Purification and properties of anionic glutathione *S*-transferase from bovine ciliary body

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An anionic glutathione *S*-transferase was purified from bovine ciliary body by DEAE-agarose chromatography and affinity chromatography on GSH-agarose and Orange A. The enzyme accounts for about 25% of total soluble glutathione *S*-transferase activity of the tissue. The purified enzyme has a molecular mass of about 50000 Da and is composed of two identical subunits of about 25000 Da. The enzyme has a pI of 5.8. The enzyme conjugates GSH with 1-chloro-2,4-dinitrobenzene, *p*-nitrobenzyl chloride, 1,2-epoxy-3-(*p*-nitrophenyl)propane, 1,2-dinitrobenzene and 3,4-dinitrobenzoic acid. The K_m values for 1-chloro-2,4-dinitrobenzene and GSH are 0.40 mM and 0.57 mM respectively. Haematin is a non-competitive inhibitor ($K_i = 4.5 \mu\text{M}$) when tested with various concentrations of 1-chloro-2,4-dinitrobenzene. The enzyme shows no glutathione peroxidase activity with either H_2O_2 or cumene peroxide as substrate. On the basis of substrate specificities, pI values, amino acid composition and peptide maps, it is concluded that the ciliary-body enzyme is probably identical with the anionic form of glutathione *S*-transferase from bovine lens and liver.

INTRODUCTION

Glutathione *S*-transferase (EC 2.5.1.18) represents a group of enzymes which catalyses the conjugation of GSH with a variety of organic compounds containing electrophilic centres (Chasseaud, 1976; Jakoby & Habig, 1980). The enzymes also bind various organic anions. Since the conjugation reaction detoxifies many xenobiotic compounds, glutathione *S*-transferase protects cellular constituents from the toxic effects of these chemicals. The glutathione *S*-transferase activity in the mammalian liver, especially in rat liver, has been investigated extensively, owing to its abundance and role of detoxification in that organ. However, the activity is not limited to the liver; it is widely distributed in non-hepatic tissues of mammals, including the eye (Shichi & Nebert, 1980) and also in a variety of species such as plants, insects and bacteria (Jakoby & Habig, 1980). More recent work shows that, in addition to its detoxification function, glutathione *S*-transferase has a role in the biosynthesis of leukotriene C_4 (Samuelsson, 1983; Bach *et al.*, 1984; Söderström *et al.*, 1985).

In previous studies we have demonstrated that drug-metabolizing and detoxifying activities in the bovine eye are highest in the uveal tissues, especially in the ciliary body (Das & Shichi, 1981; Shichi & Nebert, 1982). We have also shown the presence in the ciliary body of glutathione *S*-transferase and other enzymes involved in mercapturate synthesis (Das & Shichi, 1981). The principal function of the ciliary body is to produce the aqueous humour, which provides nutrients for the lens and other anterior tissues, and glutathione *S*-transferase activity and other detoxification enzymes are believed to play important roles in the detoxification of the aqueous humour (Das & Shichi, 1981). The enzyme may also be involved in leukotriene biosynthesis by anterior uveal tissues, because these tissues are capable of synthesizing lipoygenase products from arachidonic acid (Kulkarni *et al.*, 1984). Bovine ciliary body contains about

0.6 mM-GSH (Kishida *et al.*, 1985), a sufficiently high concentration for the compound to be involved in detoxification and leukotriene synthesis. We have therefore attempted to purify and characterize glutathione *S*-transferase in the ciliary body. We report here that bovine ciliary body contains an anionic isoenzyme of glutathione *S*-transferase which is probably identical with an anionic isoenzyme previously isolated from bovine lens (Saneto *et al.*, 1980) and liver (Asaoka, 1984).

EXPERIMENTAL

Materials

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.): acetaminophen, acetazolamide, acetylsalicylate, bilirubin, caffeine, 1-chloro-2,4-dinitrobenzene, cumene peroxide, dexamethasone, GSH, haematin, H_2O_2 , mercaptosuccinate, *p*-nitrobenzyl chloride, pilocarpin, prednisone and protoporphyrin IX. 1,2-Epoxy-3-(*p*-nitrophenyl)propane was purchased from Eastman Kodak (Rochester, NY, U.S.A.) and 1-menaphthyl sulphate was from Calbiochem (San Diego, CA, U.S.A.). The following reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.): 1,2-dinitrobenzene, 3,4-dinitrobenzoic acid, 5-nitro-2-furaldehyde, 5-nitro-2-furaldehyde diacetate and 2,3,5,6-tetrachloronitrobenzene. Acrylamide, DEAE-agarose and SDS were obtained from Bio-Rad (Richmond, CA, U.S.A.), and Sephadex G-100 was from Pharmacia Fine Chemicals (Uppsala, Sweden). Fresh bovine eyes were obtained from Monarch Meat Packing Co. (Detroit, MI, U.S.A.).

