Determination of glucuronidation in isolated rat liver cells by incorporation of $^{14}$C from fructose

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Glucuronidation constitutes a major mechanism in the deactivation and elimination of many xenobiotics [1, 2]. As a general method to study glucuronidation of a range of substrates in intact cells, we have developed an h.p.l.c.-based method for measuring the incorporation of $^{14}$C into glucuronides, following the labelling of the UDP-glucuronic acid (UDPGA) pool of isolated liver cells by incubation with $^{14}$C-fructose.

Liver cells were prepared [3] from male Wistar rats starved overnight. Starved rats were used to minimize possible effects of glycogen breakdown on the specific activity of UDPGA. The liver cells were incubated (0.5–2 mg dry weight/ml) with glucuronidation substrates dissolved in dimethylsulphoxide (final concentration 1% v/v) in Krebs buffer containing 200 μM-fructose and 0.2–0.5 μCi $^{14}$C-fructose/ml at 37°C for 20–90 min. Incubations were terminated by centrifugation of 1 ml portions through 250 μl silicone oil (Dow Corning 550:diminyl phthalate, 2:1) at 12 000 g for 30 s. Supernatants were heated in a boiling water bath for 5 min before centrifugation.

We have modified an h.p.l.c. method that has been used to separate $^{14}$C-labelled glucuronides and $^{14}$CUDPGA [4] for the separation of $^{14}$C-labelled glucuronides from $^{14}$C-fructose and other radiolabelled reaction products. Supernatants (routinely 0.1 ml) were analysed on a polar amino-cyano column at a flow rate of 1 ml/min. The mobile phase consisted of a linear gradient from 100% acetonitrile to 67% 0.01 m-tetraetylummonium hydrogen sulphate (TBAHS) in H₂O over 20 min. TBAHS at 67% was maintained for 5 min and the system returned to 100% acetonitrile over the subsequent 10 min. Fractions were collected at 0.5 min intervals for determination of the $^{14}$C content. Glucuronide peaks were identified by their disappearance following treatment with β-glucuronidase. The retention time for p-nitrophenol (PNP) and α-naphthol glucuronides was 13 min and for N-acetyl-p-aminophenol (APAP)-glucuronide, 15 min. The added $^{14}$C-fructose and $^{14}$C-glucose, produced in the liver cell incubation, eluted together at 18 min. The sensitivity was 40 pmol per injection, equivalent to 0.4 μM in the incubations. The amount of glucuronide produced was calculated from the specific activity of the $^{14}$C-fructose. The amounts of α-naphthol glucuronide formed by liver cells (100 μM-α-naphthol and 60 min incubations), quantified by the $^{14}$C-fructose method and by using $^{14}$C-naphthol and the same h.p.l.c. separation were 2.7 nmol/ml and 3.2 nmol/ml, respectively (results from one representative experiment with incubations in duplicate).

Abbreviations used: UDPGA, UDP-glucuronic acid; TBAHS, tetrabutylammonium hydrogen sulphate; PNP, p-nitrophenol; APAP, N-acetyl-p-aminophenol.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Maximum rate (pmol/min per mg dry weight)</th>
<th>EC₅₀ (μM)</th>
<th>Substrate inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP</td>
<td>42</td>
<td>750</td>
<td>Above 5 μM</td>
</tr>
<tr>
<td>PNP</td>
<td>28</td>
<td>16</td>
<td>Above 50 μM</td>
</tr>
<tr>
<td>α-Naphthol</td>
<td>94</td>
<td>14</td>
<td>Above 25 μM</td>
</tr>
</tbody>
</table>

These results confirm that the specific activity of the UDPGA pool in liver cells from fasted rats is similar to that of the $^{14}$C-fructose.

In order to examine the utility of the $^{14}$C-fructose method we have studied the concentration dependence of the glucuronidation of APAP, PNP and α-naphthol by rat liver cells (Table 1). All three dose–responses showed apparent substrate inhibition, this may be due to the toxicity of the substrates because the concentrations of substrate inhibiting glucuronidation also caused a decrease in ATP content (data not shown).

The rates of glucuronidation of APAP and PNP were lower than those reported in cells from fed rats [5, 6]. The rates of glucuronidation of α-naphthol were similar to those reported by Ullrich & Bock [7] but lower than rates reported by Schwarz [8], both measured in cells from fed rats. These differences in glucuronidation rates by cells from fed or fasted rats may be a consequence of variations in UDPGA concentrations.

These data show that this procedure, which does not require radiolabelled drug substrates or drug glucuronide standards, provides a general method for quantitative studies of the glucuronidation of a range of substrates in liver cells.


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