Biochimica et Biophysica Acta, 661 (1981) 191–198
Elsevier/North-Holland Biomedical Press

BBA 69362

SUBCELLULAR LOCALIZATION AND ISOLATION OF γ-GLUTAMYLTRANSFERASE FROM RAT HEPATOMA CELLS

J.L. DING, G.D. SMITH * and T.J. PETERS

Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Rd, Harrow, Middlesex HA1 3UJ (U.K.)

(Received February 6th, 1981)

Key words: γ-Glutamyltransferase; Subcellular localization; (Rat hepatoma cell)

Cultured rat hepatoma cells were homogenized and subjected to subcellular fractionation by analytical sucrose density centrifugation to determine the localization of γ-glutamyltransferase ((5-glutamyl)-peptide : amino-acid 5-glutamyltransferase, EC 2.3.2.2). The activity was exclusively localized to the plasma membrane. Diazotized sulphanilic acid was used as a non-penetrant membrane reagent which inactivates ectoenzymes. With both intact and sonicated cells, only 70–75% inhibition of γ-glutamyltransferase activity was observed. At least 12% of the total cell complement of γ-glutamyltransferase activity is highly resistant to inactivation by diazotized sulphanilic acid even after Triton X-100 solubilization. The enzyme was purified from hepatoma cells and its properties compared with enzyme from normal liver. Apart from the striking increase in V^{app} there were only minor differences between the enzymes from the two sources. In contrast to the complete abolition of transpeptidase activity of the purified hepatoma enzyme by diazotized sulphanilic acid, the hydrolytic activity of this preparation was only slightly inhibited.

Introduction

The enzyme, γ-glutamyltransferase ((5-glutamyl)-peptide : amino-acid 5-glutamyltransferase, EC 2.3.2.2) catalyses the transfer of γ-glutamyl groups from γ-glutamyl peptides, particularly glutathione, to various amino acids, dipeptides or water. It is a particulate glycoprotein which is widely found in the brush border membrane of kidney [1] and intestine [2].

In normal liver, γ-glutamyltransferase is primarily or even entirely associated with the plasma membrane [3] of biliary tract cells [4]. However, during carcinogenesis, and particularly in malignant hepatoma cells, the activity is markedly increased [5]. Therefore, quantitative information on the subcellular localization and properties of this induced enzyme could help to elucidate the oncogenic process. In addition, the enzyme has been purified from rat hepatoma cells and its properties compared with those of the purified enzyme from normal liver.

Materials and Methods

Reagents. L-γ-Glutamyl-7-amino-4-methyl coumarin was purchased from Uniscience, Cambridge (Bachem Fine Chemicals). 2′-Deoxy-thymidine-5′-*p*-nitrophenyl phosphate was obtained from Boehringer, Mannheim, GmbH (F.R.G.). [2-^{3}H]-Adenosine 5′-monophosphate was a product from Radiochemical Centre, Amersham (U.K.). Glycylglycine, ammediol buffer (2-amino-2-methyl-1,3 propandiol), piperazine, adenosine 5′-monophosphate, 2-glycerophosphate, sodium deoxycholate and Lubrol WX were from Sigma Chemical Co. Ltd. (Poole, Dorset). Diazotized sulphanilic acid was synthesized as outlined by DePierre and Karnovsky [6]. All other reagents were of analar grade, obtained from BDH Ltd. (Poole, Dorset, U.K.).

Hepatoma cells. These cells were isolated by Dr.

* To whom correspondence should be addressed.

G.E. Neal, MRC Toxicology Unit, Carshalton, Surrey, U.K. Male Fischer rats, aged 3 weeks were fed a diet containing 4 ppm aflatoxin for 6 weeks. After approx. 9 months on a normal MRC 41B diet, the animals developed hepatoma. The tumour cells were isolated following collagenase perfusion. γ-Glutamyltransferase-positive cells, as judged by histochemical techniques were cultured in single-strength Williams Medium E (Flow Laboratories, Scotland, U.K.), supplemented with 1.0% glutamine, 10% fetal calf serum and 0.5% gentamicin. A confluent monolayer of cells was obtained after 3–4 days.