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Fresh bovine eyes were brought to the laboratory on ice, and ciliary bodies were collected within 2 h after slaughter of the animals, frozen and stored at -20°C .

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Frozen bovine ciliary bodies (330; about 130 g wet wt.) were homogenized in 10 mM-Tris/HCl, pH 7.4 (360 ml), at 3–10 °C with a Kelmar Tissuemizer. The homogenate was centrifuged at 26000 *g* for 30 min. The supernatant was kept and centrifuged at 100000 *g* for 90 min. The supernatant (430 ml) was filtered through a Whatman cellulose filter and loaded on a DEAE-agarose column (2.6 cm × 80 cm); 1 ml fractions were collected at a flow rate of 20 ml/h. After washing of the column with 10 mM-Tris/HCl buffer, pH 7.4, the column was eluted stepwise with 0.10 M-, 0.20 M- and 0.40 M-NaCl in Tris buffer. The fractions eluted with 0.10 M-NaCl (i.e. fractions containing an anionic glutathione *S*-transferase) were dialysed against 22 mM-potassium phosphate, pH 7.0, containing 0.02% NaN₃. The dialysed sample was then placed on a GSH-agarose (Pierce Chemicals, Rockford, IL, U.S.A.) column (0.9 cm × 20 cm). After removal of non-adherent protein by washing with 50 mM-Tris/HCl, pH 9.6, the column was eluted with 5 mM-GSH to collect glutathione *S*-transferase activity. Fractions (1 ml) were collected, pooled, and concentrated with CF-25 ultrafiltration cones (Amicon, Lexington, MA, U.S.A.). The enzyme was further purified by affinity chromatography on an Orange A (Amicon) column (2 cm × 10 cm) as described by Asaoka (1984). The concentrated sample was placed on an Orange A column which had been pre-equilibrated with 0.01 M-sodium phosphate buffer, pH 6.0. The column was washed with the same buffer until non-adherent protein was completely removed, and then eluted with 50 ml of 0.05 M-sodium phosphate buffer, pH 7.0, containing 1 mM-GSH; 1 ml fractions were collected. Bovine lens anionic enzyme was purified as described by Saneto *et al.* (1980).

Determination of M_r

For determination of the M_r of holoenzyme, a purified and concentrated sample was loaded on a precalibrated Sephadex G-100 column (1.6 cm × 180 cm) and eluted with 50 mM-Tris/HCl, pH 7.5, at a flow rate of 10 ml/h; 1 ml fractions were collected. Markers used for calibration of the column were Blue Dextran (for determination of V_0), bovine serum albumin, ovalbumin, carbonic anhydrase and cytochrome *c*, all purchased from Bio-Rad. Disc-gel electrophoresis was performed in 12% polyacrylamide gel containing 0.1% SDS as described by Fairbanks *et al.* (1971). The gel was stained with Coomassie Blue. For SDS/polyacrylamide-gel electrophoresis a mixture of molecular marker proteins containing phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soya-bean trypsin inhibitor and lysozyme was purchased from Bio-Rad.

Isoelectric focusing

The purified and concentrated enzyme preparation was electrofocused in a polyacrylamide gel in Ampholyte (Pharmacia) in the pH range 4–6.5, in Bio-Rad gel tubes (5.5 mm × 125 mm). The sample in 50% sucrose (150 µl) was loaded on the alkaline end of the gel (0.02 M-NaOH). The acidic side of the gel was immersed in 0.01 M-H₃PO₄. After pre-electrophoresis at 0.16 W/tube for 1 h, the gel was subjected to electrophoresis at 0.33 W/tube for 4 h at 10 °C. The gel was sliced into discs (thickness 0.40 cm), and each disc was extracted with deionized water (0.5 ml) overnight at 3 °C. The enzyme activity of extract was assayed.

