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INHIBITION OF URIDINE DIPHOSPHOGLUCOSE DEHYDROGENASE BY GALACTOSAMINE-1-PHOSPHATE AND UDP-GALACTOSAMINE

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SUMMARY

The activity of uridine diphosphoglucose (UDPG) dehydrogenase (UDPG: NAD^+ oxidoreductase, EC 1.1.1.22) from rat liver is inhibited markedly *in vitro* by galactosamine 1-phosphate and UDPgalactosamine. The K_i value is calculated to be $6.2 \cdot 10^{-3}$ M for galactosamine-1-phosphate. The type of inhibition is non-competitive. UDPgalactosamine is effective at lower concentrations; a K_i value of $6.9 \cdot 10^{-4}$ M has been measured and the inhibition is mainly competitive in nature. This inhibition of UDPG dehydrogenase activity is regarded to be responsible for the decrease of UDPglucuronate *in vivo* after D-galactosamine administration, supporting the significance of galactosamine 1-phosphate and UDPgalactosamine in the metabolic alterations during the development of galactosamine hepatitis.

INTRODUCTION

Galactosamine 1-phosphate and UDPgalactosamine accumulate in the liver after the injection of D-galactosamine. The appearance of galactosamine metabolites is the start of a sequence of reactions[1–3] leading to galactosamine hepatitis[4,5] in rats older than three weeks[6].

In experiments with liver slices, D-galactosamine is a potent inhibitor of glucoronide synthesis[7]. Moreover, it has been shown that D-galactosamine administration causes a marked decrease of UDPglucuronate concentration[8] in rat livers.

The conversion of UDPglucose to UDPglucuronate is catalyzed by uridine diphosphoglucose (UDPG) dehydrogenase (UDPG:NAD^+oxidoreductase, EC 1.1.1.22). The enzyme is inhibited *in vitro* by UDPxylose[9] and UDPgalactose[10].

Data presented in this paper show an inhibition of both bovine and rat liver UDPG dehydrogenase by galactosamine 1-*P* and UDPgalactosamine. This inhibition is regarded as being responsible for the decrease of UDPglucuronate *in vivo* after D-galactosamine administration.

MATERIAL AND METHODS

UDPG dehydrogenase from male Wistar rats (200–220 g) was purified 80-fold as described by Strominger *et al.*[11]. Calcium phosphate gel adsorption and elution were omitted. For comparison a highly purified UDPG dehydrogenase from bovine liver was obtained from Boehringer GmbH (Mannheim). Enzyme activity was measured spectrophotometrically by following the formation of NADH at 334 nm and 25 °C. Assay mistures contained, in addition to enzyme, 1.75 mM NAD^+ and 0.01–0.5 mM UDPG in a total volume of 0.85 ml of 0.2 M glycine buffer (pH 8.7)[12].

The concentrations of galactosamine 1-phosphate and UDPgalactosamine are indicated in the figures.

Galactosamine 1-phosphate was prepared biologically as follows. Male rats received a single injection of 500 mg D-galactosamine–HCl per kg body weight. 1 h after D-galactosamine administration 25% of the injected D-galactosamine had been converted to galactosamine 1-phosphate. The livers were quickly removed under ether anesthesia, transferred to 2.5 vol. chilled 0.9 M $HClO_4$ and immediately homogenized. The suspensions were centrifuged for 15 min at 18 000 × *g*, the supernatants being carefully collected, while the sediments were rehomogenized in 1.5 vol. $HClO_4$. After a second centrifugation the supernatants were combined and neutralized with KOH. Purification was performed by column[13] and paper chromatography[14]. After hydrolysis, the purity was checked by ion-exchange chromatography[14,15] and paper electrophoresis in 0.1 M borate buffer (pH 8.8). UDPgalactosamine was synthesized enzymatically *in vitro*[14]. The determination of galactosamine metabolites was performed as described previously[14].

D-Galactosamine–HCl (puriss.) was purchased from C. Roth, OHG (Karlsruhe). All other chemicals were commercial products of analytical grade.

RESULTS

The Michaelis constants (K_m) are calculated to be $2.0 \cdot 10^{-5}$ M UDPG for rat liver dehydrogenase and $1.9 \cdot 10^{-5}$ M UDPG for bovine liver dehydrogenase. These

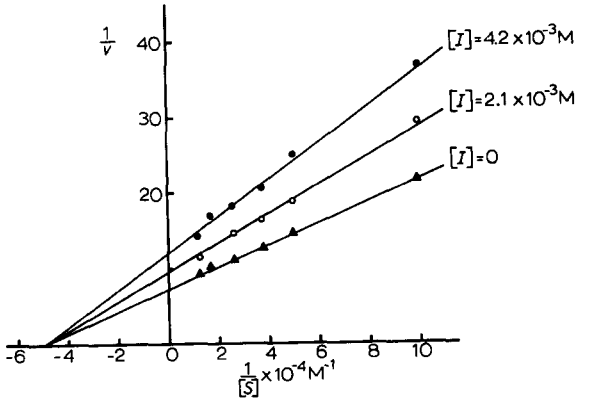

Fig. 1. Lineweaver–Burk plots of rat liver UDPG dehydrogenase activity in the presence of two concentrations of the inhibitor galactosamine 1-phosphate.

