Journal of Colloid and Interface Science 445 (2015) 127–136

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Micellar and biochemical properties of a propyl-ended fluorinated surfactant designed for membrane–protein study

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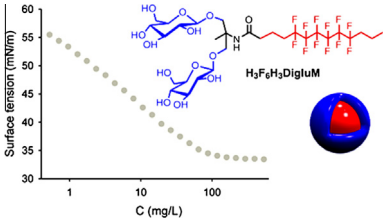
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 September 2014
Accepted 19 December 2014
Available online 3 January 2015

Keywords:
Fluorinated surfactants
Micelles
Membrane proteins
Protein stability

ABSTRACT

Our goal is to design optimised fluorinated surfactants for handling membrane proteins in solution. We report herein the self-assembling and biochemical properties of a new hemifluorinated surfactant ($H_3F_6H_3$DigluM) with a branched diglucosylated polar head group and an apolar tail consisting of a perfluorohexane core decorated with a hydrogenated propyl tip. For the sake of comparison, its fluorinated analogue without propyl tip (F_6H_3DigluM) was also studied. Isothermal titration calorimetry and surface tension showed that the addition of a propyl tip has a significant effect on the overall hydrophobicity of the surfactant, in contrast to the behaviour described when adding an ethyl tip to a fluorinated surfactant. From dynamic light scattering, analytical ultracentrifugation and small-angle X-ray scattering, both $H_3F_6H_3$DigluM and F_6H_3DigluM self-assemble into small globular micelles of 5–7 nm in diameter and have aggregation numbers of 62 ± 8 and 46 ± 2, respectively. Finally, $H_3F_6H_3$DigluM was found to be the best fluorinated surfactant developed in our group to stabilise the model membrane protein bacteriorhodopsin (bR) in aqueous solution. This study demonstrates the suitability of this new propyl-ended fluorinated surfactant for biochemical and structural applications and confirms the superiority of hemifluorinated chains over fluorinated ones.

1. Introduction

Fluorinated surfactants (FS) are amphiphilic compounds whose hydrophobic moiety consists of a perfluoroalkyl chain (F-chain), usually linked to a polar head *via* a short hydrogenated spacer (Fig. 1). In addition to being chemically and thermally stable,

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http://dx.doi.org/10.1016/j.jcis.2014.12.066
0021-9797/

F-chains are both hydrophobic and lipophobic, which confers peculiar properties to FS. The strong hydrophobic interactions among F-chains of FS result in very stable self-assemblies in aqueous solutions [1]. FS are also significantly more surface-active than their hydrogenated counterparts [2]. The tight packing of F-chains at the air/water interface results in low surface free energy and low surface tension values, and correlates with a much lower critical micellar concentration (CMC) [3]. Moreover, the larger cross-section of F-chains as compared with H-chains (30 $Å^2$ *vs.* 20 $Å^2$, respectively), and the bulkier volumes of CF_3 and CF_2 groups as compared with CH_3 and CH_2, result in higher rigidity and reduced conformational freedom of F-chains [1,4]. Therefore, FS self-assemblies are most likely to adopt the shape of cylindrical micelles or even large vesicles [5]. However, we have recently demonstrated that both shape and size of FS self-assemblies can be controlled and that their size may be reduced to small globular micelles by introducing bulky polar head groups [6,7].

Owing to their considerable therapeutic importance, MPs are the targets of more than half of all drugs on the market [8]. Thus, the elucidation of their structures and the understanding of their modes of action are two major issues in basic life sciences and drug discovery. Conventionally, MPs are extracted from their host membrane using classical surfactants, so-called detergents, which partition into lipid bilayers and solubilise them at higher concentrations. However, it has been shown that the detergent properties of classical surfactants can cause denaturation of MPs over time [9]. There are currently several approaches aiming at the development of milder compounds able to keep MPs stable and water-soluble (reviewed in *e.g.* [10–12]). The rationale behind the use of FS is that they poorly interfere with protein–lipid and protein-co-factor interactions, because bulky and stiff F-chains intrude less easily into the protein structure than the hydrogenated chains of classical detergents, thus contributing to the stability of solubilised MPs.

