Colloids and Surfaces B: Biointerfaces 123 (2014) 575–581

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Effect of choline carboxylate ionic liquids on biological membranes

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ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
11 September 2014
Accepted 28 September 2014
Available online 5 October 2014

Keywords:
Choline ionic liquids
Biological membrane
Cytotoxicity

ABSTRACT

Choline carboxylates, ChC_m, with $m = 2–10$ and choline oleate are known as biocompatible substances, yet their influence on biological membranes is not well-known, and the effect on human skin has not previously been investigated. The short chain choline carboxylates ChC_m with $m = 2, 4, 6$ act as hydrotropes, solubilizing hydrophobic compounds in aqueous solution, while the longer chain choline carboxylates ChC_m with $m = 8, 10$ and oleate are able to form micelles.

In the present study, the cytotoxicity of choline carboxylates was tested using HeLa and SK-MEL-28 cells. The influence of these substances on liposomes prepared from dipalmitoylphosphatidylcholine (DPPC) was also evaluated to provide insights on membrane interactions. It was observed that the choline carboxylates with a chain length of $m > 8$ distinctly influence the bilayer, while the shorter ones had minimal interaction with the liposomes.

1. Introduction

The investigation of the toxicity of ionic liquids and surfactants is a very important field, since it was found that commonly used cations in ionic liquids like imidazolium or pyridinium are toxic in nature [1]. Further, choline compounds are forbidden in cosmetical products according to the European Cosmetic Directive 76/768/EEC, because they are assigned as quaternary ammonium compounds, which are known as phase transfer catalysts with intrinsic irritation potential [2]. In order to boost the utilization of choline compounds in future applications, cytotoxicity tests were performed to evaluate the actual skin irritation potential. Previous toxicity experiments considered the charge, the number of ethoxy groups [3] and the hydrophilicity, but in the case of surfactants it is also very important to consider the critical micelle concentration (*cmc*) [1,3–5]. The cytotoxicity of ionic surfactants is mainly caused by the monomers [3,6] and consequently, the IC_{50} values – concentrations required to cause death in 50% of the cell population – are usually found to be below the *cmc*. To check this for our systems, we also measured the *cmc*. In a previous study Petkovic et al. [7] investigated the toxicity of choline carboxylates ChC_m with $m = 2–10$ by carrying out tests with filamentous fungi as a model for eukaryotic organisms. Choline carboxylates were found to exert only minor toxic activity on these organisms. Also Muhammad et al. found just slight cytotoxic effects on the human breast cancer cell line MCF-7 for choline carboxylates ChC_m with $m = 2, 3, 4, 6$ [8].

The present study is focused on the influence of choline carboxylates on biological cell membranes. The aim of the paper is to determine if there is a correlation between IC_{50} values and the substrate concentration at which an interaction of the substance with the liposomes is found. Although the culture and buffer media are different, the primary interaction of interest is membrane disruption caused by choline carboxylate penetration, which should be possible to discern in both environments.

The IC_{50} values were determined for two human cell lines, namely cervix carcinoma cells (HeLa) and keratinocytes (SK-MEL-28) [9–13]. Cytotoxicity was analysed for short chain choline carboxylates ChC_m with a chain length of $m = 2–10$ and their respective sodium equivalents. To further investigate the influence of the double bond in an alkyl chain on the cytotoxicity, IC_{50} values of choline oleate and choline octadecanoate were determined. Further, the impact of an additional carboxylate group was examined by analyzing the cytotoxicity of choline bicarboxylates, like choline succinate ($ChbiC_4$), choline glutarate ($ChbiC_5$) and choline adipate ($ChbiC_6$) and their sodium equivalents (Fig. 1).

To better understand interfacial interactions with membranes, the influence of the choline carboxylates ChC_m with $m = 2–10$ and

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http://dx.doi.org/10.1016/j.colsurfb.2014.09.057
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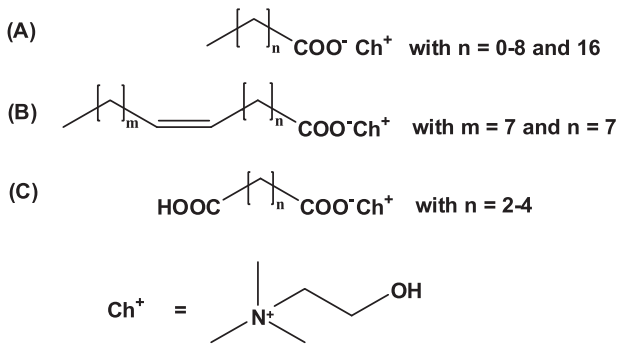

Fig. 1. Cytotoxicity was measured of choline carboxylates ChC_m with $m = 2–10$ and 18 (A), of choline oleate (B) and of choline bicarboxylate $ChbiC_m$ with $m = 4–6$ (C) and in addition of their respective sodium equivalents.

choline oleate on the gel to liquid crystalline transition of cooperativity behavior of DPPC liposomes, a basic biological membrane analog, was investigated at the biological pH of 7.4.

To ensure that the toxicity results only from the surfactant monomer, and is not influenced by the formation of micelles, the critical micelle concentration of choline octanoate, choline decanoate and choline oleate was determined by conductivity and surface tension measurements. The hydrotrope behavior of the short chain choline carboxylates like choline acetate, choline butanoate and choline hexanoate was investigated. Sodium alkylbenzene sulfonates and sodium butyl monoglycolsulfate (NaBMGS) are used to extract non-polar, water insoluble phyto-constituents selectively due to permeabilization of the cell. Also choline hexanoate is able to dissolve suberin out of cork by cleavage of the ester bonds in suberin [14].

