Subcellular distribution of selenium in deficient mouse liver

Rudolf REITER,* Rainer OTTER† and Albrecht WENDEL‡

Faculty of Biology, University of Konstanz, P.O. Box 5560, D-7750 Konstanz, Federal Republic of Germany

Selenium (Se)-deficient mice were labelled *in vivo* with single pulses of [⁷⁵Se]selenite, and the intrahepatic distribution of the trace element was studied by subcellular fractionation. At 1 h after intraperitoneal injection of 3.3 or 10 µg of Se/kg body weight, 15 % of the respective doses were found in the liver. Accumulation in the subcellular fractions followed the order: Golgi vesicular ≫ lysosomal > cytosolic = microsomal > mitochondrial, peroxisomal, nuclear and plasma-membrane fraction. At a dose of 3.3 µg/kg, more than 90 % of the hepatic Se was protein-bound. When cross-contamination was accounted for, the following specific Se contents of the subcellular compartments were extrapolated: Golgi apparatus, 7.50 pmol/mg; cytosol, 0.90 pmol/mg; endoplasmic reticulum, 0.80 pmol/mg; mitochondria, 0.49 pmol/mg; nuclei, lysosomes, peroxisomes and plasma membrane, less than 0.4 pmol/mg. At 10 µg/kg, a roughly 2–3-fold increase in Se content of all fractions was found without major changes in the intrahepatic distribution pattern. An extraordinary rise in the cytosolic fraction was due to an apparently non-protein-bound Se pool. At 24 h after dosing, total hepatic Se had decreased to 6 % of the initial dose and had become predominantly protein-bound. The 60 % decrease in hepatic Se was reflected in a similar fall in the subcellular levels of the trace element. The Golgi apparatus still had the highest specific Se content, although accumulation was 5 times less than that after 1 h. The cytosolic pool accounted for 50 % of the hepatic Se at both labelling times. After 1 h the Golgi apparatus was, with 19 %, the second largest intrahepatic pool, followed by the endoplasmic reticulum with 16 %. The high affinity and fast response of the Golgi apparatus to Se supplementation of deficient mice is interpreted in terms of a predominant function of this cell compartment in the processing and the export of Se-proteins from the liver.

INTRODUCTION

Previous work from this laboratory focused on the biochemical characterization of selenium (Se)-deficient mouse liver. During initial depletion, loss of the selenoenzyme GSH peroxidase (GSH-Px) to virtually undetectable levels was found. When dietary Se deprivation was further continued, extensive metabolic perturbations were observed [1]. Different pathways, such as drug metabolism, glucose catabolism and the tricarboxylic acid cycle were affected in severely deficient mouse liver [1,2]. Beyond this, the hepatic potential for a proliferative response was increased when partial hepatectomy was used as a stimulus [3]. Indications of a metabolic impairment of the liver as judged by increased serum transaminases or alterations in plasma protein pattern were not evident [4]. The diversity of the effects with respect to the direction of the changes and the cell compartments affected point to a highly controlled response of the liver to Se deficiency. A causatively exclusive regulatory role of GSH-Px on these metabolic modulations was challenged on the basis of the de- and re-pletion experiments [5,6]. The restoration of drug-metabolism changes to the control state obeyed a common dose-dependency and occurred at a much lower dose of Se than was needed to restore GSH-Px activity. The existence of other hepatic requirements of the trace element with an even higher affinity for Se than GSH-Px

was demonstrated by Burk *et al.* [7] with the selenoprotein ⁷⁵Se-P⁸ [8].

A more detailed understanding of the functions of Se incorporated into GSH-Px as against (a) very small pool(s) related to other hepatic functions seems to be needed. Therefore we studied the subcellular distribution of a tracer dose of Se in deficient mouse liver at dose levels below and above the threshold for reversal of enzyme modulations in drug metabolism.

MATERIALS AND METHODS

Weanling male albino mice (strain NMRI han) were made Se-deficient by feeding a low-Se diet (10 p.p.b. of Se) for 6 months [9]. Se deficiency was assessed by measuring the fall in Se-dependent GSH-Px activity and the occurrence of enzyme modulations in mouse liver [1] over Se-adequate controls. The GSH-Px activity of mice used for the labelling experiments was 1 % of that of controls, whereas glutathione S-transferase activity towards 1-chloro-2,4-dinitrobenzene was increased 2.7-fold.

