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Differential Expression of α, μ and π Classes of Isozymes of Glutathione S-Transferase in Bovine Lens, Cornea, and Retina[1]

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Isozyme characterization of glutathione S-transferase (GST) isolated from bovine ocular tissue was undertaken. Two isozymes of lens, GST 7.4 and GST 5.6, were isolated and found to be homodimers of a M_r 23,500 subunit. Amino acid sequence analysis of a 20-residue region of the amino terminus was identical for both isozymes and was identical to GST ψ and GST μ of human liver. Antibodies raised against GST ψ cross-reacted with both lens isozymes. Although lens GST 5.6 and GST 7.4 demonstrated chemical and immunological relatedness, they were distinctly different as evidenced by their p*I* and comparative peptide fingerprint. A corneal isozyme, GST 7.2, was also isolated and established to be a homodimer of M_r 24,500 subunits. Sequence analysis of the amino-terminal region indicated it to be about 67% identical with the GST π isozyme of human placenta. Antibodies raised against GST π cross-reacted with cornea GST 7.2. Another corneal isozyme, GST 8.7, was found to be a homodimer of M_r 27,000 subunits. Sequence analysis revealed it to have a blocked amino-terminus. GST 8.7 immunologically cross-reacted with the antibodies raised against cationic isozymes of human liver indicating it to be of the α class. Two isozymes of retina, GST 6.8 and GST 6.3, were isolated and identified to be heterodimers of subunits of M_r 23,500 and 24,500. Amino-terminal sequence analysis gave identical results for both retina GST 6.8 and GST 6.3. The sequence analysis of the M_r 23,500 subunit was identical to that obtained for lens GSTs. Similarly, sequence analysis of the M_r 24,500 subunit was identical to that obtained for the cornea GST 7.2 isozyme. Both the retina isozymes cross-reacted with antibodies raised against human GST ψ as well as GST π. The results of these studies indicated that all three major classes of GST isozymes were expressed in bovine eye but the GST genes were differentially expressed in lens, cornea, and retina. In lens only the μ class of GST was expressed, whereas cornea expressed α and π classes and retina expressed μ and π classes of GST isozymes.

Glutathione S-transferases (GSTs[4]; EC 2.5.1.18) play an important role in detoxification mechanisms by catalyzing the conjugation of electrophilic xenobiotics to GSH (1-5) and by covalently or noncovalently binding certain nonsubstrate ligands (2, 3, 6). In addition, some isozymes

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[4] Abbreviations used: GST, glutathione S-transferase, GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TBS, 10 mM Tris-HCl buffer, pH 7.4, containing 150 mM sodium chloride.

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of GST have been implicated in the protection of tissues from oxidative damage through GSH peroxidase II activity toward lipid hydroperoxides (7, 8). The eye frequently encounters exposure of environmental pollutants including electrophilic xenobiotics and the physiological roles of ocular GST may be important in detoxification of these toxicants. In addition, this enzyme system may play a protective role in the eye against endogenous oxidative stress (9) which was implicated in deleterious effects such as cataractogenesis in lens (10). Bovine eye, due to its easy accessibility, has been frequently used as a model to study ocular defense mechanisms against exogenous as well as endogenous chemical stress and several forms of GST have been described in bovine lens, cornea, and retina (9, 11-13).

In the studies described herein we have investigated these enzymes in regard to (a) chemical structure, (b) immunological relatedness, and (c) substrate specificities in order to elucidate their structural and functional interrelationships. In addition, using the Western blotting technique with antibodies raised against human α, μ and π classes of GST enzymes and by comparing amino acid sequences of the N-terminal region of bovine ocular GSTs with those of human GST isozymes we have studied the structural relatedness of bovine ocular GST with human GST isozymes. Immunoblotting studies have also been performed with the lens, cornea, and retina supernatants from a number of animals in order to examine if any of the three classes of GST subunits are expressed selectively in these tissues.

EXPERIMENTAL PROCEDURES

Materials. Eyes were obtained from freshly slaughtered bovine animals. The lens, cornea, and retina were removed from the eye globes and were frozen at −20°C until used. Sources for the reagents used for sequence analysis were the same as described previously (14). Sources of all other chemicals used were as we previously reported (15).

Enzyme assay. GST activity toward CDNB was determined according to the method of Habig *et al.* (16). One unit of enzyme utilized 1 μmol substrate per minute at 25°C.

