S-(1,2-Dicarboxyethyl)glutathione and Glutathione in Lens and Liver of Naphthalene-Treated Rabbits

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Summary: The determination of S-(1,2-dicarboxyethyl)glutathione and reduced glutathione (GSH) in the rabbit lens and liver was developed using an isotachophoretic analyser.

The recovery of S-(1,2-dicarboxyethyl)GSH from the rabbit liver after ion-exchange treatment was $96.8 \pm 11.3\%$ ($n = 3$). The contents of S-(1,2-dicarboxyethyl)GSH in the rabbit lens and liver were 219.9 ± 29.1 ($n = 5$) and 44.0 ± 13.5 ($n = 8$) nmol/g, respectively.

The contents of S-(1,2-dicarboxyethyl)GSH in the lens and GSH in the lens and liver of naphthalene-treated rabbits was also determined by this method 24 hours after naphthalene administration, at which time the axial opacity "spichen" was observed at the equatorial region of the lens. The content of S-(1,2-dicarboxyethyl)GSH in the lens decreased in proportion to the content of GSH.

During the further development of true lens opacity after naphthalene administration, the S-(1,2-dicarboxyethyl)GSH content further compared with that in the spichen stage, but the S-(1,2-dicarboxyethyl)GSH content of the lens that did not develop true opacity after naphthalene administration returned to the normal level. The change of S-(1,2-dicarboxyethyl)GSH content of the lens in the spichen and true opacity stages coincided with that of GSH content.

On the other hand, the content of GSH of the liver decreased markedly until 24 hours after naphthalene administration, then returned to normal, irrespective of whether true opacity did or did not subsequently develop.

Introduction

In 1963, S-(1,2-dicarboxyethyl)glutathione and S-(1,2-dicarboxyethyl)-L-cysteine were isolated from calf lens. Their chemical structures were identified by comparison with the synthetic compounds, and it has been reported that S-(1,2-dicarboxyethyl)GSH was present at a higher concentration in the lens than in the other tissues of animals (1).

S-(1,2-dicarboxyethyl)-L-cysteine has also been identified in the urine and kidney of guinea pig (2), in ox brain and spinal cord (3) and in ox liver and kidney (4).

More recently, S-(1,2-dicarboxyethyl)GSH was determined by using high performance liquid chromatography (HPLC) after pre-column reaction with 2,4-dinitrofluorobenzene, and a decrease of S-(1,2-dicarboxyethyl)GSH in rat lens during galactose cataract formation has been reported (5).

However, the physiological significance and biochemical role of S-(1,2-dicarboxyethyl)GSH are still unknown.

Herein, we describe the determination of S-(1,2-dicarboxyethyl)GSH and reduced glutathione (GSH) using an

isotachophoretic analyser (6–9), and we establish the correlation between their concentrations in the lens and cataract formation in rabbits during naphthalene treatment.

Materials and Methods

Materials

Reduced glutathione (GSH) and glutathione disulfide (GSSG) were obtained from Sigma. S-(1,2-dicarboxyethyl)-L-cysteine was synthesized as reported previously (5). S-(1,2-dicarboxyethyl)glutathione was kindly supplied from Dr K. Ogata of the Senju Pharmaceutical Co. (Osaka). All other chemicals used were of analytical grade.

Tissue preparation

Male and female albino rabbits, weighing approximately 2 kg were used. Rabbits were killed by injecting air into an ear vein, and lens and liver were then removed, blotted, weighed, and used immediately.

Assay of S-(1,2-dicarboxyethyl)glutathione

Lens (0.5 g) and liver (1.0 g) were homogenized in 3 ml of methanol-H₂O (70 : 30; by vol.) at 0 °C for 10 min, and centrifuged at 9000 g (lens) and 3000 g (liver) for 15 min.

