Journal of Colloid and Interface Science 501 (2017) 294–303

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

The *cmc*-value of a bolalipid with two phosphocholine headgroups and a C_{24} alkyl chain: Unusual binding properties of fluorescence probes to bolalipid aggregates

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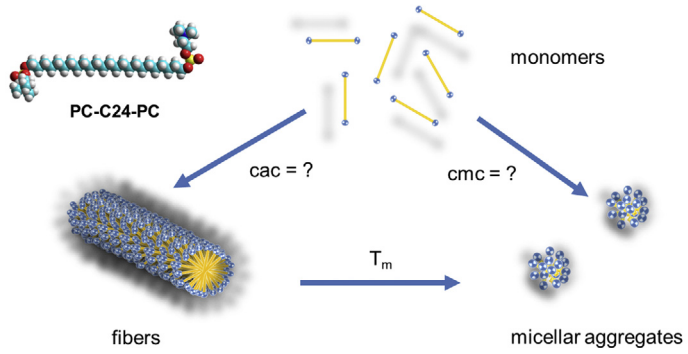
GRAPHICAL ABSTRACT

Determination of the aggregation concentration of the bolalipid PC-C24-PC



ARTICLE INFO

Article history:
Received 8 March 2017
Revised 24 April 2017
Accepted 24 April 2017
Available online 25 April 2017

Keywords:
Bolalipids
Nanofibers
Micelles
Critical aggregation concentration
Fluorescence probes
cmc-value

ABSTRACT

Bolalipids with a long alkyl chain and two phosphocholine polar groups self-assemble in water into two different types of aggregate structures, namely helical nanofibers at low temperature and two types of micellar aggregates at higher temperature. We tried to determine the critical aggregation concentration (*cac*) or critical micellar concentration (*cmc*) of the bolalipid tetracosane-1,24-bis(phosphocholine) (PC-C24-PC) by using different fluorescent probes. The use of pyrene or pyrene derivatives as fluorophores failed, whereas the probes 1,8-ANS and particularly bis-ANS gave consistent results. The structure of the bolalipid aggregates obviously hinders partitioning or binding of pyrene derivatives into the micellar interior, whereas 1,8-ANS and bis-ANS can bind to the surface of the aggregate structures. The observed large increase in fluorescence intensity of bis-ANS indicates that binding to the hydrophobic surface of the aggregates leads to a reduction of the dye mobility. However, binding of bis-ANS is relatively weak, so that the determination of a *cac*/*cmc*-value is difficult. Simulations of the intensity curves for PC-C24-PC lead to estimates of the *cac*/*cmc*-value of 0.3–1.0 $\times$ 10^{-6} M, depending on the structure of the aggregates. Single molecule fluorescence correlation spectroscopy was used to determine the mobility of bis-ANS as a function of concentration of PC-C24-PC. The dye diffusion time and the molecular brightness are lower at low bolalipid concentration, when only free dye is present, and increase at higher concentration when bis-ANS is bound to the aggregates. The experimental *cac*/*cmc*-values are higher than those estimated, using an incremental method for the change in Gibbs free energy for micellization with

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http://dx.doi.org/10.1016/j.jcis.2017.04.077

n-alkyl-phosphocholines with only one polar group as a comparison. Apparently, for PC-C24-PC in micellar or fibrous aggregates, more CH_2 groups are exposed to water than in a conventional micelle of an n-alkyl-phosphocholine.

1. Introduction

Bolaamphiphiles are a class of molecules which have been studied quite extensively in the last years. These molecules (bolalipids) are composed of one or two long hydrophobic chains with two polar headgroups at the ends. Bolalipids with two chains and ether linkages can be found in Archeae. They provide the membranes with the required rigidity and chemical stability for survival in extreme environments of high temperature and low pH. Single-chain bolalipids are much simpler and easier to synthesize and can have other functions. For instance, a bolalipid with phosphocholine (PC) headgroups at both ends of a hydrocarbon chain of only twenty-two carbon atoms (PC-C22-PC, Irlbacholine) was found in plants and was suggested to have fungicidal activity [1]. Single-chain bolalipids have been synthesized by various groups and it was found that self-assembly of these bolalipids can lead to micellar structures, vesicles with monolayer membranes, or hollow tubes when an asymmetry between the headgroups exist [2,3].

We synthesized a variety of bis-phosphocholines with one hydrocarbon chain varying in length from 22 to 36 carbon atoms (PC-Cn-PC) [4–10]. The attractive forces driving the self-assembly of these bolalipids arise exclusively from the hydrophobic effect. Due to the steric mismatch of the much larger cross-sectional area of the PC headgroup compared to the alkyl chain, an aggregation in the form of helical nanofibers was observed. These nanofibers form of a dense network leading to a very efficient gelation of the water. Upon heating, a transition at a well-defined temperature is observed, where a break-down of the fibers into micellar aggregates (type I) occurs with a loss of the gel behavior [7,8]. A further transition into another micellar aggregate (type II) with increased fluidity of the chains and slightly lower aggregation number and smaller size occurs at even higher temperature. For PC-C32-PC the first transition is observed at ca. 50 °C while for PC-C24-PC this transition is shifted to 16–17 °C. The driving force for the endothermic transition between the two types of micellar aggregates occurring at elevated temperature, namely 35 °C, in the case of PC-C24-PC is still unclear [6].

