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Bile acids: Electrochemical oxidation on bare electrodes after acid-induced dehydration

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ABSTRACT

Bile acids and sterols in general have long been considered practically inactive for direct redox processes. Herein, a novel way of electrochemical oxidation of primary bile acids is reported, involving an initial acid-induced dehydration step, as confirmed by capillary electrophoresis–mass spectrometry, thereby extending the electrochemical activity of the steroid core. Oxidation potentials were found to be ca + 1.2 V *vs.* $Ag/AgNO_3$ in acetonitrile on boron doped diamond, glassy carbon, and platinum electrodes in a mixed acetonitrile–aqueous medium employing perchloric acid as a chemical reagent, and as a supporting electrolyte for the voltammetric measurements. The chemical step proved to be effective only for primary bile acids, possessing an axial 7α-hydroxyl group, which is a prerequisite for providing a well-developed voltammetric signal. Preliminary results show that other steroids, *e.g.,* cholesterol, can also be oxidized by employing a similar approach.

1. Introduction

Biosynthesis of the bile acids (BAs) is an important pathway for the metabolism and excretion of cholesterol in mammals [1]. Depending on the place of formation, literature discerns primary BAs, which originate in the liver, and secondary BAs that are formed by bacterial transformation of the primary BAs in the gut [2]. The most common primary BAs in humans are cholic (**1**; CA) and chenodeoxycholic (**2**; CDCA) acids.

The lack of double bonds or any fluorescent or electrochemically active groups in the molecules of BAs significantly limits the range of methods useful for their determination [3]. Gas chromatography after derivatization and HPLC in combination with mass spectrometry are commonly utilized for quantitation of individual BAs [4–6]. Other methods are based on detection of the products of enzymatic reactions, frequently using 3α-hydroxysteroid dehydrogenase as the key enzyme [7]. Electrochemical biosensors detecting the enzymatically generated NADH [8,9] or hydrogen peroxide [10] represent another strategy. Other reports on utilization of electrochemical methods for quantitation of BAs are scarce, as shown in our recent review [11]. BAs give electrochemical signal on mercury electrodes at far negative potentials [12,13], presumably as a result of catalytic hydrogen evolution from the carboxyl group in the side chain. Alternatively, electrooxidation has been reported in studies employing chromatographic separation with pulsed amperometric detection on gold [14,15] or porous graphite electrodes [16]. These studies, however, are mainly focused on the chromatographic aspects of the methods, rather than on the electrochemical processes themselves. Indirect oxidation using NaCl as a mediator succeeded in conversion of the hydroxyl groups of cholic acid into keto groups [17,18]. No study sufficiently characterizing the direct electrochemical oxidation of BAs has been published to date. Reports on steroids lacking any or possessing only isolated double bonds, including cholesterol, are scarce [11].

Herein, we present anodic oxidation of primary BAs on bare platinum, glassy carbon (GC), and boron doped diamond (BDD) electrodes in a mixed medium of acetonitrile–water–perchloric acid, where perchloric acid serves as a dehydrating reagent. Such an introduction of a double bond into the steroid skeleton can potentially increase the electrochemical activity. In the case of cholesterol, the double bond, together with the respective allylic positions, was identified as one of the sites of the electrochemical attack [19]. This approach, based on acid-induced dehydration, has also enabled a spectrometric determination of cholesterol (Liebermann-Burchard reaction) [20–22]. The proposed electrochemical approach could find application in the

