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Characterization of glutathione *S*-transferases in rat kidney

Alteration of composition by *cis*-platinum

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We have developed chromatographic and mathematical protocols that allowed the high resolution of glutathione *S*-transferase (GST) subunits, and the identification of a previously unresolved GST monomer in rat kidney cytosol; the monomer was identified tentatively as subunit 6. Also, an aberrant form of GST 7–7 dimer appeared to be present in the kidney. This development was utilized to illustrate the response of rat kidney GST following *cis*-platinum treatment *in vivo*. Rat kidney cytosol was separated into three 'affinity families' of GST activity after elution from a GSH–agarose matrix. The affinity peaks were characterized by quantitative differences in their subunit and dimeric compositions as determined by subsequent chromatography on a cation-exchange matrix and specific activity towards substrates. By use of these criteria, the major GST dimers of affinity peaks were tentatively identified. The major GST dimers in peak I were GST 1–1 and 1–2, in affinity peak II it was GST 2–2, and in peak III they were GST 3–3 and 7–7. GST 3–6 and/or 4–6, which have not been previously resolved in kidney cytosol, were also present in peak II. Alterations in the kidney cytosolic GST composition of male rats were detected subsequent to the administration of *cis*-platinum (7.0 mg/kg subcutaneously, 6 days). This treatment caused a pronounced alteration in the GST profile, and the pattern of alteration was markedly different from that reported for other chemicals in the kidney or in the liver. In general, the cellular contents of the GSTs of the Alpha and the Mu classes decreased and increased respectively. It is postulated that the decrease in the Alpha class of GSTs by *cis*-platinum treatment may be related to renal cortical damage and the loss of GSTs in the urine. The increase in the Mu class of GSTs could potentially stem from a lowered serum concentration of testosterone; the latter is a known effect of *cis*-platinum treatment.

INTRODUCTION

The soluble glutathione *S*-transferases (GSTs) represent a heterogeneous family of dimeric proteins that are important for both the detoxification of various electrophilic substances including xenobiotics and lipid peroxidative products and various intermediary metabolic reactions (Mannervik, 1985). Furthermore, certain forms of the enzyme have been implicated as non-catalytic transport proteins for several types of anionic and cationic ligands, including haem and bilirubin (Ketterer *et al.*, 1976; VanderJagt *et al.*, 1982; Maruyama & Listowsky, 1984; Boyer, 1986).

Although GST activity has been demonstrated in many tissues, for the most part investigations concerning the molecular characterization of GSTs have been carried out with the liver. In comparison, reports on characterization of the kidney GSTs are few in number (Hales *et al.*, 1978; Sheehan & Mantle, 1984; Guthenberg *et al.*, 1985). In rat liver these dimeric proteins are known to be formed from binary combinations of several types of subunits, which reside in six M_r families and have been enumerated as GST 1–GST 8 according to their relative reactivity towards substrates (Habig & Jakoby, 1981; Mannervik, 1985; Meyer *et al.*, 1985). Subunits 1 (Y_a) and 2 (Y_c) have M_r values of 25000 and 28000 respectively. Subunits 3 (Y_b^1), 4 (Y_b^2) and 5 share a similar M_r of 26500. Subunits 6 (Y_n, Y_t), 7 (Y_p, Y_f) and 8 have M_r values of 26000, 24000 and 24500 respectively. Additional subunits within a particular M_r family, as well as additional subunit M_r families, have also been reported (Reddy *et al.*, 1984; Hayes & Mantle, 1986*a*,*b*). It follows, as a consequence of the various possible binary combinations, that the resulting isoenzymes have overlapping specificities for substrates (Habig & Jakoby, 1981).

In the liver, the cellular content of several GST isoenzymes is modulated by a host of chemicals (Mannervik *et al.*, 1984; Mannervik, 1985). In addition, certain disease states and hormonal manipulations alter the cellular content of the isoenzymes (Meyer *et al.*, 1985). In many cases the isoenzymic modulation has been attributed to the induction of specific subunits, followed by a random hybridization process that approximates a polynomial distribution (Mannervik *et al.*, 1984; Ding *et al.*, 1986). Limited investigations with the kidney suggest a rather refractory response of the organ to inducers of the liver isoenzymes (Benson & Barretto, 1985).

cis-Dichlorodiamineplatinum (*cis*-platinum) has been identified as a most effective chemotherapeutic agent against a variety of malignancies (Rosenberg, 1978). The kidney is the site of both accumulation and excretion of *cis*-platinum (Litterst *et al.*, 1975). Our studies have shown that *cis*-platinum preferentially exerts regulatory action on the activities of the enzymes of haem metabolic pathway in the kidney (Jollie & Maines, 1985; Maines, 1986). In addition, when GST activity was measured in

Abbreviation used: GST, glutathione *S*-transferase.
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the kidney cytosol of rats treated with 9 mg of *cis*-platinum/kg (7 days) with 1,2-dichloro-4-nitrobenzene as the general substrate, an increase of nearly 7-fold in activity was detected. The present study was undertaken to characterize GST composition in rat kidney, and to examine whether *cis*-platinum treatment causes preferential induction of specific enzyme forms.

EXPERIMENTAL

Materials

CM-Trisacryl-M was obtained from LKB Instruments. *trans*-4-Phenylbut-3-en-2-one was a product of Aldrich Chemical Co. 1,2-Epoxy-3-(*p*-nitrophenoxy)propane and 1,2-dichloro-4-nitrobenzene were products of Eastman Kodak Co. Androst-5-ene-3,17-dione was purchased from Steraloids. Other chemicals were purchased from Sigma Chemical Co. Male Sprague–Dawley rats (200–225 g) were purchased from Harlan Industries (Madison, WI, U.S.A.). The animals were allowed access to food and water *ad libitum*. Rats received a single subcutaneous injection of either *cis*-platinum (7.0 mg/kg) or 0.9% NaCl, and were killed 6 days later. The kidneys were homogenized in 5 vol. (v/w) of 20.0 mM-potassium phosphate buffer, pH 7.4, containing 0.10 mM-EDTA and 135.0 mM-KCl. The 150000 *g* supernatant fraction was prepared and was filtered through glass-wool.

