Purification and characterization of a glutathione S-transferase Omega in pig: evidence for two distinct organ-specific transcripts

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A cytosolic glutathione S-transferase (GST, EC 2.5.1.18) from the recently characterized Omega class [GSTO; Board et al. 2000, J. Biol. Chem. **275**, 24798–24806] has been identified in pig organs. It was found widely distributed in the different tissues investigated and especially abundant in liver and muscle. The hepatic enzyme has been purified to homogeneity by using its selective affinity for *S*-hexylglutathione over GSH, thus providing a simple method to isolate mammalian GSTO. The dimeric protein has a subunit molecular mass of 27328 Da as measured by electrospray ionization MS. Internal peptide sequencing and complete cDNA sequencing revealed strong similarities with its human recombinant orthologue and two rodent GST-like proteins with the ability to catalyse the GSH-dependent reduction of dehydroascorbate. Additional similarities, including the presence of a specific N-terminal extension and of immunological cross-reactivity, support the results. Moreover, this gene encoding GSTO generates two organ-specific transcripts, suggesting transcriptional mechanisms with a significance that is as yet uncharacterized.

Key words: ascorbate, dehydroascorbate, differential splicing, *Sus scrofa*.

INTRODUCTION

Glutathione S-transferases (GSTs, EC 2.5.1.18) constitute a family of multifunctional enzymes that mainly catalyse the conjugation of intracellular GSH to a wide variety of electrophilic, cytotoxic and genotoxic molecules from endogenous or exogenous origin. GSTs are expressed qualitatively and quantitatively at various constitutive basic levels in most mammalian tissues but are also inducible by many substances, including carcinogens, drugs and oxidative-stress metabolites [1,2]. In addition, their induction by anti-cancer drugs has been shown to confer drug resistance on certain tumour cells [3]. Several other functions of GSTs have been regularly discovered; for example, they are involved in the binding and transport of non-substrate hydrophobic ligands [4] and in other cell functions such as gene regulation and apoptosis [5]. Seven cytosolic GST classes (Alpha, Kappa, Mu, Pi, Sigma, Theta and Zeta) have been characterized in mammals on the basis of their molecular structure, enzymic properties and immunological reactivity [1,6,7]. A new class of GST (Omega, GSTO), very similar to proteins expressed in rat [8] and mouse [9], has been characterized more recently in human liver on the basis of structural data [10]. The human recombinant GSTO was found to be inactive towards the classical GST substrate 1-chloro-2,4-dinitrobenzene (CDNB) but exhibited (as did the rat enzyme) some enzymic characteristics of glutaredoxins, including a slight dehydroascorbate reductase activity. For these reasons, the rat enzyme had been previously identified as a cytosolic dehydrascorbate reductase. Further microsomal trimeric [11] and mitochondrial Theta-class [12] GST forms have been described.

So far, the presence of three classes of GST (Alpha, Mu and Pi) has been found in pig organs [13–17]. Pig lung GST Pi is used as a reference for the three-dimensional structure of this class in mammals [18]. GSH or *S*-hexylglutathione (ShGSH) are the most commonly used affinity ligands in GST preparations. Retention of GSH-dependent and other non-GST proteins (namely glyoxalase I, mevalonate kinase and carbonyl reductase) on these matrices has been observed [19–21]. We previously reported HPLC purification profiles of pig liver constitutive GST monomers prepared by ShGSH-affinity chromatography and then mentioned the elution of an unknown polypeptide (molecular mass 27328 Da) close to the α-class GST subunits [22]. Here we describe the purification to homogeneity of the corresponding native protein from pig liver by using its differential affinity for ShGSH and GSH. From enzymic properties, measurements of molecular mass and a structural analysis of both the amino acid and nucleotide sequences, we identify this protein as a member of the GSTO class. We also examined the organ-specific pattern of expression of both transcript and protein.

