TRYPSIN-SUSCEPTIBILITY OF UDP-GLUCURONYLTRANSFERASE

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1. Introduction

The location in liver microsomes of the membrane-bound enzyme system, UDP-glucuronyltransferase (EC 2.4.1.17) is controversial (e.g., [1–5]). Findings of Finnish workers [1,2,6–8] suggested that the transferase was not exposed on the cytoplasmic face of rat liver microsomal membranes, since tryptic digestion of 'intact' microsomes stimulated it, while trypsin strongly inhibited in disrupted microsomes. However others have reported that trypsin had little or no effect upon the transferase in rat [9–11] or guinea pig liver microsomes [5], or that it inhibited the transferase in 'intact' guinea pig liver microsomes [3,12]

We have argued on different grounds that glucuronyl-transferase is probably structurally latent [4,13,18] which is also the view of others (e.g., [1,2]). However, since some trypsin data do not fully support latency [3,5,9–12] and have been cited as evidence against it [5], the effects of trypsin upon the transferase were further studied in 'intact' and disrupted microsomes from both rat and guinea pig liver. Trypsin moderately stimulated the transferase in 'intact' microsomes from both species, but it greatly inhibited in disrupted microsomes.

2. Materials and methods

Preparation of guinea pig or rat liver microsomal fractions in 0.25 M sucrose, removal of glucuronyl-transferase latency by ultrasonication and assays of *p*-nitrophenol glucuronidation and of protein were all performed as described in [4]. Some microsomal pellets were frozen at −10°C for up to 10 days: this did not noticeably affect results. Microsomes unexposed to any further disruptive procedures are referred to as 'intact' microsomes. Significance of differences between means was assessed by *t*-test.

3. Results and discussion

Incubation at 30°C of initially 'intact' guinea pig liver microsomes with 50 μg trypsin/mg protein, increased their glucuronyltransferase activity by 150% within 40 min (fig.1(a)). In 7 similar experiments on rat or guinea pig liver microsomes, increases of 40–165% (mean 77% ± 39% SD) were seen. 'Spontaneous activation' [15] probably caused part of this increase in transferase activity, and this effect seemed unavoidable at 30°C. Further experiments were therefore done using more trypsin but at lower temperature, where 'spontaneous activation' was shown to be negligible. Thus, in an experiment on 'intact' microsomes from 8 separate guinea pigs, 150 μg trypsin/mg protein at 10°C for 60 min, significantly stimulated transferase activity by 74% ($p < 0.001$), while controls without trypsin showed no significant change in activity (2% decrease). Similarly, 250 μg trypsin/mg protein at 20°C for 60 min stimulated significantly in 'intact' microsomes from 8 rats (146% increase, $p < 0.001$) without a significant change in controls (18% increase). Increases in transferase activity (47–220%) by incubating initially 'intact' microsomes with trypsin are also shown in fig.1(b–d) and increases of 100–350% were seen in 3 other experiments at 20°C.

Disruption of initially 'intact' microsomes with

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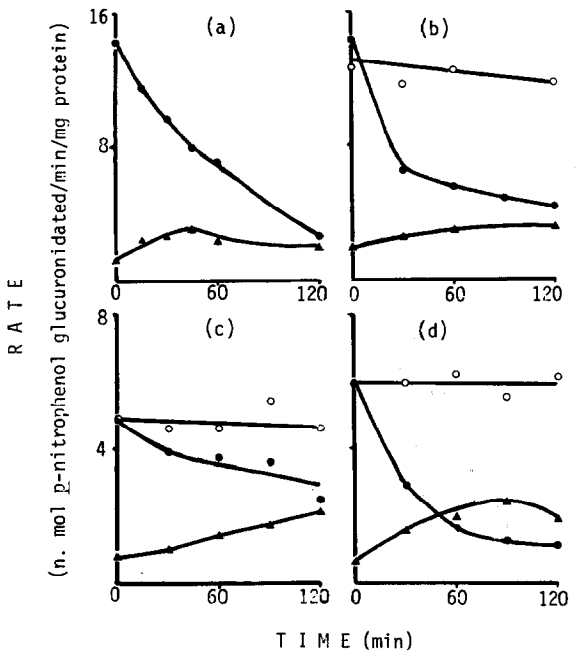