Purification of GST isozymes. GST isozymes of bovine lens, cornea, and retina pooled from several animals were purified according to procedures previously reported for GST isozymes of human liver (15) and kidney (17). Briefly, a 10% (w/v) homogenate of ocular tissues was prepared in 10 mM potassium phosphate, pH 7.0, containing 1.4 mM 2-mercaptoethanol (buffer A) using a PT 10-35 Polytron (Kinematica GmbH Litau). Homogenization was performed for 5 min and the homogenate was centrifuged at 14,000*g* for 40 min. The supernatant after dialysis against buffer A was subjected to GSH-linked epoxy-activated Sepharose 6B affinity chromatography (18). A column of GSH affinity resin was preequilibrated with 22 mM potassium phosphate, pH 7.0, containing 1.4 mM 2-mercaptoethanol (buffer B) at a flow rate of 10 ml/h and this flow rate was maintained throughout the affinity chromatography. After sample application, the unbound proteins were thoroughly washed off with buffer B and the enzyme was eluted with 5 mM GSH in 50 mM Tris-HCl, pH 9.6, containing 1.4 mM 2-mercaptoethanol. The fractions containing GST activity were pooled and dialyzed against buffer A and subjected to isoelectric focusing in a LKB 8100-1 isoelectric focusing column employing a 0 to 50% sucrose density gradient. After isoelectric focusing at 1600 V for 18 h, 0.8-ml fractions were collected and monitored for pH and GST activity using CDNB as the substrate. The enzyme preparations used for kinetic studies were dialyzed against buffer A whereas those used for structural studies were dialyzed against 0.1% aqueous acetic acid. Separate experiments involved the isoelectric focusing of the 14,000*g* supernatants of ocular tissues.

Electrophoresis. Urea/SDS/2-mercaptoethanol-PAGE was performed using the buffer system described by Laemmli (19). The concentration of urea in both stacking and resolving gels was 6 M. The stacking and resolving gels contained 5.9% (w/v) and 12.5% (w/v) of polyacrylamide, respectively. The concentrations of the crosslinker, *N,N'*-methylenebisacrylamide, in the stacking and resolving gels were 0.15% (w/v) and 0.35% (w/v), respectively.

Amino acid analysis. Samples were hydrolyzed with 5.7 N HCl *in vacuo* at 107°C for 24, 48, and 96 h. Amino acid compositional analysis was carried out on a Beckman 121M analyzer employing single column methodology on Beckman AA20 resin and on a Beckman 6300 analyzer (20).

Automated amino acid sequence analysis. The N-terminal amino acid sequence of the proteins was determined by microsequence analysis using an Applied Biosystems Model 470A protein/peptide sequencer according to the procedure we described previously (21).

Peptide mapping. Homogeneous GST isozymes were dialyzed extensively against distilled water containing 0.1% acetic acid (4 changes; 2 liters each). An

TABLE I

AMINO ACID COMPOSITIONAL ANALYSIS OF GST ISOZYMES OF BOVINE OCULAR TISSUES[a]

Amino acid	Residues/mol of enzyme					
	Lens GST 7.4	Lens GST 5.6	Cornea GST 8.7	Cornea GST 7.2	Retina GST 6.8	Retina GST 6.3
Aspartic acid	50	48	39	51	50	54
Threonine[b]	14	12	12	15	18	18
Serine[b]	20	14	31	28	30	32
Glutamic acid	42	53	55	58	46	48
Proline	28	26	31	25	24	24
Glycine	30	38	55	47	53	55
Alanine	24	21	43	39	36	34
Valine	16	16	33	23	22	22
Methionine[c]	10	14	20	5	14	16
Isoleucine[c]	26	25	36	19	16	16
Leucine	52	50	52	64	45	46
Tyrosine	18	18	23	19	12	13
Phenylalanine	26	25	30	18	20	20
Lysine	34	32	25	28	29	32
Histidine	10	8	8	9	10	10
Arginine	28	25	22	19	25	28
Total	428	425	515	467	450	468

[a] The results are based on M_r values of 47,000 for GST 7.4 and GST 5.6 of lens; 54,000 for GST 8.7 of cornea; 49,000 for GST 7.2 of cornea; 48,000 for GST 6.8 and GST 6.3 of retina. Values represent means of 24-, 48-, and 96-h hydrolyses.

[b] Extrapolated to zero time of hydrolysis.

[c] Value obtained at 96 h of hydrolysis.

aliquot containing 100 μg of protein was lyophilized and resuspended in 100 μl of 0.1 M ammonium bicarbonate, pH 7.8. Trypsin digestion was carried out by incubating the enzyme with 10 μl trypsin at a protease:substrate ratio of 1:100 (w/w) for 1 h at 37°C. The reaction was stopped by lyophilization and the resulting peptides were dissolved in 70% formic acid and analyzed by HPLC on an Ultrasphere-octyl (4.6 mm × 2.5 cm) column. The HPLC was performed on a Beckman 334 gradient liquid chromatograph connected with a Model 165 variable wavelength uv detector. The program used was essentially similar to that described by us previously (17).