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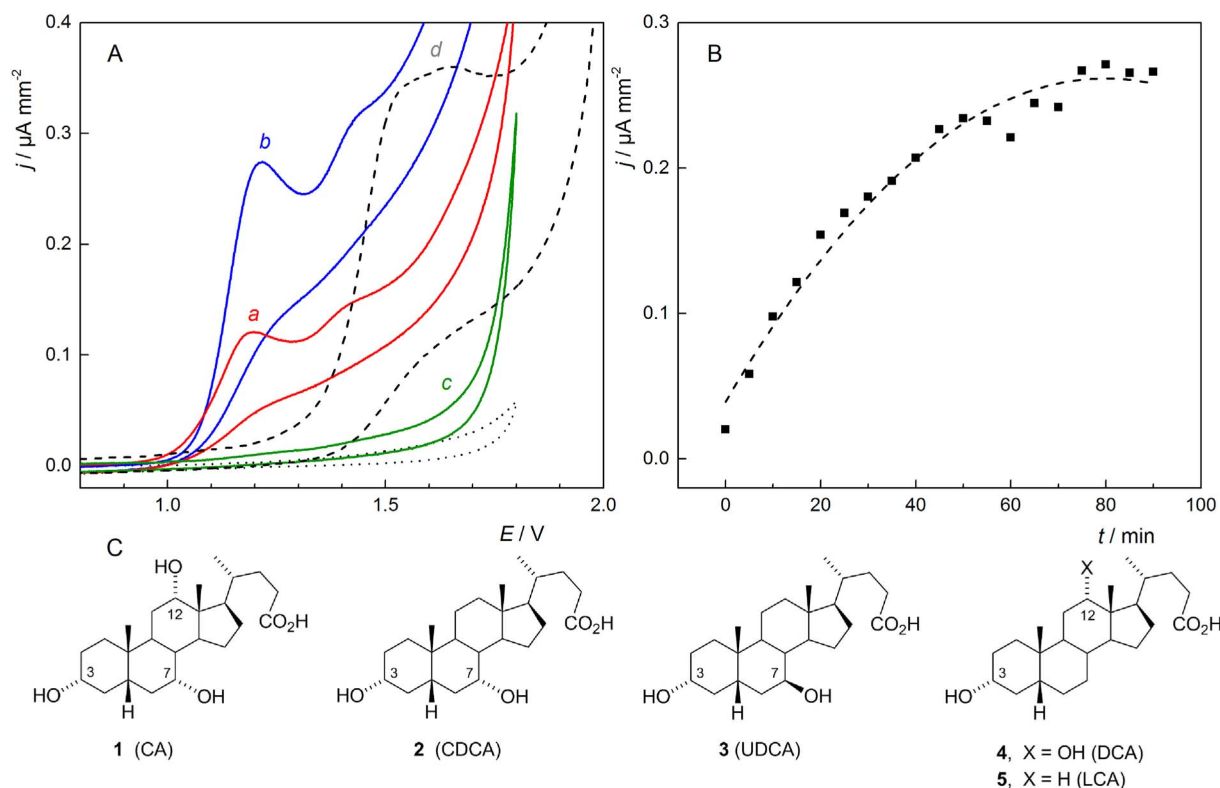

Fig. 1. (A) Cyclic voltammograms of (*a*) CA, (*b*) CDCA, (*c*) UDCA ($c = 9 \times 10^{-5}$ mol L^{-1}), and (*d*) cholesterol ($c = 5 \times 10^{-5}$ mol L^{-1}) in acetonitrile containing 0.1 mol L^{-1} $HClO_4$ and 0.43% H_2O on BDD electrode. Voltammograms recorded 90 min after the solutions were prepared from the stock solution and the supporting electrolyte. Supporting electrolyte in dotted line, scan rate 50 mV s^{-1}. (B) In-time development of the first CV peak height of CDCA ($c = 9 \times 10^{-5}$ mol L^{-1}). (C) Structural formulas of the BAs.

diagnosis of disorders of BA synthesis. A block in the biosynthesis of BAs in most cases results in a deficiency of the primary BAs [23].

2. Experimental

Cholic (**1**), chenodeoxycholic (**2**), ursodeoxycholic (**3**), deoxycholic (**4**), and lithocholic (**5**) acids, and cholesterol (all of > 99% purity, structures in Fig. 1C) were purchased from Sigma-Aldrich. All other commercially available chemicals were of analytical grade (if not stated otherwise).

