

# Purification of r-Glutamyltranspeptidase from Rat Primary Hepatoma Tissue and Preparation of a Tumor Associated Antigen\*

Sang Hwan Oh and Koo Woo

*r-Glutamyltranspeptidase (r-GT) from a rat hepatoma induced by 3'-methyl-4-dimethylaminoazobenzene (3'-Me DAB) was purified 833 fold. The purified enzyme had a specific activity of 15.0 U/mg protein with an overall yield of 3.8%. The molecular weight of native r-GT was estimated as about 350,000 daltons, which is a multicomplex of a single polypeptide having a M W of 59,000. Anti r-GT rabbit antiserum cross-reacted with kidney r-GT as well as liver r-GT. Tryptic digestion of r-GT followed by separation with Con A Sepharose column chromatography resulted in two major glycopeptides. A tumor associated antigen was prepared by the conjugation of a tryptic glycopeptide of r-GT to keyhole limpets hemocyanin, and an antibody against this antigen cross-reacted preferentially with r-GT in rat hepatoma tissue.*

**Key Words:** r-Glutamyltranspeptidase, purification, rat primary hepatoma, tumor associated antigen.

r-GT is a plasma membrane glycoprotein which catalyzes the initial step in the catabolism of glutathione in the r-glutamyl cycle for the transport of amino acids across the cell membrane (Meister and Tate 1976). r-GT is present in several tissues of adult mammals, and it is transiently increased in fetal liver (Fiala and Fiala 1973), skin (Buxman *et al.* 1979) and intestinal mucosa (Adjarov *et al.* 1979). Some tumors (skin, liver, colon, lung, kidney) have unusually high levels of r-GT, and the detection of the enzyme has been a marker for very early as well as late, putative liver preneoplastic and neoplastic cells (Fiala *et al.* 1972; Hada *et al.* 1978; Klein-Szanto *et al.* 1983; Jaken and Mason 1978).

It has been demonstrated that r-GT activity is elevated in the preneoplastic liver nodule of rats given 3'-Me DAB (Taniguchi *et al.* 1974; Suzuki *et al.* 1987), and it has been indicated that the transformational

changes of r-GT are mainly induced in the carbohydrate moieties of the enzyme molecules (Yamashita *et al.* 1983; Sawabu *et al.* 1983). r-GT from various tumors and normal tissue showed the same antigenicity and similar amino acid composition but the total number of asparagine-linked sugar chains in one molecule of AH-66 r-GT was approximately four times that in one molecule of normal liver r-GT (Taniguchi *et al.* 1983). The modified sugar residues of r-GT were clearly distinguishable by gradient polyacrylamide gel electrophoresis, and this had a diagnostic value for screening for certain cancers including hepatoma. However, immunochemical detection of the novel enzyme is unsuccessful at present time due to the similar antigenicity of r-GTs from normal and hepatoma tissue.

Although a novel type of r-GT appears in the sera of patients, the novel form can not be identified by conventional immunoassay. Therefore, more specific biochemical or immunochemical markers are necessary for the diagnosis of a tumor. In the present study, we have attempted to prepare a tumor associated r-GT antigen by conjugation of a glycopeptide containing the novel type of sugar residue in r-GT to a carrier protein, in order to produce an antibody useful in the diagnosis of hepatoma in rats.

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Department of Biochemistry, Yonsei University College of Medicine, Seoul, Korea 120

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Address reprint requests to Dr. S-H Oh, Department of Biochemistry, Yonsei University College of Medicine, Seoul, Korea 120

## MATERIALS AND METHODS

### Animals and Diets

Weanling male Albino rats (70g BW) were fed a semipurified diet (Table 1) containing 0.06% 3'-Me DAB for 16 weeks in order to induce liver tumors. The incidence of hepatoma was greater than 90% at the end of the feeding period. The control group received the same diet without 3'-Me DAB, and tap water was supplied ad libitum.

### Materials

3'-Me DAB was purchased from Eastman Kodak Co. U.S.A. DEAE-Trisacryl, Ultrogel (AcA 34), Ampholine Pag plate and agarose were obtained from LKB, Sweden. DEAE-Sephacel, Sephadex G-25 were obtained from Pharmacia Inc., Sweden. ConA Sepharose, acrylamide, N,N'-methylene-bis(acrylamide) tetramethylene diamine (TEMED), r-glutamyl-p-nitroanilide, trypsin, glycylglycine, keyhole limpets hemocyanin, 1-ethyl-3-(3'-dimethylamino propyl) carbodiimide: HCl, Tris (hydroxy-methyl) aminomethane and lauryl sulfate were purchased from Sigma Chem Co. USA, and other reagents were supplied by local suppliers.

### Enzyme assay

Assay of r-GT activity was performed according to the method described by Tate and Meister (1985). One unit of r-GT activity was defined as  $\mu$  mole of

p-nitroaniline formed per minute from L-r-glutamyl-p-nitroanilide (1mM) in the presence of glycylglycine (20mM), at pH 8.5.

### Purification of r-GT

**Preparation of liver homogenate:** Fresh rat hepatoma tissues were obtained from rats fed a diet containing 3'-Me DAB for 16 weeks. Tissues were homogenized in a glass tissue homogenizer with 5 volumes of 20mM Tris-HCl buffer, pH 8.0 (TB).

**Preparation of crude membrane fraction:** The liver homogenate was centrifuged at  $700 \times g$  for 10 min to remove the crude nuclear fraction, and the supernatant was further centrifuged at  $27,000 \times g$  for 30 min. The precipitate was collected and dissolved in TB containing 2% Triton X-100 by stirring overnight at 4°C.