Enzyme assays. γ-Glutamyltransferase was measured fluorimetrically according to Smith et al. [7]. The transpeptidase activity was estimated in the presence of acceptor, glycylglycine. The corresponding hydrolytic activity was assayed under the same conditions except that glycylglycine was omitted from the ammediol buffer. Alkaline phosphodiesterase was assayed on an automated enzyme analyzer (AURA, Pye Unicam) by a modification of the method described by Razzell [8]. 0.05 ml enzyme, was added to 0.2 ml 0.1 M Tris-HCl (pH 9.0)/10 mM $MgCl_2$/0.44 mM 2'-desoxy-thymidine-5'-*p*-nitrophenyl phosphate. The reaction was maintained at 37°C while the rate of appearance of *p*-nitrophenol was monitored. Enzyme activity was estimated from the change in absorbance at 400 nm. All other assays were performed as described by Seymour and Peters [9].

Protein estimations. Protein was determined by a modification of the biuret method [10] or by the fluorescamine assay [11] with bovine serum albumin standard.

Subcellular fractionation by sucrose density gradient centrifugation. Confluent monolayer hepatoma cells ($1.2 \cdot 10^8$) were harvested with a rubber policeman, twice-washed in phosphate-buffered saline (pH 7.4) and centrifuged at $250 \times g$ for 10 min at 4°C. The cells were then homogenized in 5 ml 0.3 M sucrose/1 mM disodium EDTA (pH 7.4)/20 mM ethanol with 35 strokes of a tight-fitting (type B) pestle in a small Dounce homogenizer (Kontes Glass Co., Vineland, NJ, U.S.A.).

Approx. 4.5 ml homogenate were layered onto a 28 ml linear sucrose density gradient ($\rho \approx 1.05–1.28$ $g \cdot cm^{-3}$) resting on 6 ml sucrose at density 1.32 $g \cdot cm^{-3}$, in a Beaufay automatic zonal rotor [12]. All solutions contained 1 mM disodium EDTA (pH 7.2)/20 mM ethanol. The rotor was then accelerated to 35 000 rev./min for 35 min. The rotor was decelerated to 8 000 rev./min, and the resulting gradient displaced with nitrogen and collected in 2-ml fractions. The percentage sucrose of each fraction was indirectly estimated with an Abbé refractometer [13]. All procedures were performed at 4°C.

Marker enzyme activities for different subcellular components were assayed in all the gradient fractions, and frequency-density histograms were constructed according to Leighton et al. [14].

Treatment of hepatoma cells with diazotized sulphanilic acid. Freshly harvested cells were washed twice in phosphate-buffered saline. Approx. 2 mg (protein) cells suspended in a final volume of 1.0 ml phosphate-buffered saline were incubated with 0–10 mM diazotized sulphanilic acid at 37°C for 10 min, after which they were immediately washed twice at 4°C with 5 ml phosphate-buffered saline to remove any unreacted diazotized sulphanilic acid. The integrity of the cells before and after treatment with diazotized sulphanilic acid was 95% as assessed by 0.15% trypan blue exclusion. Control cells were treated in the same manner, except that diazotized sulphanilic acid was omitted from the incubation medium. The control and treated cells, resuspended in 2.0 ml phosphate-buffered saline, were then disrupted at 4°C with an MSE 150 W sonicator, at amplitude 16 μ for 20 s, before biochemical analysis.

In further experiments, approx. $1.2 \cdot 10^8$ intact cells (treated with 1 mM diazotized sulphanilic acid) were homogenized in isotonic sucrose and subjected to subcellular fractionation as described above.

Purification of γ-glutamyltransferase. The techniques used for the isolation of γ-glutamyltransferase from normal rat liver have been described previously [4].

