



## Regular article

# Preparing tauroursodeoxycholic acid (TUDCA) using a double-enzyme-coupled system



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## ABSTRACT

Tauroursodeoxycholic acid (TUDCA) has been used to treat many diseases effectively. TUDCA generation *in vivo* is a complex process involving five steps catalyzed by five enzymes in the enterohepatic circulation of bile salt. In this paper, we report a novel *in vitro* TUDCA preparation technology in which the epimerization of taurochenodeoxycholic acid (TCDC) to TUDCA is directly catalyzed by immobilized 7 $\alpha$ - and 7 $\beta$ -hydroxysteroid dehydrogenases (7 $\alpha$ - and 7 $\beta$ -HSDH) in a double-enzyme-coupled system. One pair of oxidoreductases from *Clostridium absonum* DSM599, which was made up of 7 $\alpha$ - and 7 $\beta$ -HSDH, was immobilized on a modified chitosan microsphere by separate immobilization and co-immobilization. The protein loading yields of 7 $\alpha$ - and 7 $\beta$ -HSDH were 79.06% and 87.18%, and their activity yields were 57.23% and 53.97%, respectively. In the batch-wise reactions catalyzed by double-enzyme-coupled system, 72.76% of the TCDC was transformed, and only 22.08% TUDCA was obtained on the basis of separate immobilization. The TUDCA yield was 62.49% under the catalytic effects of co-immobilized 7 $\alpha$ - and 7 $\beta$ -HSDH microspheres, while it was only 41.23% based on the mixture of separate immobilized 7 $\alpha$ - and 7 $\beta$ -HSDH microspheres. The enzyme catalytic technology described in this paper offers a potential application for synthesizing TUDCA and other high-value bile acid derivatives *in vitro*.

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## 1. Introduction

Bear bile has been used in traditional Chinese medicine for thousands of years. The major constituent of bear bile is tauroursodeoxycholic acid (TUDCA), which is the physiologically active form of ursodeoxycholic acid (UDCA). Bile acids, especially TUDCA, have been effectively used to treat hepatobiliary disease [1–4]. TUDCA and its deconjugated form, UDCA, can cross the blood–brain barrier and exert effects on the central nervous system, especially in Alzheimer's disease (AD) and Parkinson's disease therapies [5–7]. Furthermore, TUDCA can slow retinal degeneration [8–10], reduce the risk of myocardial infarction [11] and enhance islet function [12].

In the past, TUDCA preparation was always considered from the perspective of chemical synthesis [13,14]. TUDCA is the

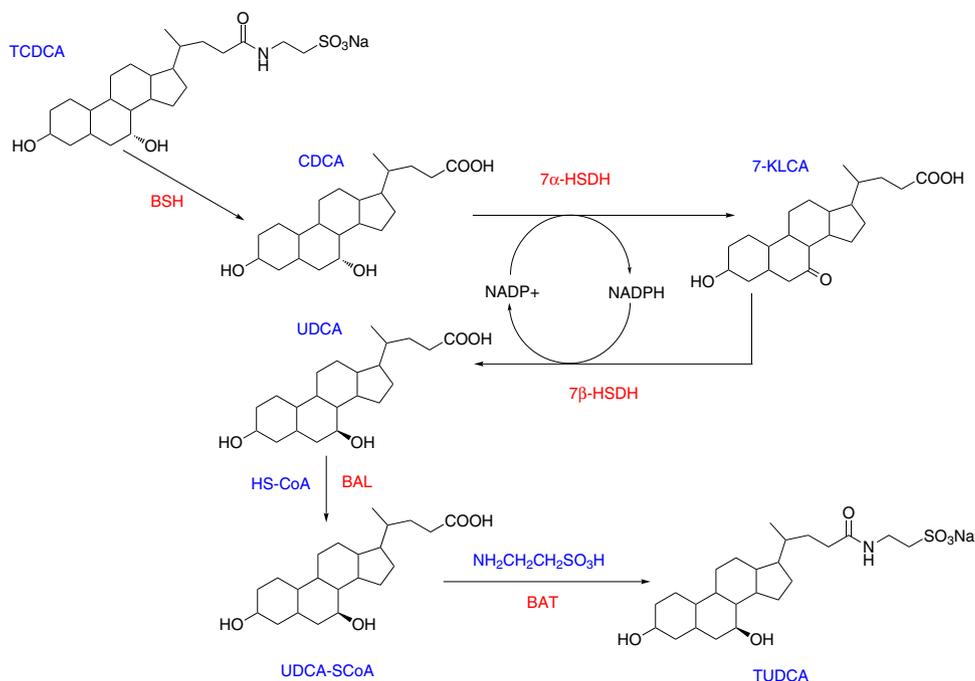
conformational isomer of taurochenodeoxycholic acid (TCDC), and TUDCA is primarily present in bear bile, making it different from other animal bile. TCDC is converted into TUDCA *in vivo* by five enzymes within a five-step process as shown in Fig. 1, which involves the deconjugation of TCDC by bile salt hydrolase (BSH) [15], the 7 $\alpha$ - or 7 $\beta$ -dehydroxylation of chenodeoxycholic acid (CDCA) [16], the ligation of UDCA and coenzyme A (CoA) thioesters by bile acid CoA ligase (BAL) [17] and the formation of TUDCA by the catalysis of amino acid N-acyltransferases (BAT) [18]. In previous study, we found that *in vitro*, TCDC could be directly transformed into TUDCA by 7 $\alpha$ - and 7 $\beta$ -hydroxysteroid dehydrogenases (7 $\alpha$ - and 7 $\beta$ -HSDH) in two steps, as shown in Fig. 2 and the other deconjugation and ligation steps were not indispensable [19]. Poultry bile is rich in TCDC, which is easy to harvest from this source [20]. We propose a novel protocol for *in vitro* TUDCA preparation using TCDC as a substrate.

