Dependence of critical micelle concentration of a zwitterionic detergent on ionic strength: implications in receptor solubilization

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Abstract The zwitterionic detergent, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), is mild, non-denaturing, and extensively used for solubilizing membrane proteins and receptors. We report here that the critical micelle concentration (CMC) of CHAPS is dependent on the concentration of NaCl in the solution. Thus, the CMC of CHAPS decreases from 6.41 mM in absence of any salt to 4.10 mM in presence of 1.5 M NaCl. The logarithm of the CMC values appear to have a linear relationship with the salt concentration. Such changes in CMC with ionic strength have important implications in solubilization of membrane-bound neuronal receptors. This is shown by optimal solubilization of the serotonin receptor type 1A (5-HT_{1A}) from bovine brain hippocampal crude (native) membrane by CHAPS at pre-micellar concentration under high salt conditions.

Key words: Critical micelle concentration; CHAPS; 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane-sulfonate; Ionic strength; 5-HT_{1A} receptor; Solubilization; Zwitterionic detergent

1. Introduction

Detergents are extremely important in studies of biological membranes due to their ability to solubilize membrane proteins and receptors [1–6]. They are soluble amphiphiles, and above a critical concentration (strictly speaking, a narrow concentration range), known as the critical micelle concentration (CMC), self associate to form thermodynamically stable, non-covalent aggregates called micelles [7–9]. CMC represents an important physicochemical characteristic of a given detergent in solution. The CMC of detergents has been measured by a number of techniques [10]. These include measurement of light scattering, surface tension, hydrodynamic properties and changes in absorbance or fluorescence upon dye solubilization.

Addition of salts is known to drastically decrease the CMC of charged detergents such as SDS [11–13] since salt would tend to reduce the repulsion between the charged headgroups, thereby helping the micelles to be formed at lower concentrations. The effect of salts on the CMC of uncharged detergents is expected to be less pronounced because of the absence of charge interactions [14]. However, such studies have been less frequent [15].

One of the most commonly used detergents in membrane biochemistry is CHAPS [3], a mild, non-denaturing and zwitterionic detergent, which is a derivative of the naturally occurring bile salts. In this paper, we report the dependence of CMC of CHAPS on NaCl concentration. Our results show that even for this zwitterionic detergent, there is a significant reduction in CMC with increasing salt concentration. These results assume significance in light of the fact that CHAPS in pre-micellar concentration (i.e., below its CMC) is shown to be very effective in solubilizing serotonin (5-hydroxytryptamine) type 1A (5-HT_{1A}) receptors from bovine brain hippocampal crude membrane under high salt conditions.

2. Materials and methods

BCA, CHAPS, EDTA, EGTA, $MgCl_2$, PEG-8000, PMSF, TRIS·HCl, iodoacetamide, polyethylenimine, serotonin, sodium azide and NaCl were obtained from Sigma Chemical Co. (St. Louis, MO). DPH was purchased from Molecular Probes (Eugene, OR). [^{3}H]OH-DPAT (147.2 Ci/mmol) was purchased from DuPont New England Nuclear (Boston, MA). Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout. Fresh bovine brains were obtained from a local slaughterhouse within 30 min of death and the hippocampal region was carefully dissected out. The hippocampi were immediately flash frozen in liquid nitrogen and stored at −70°C till further use.

Crude membranes were prepared as described by DeVinney and Wang [16] with some modifications. Hippocampal tissue (~120 g) was homogenized as 10% (w/v) in polytron homogenizer in buffer A (2.5 mM TRIS·HCl, 0.32 M sucrose, 5 mM EDTA, 5 mM EGTA, 0.02% sodium azide, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4). The homogenate was centrifuged at 900×*g* for 10 min at 4°C. The supernatant was filtered through four layers of cheese cloth and the pellet was discarded. The supernatant was further centrifuged at 50,000×*g* for 20 min at 4°C. The resulting pellet was suspended in 10 vol. of buffer B (50 mM TRIS·HCl, 1 mM EDTA, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4) using a Dounce homogenizer and centrifuged at 50,000×*g* for 20 min at 4°C. This procedure was repeated until the supernatant was clear. The final pellet (crude membrane) was resuspended in minimum volume of 50 mM TRIS·HCl buffer (pH 7.4), homogenized using a Dounce homogenizer, flash frozen with liquid nitrogen and stored at −70°C. Solubilization of crude membranes using CHAPS and NaCl was carried out as described by Ofri et al. [17] with some modifications. Crude membranes were incubated with varying concentrations of CHAPS and NaCl in buffer C (50 mM TRIS·HCl, 1 mM EDTA, 10 mM $MgCl_2$, pH 7.4) at a final protein concentration of ~2 mg/ml for 30 min at 4°C with occasional shaking. The membranes were briefly sonicated (5 s) at the beginning and the end of the incubation period using a Branson model 250 sonifier. After incubation for 30 min, the contents were centrifuged at 100,000×*g* for 1 h. The clear supernatant was carefully removed from the pellet, and either used immediately for binding assay or for PEG precipitation. PEG precipitation of the crude CHAPS extract was performed to remove NaCl from the solubilized