**Solvent extraction:** To the crude membrane extract, acetone was added dropwise to a final concentration of 60%. After centrifugation at  $4,000 \times g$  for 15 min, the precipitate was collected and dissolved in one fifth initial volume of TB containing 2% Triton X-100. The dissolved enzyme solution was mixed with same volume of diethylether and shaken vigorously for 10 min. After centrifugation at  $12,000 \times g$  for 10 min, the aqueous phase containing the enzyme was collected. Traces of diethylether remaining in the enzyme solution were evaporated by stirring at 37°C.

**DEAE-Trisacryl column chromatography:** Onto a DEAE-Trisacryl column (2.5×6.0cm) equilibrated with TB containing 0.5% Triton X-100, the enzyme solution was applied and eluted with the same buffer containing a linear gradient of 0-0.2M NaCl. Peak fractions of r-GT were pooled and precipitated with ammonium sulfate (final saturation 80%), and the precipitate was dissolved in a small volume of TB containing 0.2% Triton X-100.

**Gel filtration:** Onto an Ultrogel (AcA 34) column (1.2×80cm) equilibrated with TB containing Triton X-100 (0.5%), the concentrated r-GT solution was applied and eluted with the same buffer. Peak fractions of r-GT were pooled.

**DEAE-Sephacel column chromatography:** To a DEAE-Sephacel column, (2.5×5.0cm) equilibrated with TB containing 0.5% Triton X-100, the pooled r-GT solution was applied and eluted with the same buffer containing a step gradient of 60-80mM of NaCl. Fractions containing r-GT were pooled and used as the purified enzyme.

### Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of native r-GT

Table 1. Diet composition

Ingredient	Diet group	
	Control	3'-Me DAB
Casein, gm	180	180
Corn oil, gm	50	50
Glucose monohydrate, gm	770	770
Salt mixture, gm	40	40
Riboflavin, gm	0.001	0.001
Vitamin mixture, ml	5*	5*
3'-Me DAB, gm	—	0.6

\* Contained cod liver oil 20gm, choline chloride 1.5gm, pteroylglutamate 0.6gm, biotin 1.5mg, thiamine-HCl 20mg, pyridoxine-HCl 20mg, menadion 50mg (in 20mg of cod liver oil), nicotinamide 50mg, potassium p-aminobenzoic acid 50mg, calcium pantothenate 60mg, inositol 100mg and cyanocobalamin 40ug.

was carried out by the method of Davis (1964). SDS polyacrylamide gel electrophoresis was carried out in SDS gel (10% or 12%). Samples were mixed with 2% SDS and 5%-2mercaptoethanol (final concentration) then boiled for 2 min in a water bath. After cooling, the samples were applied to a SDS slab gel and electrophoresis was run at a constant 30mA. The subunit molecular weight of r-GT was calculated from the migration distances of r-GT and molecular weight markers.

### Amino acid analysis

Purified r-GT (100 $\mu$ g) was hydrolyzed with 6M HCl at 110°C for 18hrs under vacuum conditions, and HCl was evaporated. The hydrolysate dissolved in 100 $\mu$ l 0.2N sodium citrate buffer, pH2.2, was subjected to amino acid analysis by an amino acid analyser (LKB 4150).

### Isoelectric focusing

Isoelectric focusing of r-GT was carried out on an Ampholine Pag plate (pH 3.5-9.5). After the application of purified r-GT to the plate, current was applied to achieve an initial voltage of 800 and continued overnight. After electrophoresis, the gel plate was stained with Coomassie blue followed by destaining with 10% acetic acid containing 30% methanol, and the position of r-GT on the gel plate was identified. pH gradient on the gel plate was constructed by measuring the pHs of gel slices cut into 1 cm increments.

### Preparation of glycopeptide of r-GT

Tryptic digestion of r-GT was performed as follows. To r-GT dissolved in TB, trypsin (2% by weight of the protein) was added and incubated for 24 hours at 37°C. The digest was cooled to 47°C, then applied on a Con A Sepharose column equilibrated with TB containing 1mM CaCl<sub>2</sub>. After washing the column with the same buffer (5 times the bed volume), glycopeptides were eluted with 0.1M  $\alpha$ -D-methylmannoside. Glycopeptides were further separated by Sephadex G-25 column chromatography.

### Peptide mapping and conjugation

Glycopeptides isolated from tryptic digest of r-GT were mapped by reverse-phase high pressure liquid chromatography (Laboratory Data Control, Model I) according to the method described by Mayes (1984).

The peptide was conjugated to keyhole limpets hemocyanin by incubating at room temperature overnight in the presence of 1-ethyl-3-(3'-dimethylamino propyl) carbodiimide (10 $\times$ molar excess over the peptide) dissolved in 0.1 M phosphate buffer (pH 7.2), followed by dialysis against the buffer overnight.

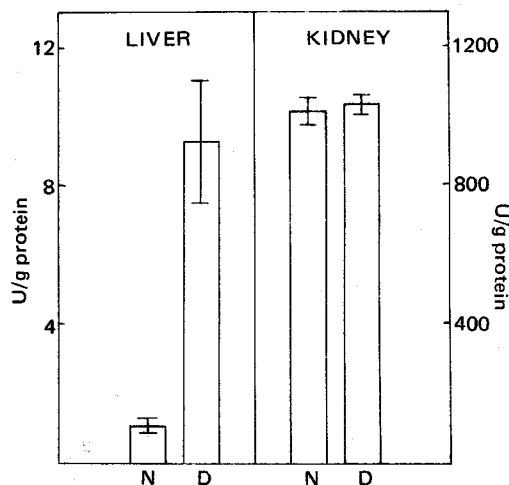
### Immunization of rabbits with r-GT and r-GT glycopeptide conjugate

For the preparation of antibodies against r-GT and tumor associated r-GT antigen, purified r-GT or glycopeptide of r-GT conjugated to keyhole limpets hemocyanin (2mg/ml) were emulsified with an equal volume of complete Freund's adjuvant and injected into rabbit lymph nodes. After 2 weeks, booster injections with the same amount of antigens were given, and 3 weeks later blood was collected from each animal. The Ouchterlony test (Ouchterlony, 1958) was carried out to identify precipitins between sera and antigens.