In previous study, to make better use of enzymes in the large-scale industrial production, enzymes were generally immobilized on an inert, insoluble carrier, which allowed the enzymes to be held in place throughout the reaction, and then they were easily separated from the products for reuse [21,22]. Upon completion, the reaction mixtures typically contain only solvent and reaction products.

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**Fig. 1.** The processes of converting TCDCA to TUDCA using five enzymes *in vivo*, including the deconjugation of TCDCA by bile salt hydrolase (BSH), the epimerization of CDCA to UDCA by 7 $\alpha$ - and 7 $\beta$ -hydroxysteroid dehydrogenases (7 $\alpha$ - and 7 $\beta$ -HSDH), the formation of UDCA coenzyme A (CoA) thioesters by a bile acid CoA ligase (BAL) and the formation of TUDCA by amino acid N-acyltransferases (BAT).

In current work, 7 $\alpha$ - and 7 $\beta$ -HSDH from *Clostridium absonum* DSM 599 (ATCC 27555) were immobilized onto a modified chitosan microsphere, and the characterizations of immobilized 7 $\alpha$ - and 7 $\beta$ -HSDH were also presented. Moreover, a double-enzyme-coupled system was established. The immobilized 7 $\alpha$ - and 7 $\beta$ -HSDH were added into the columns, where TUDCA generation occurred *via* one-step process *in vitro* as a useful alternative to the five-step process *in vivo*. We demonstrated that the novel TUDCA technology was performed in two ways, through the mixture of separately immobilized 7 $\alpha$ - and 7 $\beta$ -HSDH and through the co-immobilization of 7 $\alpha$ - and 7 $\beta$ -HSDH. The idea proposed in this study may have profound guiding significance for the potential applications of TUDCA and its derivatives.

## 2. Material and methods

### 2.1. Materials

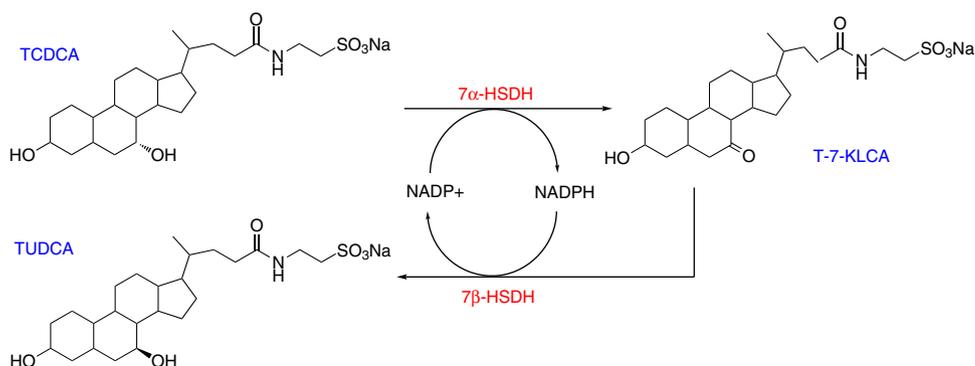
The expression and purification of 7 $\alpha$ -HSDH (EC 1.1.1.159) and 7 $\beta$ -HSDH (EC 1.1.1.201) were performed using a GST Gene Fusion System. The protocol for the expression and purification was listed

in supplementary material 1. The final protein concentration was diluted to 2 mg mL<sup>-1</sup>, and the fractions were collected and stored at -80 °C.

The chitosan (particle size, 40–100 mesh) used in the present work was purchased from Sangon Biotech (Shanghai, China), with a deacetylation degree of 95%. A BCA Protein Assay Reagent from Beyotime (Shanghai, China) was used to detect the protein concentration. TCDCA and TUDCA standards were purchased from the National Institutes for Food and Drug Control (Beijing, China). Sodium taurine-7-ketolithocholic acid (T-7-KLCA) was synthesized by our lab. NADP-Na<sub>2</sub> and NADPH-Na<sub>4</sub> (Purity  $\geq$  97%) were produced by Roche (Switzerland). Glutaraldehyde (GA) solution (concentration, 25%) was obtained from Kelong Chemicals (Chengdu, China). All the other chemicals used in this study were of analytical grade and used without further purification. Double-distilled water was used in all experiments.

### 2.2. Preparing chitosan microspheres

The chitosan microspheres were prepared according to references [23,24] with slight modifications: a chitosan solution was



**Fig. 2.** The epimerization of TCDCA to TUDCA by 7 $\alpha$ - and 7 $\beta$ -HSDH *in vitro*.

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