SUBCELLULAR DISTRIBUTION AND INTRAMITOCHONDRIAL LOCALIZATION OF THREE SULFURTRANSFERASES IN RAT LIVER

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1. Introduction

Mammalian tissues contain three enzymes involved in the transfer of bivalent sulphur to a variety of nucleophilic acceptors: rhodanese (thiosulfate sulfurtransferase, EC 2.8.1.1), 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) and thiosulfate reductase (no EC number, for definition see [1–4]). These enzymes participate in the metabolism of some amino acids and low molecular weight sulphur compounds [5–7], show detoxifying activity against sulphide [8] and cyanide [9,10], are probably involved in the formation of iron-sulfur chromophore of ferredoxin [11,12] and restoration of labile sulfur in succinate dehydrogenase (EC 1.3.99.1 [13]). Rhodanese is present solely in the mitochondria [14,15] and shows a characteristic latency [16], but the subcellular distribution of the two other sulfurtransferases is still disputed. Kun and Fanshier [17] and Van der Hamer et al. [18] observed that most of the mercaptopyruvate sulfurtransferase activity was in the soluble fraction of rat liver, while recently Taniguchi and Kimura [12] found the enzyme in both mitochondria and the cytosol of bovine adrenal cortex. Preliminary observations indicate that also thiosulfate reductase occurs in these two subcellular compartments [19]. The experiments described here confirm the bimodal intracellular localization of mercaptopyruvate sulfurtransferase and thiosulfate reductase. They also indicate that, within the mitochondria, all three sulfurtransferases are located in the matrix.

2. Materials and methods

Tissue fractionation of rat liver (adult males, Wistar strain) was performed according to Shibko et al. [20]. The liver was perfused in situ via portal vein with 20 ml of cold 250 mM sucrose in order to remove residual blood and then 10 g of the tissue were homogenized with 40 ml of 250 mM sucrose – 6 mM NaOH using a teflon-glass homogenizer. Cell debris collected after centrifuging for 10 min at 600 × *g* were rehomogenized with 10 ml sucrose and centrifuged as above (sediment = nuclear fraction). Pooled supernatants were centrifuged to obtain mitochondrial (15 min at 4 000 × *g*), lysosomal (30 min at 16 000 × *g*) and microsomal (60 min at 100 000 × *g*) fractions and the final supernatant. Each particulate fraction was suspended in 10 ml of 250 mM sucrose, frozen and thawed twice, and suitably diluted with water prior to measurement of enzymatic activity. It was shown that this treatment was sufficient to remove the latency of enzymes tested since further addition of Triton X-100 to 0.1% concentration was without significant effect on the enzymatic activity.

Rhodanese was assayed according to Sörbo [21], mercaptopyruvate sulfurtransferase according to Kun

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and Fanshier [17], thiosulfate reductase according to Koj [3], glutamate dehydrogenase (EC 1.4.1.2) according to Olson and Anfinsen [22], arylsulfatase A (EC 3.1.6.1) according to Baum et al. [23] and glucose-6-phosphatase (EC 3.1.3.9) by the method of Swanson [24]. Mitochondria were further fractionated according to Schnaitman and Greenawalt [25] using 0.16 mg digitonin/mg mitochondrial protein. The intermembrane fraction was obtained as the supernatant after suspending mitochondria in the medium and centrifuging mitoplasts at 10 000 × *g* for 15 min and outer membranes at 100 000 × *g* for 60 min. Mitoplasts were disrupted by sonication and separated into inner membrane and matrix fractions by centrifugation at 100 000 × *g* for 60 min. Cross-contamination of the intermembrane and matrix fractions was checked by determining the following marker enzymes: glutamate dehydrogenase and malate dehydrogenase (EC 1.1.1.37), the latter assayed according to Bergmeyer and Bernt [26], for the matrix, and adenylate kinase (EC 2.7.4.3) assayed as described by Sottocasa et al. [27] for the intermembrane compartment.

Enzyme activities were expressed as follows: rhodanese as μmoles SCN^- formed during 5 min incubation at 20 °C, mercaptopyruvate sulfurtransferase as μmoles pyruvate produced during 15 min at 37 °C, thiosulfate reductase as μmoles SO_3^{2-} formed during 15 min at 37 °C, glutamate dehydrogenase and malate dehydrogenase as decrease of absorbance at 340 nm during 1 min at 25 °C, adenylate kinase as increase of absorbance at 340 nm during 1 min at 25 °C, arylsulfatase A as μmoles *p*-nitrocatechol formed during 1 hr at 37 °C and glucose-6-phosphatase as μmoles phosphate formed during 10 min at 37 °C. Protein was determined by the method of Lowry et al. [28] using bovine serum albumin as standard.