[⁷⁵Se]Selenite (sp. radioactivity: 1.2 GBq/mg, 99 % radionuclidic purity) was injected intraperitoneally at doses of 3.3 or 10 µg of Se/kg body weight respectively. Each labelling experiment involved a single mouse. When the intrahepatic Se distribution was studied after 1 h, mice were fasted for 24 h before injection. After the

Abbreviations used: GSH-Px, glutathione peroxidase (EC 1.11.1.9); p.p.b., parts per billion (10⁹).

* Present address: Boehringer-Mannheim G.m.b.H., Sandhofer Strasse 116, D-6800 Mannheim 31, Federal Republic of Germany.

† Present address: BASF A.G., D-6700 Ludwigshafen, Federal Republic of Germany.

‡ To whom correspondence and reprint requests should be sent.

24 h 'in vivo' labelling interval, food was withdrawn immediately after dosing. At the end of the specified labelling period animals were killed by cervical dislocation and the liver was perfused with ice-cold saline (0.9% NaCl) before subcellular fractionation.

The experimental protocol followed essentially methods described by Fleischer & Kervina [10] and by Wattiaux *et al.* [11] for the fractionation of rat liver. A detailed description of the adaptation of the combined methods to the fractionation of a single mouse liver, yielding nuclear, plasma-membrane, mitochondrial, peroxisomal, lysosomal, microsomal, Golgi and cytosolic fractions was described in the preceding paper [12]. The purified subcellular fractions were characterized as described in [12] by the specific DNA content and specific marker-enzyme activities. Specific DNA content served as a marker of nuclei. The specific activities of 5'-nucleotidase, succinate dehydrogenase, catalase, acid phosphatase, UDP-galactosyltransferase and lactate dehydrogenase were used as markers of plasma membrane, mitochondria, peroxisomes, lysosomes, Golgi vesicles and cytosol respectively.

⁷⁵Se was determined in 500 µl aliquots of the very same subcellular fractions in a Packard model 5320 γ-radiation counter. Furthermore, protein in a 200 µl portion of each fraction was precipitated by addition of cold 10% (w/v) trichloroacetic acid. After centrifugation at 10000 g for 5 min, the precipitate and an aliquot of the supernatant were assayed for ⁷⁵Se. In some experiments, before precipitation, samples were incubated with 100 mM-dithiothreitol for 15 min at room temperature in order to release ⁷⁵Se from protein-bound mixed disulphides. Estimates of the specific ⁷⁵Se contents of the cell compartments were derived by regression analysis of the specific marker contents/activities (Table 2 below) and the ⁷⁵Se contents (Table 3 below) of the different subcellular fractions. In other words, an array of nine equations (one for each subcellular fraction) with eight variables (one for each subcellular marker) was calculated using the ⁷⁵Se-distribution data of each labelling experiment. These calculations yielded pmol of ⁷⁵Se/µg of DNA or munits of marker-enzyme activity and were

converted into specific Se content on the basis of the subcellular marker distribution. Similarly a set of eight equations with eight variables (one for the protein content of each compartment) was calculated for the subcellular markers. For simplicity reasons it was assumed that the individual subcellular markers originated specifically in different cell compartments.

RNA-free sucrose, AMP, glucosamine and UDP D-galactose were supplied by Sigma Chemical Co., München, Germany. Metrizamide and Coomassie Brilliant Blue G-250 were from Serva, Heidelberg, Germany. Pyruvate, NADH, NADP⁺ and 4-nitrophenyl phosphate were purchased from Boehringer-Mannheim G.m.b.H., Mannheim, Germany. UDP-D-[6-³H]-galactose (sp. radioactivity 65 Gbq/mmol) and sodium [⁷⁵Se]selenite (sp. radioactivity: 1.2 Gbq/mg) were from New England Nuclear, Dreieich, Germany. All other chemicals used were of analytical grade.

