. The local polarity at the nitroxide group site varies in the order SDS > DPPG > AOT, and the trend of the local viscosity change is DPPG ≫ SDS > AOT. From these findings it is concluded that in DPPG and AOT bilayers the sl-PRC molecule is oriented radially and the nitroxide group penetrates well below the Stern layer, while in SDS micelles it assumes a different location with the label inserted closer to the interface.

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1. Introduction

Although local anesthetics (LAs) have been used in medical treatment since the beginning of the 20th century, the mechanism of their action at the molecular level is still not fully understood—in spite of its importance. Indeed, by better bridging the gap between the basic and clinical sciences, issues essential for clinicians like providing the right kind and amount of anesthetic and minimizing its side effects, could be solved. Already several decades ago, it was suggested that phospholipid bilayers and the phospholipid/protein interface in the nerve membranes are specific targets of the anesthetics; a primary point of action is supposedly in the transmembrane domain of ion channels where amino acid residues confer sensitivity to anesthetics [1]. To date several fundamental aspects of the local anesthesia mechanism are still under scrutiny. In particular, it is not unambiguously explained what role in this mechanism is played by transport of the drug through the neuronal membrane to sites of sodium channels responsible for the gating properties, and whether (and how) the drug-induced changes in the physical state of membrane lipids correlate with the observed pharmacological effect [2–4].

A powerful method to study LA interactions with lipid membranes is electron spin resonance spectroscopy (ESR) with the use of spin probes such as nitroxide-substituted fatty acids or phospholipids [5,6], and/or spin labeled drugs, e.g. the nitroxide-labeled procaine (sl-PRC) of the formula given in Chart 1.

The sl-PRC features in a few studies on model and native membranes [7–10]. Conclusions from these researches are based solely on ESR line-shape analysis which delivers information on the rotational mobility of the nitroxide group and viscosity at the site of its location in the membrane. Values of ^{14}N hyperfine splitting parameters, sensitive to polarity of the microenvironment, were not reported even for observed motionally averaged signals (triplets) [8] or performed simulations of slow-motional spectra [7,10].

For an in-depth understanding of the behavior of sl-PRC in complex biomembrane systems it is essential to first know its spectral characteristics in homogeneous solutions and well-defined microheterogeneous systems that mimic membrane properties. In the present work these points in question are, for the first time, addressed in detail. We examine ESR spectra of sl-PRC in selected solvents, and next in aqueous solutions of several amphiphilic self-assemblies, viz. micelles of sodium dodecylsulfate (SDS), vesicles of sodium bis(2-ethylhexyl)sulfosuccinate

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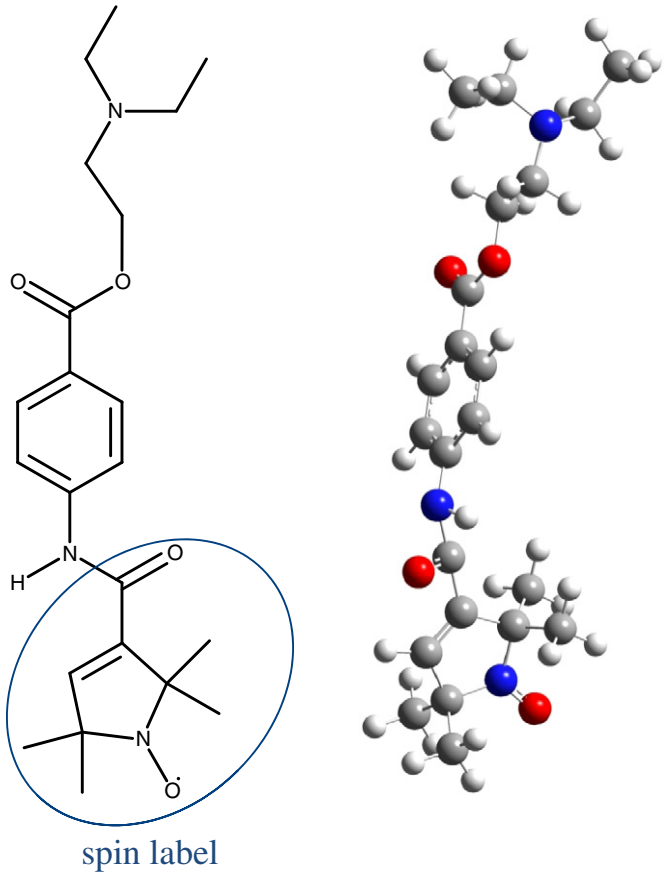

Chart 1. Chemical formula and molecular model of the spin-labeled procaine (sl-PRC) as obtained by geometry optimization (in vacuum) with Gaussian by B3LYP functional using 6–31 G(d) basis set.