Resolution of GST isoenzymes

The newly developed chromatographic procedure described here was used. The cytosol was diluted with $\frac{1}{9}$ vol. of 20.0 mM-potassium phosphate buffer, pH 7.4, containing 9.1 mM-EDTA, 0.785 M-KCl and 2.0 mM-dithiothreitol, and centrifuged at 150000 *g* for 1 h. The supernatant layer was loaded directly on to a GSH–agarose column (2.5 cm × 8.9 cm), previously equilibrated with the above buffer but containing 1.0 mM-EDTA, 0.2 M-KCl and 0.2 mM-dithiothreitol. The column was sequentially washed with 5 bed volumes (V_t) of 20.0 mM-Tris/HCl buffer, pH 7.4, containing the above concentrations of EDTA, KCl and dithiothreitol, $4 \times V_t$ of the same buffer, but adjusted to pH 9.6, and then $1 \times V_t$ of the pH 7.4 buffer. The column was eluted with a linear gradient of GSH (5–13 mM) in the Tris buffer, pH 7.8. The gradient slope was maintained at 0.5 mM-GSH/V_t. Subsequently the column was washed with 25.0 mM-GSH. The flow rate was maintained at $1 \times V_t$/h, and approx. $0.2 \times V_t$ fraction volumes were collected. The major peaks of GST activity were pooled separately and concentrated by using an Aminco PM10 Diaflo membrane. The retained fraction was washed with 10.0 mM-potassium phosphate buffer, pH 6.0, containing 0.1 mM-EDTA and 0.1 mM-dithiothreitol and reconcentrated to the approximate void volume of the ion-exchange column.

Each GST concentrate was loaded individually on a CM-Trisacryl-M ion-exchange column (1.5 cm × 12.5 cm) equilibrated with 10.0 mM-potassium phosphate buffer, pH 6.0, containing 0.1 mM-EDTA and 0.1 mM-dithiothreitol. The column was washed with $2 \times V_t$ of the equilibration buffer, and activity was eluted with a linear gradient of KCl (0–260 mM) in the equilibration buffer. The gradient slope was maintained at 20 mM-KCl/V_t. The flow rate and the fraction volume were maintained at approx. $1 \times V_t$/h and $0.1 \times V_t$ respectively. The peaks of GST activity were pooled separately, concentrated and washed, by utilizing the above-described conditions.

Assay procedures

The activity of GST towards various substrates was determined by the method of Habig & Jakoby (1981). A unit of activity was defined as the amount of enzme that catalysed the formation of 1 μmol of a GST conjugate/min. Selenium-independent peroxidase activity was determined by the method of Lawrence & Burk (1976). A unit of activity was defined as the amount of enzyme that catalysed the oxidation of 1 μmol of NADPH/min. All assays were performed at 25 °C, and changes in absorbance were recorded with an SLM Aminco DW2C spectrophotometer. The protein concentration of the cytosol was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard, and that of purified GST preparations was determined by the method of Kalckar (1947).

Polyacrylamide-gel electrophoresis

SDS/polyacrylamide-slab-gel electrophoresis was performed essentially by the method of Laemmli (1970). The separating gels contained 12.5% T and 2.67% C, and the stacking gels contained 4.0% T and 2.67% C, where T and C refer to total acrylamide concentration and cross-linker concentration respectively. Albumin (M_r 66000), ovalbumin (M_r 45000), carbonic anhydrase (M_r 29000), trypsinogen (M_r 24000) and β-lactoglobulin (M_r 18400) were used as markers. A 2.0 μg portion of GST protein obtained from the CM-Trisacryl-M fractions was applied to each slot. The gel was fixed (1 h) in an aqueous mixture containing 10.0% (w/v) trichloroacetic acid, 10.0% (v/v) acetic acid and 10.0% (v/v) methanol, and stained for at least 16 h in an aqueous mixture containing 0.05% (w/v) Coomassie Brilliant Blue R250, 7.0% (v/v) acetic acid and 12.5% (v/v) methanol. The gels were destained in 7.0% (v/v) acetic acid.

Two densitometric scans were performed on each gel; one was utilized for the determination of the modal migratory distance, and the second was utilized for the determination of the peak area. The maximum error associated with the above determination was estimated to be less than 0.6% and 8.0% respectively.

Calculations

A mathematical protocol was established to permit: (1) the determination of the fractional contribution of each GST monomer within any given chromatographic peak; (2) the assessment of the size of any given chromatographic peak; (3) the determination of the relative molar quantity of each GST subunit within a chromatographic peak; (4) the determination of the total quantity of each subunit within any given initial sample, provided that it is able to bind to the affinity column. The description of the principles applied towards developing the protocol is provided in the following.

The R_F value (Rodbard & Chrambach, 1971) was obtained for each M_r marker and GST monomer. An M_r calibration curve was constructed by the method of Shapiro *et al.* (1967). Predicted M_r values for all standards and GST monomers were obtained by least-squares analysis. The mean predicted M_r value was determined for the M_r standards and those GST monomers within a 'putative' M_r class.

The unweighted peak area under a densitometric concentration profile, which is proportional, but not equal, to the protein content (Hsieh & Anderson, 1975), was determined by the paper-weight method. The weighted peak area, which is proportional to the mole content, was the product of the unweighted peak area multiplied by the ratio of the mean predicted M_r values of the largest M_r class to the M_r class of the densitometric peak. To facilitate comparisons between the densitometric concentration profiles within and between gels, the fractional weighted peak area, $A_{d(fm)}$, which is the quotient of the weighted peak area divided by the summation of the weighted peak areas under a single densitometric concentration profile, was determined.