Approx. $7.5 \cdot 10^8$ cells containing 22 units γ-glutamyltransferase activity, suspended in 0.75 mM NaOH/80 mM $MgCl_2$ were sonicated in an ice-water bath for 30 s. The enzyme was extracted with 0.5% sodium deoxycholate/0.5% Lubrol WX and was then fractionated at 20–80% (w/v) saturation $(NH_4)_2SO_4$, and subsequently desalted on a Sephadex G-25 column. Affinity chromatography on Con A-Sepharose 4B (Pharmacia) resulted in two distinct peaks of activity: the larger (eluted with α-methylmannoside)

was pooled, concentrated and further studied. Gel filtration chromatography on Sepharose 6B (2.2 X 85 cm) was followed by preparative discontinuous polyacrylamide gel electrophoresis [15,16]. The gels were immediately cut into 1.0-mm slices, and the enzyme activity eluted overnight with 0.2% Triton X-100 in phosphate-buffered saline (pH 7.4). The enzyme recovered from 3 X 1-mm slices corresponding to the peak activity was pooled and an iodinated [17] sample was subjected to analytical gel electrophoresis.

Results

Subcellular distribution of γ-glutamyltransferase. Fig. 1 shows the distribution of the principal marker enzymes in the sucrose density gradients. The distribution of γ-glutamyltransferase activity is similar to that of the other plasma membrane markers, 5′-nucleotidase and alkaline phosphodiesterase at an equilibrium density of 1.17 g · cm^{-3}. γ-Glutamylhydrolase activity showed a similar distribution. The distributions of neutral α-glucosidase, *N*-acetyl-β-glucosaminidase, succinate dehydrogenase and catalase, marker enzymes for endoplasmic reticulum, lysosomes, mitochondria and peroxisomes have equilibrium densities of 1.19, 1.17, 1.18 and 1.21 g · cm^{-3}, respectively. The small peak of activity at density 1.28 reflects unbroken cells in the gradient.

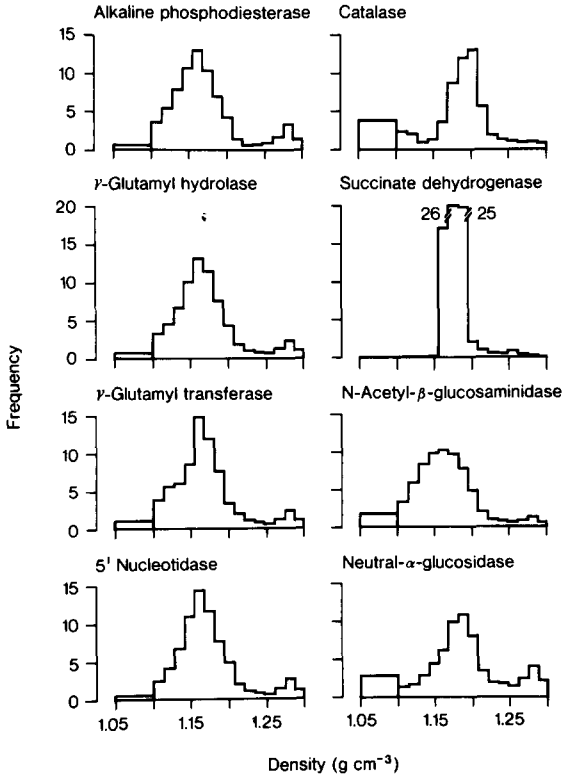

Fig. 1. Fractionation of whole homogenates of rat hepatoma cells by isopycnic centrifugation. Frequency is defined as fraction of total recovered activity present in the subcellular fractionation divided by the density span covered. The abscissa interval of densities 1.05–1.10 g · cm^{-3}, represents the activity remaining in the sample layer and is presumed to be soluble. Results show averaged data from at least two experiments.