EXPERIMENTAL

Materials

BSA, cytochrome *c*, Lys-C protease, GSH, trifluoroacetic acid (TFA), dithiothreitol, ethidium bromide and other chemicals, all of analytical grade, were purchased from Sigma-Aldrich (L'Isle d'Abeau Chesnes, France). TRIzol reagent and Moloney-murine-leukaemia virus reverse transcriptase were from Gibco BRL (Life Technologies, Cergy-Pontoise, France); *Taq* polymerase and RNAsin were from Promega (Madison, WI, U.S.A.). Ready To Go™ reverse-transcriptase-mediated PCR (RT–PCR) beads were from Amersham Pharmacia Biotech (Orsay, France). ShGSH was synthesized as described [23]. Coupling of GSH

Abbreviations used: CDNB, 1-chloro-2,4-dinitrobenzene; ESI–MS, electrospray ionization MS; GST, glutathione S-transferase; GSTO, GST Omega; RT–PCR, reverse-transcriptase-mediated PCR; RP-HPLC, reverse-phase HPLC; ShGSH, *S*-hexylglutathione; TFA, trifluoroacetic acid.

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The nucleotide sequence data reported will appear in DDBJ, EMBL and GenBank® Nucleotide Sequence Databases under the accession number AF188838.

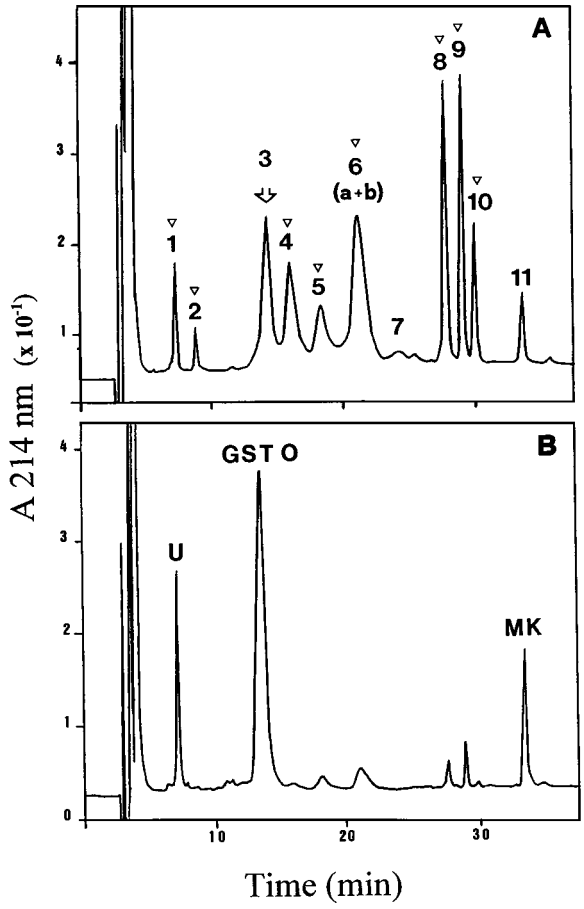

Figure 1 RP-HPLC separation of monomers of pig liver cytosolic proteins obtained by affinity purification

(**A**) ShGSH-retained proteins ('pool II' described in [22]); (**B**) successively GSH-excluded and ShGSH-retained proteins. Approximately 50 μg of protein was loaded for both profiles. Peaks in (**A**) are numbered in accordance with the previous numbering in [22]. Peak 1, unknown 20644 Da protein ('U' in **B**); peak 2, the class PI GST subunit; peaks 4–6 (peak 6 contained two isoenzymes, a and b), class Alpha GST subunits; peak 7, carbonyl reductase; peaks 8–10, class Mu GST subunits; peak 11, mevalonate kinase ('MK' in profile B). The arrow (peak 3) shows the retention of pig liver GSTO; arrowheads indicate proteins retained on both GSH and ShGSH affinity. Column: reverse-phase C_{18} Vydac 201 TP 54 (4.6 mm × 250 mm); flow rate 1 ml/min; fractions monitored at 214 nm. Gradient conditions are given in the Materials and methods section.

(Sigma) and ShGSH to epoxy-activated Sepharose CL-6B (Amersham Pharmacia Biotech) was achieved by the respective published methods [23,24]. HPLC-grade acetonitrile was from F.E.R.O.S.A (Barcelona, Spain).