Preparation of 14,000g supernatants for Western blotting. Bovine eyeballs were obtained from 10 freshly slaughtered animals and the lens, cornea, and retina were removed. A 10% (w/v) homogenate of ocular tissues was prepared in buffer A as described above and centrifuged at 14,000*g* for 40 min. Protein content in the supernatants was determined by the method of Bradford (22). An aliquot of supernatant containing 20 μg of protein from 10 different lenses, corneas, and retinas was subjected to SDS-PAGE, and Western blotting was performed as described below.

Immunological studies. Antibodies against anionic GST π of human placenta (p*I* 4.5) were raised in goat (23), whereas the antibodies against GST ψ (pI 5.5) and a mixture of the cationic GSTs (α, β, γ, δ, and ε) of human liver were raised in New Zealand rabbits (15, 23). The Western blotting was performed according to the procedure described by Towbin *et al.* (24) with slight modification. The transblotting was performed for 4 h at 25°C using the Bio-Rad transblot cell. After transblotting, the nitrocellulose paper was soaked in 5% (w/v) solution of nonfat dry milk in TBS for 30 min at 25°C. The nitrocellulose paper was then incubated overnight with appropriately diluted antiserum and rinsed with nonfat dry milk solution for 30 min (six changes of 10 ml; 5 min each). The nitrocellulose paper was incubated with 10 ml of 5% nonfat dry milk solution containing 20 μl of horseradish peroxidase-conjugated antiserum for 4 h at 25°C. Finally, the nitrocellulose paper was rinsed six times with TBS (six changes of 10 ml; 5 min each) and the peroxidase stain was developed by incubating the pa-

TABLE II

AUTOMATED AMINO ACID SEQUENCE ANALYSIS OF GST ISOZYMES OF BOVINE OCULAR TISSUES[a]

	Residues (pmol)				
Edman cycle	Lens GST p*I* 7.4	Lens GST p*I* 5.6	Cornea GST p*I* 7.2	Retina GST p*I* 6.8	Retina GST p*I* 6.3
0[b]	— (900)	— (900)	— (800)	(100)	(100)
1	Pro(608)	Pro(352)	Pro(559)	Pro(Pro) — (84)[c]	Pro(Pro) — (44)[c]
2	Met(588)	Met(193)	Pro(465)	Met(Pro) 8 (70)	Met(Pro) 10 (32)
3	Ile(462)	Ile(199)	Tyr(737)	Ile(Tyr) 13 (46)	Ile(Tyr) 13 (12)
4	Leu(362)	Leu(161)	Thr(361)	Leu(Thr) 19 (22)	Leu(Thr) 13 (5)
5	Gly(266)	Gly(145)	Ile(800)	Gly(Ile) 11 (50)	Gly(Ile) 4 (12)
6	Tyr(304)	Tyr(95)	Val(498)	Tyr(Val) 6 (53)	Tyr(Val) 7 (11)
7	Trp(50)	Trp(15)	Tyr(402)	Trp(Tyr) 13 (32)	Trp(Tyr) 1 (6)
8	Asp(228)	Asp(18)	Phe(451)	Asp(Phe) 4 (48)	Asp(Phe) 3 (12)
9	Ile(318)	Ile(75)	Pro(631)	Ile(Pro) 12 (26)	Ile(Pro) 9 (4)
10	Arg(98)	Arg(53)	Val(696)	Arg(Val) 2 (22)	Arg(Val) 1 (4)
11	Gly(114)	Gly(71)	Gln(357)		
12	Leu(192)	Leu(102)	Gly(99)		
13	Ala(130)	Ala(117)	Gly(300)		
14	His(46)	N.D.[d]	N.D.		
15	Ala(168)	Ala(78)	Glu(95)		
16	Ile(92)	Ile(13)	Ala(161)		
17	N.D.	N.D.	Ala(70)		
18	Leu(182)	Leu(77)	Leu(74)		
19	Leu(176)	Leu(70)	Met(61)		
20	Leu(210)	Leu(25)	Leu(139)		
21	Glu(34)		Leu(125)		

[a] Results were obtained with a gas-phase sequencer. Amino acid phenylthiohydantoins were quantified by HPLC.

[b] Initial picomoles applied to the sequencer.

[c] Total picomoles observed.