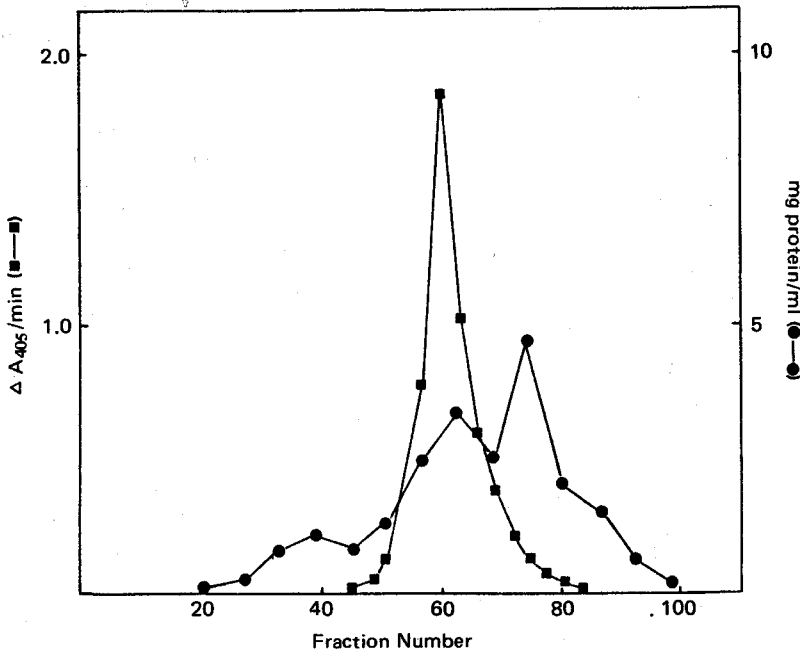
## RESULTS

### Induction of rat hepatoma by 3'-Me DAB and increase of r-GT activity in hepatoma tissue.

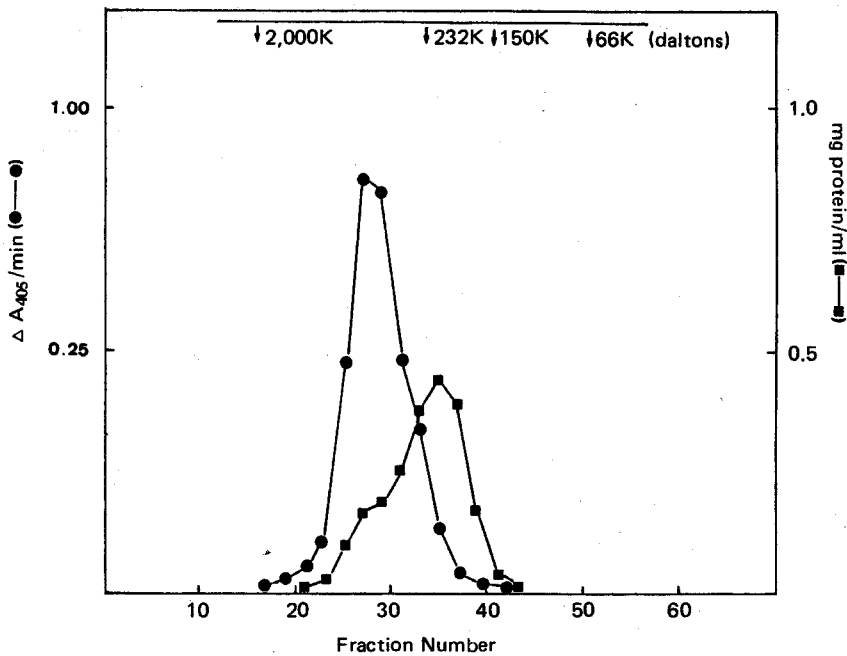
Hepatoma nodules began to appear in the liver



**Fig. 1.** r-GT activities in liver and kidney homogenates of rats with hepatoma induced by 3'-Me DAB. N, Normal rats; D, rats with hepatoma induced by a diet containing 0.06% 3'-Me DAB for 16 weeks.



