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Purification and Characterization of Glutathione S-transferase π from Human Placental Tissues[1]

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= Abstract = Human placental glutathione S-transferase(GST-π) was purified to the apparent homgeneity through salting-out with ammonium sulfate and the consecutive chromatography on carboxymethyl(CM)-, diethylaminoethyl(DEAE)-cellulose and S-hexylglutathione sepharose 6B affinity column. For the characterization of the apparently purified enzyme, sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS PAGE), kinetic studies, neuraminidase digestion, and isoelectrofocusing were performed. The yield of the enzyme was 11 percent with the 1107 fold purification and the respective specific activity to 1-chloro-2,4-dinitrobenzene(CDNB), 1,2-dichloro-4-nitrobenzene(DCNB) and p-nitrophenyl chloride was 62 IU/mg, 0.12 IU/mg, and almost non-detectable. The Km of the enzyme for reduced glutathione(GSH) was 0.085 mM at the concentration of 2 mM CDNB, while its Km for CDNB was 0.46 mM at the fixed concentration of 5 mM GSH. Calcium, magnesium, zinc, ethylenediamintertraacetic acid(EDTA) and ethylenedioxydiethylenedinitrilotetraacetic acid (EGTA) did not show any significant effect on enzyme activity. The subunit of the enzyme with a molecular weight of 25,000 did not reveal the molecular weight change after neuraminidase treatment. Isoelectrofocusing of the enzyme showed two bands, of which the pI of the major band was 4.48, while that of the minor band, 4.55. The specific antibody, raised against the purified GST-π in the rabbit serum indicated the immunologic cross-reactivity to the acidic GST from the human granulocyte.

Key words: ***Human placental glutathione S-transferase(GST-π), Purification, Characterization***

INTRODUCTION

Multiple forms of the glutathione S-transferase(GST, EC 2,5,1,18) have been found in virtually all species, among which rat and human GSTs have been well characterized(Mannervik 1985; Jakoby 1978, 1981, 1985). These are a group of dimeric enzymes that play an important role in detoxification of electrophilic substances by catalyzing the first step in mercapturic acid formation with reduced glutathione(Mannervik 1985). In addition to their catalytic activities, these proteins are involved in the cellular transport of non-substrate organic anions such as bilirubin and steroid metabolites by binding either covalently or non-covalently(Ketterer *et al.* 1967, 1971). GTSs also showed selenium independent glutathione peroxidase activity(Lawrence and Burk 1976) and isomerase activity for $\triangle^5$-3-ketosteroids(Jakoby *et al.* 1977). The major part of enzyme activity is located in the cytosol of all tissues investigated.

There are at least three distinct groups of human GSTs: basic from α to ε (Jakoby *et al.* 1975), near-neutal μ(Bahr *et al.* 1981), and acidic ρ, π, ω, ψ (Mannervik and Guthenberg 1981; Jakoby *et al.* 1978; Saneto *et al.* 1980). Jornvall *et al.* (1985) tried species-independent classification of

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major isoenzymes and afforded an unambiguos division of isoenzymes into three classes; named α, μ and π on the basis of structural analysis, kinetic properties and immunological cross-reactivity. To our interests, human placental GST(GST-π) shares similar properties with rat placental GST (GST-P or GST 7-7) immunologically, structurally and kinetically despite their different pI values.

The significances of GSTs were extended from the detoxification role of xenobiotics to cancer biology. Miller(1947) observed several aminoazo-dyes binding to liver protein in rats, while Whitehead *et al.* (1967) isolated carcinogen binding protein from dimethylaminoazobenzene(DAB)-treated rat liver, which was turned out to be a ligandin, a kind of GST isoenzymes(Kettere *et al.* 1971; Habig *et al.* 1974; Sarrif *et al.* 1976). Smith *et al.* (1977) reviewed activation and inactivation of carcinogens and commented that GSTs acted as defense molecules. The protective role of GSTs, against the binding of benzopyrene metabolites to nuclear DNA was also observed by Hesse *et al.* (1980). Moreover, in several rat chemical carcinogenesis models, a marked increase in cytosolic GST activity was one of the common features in the hyperplastic liver nodules which was considered as a physiological adaptive response and a necessary precancerous trait in carcinogenesis(Eriksson *et al.* 1983; Farber 1984).

The change in isoenzyme patterns of GST during rat chemical carcinogenesis was studied by Kitahara *et al.* (1984) and Sato *et al.* (1983). According to their works, GST-P as well as GST-A,-B showed a remarkable increase in γ-glutamyltranspeptidase-positive foci and hyperplastic nodules, produced by resistent hepatocyte model, compared with little change of GST-C,-D. GST-P was very low in normal liver and rarely induced by short-term administrtion of drugs such as 2-acetamidofluorene, in contrast to GST-A and ligandin, which led GST-P as a good preneoplastic marker of murine hepatoma(Rushmore *et al.* 1987). Its possibility as a preneoplastic marker of other cancers was reported recently in pancreas, colon, and stomach cancers by Farber(1984) and the compatible researchers(Moore *et al.* 1985). In case of human beings, increased amount of GST-π in hepatic tumors was measured by single radial immunodiffusion and also detected immuno-histochemically in stomach and colon cancers(Konishi *et al.* 1987; Sato *et al.* 1986). However, there are many disputes about the increase of GST-π in human hepatoma in contrast to the murine hepatoma model(El Mouelhi *et al.* 1987). Moreover the decrease of GST activity in the human cancer tissues raised the question of whether GST-π form could be induced in human cancers as in the murine experimental tumor models or GST-π could work as a preneoplastic marker in human cancers. (Park *et al.* 1987).

In this study, we tried to purify and characterize GST-π from human placental tissues in order to prepare the monospecific antibody and analyze its biological activity.

