THE TURNOVER OF HEPATOMA CELL γ-GLUTAMYL TRANSFERASE

Jeak L. DING, Geoffrey D. SMITH* and Timothy J. PETERS
Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England

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1. Introduction

γ-Glutamyl transferase (5-glutamyl-peptide:amino acid 5-glutamyl transferase, EC 2.3.2.2) is a plasma membrane-bound enzyme which has been implicated in amino-acid transport owing to its ability to catalyse the transpeptidation of γ-glutamyl moieties from glutathione to various amino acid acceptors [1,2]. Elevated plasma γ-glutamyl transferase (GGT) activity has been used as a diagnostic marker for hepatobiliary dysfunction [3,4]. Clinical interest has also been generated through the possibility of using the enzyme as an oncofoetal protein marker of malignancy [5–7]. Although several studies have reported a marked increase in the enzyme activity during hepatocarcinogenesis [7–9], it is not known whether this increased activity can be ascribed to an alteration in the half-life of the enzyme.

Previous studies have shown that aflatoxin-induced rat hepatoma GGT has a 16-fold increase in catalytic efficiency [10] as compared with the enzyme from normal liver [11]. Here we show that the hepatoma enzyme has a half-life of 24 h, as compared to 3 h for the normal liver enzyme [12]. This explains the additional increase in the enzyme activity of hepatoma cells.

2. Materials and methods

2.1. *Inhibition of protein synthesis by cycloheximide*

Rat hepatoma cells (JB-1 line) were cultured to confluency as in [10] in 100 × 20 mm Falcon culture dishes. The conditioned growth medium was replaced with 10 ml fresh medium containing 0.5 mM cycloheximide (Sigma Chemical Co., Poole, Dorset) [13], and incubated at 37°C for up to 30 h. Control cells were cultured in fresh growth medium alone. Attached cells were harvested in duplicate, after exposure to cycloheximide for various times.

2.2. *Inhibition of γ-glutamyl transferase by diazotised sulphanilic acid*

Diazotised sulphanilic acid (DSA) [14] is a non-penetrating reagent which covalently inactivates plasma-membrane ectoenzymes including GGT [10]. The confluent monolayer of cells was rinsed twice with phosphate-buffered saline (PBS), and irreversible inactivation of the GGT activity was carried out by incubating the cells at 37°C for 10 min in 10 ml PBS containing 3 mM DSA. Control cells were treated in the same way but with 10 ml PBS alone. Morphological studies showed that treatment of these cells, with between 1–10 mM DSA, did not affect their viability. At the end of the treatment, the cells were rinsed twice with PBS before fresh growth medium was added. Incubation at 37°C was resumed, during which the cells were harvested, in duplicate, at time intervals of up to 70 h.

2.3. *Inhibition of γ-glutamyl transferase by AT-125*

AT-125, (L-[α*S*,5*S*]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) (donated by Dr T. Connors, MRC Toxicology Unit, Carshalton) is a glutamine analog which causes specific and irreversible inhibition of GGT [15]. Preliminary studies showed that a maximum of 85–95% of the enzyme activity was inhibited by incubation with 450 μmol AT-125/l at 37°C for 30 min. Treatment of the cultured cells under these conditions was carried out as described for the DSA inhibition studies. The control and treated cells were harvested in duplicate at time intervals of up to 40 h.

2.4. *Determination of specific activity of γ-glutamyl transferase*

The harvested cells were washed twice in PBS and

* To whom correspondence should be addressed

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centrifuged at 250 × *g* for 10 min. The enzyme was solubilised with 0.5% Triton X-100 in PBS and GGT activity was measured as in [16]. DNA was assayed fluorimetrically in [17] with calf thymus DNA (type I, Sigma) as standard.

3. Results

Treatment of hepatoma cells in culture with cycloheximide resulted in a progressive detachment of the cells from the dish. The specific activity of GGT (~2000 mUnits/mg DNA) of the attached cells underwent a first-order decay. Fig.1 shows the rate of disappearance of GGT activity with $t_{1/2}$ = 23 h. An apparent lag period of ~10 h was observed.

Treatment of the cells with DSA resulted in marked loss of GGT activity. Following removal of the inhibitor, GGT activity returned to normal levels as new enzyme was synthesized. The rate of recovery of the cellular GGT level followed first-order kinetics with a $t_{1/2}$ = 22 h (fig.2). There was an initial lag period of 8–10 h preceeding the reappearance of GGT activity. The rate of reappearance of GGT after inactivation of pre-existing enzyme with AT-125 followed zero-order kinetics, with $t_{1/2}$ = 27 h (fig.3). There was no apparent lag period for the resynthesis of GGT following the withdrawal of AT-125.

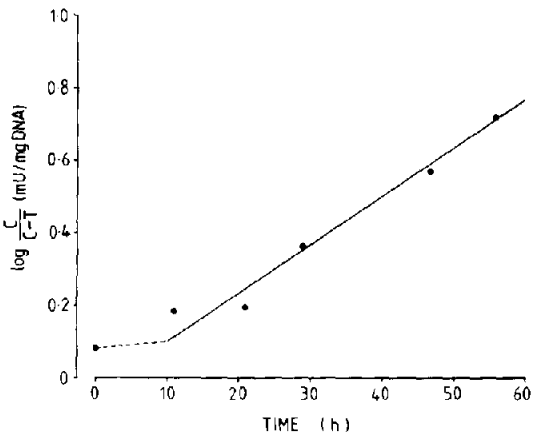

Fig.2. Inhibition of γ-glutamyl transferase (GGT) in cultured hepatoma cells by 0.3 mM diazotized sulphanilic acid. Semilogarithmic plot of the resynthesis of GGT with time, in the treated hepatoma cells: *C*, specific activity of control cells; *T*, specific activity of treated cells. From the rate of increase in the enzyme specific activity, the half-life ($t_{1/2}$) of 22 h for GGT was calculated as in fig.1.