Chromatographic elution profiles were constructed from the apparent activity of GST with the substrate 1-chloro-2,4-dinitrobenzene. The minimal area, $A_{C(pa)}$, of any given chromatographic elution peak was determined by a modification of the trapezoidal approximation method (Selby, 1973). The $A_{C(pa)}$ value was then adjusted to reflect the theoretical total area, $A_{C(aa)}$, by a modification of the method utilized by Wei & Deal (1976) to manipulate normally distributed concentration profiles. Adjustment is necessary to ensure the reliability of the peak-area estimate under those conditions when the activity within a peak does not decrease to a baseline value, or elution peaks partially overlap, or the peak activity is extremely low. Because of the fact that the various functional GST dimers exhibit different specific activities towards the substrate 1-chloro-2,4-dinitrobenzene (Mannervik, 1985) the $A_{C(aa)}$ value was transformed to reflect the protein content rather than the enzymic activity. Briefly, the theoretical total GST protein under a chromatographic peak, $A_{C(ag)}$, was the quotient of the $A_{C(aa)}$ value divided by the specific activity towards 1-chloro-2,4-dinitrobenzene, which was calculated subsequent to the consolidation and concentration of the appropriate elution fractions that comprised the chromatographic peak.

The relative mole quantity, $A_{D(rm)}$, of a GST subunit (which corresponds to a particular densitometric peak) within any given chromatographic elution peak (which corresponds to a particular densitometric concentration profile) was the product of the $A_{C(ag)}$ value multiplied by the $A_{D(fm)}$ value.

RESULTS

The results of affinity chromatography of the kidney cytosol obtained from control and *cis*-platinum-treated rats are shown in Figs. 1(*a*) and 1(*b*) respectively. As shown, the chromatographic procedure utilized in this study resolved the cytosolic GSTs into three distinct peaks, when 1-chloro-2,4-dinitrobenzene was used as the general substrate. These peaks were labelled according to the sequence of their elution as I, II and III. Peak I was eluted with 5 mM-GSH, peak II was eluted with 6.5 mM-GSH, and peak III was eluted by increasing the GSH concentration to 25 mM. Most laboratories, characterizing the liver GSTs, have detected the elution of only one protein peak from GSH affinity columns (Simons & VanderJagt, 1977; Inome *et al.*, 1981). This may well be related to the utilization of low GSH concentrations or *S*-hexylglutathione gradient to elute the enzyme from the affinity column in those studies. It follows that peak III, which requires a relatively much higher GSH concentration for elution, has not been detected in the past investigations using a GSH affinity column. Hayes *et al.* (1987) have utilized *S*-hexylglutathione gradient elution to resolve GST classes from human and mouse liver and have detected a multitude of peaks eluted from a *S*-hexylglutathione affinity column. However, the pattern of elution of GSTs substantially differed from the pattern that we have observed using a GSH affinity system. As shown, there were notable differences between the control and the *cis*-platinum-treated preparations in the total GST activity associated with the three peaks; the areas under the peaks I and II were decreased, whereas that of peak III was substantially increased.

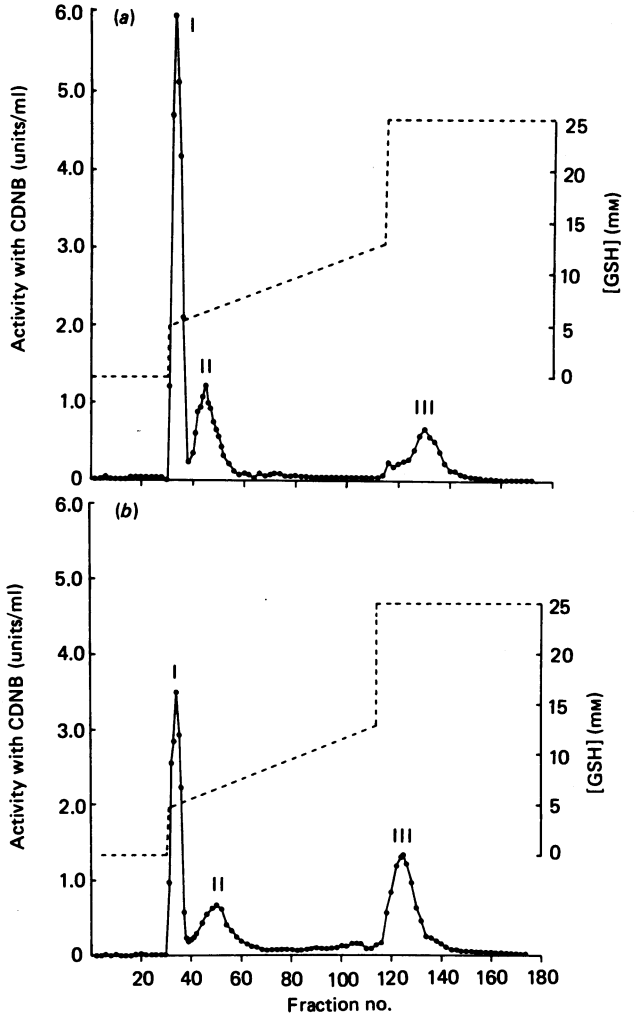

Fig. 1. Affinity-chromatographic separation of control and *cis*-platinum-treated rat kidney soluble GSTs

The cytosolic sample [1.12 g of protein, 216.8 units (activity towards 1-chloro-2,4-dinitrobenzene)] was prepared from control and *cis*-platinum-treated rats and applied to the GSH-agarose affinity column. The column was eluted with a linear gradient of GSH (5.0–13.0 mM) followed by a 25.0 mM-GSH wash. The theoretical GSH concentration in the elution fractions (------) was approximated (Lakshmanan & Lieberman, 1954). The elution profile of the GST activity was constructed with 1-chloro-2,4-dinitrobenzene (CDNB) as the test substrate. Experimental details are provided in the text. (*a*) Control rats; (*b*) *cis*-platinum-treated rats.