[d] N.D., not determined.

per in freshly prepared solution of horseradish peroxidase color development reagent (Bio-Rad) containing 4-chloro-1-naphthol (60 mg in 20 ml of ice-cold methanol) and H_2O_2 (60 μl of 30% H_2O_2 diluted to 100 ml with TBS).

RESULTS

Isolation of GST Isozymes

Upon isoelectric focusing of the 14,000*g* supernatants of 10% (w/v) homogenates of bovine ocular tissues two peaks each of GST activity were observed for lens (p*I* 7.4 and 5.6), cornea (p*I* 8.7 and 7.2), and retina (p*I* 6.8 and 6.3). The isoelectric focusing profiles of the enzymes purified by affinity chromatography from these tissues (about 70% yield) were similar to those of the corresponding crude supernatants. This indicated that during the purification by affinity chromatography followed by isoelectric focusing none of the GST isozymes of lens, cornea, and retina were selectively lost or significantly altered. The purification protocol described under Experimental Procedures yielded two apparently homogeneous GST isozymes from lens (p*I* 7.4 and 5.6), cornea (p*I* 8.7 and 7.2), and retina (p*I* 6.8 and 6.3). To distinguish the various GST isozymes of ocular tissues, they were assigned a numerical suffix which denoted their p*I* value. For example, isozymes of lens having p*I* values of 7.4 and 5.6 were designated as lens GST 7.4 and GST 5.6, re-

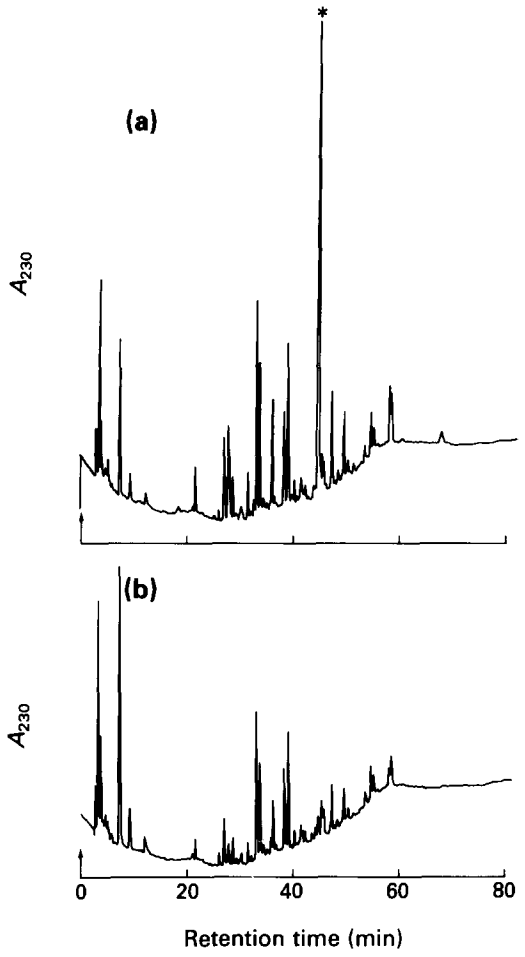

FIG. 1. HPLC of tryptic digest of (a) GST 7.4 and (b) GST 5.6 of bovine lens. Experimental details are given in the text.

spectively. The apparent molecular weight of GST preparations from bovine lens, cornea, and retina determined by Sephadex G-100 gel filtration were found to be in the range 47,000–54,000 (data not presented). The subunit compositions of these isozymes were determined by urea/SDS/2-mercaptoethanol-PAGE (data not shown). Both isozymes of bovine lens, GST 7.4 and GST 5.6, were dimers of identical sized subunits of M_r 23,500. GST 8.7 and GST 7.2 of cornea were found to be apparent homodimers of subunits of M_r 27,000 and M_r 24,500, respectively. On the other hand, GST 6.8 and GST 6.3 of bovine retina were apparent heterodimers of M_r 24,500 and M_r 23,500 subunits.

Amino Acid Composition

The amino acid compositions of GST isozymes of bovine lens, cornea, and retina are presented in Table I. The amino acid compositions of GST 7.4 and GST 5.6 of lens were found to be very similar to each other because the difference index between these two isozymes calculated by the method of Metzger *et al.* (25) was found to be 4.9. The amino acid compositions of GST 6.8 and GST 6.3 of retina were also similar to each other. On the other hand, the amino acid compositions of GST 8.7 and GST 7.2 of cornea were significantly different from each other and the difference index between these two isozymes was calculated to be 13.0. GST 7.2 of cornea had greater amounts of aspartyl, leucyl, and lysyl residues whereas GST 8.7 had relatively higher contents of prolyl, glycyl, valyl, methionyl, isoleucyl, tyrosyl, and phenylalanyl residues. The difference index values between GST 7.2 of cornea and GST 7.4 and GST 5.6 of lens were 11.1 and 10.7, respectively, indicating significant structural differences between these proteins. GST 7.2 of cornea had higher amounts of seryl, glycyl, alanyl, valyl, and leucyl residues when compared with GST 7.4 and GST 5.6 of lens which were somewhat richer in methionyl, isoleucyl, phenylalanyl, and arginyl residues. The amino acid compositions of GST isozymes of cornea and retina were also different from each other. Similarly, significant differences were observed between the amino acid compositions of GST isozymes of lens and retina.