MATERIALS AND METHODS

Reagents and enzymes

Recuced glutatione, acrylamide, N,N'-methylene-bis-acrylamide, molecular weight markers, ammonium sulfate, hexylglutathione sepharose 6B, CM cellulose and neuraminidase were purchased from Sigma Chemicals(St. Louis, Mo. U.S.A.). A pI calibration kit was obtained from Pharmacia Fine Chemicals(Uppsala, Sweden) and DEAE cellulose, from Whatman Ltd. (Maidstone Kent, England) and N,N,N'N'-tetramethylethylene diamine(TEMED) from Bio-Rad Laboratories (Richmond, Calif, U.S.A.). An ultra thin isoelectrofocusing plate was the product of Serva Feinbiochemica(Heidelberg, West Germany). All the other chemicals were of analytical grade. The purified acidic GST from human granulocytes was kindly donated by Dr. Han, J.H. from Department of Clinical Pathology, Seoul National University and the control anti GST-P antiserum was kindly donated by Professor Kiyomi Sato from Second Department of Biochemistry, Medical School, Hirosaki University, Japan.

Enzymes assay

The activity of GST-π was assayed with 1-chloro-2,4-dinitrobenzene(CDNB), 1,2-dichloro-4-nitrobenzene(DCNB) and p-nitrophenyl chloride as substrates after the method of Habig *et al.* (1974). A unit of activity was defined as the amount of enzyme catalyzing the formation of 1 μ mole of product per min under standard assay conditions. Specific activity was defined as units of enzyme activity per mg of protein, determined by Bradford dye binding method(1976) with bovine serum albumin as a standard.

Preparation of homogenate

Human placental tissues were obtained from the delivery room, immediately after parturition. All

subsequent procedures were performed at 4°C. A total of 450g of placental tissue was washed with cold 0.25M sucrose solution. The tissue was minced into small pieces with scissors and homogenized in 0.25 M sucrose by polytron homgenizer. The homogenate was diluted with sucrose solution to 30%(w/v) and centrifuged at 15,000 g for 60 min. The supernatant fraction was obtained and filtered through glass wool to remove lipid.

Salting-out and dialysis

Solid ammonium sulfate was slowly added to the supernatant to give 30% saturation. The mixture was stirred overnight and centrifuged at 10,000 g for 20 min. Pellet was discarded and the supernatant fraction was made 70% saturation of ammonium sulfate and centrifuged as above. The subsequent pellet was obtained and dissolved in and dialyzed against 10 mM sodium phosphate buffer(ph 6.2)

CM-cellulose chromatography

The dialyzed solution was applied to a column of CM-cellulose(5.1×22cm) equilibriated with the pH 6.2, 10 mM phosphate buffer. After the chromatography, the active fractions were pooled and concentrated with salt precipitation and dialyzed against 10 mM Tris-HCl(pH 7.8).

DEAE-cellulose and affinity chromatography

Concentrated active sample was applied to a column of DEAE-cellulose, equilibriated with 10 mM Tris-HCl at pH 7.8. The column was washed with 3 bed volume of the same buffer. The absorbed enzyme was eluted with a salt concentration gradient formed by linear mixing of starting buffer with the same buffer containing 0.3 M NaCl. Active fractions were collected and concentrated by Amicon centrifluomembrane cone, which were fractionated by affinity chromatography on S-hexylglutathione separose 6B, prepared after Vander Jact *et al.* (1977). Non-specifically adsorbed material was rinsed with 0.2 M NaCl in 10 mM Tris-HCl(pH 7.8). GST-π was eluted with a linear concentration gradient formed by mixing 0.2 M NaCl, 10 mM Tris · HCl with the same buffer containing 10 mM GSH. Active fractions were concentrated by Amicon centrifluomembrane cone.

Determination of molecular weight

SDS-polyacrylamide gel electrophoresis(SDS-PAGE) with a discontinuous buffer system was carried out on a 15% vertical slab gel by the method of Laemmli(1970). The sample for electrophoresis was prepared by mixing equal volume of the sample buffer containing 2% 2-mercaptoethanol and 4% sodium-dodecyl suffate. The gel was stained with coomassie blue R-250, and the relative mobility(Rf) of each protein band was calculated. The molecular weight of GST-π was determined by comparing the Rf value of the enzyme protein with those of the standard proteins; trypsin inhibitor(M.W. 20100), glyceraldehyde-3-phosphate dehydrogenase(M.W. 36000), egg albumin(M.W. 45000), bovine serum albumin(M.W. 66000)

Neuraminidase treatment

For determination of the degree of glycosylation, 10μg of GST-π was digested with 0.03 units neuraminidase and subjected to SDS-PAGE, where untreated GST-π as control and other molecular standards and the neuraminidase treated GST-π were applied on the same vertical gel.

Determination of isoelectric point

Isoelectrofocusing of GST-π in pH range of 3-10 was performed in Servalyt-Precotes of 125×125 mm. The enzyme solution of 10 mM Tris(pH 7.8), 3 mM glutathione was applied on the gel 4 cm & 6 cm apart from cathode. The solution for cathode electrode was composed of 4.0 g arginine, 0.6 g L-lysine and 120.0 g ethylene diamine per 1 liter distilled water and that for anode, 3.3 g L-aspartic acid and 3.7 g L-glutamic acid per 1 liter distilled water. Voltage was 200 V at the beginning and increased up to 2000 V over 100 min. After isoelectrofocusing, the gel was stained with 0.4% coomassie brilliant blue G in universal solvent. The pI value of the enzyme was determined by the standard pI calibration curve, with Broad pI Calibration Kit(pH 3-10) of Pharmacia Fine Chemicals which contained 11 standard proteins; amyloglycosidase (pI, 3.50), soybean trypsin inhibitor (pI 4.55), beta lactoglobulin(pI, 5.20) bovine carbonic anhydrase B(pI, 5.85), human carbonic anhydrase B(pI, 6.55), horse myoglobin-acidic band(pI, 6.85), horse myoglobin-basic band(pI, 7.3), lentil lectin-acidic band(pI, 8.15) lentil lectin-middle band(pI, 8.45), lentil lectin-basic band(pI, 8.650 and trypsinogen(pI, 9.30).

