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An ESR characterization of micelles and vesicles formed in aqueous decanoic acid/sodium decanoate systems using different spin labels

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ABSTRACT

Aqueous decanoic acid/sodium decanoate systems were studied as a function of pH and concentration, up to 0.3 M decanoic acid/sodium decanoate, by electron spin resonance (ESR) spectroscopy using three different amphiphilic spin labels. The distribution of the spin labels between vesicles and micelles as well as their dynamic properties were determined by quantitative analysis of the ESR spectra using two novel simulation software packages. Rotational correlation time of the labels in micelles was found to increase with decreasing pH, with substantial increase in the region where vesicles were formed ($7.8 < pH < 8$). In the interval $6.5 < pH < 7.8$, the coexistence of vesicles and micelles was observed. Presence of vesicles was confirmed by the captured aqueous volume, determined independently with a hydrophilic spin label. The ESR measurements indicate that decanoic acid vesicle formation observed as the concentration is increased between 0.01 M and 0.03 M at pH 7.0 most likely occurs *via* the formation of micelles which remain in coexistence with the vesicles, even if the concentration is well above these values.

1. Introduction

Fatty acid/soap systems have attracted a number of research groups in the past since fatty acids are easily available, cheap, naturally occurring and structurally simple amphiphiles (Fontell and Mandell, 1993; Small, 1986). They aggregate in different manners when brought into contact with water, e.g. hexagonal and cubic phases, micelles, extended bilayers or vesicles (Fontell and Mandell, 1993; Small, 1986). In contrast to conventional phospholipid vesicles (liposomes from different diacylphospholipids) which form in dilute aqueous solution over a wide pH range (Lasic, 1993; Barenholz and Cevc, 2000), fatty acid vesicles are formed only within a narrow pH region (Morigaki and Walde, 2007) due to significant changes in the lipophilic character of the fatty acids with pH. At high pH, the ionized form dominates and, due to electrostatic head group repulsions, only micelles form. If the pH is too low, the fatty acid separates as solid crystals or oil, depending on the fatty acid's melting temperature (Morigaki and Walde, 2007). Due to the fact that fatty acids are chemically and structurally simple amphiphiles, their potential role as membrane-forming molecules in pre-biotic times is currently discussed (Deamer and Dworkin, 2005; Walde, 2006; Thomas and Rana, 2007).

Fatty acid vesicles are able to entrap macromolecules (Apel et al., 2002) and fatty acid-based vesicles can be used for certain enzyme-catalyzed polymerization reactions in which the vesicles act as a template (Namani and Walde, 2005). The investigation of different fatty acid vesicles was originally initiated by the discovery that oleic acid (*cis*-9-octadecenoic acid) self-assembles into self-closed bilayers if the experimental conditions are appropriately chosen (Gebicki and Hicks, 1973). It was found that fatty acid vesicles form above the Krafft point in dilute aqueous solutions above a critical concentration, if a significant portion of the carboxylic acid head groups is ionized (Gebicki and Hicks, 1976; Hargreaves and Deamer, 1978; Haines, 1983; Walde et al., 2006). The vesicles thus formed are actually mixed fatty acid/soap vesicles, but for the sake of simplicity they are often just called fatty acid vesicles. A number of studies following the initial findings by Gebicki and Hicks (1973, 1976) confirmed the formation of stable bilayers from long-chain unsaturated fatty acids (Hargreaves and Deamer, 1978; Haines, 1983; Walde et al., 1994; Chen and Szostak, 2004; Namani et al., 2007) as well as from short-chain saturated fatty acids (Apel et al., 2002; Namani and Walde, 2005; Hargreaves and Deamer, 1978; Morigaki et al., 2003; Stevenson and Blanchard, 2006).

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Different aspects of fatty acid vesicles have been previously studied, for example: (i) the formation of vesicles from fatty acid anhydrides by alkaline hydrolysis (Walde et al., 1994); (ii) the changes in vesicle size and vesicle concentration that occur upon addition of fatty acids to preformed vesicles (Blöchliger et al., 1998; Stano et al., 2006); (iii) physico-chemical properties of fatty acid vesicles and their coexistence with non-associated fatty acids (Namani and Walde, 2005; Walde et al., 2006; Namani et al., 2007); (iv) kinetic and thermodynamic properties of fatty acid vesicle formation in relation to the formation of micelles (Morigaki et al., 2003) and (v) the application of Gibbs' phase rule to explain the phase behavior at thermodynamic equilibrium of aqueous mixtures of the neutral and ionized forms of fatty acids (Cistola et al., 1986, 1988).

Recently, the dynamic properties of perylene in micelles and vesicles composed of decanoic acid and sodium decanoate were investigated by a fluorescence method (Stevenson and Blanchard, 2006). A measurable difference was obtained between the motional dynamics of perylene embedded in the non-polar part of the micelles maintained in phosphate buffer at pH 9.3 and the motional dynamics of perylene embedded in the non-polar part of unilamellar vesicles maintained at pH 6.8.