Each supernatant was adjusted to pH 5.0 with 2 mol/l acetic acid, transferred to a column containing 5 ml of Amberlite IRA-68 (ac-

Fig. 1 Isotachopheric runs of authentic 10 nmol S-(1,2-dicarboxyethyl)-L-cysteine, S-(1,2-dicarboxyethyl)GSH, GSSG and GSH. Analytical conditions: the leading electrolyte was 0.01 mol/l hydrochloric acid and β -alanine pH 3.1 (a) and pH 2.8 (b). Terminal electrolyte was 0.01 mol/l caproic acid. For other conditions, see materials and methods.

tate form, 200–200 mesh), washed with 30 ml of 2 mol/l acetic acid and eluted with 50 ml of 1 mol/l hydrochloric acid. The eluate was evaporated under reduced pressure. The residue was dissolved in 2 ml of water, adjusted to pH 9.0 with 2 mol/l ammonia solution and transferred to a column containing 5 ml of Diaion SA (formic

form of sulphonated cation exchanger, mesh 100, Mitsubishi Kasei Co., Tokyo), washed with water and eluted with 30 ml of 100 g/l formic acid. The eluate was evaporated under reduced pressure. The dried residue was dissolved in 0.2 ml of water, and centrifuged at 5000 g for 5 min. Either 10 μ l or 20 μ l of the solution was directly injected onto the isotachopheric analyser.

Assay of glutathione

Lens (0.5 g) and liver (1.0 g) were homogenized and centrifuged as described above. Each supernatant was analysed directly on an isotachopheric analyser as reported previously (6).

Instrumentation

The capillary apparatus was a Shimadzu IP-3A isotachopheric analyser, Shimadzu, Seisakusho, Kyoto, Japan.

The separations were carried out in a capillary tube, 200 $\times$ 0.5 mm i.d., maintained at a constant temperature of 20 $^{\circ}$ C. The detector cell had an i.d. of 0.5 mm and length of 0.05 mm. The migration current was 100 μ A. The leading electrolyte consisted of 0.01 mol/l hydrochloric acid and β -alanine (pH 2.8). The terminating electrolyte was 0.01 mol/l caproic acid and chart speed was 10 or 20 mm/min.

Analysis of amino acids

Lenses from normal or naphthalene-treated rabbits were homogenized in four volumes of sulphosalicylic acid, and the homogenate centrifuged at 8000 g. The supernatant was applied to a column containing 5 ml of Diaion SA-100 (OH form), washed with water, and eluted with 2 mol/l hydrochloric acid.

The eluate was dried under reduced pressure, and the residue dissolved in 0.02 mol/l hydrochloric acid.

An aliquot of the solution was analysed using an amino acid analyser (Hitachi Model 835 liquid chromatograph).

Cataract formation

Male and female albino rabbits were divided into three groups, the normal group, the cataract developing group and the non-cataract developing group.

Fig. 2 Isotachopheric runs of a sample of rabbit lens (a) and the same sample fortified with authentic S-(1,2-dicarboxyethyl)GSH (10 nmol) (b).

Naphthalene (2.5 g/kg body weight) dissolved in 30 ml of liquid paraffin was administered orally to rabbits of each group on the first day or for three consecutive days.

Naphthalene cataracts were classified into the two stages of spichen and true opacity. The stage of spichen developed 24 hours after the first administration. It was characterized by the appearance of axially opacity "spichen" at the equatorial region of the lens.

The stage of true opacity developed a few weeks after the first administration.

The group that received only one administration did not develop to the stage of true opacity; this group is the non-cataract developing group. The group that developed to the stage of true opacity is the cataract developing group.

Results and Discussion

Figure 1a and 1b show the isotachopheretic runs of authentic S-(1,2-dicarboxyethyl)glutathione, S-(1,2-dicarboxyethyl)-L-cysteine, GSH and GSSG.

Under the conditions described in figure 1, GSH and GSSG were easily separated as reported previously (6), but the separation of S-(1,2-dicarboxyethyl)glutathione and S-(1,2-dicarboxyethyl)-L-cysteine was unsatisfactory, since these two compounds ran together as a mixed zone. They were separated, however, by changing the pH of the leading electrolyte from 3.1 to 2.8 as shown in figure 1b.

The calibration curves for different concentrations of S-(1,2-dicarboxyethyl)GSH and GSH showed linearity over the concentration ranges from 0 to 25 nmol. Figure 2a and 2b show the isotachopheretic runs corresponding to the sample of lens from normal rabbit and the same sample fortified with S-(1,2-dicarboxyethyl)GSH. The zone of S-(1,2-dicarboxyethyl)GSH in figure 2a was made to overlap by addition of authentic S-(1,2-dicarboxyethyl)GSH to the lens sample, resulting in an elon-

Fig. 3 Isotachopheretic runs of a sample of rabbit lens (a), a + S-(1,2-dicarboxyethyl)GSH (b), hydrolysate of a (c) and c + S-(1,2-dicarboxyethyl)-L-cysteine (d).

a

b

Fig. 4 Cataract at the spichen stage developed 24 hours after the first naphthalene administration (a) and cataract at the true

opacity stage developed a few weeks after naphthalene administration (b).

gation of the zone of S-(1,2-dicarboxyethyl)GSH in the lens sample as shown in figure 2b.