Preparation of anti-serum

A rabbit was immunized with the purified GST-π at multiple intramuscular sites with the following schedule: 0.3 mg of the enzyme was injected three

Table 1. Purification of glutathione S-transferase from human placenta

	Protein concentration (mg/ml)	Volume (ml)	Total protein (mg)	Specific activity (IU/mg)	Total activity (I.U.)	Purification	Yield (%)
Homogenate	29.5	1370	40415	0.056	2263	1	
Supernatant	12.1	970	11737	0.138	1620	2.46	72
Salting-out	25.8	170	4386	0.27	184	4.8	52
CM-cellulose	9.68	140	1355	0.70	949	12.5	42
DEAE-cellulose	2.76	35	96.6	8.37	809	146	36
Affinity Chromatography	0.43	9	3.87	62	240	1107	11

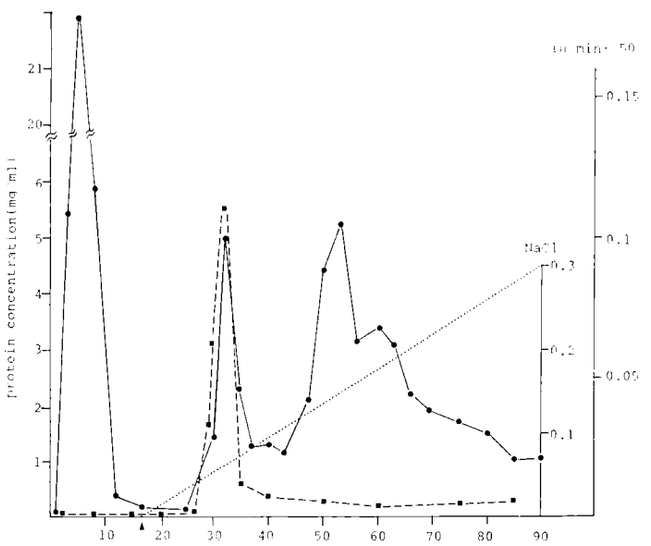

Fig. 1. Elution profile of GST-π from DEAE-cellulose column. The DEAE-cellulose column(2.6 × 17 cm), was eluted with the linear gradient from 0 to 0.3 M NaCl in 10 mM Tris-HCl buffer(pH 7.8). Closed rectangle(···■····) shows specific activity, and closed circle(—●—●—) in case of protein concentration. Gradient elution with NaCl started at the point marked as triangle(▲).

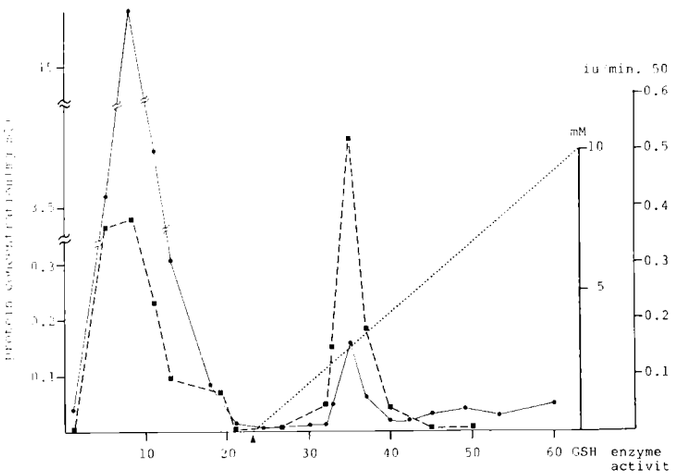

Fig. 2. Elution profile of GST-π from affinity chromatography. The affinity column(bed volume; 7 ml), was eluted with the linear gradient from 0 to 10 mM glutathione in 10 mM Tris-HCl buffer(pH 7.8) including of 0.2M NaCl. Closed rectagle (···■···■···) shows specific activity, and closed circle(—●—●—) in case of protein concentration. Gradient elution with glutathione started at the point marked as triagle(▲).

times at 10 day-interval, mixed with the same volume of complete Freund's adjuvant and booster, twice at 10 day interval with incomplete Freund's adjuvant. Then, the serum was collected and checked on the Ouchterlony double immunodiffusion plate(Hyland, USA), with serial dilution of the anti-serum.

Western blotting

Three sets of aliquats of DEAE-cellulose and affinity fraction with molecular weight standard proteins were electrophoresed on 15% SDS-polyacrylamide gel The gel was cut into three pieces. One was stained with commassie brilliant blue R. Two other pieces were transferred onto nitrocellulose paper at 4°C overnight (125 mA). The papers were washed with phosphate buffered saline(PBS) and blocked with 3% gelatin. Each of two pieces was incubated with anti GST-π antibody or anti GST-P antibody respectively. After washing with PBS, the blotted papers were reacted with second antibody (alkaline phosphatase conjugated goat anti-rabbit IgG) and were subjected with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolylphosphate

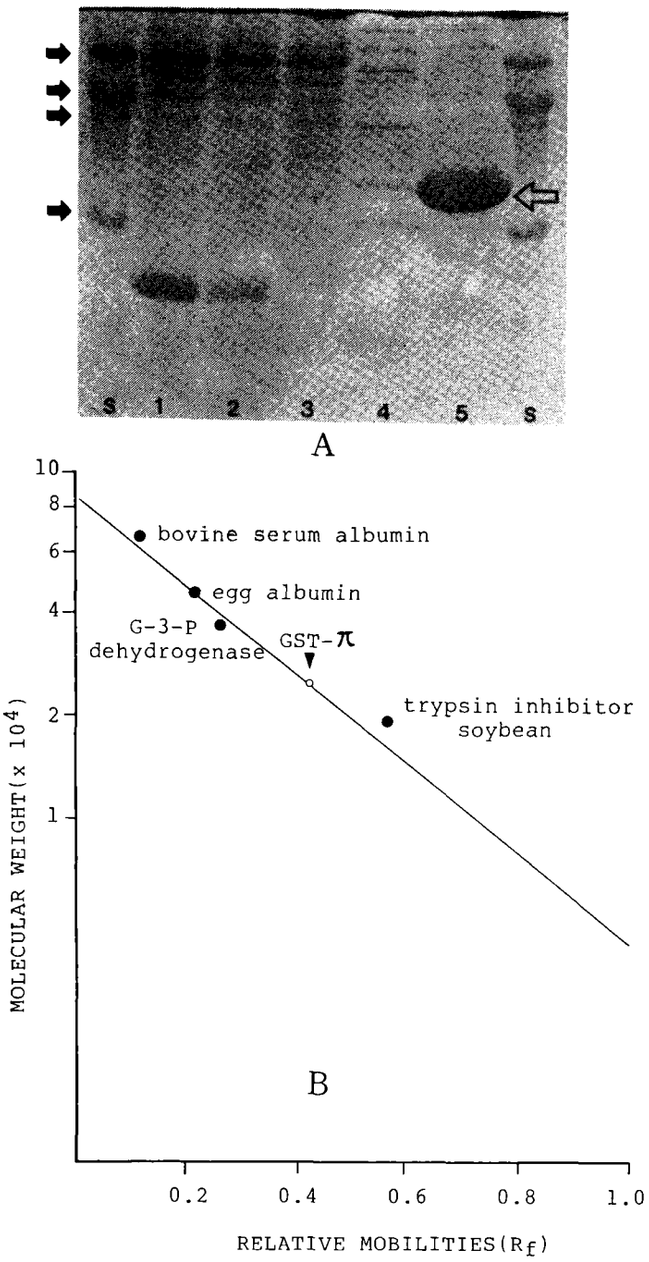

Fig. 3. SDS-polyacrylamide gel electrophoresis. The slab gel electrophoresis was performed on 15% polyacrylamide gel as described under Methods. A. Electropherogram by protein staining lane S: molecular standards, lane 1: supernatant, lane 2: dialyzate of salting out, lane 3: pooled eluates of CM-cellulose chromtography, lane 4: pooled eluates of DEAE-cellulose chromatography, lane 5: pooled eluates of affinity chromatography. B. Subunit molecular weight was obtained from molecular weight standard curve by comparing the relative mobilities.

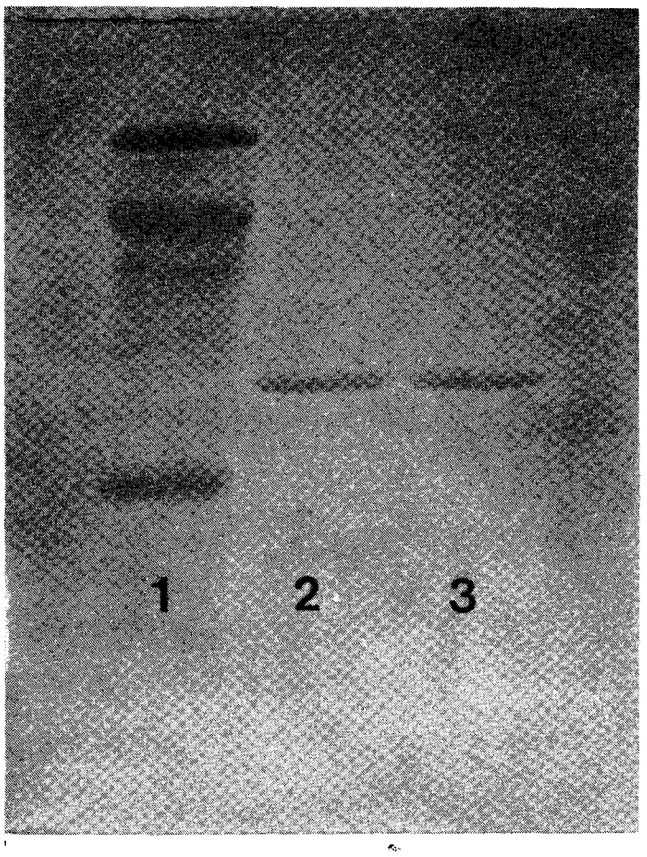

Fig. 4. SDS-polyarcylamide gel electrophoresis after neuraminidase treatment. Electrophoresis was carried out on 15% polyacrylamide slab gel. Molecular standards were applied on Lane 1 and untreated GST-π on lane 2, neuraminidase treated GST-π on lane 3, respectively.

(BCIP).

RESULTS

Purification and molecular weight of GST-π

In Table 1, the purification data of GST-π were summarized. The final enzyme preparation had the specific activity of 621 I.U./mg for CDNB, 0.12 I.U./mg for DCNB and negligible activity for p-nitrophenyl chloride. The final purification was 1107 fold with yield of 11 percent. Elution profiles on DEAE cellulose and glutathione affinity column were shown in Fig. 1, 2 respectively. Active fractions from DEAE-cellulose column were eluted between 45 mM and 70 mM NaCl as a single peak. Fractions 29 through 34 were collected. On the affinity column, fractions from 33 to 38 were collected. The subunit molecular weight of the purified enzyme was estimated to be 25,000 by SDS-PAGE(Fig. 3). No change of electrophoretic mobility after neuraminidase treatment was observed(Fig. 4).