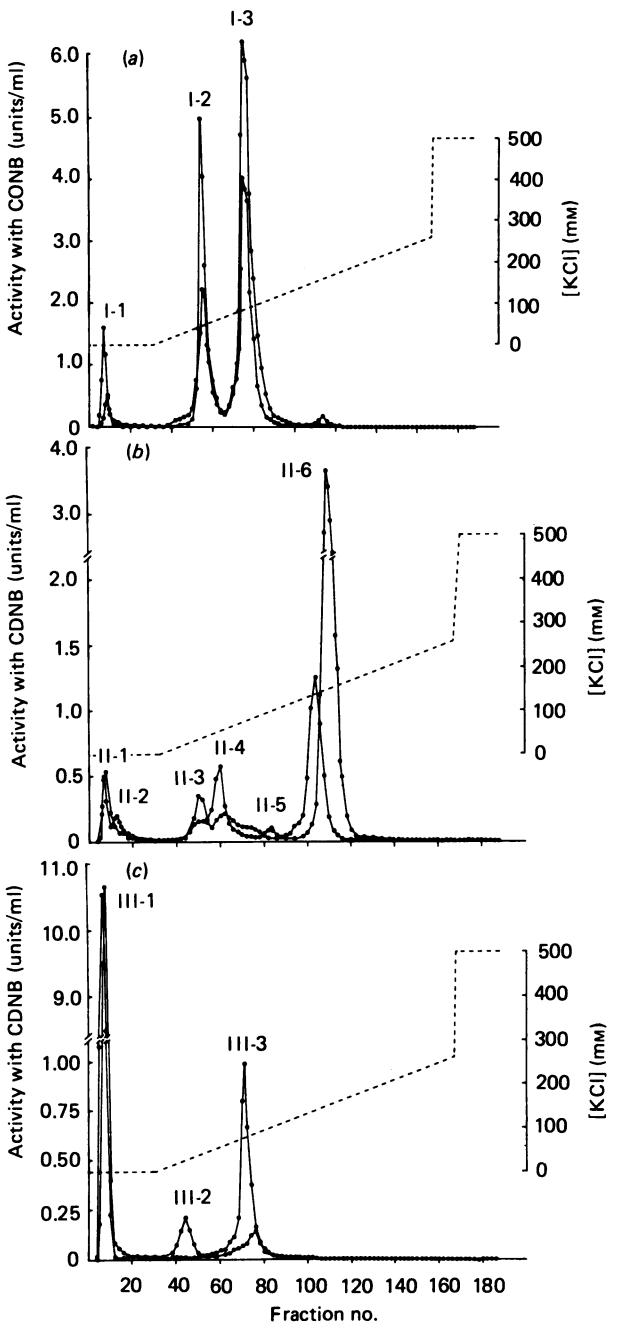

Fig. 2. CM-Trisacryl-M cation-exchange chromatographic separation of peaks I, II and III from GSH–agarose affinity chromatography of the control and the *cis*-platinum-treated preparations

Affinity peaks I–III were concentrated and applied to the ion-exchange column as described in the Experimental section. The column was eluted with a linear gradient of KCl (0–260.0 mM). The theoretical KCl concentration in the elution fractions (------) was approximated (Lakshmanan & Lieberman, 1954). The elution profile of the GST activity was constructed with 1-chloro-2,4-dinitrobenzene (CDNB) as the test substrate. (*a*) Affinity peak I; (*b*) affinity peak II; (*c*) affinity peak III. ○, Control preparation; ●, *cis*-platinum-treated preparation.

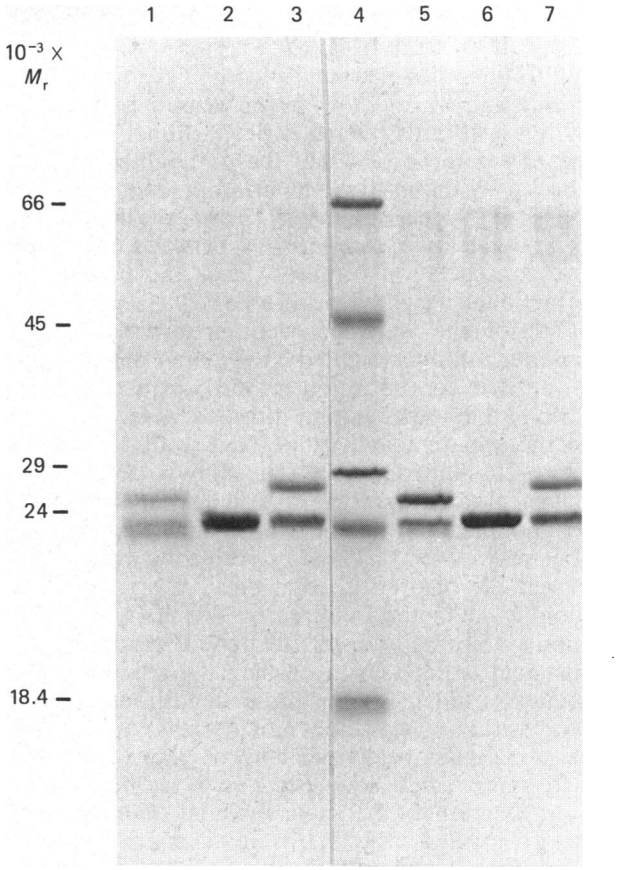

Fig. 3. SDS/polyacrylamide-gel electrophoresis of ion-exchange peaks I-1, I-2 and I-3

A 2.0 μg portion of GST protein or 0.5 μg of an M_r marker was added to the appropriate channels. The control preparations are to the left (lanes 1–3) and the *cis*-platinum-treated preparations are to the right (lanes 5–7) of the M_r marker channel (lane 4). Lanes 1 and 5, lanes 2 and 6 and lanes 3 and 7 correspond to peaks I-1, I-2 and I-3 respectively.