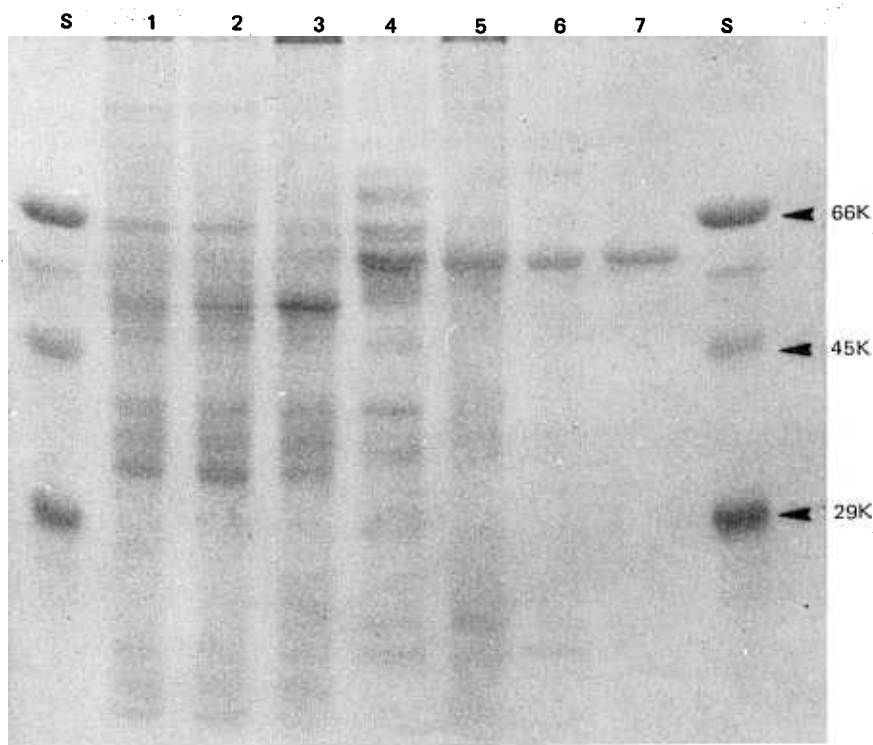
**Fig. 2.** DEAE Trisacryl column chromatography of r-GT in rat hepatoma tissue. Onto DEAE Trisacryl column (2.5 × 6.0 cm) equilibrated with 20mM Tris, buffer pH8.0 containing 0.1% Triton X-100, fractions containing r-GT were applied and washed with 2 times the bed volume of the same buffer followed by elution with the buffer containing a linear gradient of 0 to 0.2M NaCl at a flow rate of 20 ml per hour.



**Fig. 3.** Ultragel (ACA 34) column chromatography of r-GT in rat hepatoma tissue. Onto a column (1.2 × 80 cm) packed with Ultro gel equilibrated with 20mM Tris, buffer pH 8.0 containing 0.5% Triton X-100, concentrated r-GT (2.0 ml) was applied and eluted with the same buffer at a flow rate of 10 ml/hr.

**Table 2. Purification<sup>a</sup> of r-glutamyltranspeptidase from 3'-Me DAB treated rat liver.**

Step	Total activity (U)	Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Liver homogenate	1,195	65,000	0.018	100.0	1.0
Post-nuclear fraction	1,065	57,750	0.018	89.1	1.0
Crude membrane fraction	760	22,600	0.034	63.6	1.9
Solvent extraction	304	1,600	0.190	25.4	10.6
DEAE-Trisacryl chromatography	157	102	1.539	13.1	85.5
Gel filtration (AcA 34)	123	23	5.348	10.3	297.1
DEAE-Sephacel chromatography	45	3	15.000	3.8	833.3



**Fig. 4.** SDS Polyacrylamide gel electrophoresis of r-GT in rat hepatoma induced by 3'-Me DAB. SDS Polyacrylamide gel (12%) electrophoresis was run at a constant 30mA for 5 hrs and gel was stained with coomassie blue then destained with 30% methanol containing 10% acetic acid. S, molecular weight standards (bovine serum albumin 68K, ovalbumin 45K, carbonic anhydrase 29K); lane 1, liver homogenate; lane 2, postnuclear fraction; lane 3, crude membrane fraction; lane 4, solvent extraction; lane 5, DEAE Trisacryl eluate; lane 6, AcA 34 gel filtrate; lane 7, DEAE Sephacel eluate (final step).

of rats fed with a diet containing 3'-Me DAB for 12 weeks, and most of the rats exhibited hepatoma after 16 weeks of the diet. r-GT activity in the liver homogenate was markedly increased with the appearance of nodules (Fig. 1). However, r-GT activity

in the kidney of rats with hepatoma was similar to that of normal rats.

#### Purification of r-GT from rat hepatoma tissue

DEAE-Trisacryl chromatography and Ultrogel filtra-

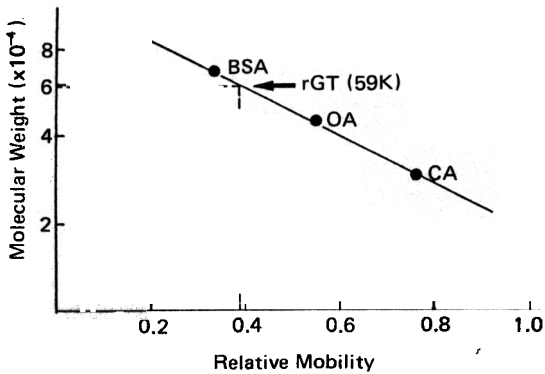


Fig. 5. Molecular weight determination of r-GT by SDS polyacrylamide gel electrophoresis. BSA, bovine serum albumin (68K daltons); OA, ovalbumin (45K daltons); CA, carbonic anhydrase (29K daltons).

tion of r-GT are shown in Fig. 2 and Fig. 3, respectively. r-GT from the hepatoma tissues of rats was 833.3 fold purified with a 3.8% yield after DEAE-Sephacel chromatography, the final purification step (Table 2).

The purified r-GT has a specific activity of 15.0 units/mg protein and the homogeneity of the enzyme was confirmed from polyacrylamidegel electrophoresis data not shown and SDS polyacrylamide gel electrophoresis (Fig. 4).

#### Physicochemical nature of r-GT

The molecular weight of the native form of r-GT was estimated to be 350,000 daltons from the Ultrogel (AcA 34) filtration chromatogram (Fig. 3). The native form of r-GT behaves as a single form of a polypeptide having a M W of 59,000 when SDS polyacrylamide gel electrophoresis was carried out (Fig. 4 and Fig. 5).