Assay of enzyme activity

Glutathione *S*-transferase activity was assayed as described by Habig *et al.* (1974), with 1-chloro-2,4-dinitrobenzene as substrate. The standard assay mixture consisted of 2 mM-1-chloro-2,4 dinitrobenzene in 0.10 M-potassium phosphate, pH 7.0 (0.5 ml), 2 mM-GSH in phosphate buffer (0.5 ml) and enzyme (0.05 ml). The reaction was followed by observing the increase in A_{340} with a Cary 17 recording spectrophotometer. The concentration of 1-chloro-2,4 dinitrobenzene was determined spectrally as follows. The substrate (0.25 ml) was mixed with 1 M-NaOH (25 ml), incubated for 1 h at 60 °C, and the A_{360} of 2,4-dinitrophenol produced was determined. The concentration of dinitrophenol was calculated from $\epsilon_{360} = 14.4 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$. GSH was slowly oxidized by air at pH 7.0; therefore a fresh GSH solution was prepared every 20 min in buffer that had been deoxygenated by bubbling prepurified-grade N₂ (Linde specialty gases; Union Carbide, Danbury, CT, U.S.A.). Protein was determined with Folin phenol reagent, with bovine serum albumin as standard (Lowry *et al.*, 1951). One unit of enzyme activity was defined as the amount of enzyme that catalyses conjugation of 1 µmol of substrate/min at 20 °C. The specific activity was expressed as units of activity per mg of protein. Compounds tested as inhibitors were dissolved in ethanol and added to the reaction mixture at ethanol concentrations not exceeding 12%. Bilirubin and protoporphyrin IX were dissolved in 10% chloroform in ethanol. Haem concentration was determined as described by Shichi & Hackett (1962). The effects of these compounds on enzyme activity were tested at different concentrations of 1-chloro-2,4-dinitrobenzene, and the results were analysed by the Lineweaver–Burk double-reciprocal plot. Leukotriene C₄ synthetase activity was assayed as described by Bach *et al.* (1984). The reaction mixture (0.6 ml) contained 5 µmol of Tris, 0.02% NaN₃, 0.3 µmol of Hepes, 0.03 µmol of EDTA, 0.6 mg of bovine serum albumin, 3 µmol of GSH and 5 nmol of leukotriene A₄.

Amino acid analysis and peptide mapping

Purified enzyme protein (0.1–0.2 mg) was hydrolysed *in vacuo* at 115 °C for 24, 48 and 72 h with 1 ml of 4 M-methanesulphonic acid containing 0.2% 3-(2-aminoethyl)indole (Pierce Chemicals) as described by Simpson *et al.* (1976). The amino acids were analysed on a Beckman automatic amino acid analyser, model 121.

Peptide mapping was carried out essentially by the method described by Bennett (1967), except that cellulose-coated plastic plates (Kodak Chromagram 13255) were used. Enzyme protein was dialysed against 0.2 M-NH₄HCO₃, pH 8.0, and digested with 1% (final concn.) of tosylphenylalanylchloromethane-treated trypsin (Sigma) for 2 h at 37 °C. More trypsin (to 2%) was added, and the mixture incubated for 2 h. Finally, the trypsin concentration was raised to 3% and the reaction mixture was incubated overnight at 37 °C. At the end of incubation, a sufficient amount (i.e. a little more than the amount of trypsin used) of soya-bean trypsin inhibitor (Sigma) was added to stop the reaction. Tryptic digests were freeze-dried, taken up in a small volume of water and applied at the origin and subjected to electrophoresis at 500 V for 40 min in acetic acid/formic acid/water (3:1:16, by vol.; pH 1.5) and to chromatographic separation in butan-1-ol/acetic acid/pyridine/water

Table 1. Glutathione *S*-transferase activity of bovine ocular tissues

	Specific activity (nmol of product/min per mg of protein)	Relative activity
Cornea	179	40
Iris	413	91
Ciliary body	454	100
Lens	48	11
Retina	124	27
Pigmented epithelium-choroid	122	27

(13:2:10:8, by vol.). The peptide spots were detected with 0.1% ninhydrin in acetone.

RESULTS

Glutathione-*S*-transferase activity of bovine ocular tissues

Glutathione *S*-transferase activities of different ocular-tissue homogenates were compared (Table 1). On the basis of specific activity, the ciliary body showed the highest activity, followed by the iris. The pigmented epithelium-choroid showed a lower activity than these tissues. The order of activities among the three uveal

tissues did not change when total activities were compared; ciliary body (23 μ mol of product/min), iris (7 μ mol of product/min) and pigmented epithelium-choroid (6 μ mol of product/min). Of all ocular tissues, the ciliary body was previously found to demonstrate highest activities of aryl hydrocarbon hydroxylase and UDP-glucuronosyltransferase (Das & Shichi, 1981).