Voltammetry was performed using a potentiostat PalmSens 2.0 with PSTrace 4.8 software. BDD ($A = 7.07$ mm^2, Windsor Scientific, UK), GC ($A = 3.14$ mm^2), or platinum ($A = 7.07$ mm^2; both Metrohm, Switzerland) working electrodes were used, routinely polished using alumina prior to each scan. Electrochemical cell with integrated reference electrode (Ag wire in 0.1 mol L^{-1} $AgNO_3$, 1 mol L^{-1} $NaClO_4$ in acetonitrile, separated from the measured solution by a salt bridge containing 0.5 mol L^{-1} $NaClO_4$ in acetonitrile) and a platinum foil counter electrode were employed. All experiments were carried out under the temperature of 21 °C. The contact time of the BA and $HClO_4$ and their concentrations are given in the caption of each voltammogram.

A modification of the previously described setup was used for capillary electrophoresis–mass spectrometry (CE-MS) [24]. The sample was hydrodynamically injected from an implemented PEEK cell: Sample volume 10 μL; injection time 2 s; separation voltage 18 kV. Parameters of fused silica capillary: Inner diameter 25 μm, outer diameter 360 μm, length 50 cm. Separation buffer: acetonitrile/1 mol L^{-1} acetic acid/10 mmol L^{-1} ammonium acetate. A Bruker microTOF (Bruker Daltonics, Germany) time-of-flight mass spectrometer equipped with a coaxial sheath liquid electrospray ionization (ESI) interface (Agilent, Waldbronn, Germany) was operated in positive ion mode; the mass range set 100–480 *m/z*; spectra rate 5 Hz. Source: ESI voltage: − 4000 V (grounded sprayer tip), plate offset: − 500 V; nebulizer: 1.0 bar; dry gas: 4.0 L min^{-1}; dry temperature: 190 °C. Transfer: capillary exit: 75.0 V; skimmer 1: 25.3 V; hexapole 1: 23.0 V; hexapole RF: 65.0 Vpp; skimmer 2: 23.0 V; lens 1 transfer: 38.0 μs; lens 1 pre pulse storage: 6.0 μs. Sheath liquid (2-propanol:water:formic acid, 49.9:49.9:0.2, v/v/v) was introduced by a syringe pump (KD Scientific, Holliston, MA, USA) with a flow rate of 0.48 mL h^{-1}.

3. Results and discussion

3.1. Voltammetric response of bile acids in the acetonitrile–water–perchloric acid medium

Electrochemical oxidation of two primary CA (**1**) and CDCA (**2**), and three secondary BAs, ursodeoxycholic acid (**3**, UDCA), deoxycholic acid (**4**, DCA), and lithocholic acid (**5**, LCA) in a mixed medium of acetonitrile–water containing perchloric acid was investigated.

Respective cyclic voltammograms on BDD electrode are presented in Fig. 1A (curves *a–c*). The overall process proved to be highly dependent on the structure. Only primary BAs with the axial 7α-hydroxyl group (CA, CDCA) afforded well-developed irreversible anodic signals at around + 1.2 V (curves *a, b*), *ca* 2.25 × higher for CDCA than CA, increasing in time (Fig. 1B). The difference in current densities for the different BAs can be rationalized by different, temperature-dependent rates at which each of the BAs undergoes the dehydration reaction. Such low oxidation potential has not been reported to date. That applies not only to BAs, but to any other steroid-based compounds, lacking any or possessing only isolated double bonds, including cholesterol. A proof of its oxidizability at + 1.5 V under the same conditions as those for BAs is presented by the voltammogram in Fig. 1A, curve *d*. The voltammograms of the secondary BAs, namely DCA and LCA lacking the 7α-hydroxyl group and UDCA possessing 7β-hydroxyl group, are featureless around this potential (shown for UDCA in Fig. 1A, curve *c*).

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