Bile acid *N*-acetylglucosaminides

Formation by microsomal *N*-acetylglucosaminyltransferases in human liver and kidney

H. Matern, R. Bolz, H.-U. Marschall, J. Sjövall and S. Matern

Department of Internal Medicine III, Aachen University of Technology, Aachen, FRG and *Department of Physiological Chemistry, Karolinska Institutet, Stockholm, Sweden*

Received 13 July 1990

Bile acid *N*-acetylglucosaminyltransferase activity has been identified in microsomes from human liver and kidney. In both organs the transferases required UDP-*N*-acetylglucosamine as sugar donor and were mainly active towards ursodeoxycholic acid. Minor activities were observed towards amidated ursodeoxycholic, hyodeoxycholic and β-muricholic acids. No *N*-acetylglucosaminidation was detectable with the major primary and secondary bile acids suggesting a specific requirement of the enzymes for bile acids containing 7β- or 6α-hydroxyl groups. Kinetic parameters and other catalytic properties of liver and kidney microsomal *N*-acetylglucosaminyltransferase activities towards ursodeoxycholic acid are described.

Glycosidic conjugation; Bile acid; Glycosyltransferase

1. INTRODUCTION

N-Acetylglucosaminides of bile acids were recently identified in normal human urine [1]. The daily excretion of these novel bile acid conjugates was comparable to the urinary daily excretion of bile acid glucosides [1,2] and bile acid glucuronides [3]. Whereas characteristics of the biosynthesis of bile acid glucosides [4,5] and glucuronides [6–9] could be elucidated from studies of the corresponding glycosyltransferases in man, nothing was known on the enzyme(s) catalyzing bile acid *N*-acetylglucosaminidation. The present report describes a rapid and sensitive assay procedure which enabled the characterization of the biosynthesis of bile acid *N*-acetylglucosaminides.

2. MATERIALS AND METHODS

Sources of chemicals were the same as described in previous papers [4,6]. UDP-*N*-acetyl-D-glucosamine [glucosamine-^{14}C (U)], 284 mCi/mmol, and UDP-*N*-acetyl-D-glucosamine [glucosamine-6-^{3}H (N)], 19 Ci/mmol were obtained from NEN chemicals, Dreieich. Human tissue specimens were obtained from a similar collective of surgical patients or organ donors as described [5]. Kidney samples were restricted to the cortical region. Only tissues with apparently normal histology were used.

Human tissue specimens could be stored for maximally 4 weeks at −30°C without a marked loss of bile acid *N*-acetylglucosaminyltransferase activity. Homogenization of tissues and preparation of microsomes by differential centrifugation was performed as described in a previous report [6].

Unless otherwise stated, bile acid *N*-acetylglucosaminyltransferase activity in human kidney or liver towards ursodeoxycholic acid was determined as follows: approximately 10–30 μg of microsomal protein was incubated in a reaction mixture which contained in a total volume of 30 μl 0.1 mM ursodeoxycholic acid, 50 μM UDP-*N*-acetyl-D-[^{3}H]glucosamine (0.05 μCi), 0.05 mM ADP for inhibition of nucleotide pyrophosphatase and the following additions for incubations with renal or hepatic microsomes, respectively: 0.1 M sodium acetate (pH 6.2), 2 mM $MgCl_2$ and 0.002% (w/v) Brij 58 with renal microsomes; 0.1 M 4-morpholineethanesulfonic acid (Mes)-KOH (pH 6.4) and 1 mM $MgCl_2$ with hepatic microsomes. ADP (0.05 mM) had no inhibitory effect on bile acid *N*-acetylglucosaminidation and maintained UDP-*N*-acetylglucosamine levels in 15-min incubations at about 95% and 99% of the initial values as compared to 77% and 93% without ADP in kidney and liver microsomes, respectively, as determined by thin layer chromatography (cf. below). After 15 min at 37°C the reactions were terminated with 30 μl 0.7 M Glycine-HCl (pH 2.7) containing 0.8 M sodium chloride. The water phase was extracted for 5 min with 300 μl water-saturated ethyl acetate resulting in transfer of about 95% of the reaction product into the organic phase. The unreacted UDP-*N*-acetylglucosamine remained in the water phase (> 99.9%). A 200 μl aliquot of the organic phase was counted for radioactivity in 5 ml Optifluor (Packard) scintillation cocktail.