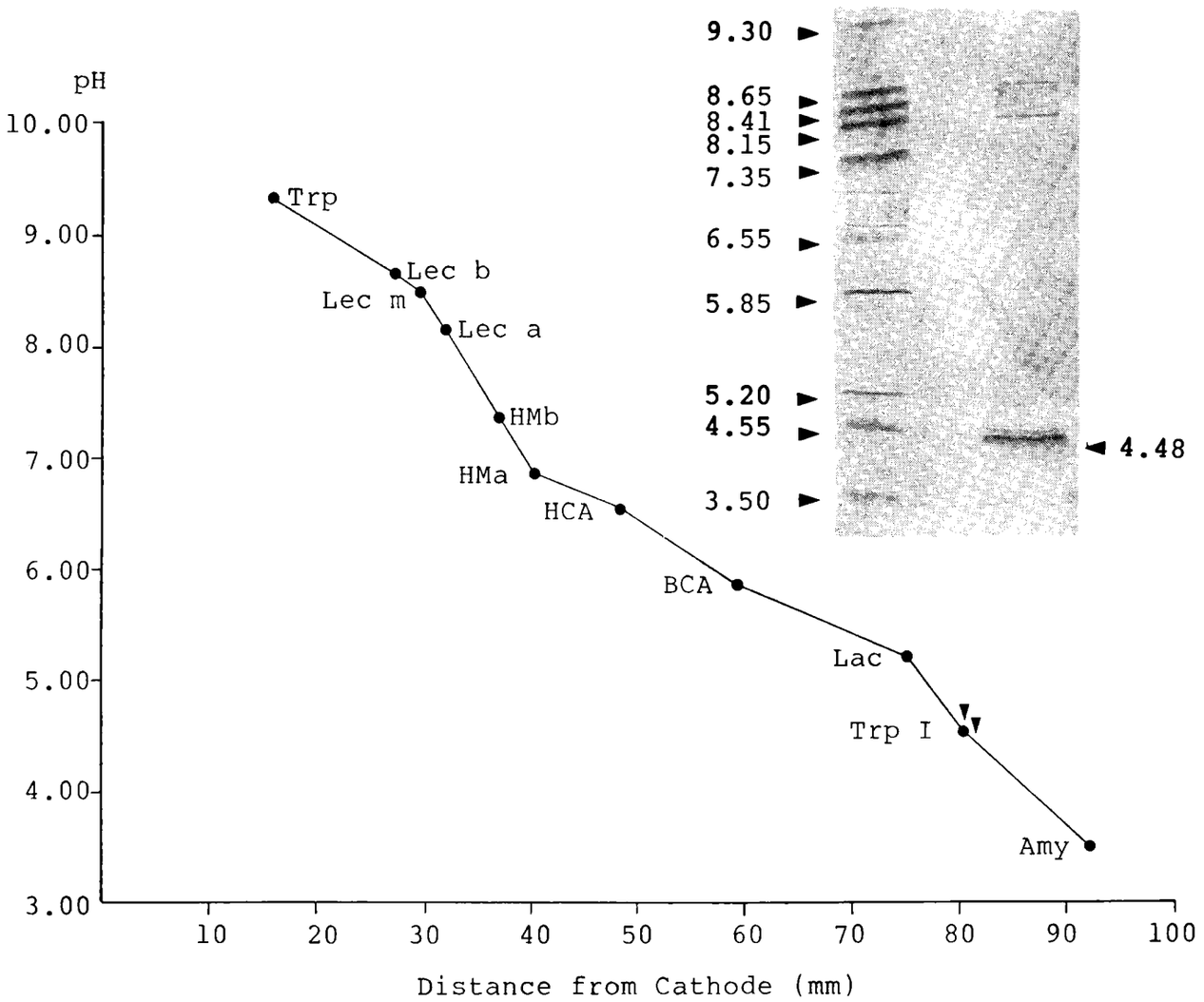

Fig. 5. Isoelectrofocusing of GST-π. A. isoelectrofocusing in the pH range of 3-10 was performed as described under Materials and Methods. The pI standard markers are shown on lane 1(pH increase from 3.50 to 9.30) and GST-π on lane 2. B. Estimation of pI value of GST-π. Two arrows indicate two isomers of GST-π.

Isoelectric point of GST-π

Isoelectrofocusing of the purified GST-π on SERVALYT-precotes with pH 3-10 ampholytes showed two closely spaced bands of protein. The pI values for major and minor bands were 4.48, 4.55 respectively (Fig. 5).

Kinetic properties

The reaction velocity of GST-π as a function of substrate concentration was studied with various concentration of reduced glutathione from 0.042 to 1 mM, of CDNB from 0.065 to 1 mM, of DCNB from 0.25 to 4 mM, and of p-nitrophenyl chloride from 0.25 to 2 mM. For glutathione and CDNB, we could obtained apparent Km under the assumption of Michaelian kinetics over assay range(Fig. 6A,B). But in case of DCNB, the curve was slightly concave upward as shown in Fig. 6C. The Km for glutathione, CDNB were 0.085 mM, 0.046 mM respectively. Kinetic parameters for p-nitrophenyl chloride were not determined due to low specific activity and high absorbance of substrate over the above assay range.

Effect of heavy metals

Calcium, magnesium, zinc, EGTA and EDTA had no noticeable effects on GST-π activity as shown in Table 2.(CDNB & GSH were used as substrates.)

Anti-GSTπ serum

The rabbit anti GSTπ serum was characterized for the immunologic property to the purified GST-π and acidic GST from human granulocytes. In

Table 2. Effects of heavy metals on GST-π activity

Heavy metals & their chelators	Relative activity	
Control	—	100%
Ca^{a+}	1 mM	104%
Mg^{a+}	1 mM	98%
Zn^{a+}	1 mM	87%
ECTA	1 mM	99%
EDTA	1 mM	96%

the 1st center well, the purified GST-π was applied and the respective dilution(neat, 1/5, 1/10, 1/50, 1/250) of the rabbit serum was pipetted into the surrounding wells(Fig. 7A). In the 2nd center well 5 μl of anti-serum was applied, while in the surrounding wells, the purified GST-π, GST from human WBC, active GST fractions after DEAE-cellulose chromatography, cytosol fraction of the placental homogenate and the liver transglutaminase were applied in turn(Fig. 7B). We could observe the immunoprecipitation line up to 1/5 dilution of the anti-serum (Fig. 7A) which indicated the low titer of anti GST-π. The fusion of precipitation line with human granulocyte GST indicated the immunologic identity of those acidic GSTs, but the spike formation and the extra immunoprecipitation line with the DEAE-cellulose fraction and homogenate suggested the existence of other type of GST or other proteins.

