Colloids and Surfaces A 555 (2018) 332–338

Contents lists available at ScienceDirect

Colloids and Surfaces A

journal homepage: www.elsevier.com/locate/colsurfa

# Characterization of the aggregated pattern of CHAPS using solvent paramagnetic relaxation enhancements

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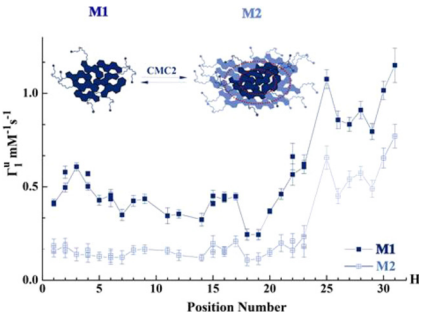
## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Keywords:*
CHAPS
Detergent
Solvent paramagnetic relaxation enhancement
Saturation transfer difference
Aggregated pattern

## ABSTRACT

CHAPS is a typical zwitterionic detergent widely used in protein purification. It has two peculiar aggregation states, whose aggregation patterns are still controversial and uncertain. In this paper, we analyzed the aggregation pattern of CHAPS by $^{1}$H solvent Paramagnetic Relaxation Enhancement (sPRE) experiment. It was found that the $^{1}$H sPREs of head groups of CHAPS are much smaller than those of the tail groups, and all of them become smaller simultaneously when the concentration of CHAPS is above CMC2. In addition, the sPREs of those protons in the steroid ring were found to be very similar to each other in both micellar states (M1 and M2). These results indicated that the micellar core of CHAPS is mainly formed by its steroid head which aggregate disorderly. On the other hand, a transition from single to double layer micelle is most likely to happen when the concentration of CHAPS is above CMC2. The micelle in M2 state is formed by the aggregation of the partially disordered primary ones as well. Furthermore, the STD (Saturation Transfer Difference) experiment showed that the apparent coherence transfer rates between water and those OH near protons at the steroid head are slower in M2 state, revealing that the CHAPS aggregate loosely in M1 state, but more tightly in M2 state, which may be driven by the increased hydrophobic interactions between the steroid groups of nearby CHAPS molecules, as well as the hydrogen bond between OH in the hydrophobic side and $SO_3^{-1}$, CO, or HN in the hydrophilic side of different molecules, which consequently extrude the water and stabilize the micelle.

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https://doi.org/10.1016/j.colsurfa.2018.07.008
Received 14 May 2018; Received in revised form 3 July 2018; Accepted 5 July 2018
Available online 10 July 2018
0927-7757/ 