RESULTS

Severely Se-deficient mice were labelled *in vivo* with a single pulse of [⁷⁵Se]selenite, and its intrahepatic distribution was studied by subcellular fractionation. Interest was focused on obtaining highly enriched nuclear, plasma-membrane, mitochondrial, lysosomal, peroxisomal, microsomal, Golgi and cytosolic fractions rather than giving a quantitative account of the hepatic protein distribution. A characterization of the various subcellular fractions, i.e. the distribution of specific DNA content and marker-enzyme activities in the subcellular fractions, is given in Table 1. Purity of the individual fractions can be estimated as specific cross-contamination, i.e. % residual marker-enzyme activity in the contaminated fraction relative to the maximal marker enzyme activity in the highly purified subcellular fraction from which the enzyme originated. In the nuclear, mitochondrial, peroxisomal and cytosolic fractions, only minor contamination by other cellular compartments was detected. In contrast, the plasma-membrane and microsomal fractions showed a roughly 20% specific contamination by the mitochondrial marker. Furthermore, the Golgi and the lyso-

Table 1. Specific DNA content and marker-enzyme activities in subcellular fractions of Se-deficient mouse liver

Results are means ± S.D. for three observations. Abbreviations: 5'-Nucl., 5'-nucleotidase; SDH, succinate dehydrogenase; Ac-phos., acid phosphatase; NADPH-red., NADPH:cytochrome *c* reductase; Gal-trans., UDP-galactosyltransferase; LDH, lactate dehydrogenase; n.d., not detectable.

Fraction	DNA content (µg/mg)	Marker enzyme...	Activity (munits/mg*)						
			5'-Nucl.	SDH	Catalase	Ac-phos.	NADPH-red.	Gal-trans.	LDH
Homogenate	29.4 ± 2.8		8.5 ± 0.7	18.8 ± 2.8	33.4 ± 2.1	12.8 ± 3.1	28.5 ± 7.2	0.07 ± 0.01	896 ± 70
Nuclei	189 ± 8		n.d.	7.2 ± 6.9	6.7 ± 2.5	4.4 ± 1.4	5.6 ± 1.4	0.05 ± 0.03	46.9 ± 12.2
Plasma membranes	n.d.		234 ± 10	16.7 ± 9.4	10.3 ± 8.3	40.8 ± 5.4	7.4 ± 0.2	0.17 ± 0.02	39.2 ± 12.4
Mitochondria	n.d.		1.9 ± 2.2	72.0 ± 8.0	16.2 ± 3.8	19.8 ± 3.9	16.8 ± 12.4	0.02 ± 0.01	16.1 ± 17.6
Peroxisomes	n.d.		8.8 ± 3.6	6.4 ± 4.0	462 ± 16	12.3 ± 1.3	1.5 ± 0.4	n.d.	n.d.
Lysosomes	n.d.		16.6 ± 2.9	5.6 ± 1.7	4.7 ± 0.6	776 ± 69	42.8 ± 15.1	0.46 ± 0.13	31.1 ± 7.9
Microsomes	n.d.		5.4 ± 0.9	15.3 ± 6.6	10.6 ± 5.8	18.6 ± 4.6	185 ± 6	0.11 ± 0.11	18.8 ± 14.7
Golgi vesicles	n.d.		32.9 ± 8.2	2.5 ± 2.3	4.9 ± 2.2	80.9 ± 29.3	13.1 ± 4.6	3.6 ± 0.4	13.2 ± 12.6
Cytosol	n.d.		4.1 ± 1.9	1.3 ± 1.1	4.9 ± 2.4	7.1 ± 3.1	2.1 ± 0.4	n.d.	2888 ± 299

* Except for catalase activity, the units for which are kat/mg.