Primary Structure of Bovine Ocular GST

Amino acid sequence analyses of the N-terminal region of GST isozymes of bovine ocular tissues are presented in Table II. Single N-terminal polypeptide sequences were obtained for GST 7.4 and GST 5.6 of lens and these sequences were identical for the first 20 amino acid residues determined in this study. This indicated that both GST

TABLE III

COMPARISON OF THE N-TERMINAL REGION OF AMINO ACID SEQUENCES OF GST ISOZYMES OF BOVINE OCULAR TISSUES WITH THOSE OF OTHER MAMMALIAN GST ISOZYMES[a]

	1									10										20
Bovine lens GST 7.4[b]		P	M	I	L	G	Y	W	D	I	R	G	L	A	H	A	I	–	L	L	L	E/
Bovine lens GST 5.6[b]		P	M	I	L	G	Y	W	D	I	R	G	L	A	–	A	I	–	L	L	L/	
Bovine cornea GST 7.2[b]	P	P	Y	T	I	V	Y	F	P	V	Q	G	G	–	E	A	A	L	M	L	L/	
Human liver GST Ha[c]	A	E	K	P	K	L	H	Y	F	N	A	R	G	R	M	E	S	T	R	W	L/	
Human liver GST ψ[d]		P	M	I	L	G	Y	W	D	I	R	G	L	A	H	A	I	R	L	L	L	E/
Human placenta GST π[e]	P	P	Y	T	V	V	Y	F	P	V	R	G	R	C	A	A	L	R	M	L	L	A/
Rat liver GST Yb_1[f]		P	M	I	L	G	Y	W	N	V	R	G	L	T	H	P	I	R	L	L	L	E/
Rat liver GST Yb_2[g]		P	M	T	L	G	Y	W	D	I	R	G	L	A	H	A	I	R	L	F/		
Rat liver GST Yb_3[h]		P	M	T	L	G	Y	W	D	I	R	G	L	A	H	A	I	R	L	L	L	E/
Rat placenta GST P[i]	P	P	Y	T	I	V	Y	F	P	V	R	G	R	C	E	A	T	R	M	L	L	A/

[a] For best alignment the starting positions have been shifted.
[b] Present study.
[c] Tu and Qian (36).
[d] Singh *et al.* (14).
[e] Dao *et al.* (23).
[f] Ding *et al.* (28).
[g] Ding *et al.* (29).
[h] Abramovitz and Listowsky (35).
[i] Knoll *et al.* (30).

isozymes of bovine lens were closely related proteins. However, despite the fact that their N-terminal region of 20 amino acids was identical, the two GST isozymes of lens had significant differences. Specifically, they differed in their observed p*I* values and peptide maps (Fig. 1). The HPLC profiles of tryptic digests of GST 7.4 and GST 5.6 of lens (Fig. 1) were similar to each other except that a peak with a retention time of 44.2 min was prominent in the profile of GST 7.4 which was practically absent in the HPLC profile of GST 5.6. These results suggested that GST 7.4 and GST 5.6 of lens were closely related yet distinct proteins.

The N-terminal region sequence analysis of GST 7.2 of cornea also yielded a single amino acid sequence which was significantly different from that of lens GST isozymes. GST 7.2 of cornea showed only about 29% sequence identity with bovine lens GST isozymes in this region. The N-terminal region amino acid sequence of GST 8.7 of cornea could not be determined probably due to the occurrence of a blocked N-terminal. During amino acid sequence analysis of GST 6.8 and GST 6.3 of retina, two amino acid sequences were observed for each of these isozymes. One sequence common to both retina isozymes was identical to those of bovine lens GSTs whereas the other was identical to that of cornea GST 7.2.