Purification of anionic glutathione *S*-transferase from bovine ciliary body

When soluble extracts of bovine ciliary-body homogenate were chromatographed on a DEAE-agarose column and the column was washed with 10 mM-Tris

Fig. 1. DEAE-agarose chromatography of ciliary-body glutathione *S*-transferase

Washing of the column with 10 mM-Tris/HCl began at the first arrow. Stepwise elution with 0.10 M-, 0.20 M- and 0.40 M-NaCl was effected at points shown by the respective arrows. ●, A_{280} ; ○, enzyme activity.

Table 2. Purification of anionic *S*-transferase from bovine ciliary body

Procedure	Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
Tris buffer (10 mM, pH 7.4) extraction	860	2546	135	0.142	100
DEAE-agarose, elution with 0.1 M-NaCl	220	218	39	0.180	29
GSH-agarose	8	4.1	13.5	3.30	10
Orange A	4	1.8	12	6.60	8.9

Fig. 2. Determination of molecular mass

SDS/polyacrylamide-gel electrophoresis of purified enzyme is shown at the top. The molecular mass of subunits (○) was estimated by using the following marker proteins: Ph, phosphorylase *b* (92 500 Da); BSA, bovine serum albumin (66 200 Da); Ov, ovalbumin (45 000 Da); CA, carbonic anhydrase (31 000 Da); TI, soya-bean trypsin inhibitor (21 500 Da); Ly, lysozyme (14 400 Da).

buffer, cationic glutathione *S*-transferase isoenzymes were collected in the non-adherent fractions (Fig. 1). The major anionic isoenzyme was eluted from the column with 0.1 M-NaCl. Subsequent elution of the column with 0.2 M-NaCl separated the minor anionic isoenzyme. From the integrated area of each activity peak, the anionic isoenzyme (i.e. adherent fraction) was estimated to account for about 25% of total soluble glutathione *S*-transferase activity. The major anionic enzyme was then loaded on a glutathione-agarose column and eluted

with GSH (chromatogram not shown). The specific activity of the enzyme was markedly increased by the affinity-chromatography procedure (Table 2). The enzyme thus purified contained a protein of molecular mass about 25 000 Da and an additional protein of molecular mass about 24 000 Da as examined by SDS/polyacrylamide-gel electrophoresis. We thought at this point that the enzyme was composed of two subunits with slightly different molecular masses. However, the 24 000-Da protein could be removed after further purification by affinity chromatography on Orange A. Purification of the anionic isoenzyme is summarized in Table 2.

Properties of purified anionic *S*-transferase

The anionic isoenzyme purified to homogeneity by affinity chromatography on Orange A was found to migrate as a single band in SDS/polyacrylamide-gel electrophoresis (Fig. 2). A comparison of the electrophoretic mobility of the subunit with proteins of known molecular mass indicated that the subunit has a molecular mass of about 25 000 Da. The molecular mass of holoenzyme was estimated to be about 50 000 Da on a precalibrated Sephadex G-100 column. Therefore the anionic isoenzyme consists of two subunits of the same molecular mass.

The pI of the purified anionic enzyme was determined to be about 5.8 by electrofocusing in polyacrylamide gel. Two independent measurements gave essentially identical results.

The anionic enzyme was active with the following substrates: 1-chloro-2,4-dinitrobenzene (6.6 $\mu\text{mol/min}$ per mg of protein), *p*-nitrobenzyl chloride (0.11 $\mu\text{mol/min}$ per mg of protein), 1,2-epoxy-3-(*p*-nitrophenyl)-propane (0.11 $\mu\text{mol/min}$ per mg of protein), 1,2-dinitrobenzene (0.05 $\mu\text{mol/min}$ per mg of protein) and 3,4-dinitrobenzoic acid (0.15 $\mu\text{mol/min}$ per mg of protein). Little or no activity was detected with 1,2-dichloro-4-nitrobenzene, 1-menaphthyl sulphate, 2,3,5,6-tetrachloronitrobenzene, 5-nitro-2-furaldehyde and 5-nitro-2-furaldehyde diacetate. The estimated values of K_m (mM) and V_{max} [in mol/min per mol of enzyme (50 000 Da)] were 0.40 and 560 for 1-chloro-2,4-dinitrobenzene, and 0.57 and 340 for GSH, respectively. The K_m for 1-chloro-2,4-dinitrobenzene was determined at 5 mM-GSH at various concentrations of chlorodinitrobenzene. On the other hand, the K_m for GSH was determined at 2 mM-chlorodinitrobenzene because of limited solubility of the aromatic substrate in aqueous medium. It is therefore possible that the enzyme was not fully saturated by this substrate at the concentration. This may explain the slightly lower V_{max} value with 2 mM-chlorodinitrobenzene than the V_{max} with 5 mM-GSH.