somal fractions were subject to considerable cross-contamination. Enrichment as compared with the homogenate was highest for the lysosomal (60-fold), followed by the Golgi vesicles (51-fold), the plasma-membrane (27-fold), peroxisomal (14-fold) and microsomal as well as nuclear fraction (6.5-fold). The enrichment indices of the mitochondrial and cytosolic fraction were 4 and 3 respectively. The intrahepatic Se distribution was determined concomitantly in the very same subcellular fractions. In Table 2 the conditions of the three labelling experiments differing in dose and/or labelling time, and the hepatic recoveries, are summarized. About 15% of the dose was found in mouse liver after 1 h, regardless of whether a pulse of 3.3 or 10 μg of ^{75}Se /kg had been given. The specific ^{75}Se contents of the subcellular fractions are given in Table 3. At 1 h after injection of 3.3 μg of ^{75}Se /kg, 0.50–0.65 pmol/mg of protein was found in the nuclear, plasma-membrane, mitochondrial and peroxisomal fractions of the liver. Approx. 1 pmol of ^{75}Se /mg of protein was present in the microsomal and cytosolic fractions. The Golgi fraction had the highest specific Se content, with 8.16 pmol/mg, followed by the lysosomal fraction, with 2.42 pmol/mg. When aliquots of the different subcellular fractions were treated with 10% trichloroacetic acid, over 90% of the label remained in the

precipitate. The Golgi fraction was characterized further. Treatment with dithioerythritol at 100 mmol/litre, in order to release Se from mixed disulphides, did not result in a significantly decreased ^{75}Se content of the precipitate. In the liver of the animal which had received 10 μg of Se/kg, a 2–3-fold rise in the ^{75}Se content of all fractions as compared with the 3.3 μg /kg dose was observed without major changes in the intracellular ^{75}Se distribution pattern. Only the cytosolic fraction showed an extraordinary increase in total ^{75}Se content. However, in contrast with the remaining subcellular fractions, approx. 50% of the cytosolic and 26% of the ^{75}Se in the homogenate was soluble in 10% trichloroacetic acid. At 24 h after injection, the hepatic ^{75}Se content had decreased to 6% of the 10 μg /kg dose and was predominantly precipitated by trichloroacetic acid. In most fractions the specific Se content was reduced by 50% compared with the levels accumulated after a 1 h labelling time. The Golgi fraction still retained the highest specific ^{75}Se content among the subcellular fractions, although accumulation was 5 times less than that after 1 h. By the same token, protein-bound ^{75}Se in the cytosolic fraction was decreased only by 30%, despite the 60% overall fall in hepatic Se content after 24 h.

From the specific markers (Table 1) and the ^{75}Se distribution (Table 3) among the subcellular fractions, estimates of the specific ^{75}Se contents and the pool sizes of the cell compartments were derived. As shown in Table 4, approx. 80% of the Se content of the lysosomal fraction and roughly 40–50% in the plasma-membrane, peroxisomal fraction originated in other cell organelles, especially the Golgi apparatus. The specific Se content among the different subcellular compartments decreased in all experiments uniformly in the order: Golgi apparatus $\gg$ cytosol = endoplasmic reticulum $>$ mitochondria $>$ nuclei, plasma membranes, peroxisomes and lysosomes. Under all conditions studied the cytosol accounted for roughly 50% of the hepatic Se. The Golgi apparatus was the second largest intrahepatic pool after 1 h, but declined from 19 to 8% within 24 h. Similarly the endoplasmic-reticular pool decreased from 16 to 12% during this time. In contrast, the relative contributions of the mitochondrial and nuclear pools to

Table 2. Accumulation of tracer doses of ^{75}Se in deficient mice after different times of intraperitoneal administration

Each labelling experiment involved a single mouse.

Parameter (units)	Labelling expt.	I	II	III
Dose ($\mu\text{g}/\text{kg}$)		3.3	10	10
Labelling time (h)		1	1	24
Body weight (g)		34	34	35
^{75}Se injected (pmol)		1540	4467	4467
Liver wet weight (g)		1.3	1.2	1.4
Hepatic protein content (mg/g)		180	176	178
Hepatic ^{75}Se content (pmol/liver)		234	701	277

Table 3. ^{75}Se distribution in subcellular fractions of deficient mouse liver after single injections of two different doses of $\text{Na}_2^{75}\text{SeO}_3$

Each labelling experiment involved a single mouse. Abbreviations: n.d., not detectable; TCA, trichloroacetic acid.