Effects of digitonin. To confirm the plasma membrane location of γ-glutamyltransferase, cell homogenates, prepared in isotonic sucrose containing 0.24 mM digitonin, were subjected to analytical subcellular fractionation. Digitonin is a selective membrane

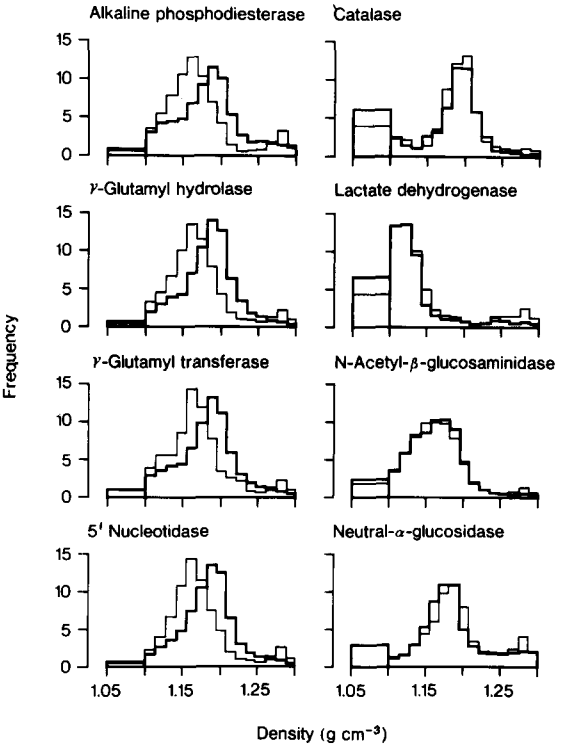

Fig. 2. Fractionation of whole homogenates of rat hepatoma cells prepared in the presence of digitonin by isopycnic centrifugation. Frequency-density distributions of some organelle marker enzymes in control (thin lines) and digitonin (thick lines) homogenates. Further details as Fig. 1.

perturbant which specifically complexes with cholesterol, thus increasing the equilibrium density [18]. As found with 5′-nucleotidase and alkaline phosphodiesterase, γ-glutamyltransferase activity is also shifted to the same extent towards the dense end of the gradient (Fig. 2). In contrast, neutral α-glucosidase, *N*-acetyl-β-glucosaminidase, catalase and lactate dehydrogenase are unaffected by the digitonin treatment.

Subcellular fractionation of cells treated with diazotized sulphanilic acid. Intact cells were treated with 1 mM diazotized sulphanilic acid for 10 min at 37°C, washed, homogenized and subjected to subcellular fractionation. The distribution of residual enzyme activity for plasma membrane and cytosolic marker enzymes are shown in Fig. 3 as relative frequency-density histograms. Lactate dehydrogenase, an inctracellular enzyme, was unaffected by diazotized sulphanilic acid. In contrast, the two plasma membrane enzymes show a 70–75% decrease in activity but the residual activity had a similar distribution to that in untreated cells.

Effects of diazotized sulphanilic acid on intact and sonicated cells. Fig. 4a and b compares the inhibition

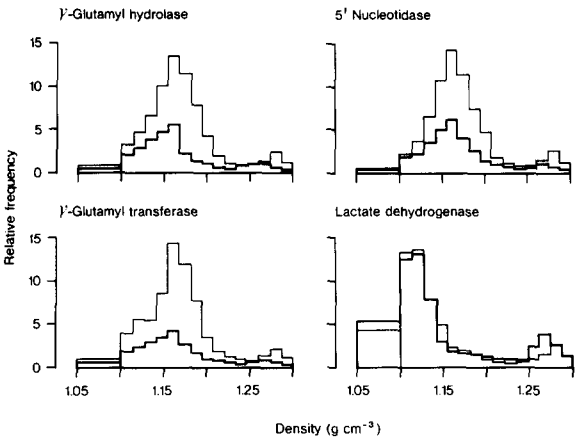

Fig. 3. Fractionation of whole homogenates of rat hepatoma cells after treatment of the intact cells with diazotized sulphanilic acid (DSA). Relative frequency-density distributions for the principal membrane marker enzymes in control (thin lines) and test (thick lines) following isopycnic centrifugation in sucrose density gradient. Relative frequency is calculated by multiplying frequency values for the treated cells by the ratio of the specific activity of the treated cell homogenate to that of the control homogenate. Further details as in Fig. 1.

by diazotized sulphanilic acid of various enzymes from either intact or disrupted cells. In intact cells, γ-glutamyltransferase and 5′-nucleotidase activities were inhibited to the same extent (70%) but alkaline phosphodiesterase activity showed less than 50% inhibition. Although 5′-nucleotidase and alkaline phosphodiesterase activitites were completely inhibited in the disrupted cells, there was no further inhibition of γ-glutamyltransferase activity even with 10 mM diazotized sulphanilic acid. In contrast, the cytosolic enzyme, lactate dehydrogenase was unaffected by diazotized sulphanilic acid unless the cells were disrupted.