For identification of bile acid *N*-acetylglucosaminides the reaction products were purified on a Sep-Pak C_{18} cartridge (see legend to Table I) and were incubated with or without *N*-acetylglucosaminidase as described [1]. Ethanol extracts of these incubation mixtures were applied to silica gel 60 thin-layer plates which were developed with *n*-butanol/acetic acid/water (50/5/10, v/v) and analyzed in a thin-layer chromatogram scanner (Berthold, Wildbad). When incubations were performed in the absence of *N*-acetylglucosaminidase single spots of the following *N*-acetylglucosaminides could be detected: *N*-acetylglucosaminides of ursodeoxycholic, R_F 0.609; glycoursodeoxycholic, R_F 0.235; tauroursodeoxycholic, R_F 0.158; hyodeoxycholic, R_F 0.591 and β-muricholic acids, R_F 0.535, respectively. After incubation of reaction products in the presence of *N*-acetylglucosaminidase these spots were shifted to a position identical to that of authentic *N*-acetyl[^{14}C]glucosamine (R_F 0.266).

Protein was determined as described in a previous report [9].

Correspondence address: H. Matern, Medizinische Klinik III, RWTH Aachen, D-5100 Aachen, FRG

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

3. RESULTS AND DISCUSSION

3.1. *Substrate specificity and tissue distribution*

Analysis of reaction mixtures after incubation of renal or hepatic microsomes with various bile acids as acceptor substrates and UDP-*N*-acetylglucosamine as donor substrate showed that formation of *N*-acetylglucosaminides mainly occurred in the presence of ursodeoxycholic acid (Table I). Amidation of ursodeoxycholic acid with glycine or taurine, change of the position and configuration of the hydroxyl group from 7β to 6α (hyodeoxycholic acid versus ursodeoxycholic acid) or the presence of a third hydroxyl group in the bile acid molecule as in β-muricholic acid led to a marked decrease in the rate of *N*-acetylglucosaminidation (Table I). No reaction was detectable (< 1 pmol/min/mg protein) with the primary bile acids cholic and chenodeoxycholic acids, the major secondary bile acids lithocholic and deoxycholic acids and the following other bile acids: 3α, 6β-di-hydroxy-5β-cholanoic, ursocholic (3α, 7β, 12α-trihydroxy-5β-cholanoic) and hyocholic (3α, 6α, 7α-trihydroxy-5β-cholanoic) acids. These results suggest a specificity of the enzymes from liver and kidney towards the 7β, or 6 α-hydroxyl group of preferentially dihydroxy as compared to trihydroxy bile acids. The restricted bile acid specificity of *N*-acetylglucosaminyltransferases in in vitro assays is in accordance with in vivo findings since ursodeoxycholic acid was the only bile acid that could be identified as *N*-acetylglucosaminide from human urine [1].

In contrast to the activities observed in kidney and liver (Table I), no *N*-acetylglucosaminyl transfer from UDP-*N*-acetylglucosamine (50 μM or 6 μM) to ursodeoxycholic acid (0.1 mM) was detectable with homogenate or microsomes from different segments of human intestinal mucosa (ileum, colon, sigmoid and rectum).

Table I

Bile acid substrate specificity of *N*-acetylglucosaminyltransferases

Bile acid	Relative activity	
	Kidney (%)	Liver (%)
Ursodeoxycholic acid (3α, 7β)	100[a]	61
Glycoursodeoxycholic acid (3α, 7β)	19	3.1
Tauroursodeoxycholic acid (3α, 7β)	35	12
Hyodeoxycholic acid (3α, 6α)	9.4	13
β-Muricholic acid (3α, 6β, 7β)	7.4	4.0

Enzyme activities were determined as described in Materials and Methods at bile acid concentrations of 0.1 mM with the following modifications: microsomal protein (100 μg) was incubated in a total volume of 180 μl; the sugar donor was UDP-*N*-acetyl-D-[^{14}C]glucosamine (6 μM, 0.3 μCi); the incubations were terminated with 800 μl ethanol and protein was removed by centrifugation; the reaction products were purified from the supernatants by adsorption to a Sep-Pak C_{18} cartridge [10] and analyzed by thin-layer chromatography as described in Materials and Methods. Position and configuration of hydroxyl groups in the bile acid skeleton are given in parentheses.

[a] Definition of 100% value: 13.5 pmol of product formed per min per mg of protein

3.2. *Characteristics of* N-*acetylglucosaminyltransferases in liver and kidney*

The influence of pH on the formation of ursodeoxycholic acid *N*-acetylglucosaminide was similar for the enzymes from liver and kidney. Enzyme activities were optimal between pH 6.1–6.4 in renal microsomes and pH 6.1–6.7 in hepatic microsomes and showed a steep decline of activity towards pH 5.0 and 8.0 (not shown).