3. Results and discussion

Three experiments on the subcellular fractionation of rat liver homogenate were carried out with subsequent determination of protein content and enzyme activities. The highest specific activities of all sulfurtransferases were found in the mitochondrial fraction, similarly to the pattern observed for glutamate dehydrogenase (table 1). The results were fairly reproducible as indicated by a rather small scatter of values in individual experiments. A relatively high specific activity of all three sulfurtransferases in the nuclear

Table 1
Specific activities of some enzymes in subcellular fractions of rat liver

Enzyme	H	N	M	L	Mic	S
Rhodanese	9.25 8.11–10.30	15.31 12.63–17.40	22.23 21.00–24.30	5.57 5.20–6.00	0.29 0.16–0.48	0.95 0.61–1.60
Thiosulfate reductase	0.069 0.066–0.077	0.101 0.098–0.107	0.132 0.122–0.153	0.042 0.033–0.054	0.014 0.005–0.030	0.044 0.032–0.055
Mercaptopyruvate sulfurtransferase	14.11 12.15–16.86	18.33 14.55–24.35	24.27 18.09–32.67	10.30 4.77–13.79	2.66 1.14–4.32	10.48 7.01–12.29
Glutamate dehydrogenase	0.845 0.746–0.925	1.161 0.966–1.282	1.744 1.143–2.160	0.378 0.330–0.446	0.104 0.100–0.108	0.008 0.006–0.010
Arylsulfatase A	0.452 0.360–0.566	0.257 0.185–0.305	0.785 0.541–1.126	2.108 1.727–2.617	0.242 0.228–0.355	0.063 0.055–0.079
Glucose-6-P phosphatase	0.924 0.760–1.100	0.766 0.670–0.800	0.564 0.250–0.734	2.250 1.450–2.800	2.733 2.300–3.180	0.071 0.040–0.125

Enzyme activities (mean values of 3 experiments) are expressed in arbitrary units (described under Materials and methods) per mg protein; the range form individual experiments is given in parentheses. H, whole homogenate; N, nuclear fraction; M, mitochondrial fraction; L, lysosomal fraction; Mic, microsomal fraction; S, final supernatant.

Table 2
Distribution of some enzymes in submitochondrial fractions

Fraction	Protein	Adenylate kinase	Malate dehydrogenase	Glutamate dehydrogenase	Rhodanese	Thiosulfate reductase	Mercaptopyruvate sulfurtransferase
Outer membrane	12.6= 5.8%	ND	33= 1.4%	9.1= 1.3%	163= 2.3%	0.60= 1.6%	79= 0.8%
Inner membrane	61.5=28.4%	ND	65= 2.8%	42.0= 6.0%	117= 1.6%	0.23= 0.6%	132= 1.4%
Inter-membrane space	40.5=18.7%	230=94.0%	240=10.2%	89.7=12.8%	969=13.3%	4.42=11.5%	1189=12.6%
Matrix	102.0=47.1%	15= 6.0%	2020=85.6%	559.2=79.9%	6021=82.8%	33.12=86.3%	8068=85.2%

Protein content is expressed in mg and enzyme activities in arbitrary units as specified under Materials and methods. The percentage is calculated with respect to the sum of fractions taken as 100%. This recovery for protein content and enzyme activities ranged from 85% to 105% of values found for whole mitochondria. ND = not determined.

fraction may be explained by the high contamination of this fraction by mitochondria as indicated by high specific activity of glutamate dehydrogenase.

An even more clear picture was obtained after graphical presentation of the distribution pattern of investigated enzymes in terms of relative specific activities (fig.1). Rhodanese and glutamate dehydrogenase exhibit one maximum in the mitochondrial fraction, while thiosulfate reductase and mercaptopyruvate sulfurtransferase show a bimodal distribution with a significant proportion of activity recovered in the final supernatant. This can be explained either by the presence of independent mitochondrial and cytosolic isoenzymes of these sulfurtransferases or by their easier release from mitochondria during homogenization, especially if they were present in the intermembrane space.

To test the latter possibility mitochondria were further fractionated and the enzymes determined in

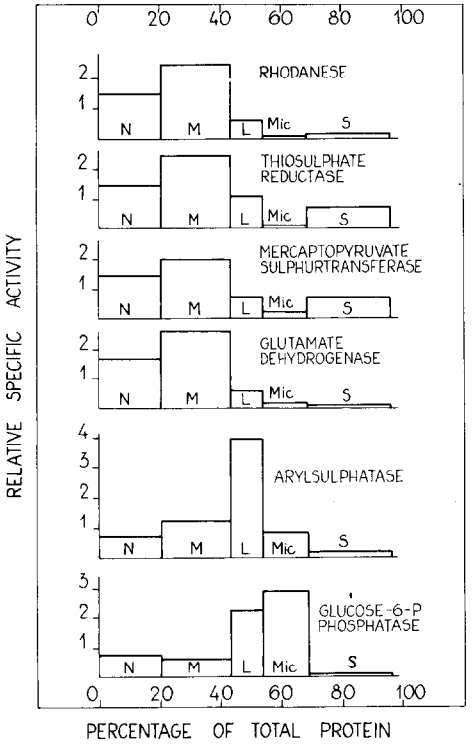

Fig.1. Distribution patterns of three sulfurtransferases and some marker enzymes in rat liver. Symbols of subcellular fractions as in table 1. The diagram represents a typical experiment out of three.