Fraction	Distribution (pmol or %)					
	75 μg of $^{75}\text{Se}/\text{kg}$, 1 h		10 μg of $^{75}\text{Se}/\text{kg}$, 1 h		10 μg of $^{75}\text{Se}/\text{kg}$, 24 h	
	Total ^{75}Se (pmol/mg of protein)	TCA-soluble ^{75}Se (%)	Total ^{75}Se (pmol/mg of protein)	TCA-soluble ^{75}Se (%)	Total ^{75}Se (pmol/mg of protein)	TCA-soluble ^{75}Se (%)
Homogenate	1.00	n.d.	3.32	26	1.11	6
Nuclei	0.50	n.d.	0.78	n.d.	0.69	n.d.
Plasma membranes	0.59	5	2.03	10	0.76	n.d.
Mitochondria	0.54	2	2.74	8	0.76	12
Peroxisomes	0.75	4	2.09	9	1.05	10
Lysosomes	2.42	4	5.55	4	2.40	1
Microsomes	1.21	n.d.	2.91	10	1.50	1
Golgi vesicles	8.16	2	20.74	3	3.97	n.d.
Cytosol	0.93	2	4.42	50	1.52	4

Table 4. Estimated protein and specific as well as total ^{75}Se content of subcellular compartments in deficient mouse liver after pulse-labelling with $\text{Na}_2^{75}\text{SeO}_3$

Compartment	Protein content (mg/g)	^{75}Se cross- contamination (%)	Specific (pmol/mg of protein) and total (pmol/g of liver) ^{75}Se content of the compartment					
			3.3 μg of Se/kg, 1 h		10 μg of Se/kg, 1 h		10 μg of Se/kg, 24 h	
			Specific	Total	Specific	Total	Specific	Total
Homogenate	178	—	1.0	178	3.3	591	1.1	198
Nuclei	27.6	17	0.39	10.7	0.78	21.5	0.48	13.2
Plasma membranes	6.5	49	0.25	1.6	0.96	6.2	0.68	4.4
Mitochondria	46.5	18	0.49	22.8	1.79	83.2	0.68	31.6
Peroxisomes	12.9	48	0.38	4.9	1.11	14.3	0.54	7.0
Lysosomes	2.9	82	0.37	1.1	1.00	2.9	0.48	1.4
Endoplasmic reticulum	27.3	39	0.80	21.8	1.52	41.5	0.78	21.3
Golgi apparatus	3.5	6	7.50	26.3	20.74	72.6	3.50	12.3
Cytosol	55.2	3	0.90	49.7	4.02	221.9	1.57	86.7

overall hepatic Se content were independent of the labelling time, accounting for 17 and 7% respectively. The lysosomes, plasma membranes and the peroxisomes contained only traces of Se.

DISCUSSION

^{75}Se was recovered in all subcellular fractions studied, and estimates of its distribution in the subcellular compartments were derived, cross-contaminations being taken into account. A dependence of the extent of Se accumulation in the compartments on total hepatic Se content was observed, suggesting at least some unspecific binding of the label. This limits in particular the assessment of cell compartments with very low specific Se contents, e.g. nuclei, plasma membranes, peroxisomes and lysosomes. Minute Se pools with important functions may have been obscured by an excess of unspecifically bound Se and thus may have been not resolved under the conditions applied.

The observed moderate accumulation of Se in mitochondria, endoplasmic reticulum and cytosol are in agreement with previous studies from other laboratories [13–15]. A cytosolic trichloroacetic acid-soluble Se pool was detected after 1 h labelling with 10, but not 3.3, μg of $^{75}\text{Se}/\text{kg}$ body weight. The solubility in cold 10% trichloroacetic acid seems to exclude a protein-bound form of Se, but could be explained by selenotrisulphide formation [16] or incorporation into tRNA species [17]. The threshold of the appearance of this pool can be estimated to be similar to that reported for reversal of certain metabolic perturbations in deficient mouse liver [6]. A common link between both events seems conceivable, although a cause-and-effect relationship is not compelling, since two independent events may be triggered by the same mechanism. The size of this pool and its transient nature suggest as an alternative some kind of intermediary storage of Se. Compared with this cytosolic pool, the Golgi apparatus accumulated Se with distinctly higher affinity. Even with the 3.3 $\mu\text{g}/\text{kg}$ pulse, it enriched Se 20-fold over several other cellular compartments and accounted for 19% of the hepatic content after 1 h. The pronounced fall within 24 h suggests a rapid turnover of this pool. The chemical form of Se and its function are a