The lens sample containing S-(1,2-dicarboxyethyl)GSH was hydrolysed in 6 mol/l hydrochloric acid for 24 hours at 110 °C, the hydrolysate was evaporated under reduced pressure and the dried residue was injected onto the isotachophoretic analyser.

After hydrolysis, the zone of S-(1,2-dicarboxyethyl)GSH in the lens sample disappeared completely and was detected as a zone of S-(1,2-dicarboxyethyl)-L-cysteine (fig. 3c).

The zone of S-(1,2-dicarboxyethyl)-L-cysteine in figure 3c was made to overlap by addition of authentic S-(1,2-dicarboxyethyl)-L-cysteine to the hydrolysate of lens sample, resulting in an elongation of the zone of S-(1,2-dicarboxyethyl)-L-cysteine in the hydrolysate as shown in figure 3d.

These findings indicate that the zone of S-(1,2-dicarboxyethyl)GSH in the lens sample in a single zone, and the amount of S-(1,2-dicarboxyethyl)GSH in the lens sample can be determined by measuring the zone length. Various amounts of S-(1,2-dicarboxyethyl)GSH were added to

rabbit liver homogenate and their recoveries were measured in a separate run by the procedure described above. The recovery of S-(1,2-dicarboxyethyl)GSH in rabbit liver after cation exchange resin treatment was $96.8 \pm 11.3\%$ ($n = 3$). The contents of S-(1,2-dicarboxyethyl)GSH in normal rabbit lens and liver were 219.9 ± 29.1 ($n = 5$) and 44.0 ± 13.5 ($n = 8$) nmol/g, respectively.

The results of the determination of S-(1,2-dicarboxyethyl)GSH in normal rabbit lens and liver reported in this paper are in good agreement with an earlier report (5). The findings described above demonstrate that the method for measurement of S-(1,2-dicarboxyethyl)GSH using an isotachophoretic analyser was satisfactory. This isotachophoretic method offers several advantages over previously described techniques.

We studied the changes in the levels of S-(1,2-dicarboxyethyl)GSH and GSH in the rabbit lens and liver during the naphthalene cataract formation. Naphthalene at a dose of 2.5 g/kg body weight was administered orally to rabbits.

Naphthalene cataracts were classified into the two stages of spichen and true opacity. Cataracts at the spichen

Fig. 5 Changes of GSH and S-(1,2-dicarboxyethyl)GSH (DCEG) in the lens during the 24 hours after naphthalene administration (a), and contents of GSH and S-(1,2-dicarboxyethyl)GSH in nor-

mal lens (control), in the lens in the cataract developing group (cataract) and in the lens in the non-cataract developing group (non-cataract) (b).

Fig. 6 Changes of GSH in the liver during the 24 hours after naphthalene administration (a), and contents of GSH in normal liver (control), in the liver in the cataract developing group (cata-

ract) and in the liver in the non-cataract developing group (non-cataract) (b).

Tab. 1 Contents of amino acids in lens of normal rabbits and rabbits after naphthalene feeding (n mol/g).