In order to identify the specific GSTs affected by *cis*-platinum treatment, the fractions corresponding to the three GST peaks of the control and the *cis*-platinum-treated samples were further resolved by chromatography on a CM-Trisacryl-M ion-exchange column. The results are shown in Figs. 2(*a*), 2(*b*) and 2(*c*) for peaks I, II and III respectively. For comparative purposes, the chromatograms of the *cis*-platinum-treated and the control preparations are superimposed. As noted in the following, for the most part the patterns of resolution of the control and the *cis*-platinum-treated preparations differed extensively. Fig. 2(*a*) shows that the affinity peak I was resolved into three ion-exchange peaks, enumerated as I-1, I-2, and I-3. The small peak present in the control preparation in fractions 115–120 was not analysed. This peak, however, was not detected in the *cis*-platinum-treated preparation. Peak I-1, which was only a minute peak in the control fraction, was increased notably in the treated preparation. On the other hand, peaks I-2 and I-3 were decreased in the treated preparation. The result of the ion-exchange chromatography of affinity peak II is shown in Fig. 2(*b*). In all, six peaks were observed in the treated sample; these were enumerated according to the sequence of elution as II-1 to II-6. When compared with the chromatogram of the control sample, the following observations were made: (*a*) peaks II-2 and II-5 were

Table 1. Area and densitometric measurement of the subunit composition of peaks from CM-Trisacryl-M ion-exchange chromatography of peak I from GSH–agarose affinity column chromatography of the control and *cis*-platinum-treated rat kidney GSTs

Kidney cytosol fraction was prepared from control and *cis*-platinum-treated rats, and subjected to GSH–agarose affinity chromatography. Subsequently, peak I of the affinity column was chromatographed on a CM-Trisacryl-M ion-exchange column. The three fractions were analysed for the total area and were concentrated and subjected to SDS/polyacrylamide-gel electrophoresis. GST bands were densitometrically quantified. Experimental details are provided in the Experimental section. $A_{c(ag)}$ is the chromatographic peak area in terms of theoretical total GST protein. $A_{D(rm)}$ is the relative mole quantity of a GST subunit of a given M_r family.

		$A_{D(rm)}$			
Chromatographic peak	$A_{c(ag)}$	Subunit M_r family ... Proposed GST subunit ... 27600 (GST 2)	26700 (GST 3 or 4)	24700 (GST 1 or 8)	23800 (GST 7)
Control:					
I-1	0.50	0	0.18	0.17	0.16
I-2	3.07	0	0	3.07	0
I-3	7.48	2.89	0	4.59	0
cis-Platinum-treated:					
I-1	1.60	0	0.79	0.67	0.14
I-2	2.19	0	0	2.19	0
I-3	4.58	1.63	0	2.95	0
Ratio of *cis*-platinum-treated to control:					
I-1	3.19	–	4.45	3.98	0.90
I-2	0.71	–	–	0.71	–
I-3	0.61	0.56	–	0.64	–

clearly observable in the treated preparation but not in the control; (*b*) the relative areas under peaks II-3 and II-4 were more or less reversed in the two preparations, with *cis*-platinum treatment increasing that of II-4 and decreasing that of II-3; (*c*) *cis*-platinum treatment caused a marked decrease in the area corresponding to peak II-6. The results of ion-exchange chromatography of affinity peak III are shown in Fig. 2(*c*). In the control preparation three distinct peaks were observed, and were enumerated as III-1–III-3. In the treated preparations peak III-2 was undetectable. On the other hand, in the treated sample a major increase in the area under the peak III-3 was detected. The area under peak III-1 was similar in both samples.

The subunit composition of peaks I-1 to I-3 of the control and treated samples was examined by SDS/polyacrylamide-gel electrophoresis (Fig. 3). Peak I-1 was resolved into three M_r bands, peak I-2 consisted of one band, and peak I-3 resolved into two bands. As shown, *cis*-platinum treatment caused increases in subunits with M_r values of 26700 and 24700 in peak I-1, and decreases in subunits with an M_r value of 24700 in peak I-2 and in those with M_r values of 27600 and 24700 in peak I-3. A major change in subunits with an M_r value of 23800 in peak I-1 was not observed. By using the above information, i.e. the chromatographic peak area calculated from Figs. 1 and 2, and the apparent subunit M_r, obtained from Fig. 3, the relative composition of the putative subunits in each peak was determined (Table 1). This Table also shows the comparative values for these parameters for the control and the *cis*-platinum-treated preparations. Similarly, column fractions corresponding to peaks II-1, II-2 and II-6 were subjected to electrophoresis. The results are shown in Fig. 4. Peak II-1 in both samples consisted of subunits with M_r values of

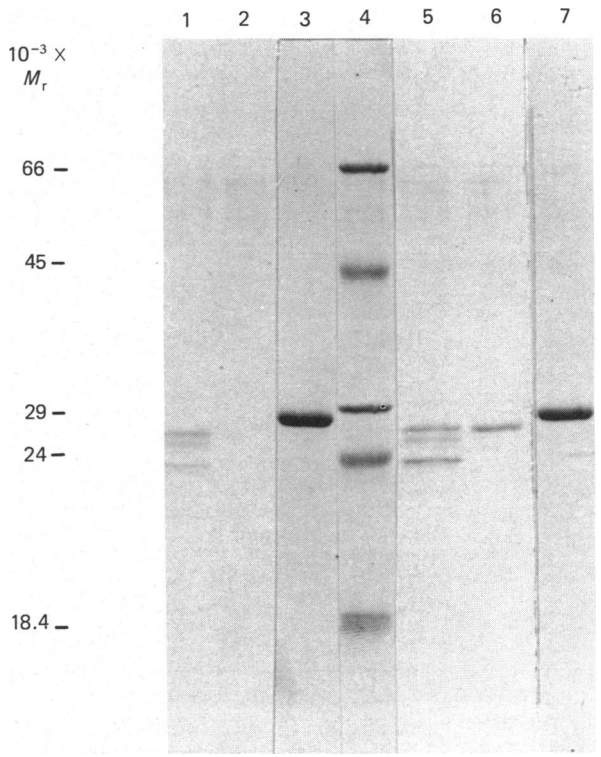

Fig. 4. SDS/polyacrylamide-gel electrophoresis of ion-exchange peaks II-1, II-2 and II-6

Electrophoretic conditions were similar to those described for Fig. 3. Control preparations are to the left (lanes 1–3) and the *cis*-platinum-treated preparations are to the right (lanes 5–7) of the M_r marker channel (lane 4). Lanes 1 and 5, lanes 2 and 6 and lanes 3 and 7 correspond to peaks II-1, II-2 and II-6 respectively.