Subcellular fractionation and GSTO purification procedure

Cytosol preparation, storage and GSH-affinity or ShGSH-affinity chromatography, used sequentially in GSTO purification, were performed in accordance with procedures described previously [22]. In brief, cytosol equilibrated with 10 mM Tris/HCl (pH 7.8)/1 mM EDTA/0.2 mM dithiothreitol (buffer A) on a Sephadex G-25 column was loaded on an affinity chromatography column equilibrated with the same buffer. The unretained fraction on a GSH matrix was collected and immediately loaded on an ShGSH-affinity column, which was rinsed with buffer A plus 0.2 M NaCl (buffer B) until no protein could be detected in the effluent, as estimated from A_{280}. Elution was performed with 5 mM ShGSH in buffer B. The fraction retained on the GSH-affinity column was eluted in a single step by 200 mM Tris/HCl (pH 9.2)/5 mM GSH/0.2 mM dithiothreitol after extensive washing with buffer A. Subsequent gel filtration was performed at 4 °C on a 1.6 cm × 100 cm Sephacryl S200 (Pharmacia) column equilibrated in buffer A. Proteins retained on ShGSH were concentrated to 0.8 ml before being loaded on the column and eluted with the same buffer. The molecular mass of native GSTO was estimated after column calibration with molecular mass standards (Blue Dextran, BSA, β-lactoglobulin, cytochrome *c* and cytidine). Protein purity in the fractions was checked by HPLC monomer profiling as described [22]. In brief, GST subunits were separated at room temperature on a C_{18} Vydac 201 TP54 reverse-phase HPLC (RP-HPLC) column (4.6 mm × 250 mm) with a Waters 625 system (Waters S. A., St Quentin-en-Yvelines, France) equipped with UV detection at 214 nm. Mobile phases were mixtures of acetonitrile in water (A, 30:70; B, 70:30; v/v) containing 0.1 % (v/v) TFA. Elution was performed at a flow rate of 1 ml/min with successive linear increases of B in A: 25 % at 0 min, 38 % at 5 min, 40 % at 20 min, 80 % at 35 min, 100 % at 35.1 min and isocratic 100 % B for 10 min. Protein was determined by staining with Amido Black [25]. The isoelectric point of native GSTO was estimated by isoelectric focusing with precast gels (pH 3–9) on a PHAST System (both from Pharmacia) in accordance with the manufacturer's instructions.

Enzyme assays

The CDNB-conjugating activity of GSTs was determined as described by Habig et al. [26]. Dehydroascorbate reductase activity was measured by the method of Mallero et al. [27] with freshly prepared dehydroascorbate.

Molecular mass determination of HPLC-isolated proteins by electrospray ionization MS (ESI–MS)

Analysis of protein by ESI–MS was performed with a Finnigan LCQ quadrupole ion-trap mass spectrometer (Thermoquest, Les Ulis, France) as described previously [22]. In brief, protein samples were dried in a SpeedVac evaporator (Savant Instruments, Farmingdale, NY, U.S.A.) and solubilized in water/methanol/acetic acid (49:49:2, by vol.) to a final concentration of 20–40 μM before infusion into the electrospray interface. Molecular masses were deduced from at least three measurements.

N-terminal and C-terminal amino acid sequence determination

Automated sequence analysis was performed on a Procise 494A sequencer (ABI, Foster City, CA, U.S.A.). The RP-HPLC-purified GSTO was dried and solubilized in acetonitrile/water/TFA (49:49:2, by vol.) before sequencing was performed. The N-terminus was found blocked; the protein was therefore digested with Lys-C endoproteinase for 4 h at 37 °C in 0.1 M Tris/Tricine/HCl, pH 8.2, containing 2 M urea (enzyme-to-substrate ratio 1:25, w/w). Digests were fractionated by RP-HPLC on a Vydac 218TP54 column (4.6 mm × 250 mm) at a flow rate of 1.0 ml/min with a linear gradient of 5–80 % acetonitrile and 0.1–0.096 % TFA over 80 min. Isolated peptides were analysed as described above and compared with GenBank and Swiss-Protein sequence data by using the Blast program.