Divalent metal ions exhibited a weak stimulatory effect on enzyme activities towards ursodeoxycholic acid. Compared to the control without metal ion and containing 10 mM EDTA, the kidney enzyme was maximally stimulated to about 140% in the presence of 2 mM Mg^{2+}, 2 mM Mn^{2+} or 10 mM Ca^{2+}. The liver enzyme showed maximal activation to about 160% in the presence of 1 mM Mg^{2+}, 0.1 mM Mn^{2+} or 0.5 mM Ca^{2+}. In contrast to the enzyme from renal microsomes, the metal ion dependence of the liver enzyme was only observed at a low concentration of UDP-*N*-acetylglucosamine (6 μM). No influence of these metal ions on hepatic enzyme activity was detectable at 50 μM UDP-*N*-acetylglucosamine. Zn^{2+} was not stimulatory but inhibitory to the enzymes from liver and kidney in concentrations >0.01 mM.

Since membrane-bound enzymes may be activated by the addition of detergents [11] the influence of the nonionic detergents Brij 58 and Triton X-100 (0.001–0.2%, w/v or v/v, respectively) was studied on ursodeoxycholic acid *N*-acetylglucosaminidation in liver and kidney microsomes. Whereas the effect of Triton X-100 on both hepatic and renal enzymes and Brij 58 on the liver enzyme was not activatory but inhibitory with increasing concentration (> 0.002%), Brij 58 led to a 1.5-fold activation of the renal enzyme at a concentration of 0.002%.

Double-reciprocal plots of *N*-acetylglucosaminyltransferase activities towards ursodeoxycholic acid with UDP-*N*-acetylglucosamine as varied substrate yielded a straight line with renal microsomes whereas with hepatic microsomes plots were concave downwards (Fig. 1). The same curved double-reciprocal plots were obtained with liver microsomes in the presence or absence of the detergent Brij 58 (0.002%, w/v). The data in Fig. 1 indicate that rates of ursodeoxycholic acid *N*-acetylglucosaminidation in hepatic microsomes are higher at low concentrations of UDP-*N*-acetylglucosamine than calculated from extrapolation of the plots at high concentrations of the sugar donor. Therefore, destruction of UDP-*N*-acetylglucosamine in other metabolic microsomal pathways cannot explain these results. Whether or not nonlinearity of double-reciprocal plots could be explained by a negatively cooperative binding mechanism [12] or UDP-*N*-

Table II

Kinetic constants of bile acid-*N*-acetylglucosaminyltransferases in kidney and liver

Kinetic constants	Kidney	Liver	
		Low UDPGNAc[a]	High UDPGNAc[a]
K_m for ursodeoxycholic acid	93.0 ± 23.5	27.4 ± 5.3	31.8 ± 8.9
K_m for UDP-*N*-acetylglucosamine	409.8 ± 138.9	2.2 ± 1.3	51.2 ± 26.2
V_{max}	295.6 ± 95.5	1.0 ± 0.5	3.9 ± 0.8

Values are given as mean ± SD which were obtained from the following number of organ samples: kidney, $n = 5$; liver, $n = 4$. The kinetic parameters for the kidney enzyme were calculated according to Florini and Vestling [13] from double-reciprocal plots of initial rates as a function of the concentration of ursodeoxycholic acid (0.01–0.1 mM) at various fixed concentrations of UDP-*N*-acetylglucosamine (25 μM, 35 μM, 50 μM, 100 μM) yielding an intersecting pattern of straight lines. The kinetic data for the liver enzyme were determined from Lineweaver-Burk plots as follows: with ursodeoxycholic acid (0.01–0.1 mM) as variable parameter at either low (6 μM) or high (50 μM) concentration of UDP-*N*-acetylglucosamine yielding the K_m-values for ursodeoxycholic acid; with UDP-*N*-acetylglucosamine as variable parameter by extrapolating the regions in double reciprocal plots tending towards linearity at low or high concentrations of UDP-*N*-acetylglucosamine (see Fig. 1) yielding the K_m-values for UDP-*N*-acetylglucosamine and the V_{max}-values. [a]UDPGNAc, UDP-*N*-acetylglucosamine; K_m, μM; V_{max}, pmol/min/mg protein.

acetylglucosamine to the liver enzyme or by the existence of two hepatic isoenzymes for the synthesis of ursodeoxycholic acid *N*-acetylglucosaminide should be analyzed with a purified enzyme preparation and not with a heterogenous microsomal fraction.

The apparent kinetic constants for microsomal *N*-acetylglucosaminyltransferase activities from kidney and liver towards ursodeoxycholic acid are summarized in Table II. Due to the nonlinearity of double-reciprocal plots with liver *N*-acetylglucosaminyltransferase (Fig. 1) the kinetic parameters for this enzyme were calculated at low and high concentrations of UDP-*N*-acetylglucosamine (see legend to Table II). As may be seen from Table II the enzymes from kidney and liver show marked differences in kinetic parameters especially with respect to the K_m-values for UDP-*N*-acetylglucosamine and the V_{max}-values. From these kinetic parameters, however, a similar efficiency of ursodeoxycholic acid *N*-acetylglucosaminidation in kidney and liver can be calculated based on the ratio of the V_{max}- to K_m-values for UDP-*N*-acetylglucosamine at saturating concentrations of ursodeoxycholic acid: 0.7 for the renal enzyme and 0.45 for the hepatic enzyme at low concentrations of UDP-*N*-acetylglucosamine.