In previous work, we have demonstrated that the addition of a short hydrocarbon ethyl tip to FS, resulting in so-called hemifluorinated surfactants, increases their affinity toward the hydrophobic domain of MPs, thus helping to reduce their aggregation, while preserving the protective effect of the fluorocarbon chains [7,13,14]. We found that fluorinated and hemifluorinated surfactants bearing two branched glucose moieties, such as the F_6Diglu, F_6DigluM and H_2F_6Diglu derivatives (Fig. 1), self-assemble into small and monomodally distributed globular micelles. They were found to stabilise the native structure of solubilised bacteriorhodopsin (bR) and cytochrome b_6f complex [7,15]. In addition, F_6DigluM has recently proven very useful for investigating the solution structure of a deuterated protein within a membrane–protein complex by small-angle neutron scattering (SANS) [17,17]. There is thus a strong interest in confirming the potential of such FSs for *in vitro* studies of MPs and in extending the understanding of the effect of varying the length of the alkyl and fluorinated segments, or modulating the structure of the hydrophilic head.

We have recently put forward a convenient synthetic route to propyl-ended surfactants using radical addition of two alkenes onto commercially available 1,6-diiodoperfluorohexane [19]. We also showed that the use of a diglucosylated methyl polar head group (DigluM) significantly shortened the preparation of the branched diglucosylated polar head when compared with the previously described Tris-based head group Diglu [16]. In the present work, the diglucosylated methyl polar head group (DigluM) was grafted onto the propyl-ended fluorinated chain, leading to a new hemifluorinated surfactant dubbed $H_3F_6H_3$DigluM (Fig. 1). For the sake of comparison, its fluorinated analogue F_6H_3DigluM without propyl tip was also studied (Fig. 1). The self-assembly and biochemical properties of these new surfactants were investigated. Micelle formation was studied by a combination of isothermal titration calorimetry (ITC) and surface tension (ST), while micelle size and shape were determined by dynamic light scattering (DLS), analytical ultracentrifugation (AUC), and small-angle X-ray scattering (SAXS). Finally, the homogeneity and biochemical stability of complexes of bR in $H_3F_6H_3$DigluM and F_6H_3DigluM were investigated.

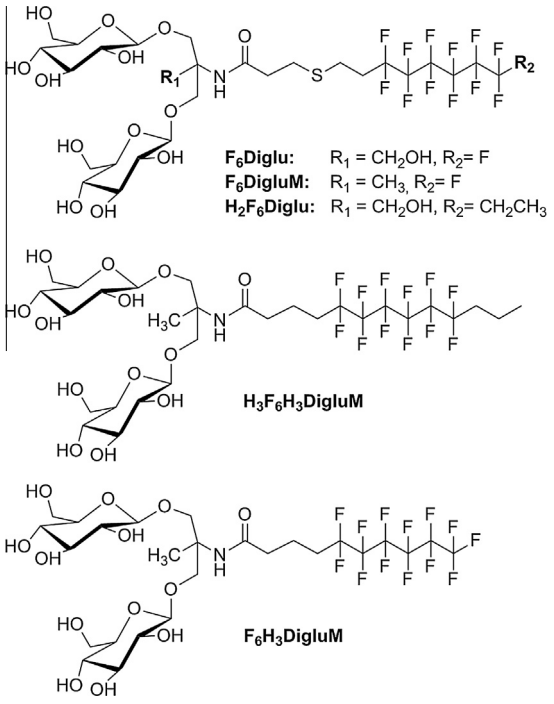

Fig. 1. General structure of previously reported branched glycosylated surfactants and chemical structures of the two surfactants studied in this work, $H_3F_6H_3$DigluM and F_6H_3DigluM.

2. Experimental

2.1. Synthesis

Materials, instrumentation, and procedures for the synthesis of $H_3F_6H_3$DigluM and F_6H_3DigluM are described in the Supplementary data section.

2.2. Isothermal titration calorimetry

High-sensitivity microcalorimetry was performed at 25 °C on a VP-ITC (GE−Healthcare, Freiburg, Germany). For demicellisation experiments, 5-μL aliquots of 15 mM F_6H_3DigluM in water, or 10-μL aliquots of 3.5 mM or 4 mM $H_3F_6H_3$DigluM in water, were injected into water. Time spacings between injections were chosen long enough to allow for complete re-equilibration. Baseline subtraction and peak integration were performed using NITPIC [20]. All reaction heats were normalised with respect to the molar amount of surfactant. Nonlinear least-squares fitting was performed in an Excel (Microsoft, Redmond, USA) spreadsheet using the Solver add-in (Frontline Systems, Incline Village, USA), as explained elsewhere [21].

2.3. Surface tension measurements

The surface activity of surfactants in solution at the air/water interface was determined using a K100 tensiometer (Kruss, Hambourg, Germany). Surface tensions were determined by dilution