KINETIC PROPERTIES OF MICROSOMAL UDP-GLUCURONYLTRANSFERASE. REGULATION BY METAL IONS*

David Zakim, Jovita Goldenberg, and Donald A. Vessey

Molecular Biology Division and Department of Medicine, Veterans Administration Hospital, San Francisco, California, 94121, and the Departments of Medicine and Biochemistry and Biophysics, University of California Medical Center, San Francisco, California, 94122

Received March 22, 1973

SUMMARY

Treatment of microsomes with EDTA abolishes the stimulation of glucuronidation produced by UDP-N-acetylglucosamine. Addition of divalent metal ions to EDTA-treated microsomes restores the sensitivity of UDP-glucuronyltransferase to UDP-N-acetylglucosamine. Regulation of the activity of this enzyme by UDP-N-acetylglucosamine depends, therefore, on the presence of divalent metal ions. In addition, divalent metals increase the rate of glucuronidation of p-nitrophenol at V_{max}. The data indicate that metals are essential for the efficient function of UDP-glucuronyltransferase.

Several endogenous and exogenous compounds are detoxified in the liver via the formation of glucuronide derivatives (1). The significance of this detoxification mechanism is underlined by the occurrence of serious disease in the absence of normally functioning microsomal UDP-glucuronyltransferase (E. C. 2.4.1.17) (2). It is somewhat unexpected, therefore, that the concentration of UDP-glucuronic acid in intact liver is only 1 to 2 per cent (3, 4) of that needed for half-maximal rates of glucuronidation *in vitro* (5-7), and that the specific activity of UDP-glucuronyltransferase is quite low even at V_{max}. It would seem that the formation of glucuronide derivatives is either an inefficient mechanism for detoxification or the kinetic parameters of UDP-glucuronyltransferase as determined *in vitro* do not reflect those prevailing *in vivo*. Recent observations imply that the latter possibility is the case. UDP-N-acetylglucosamine, a physiological metabolite, enhances the affinity of UDP-glucuronyltransferase for UDP-glucuronic acid (6); and the concentration of UDP-glucuronic acid required for half-maximal rates of glucuronidation *in vitro* is reduced in the presence of UDP-N-acetylglucosamine to levels approaching those in liver *in vivo*. This result suggests that UDP-N-acetylglucosamine could be a physiological regulator of the activity of UDP-glucuronyltransferase. As shown in this paper, regulation of UDP-glucuronyltransferase by UDP-N-acetyl-

*This work was supported in part from grants from the U. S. Public Health Service (HL-10027), and the National Science Foundation (GB 20814) to Dr. T. P. Singer.

glucosamine depends on the presence of divalent metal ions. Thus, prior treatment of microsomes with EDTA prevents activation of UDP-glucuronyltransferase by UDP-N-acetylglucosamine, whereas addition of Mg^{2+} or Mn^{2+} to EDTA-treated microsomes restores the response of the enzyme to UDP-N-acetylglucosamine. Metals also have a second effect on the form of UDP-glucuronyltransferase which metabolizes p-nitrophenol in that they increase activity at V_{max}, independently of their effect on activation by UDP-N-acetylglucosamine.

MATERIALS AND METHODS

Untreated microsomes were prepared from guinea pig livers and stored as described previously (5). For the preparation of EDTA-treated microsomes livers from guinea pigs were homogenized in 0.25 M sucrose, 0.005 M EDTA pH 6.5 and the microsomal fraction isolated as in ref. 5. Microsomes were resuspended and washed twice in 0.25 M sucrose. UDP-glucuronic acid and UDP-N-acetylglucosamine (SIGMA) were treated with Chelex (Biorad) in order to remove trace amounts of heavy metals. Enzyme activity was assayed with p-nitrophenol as aglycone at 37° in 50 mM phosphate or Tris buffer, pH 7.6, using standard techniques (10). The concentrations of substrates and effectors are noted in the text and legends. Activities are expressed as mμ moles of p-nitrophenol metabolized per min per mg of protein. Protein was measured with the biuret method (11).