values are in agreement with data from the literature[16]. Typical Lineweaver–Burk plots (Fig. 1), obtained with UDPG as substrate and galactosamine 1-phosphate as inhibitor, show that galactosamine 1-phosphate does effectively inhibit the enzyme *in vitro* at concentrations which are present *in vivo* after D-galactosamine administration (see Table I). Measurements with different concentrations of inhibitor have shown that all lines of Lineweaver–Burk transformation cross the ordinate and intersect the negative abscissa. Therefore, the type of inhibition is non-competitive.

TABLE I

CONCENTRATION OF GALACTOSAMINE METABOLITES

Time-dependent changes of galactosamine 1-phosphate and UDPhexosamine concentrations after intravenous injection of 375 mg D-galactosamine–HCl per kg body weight. The results are expressed as μmoles/g fresh liver, $\pm$S.D. ($n = 3$). The average ratio of UDPgalactosamine to UDPglucosamine is 1:1. Results are expressed in μmoles/g.

Time after D-galactosamine administration (h)	*Sum of galactosamine metabolites*	*Galactosamine 1-phosphate*	*UDPgalactosamine*	*UDPglucosamine*
0.5	4.4 ± 0.16	2.9 ± 0.37	0.25 ± 0.01	0.23 ± 0.01
1	11.5 ± 0.50	8.5 ± 0.31	0.44 ± 0.01	0.38 ± 0.01
3	9.9 ± 0.26	5.6 ± 0.28	0.52 ± 0.02	0.54 ± 0.01
6	9.4 ± 0.41	3.8 ± 0.08	0.51 ± 0.02	0.48 ± 0.02
12	4.5 ± 0.12	0.4 ± 0.03	—	—

There is only a slight difference between the K_i values for the rat ($6.2 \cdot 10^{-3}$ M) and bovine enzyme ($7.6 \cdot 10^{-3}$ M) UDPgalactosamine causes an inhibition at concentrations lower than that for the precursor, galactosamine 1-phosphate. At low concentrations of UDPgalactosamine, the inhibition can be overcome by addition of UDPG. The type of inhibition seems to be mainly competitive (Fig. 2). For rat UDPG dehydrogenase a K_i value of $6.9 \cdot 10^{-4}$ M was measured.

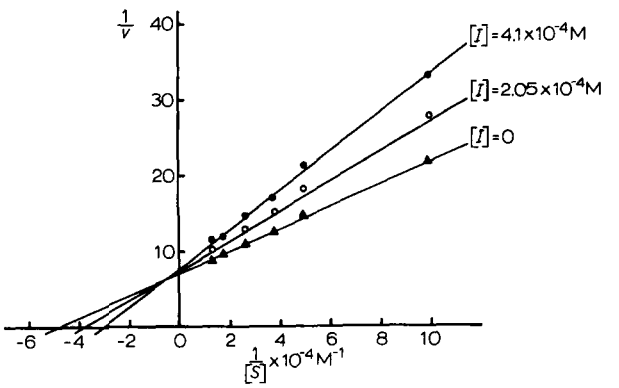

Fig. 2. Double-reciprocal plot of UDPG dehydrogenase activity in the presence of UDPgalactosamine. Each point represents the mean value of duplicate determinations at that concentration.

DISCUSSION

The enzymatic activity of UDPG dehydrogenase is inhibited by both galactosamine 1-phosphate and UDPgalactosamine *in vitro*. The decreased level of UDPglucuronate in the liver[8] and the inhibition of the synthesis of glucuronides in liver slices[7] can be partially explained by the diminished level of UDPG after D-galactosamine injection[8]. Yet, studies with D-glucosamine instead of D-galactosamine indicate that factors other than the lowered concentration of UDPG must be regarded responsible for the inhibition of UDPglucuronate synthesis[8]. Galactosamine 1-phosphate and UDPgalactosamine reach high levels after D-galactosamine administration and remain at this level for 3 to 6 h. At this time the first signs of a liver injury are seen[3,17]. From the data presented it can be concluded that these metabolites are mainly responsible for the diminished concentration of UDPglucuronate. Inhibition of enzymatic activity by galactosamine metabolites, especially by galactosamine 1-phosphate, has been shown for UDPG pyrophosphorylase (EC 2.7.7.9)[2] supporting the significance of these metabolites in metabolic alterations during the development of galactosamine hepatitis.

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