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REGULATION OF MICROSOMAL UDP-GLUCURONYLTRANSFERASE—

MECHANISM OF ACTIVATION BY UDP-*N*-ACETYLGLUCOSAMINE*

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Abstract—UDP-*N*-acetylglucosamine, *in vitro*, increases the rate of glucuronidation of *p*-nitrophenol by microsomal UDP-glucuronyltransferase. In contrast, UDP-*N*-acetylglucosamine inhibits the reverse reaction. Inhibition is competitive with respect to UDP. UDP-*N*-acetylglucosamine does not compete, however, with UDP-glucuronic acid in assays in the forward direction. Inhibition of the reverse reaction by UDP-*N*-acetylglucosamine must be due, therefore, to an allosteric effect. This was verified in studies of the extent of end-product inhibition by UDP in the presence and absence of UDP-*N*-acetylglucosamine. The mechanism of activation by UDP-*N*-acetylglucosamine is suited ideally for efficient function of this enzyme.

Usually a variety of non-physiological agents can modify the properties of an enzyme *in vitro*. It is often difficult to determine whether modifiers of enzyme activity *in vitro* are significant for function *in vivo*. This is especially true with regard to the activity of microsomal UDP-glucuronyltransferase‡ (EC 2.4.1.17), an enzyme which is important for the detoxification of many compounds [1]. The activity of the enzyme *in vitro* is relatively low and its affinity for UDP-glucuronic acid is poor [2–4]. It is potentially of great importance, therefore, that a variety of different treatments increase the activity of UDP-glucuronyltransferase several-fold *in vitro* [2–9]. Certain of these activators may be important *in vivo* for the maintenance of adequate rates of conjugation. Not all activators produce forms of the enzyme, however, that would function well under conditions presumed to exist *in vivo*. Understanding of the exact kinetic basis of activation is essential for assessing the physiological usefulness of treatments that modulate the activity of UDP-glucuronyltransferase.

UDP-*N*-acetylglucosamine, a naturally occurring metabolite, is an activator of UDP-glucuronyltransferase *in vitro* [5, 7, 9]. Treatment with this compound increases the apparent affinity of UDP-glucuronyltransferase for UDP-glucuronic acid [9]. Since the concentration of this substrate *in vivo* is quite low as compared with the concentration needed *in vitro* for half-maximal rates of glucuronidation [3], activation by UDP-*N*-acetylglucosamine seems important for the function of UDP-glucuronyltransferase under conditions presumed to exist *in vivo*. In view of the potential importance of this type of activation we considered it essential to study in more detail changes in the properties of UDP-glucuronyltransferase induced by UDP-*N*-acetylglucosamine. We have investigated accordingly the effects of UDP-*N*-acetylglucosamine on product inhibition by UDP and on the rate of the reverse reaction catalyzed by UDP-glucuronyl-transferase.

MATERIALS AND METHODS

Liver microsomes from retired male breeder guinea pigs were used as the source of UDP-glucuronyltransferase. Microsomes were isolated in 0.25 M sucrose and stored as described previously [10]. UDP-glucuronic acid (ammonium salt), *p*-nitrophenyl-β-D-glucuronide, and UDP-*N*-acetylglucosamine were purchased from Sigma Chemical Co., and UDP from PL Biochemical. Trace amounts of heavy metals were removed from the nucleotides by treatment with an ion exchange resin [4].

Enzyme assays were carried out in 50 mM Tris–HCl, pH 7.6, at 37°. The concentrations of UDP-glucuronic acid, UDP-*N*-acetylglucosamine, UDP, *p*-nitrophenol and *p*-nitrophenylglucuronic acid are indicated in the legends of Fig. 1 and Table 1 and in the text. Assays contained approximately 1 mg of microsomal protein. Initial rates of activity were measured for each assay by removal of serial aliquots from the assay media and determination of the rate of disappearance of *p*-nitrophenol (forward reaction) or the rate of appearance of *p*-nitrophenol (reverse reaction), using standard colorimetric techniques [10]. Glucaro acid-1,4-lactone was added to assays of the reverse in order to inhibit β-glucuronidase [11]; 10 mM lactone gave complete inhibition of β-glucur-

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‡ The number of substrate specific forms of UDP-glucuronyltransferase is uncertain. As used in this paper the activity of UDP-glucuronyltransferase refers only to the properties of the enzyme catalyzing the glucuronidation of *p*-nitrophenol.

onidase at concentrations of *p*-nitrophenylglucuronic acid as high as 20 mM. Activities are expressed as nmoles substrate metabolized/min/mg of protein. Protein was determined by the biuret method [12].

