BILE ACID UDP-GLUCORONYLTRANSFERASE FROM HUMAN LIVER

Properties and studies on aglycone substrate specificity

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1. Introduction

Glucuronidation has been shown to be a common detoxication mechanism for a variety of foreign and endogenous compounds [1]. However, except for the enzyme(s) catalyzing the glucuronidation of bilirubin [2], 1-naphtol and morphine [3], knowledge about the characteristics of UDP-glucuronyltransferase(s) in man is scanty. Since bile acid glucuronides were detected about 6 years ago [4,5], bile acid glucuronidation seems also to be a detoxication mechanism in addition to the well-known bile acid sulphation (review [6]). However, nothing is known about the bile acid UDP-glucuronyltransferase in man. Only some properties of this enzyme from rat liver have been reported [7]. In this paper catalytic properties, the effect of detergents on enzyme activity and studies on aglycone substrate specificity of the bile acid UDP-glucuronyltransferase from human liver are described. The enzyme was competitively inhibited by bilirubin and estradiol, noncompetitively by testosterone and uncompetitively by 4-nitrophenol, whereas morphine had no effect on bile acid UDP-glucuronyltransferase activity, suggesting that in human liver bile acids, bilirubin and estradiol may be glucuronidated by the same enzyme.

2. Materials and methods

Specimens of human liver were obtained for histological examination from surgical patients who had undergone laparotomy for cholecystectomy because of cholelithiasis. Only laparotomy specimens with normal hepatic histology were used. The liver tissue was immediately frozen at −20°C. The activity of bile acid UDP-glucuronyltransferase was found to be stable for at least 4 weeks under these conditions. A 10% homogenate (wet wt/vol.) was prepared with 0.25 M sucrose in 5 mM Tris–HCl (pH 7.4) using a glass homogenizer with a teflon pestle.

For the determination of bile acid UDP-glucuronyltransferase activity, human liver homogenate corresponding to ~2 mg liver (wet wt) was incubated for 30 min at 37°C in a reaction mixture containing 0.5 mM [^{14}C]chenodeoxycholic acid (0.8 μCi/μmol), 9 mM UDP-glucuronic acid, 5 mM $MgCl_2$, 5 mM saccharolactone and 0.1 M imidazole–HCl (pH 6.8). Details of the assay and product identification are in [8]. Bilirubin UDP-glucuronyltransferase was determined as in [2] with the following modifications: the final concentrations of bilirubin and of UDP-glucuronic acid were 0.17 mM and 9 mM, respectively; the reaction mixture contained 0.1 M imidazole–HCl (pH 6.7) as buffer.

UDP-glucuronic acid was obtained from Boehringer (Mannheim); [24-^{14}C]chenodeoxycholic acid from NEN Chemicals (Dreieich); saccharolactone from Sigma (München) and bile acids from Steraloids (Wilton, USA). All other chemicals were from Merck (Darmstadt).

3. Results and discussion

3.1. *Effect of detergents*

Since UDP-glucuronyltransferases are predominantly microsomal enzymes [1] surface-active agents activate these membrane-bound enzymes by altering the membrane structure [10]. Therefore, the effect of detergents on the activity of bile acid UDP-

glucuronyltransferase from human liver homogenate has been investigated. The enzyme was activated 1.6-fold by 0.01% (v/v) Triton X-100 and 1.8-fold by digitonin (0.6 mg/mg liver protein) when a non-saturating substrate concentration (0.01 mM chenodeoxycholic acid) was used. Higher concentrations of Triton X-100 or digitonin inhibited the enzyme. At saturating substrate concentration (0.5 mM chenodeoxycholic acid) Triton X-100 and digitonin had no activating effect on enzyme activity indicating that the enzyme is fully activated in the presence of 0.5 mM chenodeoxycholic acid due to the surface-active effect of the bile acid. Therefore, the enzyme has only been assayed in the fully activated state which was achieved either by 0.5 mM chenodeoxycholic acid as substrate or by the presence of 0.01% Triton X-100 at <0.5 mM chenodeoxycholic acid.

3.2. *Catalytic properties*

Lineweaver-Burk plots of the initial rates of enzyme activity as a function of varying concentrations of chenodeoxycholic acid in the presence of 0.01% Triton X-100 yielded a straight line from which the app. K_m was calculated to be 0.083 mM chenodeoxycholic acid. The V_{max} was 2.4 nmol. h^{-1}. mg protein^{-1}. When UDP-glucuronic acid was used as the variable substrate and chenodeoxycholic acid was kept constant at 0.5 mM, the app. K_m for UDP-glucuronic acid was determined to be 0.96 mM (not shown).