matter of conjecture. The precipitation by trichloroacetic acid, but resistance to thiol treatment, point towards Se bound to the protein backbone, which could be formed, despite the very short time span, by protein synthesis *de novo* [18]. The transient nature of the pool argues against a Se-containing constituent in this cell organelle. The role of the Golgi apparatus in modifying and exporting proteins from the cell rather suggests an association with the processing and the export of Se-proteins like $^{75}\text{Se-P}$ [8,9]. The flow through the Golgi apparatus is difficult to estimate, but it seems likely that a considerable portion of the 60% decrease in hepatic Se content within 24 h was processed via this route.

During deficiency the liver could play a central distributor function in supplying Se specifically to tissues with a more stringent need for the element, such as, for example, the testis. The redistribution of Se in deficient rat tissues, reported by Behne *et al.* [19], corroborate this view. The unique blood supply and the high metabolic activity make the liver an ideal organ to screen for and recycle dietary and endogenous Se. Thus the loss of the trace element can be minimized provided the liver is fairly insensitive to any damaging effects of pure Se deficiency or can cope with them.

This work was supported by the Deutsche Forschungsgemeinschaft (grant We 686/8-3). The technical expertise of Mr. Bierbaum and discussions with Professor Dr. P. Bohley, University of Tübingen, are gratefully acknowledged.

REFERENCES

1. Reiter, R. & Wendel, A. (1983) *Biochem. Pharmacol.* **32**, 3063–3067
2. Wendel, A. & Otter, R. (1987) *Biochim. Biophys. Acta* **925**, 94–100
3. Otter, R., Goldberg, M., Vogl, S. & Wendel, A. (1986) *Chem.-Biol. Interact.* **59**, 295–300
4. Vogl, S., Goldberg, M., Hoffmann, G., Ruhenstroth-Bauer, G., Otter, R. & Wendel, A. (1987) *J. Hepatol.* **4**, 212–217
5. Reiter, R. & Wendel, A. (1984) *Biochem. Pharmacol.* **33**, 923–928

6. Reiter, R. & Wendel, A. (1985) *Biochem. Pharmacol.* **34**, 2287–2290
7. Burk, R. F. & Gregory, P. E., (1982) *Arch. Biochem. Biophys.* **213**, 73–80
8. Yang, J.-G., Plummer, J. M. & Burk, R. F. (1987) *J. Biol. Chem.* **262**, 13372–13375
9. Burk, R. F. (1987) *Methods Enzymol.* **143**, 307–313
10. Fleischer, S. & Kervina, M. (1974) *Methods Enzymol.* **31**, 6–40
11. Wattiaux, R., Wattiaux-de Coninck, S., Ronveaux-Dupal, M.-F. & Dubois, F. (1978) *J. Cell. Biol.* **78**, 349–368
12. Otter, R., Reiter, R. & Wendel, A. (1989) *Biochem. J.* **258**, 535–540
13. Burk, R. F., Mackinnon, A. M. & Simon, F. R. (1974) *Biochem. Biophys. Res. Commun.* **56**, 431–436
14. Hawks, W. C., Wilhelmsen, E. C. & Tappel, A. L. (1985) *J. Inorg. Biochem.* **23**, 77–92
15. Beilstein, M. A. & Whanger, P. D. (1985) *Biochem. Arch.* **1**, 153–162
16. Ganther, H. E. (1971) *Biochemistry* **10**, 4089–4098
17. Ching, W.-M. (1986) *Arch. Biochem. Biophys.* **244**, 137–146
18. Wheatley, D. N. & Inglis, M. S. (1986) *Curr. Top. Cell. Regul.* **28**, 107–182
19. Behne, D., Duk, M. & Elger, W. (1986) *J. Nutr.* **116**, 1442–1447

Received 28 April 1988/24 June 1988; accepted 13 July 1988