Fig.1. Effects of trypsin upon UDP-glucuronyltransferase in 'intact' and disrupted microsomes. Microsomes were resuspended in cold 0.25 M sucrose at 5–10 mg protein/ml, immediately prior to an experiment. Part was disrupted with 0.05% Triton X-100 or by intermittant ultrasonication with cooling for 6 min [4]. 'Intact' or disrupted microsomes, buffered at pH 7.5 with Tris–HCl, were incubated at 20–30°C for up to 120 min, with or without the indicated concentrations of bovine trypsin (Sigma, Type 1). Samples were removed at timed intervals and *p*-nitrophenol glucuronidation was measured at 37°C [4]. Soy bean trypsin inhibitor (Sigma, Type 1s) which was present at twice the concentration of trypsin in these assays, itself had no detectable effect upon glucuronyltransferase. (a) Guinea pig liver microsomes incubated with 50 μg trypsin/mg protein at 30°C. (b) Guinea pig livermicrosomes incubated with 300 μg trypsin/mg protein at 20°C. (c) Rat liver microsomes incubated with 100 μg trypsin/mg protein at 20°C. (d) Rat liver microsomes incubated with 400 μg trypsin/mg protein at 20°C. (▲) 'Intact' microsomes + trypsin. (○) Disrupted microsomes. (●) Disrupted microsomes + trypsin.

Triton X-100 (fig.1(a), (b), (d)) or by ultrasonication (fig.1(c)) unmasked much more glucuronyltransferase activity than did trypsin treatment, which is therefore a relatively inefficient way of removing the latency of the transferase. However, subsequent exposure of disrupted microsomes to trypsin (50–400 μg/mg protein) caused major decreases in the high transferase activity elicited by detergent or ultrasonic treatment (fig.1(a–d)), inhibitions of 50–90% (mean 65%) were seen. In fig.1(b–d), incubated controls in which trypsin was omitted showed only small (7–8%) decreases.

Our results for rat agree well with those of others [1,2,6–8]. In 'intact' guinea pig liver microsomes, trypsin stimulated the transferase, just as in rat [1,2, 6–8], which is contrary to reports that the transferase was unaffected [5] or inhibited by trypsin [3,12]. In the latter studies [3,12] the guinea pig liver transferase may have been 'spontaneously activated' (see [5]) perhaps by preparation and repeated washing of microsomes in KCl. In support of this possibility, such preparations from guinea pig liver (but not rat) exhibit much greater transferase activity than do microsomes prepared in sucrose [16].

Absence of marked trypsin effects upon the rat transferase in other studies [9–11] is probably due to treating with trypsin (50 μg/mg protein) for only 10 min at 30°C. This substantially inhibited 5 other microsomal enzymes in disrupted microsomes [9,10], but glucuronyltransferase is somewhat trypsin resistant. In disrupted microsomes, we saw noticeable inhibition by 50 μg of trypsin in 10 min at 30°C, but substantial transferase inhibition or substantial stimulation in 'intact' microsomes required longer trypsin treatments. Insufficient treatment may account for another report that glucuronyltransferase is not affected by trypsin [5].

Trypsin susceptibility of UDP-glucuronyltransferase can probably be interpreted as follows. In 'intact' microsomes, the transferase is inaccessible to trypsin and poorly accessible to its own substrates, because it is embedded in the cisternal face of the endoplasmic reticular membranes [4,14]. Proteolysis of the cytoplasmic face of 'intact' microsomes may increase their permeability to transferase substrates, moderately stimulating glucuronidation. Longer trypsin treatment of 'intact' microsomes sometimes caused transferase activity to decrease slightly again (e.g., fig.1(a), (d)). It is unclear if this inhibition was due to some membrane disruption, allowing trypsin direct access to some of the transferase. However, the extensive transferase inhibition caused by trypsin treatment of microsomes previously disrupted by detergents or by ultrasonication, does seem to be due to trypsin being allowed direct access to the transferase, which it proteolytically destroys.

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