Structural Relatedness with Other Mammalian GST Isozymes

In order to examine the structural interrelationship of bovine ocular GST isozymes with other mammalian GST isozymes, the N-terminal regions of bovine ocular GST were compared with those of other reported GST isozymes (Table III). Both GST 7.4 and GST 5.6 isozymes of bovine lens had an identical N-terminal region of 20 residues which was also identical to GST ψ (14) and GST μ (26) of human liver. These isozymes had 80 and 89% identities at their N-terminal when compared with rat liver Yb_1 and Yb_2 subunits, respectively (27-29). GST 7.2 of bovine cornea, on the other hand, shared about 67% and 76% sequence identities at its N-terminal with that of GST π of human placenta (23) and GST P of rat placenta (30), respectively (Table III).

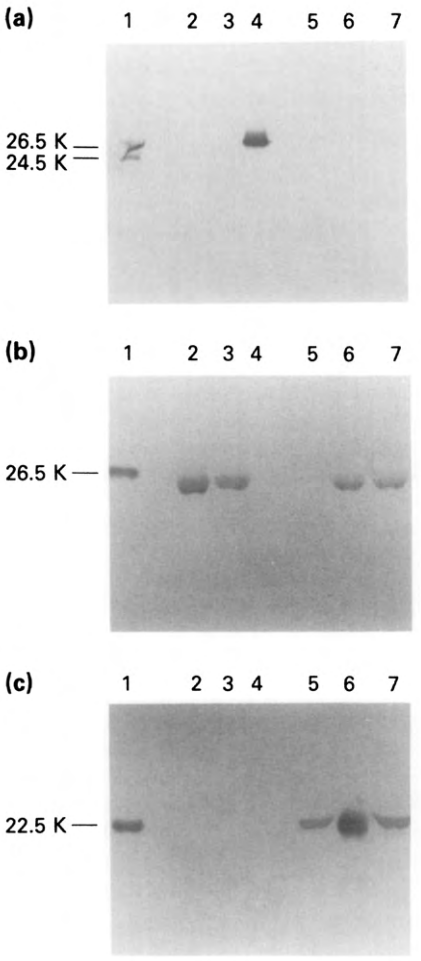

FIG. 2. Western blotting of apparently homogeneous GST isozymes of bovine ocular tissues using antibodies raised against (a) cationic GST α through ϵ of human liver; (b) GST ψ of human liver; and (c) GST π of human placenta. Every lane in all the three plates contained 5 μg of purified respective isozyme. (a) Lane 1, GST α through ϵ; lane 2, lens GST 7.4; lane 3, lens GST 5.6; lane 4, cornea GST 8.7; lane 5, cornea GST 7.2; lane 6, retina GST 6.8; and lane 7, retina GST 6.3. Lane 1 in b and c contained GST ψ of human liver and GST π of human placenta, respectively. Lanes 2 to 7 in plates (b) and (c) contained enzymes similar to those in a. The indicated molecular weight values in lane 1 of all three plates are for the corresponding positive control.

Immunological Relationship between Bovine Ocular GST and Human GST Isozymes

In Western blot analysis, the antibodies raised against the cationic GST α through ϵ of human liver (15) cross-reacted only with GST 8.7 of cornea (Fig. 2a; lane 4) indicating it to be immunologically related to the α class of human GST. The apparent molecular weights of the subunits of GST 8.7 of cornea were higher than that reported for the cationic GST isozymes of human liver (15). Antibodies raised against GST ψ of human liver cross-reacted with each of the isozymes of bovine

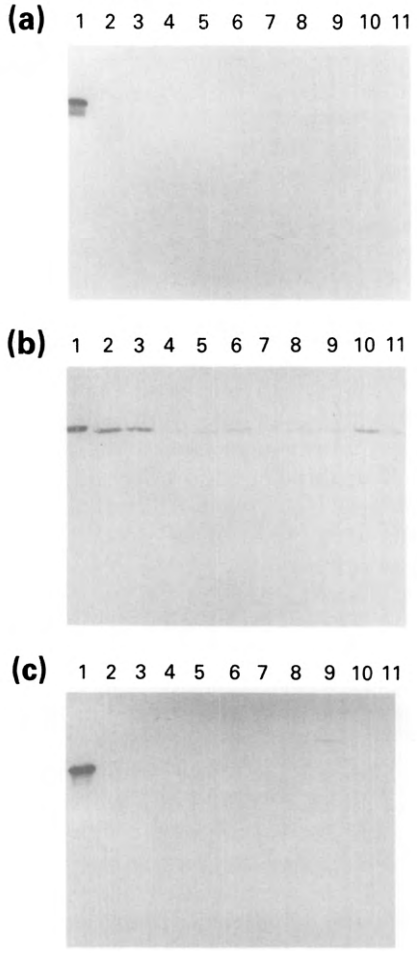

FIG. 3. Western blot analysis of GST in 14,000*g* supernatants of 10% (w/v) homogenates of lenses from 10 different cows using antibodies raised against (a) GST α through ϵ; (b), GST ψ and; (c) GST π. Lane 1 in a, b, and c contained 5 μg of homogeneous preparations of GST α through ϵ, GST ψ, and GST π, respectively. Lanes 2–11 in all three plates contained aliquots of supernatants (20 μg of protein) of lenses from 10 different animals.