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Abbreviations: BCA, bicinchoninic acid; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; CMC, critical micelle concentration; DPH, 1,6-diphenyl-1,3,5-hexatriene; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl-ether)-*N,N,N′,N′*-tetraacetic acid; 5-HT, 5-hydroxytryptamine; OH-DPAT, 8-hydroxy-2-(di-*N*-propylamino)tetralin; PEG, polyethylene glycol; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; THF, tetrahydrofuran; TRIS, *tris*-(hydroxymethyl)aminomethane

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extract since the agonist binding of 5-HT_{1A} receptor is inhibited by NaCl [18]. This procedure is believed to remove the salt [19,20]. PEG precipitation was carried out by diluting the extract with equal volume of 40% PEG-8000 in buffer C [17]. Following a vigorous vortexing and incubation for 10 min on ice, the samples were centrifuged at 15,000×g for 10 min at 4°C. The pellet was carefully rinsed twice with buffer C and finally resuspended in the same buffer. The suspension was homogenized using a Dounce homogenizer and used for ligand binding assays. Radioligand binding assays were performed as follows. Tubes in triplicate containing 1–1.2 mg of total protein (for crude membrane) or 500 μl of the PEG precipitated extract in a total volume of 1 ml of buffer C were incubated with 0.29 nM [^{3}H]OH-DPAT (sp. activity: 147.2 Ci/mmol) for 1 h at room temperature in the presence and absence of 10 μM 5-HT. The incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters which were presoaked in 0.3% polyethylenimine for 3 h [21]. The filters were then washed 3 times with 3 ml of ice-cold water, dried, and the retained radioactivity was measured in Packard Tri-Carb 1500 scintillation counter using 5 ml of scintillation fluid. Protein concentration was determined using BCA reagent [22].

The CMC of CHAPS at different concentrations of NaCl were determined fluorimetrically using a method previously developed by one of us which utilizes enhancement of DPH fluorescence upon micellization [13]. Fluorescence measurements were performed with a Hitachi F-4010 spectrofluorometer using 1 cm path-length quartz cuvettes. The excitation wavelength was 358 nm and the emission wavelength was 430 nm. The excitation and emission slits were set at bandwidths of 1.5 and 5 nm, respectively. Fluorescence was averaged over 5 s for each sample reading. The protocol for determination of CMC was as follows [13]: 1 μl of 10 mM DPH dissolved in THF was added to various amounts of CHAPS dissolved in a total volume of 1.5 ml of aqueous solution containing varying concentrations (0–1.5 M) of NaCl. Tubes were vortexed well and incubated in dark for 30 min at room temperature before measurement of fluorescence. Background samples lacking DPH were prepared in all cases, and their fluorescence intensity was subtracted from reported values. Duplicate sets of samples were prepared in each case and average fluorescence is shown in Fig. 1. To reverse any photoisomerization of DPH, samples were kept in dark in the fluorimeter for 30 s before the excitation shutter was opened and fluorescence measured.

3. Results

The principle of the DPH assay of CMC is that DPH fluorescence will be greatly enhanced above the CMC due to its incorporation into the hydrophobic interior of the micelle. A unique advantage of this method is that, unlike some other methods which employ charged probes [23,24], the CMC determined using this approach is reliable even for charged detergents. This is in contrast to methods which employ charged fluorescent probes where it has been demonstrated that such assays usually do not work if probe and detergent have opposite charges [23,24]. Fig. 1 shows the dependence of DPH fluorescence upon detergent concentration for CHAPS under three different salt concentrations. Fluorescence is weak at low CHAPS concentrations followed by a rapid rise in all the cases shown. The rapid rise in DPH fluorescence takes place at and above the CMC of CHAPS under the given condition. As the amount of CHAPS is increased, the number