Table 1
Effect of divalent metal ions on bile acid UDP-glucuronyltransferase activity

Addition to assay	Bile acid UDP-glucuronyltransferase activity (% control)[a]
None	100
10 mM EDTA	97
5 mM Mg^{2+}	138
5 mM Mn^{2+}	134
5 mM Ca^{2+}	135
5 mM Co^{2+}	35
5 mM Ni^{2+}	44
5 mM Zn^{2+}	6
5 mM Fe^{2+}	13

[a] Control: Enzyme activity without addition of EDTA and metal ions

For the determination of enzyme activity, see section 2

The effect of divalent metal ions on the activity of bile acid UDP-glucuronyltransferase is shown in table 1. The enzyme was stimulated to ~140% by 5 mM Mg^{2+} in comparison to the control value. No further increase or decrease in enzyme activity was observed when Mg^{2+} was 4–15 mM (not shown). The glucuronidation of bilirubin in human liver was also stimulated by the addition of Mg^{2+} [2]. Mn^{2+}

Table 2
Effect of bile acids on the glucuronidation of chenodeoxycholic acid

Bile acid	Bile acid (mM)	% Inhibition of chenodeoxycholic acid glucuronidation in the presence of		
		Unconjugated bile acid	Glycine conjugated bile acid	Taurine conjugated bile acid
Lithocholic acid (3α-ol)	0.1	62	51	58
	0.5	–	91	98
Chenodeoxycholic acid (3α,7α-ol)	0.1	–	28	33
	0.5	–	76	90
Ursodeoxycholic acid (3α,7β-ol)	0.1	27	17	–
	0.5	69	25	–
Deoxycholic acid (3α,12α-ol)	0 1	42	45	51
	0.5	99	91	99
Cholic acid (3α,7α,12α-ol)	0.1	16	15	19
	0.5	21	24	44

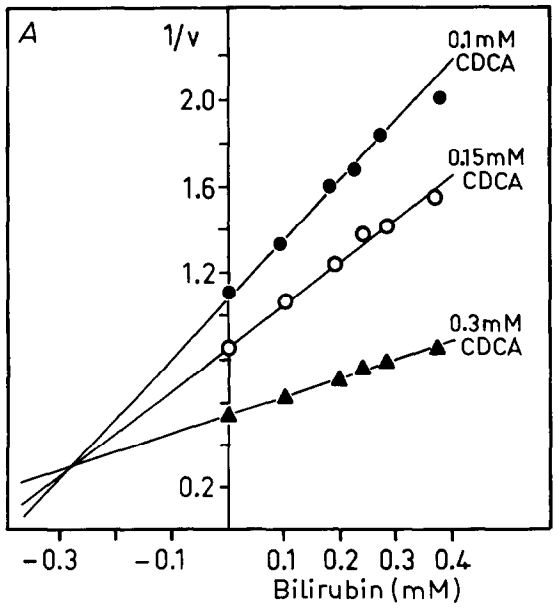
Enzyme activity was assayed in the presence of 0.5 mM chenodeoxycholic acid as in section 2. Lithocholic acid (unconjugated or conjugated with glycine or taurine) was dissolved in ethylene glycol containing 1% (v/v) ethanolamine (final conc. solvent 19.2% (v/v)). The position and configuration of the hydroxyl groups in the bile acid skeleton is given in brackets

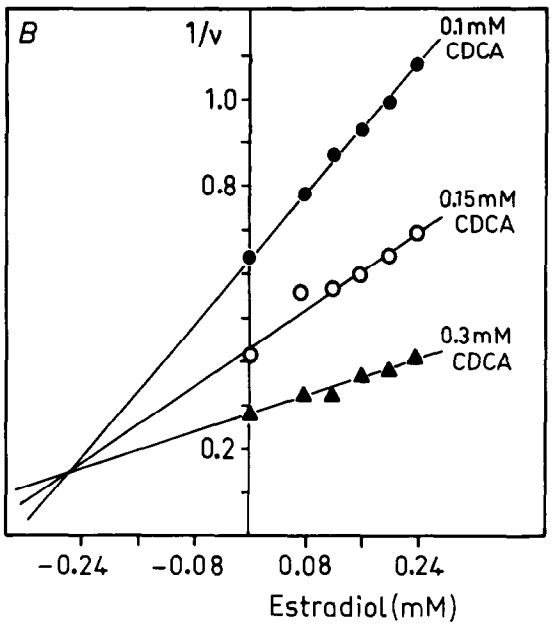
and Ca^{2+} could replace Mg^{2+} in the stimulation of bile acid UDP-glucuronyltransferase, whereas Co^{2+}, Ni^{2+}, Zn^{2+} or Fe^{2+} inhibited the enzyme (table 1).

The influence of the pH upon the activity of bile acid UDP-glucuronyltransferase was investigated at pH 6.0–8.0 in 0.1 M potassium phosphate buffer. The enzyme activity was optimal at pH 6.7 and declined steeply towards pH 6.0 and 8.0.