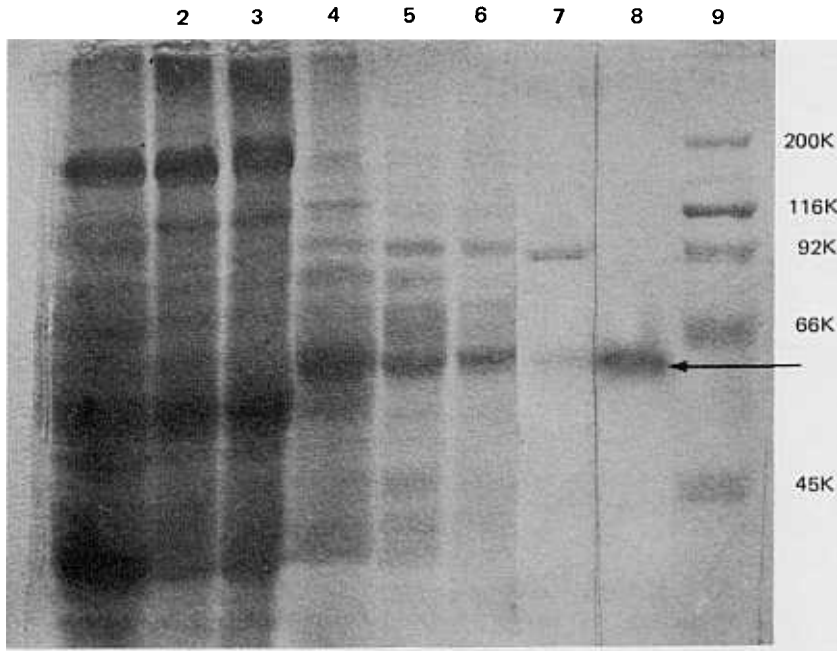


Fig. 6. SDS polyacrylamide gel electrophoresis of r-GT in rat hepatoma tissue and identification of the enzyme by activity staining.

Each sample was treated with 0.1% SDS without heat treatment (no boiling) and applied on the SDS polyacrylamide gel (10%). Electrophoresis was run at a constant 30 mA for 5 hrs. After electrophoresis, the gel (lane 8) was cut out for activity staining of r-GT (Arrow indicates yellow color for activity). The rest of the gel was stained followed by destaining as in Fig. 4. 1, homogenate; 2,3, postnuclear fraction; 4, crude membrane fraction; 5, solvent extraction; 6, DEAE Trisacryl eluate; 7, Ultrogel filtrate; 8, Activity staining for r-GT. Gel was incubated at 30°C for 10 min in a buffer solution (Tris HCl, pH 8.0) containing 1mM r-glutamyl p-nitroanilide; 9, Molecular weight standards.

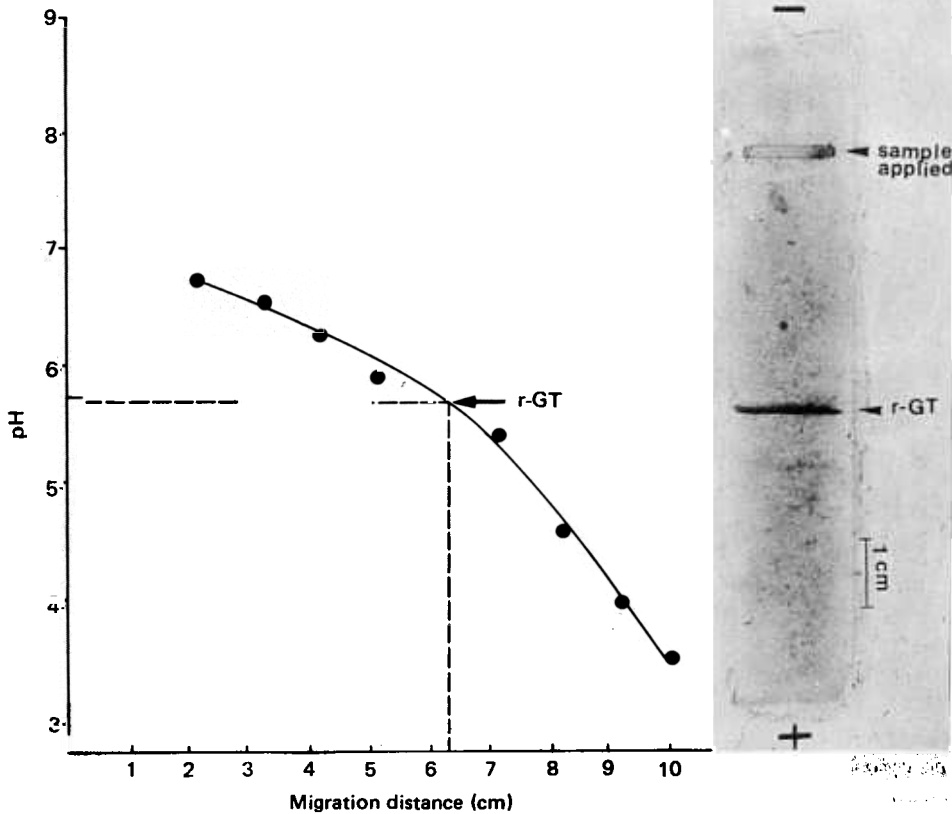


Fig. 7. Isoelectric focusing of r-GT purified from rat hepatoma induced by 3'-Me DAB. On an Ampholine Pag plate (pH 3.5-9.5), r-GT was applied to the cathode side of the gel plate, and an initial 800 voltage was attained. After electrophoresis for over 12 hours, the protein was fixed on the gel with 20% trichloroacetate, then the gel was soaked overnight and stained with Coomassie blue followed by destaining as in Fig. 4. A pH gradient was constructed after the measurement of pH in the extract from each piece of gel cut into 1cm increments. The photograph in the box shows the position of r-GT.