Table 2. Area and densitometric measurement of the subunit composition of peaks from CM-Trisacryl-M ion-exchange chromatography of peak II from GSH–agarose affinity column chromatography of the control and *cis*-platinum-treated rat kidney GSTs

An experimental protocol similar to that described in the legend to Table 1 was used except that peak II of the affinity column was analysed. $A_{c(ag)}$ is the chromatographic peak area in terms of theoretical total GST protein. $A_{D(rm)}$ is the relative mole quantity of a GST subunit of a given M_r family.

Chromatographic peak	$A_{c(ag)}$	$A_{D(rm)}$ Subunit M_r family ...	27600	26700	25600	24700	23800
		Proposed GST subunit ...	(GST 2)	(GST 3 or 4)	(GST 6)	(GST 1 or 8)	(GST 7)
Control:							
II-1	0.16		0	0.06	0.05	0	0.05
II-2	0		0	0	0	0	0
II-6	2.72		2.72	0	0	0	0
cis-Platinum-treated:							
II-1	0.16		0	0.05	0.04	0	0.07
II-2	0.23		0	0.23	0	0	0
II-6	1.46		1.46	0	0	0	0
Ratio of *cis*-platinum-treated to control:							
II-1	0.99		–	0.77	0.82	–	1.43
II-2	0		–	∞	–	–	–
II-6	0.54		0.54	–	–	–	–

Table 3. Area and densitometric measurement of the subunit composition of peaks from CM-Trisacryl-M ion-exchange chromatography of peak III from GSH–agarose affinity column chromatography of the control and *cis*-platinum-treated rat kidney GSTs

An experimental protocol similar to that described in the legend to Table 1 was used except that peak III of the affinity column was analysed. $A_{c(ag)}$ is the chromatographic peak area in terms of theoretical total GST protein. $A_{D(rm)}$ is the relative mole quantity of a GST subunit of a given M_r family.

Chromatographic peak	$A_{c(ag)}$	$A_{D(rm)}$ Subunit M_r family ..	27600	26700	24700	23800
		Proposed GST subunit ..	(GST 2)	(GST 3 or 4)	(GST 1 or 8)	(GST 7)
Control:						
III-1	4.90		0	0	0	4.90
III-2	0.32		0	0	0	0.32
III-3	0.19		0	0.19	0	0
cis-Platinum-treated:						
III-1	6.47		0	0	0	6.47
III-2	0		0	0	0	0
III-3	0.52		0	0.52	0	0
Ratio of *cis*-platinum-treated to control:						
III-1	1.32		–	–	–	1.32
III-2	0		–	–	–	–
III-3	2.78		–	2.78	–	–

26700, 25500 and 23800. To our knowledge, the species of M_r 25500, which was detected in peak II-1 of both the control and the treated samples, has not been unequivocally described in the kidney, or resolved by chromatographic procedures in this organ, by other laboratories. As becomes more apparent by the activity data described below, it is likely that this band is a newly chromatographed transferase subunit in the kidney cytosol. Moreover, in peak II-2 of the treated preparation a large amount of GST with an M_r value of 26700 was detected. We could not detect this peak in the control preparation. Peak II-6 in both preparations contained proteins of M_r 27600 only. The chromatographic peak areas, the apparent M_r values and the putative subunit compositions of peaks II-1, II-2 and II-6 are shown in Table 2. As suggested by electrophoretic data (Fig. 4), *cis*-platinum treatment did not alter quantitatively the amount of transferases in peak II-1, but rather changed the subunit composition within the peak. Moreover, the area under peak II-6 was reduced by half in the treated

Table 4. Relative substrate specificities of kidney GSTs in the control and *cis*-platinum-treated rats

The GST activity of CM-Trisacryl-M ion-exchange chromatographic peaks was assessed with the indicated substrates. Experimental details are provided in the text. In all cases relative activity towards 1-chloro-2,4-dinitrobenzene was designated as 100%. Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; EpNPP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; tPBO, *trans*-4-phenylbut-3-en-2-one; EAA, ethacrynic acid; 5ADO, androst-5-ene-3,17-dione; CHP, cumene hydroperoxide.

	Putative composition			Relative activity (% of that with CDNB)						
Chromatographic peak	Monomeric	Dimeric	Substrate...	CDNB	DCNB	EpNPP	tPBO	EAA	5ADO	CHP
Control:										
I-1	4, 7, 8	4–4, 7–7, 8–8		100	0.71	5.12	7.64	52.58	0.44	12.90
I-2	1	1–1		100	0.15	0.84	0.03	0.20	19.82	16.36
I-3	1, 2	1–2		100	0.18	0.24	0.04	9.54	10.14	33.96
cis-Platinum-treated:										
I-1	4, 7, 8	4–4, 7–7, 8–8		100	0.48	4.43	11.16	44.78	0.51	11.87
I-2	1	1–1		100	0.48	0	0.10	1.23	15.43	17.73
I-3	1, 2	1–2		100	0.28	0	0.06	10.25	7.19	33.13
Control:										
II-1	*, 6, 7	*–6, 7–7		100	10.73	0	2.25	3.27	0.66	3.70
II-2	–	–		0	0	0	0	0	0	0
II-6	2	2–2		100	0.23	0	0	3.94	0.05	35.66
cis-Platinum-treated:										
II-1	*, 6, 7	*–6, 7–7		100	6.50	3.73	1.46	4.48	0.44	1.87
II-2	3, 4	3–4		100	8.25	0	1.40	0	0.04	1.94
II-3	*	*		100	11.46	0	4.81	0.74	0.31	3.12
II-4	*	*		100	9.60	0	3.65	1.38	0.25	4.35
II-5	*	–		100	19.55	0	0.32	12.40	0.47	34.02
II-6	2	2–2		100	0.29	0	0.05	4.92	0	40.29
Control:										
III-1	7	7–7		100	0.19	0.23	0.20	10.25	0.40	0.57
III-2	7	7–7		100	0.43	0	0.60	13.99	1.66	0.29
III-3	3	3–3		100	11.78	0	0.05	3.98	0.27	6.12
cis-Platinum-treated:										
III-1	7	7–7		100	0.24	0	0.46	13.65	0.25	0.69
III-2	–	–		100	0	0	0	0	0	0
III-3	3	3–3		100	8.54	0	0.31	0.13	0.03	0.38