Immunoblot analysis

A polyclonal antibody was raised in rabbits against the pig liver GSTO subunit purified by HPLC (peak GSTO in Figure 1B). No

cross-reactivity of this antibody with various HPLC-purified pig and rat Alpha, Mu or Pi GSTs or a wheat GST Theta was observed (results not shown). The antibody was used at a dilution of 1:250 in Western blot analyses of pig tissues and liver cytosolic proteins from various species (rat, mouse and trout). Equal quantities of protein were separated by SDS/PAGE [28] and transferred to a 0.45 μm pore-size Protran nitrocellulose membrane (Schleicher & Schüll, Dassel, Germany) as described [29]. Immunoreactive species were revealed with SuperSignal™ chemiluminescent substrate (Pierce, Rockford, IL, U.S.A.) after reaction with a peroxidase-coupled goat anti-rabbit secondary antibody (Sigma-Aldrich) (1:500 dilution). After autoradiography the resulting band was evaluated by densitometry with a GelDoc 2000 scanner with the Quantity One analysis program (Bio-Rad, Ivry-Sur-Seine, France).

RNA isolation, RT–PCR and cDNA cloning and sequencing of pig liver GSTO

Fresh pig organs were obtained from the local slaughterhouse, snap-frozen and stored at −80 °C. Total RNA isolation was performed with the improved acid guanidinium thiocyanate/phenol/chloroform method [30] with the use of TRIzol reagent. RNA yield and purity was assessed by determining the A_{260}-to-A_{280} ratio; integrity was assessed by comparing the relative intensities of the 18 S and 28 S ribosomal RNA bands as detected under UV radiation after electrophoresis and staining with ethidium bromide. Initial RT–PCR was performed with 5 μg of total RNA and primers (P1, sense, 5′-ATGTCCGGGGAGTC-AGCCAGGAGCTTGGGGAAGG-3′; P2, anti-sense, 5′-TCA-GAGCCCATAGTCACAGGCCTC-3′) corresponding to 5′ and 3′ sequences of the cDNA encoding the human GSTO (accession numbers U90313 and AF212303). PCR cycling conditions were 2 min at 95 °C, followed by 40 cycles of 1 min at 95 °C, 1 min at 57 °C, 1 min at 72 °C and a final step of 6 min at 72 °C. The size and purity of PCR products were evaluated on an (ethidium bromide)-agarose gel with appropriate molecular mass markers. The amplification product was cloned in a pCR®TOPO plasmid. The resulting plasmid was used to transform an *Escherichia coli* strain with the TA-Cloning® kit (Invitrogen, Leek, The Netherlands) in accordance with the manufacturer's instructions. After bacterial amplification the plasmid containing the cDNA insert was extracted from cell lysate by using a plasmid purification kit from Qiagen (Courtaboeuf, France) and directly submitted for sequencing of both strands to Genome Express (Grenoble, France). A third RT–PCR primer (P3), starting at the putative second ATG site in the cDNA sequence, was designed for experiments aiming at identifying possible variants of GSTO transcripts in pig organs (see the text). Its sequence was 5′-ATGAGATTCTGTCCTTTCGCCCAGAGG-ACTCTCC-3′ (sense). The sequence upstream of the start codon was obtained using primers 5′-CGAGGAGAGAGTGGCGAC-3′ (sense) and 5′-GTTGATGACTTGATGCCGGAT-3′ (reverse) specific for partial determination (accession number F14520) and the pig cDNA obtained as described above. The sequence from the stop codon to poly(A) was obtained by PCR with rapid amplification of cDNA ends by using the following primers: sense, 5′-CATACTTTGGTGGAAGTTCTCTTTC-3′; reverse, 5′-GACTCGAGAGTCGACATCGAT$_{18}$-3′; adaptor sense, 5′-GACTCGAGAGTCGACATCGA-3′.

Northern blot analysis

Total RNA (15 μg) prepared from various pig organs was loaded on agarose gels containing 2.2 M formaldehyde [31]. After electrophoresis, RNA species were subjected to capillary transfer to a positively charged nylon membrane (Biodyne B; Pall, Saint-Germain-en-Laye, France). The complete cDNA of GSTO was used as a probe after random priming labelling (Amersham-Pharmacia Biotech, Orsay, France) with [α-^{32}P]dCTP (Isotop-chim, Ganagobie-Peyruis, France). Unincorporated nucleotides were removed with a Micro Bio-Spin 6 column (Bio-Rad). Hybridization and washing conditions were in accordance with the method described by the membrane manufacturer. Quantitative expression of GSTO mRNA was evaluated after final autoradiography of the blots on Biomax MR films (Kodak, France) by using the tools previously described for Western blots.