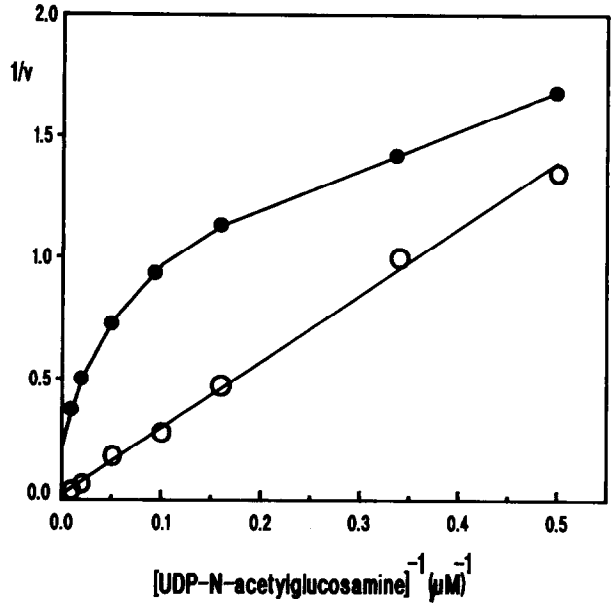

Fig. 1. Double reciprocal plots of initial rates of *N*-acetylglucosaminidation as a function of the concentration of UDP-*N*-acetylglucosamine at an ursodeoxycholic acid concentration of 0.1 mM with microsomes from liver (•) and kidney (○). The depicted plots show typical results from a single microsomal preparation of each organ; v, pmol/min/mg protein.

The results of the present study show that the occurrence of bile acid *N*-acetylglucosaminides in urine of man [1] can be explained by the existence of bile acid *N*-acetylglucosaminyltransferases in liver and kidney. The bile acid specificity of these enzymes is interesting because ursodeoxycholic acid was the preferred substrate in *N*-acetylglucosamine conjugation, and this bile acid was recently shown to improve liver function in patients with cholestatic liver disease [14]. Further studies are needed to elucidate whether or not a correlation exists between *N*-acetylglucosaminidation and the pathophysiological effects of ursodeoxycholic acid in liver disease.

REFERENCES

[1] Marschall, H.-U., Egestad, B., Matern, H., Matern, S. and Sjövall, J. (1989) J. Biol. Chem. 264, 12989–12993.
[2] Marschall, H.-U, Egestad, B., Matern, H., Matern, S. and Sjövall, J. (1987) FEBS Lett. 213, 411–414.
[3] Almé, B. and Sjövall, J. (1980) J. Steroid Biochem. 13, 907–916.
[4] Matern, H., Matern, S. and Gerok, W. (1984) Proc. Natl. Acad. Sci. USA 81, 7036–7040.
[5] Matern, H. and Matern, S. (1987) Biochim. Biophys. Acta 921, 1–6.
[6] Matern, S., Matern, H., Farthmann, E.H. and Gerok, W. (1984) J. Clin. Invest. 74, 402–410.
[7] Parquet, M., Pessah, M., Saquet, E., Salvat, C., Raizman, A. and Infante, R. (1985) FEBS Lett. 189, 183–187.
[8] Radomińska-Pyrek, A., Zimniak, P., Irshaid, Y.M., Lester, R., Tephly, T.R. and Pyrek, J.St. (1987) J. Clin. Invest. 80, 234–241.
[9] Marschall, H.-U., Matern, H., Egestad, B., Matern, S. and Sjövall, J. (1987) Biochim. Biophys. Acta 921, 392–397.

[10] Takikawa, H., Otsuka, H., Beppu, T., Seyama, Y. and Yamakawa, T. (1982) J. Biochem. 92, 985-998.
[11] Coleman, R. (1973) Biochim. Biophys. Acta 300, 1-30.
[12] Levitzki, A. and Koshland, Jr., D.E. (1969) Proc. Natl. Acad. Sci. USA 62, 1121-1128.
[13] Florini, J.R. and Vestling, C.S. (1957) Biochim. Biophys. Acta 25, 575-578.
[14] Poupon, R., Poupon, R.E., Calmus, Y., Chrétien, Y., Ballet, F. and Darnis, F. (1987) Lancet i, 834-836.