(aerosol OT, AOT), and small unilamellar liposomes of synthetic lipid 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (or dipalmitoyl-phosphatidylglycerol; DPPG, sodium salt), cf. Chart 2. While phosphatidylglycerols are components of many biological membranes, both the liposomes and surfactant aggregates are also promising delivery systems for local anesthetics [11]. Our main objective was to assess how the hyperfine splitting parameters and correlation times of rotational diffusion of the spin label depend on relevant solvent properties (polarity, H-bonding ability, viscosity), and how procaine binding to amphiphilic aggregates is affected by their structure and electric charge.

2. Experimental

2.1. Materials

sl-PRC was synthesized according to Hideg et al. [12]. SDS, AOT (Sigma-Aldrich), DPPG (Avanti, Polar Lipids Inc.) and other chemicals were used as received. Organic solvents include n-hexane ≥95% for HPLC, methylcyclohexane ≥98% and dimethylformamide ≥99.8% for UV spectroscopy from Fluka; benzene 99.9%, chloroform 99.8%, 2-propanol ≥99.9%, ethyl acetate ≥99.9% and anhydrous ethylene glycol 99.8% from Sigma-Aldrich; ethanol 99.8% and methanol 99.9% for spectroscopy from Chempur, Poland. Millipore deionized water (pH≈6) was used for preparation of the aqueous systems, and anhydrous LiCl (99.9%, Wako Pure Chemical Industries) or NaCl (99.5%, Sigma-Aldrich) were added where required.

2.2. Sample preparation

Proper amounts of stock solutions of sl-PRC in chloroform were transferred into vials, the solvent was evaporated under a stream of nitrogen, and the obtained films were dried in vacuum for at least 2 h. Then, a desired amount of the argon-saturated solvent or surfactant solution (0.1 M SDS, or 0.028 M AOT) was added to the vial in an Ar-filled glove box, and the sample was stirred with a magnet bar at room temperature until dissolution (homogeneous solutions) or at 40 °C for 2 h (surfactant solutions). The drug concentration was ≤0.25 mM; drug/surfactant molar ratios were $2.5\cdot10^{-3}$ and $5\cdot10^{-3}$ for SDS and AOT, respectively. To obtain liposome samples the sl-PRC films were prepared in centrifuge tubes as described above, stirred with the proper amount of a DPPG stock solution in chloroform, and the solvent was evaporated

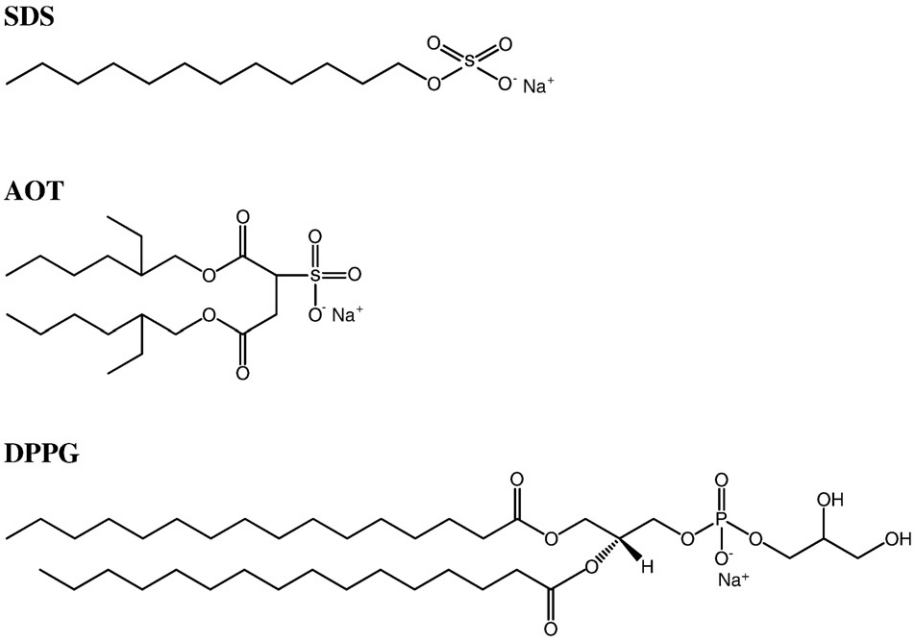

Chart 2. Structural formulae of amphiphilic compounds.

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