Journal of Colloid and Interface Science 453 (2015) 79–89

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Determination of thermodynamic potentials and the aggregation number for micelles with the mass-action model by isothermal titration calorimetry: A case study on bile salts

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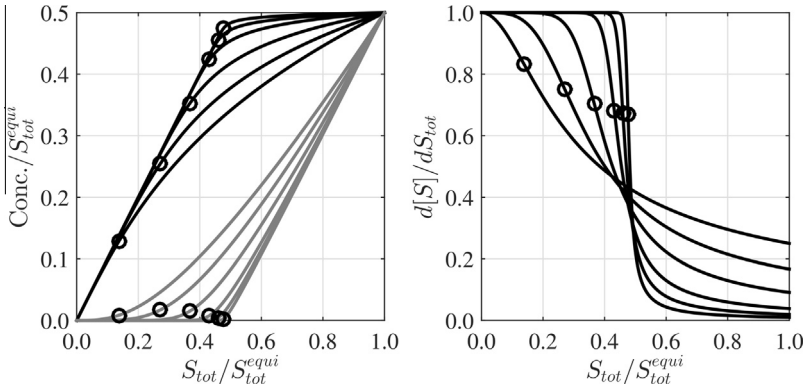
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 January 2015
Accepted 30 March 2015
Available online 2 May 2015

Keywords:
Bile salts
Micelle
Homo-oligomer
Aggregation number
Critical micelle concentration
Mass-action model
Isothermal titration calorimetry

ABSTRACT

The aggregation number (n), thermodynamic potentials (ΔG, ΔH, ΔS) and critical micelle concentration (CMC) for 6 natural bile salts were determined on the basis of both original and previously published isothermal titration calorimetry (ITC) data. Different procedures to estimate parameters of micelles with ITC were compared to a mass-action model (MAM) of reaction type: $n{\cdot}S \rightleftharpoons M_n$. This analysis can provide guidelines for future ITC studies of systems behaving in accordance with this model such as micelles and proteins that undergo self-association to oligomers. Micelles with small aggregation numbers, as those of bile salts, are interesting because such small aggregates cannot be characterized as a separate macroscopic phase and the widely applied pseudo-phase model (PPM) is inaccurate. In the present work it was demonstrated that the aggregation number of micelles was constant at low concentrations enabling determination of the thermodynamic potentials by the MAM. A correlation between the aggregation number and the heat capacity was found, which implies that the dehydrated surface area of bile salts increases with the aggregation number. This is in accordance with Tanford's principles of opposing forces where neighbouring molecules in the aggregate are better able to shield from the surrounding hydrophilic environment when the aggregation number increases.

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http://dx.doi.org/10.1016/j.jcis.2015.03.069
0021-9797/

1. Introduction

Bile salts are important in both basic science and clinical applications and have recently attracted renewed interest [1–3]. Bile salts are the main catabolic product of cholesterol and functions as detergents in solubilization and absorption of dietary fats and lipophilic vitamins. In addition, bile salts are known to have significant impact of the bioavailability of drugs [4], they are under current investigation as drug delivery systems of non-soluble compounds [5,6] and certain bile acids have been employed as therapeutic agents for treatment of liver and metabolic disorders [2,7].

Owing to their amphiphilic nature bile salts are able to self-associate into micelles and a large number of studies have addressed different thermodynamic and structural aspects of this [2,8]. One key parameter in this area is the critical micelle concentration (CMC), which has been measured by a number of methods. Much of this work has been reviewed recently [2,3,8] and it has been emphasized that bile salt micelles have much smaller aggregation numbers compared to micelles composed of surfactants with straight-chain hydrophobic moieties. The small size and aggregation numbers raises a number of challenges regarding both analytical techniques and the thermodynamic interpretation of the experimental data. It is therefore still debated whether bile salt-aggregates are true micelles and controversies regarding properties such as size, structure, stabilizing forces and polydispersity remain [9]. Some authors prefer to characterize the aggregation as a multimerization reaction and therefore only define CMC as a concentration range [3,10–15] or uses the concept of a noncritical multimerization concentration [16]. Thermodynamic descriptions of the formation of bile salt micelles may also become problematic inasmuch as the widely applied pseudo-phase separation model (PPM) is not suitable for micelles with such small aggregation numbers. Bile salt micelles have often been reported to contain well under a dozen monomers, which is not immediately reconcilable with the idea of a separate phase. However, due to their small size the aggregation number is hard to determine by standard analytical techniques and a vast amount of methods have been used at a concentration above CMC where the aggregation number is increased and depends on the concentration [2].