Table 1
CMC of CHAPS as a function of salt concentration

NaCl concentration(mM)	CMC (%)	CMC (mM)
0	0.394	6.41
100	0.382	6.21
200	0.365	5.94
500	0.347	5.64
1000	0.267	4.34
1500	0.252	4.10

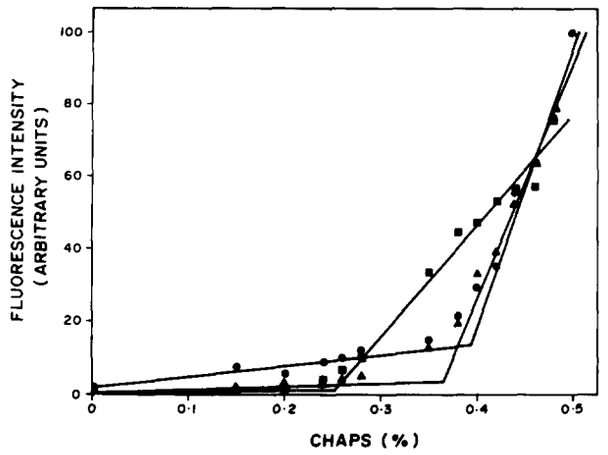

Fig. 1. DPH fluorescence as a function of concentration of CHAPS for 0 (●), 0.2 (▲), and 1.5 M (■) NaCl. The fluorescence scale is in arbitrary units, and the maximum fluorescence for each concentration of NaCl has been assigned a value of 100. See Section 2 for other details.

of micelles increase with a concomitant increase in the amount of bound DPH causing an increase in fluorescence. The CMC is obtained from the intersection of the straight line through the fluorescence at low detergent concentrations with the straight line through the fluorescence values in the region of rapid intensity increase [13]. The CMC of CHAPS determined by this method is 6.41 mM, in the absence of any added salt (see Fig. 1 and Table 1). This is in excellent agreement with the literature value of 6.50 mM as the CMC of CHAPS [5]. However, the CMC progressively decreases to 5.94 mM and 4.10 mM when the concentration of NaCl is increased to 200 mM and 1.5 M, respectively (Fig. 1 and Table 1). Such dependence of CMC on salt concentration, though common for charged detergents [11–13], has not been previously reported for zwitterionic CHAPS. Although the mechanism for such salt-dependent CMC for CHAPS needs to be worked out, it could be due to the salting out of the non-polar moiety of CHAPS, as has been shown for other uncharged detergents [11].

The steady decrease in CMC of CHAPS with increasing salt concentration is shown in Table 1. As the salt concentration is increased up to 1.5 M (this range of salt concentration is commonly used while solubilizing membrane-bound receptors with CHAPS, see later), the CMC decreases by 36% from 6.41 mM to 4.10 mM. Fig. 2 shows the variation of the logarithm of the CMC with salt concentration. Regression analysis shows that the logarithm of the CMC varies linearly with salt concentration (r = 0.98) and the following empirical relationship is obeyed:

$$\log \mathrm{CMC} = \mathrm{constant} - k_s C_s \quad (1)$$

where C_s = concentration of the salt and k_s = the salt effect constant. Such a relationship has previously been reported for the zwitterionic alkylbetaines [15].

The solubilization of crude (native) membranes containing the 5-HT_{1A} receptor as a function of CHAPS and NaCl concentration is shown in Fig. 3. It is apparent from the figure that the solubilization efficiency is low without any added salt and that the solubilization efficiency of CHAPS changes dis-

continuously around its CMC at various salt concentrations. The solubilization efficiency increases, specially at pre-micellar concentrations, when NaCl is used. We attribute this to the lowering of CMC in presence of added salt as discussed above. Thus, optimal solubilization is achieved with 5 mM CHAPS (i.e., at pre-micellar concentration judged by its CMC in water) in buffer containing 1 M NaCl.

4. Discussion

CHAPS is a non-denaturing zwitterionic detergent that combines useful features of both the bile salt hydrophobic group and the *N*-alkyl sulfobetaine-type polar group [3,25]. It is more efficient in solubilizing proteins than the structurally related carboxylic acid anions and is much more effective in breaking protein–protein interactions than either sodium cholate or Triton X-100. In addition, CHAPS has very low absorbance at 280 nm (unlike Triton X-100) and does not have circular dichroic activity in the far UV region, making it ideal for optical studies of proteins. These factors have led to extensive use of CHAPS in solubilization of membrane proteins and receptors [17,26–31].