Western blotting

Molecular standard marker proteins, DEAE and affinity fractions were applied on lane S, lane 1, and lane 2 respectively. (Fig. 8). Coomassie brilliant blue R staining of SDS-gel, alkaline phosphatase immunodetection of nitrocellulose papers after anti GST-π antibody and anti GST-P antibody treatment were illustrated in Fig. 8(A), (B), (C) in order. As shown in these figures both antibodies were immunologically similar and had high specificity against GST-π

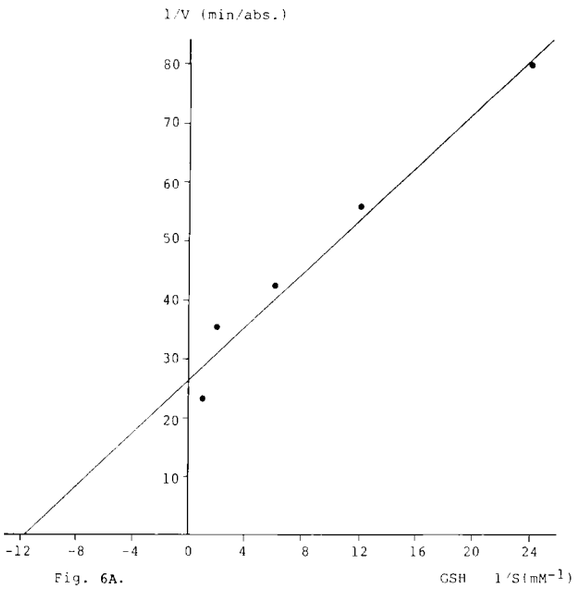

Fig. 6A.

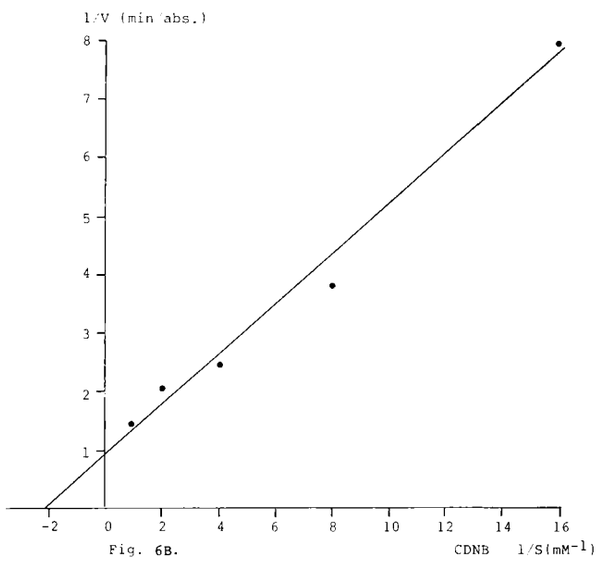

Fig. 6B.

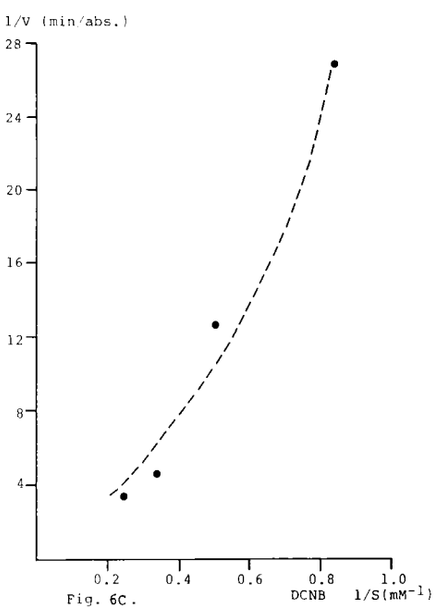

Fig. 6C.

Fig. 6. Kinetic studies of GST-π. A. Effect on GST-π activity of various glutathione concentration(0.042 -1 mM) at fixed concentration(2 mM) of CDNB. B. Effects on GST-π activity of various CDNB concentration(0.065-1 mM) at fixed concentration (5 mM) of glutathione. C. Effects on GST-π activity of various DCNB concentration(0.5-4 mM) at fixed concentration(5 mM) of glutathione.