Effect of diazotized sulphanilic acid on γ-glutamyltransferase activity. Rat hepatoma cells were solubilized by suspension in phosphate-buffered saline containing 0.5% Triton X-100 for 5 h at 4°C. Treatment of the solubilized material with diazotized sulphanilic acid as before, gave a maximum inactivation of 88% of the γ-glutamyltranspeptidase activity although at this concentration of diazotized sulphanilic acid, the γ-glutamylhydrolase activity was completely inhibited (Fig. 5a and b). It is apparent therefore, that even under these conditions, approx. 12% of the γ-glutamyltranspeptidase activity is insensitive to diazotized sulphanilic acid.

Purified γ-glutamyltransferase was incubated with various concentrations of diazotized sulphanilic acid. Assay of both hydrolase and transpeptidase activities showed that γ-glutamyltranspeptidase could be inhibited by up to 98%, whereas γ-glutamylhydrolase activity was only inhibited by 28%, contrasting markedly with the behaviour of the Triton X-100 solubilized preparation.

Effect of diazotized sulphanilic acid on the kinetic parameters of γ-glutamyltranspeptidase activity. The K_m^{app} and V^{app} of γ-glutamyltranspeptidase for L-γ-glutamyl-7-amino-4-methyl-coumarin was estimated in both control and diazotized sulphanilic acid-treated cells. No significant difference was found in the K_m^{app} values (0.34 and 0.29 mM for control and treated cells, respectively). However, the V^{app} of 46 nmol · min^{-1} · mg^{-1} with the intact control cells was reduced to 8 nmol · min^{-1} · mg^{-1} in the diazotized sulphanilic acid-treated cells.

Purification of hepatoma γ-glutamyltransferase. Table I summarizes the steps in the purification of γ-glutamyltransferase from these cultured hepatoma

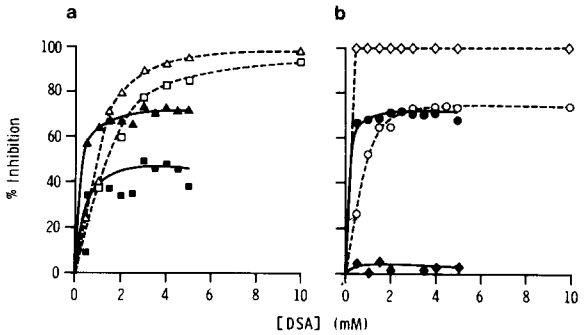

Fig. 4a and b. Percentage inhibition of plasma membrane enzymes after treatment of intact (▲———▲, 5'-nucleotidase; ■———■, alkaline phosphodiesterase; ●———●, γ-glutamyltransferase; ◆———◆, lactate dehydrogenase) and sonicated (△ - - - - - - △, 5'-nucleotidase; □ - - - - - - □, alkaline phosphodiesterase; ○ - - - - - - ○, γ-glutamyltransferase; ◇ - - - - - - ◇, lactate dehydrogenase) cells with diazotized sulphanilic acid (DSA).