Fig. 3. Effects of haematin, protoporphyrin IX and bilirubin on the activity of anionic glutathione *S*-transferase

○, experiments without inhibitors; ■, ▲, experiments with inhibitors. Two concentrations (7.5 μM and 14.6 μM) of haematin were tested. Protoporphyrin IX was at 5 μM and bilirubin at 37 μM . GSH concentration was maintained at 2 mM, and that of 1-chloro-2,4-dinitrobenzene was varied from 1 to 0.1 mM in the presence or absence of inhibitor.

The enzyme did not show glutathione peroxidase activity with either H_2O_2 or cumene peroxide. Mercapto-succinate, a potent inhibitor of selenium-dependent glutathione peroxidase (Chaudiere *et al.*, 1984), had no effect on the activity of anionic glutathione *S*-transferase. Prednisone, dexamethasone, pilocarpine, acetazolamide, acetaminophen, acetylsalicylate and caffeine (all at 2 mM) were without effect. Haematin was found to be a non-competitive inhibitor of the enzyme (Fig. 3); the K_i value was estimated to be 4.5 μM . Neither protoporphyrin IX nor bilirubin seemed to have any effect on the enzyme (Fig. 3). The K_i of 4.5 μM is one order of magnitude higher than the K_i for the competitive inhibition by haematin of human erythrocyte glutathione *S*-transferase (Harvey & Beutler, 1982). Rat liver basic *S*-transferases are known to bind haematin with different affinities (Sugiyama *et al.*, 1984).

As analysed by radioimmunoassay, the purified enzyme did not show any appreciable activity of converting leukotriene A₄ into leukotriene C₄.

Amino acid composition and peptide map

The amino acid composition of the anionic glutathione *S*-transferase is shown in Table 3. For comparison with the ciliary-body enzyme, the amino acid composition of the anionic form of glutathione *S*-transferase from bovine lens and liver is also included in Table 3. The total 'mol %' value is not 100, because the values for the individual amino acids were rounded. It is evident that the anionic enzymes from the three different tissues are very similar in amino acid composition. To evaluate whether the similarities of two proteins (having the same number of residues, N), are statistically significant, Cornish-Bowden (1981) proposed a difference index ($S\Delta n$), given by $S\Delta n = \frac{1}{2}\sum(n_{iA} - n_{iB})^2$, where n_{iA} is the number of residues of the i th type in protein A and n_{iB} is the corresponding number in protein B. Any pair of proteins can be

Table 3. Comparison of the amino acid composition of anionic glutathione *S*-transferases from bovine ciliary body, lens and liver

Values for ciliary body are means of three independent analyses (three hydrolysis times for each analysis). Data on lens enzyme were calculated from Saneto *et al.* (1980) and data on liver enzyme from Asaoka (1984).

Amino acid	Content (mol %)		
	Ciliary body	Lens	Liver
Asp	10.3	10.8	10.6
Thr	4.0	2.6	3.0
Ser	4.8	3.2	5.7
Pro	5.4	5.8	4.6
Gly	8.5	8.7	7.3
Ala	5.3	4.7	5.5
Cys	1.6	3.0	0.4
Val	4.0	3.5	3.7
Met	2.7	3.0	2.2
Ile	5.7	5.5	6.0
Leu	10.9	11.5	12.1
Glu	10.6	11.9	10.2
Tyr	3.2	3.2	3.5
Phe	6.8	5.6	7.9
Lys	7.6	7.2	8.1
His	1.7	1.8	1.9
Arg	5.3	5.5	5.7
Trp	1.2	1.7	0.8
Total	99.6	99.9	99.2

classified as related if $S\Delta n < 0.42N$, inconclusive if $0.42N < S\Delta n < 0.93N$, and probably unrelated if $S\Delta n > 0.93N$. Assuming $N = 440$, the similarities between ciliary-body enzyme and lens enzyme and between ciliary-body enzyme and liver enzyme were estimated

from the above equation. $S\Delta n = 90$ for comparison between ciliary-body enzyme and lens enzyme, and $S\Delta n = 80$ for comparison between ciliary-body enzyme and liver enzyme. $0.42N$ was calculated to be 185. The $S\Delta n$ values are considerably smaller than $0.42N$. The similarities among the three enzymes are therefore statistically highly significant.