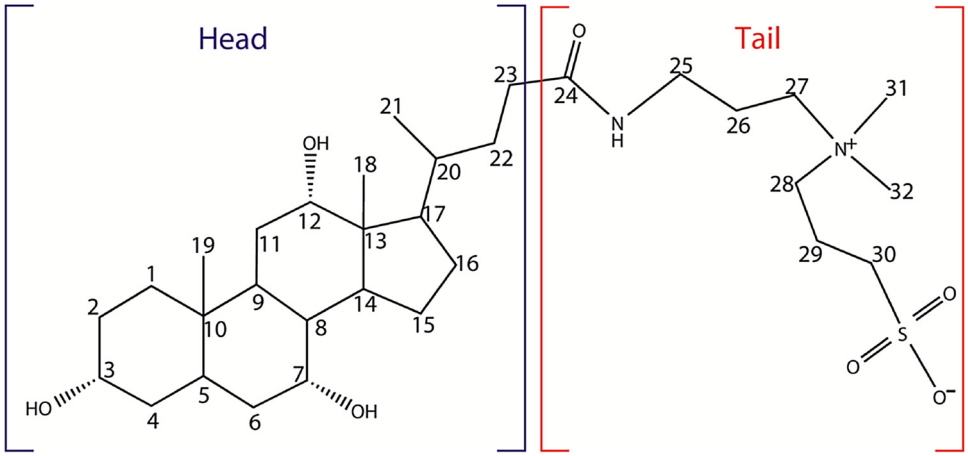


**Fig. 1.** 2D structure diagram of CHAPS molecule with atom positions included.

## 1. Introduction

CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) is a typical zwitterionic detergent, which was designed for membrane protein purification in the early 1980s [1]. As a derivative of bile salts, the steroid head of CHAPS has four fused rings with a hydrophilic concave side (α-plane or face), where three hydroxyl groups protrude, and a hydrophobic convex side (β-plane or back). The mobile tail of CHAPS contains a zwitterionic amidosulfobetaine group (Fig. 1 shows the 2D structure diagram of CHAPS molecule). Due to its electrical neutrality, and zwitterionic properties, CHAPS can extract proteins without denaturation or aggregation. It was found that CHAPS can inhibit the dimerization of EnvZ-PD [2], and plays a crucial role in blocking the interaction between mS100A9 and RAGE V domain [3]. However, unlike other detergents, a number of researches have revealed that the applications of CHAPS are concentration related. CHAPS can minimize the degree of aggregation of calcineurin B with optimized concentration of about 20 mM [4]. The most effective concentration of CHAPS to prevent self-association of MLC2 [5] was found to be 22.5 mM. McGuire et al. have revealed that adding 25–50 mM CHAPS increased the solubility and stability of yeast eIF4E [6]. Garner et al. have found the disordered lipid phase could be selectively solubilized after the addition of 6 mM CHAPS, whereas the whole phase-separated supported lipid bilayers is solubilized within 30 min in 30 mM CHAPS [7].

The concentration related applications of CHAPS have been found to be attributed to its peculiar aggregation properties. Using 2D NMR experiments, Funasaki et al. have found that there are possible associations of dimeric fragments in CHAPS micelle, and the mainly associations of dimeric fragments is back-to-back (antiparallel or parallel) [8]. Qin et al. have investigated the characterization of CHAPS using 1D selective NOESY, and revealed a transition from single to double layer micelle may performs when the concentration of CHAPS increases [9]. Using SAXS, Lipfert et al. have suggested the contrast difference between hydrophobic core and hydrophilic head groups is much less pronounced than that for other detergents [10]. Kroflic et al. have concluded that CHAPS micelle can be regarded as a small and loose aggregates with lots of water molecules still in contact with the hydrocarbon skeleton by isothermal titration calorimetry [11]. By adding stearic acid spin labels to CHAPS, Rodi et al. have reported that the primary micelles of CHAPS are barrel-shaped with a minimum mean radius of 1.46 nm, and the secondary micelle are formed by aggregation of primary ones as well, besides, there are several elongated hydrophobic pockets, with similar dimensions for all aggregate sizes [12]. Herrera et al. found that CHAPS show a grain like heterogeneity with hydrophobic micropockets by molecular dynamics simulation [13].

In summary, though the aggregation characterizations of CHAPS, such as aggregation number and Critical Micelle Concentration (CMC) have been extensively studied, the aggregated pattern of CHAPS is still controversial and uncertain. Some results suggested that the micelles of CHAPS have no clear hydrophobic core, and maybe typically loose and heterogeneity inside [10,11,13]. Whereas, others inferred that the micelle has a symmetry structure with barrel-shaped or spherical layered hydrophobic region [9,12].

In this manuscript, the aggregated pattern of CHAPS has been further investigated by using sPRE NMR experiment. Compared with normally used NOE NMR experiment, the PRE experiment can give distance between nuclei extending over much larger distances and has little spin diffusion artifacts as well. PRE has been widely used to study conformation of hydrocarbon chain [14–16] and evaluate location of solubilizates within micelles [17–20]. Recently, sPRE which use solvent paramagnetic probe has been applied to investigate orientation and insertion depths of peptides [21,22] and receptors incorporated into micelles [23]. Herein, sPRE along with STD (Saturation Transfer Difference) NMR experiments were applied to estimate the detailed insertion depth of CHAPS inside its micelle in order to reveal the aggregated pattern of the micelle accordingly.

## 2. Materials and methods

CHAPS (98%), $D_2O$ (99.9%) and the external reference TSP ($Me_3Si$-$CD_2CDCO_2Na$, 99%) were all purchased from Sigma-Aldrich (USA). The paramagnetic probe Gd(DTPA-BMA) (98%) were purchased from LiTTLE-PA Sciences (CHINA). EDTA-$Na_2$ (99%), $MnCl_2$ (99%), and $DyCl_3$ (98%), which were used to generate the other two paramagnetic probes EDTA-Mn and EDTA-Dy through chelate reaction, respectively, were purchased from Baitg (CHINA). All the reagents were utilized without any further purification.

### 2.1. Sample preparation

99.9% $D_2O$ were used as the solvent for all experiments except the STD experiments (10.0% $D_2O$, 90.0% $H_2O$). To identify the CMC, CHAPS was configured to 185.2 mM (pD 6.9), and then gradually diluted to all concentrations required. The exact same sample preparation process is applied to the STD experiments. In the sPRE experiments, 5 different concentrations (2.8 mM, 19 mM, 38 mM, 114 mM, and 152 mM) of CHAPS were configured, each of them was titrated with those paramagnetic probes mentioned above. The final concentrations of the titrating Gd(DTPA-BMA) range from 1.1 mM to 8.9 mM. The final concentrations of the titrating EDTA-Mn and EDTA-Dy are 5.6 mM and 33.3 mM, respectively. 10 min sonication was performed for all the samples to remove the dissolved paramagnetic oxygen and make the solution homogeneous. The solutions were transferred into 5 mm NMR