RESULTS

Protein purification and monomer isolation by HPLC

As supported by measurements of dehydroascorbate reductase and CDNB-conjugating activities and a comparison of HPLC–MS profiling with previous data [22], pig liver GSTO was shown to pass through GSH-affinity columns but to be retained on the ShGSH-affinity matrix (Table 1, Figure 1). Thus sequential affinity chromatography performed with both matrices followed by Sephacryl S200 filtration completed the purification of native pig liver GSTO (see the methods section). The enzyme exhibited a molecular mass of approx. 50 kDa and an isoelectric point of 6.3 as estimated by gel filtration and isoelectric focusing electrophoresis respectively (results not shown). These results partly support others obtained with the human enzyme [10]. In addition, RP-HPLC experiments showed pig GSTO to be composed of a single monomer that was eluted at 13 min (Figure 1), indicating that it should also be homodimeric. Consequently, the discrepancy observed between the measured (native enzyme) and calculated (two monomers) molecular masses will require further examination.

Peptide and nucleotide sequence analysis

RT–PCR with pig liver mRNA produced a single cDNA that was fully sequenced from both ends (Figure 2). It encoded a 241-residue polypeptide with a predicted molecular mass of 27419 Da. Direct Edman degradation of the HPLC-purified protein gave no result, probably because of the presence of a blocked N-terminus. Internal peptide sequencing enabled us to confirm more than half of the cDNA-deduced primary structure of pig GSTO (161 determinations) including 7 of the 11 residues encoded by the sense human primer used in RT–PCR (see the Experimental section and Figure 3). Two discrepancies were found between the peptide and the corresponding cDNA-deduced sequence (Cys instead of Tyr at position 139, and Thr instead of Gln at position 158). Repetition of RT–PCR and controls performed on the nucleotide sequence confirmed the cDNA-deduced sequence, which was retained. This choice was supported by MS analysis of the protein (see below).

When the sequence of the pig enzyme was compared with published sequences, it seemed substantially different from classical mammalian GSTs but was strongly similar to that of human liver GSTO (accession number AF212303) [10], its previously described orthologues as a GSH-dependent dehydroascorbate reductase in rat liver (AB008807) [8] and a GST-like stress-induced protein in mouse lymphoma cells resistant to apoptosis induced by ionizing radiation (U80819) [9]. Thus, the pig enzyme displayed 82 %, 77 % and 71 % identities with the human, rat and mouse sequences respectively (Figure 3). Similarities with plant dehydroascorbate reductases and GSTs were also observed but to a much smaller extent. A partial

Table 1 Purification of pig liver GSTO

Abbreviation: n.d., not determined.

		Activity with CDNB		Dehydrascorbate reductase activity			
Purification step	Total protein (mg)	Total (μmol/min)	Specific (μmol/min per mg)	Total (nmol/min)	Specific (nmol/min per mg)	Purification (fold)	Yield (%)
Cytosol	775	2327	3.0	1093	1.4	1.0	100.0
Sephadex G25	701	2035	2.9	1010	1.4	0.9	
Affinity							
GSH retained	26.2	1224	46.7	n.d.	n.d.	n.d.	n.d.
GSH unretained and ShGSH retained	4.9	0.42	0.09	535	108.6	77.0	48.9
Sephacryl S200	2.9	0.49	0.17	504	174.4	123.7	46.0