The peptide maps prepared by electrophoresis and chromatography of tryptic digests of ciliary-body and lens enzymes are presented in Fig. 4. On the basis of 11 peptides clearly separated and identified, the two enzymes are considered to be very similar. In addition, six barely detectable peptide spots (indicated by dotted circles in Fig. 4) appeared in the peptide map of the lens enzyme. Three barely detectable spots corresponded to those which appeared in the peptide map of the ciliary-body enzyme. One peptide in the map of the lens enzyme was intensely stained by ninhydrin. We suspect that the spot might be attributed to enzyme-bound GSH; free GSH migrates to this region in the peptide-mapping system. Control (no protein) treated likewise with trypsin and trypsin inhibitor did not yield peptide spots detectable by the method. The 'mol %' values indicate that the enzyme contains about 25 residues of lysine and arginine combined per subunit. Nevertheless, no more than

two-thirds of the peptides that should have been produced were detected in the map. The reason for this is not known. It is possible that the enzyme protein was not completely digested by trypsin even after overnight incubation. It is also possible that some of the peptides were electrophoresed out toward the anode.

DISCUSSION

Cationic isoenzymes of rat liver glutathione *S*-transferase (i.e. those with alkaline pI) have been separated on the cationic exchanger CM-cellulose (Habig *et al.*, 1974). More recently, an increasing number of papers have reported the isolation and characterization of anionic isoenzymes (i.e. those with acidic pI), which bind to the anionic exchanger DEAE-cellulose (Awasthi *et al.*, 1980; Friedberg *et al.*, 1983; Guthenberg *et al.*, 1979, 1983; Marcus *et al.*, 1978; Maruyama *et al.*, 1983; Partridge *et al.*, 1984, 1985; Reddy *et al.*, 1983; Saneto *et al.*, 1980, 1982; Warholm *et al.*, 1983). The major anionic glutathione *S*-transferase of bovine ciliary body was purified and characterized in the present work. On the basis of molecular mass, the ciliary-body enzyme is similar to one of the rat liver enzymes (Reddy *et al.*, 1983) and human liver enzyme μ (Warholm *et al.*, 1983), but these enzymes are different from the ciliary-body enzyme in their substrate specificities and pI values. Rat testis enzyme (Guthenberg *et al.*, 1983) and human placenta enzyme (Guthenberg *et al.*, 1979) have a pI (5.9) close to that of the ciliary-body enzyme, but are much more active toward 1-chloro-2,4-dinitrobenzene than the ciliary enzyme. Human retina enzyme (pI 4.5) (Singh *et al.*, 1984) and human cornea enzyme (pI 4.4) (Singh *et al.*, 1985) are more anionic than bovine ciliary-body enzyme. Several glutathione *S*-transferase isoenzymes have been isolated from bovine tissues. Bovine retina contains two anionic isoenzymes (Saneto *et al.*, 1982). The more anionic form (pI 6.34) is similar to the ciliary-body enzyme in molecular mass, but is different in having glutathione peroxidase activity. Saneto *et al.* (1980) isolated from bovine lens an anionic isoenzyme (pI 5.6) which has no glutathione peroxidase activity. More recently, Asaoka (1984) purified an anionic isoenzyme (pI 6.1) from bovine liver. We have found in the present study that the three isoenzymes are very similar in amino acid composition. The peptide maps prepared from tryptic digests of lens enzyme and ciliary-body enzyme are essentially identical. The three isoenzymes show very similar substrate specificities and molecular masses. Saneto *et al.* (1980) describe that antisera raised against the bovine lens anionic isoenzyme cross-reacts with the liver anionic isoenzyme. On the basis of these findings we conclude that the anionic isoenzymes isolated from bovine liver, lens and ciliary body are probably identical. A new nomenclature system has been proposed for glutathione *S*-transferase isoenzymes mainly based on the properties of rat isoenzymes (Jakoby *et al.*, 1984). Although species are different, according to the nomenclature, the anionic isoenzyme purified from bovine ciliary body in the present work has 1-1 subunit composition on the basis of molecular mass and is similar to a rat isoenzyme.

Fig. 4. Peptide maps of tryptic digests of ciliary-body enzyme (a) and lens enzyme (b)

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