Table 3. Amino acid composition of r-glutamyltranspeptidase purified from rat hepatoma induced by 3'-Me DAB

Amino acid	Mole %	Residue	Amino acid	Mole %	Residue
Asx*	10.02	(49)	Met	2.07	(10)
Thr	5.97	(29)	Ile	4.25	(21)
Ser	6.86	(34)	Leu	9.52	(47)
Glx*	11.05	(54)	Phe	3.31	(16)
Pro	7.84	(39)	Thr	5.01	(25)
Gly	8.04	(40)	His	2.40	(12)
Ala	7.39	(36)	Lys	5.89	(29)
Cys	0.56	(3)	Arg	2.74	(29)
Val	7.08	(35)			

\* Asx : Asp + Asn, Glx : Glu + Gln.

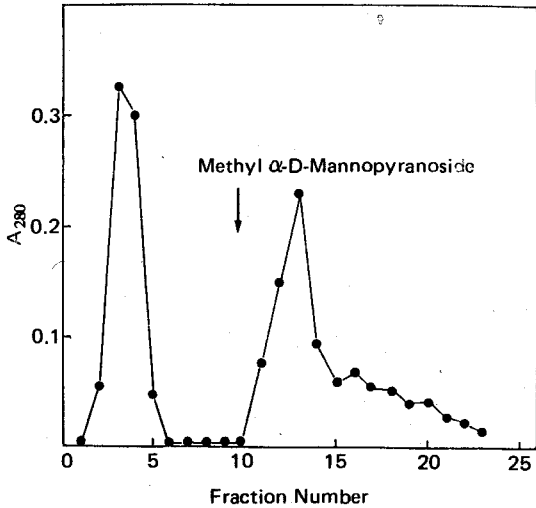
This single form of polypeptide had a catalytic activity when the enzyme was not treated with heat during the course of SDS polyacryl amide gel electrophoresis (Fig. 6).

The isoelectric point of purified r-GT was determined as 5.7 by isoelectric focusing of the enzyme on an Ampholine Pag plate (Fig. 7).

Amino acid compositions of purified r-GT are shown in Table 3. Acidic amino acid residues are relatively richer than basic amino acid residues. Valine and leucine residues are fairly rich.

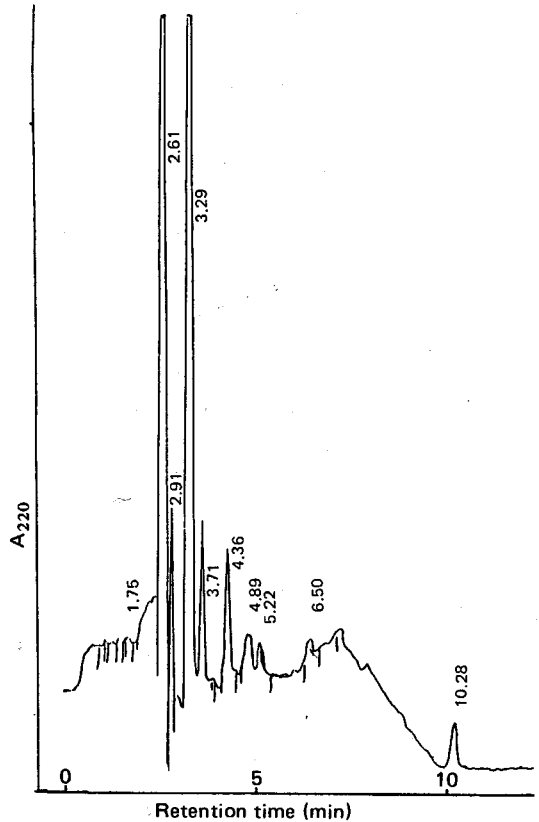
#### Preparation of glycopeptides of r-GT

Separation of glycopeptide from the tryptic digest of purified r-GT by ConA Sepharose column chromatography is shown on Fig. 8. Pooled fractions



**Fig. 8.** ConA-Sepharose column chromatography of tryptic digest of r-GT purified from rat hepatoma induced by 3'-Me DAB.

Onto a ConA Sepharose column (1 × 5 cm) equilibrated with 20 mM Tris, pH 8.0 buffer containing 1 mM  $\text{CaCl}_2$ , a tryptic digest of r-GT was applied then washed with the same buffer until no detectable peptides were eluted. After sufficient washing of the column, glycopeptides were eluted by the same buffer containing 0.1M methyl  $\alpha$ -D-mannopyranoside. 3 ml of each fraction was collected.



**Fig. 9.** High pressure liquid chromatography of glycopeptides obtained from the tryptic digest of the purified rat r-GT. 20  $\mu$ l of glycopeptide fractions obtained from ConA-Sepharose column-chromatography were injected into Spherisorb C6 column and separated by a linear gradient of 0 to 60% acetonitril in 0.05M ammonium acetate buffer (pH 6.0). Chromatography was run at a flow rate of 1ml/min and with a pressure of 1,700 PSI.

(No. 11-20 in Fig. 8) of glycopeptides were freeze dried and further separated by HPLC (Fig. 9) and by Sephadex G-25 column chromatography (Fig. 10).