RESULTS AND DISCUSSION

Addition of divalent metals to microsomes prepared in 5 mM EDTA increases the rate of synthesis of p-nitrophenylglucuronide (Fig. 1). This effect of metals on activity is seen at infinite concentrations of UDP-glucuronic acid and p-nitrophenol, indicating that they increase the catalytic rate constant for the form of UDP-glucuronyltransferase which metabolizes p-nitrophenol. The significance of non-linearity of the data in Fig. 1, which has

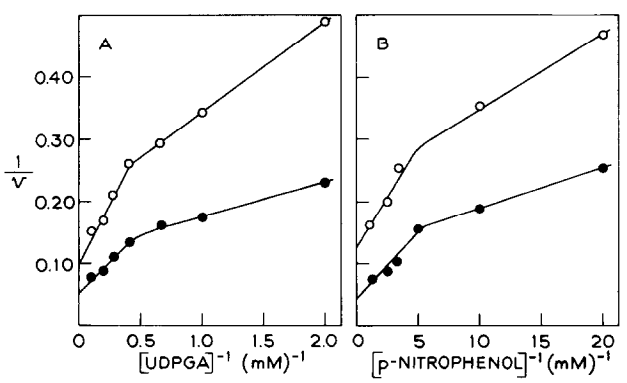


Fig. 1. Effect of $MnCl_2$ on the rate of synthesis of p-nitrophenylglucuronide. Initial rates of activity were measured in EDTA-treated microsomes as in Table 1 as a function of variable concentrations of UDP-glucuronic acid (A) or p-nitrophenol (B) in presence (●) or absence (○) of 1 mM $MnCl_2$.

Table 1

Effect of UDP-N-acetylglucosamine on the rate of glucuronidation by EDTA-treated microsomes.

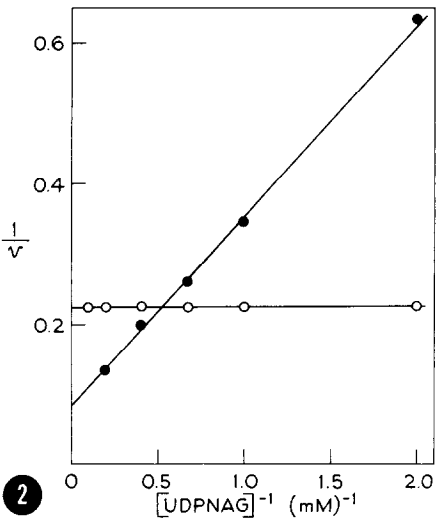
Addition to assay	Activity
None	3.00
2 mM UDP-N-acetylglucosamine	3.00
1 mM $MnCl_2$	6.00
1 mM $MnCl_2$ + 2 mM UDP-N-acetylglucosamine	16.9

Initial rates of activity were measured at 37° as in Methods in 50 mM Tris, pH 7.6, containing 0.2 mM p-nitrophenol, 1.0 mM UDP-glucuronic acid and the indicated amounts of $MnCl_2$ and UDP-N-acetylglucosamine.

been discussed at length in a previous publication (6), is thought to reflect cooperativity in the binding of substrates.

In addition to the effect of metals on the catalytic rate constant of UDP-glucuronyltransferase, the data in Table 1 show that metal ions are an absolute requirement for the stimulation of this enzyme by UDP-N-acetylglucosamine. UDP-N-acetylglucosamine (2 mM) increases the rate of synthesis of p-nitrophenylglucuronide by untreated-microsomes 3 - 4 fold (6). In contrast, 2 mM UDP-N-acetylglucosamine is without effect on the activity of UDP-glucuronyltransferase when EDTA-treated microsomes are used as the source of the enzyme (Table 1). Addition of Mn^{2+} restores the responsiveness of EDTA-treated microsomes to UDP-N-acetylglucosamine. Though the data are not shown in Table 1, Mg^{2+} has effects similar to those for Mn^{2+}. Further evidence for the influence of metal ions on the regulation of UDP-glucuronyltransferase by UDP-N-acetylglucosamine is apparent in studies with untreated microsomes (Table 2). Mg^{2+} potentiates the effect of UDP-N-acetylglucosamine on the activity of the untreated form of UDP-glucuronyltransferase. Mn^{2+} has a similar effect.