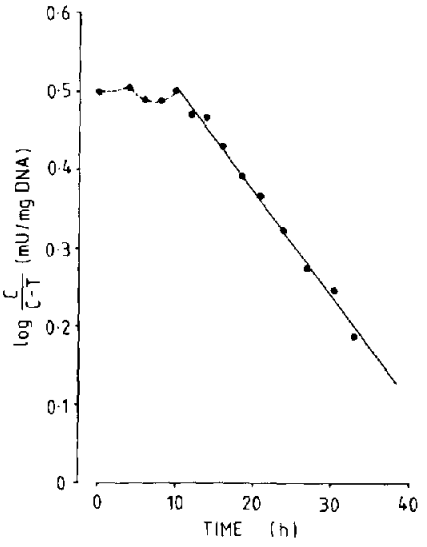

Fig.1. Inhibition of protein synthesis in cultured hepatoma cells, by 0.5 mM cycloheximide. Semilogarithmic plot of the decrease in specific activity of γ-glutamyl transferase (GGT) against time of treatment with cycloheximide: *C*, specific activity of control culture; *T*, specific activity of treated culture. The slope is $k/2.303$, where k is the first-order rate constant with the units of time^{-1}. The half-like ($t_{1/2}$) of 23 h for GGT was calculated from: $t_{1/2} = 0.693/k$.

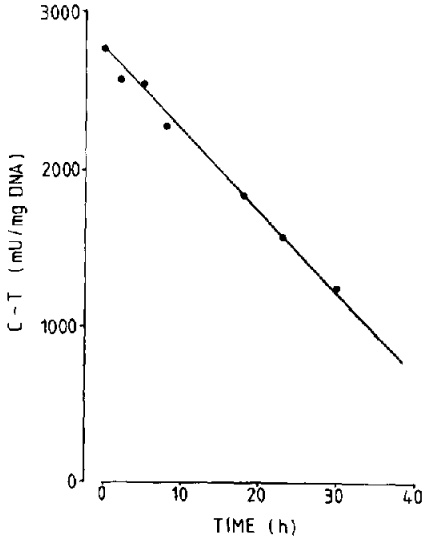

Fig.3. Specific inhibition of γ-glutamyl transferase (GGT) in cultured hepatoma cells, by 450 μm L-[α*S*,5*S*]-α-amino-3-chloro-4,5-dihydro-5-isoxazolacetic acid (AT-125). Rate of resynthesis (*C*−*T*) plotted against time, where *C* is specific activity of control, and *T* is the specific activity of the AT-125 treated cells. The slope (k) is the zero-order rate constant of the resynthesis of GGT. The half-life ($t_{1/2}$) of 27 h for GGT was obtained from the formula: $t_{1/2} = C/2k$.

4. Discussion

Here, the rate of breakdown of GGT in the cultured hepatoma cells was followed during the inhibition of protein synthesis by cycloheximide. Also, the rate of synthesis of the enzyme was monitored after covalent inactivation of the pre-existing levels of GGT with DSA or AT-125. Because the $t_{1/2}$-values, as determined by measurements of both synthesis and degradation rates, are similar, it is suggested that these techniques give an accurate reflection of the half-life of the enzyme.

The lag period observed in cycloheximide studies is probably the time taken for this compound to exert its effect on protein synthesis and translocation systems within the cell and has been similarly observed in cultured fibroblasts [13]. Following the inhibition of GGT with DSA, a similar lag period was apparent. Since DSA is a non-specific inhibitor, it would be expected to inactivate other ecto-plasma membrane enzymes. This may lead to disturbance, or loss, of some other membrane-bound proteins that are essential for metabolite transport. However, the lag period could be partly explained by the time taken for the processing of newly formed proteins from rough endoplasmic reticulum to the Golgi complex with the subsequent insertion of the glycoproteins into plasma membrane. The absence of a lag period during the turnover studies with AT-125, a highly selective inhibitor of GGT, suggests that the lag phase observed with DSA relates to inactivation of non-GGT proteins.

The synthesis of proteins is generally thought of as a zero order process, and indeed the recovery of GGT activity after inactivation with AT-125 follows this pattern. However, after inactivation with DSA, recovery follows first-order kinetics. This may result from a limiting concentration of certain amino acids, since the amino acid transport mechanisms would also be expected to be inactivated by this reagent.

The half-life derived from these studies is shorter than the 100 h reported for plasma membrane glycoproteins of a cloned line of cultured hepatoma cells [18]. However, as compared with the half-life of 3 h obtained for the normal rat liver GGT [12], the aflatoxin-induced GGT has a substantially prolonged (8-fold) half-life. The longer life-span of the hepatoma cell GGT might be explained by the increase in the sialylation of the enzyme [10], which is a feature of hepatocarcinogenesis [19]. By analogy with circulating glycoproteins [20], this modification may protect the enzyme and delay its clearance rate.

Acknowledgements

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