RESULTS

Liver microsomes from most animals contain an active pyrophosphatase which hydrolyzes the pyrophosphate bond of UDP-glucuronic acid. Many other nucleotide-sugars and nucleotides are either substrates or inhibitors of this enzyme [13]. Assays of UDP-glucuronyltransferase may be invalidated by failure to consider that modifiers of the pyrophosphatase-catalyzed reaction can appear to alter the activity of UDP-glucuronyltransferase. This technical problem is avoided by using microsomes from guinea pig liver as the source of UDP-glucuronyltransferase, because these microsomes have negligible pyrophosphatase activity with UDP-glucuronic acid as substrate [14], and by measuring initial rates of activity.

In contrast to its effect on the forward reaction [5, 7, 9], UDP-*N*-acetylglucosamine decreases the rate of the reverse reaction. Inhibition of the reverse reaction by UDP-*N*-acetylglucosamine is competitive with respect to UDP (Fig. 1). Bisubstrate kinetic analysis [15] of the reverse reaction revealed that K_{UDP} was 2.0 mM in untreated microsomes, and 6.0 mM in the presence of 2.5 mM UDP-*N*-acetylglucosamine. Addition of this modifier had no effect on the activity at V_{max} of the reverse reaction.

There are two possible explanations for the pattern of inhibition in Fig. 1. UDP-*N*-acetylglucosamine could compete directly with UDP for binding at the active site of UDP-glucuronyltransferase. Alternavely, prior binding of UDP-*N*-acetylglucosamine at an allosteric site could decrease the affinity of UDP-glucuronyltransferase for subsequent binding of UDP at the active site. The data in Fig. 1 cannot differentiate between these mechanisms. Data from other experiments exclude the possibility that UDP and UDP-*N*-acetylglucosamine compete for binding at the active site. UDP-*N*-acetylglucosamine does not compete with UDP-glucuronic acid for binding at the active site in assays in the forward direction [9]. The effect of UDP-*N*-acetylglucosamine on the binding of UDP results, therefore, from allosteric modification of UDP-glucuronyltransferase. This is compatible with UDP-*N*-acetylglucosamine-induced activation of the forward reaction.

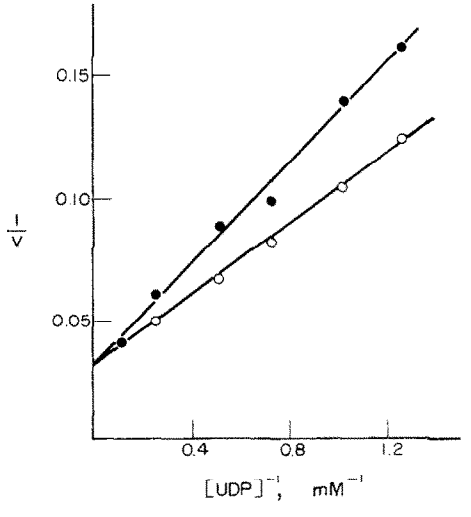

Fig. 1. Effect of UDP-*N*-acetylglucosamine on the rate of the reverse reaction of UDP-glucuronyltransferase. Initial rates of activity of UDP-glucuronyltransferase were determined with 4 m*M* *p*-nitrophenylglucuronic acid and the indicated concentrations of UDP as substrates, as described in Materials and Methods, in the presence (●) or absence (○) of 2.5 mM UDP-*N*-acetylglucosamine. Activities are expressed as nmoles *p*-nitrophenol liberated/min/mg of protein.