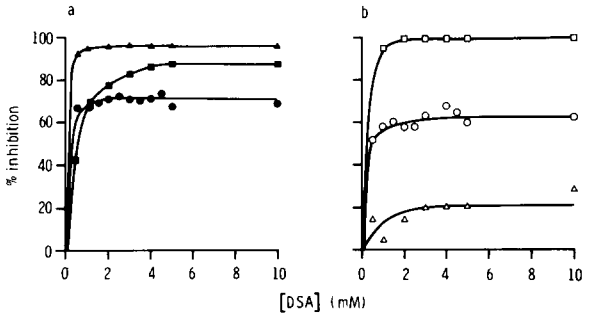

Fig. 5a and b. Effects of diazotized sulphanilic acid (DSA) on three different preparations of γ-glutamyltranspeptidase (●, intact cells; ■, Triton X-100 solubilized; ▲, purified), and its hydrolytic activities (○, intact cells; □, Triton X-100 solubilized; △, purified).

TABLE I

PURIFICATION OF γ-GLUTAMYLTRANSFERASE FROM CULTURED RAT HEPATOMA CELLS

	Volume (ml)	Enzyme activity		Protein		Specific activity (mU/mg protein)	Yield (%)	Purification (-fold)
		(mU/ml)	(total mU)	(mg/ml)	(total mg)			
Homogenization	18	1 253	22 554	13.82	249.00	91	100	1
Detergent extraction	39	418	16 300	2.90	113.00	144	72	2
$(NH_4)_2SO_4$ precipitation	8	1 910	15 280	5.34	43.00	358	68	4
Con A-Sepharose	4	664	2 656	0.16	0.64	4 150	12	46
Sepharose 6B	2	529	1 058	0.09	0.18	5 878	5	65

TABLE II

COMPARISON OF PROPERTIES BETWEEN γ-GLUTAMYLTRANSFERASE FROM HEPATOMA CELLS AND FROM NORMAL LIVER

Non-parametric 95% confidence limits [28] on the kinetic parameters are shown in parentheses. GGT: γ-Glutamyltransferase; GGH: γ-Glutanylhydrolase.

	Hepatoma cell enzyme	Normal liver enzyme
Specific activity of cell homogenate (mU/mg protein) and purification achieved	90 (65-fold)	0.35 (680-fold)
Concanavalin A binding (%) [a]	65	93
Molecular weight (apparent)	235 000	250 000
K_m^{app} (mM)	0.51 (0.42–0.63)	0.39 (0.32–0.49)
V^{app} (nmol · min^{-1} · mg^{-1} protein)	11 700 (10 400–13 400)	723 (634–835)
Turnover number (min^{-1})	2 760	181
GGH/GGT (%)	8.5	7.6
$ZnCl_2$ inhibition (50%)	0.55 mM	0.40 mM
pCMB (0.1 mM) inhibition	18%	15%

[a] Refers to lectin binding step in purification procedure.

cells. A 65-fold purification with 5% yield was achieved up to the gel filtration step. On polyacrylamide gel electrophoresis, there were two bands of protein, the major of which (approx. 95% of the total protein) corresponded to γ-glutamyltransferase activity. Preparative polyacrylamide gel electrophoresis gave rise to homogenous enzyme as judged by analytical polyacrylamide gel electrophoresis of a sample of radioactively-labelled protein. At this stage, it was however not possible to determine the protein content of the enzyme, because of the very small amounts available. The presence of Tris interfered with the fluorescamine protein assay.

Some properties of the purified hepatoma enzyme were measured and compared to that of the enzyme from normal liver [4] (Table II). There are distinct differences between the two purified enzyme preparations. The hepatoma cell enzyme shows a strikingly higher activity than the enzyme from normal liver. This reflects not only a higher level in the homogenate but also a markedly increased turnover number of the purified enzyme. The hepatoma cell enzyme has a slightly lower apparent molecular weight than the normal liver enzyme. It is also clear that significantly less activity from the hepatoma cell binds to Con A-Sepharose, thus reflecting differences in the carbohydrate content of at least a portion of the enzyme. Other kinetic and inhibition characteristics of the two enzymes are similar.

Discussion

The subcellular fractionation studies suggest that γ-glutamyltransferase is entirely localized to the plasma membrane of hepatoma cells. This finding is in agreement with the localization of γ-glutamyltransferase in normal rat liver [3]. Having localized the enzyme, its disposition within the plasma membrane was examined. The definition and characterization of an ectoenzyme relies mainly on the comparison of whole cell and homogenate enzyme activities, and the use of extracellular non-penetrating inhibitors [6,19]. In this study, diazotized sulphanilic acid was used as a structural probe to assess the fraction of γ-glutamyltransferase activity which was located on the external aspect of the plasma membrane.