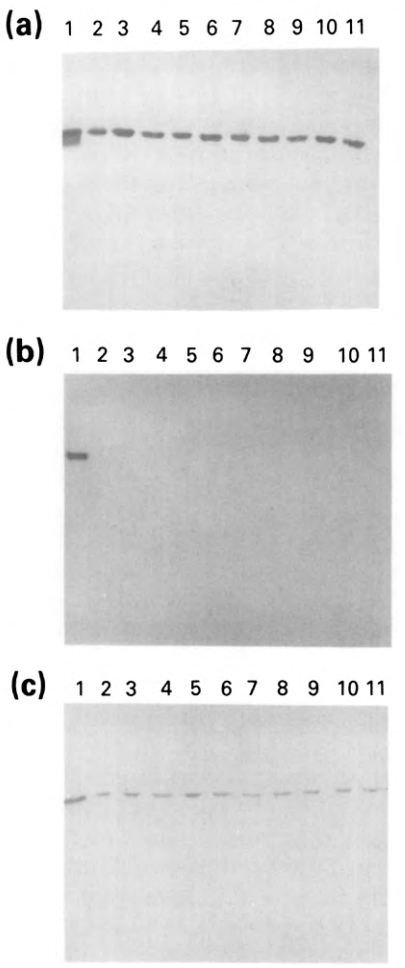

FIG. 4. Western blot analysis of GST in 14,000*g* supernatants (20 μg of protein) of 10% (w/v) homogenates of cornea from 10 different cows using antibodies raised against (a) GST α through ε; (b) GST ψ; and (c) GST π. Lane 1 in all three plates contained samples similar to those described in Fig. 3.

lens and retina (Fig. 2b) but did not cross-react with either of the cornea isozymes. But the apparent molecular weights of the subunits (M_r 23,500) of lens and retina enzymes cross-reacting with these antibodies was lower than that reported (14, 15) for GST ψ (M_r 26,500). The antibodies against GST π of human placenta cross-reacted with GST 7.2 of cornea (Fig. 2c; lane 5) as well as GST 6.8 and GST 6.3 of retina (Fig. 2c; lanes 6 and 7) but did not cross-react with the remaining isozymes. The molecular weights of the subunits cross-reacting with these antibodies were, however, higher (M_r 24,500) than that reported (23) for GST π of human placenta (M_r 22,500). These results were consistent with the amino acid sequence analysis and indicated that bovine ocular GST isozymes may have significant structural similarities with corresponding human GST.

Expression of GST Isozymes in Lens, Cornea, and Retina

Since immunological and structural studies indicated that all three major

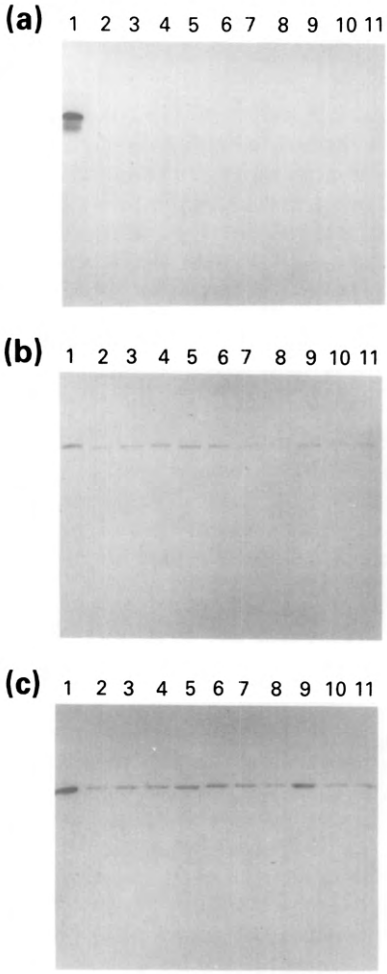

FIG. 5. Western blot analysis of GST in 14,000*g* supernatants (20 μg of protein) of 10% (w/v) homogenates of retina from 10 different cattle using antibodies raised against (a) GST α through ε; (b) GST ψ; and (c) GST π. Lane 1 in all three plates contained samples similar to those described in Figs. 3 and 4.

