HEPATIC MITOCHONDRIAL AND CYTOSOLIC GLUTATHIONE CONTENT AND THE SUBCELLULAR DISTRIBUTION OF GSH-*S*-TRANSFERASES

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1. Introduction

Glutathione participates in a number of redox and alkylation reactions in the hepatocyte which have been studied either in intact cells or in isolated subcellular fractions (cf. recent symposium [1]). However, information on the relevant subcellular concentrations of glutathione in the intact cell is lacking. Therefore, we have studied here the subcellular distribution of glutathione between the mitochondrial matrix (M) and cytosolic (C) spaces. In addition, the activity of the glutathione(GSH)-*S*-transferases was also measured, since it is known that the protective action of glutathione against certain types of liver damage is due to this S-conjugating activity [2].

2. Materials and methods

2.1. *Non-aqueous subfractionation of freeze-quenched perfused rat liver*

Livers from male Wistar rats of 150–200 g body wt, fed on stock diet, were perfused [3] for ~30 min and freeze-quenched. The subfractionation procedure [4] was carried out as in [5].

2.2. *Assays*

Marker enzymes for the mitochondrial matrix (citrate synthase [6]) and cytosolic (phosphoglycerate kinase [7]) compartments (cf. [4,5]), protein [8] and GSH-*S*-transferase were determined in each fraction obtained from the density gradient centrifugation.

Enzyme activities in the extracts obtained after homogenisation in heptane/CCl_4 can be up to 30% lower than without this procedure. It is assumed that this has no effect on the subcellular distribution. The GSH-*S*-transferases were assayed according to [9] except that the GSH concentration was 5 mM, formation of the conjugate with 1-chloro-2,4-dinitrobenzene (1 mM), *S*-(2,4-dinitrophenyl)glutathione, was followed at 334 nm in an Eppendorf model 6114 photometer. 1 U is 1 μmol conjugate formed/min at 25°C.

Glutathione was assayed either as the sum of the reduced and oxidized forms expressed in GSH equivalents (GSH + 2 GSSG) using the catalytic assay with 5,5′-dithiobis(nitrobenzoate) and glutathione reductase [10], or as GSSG alone [11], so that GSH could be calculated by difference. This procedure gave results for GSH identical to the separate GSH measurement [12].

2.3. *Calculations*

Mitochondrial and cytosolic contents of GSH (nmol/mg protein) or GSH-*S*-transferase activity (U/mg protein) were obtained by extrapolating the values for the fractions of the density gradient to fractions of pure mitochondria and cytosol according to [4]. The contents were converted to concentrations assuming water contents of 0.8 and 3.8 μl/mg protein in the mitochondrial and cytosolic compartments, respectively [5].

2.4. *Chemicals and biochemicals*

These were purchased from Merck (Darmstadt), Boehringer (Mannheim) and Sigma (München).

3. Results and discussion

3.1. *Glutathione*

The subcellular distribution of glutathione is shown in table 1: 87% of the cellular reduced and oxidized glutathione is present in the cytosol whereas 13% is mitochondrial. The glutathione concentrations, calculated from the respective water contents, are ~10 mM, with a slightly higher mitochondrial value. The *M/C* concentration ratio is 1.34.

The results of separate measurements of the GSSG concentration obtained from the same extracts are also shown in table 1. The compartmentation between mitochondria and cytosol is similar to that found for GSH + 2 GSSG, so that the values obtained for GSH calculated by difference also give a similar compartmentation, being 13.4 ± 0.6% mitochondrial. The GSH concentrations are 10.7 ± 2.7 and 7.2 ± 0.8 mM for the mitochondrial and cytosolic spaces, respectively. From these data, the redox ratios, [GSSG]/[GSH], were calculated, giving values of ~0.07 as shown in table 1. However, as stated above, free solution of GSSG and GSH (versus possible bound forms) in the water spaces represents an assumption which must be investigated further.