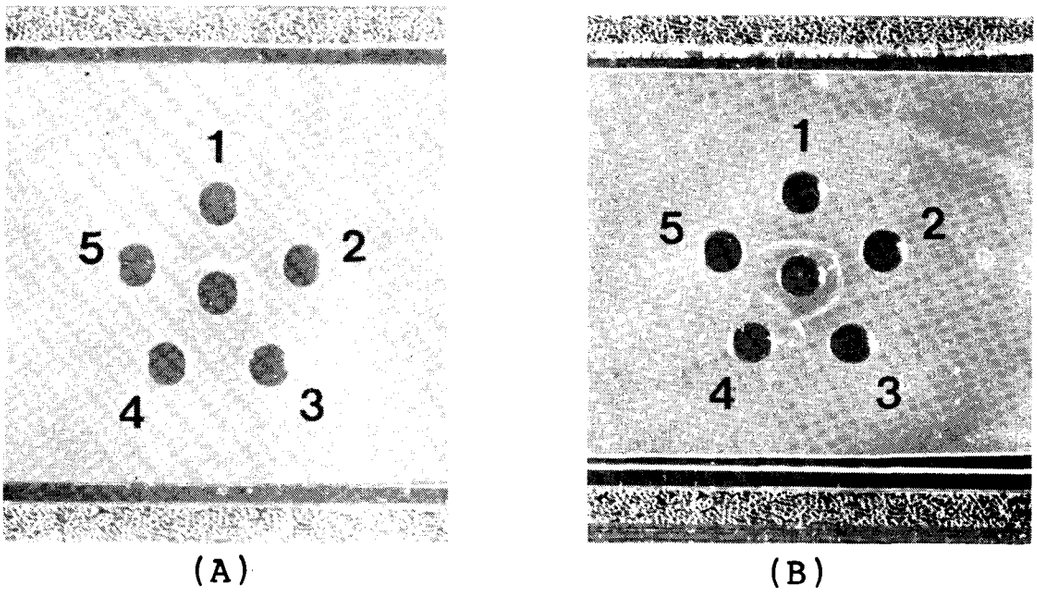

Fig. 7. Double immunodiffusion. A. Center well was filled with purified GST-π and peripheral wells with anti GST-π antibody diluted serially (neat, 1/5, 1/10, 1/50, 1/250). B. Center well was filled with anti GST-π antibody and peripheral wells with purified GST-π (1), acidic GST from WBC (2), DEAE fraction (3), supernatant (4), transglutaminase from guinea pig (5).

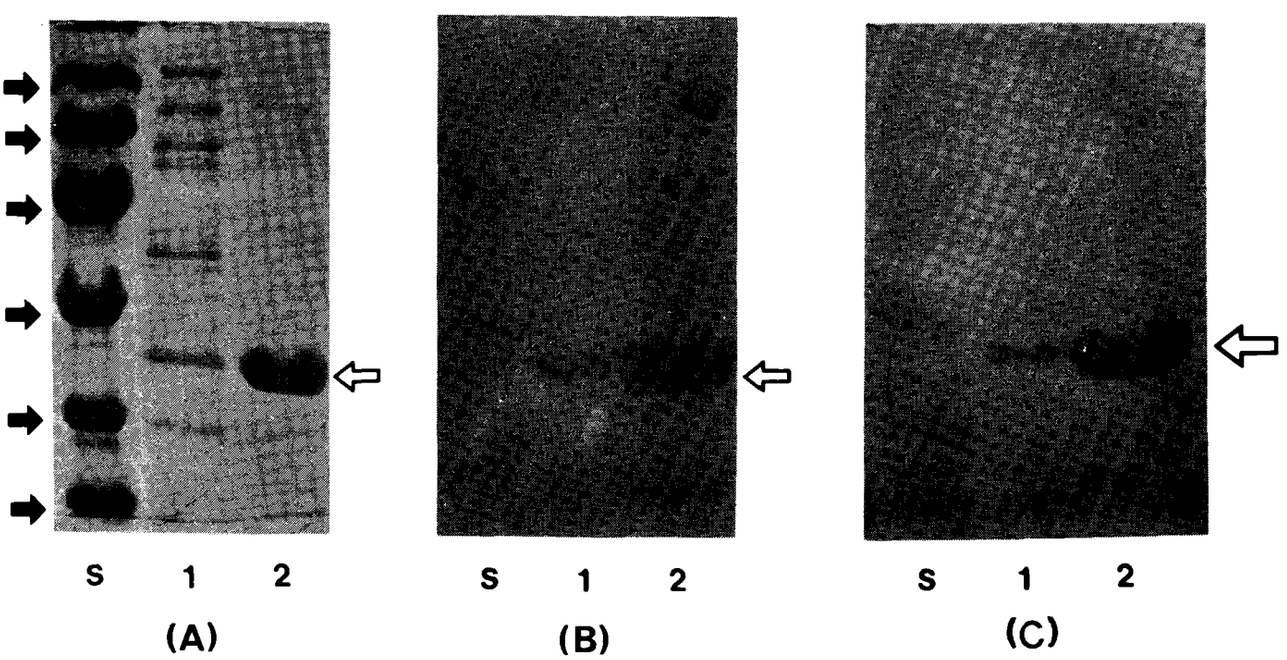

Fig. 8. Western blotting of purified GST. Molecular weight standards were applied on lane S, the pooled eluates of DEAE-cellulose chromatography were on lane 1 and the purufied GST-π fraction on lane 2. The western blotting was performed by the consecutive treatment of 1st antibody(Fig. 8-B with anti GST-π antibody, Fig. 8-C with anti GST-P antibody.) and 2nd antibody conjugated with alkaline phosphatase, which was visualized by formazan reaction using nitroblue tetrazolium and bromochloroindolylphosphate. The proteins of SDS-PAGE was stained with coomassie brilliant blue R(Fig. 8-A).

DISCUSSION

The substrate-rate saturation curve of GST was reported to be non-Michaelian(nonhyperbolic) (Habig *et al.* 1974). Many researchers suggested complex kinetic mechanisms, among which steady-state random sequential mechanism(Mannervik *et al.* 1977) and hybrid mechanism(Pabst *et al.* 1974) were good representatives. Actually, the versatility of GST in structure and function confused the biochemists, but recently relatively distinctive substrate specificities of GST isoenzymes were used to classify and identify them. Human basic GST has high GSH peroxidase activities with organic hydroperoxides and high isomerase activity with $\triangle^5$-androsten-3,17-dione, but which is inhibited strongly($I_{50}=0.1\,\mu M$) by tributyltin acetate. Near-neutral human GST is characterized by high activity with trans-4-phenyl-3-buten-2-one and benzo(a)pyrene-4,5-oxide and to which Cibacron Blue is a strong inhibitor ($I_{50}=0.05\,\mu M$). The substrate most characteristic for the acidic transferase is ethacrynic acid, while its inhibitor found so far is Cibacron Blue($I_{50}=0.5\,\mu M$). And pI values of human basic GST were 7.5-9, while near-neutral GST, 6.6 with subunit molecular weight 25K-26K(Jakoby 1981; Ito *et al.* 1984; Mannervik 1985).

