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Determination of number-average aggregation numbers of bile salts micelles with a special emphasis on their oxo derivatives—The effect of the steroid skeleton

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ABSTRACT

Background: The special geometry of the steroid skeleton causes that bile acid anions, in contrast to aliphatic amphiphiles, form micelles with a small aggregation number.
Methods: The number-average aggregation numbers ($\overline{n}$) are determined using Moroi–Matsuoka–Sugioka thermodynamic method. Also, for analysed bile acid sodium salts functions between spin–lattice relaxation time (T_1) and concentration of monomers (c_{BA^-}) are determined.
Results: For 7-oxodeoxicholic (7-ODC) acid and hyodeoxicholic acid (HD) monomers, curve $T_1 = f(c_{BA^-})$ contains two inflexion points. Mentioned monomers and cholic acid anion (C) are influential observations in relation to a line of linear regression between $\overline{n}$ and para\meter of monomer hydrophobicity (ln*k*, retention capacity from RPHPLC). This suggests that, in micelles of bile acid anions: 7-ODC, HD and C, beside main, hydrophobic interactions, hydrogen bonds are also possible between building units.
Conclusion: The increase in the number of oxo groups in the molecule is accompanied with a decrease in the hydrophobicity of the convex side of the steroid skeleton of the bile acid anion, resulting in a lower aggregation number. Obtained results indicate that C12 and C7 α-axial OH and oxo groups on the same C atoms of the investigated bile acid molecules have different spatial environment, which is confirmed by conformational analysis.
General significance: Deviation from the linear model: number-average aggregation numbers with hydrophobicity of monomers, suggests the existence of additional, intermolecular interactions beside hydrophobic in micelles.

1. Introduction

Bile acid salts are ionic amphiphilic compounds with a steroid skeleton. They represent a special group of amphiphilic compounds as they belong to biplanar amphiphiles. This is due to molecular geometry of cholanoic acid, of which all other bile acids of the 5β series can be derived. The concave (α) side of the steroid skeleton of bile acid molecules is hydrophilic (presence of OH groups), whereas the convex side with its angular methyl groups is hydrophobic. Bile acid salts in concentrations above critical micelle concentration form aggregates—micelles in water solutions [1–3]. According to the oldest, Smalls concept, primary micelles whose building units are connected over their steroid skeletons β sides (hydrophobic binding) are formed first. At higher concentrations, primary micelles form secondary micelles by hydrogen bonds [4,5]. Bile acid anions aggregation numbers range from 2 (Smalls primary micelles) to 15 (secondary micelles), so are far behind aggregation numbers of alkylsulfates (sodium-dodecylsulfat can have aggregation numbers up to 120) [6].

Primary bile acids (cholic and chenodeoxycholic) are synthesised in the liver of vertebrates, where they conjugate with glycine and taurine. The main physiological role of bile salts is emulsification of fats in the intestinal lumen, which enhances efficiency of the hydrolysis of triglycerides with the enzyme lipase. Bile salts form mixed micelles with monoacylglycerol, long-chain fatty acids and cholesterol, which facilitates transport of lipid components from the lumen to the intestinal epithelium. Bile acids also participate in the regulation of cholesterol homeostasis. Namely, in the gall bladder, salts of conjugated bile acids form mixed micelles with phospholipids and cholesterol, that are excreted to the duodenum [7,8]. Bile acids are endogen ligands for nuclear FXR receptor and G-protein coupled TGR5/Gpbrar1 receptor. By modulation of signal paths where higher receptors are included, bile acids modulate their biosynthesis *i.e.* participate in regulation of homeostasys of fat and glucose [9–12].

Sodium salts of chenodeoxycholic and ursocholic acids are of importance in the pharmacological industry, as their preparations are used for dissolution of cholesterol gallstone [8]. Generally, the more hydrophobic is the bile acid anion, the greater is its solubilisation effect. However, at the same time its membranotoxicity is increased [13–16]. Due to a lower hydrophobicity of the β (convex) side of the steroid skeleton, bile acid oxo derivatives exhibit a lower toxicity to membranes [17]. In the *in vitro* conditions, these compounds show a promotive action

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on the transport of some drugs (morphine, lidocaine, verapamil, etc.) through the hydrophobic barriers (depot effect) [18,19]. In the pharmacological experiments on animals, they lowered the glucose blood level and increased permeability of the blood–brain barrier, etc. [20–26]. Many pharmacological effects of bile oxo derivatives are due to interaction of their mixed micelles and the drug (biomolecule) [16,18,27–31]. Because of that, it is necessary to acquire detailed knowledge of micelle parameters such as critical micelle concentration (CMC) and aggregation number (n). CMC values of bile acid oxo derivatives have been determined [1,17]. However, data about their aggregation numbers lack in literature. The knowledge of these characteristics of bile acids oxo derivatives is of importance in view of their application in the pharmacological industry. Namely, a higher value of the aggregation number means a higher probability of being accepted of a hydrophobic guest species.