* In these instances, although the monomeric M_r was consistent with GST subunits of Yb type, the activity profile was not consistent with such assignment.

enzyme source. The M_r values, the putative subunit compositions and the chromatographic peak areas for ion-exchange peaks III-1 to III-3 are shown in Table 3. Peak III-1 in both preparations contained only transferases with an M_r value of 23800. The prominent band with an M_r value of 23800, noted in peak III-2 of the control preparation, was not detected in the treated sample. The band with an M_r value of 26700, which was abundant in peak III-3 of the treated sample, was less prominent in the control preparation.

Experiments were conducted to characterize further the subunits of transferases affected by *cis*-platinum treatment on the basis of their relative specific activity. Table 4 shows the activities of CM-Trisacryl-M ion-exchange chromatography peaks of the control and the treated samples towards seven GST substrates. The activity towards each substrate is expressed in terms of percentage of the activity with 1-chloro-2,4-dinitrobenzene. In this Table also the putative monomeric and dimeric compositions of the chromatographic peaks are shown.

On the basis of the published reports on the relative specific activities of highly purified GSTs towards different substrates, and the presently observed activities of the various chromatographic peaks, the assigned monomeric and dimeric compositions of transferases appear plausible. There were, however, instances that the estimated M_r of a subunit and its relative specific activity did not correspond to any previously characterized functional dimer. This could reflect the detection of aberrant forms of dimers previously undescribed in kidney. Alternatively, it may reflect experimental anomalies. In general, however, these anomalies were associated with minor GST fractions.

Following the tentative identification of the GST dimers from their relative specific activities and M_r values, and calculation of the corresponding $A_{C(sg)}$ values along with the $A_{D(rm)}$ values of the component subunits (Tables 1–3), the effect *in vivo* of *cis*-platinum treatment on GST monomers and dimers was evaluated. Surprisingly, the relative mole quantity of most GST monomers and dimers was altered by the treatment. In general, *in vivo cis*-platinum treatment decreased transferases belonging to the Alpha class, but increased transferases belonging to the Mu class (Mannervik *et al.*, 1984). GST subunits 1 and 2 decreased by 33% and 45%

respectively, whereas subunit 8 increased by 4-fold. Also, subunits 3 and 4 increased by 3- and 4.5-fold respectively. Subunit 6, which has the ability to form heterodimers with either subunit 3 or 4 (Hayes, 1984), decreased by 20%, and subunit 7 increased by 1.2-fold. Alterations in the relative mole quantities of the subunits yielded the expected modification in dimeric content. GST 1–1 and GST 1–2 decreased by 30% and 40% respectively, and GST 2–2 decreased by 46%. GST 8–8 increased by 4-fold, and GST 3–3 and 4–4 increased by 3- and 4.5-fold respectively. GST 3–4, which was present in the *cis*-platinum-treated sample, was not detectable in the control preparation. Marginal (20%) increase in GST 7–7 was also observed.

DISCUSSION

The new chromatographic protocol that we have developed has enabled us to identify a previously unresolved subunit of GST in rat kidney. This protocol, along with mathematical transformations, permitted ready detection and assessment of the absolute amounts and the magnitude of changes in the concentration of GST subunits in the kidney cytosol. Presently, we have utilized this protocol to assess the response of particular subunits and functional GST dimers in the kidney to *cis*-platinum treatment *in vivo*.

We observed that when a cytosol sample load of approx. 15 units of GST (activity towards 1-chloro-2,4-dinitrobenzene)/ml gel volume was used, and eluted with 5 mM-GSH, the adsorbed GST activity emerged from the column as a single but highly skewed peak. Under this condition 59% of the activity applied to the column could be accounted for by GSTs that were adsorbed on the column. However, when the cytosol sample load was decreased to less than 5 units of GST (activity towards 1-chloro-2,4-dinitrobenzene)/ml gel volume only 1.5% of the sample was not adsorbed on the column. Further, two non-overlapping peaks of GST activity emerged from the column. Prolonged elution with 5 mM-GSH slowly removed additional GST activity from the column. When the GSH concentration was increased to 25 mM an additional GST peak emerged from the column, which accounted for approx. 30% of the activity that was initially applied to the column. Thus it appears that the previously reported (Guthenberg *et al.*, 1985) recovery of only about 70% of GSTs from the affinity column may perhaps reflect the requirement for exceedingly high concentrations of GSH for successful desorption of the third GST peak. Moreover, it appears that this has led to a certain degree of misinterpretation of data. For example, it has been reported that approx. 25% of the GST activity in rat kidney cytosol does not bind to an *S*-hexylglutathione affinity matrix (Guthenberg *et al.*, 1985). This result has been interpreted to suggest a relatively large amount of GST 5–5 in kidney cytosol. Our findings indicated that only a minute amount (1.5–3.5%) of the total GST activity was unable to bind to the GSH affinity matrix. Hence it appears that GST 5–5 constitutes a much smaller percentage of the total GST activity of kidney cytosol than was previously suspected.