intramitochondrial compartments. It appeared (table 2) that over 80% of the activity of all three sulfurtransferases was recovered in the matrix fraction, similarly to malate and glutamate dehydrogenases. There is no indication that the sulfurtransferases are located either in the intermembrane space or the membranes since their activities in these fractions are the same as those of the two marker enzymes for the matrix.

Thus it seems clear that the presence of thiosulfate reductase and mercaptopyruvate sulfurtransferase in the cytosolic fraction does not result from the leakage from the intermembrane compartment of mitochondria during the separation procedure but reflects a real bimodal localization of these enzymes in liver cell. Nevertheless, a further proof for the existence of separate mitochondrial and cytosolic mercaptopyruvate sulfurtransferase and thiosulfate reductase is required and may involve purification of these enzymes and comparison of their kinetic parameters. In case of mercaptopyruvate sulfurtransferase the situation is additionally complicated by the fact that the enzyme also occurs in erythrocytes [17], and hence the activity found in the cytosol may derive, at least partly, from residual blood left in the liver despite perfusion carried out before homogenization.

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References

[1] Sörbo, B. (1964) Acta Chem. Scand. 18, 821–823.
[2] Koj, A. and Frendo, J. (1967) Folia Biol. (Krakow) 15, 49–63.
[3] Koj, A. (1968) Acta Biochim. Polon. 15, 161–169.
[4] Sido, B. and Koj, A. (1972) Acta Biol. Cracov., Ser. Zool. 15, 97–103.
[5] Koj, A., Frendo, J. and Janik, Z. (1967) Biochem. J. 103, 791–795.
[6] Roy, A. B. and Trudinger, P. A. (1970) The Biochemistry of Inorganic Sulphur Compounds, Cambridge University Press.

[7] Westley, J. (1973) Adv. Enzymol. 39, 327–368.
[8] Szczepkowski, T. W. and Wood, J. L. (1967) Biochim. Biophys. Acta 139, 469–476.
[9] Sörbo, B. (1957) Acta Chem. Scand. 11, 628–633.
[10] Auriga, M. and Koj, A. (1975) Bull. Acad. Polon. Sci. Ser. Sci. Biol. 23, in press.
[11] Finazzi-Agrò, A., Cannella, C., Graziani, M. T. and Cavallini, D. (1971) FEBS Lett. 16, 172–174.
[12] Taniguchi, R. and Kimura, T. (1974) Biochim. Biophys. Acta 364, 284–295.
[13] Pagani, S., Cannella, C., Cerletti, P. and Pecci, L. (1975) FEBS Lett. 51, 112–115.
[14] Sörbo, B. (1951) Acta Chem. Scand. 5, 724–729.
[15] DeDuve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604–617.
[16] Greville, G. D. and Chappell, J. B. (1959) Biochim. Biophys. Acta 33, 267–269.
[17] Kun, E. and Fanshier, D. W. (1959) Biochim. Biophys. Acta 32, 338–348.
[18] Van der Hamer, C. J., Morell, A. G. and Scheinberg, I. H. (1967) J. Biol. Chem. 242, 2514–2517.
[19] Koj. A. (1969) Post. Biochem. 15, 357–369.
[20] Shibko, S., Koivistoinen, P., Tratnyek, C. A., Newhall, A. R. and Friedman, L. (1967) Analyt. Biochem. 19, 514–528.
[21] Sörbo, B. (1955) in: Methods in Enzymology, Vol. 2, pp. 334–337. (eds. S. P. Colowick and N. O. Kaplan). Academic Press, New York.
[22] Olson, J. A. and Anfinsen, C. B. (1952) J. Biol. Chem. 197, 67–68.
[23] Baum, H., Dodgson, K. S. and Spencer, E. (1959) Clin. Chim. Acta 4, 453–455.
[24] Swanson, M. A. (1955) in: Methods in Enzymology, Vol. 2, pp. 541–544. (eds. S. P. Colowick and N. O. Kaplan). Academic Press, New York.
[25] Schnaitman, C. and Greenawalt, J. W. (1968) J. Cell. Biol. 38, 158–175.
[26] Bergmeyer, H. V. and Bernt, E. (1963) in: Methods of Enzymatic Analysis pp. 757–760 (ed. H. V. Bergmeyer). Verlag Chemie, Weinheim and Academic Press, New York.
[27] Sottocasa, G. L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) Methods Enzymol. 448–463.
[28] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.