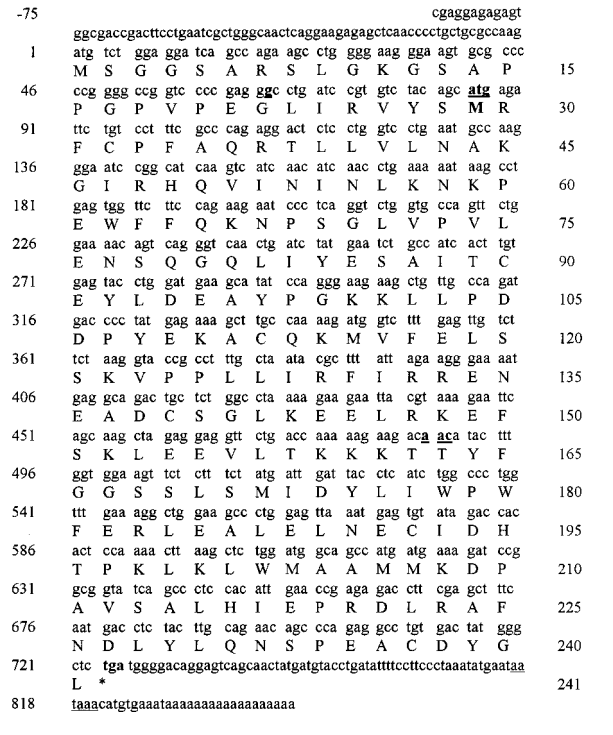

Figure 2 Nucleotide sequence and deduced amino acid sequence of pig liver cytosolic GSTO

Underlined bold nucleotides correspond to specific positions in similar sequences (see the text and the legend to Figure 3): **g** at position 65 was omitted from the rat sequence in adjusting the reading frame; codon **atg** at position 85–87 corresponds to the initiator in the rat published sequence (AB008807); **aca** at position 486–488 is deleted in the mouse sequence (U80819). The consensus polyadenylation signal (position 816–821) is underlined.

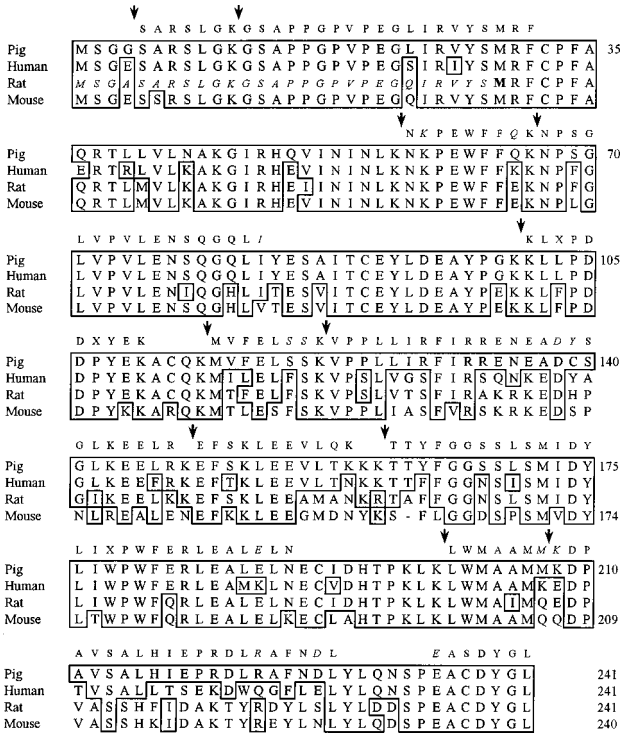

Figure 3 Comparison of the amino acid sequence of pig GSTO with its human, rat and mouse orthologues

The cDNA-deduced sequences of Omega class GSTs from pig liver (accession number AF188838), human placenta (U90313) or liver (AAF73376), rat liver (AB008807) and mouse lymphoma cells (U80819) were aligned. Numbering is in accordance with human and pig sequences. Structural identities with the pig sequence are boxed. The top line is an amino acid sequence determination of peptides obtained after digestion of the pig liver enzyme with Lys-C (arrows are cleavage points). Amino acids at position 235–241 were determined by C-terminal automated sequence analysis. Uncertain and unidentified amino acids are represented by italic letters and X respectively. The smaller italic letters in the rat sequence correspond to a translation of the reported nucleotide sequence with a different reading frame (see the text). The bold M corresponds to the putative alternative initiator methionine.

sequence obtained from the characterization of a pig small intestine cDNA library [32] (accession number F14520) was almost identical with the 5′ third of our cDNA, with the exception of an undetermined nucleotide. RT–PCR analysis confirmed these results and thus ruled out possible inter-organ variability in this region from liver to intestine in pig. PCR with 3′ rapid amplification of cDNA ends showed identity between the human and pig sequences at the 3′ end and allowed characterization of the polyadenylation site 71 nt downstream of the stop codon, which was preceded by a consensus signal at position 816–821 (Figure 2). The sizes of the coding sequence were identical between pig and human. The reported rat sequence was found to be shorter [8], starting at a putative second, in-frame, start codon that was also present at position 85–87 in other species. Nevertheless, a shift in the translation reading frame resulting from the deletion of G at position 65 made it possible to align the rat sequence with others from the upstream common start codon (Figure 2). Under these conditions, the rat GSTO was identical in size with the human and pig proteins. A three-nucleotide