In rats, the classification and nomenclature of GST are different from those of human GST. B-L groups have high glutathione peroxidase activity but negligible activity with bromosulfophthalein. The Ya subunit has high activity with $\triangle^5$ androsten-3,17-dione and low activity with ethacrynic acid but in Yc subunit vice versa. A-C groups have low glutathione peroxidase activity and high catalytic activity with either bromosulfophthalein or trans-4-phenyl-3-buten-2-one. The Yb and Yb' subunits are distinguished by giving high activity with bromosulfophthalein and trans-4-phenyl-3-buten-2-one respectively. The pIs of L_2(YaYa 1-1), BL(YaYc, 1-2), B_2(YcYc, 2-2), A_2 (YbYb, 3-3), AC(YbYb', 3-4), C_2(Yb'Yb', 4-4) and P(YpYp, 7-7) were reported to be 10, 9.9, 9.8, 8.4, 8.1, 6.9 and 6.7, respectively, while the molecular weights of GST subunits were in the range of 22K-28K. These multiple isoenzymes can afford broader coverage of hazardous xenobiotics than a single enzyme.

Among the variety of GST isoenzymes, the acidic GST was of special concern because of its nature as a preneoplastic marker. The increase of gamma-glutamyltranspeptidase(GGTP) in the experimetal murine hepatic tumor model, especially in the preneoplastic stage, allowed it to be used as a preneoplastic marker(Kitahara *et al.* 1984 Moure *et al.* 1985). The concomittant increase of GST with GGTP in the carcinogenic model suggested GST as a new preneoplastic marker(Sato 1986). But in case of GST, the newly-induced GST was found to be a new acidic isoenzyme, which was rarely detected in the normal liver, therefore, it would be recommended as the better index of preneoplastic change than GGTP. Since the new GST isoenzyme was proven to be immunologically identical to the placental type of acidic GST(GST-P), the significances of GST in carcinogenesis was evaluated again. The GST increase might be related to the multi-drug resistence of the cancer via increase in detoxification and inactivation of the chemicals. In contrast, the biochemical study in human cancers showed a deviation from the results of the animal study; that is, GST activity in human cancer tissues was lower than the control normal tissues(El Mouelhi *et al.* 1987, Park *et al.* 1987). But it is not clear yet whether the acidic GST was induced in human cancer tissues depsite the decrease in total GST activity.

In our kinetic condition, Lineweaver-Burk plots for GSH and CDNB show above 98 percent linear regression reliabilities, but upward concave curvature was noticed for DCNB(Fig. 6C). The values cited for the molecular weight of the GST-π vary from 45,000 to 60,000 daltons and those for the subunit range from 22,000 to 24,500. A pI of 4.5 to 4.9 has been observed(Radulovic and Kulkarni 1985; Guthenberg and Mannervik 1981; Sato *et al.* 1986; Federici *et al.* 1980). In our work, the subunit had a molecular weight of 25,000 and pIs were 4.48, 4.55 respectively. (Fig. 3A, B, Fig. 4, Fig. 5A, B) This variability may in part be attributed to the possible heterogeneity of the preparation used. The presence of multiple conformers or charge isomers was suggested and its catalytic activity dependence on glutathione concentration makes reproducibility of kinetic assay difficult (Radulovic and Mannervik 1985; Ito *et al.* 1984; Kulkarni and Radulovik 1986). Differences in these parameter may also arise from different portions of multiple conformers present. Heavy metals and their chelators had little effects on GST-π activity. This result was already expected during purification

steps which contained EDTA to prevent protease activity. According to the result of neuraminidase treatment, this enzyme may have little, if any, sialic acid content. The apparently purified GST-π sufficed as an antigen to prepare the anti GST-π antibody in the rabbit. The antibody prepared in our laboratory showed the cross reactivity to other acidic GST, human granulocyte GST(Fig. 7). The western blot analysis showed the monospecificity of the antibody and the same immunologic patterns with anti GST-P antibody, which has been well used in monitoring the preneoplastic lesion in murine tumor model(Ito *et al.* 1984; Sato *et al.* 1986). From these results, it would be sufficient to apply this anti GST-π antibody for the study of the preneoplastic and neoplastic change of human tissues. Anti GST-π antibody produced in our laboratory was immunologically same with anti GST-P antibody. (Fig. 8A, B, C)

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= 국문초록 =

인체 태반조직 glutathione S-transferase π의 정제 및 특성구명

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흰쥐 태반에서 분리한 GST isoenzyme은 여러 동물실험 암모델에서 전암성 지표로서 높은 특이도 및 민감도를 나타내는 것으로 보고되었다. 저자들은 인체암의 동정 및 발암기전에 관한 연구를 위한 목적의 일환으로 인체태반으로부터 GST-π를 분리하여 생화학적 특성을 구명하고 가토로부터 항체를 조제하여 다음과 같은 결과를 얻었다.

1. 인체태반으로부터 homogenization, centrifugation, ammonium sulfate염석, CM-cellulose chromatography, DEAE-cellulose chromatography, hexyl-glutathione affinity chromatography의 과정을 거쳐 정제율 1100배 정도의 순도높은 단백질 단일분획을 얻었다.

2. Neuraminidase를 이용한 실험에서 본 효소의 sialic acid 함유정도는 미미한 것으로 나타났으며 subunit의 분자량이 25,000 dalton이었고 isoelectrofocusing을 통하여 pI가 4.48인 주분획과 4.55인 부분획의 두가지 charge isomers를 확인하였다.

3. 효소 역동학의 실험결과 GSH, CDNB에 대한 Km값은 각각 0.085 mM, 0.46 mM이었고, 효소반응계에 금속이온의 첨가 및 제거는 효소의 활성도에 유의한 변화를 주지 못했다.

4. 가토에 면역하여 생성된 항 GST-π 항체로 검정해 본 GST-π는 인체 백혈구의 acidic GST와 면역학적으로 동일하였으며, 이 항체는 western blotting결과로 보아 GST-π에 대하여 단일 특이성(monospecificity)을 보여주었고 항 GST-P 항체와 동일한 pattern을 나타내었다.