In contrast, ITC enables measurements of the thermodynamic potentials at the CMC and has therefore previously been applied to investigate bile salt micellization [17–19]. Recently, a large study investigating bile salts by ITC was reported by Maestre et al. for a range of temperatures and at two different salt concentrations [20]. Maestre et al. made a thermodynamic analysis based on the PPM, which does not enable determination of aggregation numbers. These are, however, needed to make an accurate description of the stability of micelles with aggregation numbers, as bile salts. Currently, many published ITC studies on micellization do not determine the aggregation number [21–24], possible due to the complicated estimation process and the absence of pre-implemented protocols in standard software. To facilitate this we have recently, proposed a simplified estimation method, to determine the aggregation number from ITC-data in a fast and reliable way [25]. The present work shows, in line with our previous study [25], that ITC was well suited for determination of the aggregation number of micelles. As the MAM only assumes a concentration-independent aggregation number $n{\cdot}S \rightleftharpoons M_n$ it can be applied to other systems, which behave in accordance with this model such as proteins that undergo self-association to homo-oligomers of distinct molecularity [26].

In this work the thermodynamic potentials for bile salt micelles were solely based on the MAM, therefore different values were determined compared to the results from the PPM obtained by Maestre et al. To convey comparison between the estimates from PPM and the MAM, an in-depth analysis of the differences is made in the theory section. By analyzing the data in accordance with the MAM, the parameter estimates were improved and new light can thereby be shed on the correlation between the structure and the thermodynamic potentials of micelles with low aggregation numbers, here represented by bile salt.

2. Experimental section

2.1. Materials

The sodium salts of the six bile acids were purchased from various sources. Taurocholate (TC; CAS no. 83830-80-2) was purchased from Fluka (Buchs, Switzerland). Taurochenodeoxycholate (TCDC, CAS no. 516-35-8), taurodeoxycholate (TDC; CAS No. 516-50-7), glycocholate (GC; CAS No. 475-31-0), glycodeoxycholate (GDC; CAS No. 16409-34-0), and glycochenodeoxycholate (GCDC; CAS No. 640-79-9) were purchased from Sigma Aldrich (St. Louis, MO, USA). All bile salts were dried at least 24 h in a vacuum oven before use. Bile salt solutions of predefined concentration were prepared by weighing and dissolution in 50 mM phosphate buffer with a cat-ion concentration of 78.3 mM, pH 7.0.

2.2. Isothermal titration calorimetry

Microcalorimetric titrations were performed at atmospheric pressure at 25 °C and 37 °C, in a MicroCal VP-ITC titration micro calorimeter (MicroCal, Northampton, MA, USA). The reaction cell (V = 1.4442 mL) was filled with degassed buffer. Aliquots of degassed bile salt solutions were subsequently injected into the filled reaction cell during stirring at 307 rpm. The concentration of bile salt in the syringe was respectively 40 mM (TCDC), 40 mM (GCDC), 40 mM (TDC), 50 mM (GDC), 200 mM (TC) and 200 mM (GC) to ensure that the CMC was reached in the reaction cell during the experiment.

3. Theory

Micellization can be investigated by ITC in a so-called dilution experiment, where a solution of concentrated micelles above CMC is titrated into a buffer. To extract information from an ITC experiment a functional relationship – known as a binding isotherm – is applied. Mathematically this is expressed as a system of two coupled equations. The first is a constitutive equation relating the experimentally controlled variable (i.e. the total surfactant concentration in the reaction cell, S_{tot}) to the measured response from the ITC. The second equation is a model of the chemical reaction incorporating conservation of mass and the reaction mechanism in terms of the micellization constant.

The constitutive equation

The constitutive equation can be derived by considering that the heat detected by the ITC is proportional to the change in number of moles of micelles, i.e. $dQ = \Delta H_{mic} \cdot V_{cell} \cdot n \cdot d[M_n]$.

Usually the heat is normalized by the increase in total surfactant concentration, and we therefore write

$$\frac{dQ}{dS_{tot}} = V_{cell} \cdot \Delta H_{mic} \cdot n \cdot \frac{d[M_n]}{dS_{tot}} \tag{1}$$

where dQ is the heat, ΔH_{mic} is the enthalpy of micellization, V_{cell} is the volume of the reaction cell, S_{tot} is the total surfactant concentration and $[M_n]$ is the concentration of micelles each with an aggregation number of n monomers.