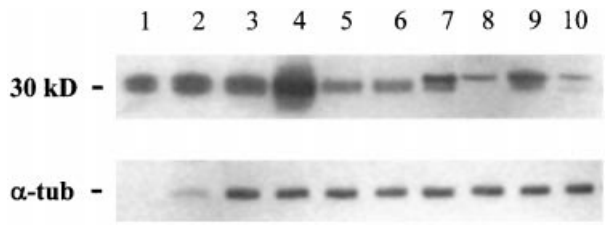

Figure 4 Western blot analysis of GSTO expression in various organ and species cytosols with an antibody directed against pig liver GSTO

Apart from pig liver GSTO (lane 1), control and 10-fold diluted pig liver cytosol (lane 2), 50 μg of cytosolic proteins were loaded. Lane 3, pig heart; lane 4, pig skeletal muscle; lane 5, pig small intestine; lane 6, pig kidney; lane 7, Sprague–Dawley rat liver; lane 8, rainbow trout liver; lane 9, Fischer rat liver; lane 10, mouse liver. Abbreviation: kD, kDa.

deletion observed at position 536–538 shortened the mouse sequence.

Molecular mass determination of pig liver GSTO by ESI–MS

A single polypeptide with a molecular mass of 27328 ± 3 Da was detected in the RP-HPLC fraction containing the pig liver GSTO monomer (Figure 1). Moreover, this value was identical for proteins purified by both ShGSH affinity and successive GSH affinity and ShGSH affinity. This indicated the absence of monomer variants of pig liver GSTO. In addition, the molecular mass corresponded exactly to the cDNA-deduced sequence [corrected for Gly instead of Glu at position 4 of the human primer (see above)] deprived of the initiator methionine residue and with the addition of an N-terminal acetyl blocking group. Two proteins co-purified with GSTO in successive affinity experiments (Figure 1B). They had molecular masses of 20644 ± 2 and 42361 ± 4 Da, corresponding to previous determinations for an unknown polypeptide and mevalonate kinase respectively [22]. Whether or not the unknown protein was non-covalently linked to GSTO in the native state remains to be investigated.

Immunoblot analysis

With the use of our polyclonal antibody directed against the HPLC-purified pig liver GSTO subunit, Western blot analysis revealed the presence of immunoreactive proteins in the cytosolic fraction of various species (Figure 4). As observed previously, the subunit migrated slightly above the 30 kDa molecular mass marker. Cross-reaction of the antibody with a minor band approx. 2–3 kDa smaller than the main band was visible in the two rat strains (namely Sprague–Dawley and Fischer) and in the mouse liver cytosolic fractions examined. In contrast, the antibody seemed to recognize a single protein in trout liver. The presence of this minor protein in pig samples could account for the diffuse aspect of certain bands. Indeed, we cannot completely exclude the possibility of contamination of the GSTO monomer in the HPLC fraction, used in antiserum preparation, with a very minor undetected protein. Although present in quantities approx. 5-fold smaller than in liver, the expression of pig GSTO in skeletal muscle seemed substantial. The lowest expression of the protein in pig organs was found in the small intestine and kidney.

Tissue-specific expression of GSTO mRNA in pig tissues

Equal amounts of total RNA prepared from various pig organs and tissues (liver, spleen, pancreas, thymus, mature and immature ovary, heart, skeletal muscle, diaphragm, lung, kidney cortex and medulla, colon, small intestine) were analysed by Northern blotting (Figure 5). The complete liver GSTO cDNA probe was able to hybridize with mRNA in each sample tested, showing a widespread expression of GSTO transcripts in pig tissues. This expression was especially high in liver and muscle. In the organs tested, we observed the unexpected occurrence of two different transcripts of approx. 1.3 and 1.1 kb, which were never present simultaneously in the same tissue. The smaller transcript occurred in muscular tissues (namely skeletal muscle, heart and diaphragm) and the larger molecule was observed in all other organs tested. Additional RT–PCR analysis of liver and muscle mRNA species with combinations of the forward or reverse primer with the internal-sequence primer described in the Experimental section gave similar sizes of amplification products, showing that the coding sequence of pig GSTO was not affected in these organs (results not shown). This was confirmed at the protein level in a Western blot analysis (Figure 4).