The objective of this work is determination of unknown number-average aggregation numbers ($\overline{n}$) [32] of bile salts oxo derivatives micelles (Fig. 1) using the thermodynamic Moroi–Matsuoka–Sugioka pH-metric method in a system consisting of the bile acid solid phase and the aqueous solution of its sodium salt (in monomer and micellar forms) [33,34]. The above mentioned method is chosen since it is non-invasive and can be applied for micelles with relatively small aggregation numbers as bile acid anion micelles. For example, static light scattering method for bile acid salts gives rough estimation of $\overline{n}$ [34] (which is not the case for conventional surfactants as alkylsulfates with micelles of 80 and more building units). The aim as well is to obtain the curve of dependence between spin–lattice relaxation time (T_1) and concentration of bile acid sodium salt (c_{BA^-}). The shape of the curve $T_1 = f(c_{BA^-})$ may help to shed light on aggregation processes of bile acid anions (number of jumps *i.e.* inflexion points on the curve, stretched or sharp changes) [35]. It is known that there is a good correlation between critical micelle concentration (CMC) of bile acid salts and reversed phase chromatographic parameters [36–41]. Also it is known that for aliphatic surface active molecules aggregation number grows with increase in the length of hydrocarbon series (on CMC values of sodium dodecylsulfat n = 33, while for sodium-octadecylsulfat $n = 78$ [42]). Thus, in order to examine dependence between $\overline{n}$ and hydrophobicity of bile acid sodium salts, capacity factors (k, measure for hydrophobicity of a molecule) in reversed phase HPLC are determined. The emphasis in the discussion is on the analysis of the effect of the structure of bile acid molecules steroid skeleton on the value of the micelle aggregation number (using new molecular referent system based on molecular graph [43]).

2. Materials and methods

2.1. Synthesis of oxo derivatives of cholic, deoxycholic and chenodeoxycholic acids

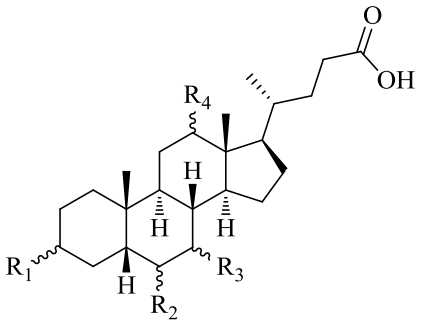
Cholic, deoxycholic and chenodeoxycholic acids (Sigma, New Zealand) were used as starting compounds for the synthesis of their oxo derivatives.

3α-Hydroxy-12-oxo-5β-cholanoic acid (12-OL) and 3α, 7α-dihydroxy-12-oxo-5β-cholanoic acid (12-OCD) were prepared according to the procedure of Miljković et al. [44], while 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid (7-ODC) and 3α-hydroxy-7-oxo-5β-cholanoic acid (7-OL) were obtained according to Tullar [45]. 3α-Hydroxy-7, 12-dioxo-5β-cholanoic acid (7,12-DOC) was synthesised by a selective oxidation of the 7α-hydroxy group of 3α,7α-dihydroxy-12-oxo-5β-cholanoic acid following the procedure of the same author (Tullar). 3,12-dioxo-5β-cholanoic acid (3,12-DOC) and 3,7-dioxo-5β-cholanoic acid (3,7-DOC) were obtained according to Fieser and Rajagopalan [46] Hyodeoxycholic acid was purchased from Sigma, New Zealand. All bile acids were transformed to their sodium salts by known procedure [3].

2.2. ^{1}H NMR studies

Stock solution of bile acid salts (10, 20, 40, 100, 140 or 300 mM in D_2O; dependent of the studied bile acid salts solubility) was diluted with D_2O to cover the appropriate concentration range. Measurements were performed at 25 °C on a Bruker Spectrospin-500 instrument with standard Bruker software. The ^{1}H NMR spectra were recorded using a spectral window of 3200 Hz. Spin–lattice relaxation times T_1 were determined by the inversion recovery experiments (180°-τ-90°-AQC) [35,47]. Selected peak areas for nine different interpulse delays τ were determined.

The spin–lattice relaxation time T_1 was determined for the singlet of the CH_3-18 methyl group of sodium salt's of the following bile acids: C; CDC; 7-ODC; 7-OL; 3,7-DOC and HD at 0.68–0.80 ppm in D_2O, and at 1.00–1.13 ppm in D_2O for the two overlapping singlets belonging



$R_1 = R_3 = R_4 = OH$; $R_2 = H$	3α,7α,12α-trihydroxy-5β-cholanoic acid (cholic a.); **C**
$R_1 = R_3 = OH$; $R_2 = R_4 = H$	3α,7α-dihydroxy-5β-cholanoic acid (chenodeoxycholic a.); **CDC**
$R_1 = R_4 = OH$; $R_3 = =O$; $R_2 = H$	3α,12α-dihydroxy-7-oxo-5β cholanoic acid (7-oxodeoxycholic a.); **7-ODC**
$R_1 = R_3 = OH$; $R_4 = =O$; $R_2 = H$	3α,7α-dihydoxy-12-oxo-5β-cholanoic acid (12-oxochenodeoxycholic a.); **12-OCD**
$R_1 = OH$; $R_3 = R_4 = =O$; $R_2 = H$	3α-hydroxy-7,12-dioxo-5β-cholanoic acid; **7,12-DOC**
$R_1 = OH$; $R_4 = =O$; $R_2 = R_3 = H$	3α-hydroxy-12-oxo-5β-cholanoic acid (12-oxolithocholic a.); **12-OL**
$R_1 = OH$; $R_3 = =O$; $R_2 = R_4 = H$	3α-hydroxy-7-oxo-5β-cholanoic a. (7- oxolithocholic a.); **7-OL**
$R_1 = R_4 = =O$; $R_2 = R_3 = H$	3,12-dioxo-5β-cholanoic acid; **3,12-DOC**
$R_1 = R_3 = =O$; $R_2 = R_4 = H$	3,7-dioxo-5β-cholanoic acid; **3,7-DOC**
$R_1 = R_2 = R_4 = OH$; $R_3 = H$	3α,6α-dihydroxy-5β-cholanoic acid (hyodeoxycholic acid); **HD**

Fig. 1. Structures of tested bile acids.