The concept of micelle formation is relevant to solubilization and reconstitution studies of membrane proteins since it appears that there is some correlation between the ability to form micelles and the concentration of detergent required for solubilization [32]. The CMC is an important parameter for a given detergent since at this concentration the detergent will start to accumulate in the membrane. Studies on several receptors including the insulin receptor, opioid receptor, and angiotensin II receptor indicate that successful solubilization is achieved only with high (> 1 mM) CMC detergents such as CHAPS and octyl glucoside at concentrations below the CMC [33]. Concentrations of detergents above the CMC invariably led to loss of function in these cases. The mechanism by which these detergents solubilize membranes at concentrations below the CMC, and the related loss of function above the CMC remain largely unexplored. This has given rise to the concept of 'effective CMC' (the concentration of detergent existing as monomers at any given condition) [32,34] which takes into account contributions from all the components (lipids, proteins, pH, temperature and ionic strength) in the specific system under study. Determination of the effective CMC could serve as a useful indicator in solubilization of membrane proteins under various experimental conditions. Thus, solubilization could occur below the CMC if the effective CMC is low.

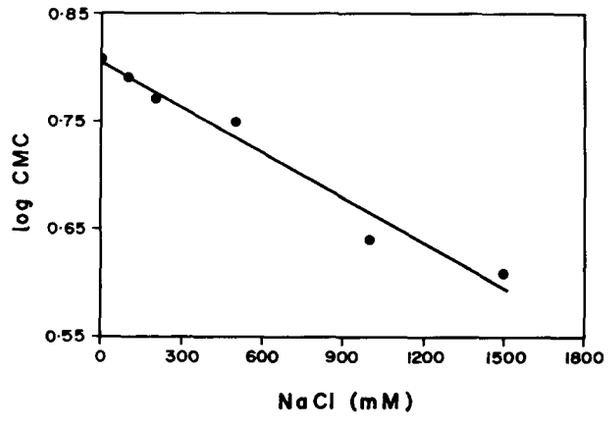

Fig. 2. Dependence of the logarithm of the CMC of CHAPS on salt concentration. All other conditions are as in Fig. 1. See Section 2 for other details.

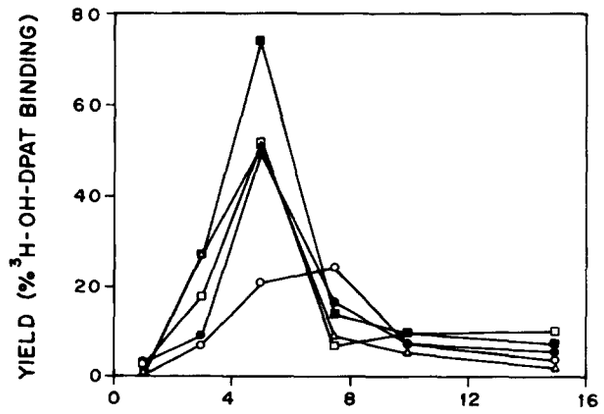

Fig. 3. Solubilization of crude membranes containing the 5-HT_{1A} receptor with different concentrations of CHAPS and NaCl. The concentration of NaCl used was 0 (○), 0.25 M (●), 0.5 M (□), 1 M (■), and 1.5 M (△) Results are expressed as percentage of binding for an equal volume of crude membrane. See Section 2 for other details.

In this report, we have shown that the CMC of CHAPS is dependent on the salt concentration of the medium, and that optimal solubilization of the 5-HT_{1A} receptor can be achieved with pre-micellar concentration of CHAPS under high salt concentration. Although salt dependence of CMC for charged detergents is well documented [11–13], to the best of our knowledge, this is the first report describing such salt dependence of CMC for detergents such as CHAPS. This observation is significant in view of our present results on solubilization of 5-HT_{1A} receptor, and the fact that the efficiency of CHAPS in solubilizing a number of membrane-bound receptors from the central and peripheral nervous systems such as the dopamine receptors [26], and opioid receptors [17,28] have been reported to be dependent on salt concentration with the efficiency increasing with higher salt concentrations. In many of these studies, the concentration of CHAPS needed for efficient solubilization is pre-micellar, as judged by its CMC in water. Our present results show that under such solubilization conditions, the CMC of CHAPS is significantly lowered due to the presence of salt (see Table 1). This explains the increased efficiency of solubilization at the pre-micellar concentrations.

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