TABLE IV

OCCURRENCE OF GST ISOZYMES IN BOVINE OCULAR TISSUES CORRESPONDING TO α, μ, AND π CLASSES OF HUMAN GST[a]

	Expression of GST isozymes corresponding to human GST		
Tissue	α class	μ class	π class
Lens	−	+	−
Cornea	+	−	+
Retina	−	+	+

[a] The data in this table are based on amino acid sequence analysis and immunological characterization.

classes (α, μ, and π) of GST isozymes were present in bovine ocular tissues, we examined the expression of these isozymes in lens, cornea, and retina in 10 individual animals. Immunoblotting studies indicated that the lenses from all 10 animals expressed only the μ class subunits (Fig. 3b). On the other hand, in all 10 animals, cornea expressed both α and π classes (Figs. 4a and 4c) and retina expressed μ and π classes (Figs. 5b and 5c) of GST subunits. The expression of the μ type subunits in lenses of all 10 animals used in this study was interesting because in the human population μ class subunits are expressed only in approximately 60% of the subjects (4). These results taken together with the structural and immunological data presented earlier in this section indicated that the expression of the different classes of GST subunits in bovine eye was tissue specific and unlike human liver the μ locus (GST1 locus) did not exhibit polymorphism in the 10 animals studied.

DISCUSSION

This study documents the occurrence of several structurally and immunologically distinct subunits among bovine ocular GST isozymes. In this respect bovine ocular isozymes resemble other mammalian GST isozymes (4, 5). Based on structural and functional characteristics, human and other mammalian GST isozymes have been categorized into three major classes designated as α, μ, and π (31). The results presented herein demonstrate that the GST isozymes of bovine eye have significant primary structure homology at their N-termini with human GSTs and that the isozymes corresponding to all three major classes of GST are differentially expressed in bovine ocular tissues (Table IV). In lens only the μ class of isozymes was expressed (Table IV and Fig. 3); however, in cornea this class of isozyme was missing (Fig. 4). In retina, the μ and π classes of isozymes were expressed and the α class was absent (Table IV, Fig. 5). It had been reported that the μ class isozymes in human liver are products of a polymorphic GST1 locus (32) and that this class of isozyme is expressed only in about 60% population (4) due to the presence of a null allele at this locus (32). All 10 cows examined in this study expressed the μ type subunit in their lens and no evidence of variable expression of this subunit was indicated. However, this sample size was not significantly large. Also, the species of cow was unknown.

A number of recent studies suggest tissue-specific expression of GST isozymes in rats and humans (33–35). The results of the present study provides definitive evidence for tissue-specific expression of GST isozymes in bovine ocular tissues. The tissue-specific expression of GST isozymes in bovine ocular tissues might have evolved to meet the special detoxification needs of ocular tissue. For example, the isozymes corresponding to the α class of human GST have been implicated to be important in the protection of tissues from oxidative damage through GSH peroxidase II activity (4, 15). The expression of an isozyme corresponding to this class only in cornea may be important in the protection of this tissue from oxidative damage due to frequent ultraviolet irradiation from the sun.

Individually, each of the isozymes characterized in this study appeared to be distinct proteins sharing varying degrees of structural relatedness among each other. The N-terminal sequence of both the lens isozymes was identical to that of the μ class of human GST. However, both these isozymes were structurally distinct from each other as evidence by peptide finger-

print analysis and a marked difference in their p*I* values. The major isozyme of cornea (p*I* 7.2) had a significant N-terminal region sequence homology and immunological identity with the π class of human and rat GST (Table III). It was interesting to note that unlike the human π class isozymes, which are anionic (4, 15, 17, 23), this isozyme was cationic. The other isozyme of cornea (GST 8.7) was also cationic but probably belongs to the α class because it had a blocked N-terminal (same as the α class GST of human liver) (36) and was immunologically similar to cationic GST of human liver. Both retina isozymes, GST 6.8 and GST 6.3, were heterodimers and cross-reacted with the antibodies against the μ as well as the π class of GST isozymes, indicating the occurrence of both these types of subunits. These results were in agreement with amino acid sequence analysis because both retina isozymes exhibit two sequences, one identical to lens GSTs and the other identical to that of GST 7.2 of cornea. It has been proposed by some investigators that subunits of two different classes do not hybridize (31). On the contrary, in other reported studies such cross-hybridization has been shown and the presence of anionic isozymes which are heterodimers of α and π class subunits has been demonstrated in human liver (15, 37), brain (38), and cornea (39). The results of the present studies provide added support to the contention that hybridization of subunits of different classes of GST is a likely occurrence. Since considerable structural homologies exist among the various subunits it is conceivable that hybridization may occur between some of the subunits belonging to different classes.

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