There were two major peaks (GPI, GPII) on the chromatogram when the absorbance of fractions at 280 nm was measured. The first peak (GPI) corresponding to a MW of 6,000, absorbed more light at 220 nm than the second peak (GPII) did, and the position of GPII peak ( $A_{280}$ ) did not coincide with GPII peak ( $A_{220}$ ), indicating that this peak is heterogeneous.

#### Immunoprecipitation of tumor associated antigen

Antibodies against r-GT isolated from rat hepatoma and a glycopeptide of r-GT conjugated to keyhole limpets hemocyanin were produced in rabbits. A single precipitin was formed between r-GT and antiserum (Fig. 11, A).

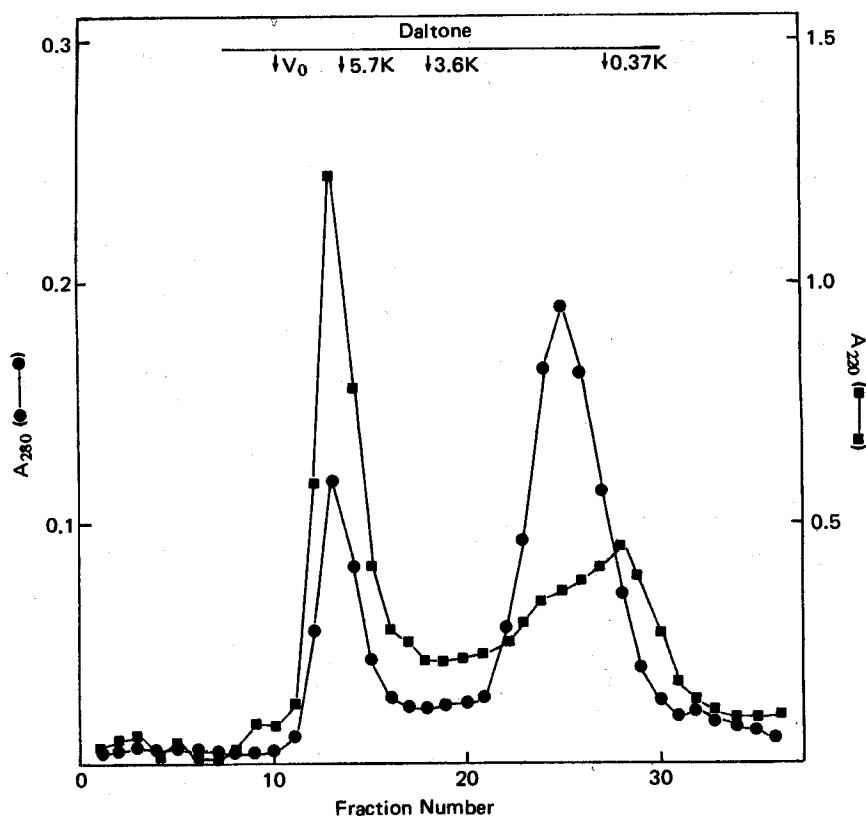
Anti r-GT rabbit antiserum cross-reacted with r-GT in rat kidney as well as that in rat liver, but the positions of precipitins formed were not identical between

liver and kidney (Fig. 11, B Fig. 11, C).

Anti-r-GT antiserum also cross-reacted with tryptic digest (Fig. 11, C) and with glycopeptide (GPI) (Fig. 11, D). However, their precipitin boundaries were heterogeneous. Anti r-GT antiserum cross reacted with glycopeptide conjugated to keyhole limpets hemocyanin, but precipitins did not form a hexagon (Fig. 11, E) indicating the nonidentity between r-GT and the glycopeptide conjugate.

Rabbit antiserum against GPI-conjugated antigen cross reacted with r-GT of rat hepatoma more preferentially than with r-GT from normal rat liver (Fig. 11F).





**Fig. 10.** Sephadex G-25 column chromatography of glycopeptides obtained by tryptic digestion of r-GT in rat hepatoma. Glycopeptide fraction from ConA Sepharose chromatography were concentrated by freeze drying and dissolved in a small volume (2ml). The sample was applied to a Sephadex G-25 column ( $1.2 \times 60$  cm) equilibrated with ammonium acetate buffer (pH 6.0) then eluted with the same buffer at a flow rate of 10 ml/hr.

## DISCUSSION

The activity of r-GT increased up to 100 fold in rat liver after the induction of hepatoma by chemical carcinogens (Fiala *et al.* 1972; Conway *et al.* 1987). The same localization of r-GT and multiple hyperplastic nodules has been observed in an immunohistochemical study of r-GT during the early stages of hepatocarcinogenesis induced by 3'-Me DAB (Suzuki *et al.* 1987). A diet of 3'-Me DAB, a potent hepatocarcinogen, induced hepatoma in rats with an 8 fold increase in r-GT activity in the present study, and the elevation of r-GT activity was associated with the appearance of nodules in the liver.

r-GT isolated from rat kidney by detergent extrac-

tion and affinity chromatography behaved as an aggregate of MW greater than 200,000, and the treatment of the aggregate by bromelain yielded a light form of enzyme having a molecular weight of 68,000 composed of two non identical subunits of 46,000 and 22,000 daltons (Tate and Meister 1976). Nash and Tate (1982) had reported the MW of rat kidney r-GT as 78,000 daltons composed of two subunits of MW of 51,000 and 22,000.

The MW of r-GT purified from azo dye-induced rat hepatoma has been reported as 113,000 (Taniguchi 1974), and that from human adult liver, fetal liver, and primary hepatoma as 84,000. Recently, it has been reported that a human hepatoma cell line expresses a single-chain form of r-GT which has a MW of 120,000 (Tate and Galbraith 1987).