In this paper we address another, also previously unresolved question, namely the critical aggregation or micellization concentration (*cac*/*cmc*) of these single chain bolalipids of the type PC-Cn-PC. For single chain amphiphiles with one headgroup, it is well known that the logarithm of the *cmc* decreases with the number of carbon atoms n in the chains in a linear fashion [11]:

$$\ln(cmc') = \ln\left(\frac{cmc}{55.55}\right) = b_0 - b_1 n = \frac{\Delta G_{mic}}{RT} \quad (1)$$

with *cmc'* being the critical micellization concentration expressed in mole fraction units, the paramter b_0 being dependent on the nature of the amphiphile headgroup and b_1 describing the slope [11,12]. The ln *cmc'* is therefore directly proportional to the change in Gibbs free energy ΔG_{mic} for micellization, the value of $b_1 \cdot RT$ corresponding to the incremental change in ΔG_{mic} per additional CH_2 group [12]. For a number of different ionic surfactant systems this value was found to be ca. 1.7–1.8 kJ mol^{-1} and for some nonionic and zwitterionic surfactants ca. 2.8–2.9 kJ mol^{-1} per CH_2 group. These values are slightly lower than the CH_2 incremental value for transfer from water to a pure hydrocarbon (3.7 kJ mol^{-1} per CH_2) [13]. As a rule of thumb, the elongation of an alkyl chain of a nonionic or zwitterionic surfactant by two CH_2 groups usually decreases the *cmc* by a factor of 10. For instance, for the zwitterionic lipid lyso-phosphatidylcholine, the *cmc* decreases from 7×10^{-3} M to 7×10^{-6} M, i.e. a factor of 1000, when the chain is elongated by 6 CH_2 groups from 10 to 16 carbon atoms. For the amphiphile decyl-phosphocholine (C10-PC) with 10 carbon atoms, a value of $\sim 11 \times 10^{-3}$ M was reported for the *cmc* [14] and for the same amphiphile with 16 carbon atoms (C16-PC), the *cmc* decreased to $\sim 12 \times 10^{-6}$ M, again a factor of ca. 1000 for additional 6 CH_2 groups [15–17]. For diacyl phosphatidylcholines, however, the increment in ΔG_{mic} per additional CH_2 group decreases to 2.1 kJ mol^{-1}, because for the double-chain amphiphiles the two acyl chains seem to be in contact already in the monomeric state, thus reducing the solvent exposed surface area. Therefore, the absolute value of the *cmc* is higher for a diacyl phosphatidylcholine with the same number of carbon atoms as the corresponding lyso-phosphatidylcholine. This also leads to a reduced dependence of the *cmc* on chain length as not the complete surface of each additional CH_2-group is exposed to water [13,16].

In the case of bolalipids PC-Cn-PC only one chain is present, but there are two polar groups located at opposite ends of the chain. The question arises how the *cmc* is influenced by this additional polar group. From the chain length dependence of the related phosphocholines with only one polar group one can calculate the free energy contribution of the headgroup to the parameter b_0 using Eq. (1). For the bolalipids PC-Cn-PC with two identical headgroups one would therefore expect a relation for the *cmc'* according to Eq. (2):

$$\ln(cmc') = \ln\left(\frac{cmc}{55.55}\right) = 2b_0 - b_1 n = \frac{\Delta G_{mic}}{RT} \quad (2)$$

A prerequisite for the application of this equation using b_0 and b_1 values obtained from Cn-PCs is that the chain of the bolalipid is fully accessible to water, *i.e.* that for the monomeric state no back-folding of the chain occurs, reducing the water accessible surface area of the alkyl chain. The *cmc* of bolalipids with two charged headgroups, *i.e.* alkyl-α,ω-bis(trimethylammonium) dibromides, have been measured before in the group of Zana [18,19] and by Yasuda et al. [20] From the chain length dependence of the *cmc* it was found that the incremental value of the free energy of transfer per CH_2 group was slightly lower as compared to the values found for the ionic surfactant with just one cationic headgroup, the alkyltrimethylammonium bromides [21]. As this class of bola-surfactant has charged headgroups, a comparison with the bolalipids with zwitterionic headgroups investigated here is difficult, as the charged headgroups have a strong influence on the aggregation properties [13].

We have tried to determine the *cmc* of the bolalipid PC-C24-PC (see Fig. 1) at three different temperatures, where aggregation occurs either to nanofibers or to two different types of micellar aggregates with different fluidity of the alkyl chains. We used dodecyl-phosphocholine (C12-PC) as a reference compound representing half of the bolalipid PC-C24-PC (see Fig. 1). For the determination of the *cmc* we used fluorescence spectroscopy and tested several fluorescence probes, such as pyrene, pyrene-3-carboxyaldehyde [22,23], 1,8-ANS [24,25] and bis-1,8-ANS (bis-ANS) [26–28]. It turned out that the structure of the fiber and micellar aggregates of PC-C24-PC must be different compared to conventional micellar structures formed by simple amphiphiles with alkyl chains, as many of the fluorescence probes