3.3. *Studies on aglycone substrate specificity*

The affinity of the enzyme to different bile acids has been investigated by studying the influence of various bile acids on the enzymatic glucuronidation of chenodeoxycholic acid (table 2). The affinity of the unconjugated bile acids to the enzyme decreased with increasing number of hydroxyl groups in the bile acid skeleton. The glucuronidation of chenodeoxycholic acid was inhibited in the presence of 0.1 mM lithocholic acid, a monohydroxylated bile acid, by 62%, whereas only 16% inhibition of chenodeoxycholic acid glucuronidation was observed by 0.1 mM cholic acid, a trihydroxylated bile acid. The degree of inhibition of chenodeoxycholic acid glucuronidation observed in the presence of dihydroxylated bile acids shows, that the affinity of the bile acids to the enzyme seems to depend not only on the number but also on the position and configuration of hydroxyl groups in the bile acid skeleton. Compared to the corresponding unconjugated bile acids, the affinity of the glycine conjugat-





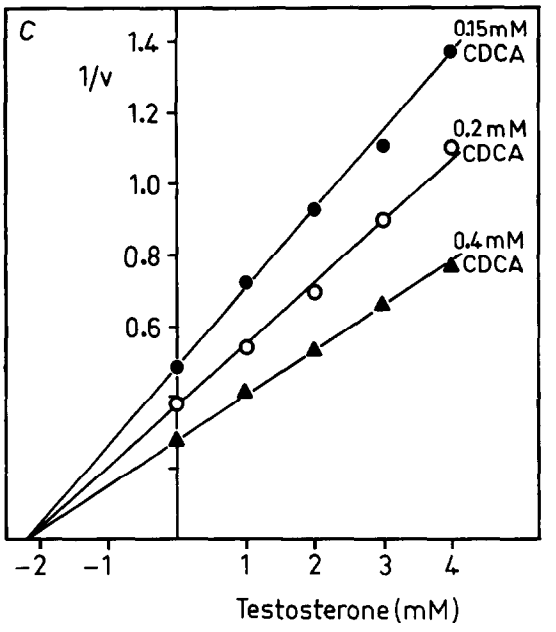


Fig.1. Inhibition of bile acid UDP-glucuronyltransferase by bilirubin (A), estradiol-17β (B) and testosterone (C) (Dixon plots). The concentrations of chenodeoxycholic acid (CDCA) used are shown in the figure. Bilirubin, estradiol and testosterone were dissolved as described in the legend to table 2. For the determination of enzyme activity, see section 2. Triton X-100 was added to the reaction mixtures in final conc. 0.01% (v/v). v = nmol. h^{-1}. mg $protein^{-1}$.

ed bile acids to the enzyme was similar or lower, whereas the taurine conjugated bile acids showed a similar or higher affinity to the enzyme (table 2).

In human liver evidence exists that there is more than one UDP-glucuronyltransferase. In human liver 1-naphtol, morphine and bilirubin may be glucuronidated by separate forms of enzymes [3]. In rat liver the existence of at least two different UDP-glucuronyltransferases could be shown by isolation of two enzymes which differed in the substrate specificity towards planar compounds like 4-nitrophenol and substrates containing non-planar portions like morphine [11]. Whether or not the recently purified bilirubin UDP-glucuronyltransferase from rat liver [12] is identical with the enzyme specific for non-planar substrates isolated in [11] remains to be established.

In order to obtain evidence for the aglycone substrate specificity of the bile acid UDP-glucuronyltransferase from human liver the influence of the following foreign and endogenous substrates on the glucuronidation of chenodeoxycholic acid was investigated: 4-nitrophenol and morphine as foreign substrates, and bilirubin, estradiol as well as testosterone as endogenous substrates. Whereas 15 mM morphine had no effect on the glucuronidation of chenodeoxycholic acid, the activity of bile acid UDP-glycuronyltransferase was inhibited by ~60% in the presence of 5 mM 4-nitrophenol or 4 mM testosterone and by ~40% in the presence of 0.25 mM bilirubin or 0.25 mM estradiol. Inhibition kinetics were performed with these substrates according to Dixon (fig.1) and to Lineweaver-Burk (not shown). Bilirubin (fig.1A) and estradiol (fig.1B) competitively inhibited the glucuronidation of chenodeoxycholic acid with app. K_i 0.28 mM for bilirubin and 0.26 mM for estradiol. Testosterone exhibited a noncompetitive inhibition pattern with an app. K_i of 2.2 mM testosterone (fig.1C) and 4-nitrophenol showed an uncompetitive inhibition type (not shown). Furthermore, it could be shown, that the glucuronidation of chenodeoxycholic acid was not only inhibited by bilirubin, but that conversely, the glucuronidation of bilirubin was inhibited by 40% in the presence of 1 mM chenodeoxycholic acid at pH 6.7. These studies suggest that in human liver bile acids, bilirubin and estradiol may be glucuronidated by the same enzyme, whereas morphine, 4-nitrophenol and testosterone may be substrates for a separate or different form(s) of UDP-glucuronyltransferase(s). However, definite proof can only be obtained from specificity studies with the purified UDP-glucuronyltransferase. Since an enzyme purification can only be performed when large amounts of starting material are available, purification of the bile acid UDP-glucuronyltransferase from rat liver is now in progress.

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