An alternative possibility that may explain the difference between previous reports and our findings may be related to the pre-affinity column treatment of the cytosol. The procedure that we have used substantially differs from that used in the past (Guthenberg *et al.*, 1985). Alternatively, the affinity matrix itself may have been responsible for the better resolution of GSTs. In other studies the affinity matrix has been prepared from a GSH derivative and epoxy-activated agarose (Simons & VanderJagt, 1977; Mannervik & Guthenberg, 1981; Hayes *et al.*, 1987); we used commercially available affinity matrix.

Following the ion-exchange chromatographic separation of all affinity peaks, we attempted to characterize the various GST subunits and dimers within the fractions on the basis of the subunit M_r (Tables 1–3), and the relative specific activity towards a variety of substrates (Table 4). The major GST dimers and hence the corresponding subunits of affinity peak I were identified tentatively as GST 1–1 (peak I-2) and GST 1–2 (peak I-3), with small amounts of dimers containing subunit 4 (peak I-1), subunit 7 (peak I-1) and subunit 8 (peak I-1). On the basis of subunit M_r, affinity and ion-exchange chromatographic behaviours of the parent dimer, an anionic subunit that was detected in affinity peak I appeared to be similar to, but not identical with, subunit 1. Indeed, the anionic subunit could be equivalent to the Yk and GST 8 subunits of rat liver that have been characterized by Hayes (1986) and Jensson *et al.* (1986) respectively. Subunits 1 and Yk have been shown to have immunochemical cross-reactivity (Hayes & Mantle, 1986*a*), owing to a high degree of sequence homology (Hayes, 1986). However, unlike previous reports (Hayes & Mantle, 1986*b*), we were unable to demonstrate a statistically significant M_r difference between subunits 1 and 8. Nonetheless the close similarity of the presently identified anionic subunit and Yk subunit explains the exceptionally high relative specific activity that we observed towards ethacrynic acid in peak I-1. Accordingly, we suspect that the subunit-8-containing dimer in affinity peak I is GST 8–8.

The major GST dimers of affinity peak II have been identified tentatively as GST 2–2 (peak II-6) and GST 3–4 (peak II-2), with small amounts of dimers containing subunit 6 (peak II-1) and subunit 7 (peak II-1). On the basis of the relative activity towards substrates, the band with M_r 25500 detected in peak II-1 appears to be a dimeric complex of subunit 6 (Hayes, 1984; Boyer & Kenney, 1985). Should this assignment be correct, the present paper would constitute the first reporting of a chromatographic technique that would allow resolution of subunit 6 in the kidney.

The major GST dimers of affinity peak III have been identified as GST 3–3 (peak III-3), GST 7–7 (peak III-1) and a second form of GST 7–7 in peak III-2. The two GST 7–7 forms were similar with respect to subunit M_r (Table 3) and catalytic behaviour towards ethacrynic acid (Table 4). However, differences were detected between these homodimers with respect to both catalytic behaviour towards the other substrates and their ion-exchange properties. The presence of alternative forms of the GST 7–7 dimer in ion-exchange peaks III-1 and III-2 in addition to their likely presence in ion-exchange peaks I-1 and II-1 is enigmatic. Several possibilities exist to explain their seemingly non-restrictive affinity-chromatographic peak location. One possibility is that the different GST 7–7 dimers represent autoxidative products of a single GST 7–7 dimer. Indeed, such a phenomenon has been observed with rat liver GST 3–3

and GST 8–8 (Hayes, 1986; Hayes & Clarkson, 1982). Another possibility is that the different GST 7–7 dimers represent net charge isomers, not unlike the variants that have been identified in rat placenta (Sato *et al.*, 1984) and neoplastic tissue of rat liver (Satoh *et al.*, 1985).

The presently observed *cis*-platinum-mediated pattern of alteration in the contents of the various rat kidney cytosolic GST subunits, and the corresponding dimers, is markedly different from the response observed in the rat liver following treatment with other chemicals *in vivo*. Inducers such as phenobarbital, 3-methylcholanthrene and *trans*-stilbene oxide have been shown to increase GST 1–1, 1–2, 3–3 and 3–4 and decrease GST 2–2 and 4–4 in rat liver cytosol (Mannervik *et al.*, 1984; Mannervik, 1985). The response is apparently the consequence of the specific induction of subunits 1 and 3 (Mannervik *et al.*, 1984). However, the present findings indicate that *cis*-platinum not only increased subunits 3 and 4, as well as GST 3–3, 3–4 and 4–4, but also decreased the contents of subunits 1 and 2 and GST 1–1, 1–2 and 2–2. Curiously, this pattern resembles that observed in the liver cytosol subsequent to the induction of primary hepatomas (Meyer *et al.*, 1985).

The possibility exists that the decrease in the renal cytosolic contents of GST subunits 1 and 2 following *cis*-platinum treatment *in vivo* results from the repression of the GST 1 and 2 loci. However, it would appear most likely that the observed decrease is the consequence of renal damage caused by *cis*-platinum. The drug has been shown to cause necrosis of the proximal tubular cells (Safirstein *et al.*, 1981). Indeed, the quantitative determination of urine GST B (i.e. GST 1–1 and/or GST 1–2) has been used to assess the degree of proximal tubular damage.

On the basis of the following reported findings, it is plausible that the increase in GST subunits 3 and 4 in rat kidney cytosol subsequent to *cis*-platinum treatment *in vivo* may be the direct result of alterations in the circulating concentrations of testosterone. *cis*-Platinum has been shown to deplete circulatory concentrations of testosterone (Maines & Mayer, 1985). Also, orchidectomy has been reported to increase the hepatic cytosolic GST activity towards certain substrates, and administration of testosterone, or other androgenic steroids, to the orchidectomized animals fully reversed the observed effect (Hales *et al.*, 1982).

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