2. Materials and methods

2.1. Synthesis

The synthesis of choline acetate was done by neutralization of acetic acid (Merck, purity 100%) with 45% methanolic choline hydroxide solution (Sigma–Aldrich). The obtained salt was recrystallized twice in diethylether. A white salt was obtained after lyophilization and drying under vacuum atmosphere.

Choline carboxylates (ChC_m) with $m = 4, 5, 6, 7, 8, 9, 10$, choline oleate and choline bicarboxylates ($ChbiC_m$) with $m = 4, 5, 6$ were synthesized according to the synthesis route of Petkovic et al. but with minor modifications [7]. In contrast to the synthesis of Petkovic et al. [7] the choline carboxylate ionic liquid was lyophilized and then dried for more than two weeks on a high vacuum pump. Heating during this procedure was skipped to avoid decomposition of the choline cation.

The following chemicals were used: Oleic acid (Sigma–Aldrich, purity ≥ 99%), propionic acid (Sigma–Aldrich, purity ≥ 99.5%), butyric acid (Sigma–Aldrich, purity ≥ 99%), valeric acid (Sigma–Aldrich, purity ≥ 99%), hexanoic acid (Sigma–Aldrich, purity ≥ 99%), heptanoic acid (Merck, purity ≥ 99%), octanoic acid (Sigma–Aldrich, purity ≥ 99%), nonanoic acid (Sigma–Aldrich, purity ≥97%), decanoic acid (Alfa Aesar, purity = 99%), adipic acid (Alfa Aesar, purity ≥ 99%), glutaric acid (Alfa Aesar, purity ≥99%), succinic acid (Alfa Aesar, purity ≥ 99%) and 80 wt% aqueous choline bicarbonate solution (Sigma–Aldrich, stored at 2 °C to avoid decomposition and without stabilizer).

The purity of the choline carboxylates was evaluated with electro-spray mass spectroscopy (ThermoQuest Finnigan TSQ 7000 instrument) (ES-MS) and ^1H and ^{13}C NMR measurements (Bruker Avance 300 spectrometer at 300 MHz using tetramethylsilane (TMS) as internal standard) were performed. Coulometric Karl-Fischer titration was performed on an Abimed MCI analyzer (Model CA-02) to determine the water content.

The sodium salts were prepared by adding an equimolar amount of 1 M sodium hydroxide solution (Merck) to the corresponding carboxylic acid. The sodium salts were lyophilized (>24 h) and dried under vacuum atmosphere. For the synthesis the above mentioned carboxylic acids were used. Sodium octanoate (Sigma–Aldrich, purity ≥ 99%), sodium decanoate (Sigma–Aldrich, purity ≥ 98%) and sodium oleate (Sigma–Aldrich, purity = 99%) were bought. The melting temperatures of the choline based ionic liquids are given in the SI.

2.2. UV–Vis measurements

To determine the concentration of hydrotrope in water at which the solubilization of hydrophobic dye in the aqueous hydrotrope solution of choline acetate, choline butanoate or choline hexanaote increase, Disperse Red 13 was solubilized as hydrophobic dye. All the solubilization experiments and the UV–Vis measurements were performed in a thermostated room at 25 ± 0.2 °C. Different concentrated aqueous hydrotrope solutions were prepared and saturated with Disperse Red 13. After stirring for 24 h the excess of dye was removed. The amount of the dissolved Disperse Red 13 was determined with UV–Vis measurements on a Varian Cary 3E spectrophotometer. The absorption was measured at the wavelength of 503 nm [15]. The calibration curve with defined amounts of Disperse Red 13 was prepared by dissolving Disperse Red 13 in absolute ethanol (Baker, purity ≥ 99.9%).

2.3. Conductivity

A valuable method to determine the critical micelle concentration *cmc* and also to determine the micelle ionization degree α at the *cmc* is to measure the concentration dependent specific conductivity κ of an aqueous choline carboxylate solution ChC_m with $m = 8$, 10 and oleate. An autobalance conductivity bridge (Konduktometer 702, Knick), arranged with a Consort SK41 T electrode cell was used to measure the specific conductivity. For the calibration 0.01 M, 0.1 M and 1 M potassium chloride solutions were needed to determine the cell constant at 25 °C and 40 °C [16]. The concentration dependent conductivity was evaluated at 25 °C and 40 °C. A break in the slope of the plot of concentration versus the specific conductivity κ marks the *cmc* of the respective choline carboxylates (see Supplementary Information (SI)). The accuracy of the *cmc* values is ±4%. We are aware that the culture medium would cause a minor decrease in the *cmc* because of the high ionic strength. However, this slight shift would not change our qualitative conclusions.

For the calculation of the micelle ionization degree α at the *cmc* Eq. (1) presented by Evans [17] was used:

$$0 = \left[N^{\frac{2}{3}}(1000 \cdot S_1 - \lambda_{Ch+})\right] + \alpha - \quad (1)$$

λ_{Ch+} is the counterion conductivity given by Fleming as 42.0 S cm^2 [18]. N represents the amount of surfactant molecules in the micelle and S_1 the slope before the *cmc* and S_2 the slope above the *cmc*. The micelle ionization degree α could only be calculated for choline oleate (Table 1). Choline octanoate and choline decanoate are at the limits of Eq. (1) and present too small slopes.

2.4. Surface tension

To determine the critical micelle concentration of choline octanoate, choline decanoate and choline oleate at 25 °C and 40 °C the concentration dependent surface tension σ was measured. The temperature accuracy was 25 °C ± 0.1 °C and 40 °C ± 0.1 °C. The measurements were performed with a Krüss tensiometer (model