	Normal	Spichen developing group (A, n = 4)	Cataract developing group (B, n = 4)	Non-cataract developing group (C, n = 4)
Asp	52.1 ± 76.0	18.8 ± 3.8	254.9 ± 13.1	54.3 ± 1.1
Thr	142.4 ± 11.5	87.4 ± 18.8	420.9 ± 4.8	190.5 ± 7.5
Ser	347.8 ± 12.8	151.8 ± 32.7	1174.5 ± 6.9	638.6 ± 23.1
Glu	2256.9 ± 133.9	1090.9 ± 127.8	2819.1 ± 136.1	2338.5 ± 58.0
Gly	482.1 ± 61.2	256.8 ± 41.1	1265.8 ± 56.0	1223.1 ± 53.3
Ala	1202.7 ± 206.0	494.9 ± 125.3	1157.0 ± 27.5	758.1 ± 7.6
Val	254.0 ± 23.2	132.0 ± 13.8	670.0 ± 21.7	325.1 ± 15.2
Met	62.3 ± 27.1	26.6 ± 0.5	191.0 ± 9.7	68.8 ± 0.6
Cys/2	138.9 ± 24.4	90.7 ± 20.3	217.7 ± 89.8	253.3 ± 79.5
Leu	106.1 ± 5.3	81.5 ± 8.2	601.9 ± 13.6	187.6 ± 7.0
Ile	69.8 ± 5.7	38.4 ± 4.9	341.3 ± 8.0	106.7 ± 6.7
Tyr	241.5 ± 108.5	101.7 ± 30.1	482.0 ± 13.6	197.6 ± 15.3
Phe	135.8 ± 13.1	66.0 ± 9.3	359.4 ± 8.8	120.5 ± 1.1
Lys	209.0 ± 23.5	122.1 ± 8.2	880.1 ± 20.3	383.1 ± 17.8
Arg	44.9 ± 10.7	67.6 ± 8.1	444.1 ± 32.3	122.3 ± 11.1
His	169.5 ± 21.8	53.0 ± 13.9	388.4 ± 15.8	202.2 ± 8.2
Taurine	2955.9 ± 422.0	847.2 ± 77.1	530.1 ± 70.7	3306.0 ± 178.1
GSH + GSSG	11025.5 ± 257.1	3638.6 ± 257.1	3689.3 ± 439.6	12105.3 ± 1085.6

A: spichen developing lens at 24 hours after naphthalene administration.

B: cataract developing lens at a few weeks after naphthalene administration (three times).

C: non-cataract developing lens at a few weeks after naphthalene administration (three times).

stage developed 24 hours after the first administration. They were characterized by appearance of axialed opacity "spichen" at the equatorial region of lens (fig. 4a). Cataracts of the true opacity stage developed a few weeks after administration for three consecutive days (fig. 4b), but the rabbit which had received only once administration did not develop true opacity cataracts.

We examined the time course of the levels of S-(1,2-dicarboxyethyl)GSH and GSH for 24 hours after naphthalene administration. Results are shown in figure 5a.

The content of S-(1,2-dicarboxyethyl)GSH in the lens decreased until 24 hours after naphthalene administration in proportion to the content of GSH.

Raddy et al. (7) reported that the level of GSH in rat lens decreased rapidly during cataract formation, and S-(1,2-dicarboxyethyl)GSH in rat lens also decreased rapidly during cataract formation by feeding galactose (5).

During the further development of true lens opacity after naphthalene administration, the S-(1,2-dicarboxyethyl)GSH content decreased further compared with that in the spichen stage, but the lens content of S-(1,2-dicarboxyethyl)GSH in the group that did not develop cataract after naphthalene administration returned to the normal level (fig. 5b).

The content of GSH in liver decreased markedly until 24 hours after naphthalene administration (fig. 6a).

The content of GSH in the liver in both the cataract developing group and the non-cataract developing group returned to the normal level (fig. 6b).

Table 1 shows the contents of amino acids in the lens of normal and naphthalene-treated rabbits.

The levels of the sum of reduced and oxidized glutathione, taurine and other free amino acids in the lens in the spichen stage decreased markedly compared with their levels in the lens of normal rabbits, while the levels of glutathione and taurine in the lens of the cataract developing rabbits a few weeks after naphthalene administration remained lower than those in the normal lens; but the levels of other amino acids returned to normal. On the other hand, glutathione, taurine and other amino acids in the lens in the non-cataract developing rabbits after naphthalene administration recovered to the same levels as those in the normal lens. It has been reported that the free amino acids are decreased in the lens of rats with galactose-generated cataract (11, 12).

In conclusion, these findings indicate that the changes in concentration of S-(1,2-dicarboxyethyl)GSH and GSH in the lens of naphthalene-treated rabbits are similar to those reported during cataract formation after galactose administration (5). The findings also indicate that the ratio of the synthesis and the degradation of GSH in rabbit lenses during cataract formation after naphthalene administration may be the same as that of S-(1,2-dicarboxyethyl)GSH.

The effect of GSH on prevention of cataract formation has been reported in previous papers (11, 12), but these findings suggest that taurine and S-(1,2-dicarboxyethyl)GSH may also prevent cataract formation in the lens of rabbits.

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