Several conclusions can be drawn from these results:

1. Regarding the method of subfractionation, it is noteworthy that neither the contents nor the redox state of glutathione are affected by the non-aqueous fractionation procedure. Furthermore, the ratio of contents, GSSG/GSH, obtained from freeze-quenched rat liver in situ was 0.051 [13], a value similar to that obtained for the perfused liver in this study.
2. The mitochondrial matrix water content is ~10% of the total cellular water [4,14], so that the value of 13% total glutathione in the mitochondrial matrix (table 1) indicates a similar concentration of glutathione in cytosolic and mitochondrial matrix water spaces, with possibly a slight preponderance in the mitochondrial space (*M/C* ratio = 1.34). Since at present there is no information on a transport system for glutathione from the cytosol to the mitochondria, the possibility exists that a separate mitochondrial glutathione-synthesizing system is utilized for the maintenance of the matrix glutathione concentration.
3. The value of 8.8 nmol glutathione/mg protein for the mitochondrial matrix is only slightly higher than those obtained with isolated mitochondria ([15], A. W., K. M. Moss, unpublished observations), so that one can assume that little glutathione is lost from the matrix during isolation of the mitochondria. Mitochondria have also been shown to retain most of their glutathione upon incubation [16].

3.2. *Glutathione-S-transferases*

The activity of glutathione-*S*-transferases as assayed with 1-chloro-2,4-dinitrobenzene largely represents the transferases A and B [9], at present, a substrate suitable for the assay of one specific enzyme is unknown due to the general overlapping substrate specificity. Therefore, the activity data shown in table 2 represent one of a set of similar data obtainable with other sub-

Table 1
Subcellular distribution of glutathione in liver

	Glutathione (GSH + 2 GSSG)			GSSG		[GSSG]/[GSH]
	nmol/mg protein	mM	% total content	mM	% total content	
Cytosolic	31 0 ± 3 8	8.2 ± 1 0	87.2 ± 0.8	0.47 ± 0.10	85 9 ± 1 8	0.065 ± 0 015
Mitochondrial (matrix)	8 8 ± 1.2	11.0 ± 1 5	12.8 ± 0.8	0 70 ± 0 11	14 1 ± 1 8	0.074 ± 0 018

The isolated perfused rat liver was freeze-quenched and further processed by the non-aqueous fractionation procedure [4,5]. Data are means ± SEM (n = 4), obtained from the results of 4–6 different density gradients analyzed for each of the 4 different livers Total glutathione content was 23 1 ± 3.0 nmol/mg protein. The ratio of contents, GSSG/GSH, was 0 072 ± 0 014

Table 2
Subcellular distribution of GSH-*S*-transferase activity, assayed with 1-chloro-2,4-dinitrobenzene as substrate

	GSH-*S*-transferase activity		
	U/mg protein	U/μl H_2O	% total act
Cytosolic	0 69 ± 0.11	0 18 ± 0.03	93.4 ± 2 4
Mitochondrial	0.09 ± 0 03	0.11 ± 0.04	6.6 ± 2.4

Data are means ± SEM (n = 4) obtained from the results of 4–6 different density gradients analyzed for each of the 4 different livers. Total activity was 0.43 ± 0.04 U/mg liver protein as measured after homogenisation in heptane/CCl_4

strates of possibly different substrate specificity. Nevertheless, table 2 indicates that ~7% of the total activity is present in the mitochondrial matrix space. This result supports the report [17] of a mitochondrial glutathione-*S*-transferase activity as deduced from measurements with isolated mitochondrial fractions after repeated washings. Further evidence for a mitochondrial GSH-*S*-transferase activity may be deduced from our recent finding of a non-selenium-dependent glutathione peroxidase activity assayed in isolated mitochondria from selenium-deficient rats [18], ~5% of the control activity was found with *t*-butyl hydroperoxide as substrate, but none with H_2O_2 as substrate. Glutathione-*S*-transferase B has been identified with the non-selenium-dependent glutathione peroxidase activity [19,20]. However, in a study on the subcellular distribution of non-selenium-dependent glutathione peroxidase [21] it was recently concluded that the activity was not present in subcellular organelles, the data in [21], however, do not exclude a mitochondrial localization of the order of 5%.

If it is assumed that the mitochondrial and cytosolic glutathione-*S*-transferases are distributed in the respective water spaces, a concentration of 0.11 and 0 18 U/μl is calculated for these two spaces (table 2), the *M/C* ratio being 0.63. Clearly, these numbers are subject to a correction to account for (low) membrane-bound activities [17,22] and for the recovery, as mentioned above. However, it may be concluded from this study that the potential for protection by S-conjugation is similar in cytosol and mitochondrial matrix, both with respect to the *S*-transferase activity and the glutathione concentration available.

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