The conformation of UDP and UDP-glucuronic acid in solution is unknown. It is not possible to conclude with certainty the mechanism by which UDP-*N*-acetylglucosamine alters the binding of UDP-glucuronic acid to UDP-glucuronyltransferase. If it is assumed, on the other hand, that the UDP and glucuronic acid moieties contribute to binding, then UDP-*N*-acetylglucosamine would appear to increase the affinity of the enzyme for the glucuronic acid portion of UDP-glucuronic acid, i.e. UDP-*N*-acetylglucosamine decreases K_{UDPGA} [9] but increases K_{UDP}.

The extent of end-product inhibition of the forward reaction by UDP depends on the relative affinities of UDP-glucuronyltransferase for substrate and products. The data in Fig. 1 predict, therefore, that UDP-*N*-acetylglucosamine should limit the extent of end-product inhibition of the forward reaction by UDP in addition to increasing the rate of the forward reaction. The data in Table 1 show that UDP-*N*-acetylglucosamine reduces the extent of end-product inhibition of UDP-glucuronyltransferease by UDP from 70 to 10 per cent.

DISCUSSION

The relationship between activations observed *in vitro* and regulation of an enzyme under conditions likely to exist *in vivo* is a question that is difficult to resolve directly. It is possible to examine the biological significance of enzyme activations observed *in vitro* by careful study of all the changes in kinetic properties associated with activation. Activation of UDP-glucuronyltransferase by treatment with phospholipase A is associated, for example, with a loss

Table 1. Effect of UDP-*N*-acetylglucosamine on the rate of synthesis of *p*-nitrophenyl-glucuronic acid*

Addition	Activity†
None	2.44
UDP-*N*-acetylglucosamine	9.80
UDP	0.68
UDP plus UDP-*N*-acetylglucosamine	8.30

* Initial rates of activity of UDP-glucuronyltransferase were determined as described in Materials and Methods in 1.0 mM UDP-glucuronic acid and 0.2 mM *p*-nitrophenol. When added, the concentrations of UDP-*N*-acetylglucosamine and UDP were 2.5 and 1.0 mM respectively.

† Activity is expressed as nmoles *p*-nitrophenol metabolized/min/mg of protein.

of substrate specificity of the UDP-glucuronic acid site [16], enhanced affinity of the enzyme for products of the reaction relative to substrates, and an increase in activity at V_{max} of the reverse reaction, which is greater than the increase in activity at V_{max} of the forward reaction [16, 17]. Activation of this type is unlikely to enhance the efficiency of function under conditions presumed to exist *in vivo*. In contrast, activation of UDP-glucuronyltransferase by UDP-*N*-acetylglucosamine enhances the affinity of the enzyme for UDP-glucuronic acid, but decreases affinity for the UDP-moiety. This mechanism should allow for efficient function even when the concentration of UDP is relatively high. Certainly, the most useful manner for modulating the interactions between UDP-glucuronyltransferase and UDP-glucuronic acid is to maximize affinity for the glucuronic acid portion, and minimize affinity for UDP. A practical limitation to the physiological usefulness of this type of regulation is retention of specificity for the sugar moiety of the sugar nucleotide because the total concentration of UDP-sugars in liver is greater than the concentration of UDP-glucuronic acid [18]. Activation of UDP-glucuronyltransferase by UDP-*N*-acetylglucosamine does not alter enzyme–glucuronic acid interactions sufficiently to reduce specificity of the binding of UDP-sugars [16]. The properties of the UDP-*N*-acetylglucosamine-modified form of UDP-glucuronyltransferase are suited ideally for function under conditions presumed to exist *in vivo*. Affinity for substrate is enhanced, specificity is conserved, and affinity for end-product is diminished. It is not certain as yet whether this is a general mechanism for UDP-*N*-acetylglucosamine-induced activation of UDP-glucuronyltransferase, or whether it applies only to the glucuronidation of *p*-nitrophenol in microsomes from guinea pig liver.

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