In this paper, we report about *decanoic acid vesicle* formation from micelles, studied as a function of pH and decanoic acid concentration. The method used to characterize decanoic acid micelles and vesicles is electron spin resonance (ESR) spectroscopy with spin labels. ESR spectroscopy has been applied extensively in studies of a wide range of micelle-forming surfactants such as sodium dodecylsulphate (Szajdzinska-Pietek et al., 1985), dodecyltrimethylammonium bromide (Baglioni and Kevan, 1995) and amphiphilic tri-block copolymers (Caragheorgheopol et al., 1997). A few years ago, an ESR method with spin labels was developed for determining changes in the aggregation number of sodium dodecylsulphate micelles with remarkably high precision (Bales et al., 1998; Lebedeva and Bales, 2006). With respect to fatty acid systems, ESR spectroscopy has been used recently to investigate the pH-induced transformation of oleate micelles into mixed oleic acid/oleate vesicles, using the fatty acid spin label 16-doxylstearic acid (Fukuda et al., 2001). It was found that the experimental ESR spectra of 16-doxylstearic acid in oleic acid vesicle suspensions could only be simulated if the presence of two different environments of the doxyl group were considered, one in vesicle bilayers and one in non-vesicular (micellar) aggregates (Fukuda et al., 2001). This finding was in apparent contradiction to expectations that arose from the application of Gibbs' phase rule (Cistola et al., 1986, 1988) and it was pointed out that further studies are needed.

The main aim of the present work was to clarify whether two micro-phases (micelles and vesicles), can be found to coexist in fatty acid systems composed of alkyl chains shorter than oleic acid (Fukuda et al., 2001) (i.e. lower lipophilicity) as well as to determine how dynamic properties of micelles and vesicles in the decanoic acid/sodium decanoate system vary with pH, decanoic acid concentration and temperature. Concentration and pH dependent formation of vesicles from micelles was investigated by ESR spectroscopy using three lipophilic spin labels: (i) the methyl ester of 7-doxyldodecanoic acid (MeFASL (4,5)), (ii) (2,2,6,6-tetramethylpiperidine-1-oxyl-4-yl)-octanoate (TEMPO-octanoate), and (iii) (2,2,6,6-tetramethylpiperidine-1-oxyl-4-yl)-decanoate (TEMPO-decanoate) (Fig. 1). TEMPO-octanoate and TEMPO-decanoate, with their nitroxide moiety covalently attached at the polar end of the spin label molecule, report on the surface properties of the micelles and/or vesicles. The doxyl group of MeFASL (4,5) is expected to give information about the hydrophobic interior of the bilayer formed only from vesicles, while in micelles it will report on the Stern layer, as has been recently suggested (Lebedeva and Bales, 2006).

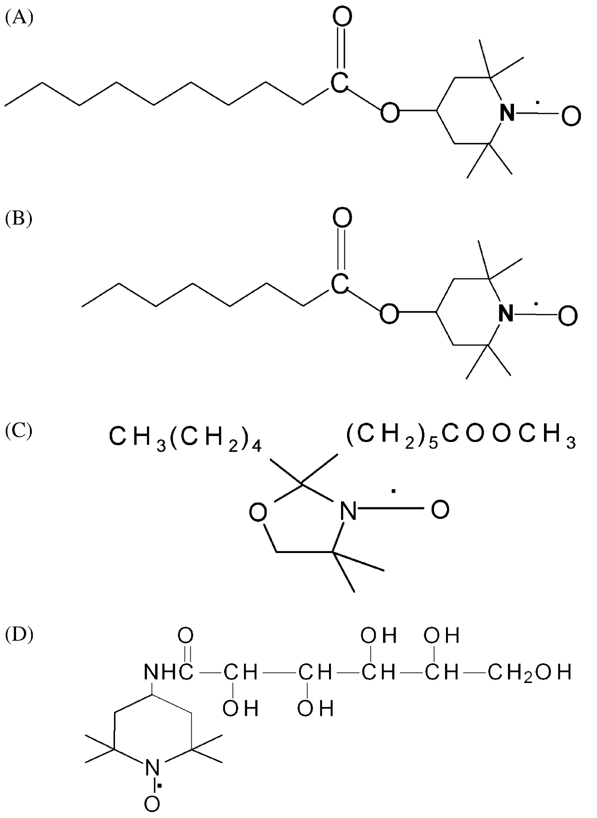

Fig. 1. Structural formulae of lipophilic (A–C) and hydrophilic (D) spin labels used in this study. TEMPO is 2,2,6,6-tetramethylpiperidine-1-oxyl-4-yl. (A) TEMPO-decanoate; (B) TEMPO-octanoate; (C) 7-doxyl-dodecanoic acid methyl ester (MeFASL (4,5)); (D) 1-oxyl-2,2,6,6-tetramethyl-4-(2′,3′,4′,5′,6′-pentahydroxyhexanoyl-1′-amino)-piperidine (GluSL).

Experimental ESR spectra of the spin labels in micelles and vesicles are analyzed using two different software programs (Stoll and Schweiger, 2006; Štrancar et al., 2003) both enabling a quantitative description of the dynamic characteristics of spin labels in micelles and vesicles. Data obtained for these lipophilic spin labels are correlated with measurements of the vesicles' captured volume.

To the best of our knowledge, the decanoic acid system has not been studied by ESR before. Furthermore, the dynamic characteristics of fatty acid micelles and vesicles throughout the entire pH region where micelles and vesicles are formed (i.e. from pH 11.6 to 6) have also not been investigated previously.

2. Materials and methods

2.1. Materials

Decanoic acid (>98%) and sodium ascorbate were obtained from Fluka (Buchs, Switzerland); sodium oleate (99%) and bicine (*N*,*N*-bis(2-hydroxyethyl)glycine, 99%) were purchased from Sigma–Aldrich (St. Louis, USA); sodium dihydrogen-phosphate (p.a.), sodium hydroxide (p.a.) and hydrogen chloride (p.a.) were from Kemika (Zagreb, Croatia). All aqueous solutions were prepared in bi-distilled water.

Two groups of lipophilic spin labels were synthesized by one of us (S. Pečar): (i) fatty acids that were esterified with 4-hydroxy-1-oxyl-2,2,6,6-tetramethylpiperidine (Rozantsev, 1970), i.e. octanoic acid, resulting in TEMPO-octanoate (Fig. 1B), and decanoic acid,