The 70% inhibition of γ-glutamyltranspeptidase activity in intact cells, might at first suggest that 30% of the activity was intracellular. However, only 75% inhibition was obtained with disrupted cells. In contrast, the 5′-nucleotidase and alkaline phosphodiesterase of disrupted cells were almost completely inactivated by diazotized sulphanilic acid. This suggests that 20–30% of the γ-glutamyltranspeptidase is embedded within the hydrophobic environment of the plasma membrane. When the cells were disrupted in the presence of detergent and the lysate exposed to diazotized sulphanilic acid, only 50% of the residual γ-glutamyltranspeptidase activity was further inactivated. Therefore, at least 12% of the total cell complement of γ-glutamyltranspeptidase activity remained inaccessible to diazotized sulphanilic acid. In contrast, in the presence of detergent, 5′-nucleotidase and alkaline phosphodiesterase were completely inactivated by diazotized sulphanilic acid. The same observation has been reported for 5′-nucleotidase in mouse peritoneal macrophages [20]. However, the apparent K_m for L-γ-glutamyl-7-amino-4-methyl-coumarin of the residual γ-glutamyltranspeptidase, after diazotized sulphanilic acid treatment of hepatoma cells, is similar to that of the uninhibited enzyme, suggesting that there are not two distinct enzymes located both superficially and within the plasma membrane. The fact that a large percentage of the total cell complement of γ-glutamyltranspeptidase is located on the external aspect of the plasma membrane, argues against participation of γ-glutamyltranspeptidase, as envisaged by Meister [21], in the transport of amino acids in this cell type.

A comparison of hepatoma cell γ-glutamyltransferase with that from normal liver showed that during purification, 90% of the normal liver γ-glutamyltransferase was bound to concanavalin A, whereas only 65% of the hepatoma cell enzyme was bound. This indicates that a significant proportion of the hepatoma enzyme was sialylated [22]. This modification may protect the enzyme from being degraded, and by analogy with circulating glycoproteins, prolong its half life [23]. Further investigation into the physical properties of the enzyme was carried out on a purified preparation. Comparison of hepatoma cell γ-glutamyltransferase with that from normal liver showed close similarities with respect to apparent molecular weight, K_m and the effect of various inhibitors. However, kinetic studies revealed that the

turnover number of the hepatoma cell enzyme was approx. 15-fold greater than that of normal liver enzyme. This means that although increased amounts of enzyme are present in the hepatoma cell, the increase in specific activity (16-fold of this enzyme) is due, in part, to an increase in the catalytic efficiency, suggesting some structural or conformational alteration to the enzyme during the carcinogenic transformation of the liver cell.

γ-Glutamyltransferase may also function as a non-specific hydrolase, depending on the conditions of assay. In the absence of glycylglycine acceptor, hydrolysis predominates. McIntyre and Curthoys [24] have shown that at physiological pH, γ-glutamyltransferase efficiently catalyses hydrolysis in preference to transpeptidation. In the present study, treatment of the intact cells with diazotized sulphanilic acid revealed that nearly 40% of the hydrolytic activity remained inaccessible, although Triton X-100 solubilization allowed inhibition of all the hydrolytic activity. In contrast, when the purified enzyme was treated with 0.5 mM diazotized sulphanilic acid, the hydrolytic activity was affected to a much lesser extent than transpeptidase activity. One possible explanation for this behaviour is that the process of purification of the enzyme may have resulted in the loss of a protein factor conferring sensitivity of the hydrolase towards diazotized sulphanilic acid inhibition, analogous to the oligomycin sensitivity conferring protein of mitochrondrial adenosine triphosphatase [25].