In order to investigate whether metals simply alter the affinity of UDP-N-acetylglucosamine for UDP-glucuronyltransferase, the rate of synthesis of p-nitrophenylglucuronide was studied as a function of variable concentrations of UDP-N-acetyl-



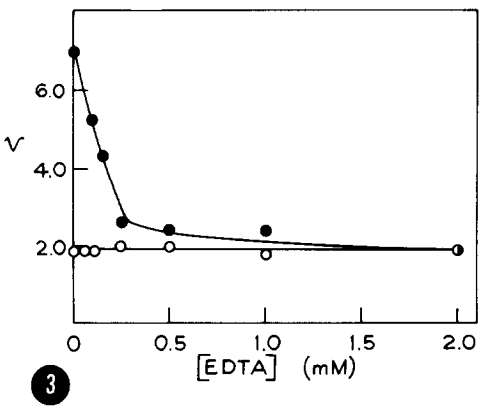


Fig. 2. Rate of glucuronidation as a function of the concentration of UDP-N-acetylglucosamine (UDPNAG) in presence (●) and absence (○) of 1 mM $MgCl_2$. Initial rates of activity were measured in EDTA-treated microsomes as in Table 2 in the indicated concentrations of UDP-N-acetylglucosamine. The data obtained in the presence of $MgCl_2$ have been corrected by subtracting the rate of synthesis of p-nitrophenylglucuronide in the absence of UDP-N-acetylglucosamine from the rate obtained in the presence of each concentration of this effector.

Fig. 3. The effect of EDTA on the rate of synthesis of p-nitrophenylglucuronide in untreated microsomes. Activities were determined as in Methods and Fig. 1. in the presence (●) or absence (○) of 2 mM UDP-N-acetylglucosamine.

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glucosamine at fixed concentrations of substrates, using EDTA-treated microsomes as the source of UDP glucuronyltransferase. As shown in Fig. 2, concentrations of UDP-N-acetylglucosamine as high as 10 mM did not enhance the rate of synthesis of p-nitrophenylglucuronide by EDTA-treated microsomes. After addition of Mg^{2+} to EDTA-treated microsomes, there is the expected dependence of rate on the concentration of UDP-N-acetylglucosamine (Fig. 2). It should be noted that the data plotted in Fig. 2 for assays in the presence of Mg^{2+} are corrected for the fact that p-nitrophenylglucuronide is synthesized in the absence of UDP-N-acetylglucosamine.

There are two possible interpretations for the effect of metals on UDP-N-acetylglucosamine stimulation of UDP-glucuronyltransferase. Metal ions may be required for the binding of UDP-N-acetylglucosamine, as for example in an ordered kinetic mechanism; or bound UDP-N-acetylglucosamine may have no effect on the configuration of UDP-glucuronyltransferase in the absence of metal ion. Irrespective of the exact mechanism, however, the data indicate that metal ions are important modifiers of the activity of

Table 2

Effect of Mg^{2+} on UDP-N-acetylglucosamine stimulation of UDP-glucuronyltransferase.

Addition to assay	Activity
None	1.25
5.0 mM $MgCl_2$	1.60
2.0 mM UDP-N-acetylglucosamine	3.30
5.0 mM $MgCl_2$ + 2.0 mM UDP-N-acetylglucosamine	9.1

Initial rates of activity were measured at 37° as in Methods in 50 mM phosphate buffer, pH 7.6, containing 0.2 mM p-nitrophenol, 1.0 mM UDP-glucuronic acid and the indicated concentrations of $MgCl_2$ and UDP-N-acetylglucosamine. Microsomes prepared in 0.25 M sucrose (untreated microsomes) were the source of UDP-glucuronyltransferase.

UDP-glucuronyltransferase, and that interactions between metal ions and UDP-N-acetylglucosamine may be essential for the efficient detoxification via the synthesis of glucuronide derivatives.

Although it was not possible to delineate the mechanism of the effect of metal ions on UDP-N-acetylglucosamine stimulation of UDP-glucuronyltransferase, it is clear that the effect of metals on activity at V_{max} is independent from their effects on regulation by UDP-N-acetylglucosamine. Thus, addition of EDTA to untreated microsomes does not decrease the rate of synthesis of p-nitrophenylglucuronide in the absence of UDP-N-acetylglucosamine; but EDTA decreases the rate of glucuronidation in the presence of UDP-N-acetylglucosamine (Fig. 3). There must be, therefore, two distinct sites at which divalent metal ions act to alter the kinetic properties of UDP-glucuronyltransferase. Microsomes prepared in 0.25 M sucrose have endogenous metal ions still attached at only one of these sites.

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