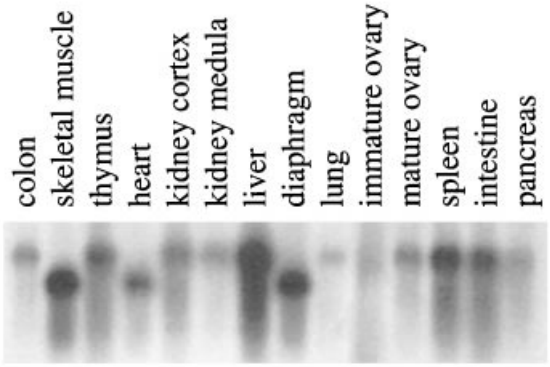

Figure 5 Northern blot showing the abundance of GSTO mRNA species in various pig tissues

Total RNA (10 μg) from the various pig organs was transferred from agarose gel to a nylon$^+$ membrane by capillarity. GSTO mRNA species were hybridized with the ^{32}P-labelled pig liver coding sequence obtained by RT–PCR. The sizes of the major and minor transcripts were approx. 1.3 kb and approx. 1.1 kb respectively.

DISCUSSION

The recently discovered Omega class represents a particular member of the GST family that possesses specific structural features as a novel unit formed by the proximity of a specific N-terminal extension to the C-terminus and a large opened H site, but also the ability to catalyse the GSH-dependent reduction of dehydroascorbate [10]. Initially identified in rat liver as a cytosolic dehydroascorbate reductase on the basis of its catalytic properties [33], it has also been considered by others to be a GST Theta-like stress response protein named p28 in mouse lymphoma cells [9]. Determination of the crystal structure of the human enzyme brought these proteins into a new class of GSTs named Omega [10]. In the present study we investigated the expression of an unknown protein in pig organs and characterized the hepatic form at the transcriptional and translational levels as a member of the new GSTO class. Two distinct organ-specific transcript sizes, not observed in human tissues, were found clearly expressed in pig organs, suggesting a possible opportunity for differential mRNA splicing. Similar results attributed to an alternative location of mRNA polyadenylation have been mentioned for p28 in mouse, except that both forms were detected together in the

different tissues [9]. Differential transcription in pig organs should account for the occurrence of an organ-specific (i.e. skeletal muscle, heart and diaphragm) regulatory mechanism that seems to be related to muscular function. The demonstration that other GSTs [34] and the mouse GSTO [9] could be involved in the response to oxidative stress suggests that GSTO might be involved in oxidative metabolism, which is important in muscular tissue.

In a previous study Hussey et al. [35] described a CDNB-inactive 31 kDa protein among human skeletal muscle cytosolic GSTs eluted from an ShGSH-affinity column. We have described the presence of such a protein (identified here as GSTO) in pig liver and shown that it was selectively retained on ShGSH-affinity matrix and not on the commonly used GSH-affinity matrix. The few mammalian GSTO purifications reported in the literature did not use an affinity strategy and required at least four purification steps. Because the reported binding of mouse GSTO to GSH–Sepharose still seems to be controversial [9,10], our proposed strategy provides the simplest method of purifying this enzyme. It is not yet known why GSTO binds to ShGSH and not GSH but it could be that the enzyme is able to recognize cellular hydrophobic molecules and/or thiol-targeted adducts to GSH or proteins. The ability of human GSTO to accept large molecules as substrate has been suggested by the opened conformation of its H site [10]. A possible role for GSTO could be to restore the function of enzymes inactivated by the adduction of lipid peroxidation products. This hypothesis is in accordance with the induction of GSTO among stress proteins observed in mouse lymphoma cells exposed to ionizing radiation [9] and provides a new field of investigation into this enzyme.

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