On the other hand, the transpeptidase activity of purified enzyme was completely inactivated by diazotized sulphanilic acid. This observation suggests that chemical modification of its accessible active centre by the diazonium salt of sulphanilic acid has affected the enzyme differentially in respect to its resulting activity towards different acceptor substrates (H_2O or glycylglycine). The diazonium group may have modulated the enzyme so as to uncouple or dissociate the transpeptidation function from its ability to use glycylglycine, but still allows access of H_2O to the active site. Such an effect is consistent with reports on the ability of maleate [26] and hippurate [27] to enhance hydrolysis of γ-glutamyl compounds at the expense of transpeptidation. In this instance, detailed kinetic investigations of the remaining activity will be required to give further indications of effects on activity through modification either of catalytic or substrate-binding residues. A more direct approach to this problem may be to use monospecific antibodies raised against the homogenous enzyme to differentiate the two activities based on their possible immunological differences.

Acknowledgments

We thank Dr. G.E. Neal for providing hepatoma cells, and details of the culture technique. We are grateful to Dr. D.S. Leake for advice on cell culture, to Mr. P. White and Ms. T. Shah for expert technical assistance and to Ms. R. Greensted for typing the manuscript. This work is supported by the Cancer Research Campaign.

References

1 Glossman, H. and Neville, D.M. (1972) FEBS Lett. 19, 340–344
2 Peters, T.J. (1976) Clin. Sci. Mol. Med. 51, 557–574
3 Smith, G.D. and Peters, T.J. (1980) Eur. J. Biochem. 104, 305–311
4 Ding, J.L., Smith, G.D. and Peters, T.J. (1981) Biochim. Biophys. Acta 657, 334–343
5 Fiala, S. and Fiala, E.S. (1973) J. Natl. Cancer Inst. 51, 151–158
6 DePierre, J.W. and Karnovsky, M.L. (1974) J. Biol. Chem. 249, 7 111–7 129
7 Smith, G.D., Ding, J.L. and Peters, T.J. (1979) Anal. Biochem. 100, 136–139
8 Razzell, W.E. (1963) Methods Enzymol. 6, 238
9 Seymour, C.A. and Peters, T.J. (1977) Clin. Sci. Mol. Med. 52, 229–239
10 Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766
11 Bohlen, P., Stein, S., Dairman, W. and Udenfriend, S. (1973) Arch. Biochem. Biophys. 155, 213–220
12 Beaufay, H. (1966) Thèse d'Aggrègation de L'Enseigment Supérieur, Universitè Catholique de Louvain, Louvain, Belgium
13 De Duve, C., Berthet, J. and Beaufay, H. (1959) Progr. Biophys. Biophys. Chem. 9, 325–369
14 Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, S. and De Duve, C. (1968) J. Cell Biol. 37, 482–513
15 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
16 Brooks, T.J., and Sammons, H.G. (1980) Ann. Clin. Biochem. 17, 15–19
17 Jorgensen, K.H. and Larsen, U.D. (1972) Horm. Metab. Res. 4, 224–225

18 Beaufay, H., Amar-Costesec, A., Thinès-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) J. Cell. Biol. 61, 213–231
18 Berg, H.C. (1969) Biochim. Biophys. Acta 183, 65–78
19 Edelson, P.J. and Cohn, Z.A. (1976) J. Exp. Med. 144, 1 595–1 608
21 Meister, A. (1973) Science 180, 33–39
22 Kottgen, E., Reutter, W. and Gerok, W. (1976) Biochem. Biophys. Res. Commun. 72, 61–66
23 Ashwell, G. and Morell, A.G. (1974) Adv. Enzymol. 41, 99–128
24 McIntyre, T.M. and Curthoys, N.P. (1979) J. Biol. Chem. 254, 6 499–6 504
25 Racker, E. (1963) Biochem. Biophys. Res. Commun. 10, 435
26 Tate, S.S. and Meister, A. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3 329–3 333
27 Thompson, G.A. and Meister, A. (1980) J. Biol. Chem. 255, 2 109–2 133
28 Cornish-Bowden, A., Porter, W.R. and Trager, W.F. (1978) J. Theor. Biol. 74, 163–175