Most mammalian r-GTs have been shown to be

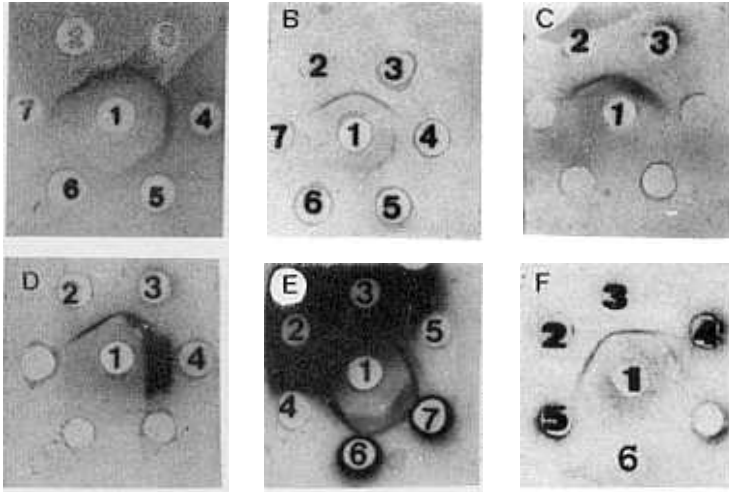


Fig. 11. Ouchterlony double immunodiffusion of rabbit anti r-GT antiserum or rabbit anti conjugated r-GT glycopeptide antiserum with r-GT from various rat tissues.

- A) 1, purified rat liver r-GT.  
2, rabbit anti-r-GT antiserum (1 × dilution).  
3, antiserum (2 × dilution).  
4, antiserum (4 × dilution).  
5, antiserum (8 × dilution).  
6, antiserum (16 × dilution).  
7, antiserum (32 × dilution).
- B) 1, rabbit anti r-GT antiserum.  
2,3, rat hepatoma extract.  
4,5, rat kidney extract.
- C) 1, rabbit anti r-GT antiserum.  
2, r-GT purified from rat hepatoma.  
3, tryptic digest of r-GT purified from rat hepatoma.
- D) 1, rabbit anti r-GT antiserum  
2, r-GT purified from rat hepatoma.
- 3, tryptic glycopeptide of purified r-GT.  
4, tryptic non-glycopeptide of purified r-GT.
- E) 1, rabbit anti r-GT antiserum.  
2,3, normal rat liver extract.  
4,5, rat hepatoma extract.  
6,7, glycopeptide conjugated to keyhole limpets hemocyanin.
- F) 1, rabbit antiserum against glycopeptide of r-GT conjugated to keyhole limpets hemocyanin.  
2, purified r-GT  
3, rat hepatoma extract  
4, hepatoma bearing rat serum  
5, normal rat liver extract  
6, normal rat serum

heterodimeric glycoproteins (Tate and Meister 1982). However, the present results show the presence of a single form of r-GT (MW 59,000) in rat hepatoma tissues as evidenced by activity staining (Fig. 6) and SDS polyacrylamide gel electrophoresis (Fig. 4). The discrepancy between these results and previous reports might arise from differences in animal strain, diet composition and tumor states of tissues or cells, as indicated by Russell *et al.* (1987). Moreover, no agreement on the MW of r-GT from various sources has yet been reached.

It has been demonstrated that transformation of liver cells accompanies the change in carbohydrate moieties of r-GT (Yamashita *et al.* 1983; Taniguchi *et al.* 1985). The sugar chains of the hepatoma r-GT is

composed of the high mannose type with more bisecting N-acetyl glucosamine residues which are not found in those of the normal liver enzyme.

It has been also indicated that r-GT produced in rat hepatoma, rat mammary tissues and human renal carcinoma had a more acidic pI value than those of r-GT in the respective normal tissues (Taniguchi 1974; Jaken and Mason 1978; Hada *et al.* 1978). r-GT in rat kidney exists in 12 different isozymic forms depending on the sialic acid content in the enzyme (Tate and Meister 1976) and the pI values for these enzymes were between 5 and 8. The pI value (5.7) of the r-GT prepared in this study indicated that a single form of r-GT having a more acidic pI is predominant in hepatoma tissues.

Since the recognition of the diagnostic value of r-GT activity for hepatoma (Taniguchi 1974), extensive studies on the use of r-GT as a histochemical or immunochemical marker for neoplasia in different tissues of animals have been carried out (De Young *et al.* 1979; Suzuki *et al.* 1987). Studies with monoclonal antibodies for a family of blood group-related antigens based on the carbohydrate backbone sequence have shown that the carbohydrate sequences behave as tumor associated antigens in certain cell types (Feizi and Childs 1985).

A monoclonal antibody against r-GT from human primary-hepatoma preferentially bound to the small subunit of r-GT from human hepatoma and weakly bound to the normal liver enzyme (Taniguchi *et al.* 1987).

However, these monoclonal antibodies were neither tumor specific nor common to tissues.

GPI of r-GT conjugated to keyhole limpets hemocyanin in this study has been evaluated for a tumor associated antigen. The preferential cross-reaction between antibody against the conjugated glycopeptide and r-GT from hepatoma as well as sera from hepatoma rats indicates the usefulness of this conjugated antigen in the production of monoclonal antibody for the diagnosis of certain tumors.

The site of the antigen determinant of the glycopeptide of r-GT has not been determined yet, and further study is required for the identification of the epitope of this antigen to discriminate tumors.

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