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Hepatic iodothyronine 5′-deiodinase

The role of selenium

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Selenium (Se) deficiency decreased by 8-fold the activity of type I iodothyronine 5′-deiodinase (ID-I) in hepatic microsomal fractions from rats. Solubilized hepatic microsomes from rats injected with ^{75}Se-labelled Na_2SeO_3 4 days before killing were found by chromatography on agarose gels to contain a ^{75}Se-containing fraction with ID-I activity. PAGE of this fraction under reducing conditions, followed by autoradiography, revealed a single ^{75}Se-containing protein (M_r 27400 ± 300). This protein could also be labelled with ^{125}I-bromoacetyl reverse tri-iodothyronine, an affinity label for ID-I. The results suggest that hepatic ID-I is a selenoprotein or has an Se-containing subunit essential for activity.

INTRODUCTION

The only well-characterized function for selenium (Se) in animal cells is as a component of the glutathione peroxidase [1,2], although at least 13 selenoproteins have been identified in animal cells. It has been suggested that some of these have a role in endocrine-organ metabolism, particularly in the thyroid gland [3,4].

Thyroxine (T_4) is produced in the thyroid gland and is considered to be a prohormone, since it is converted into the more metabolically active 3,3′,5-tri-iodothyronine (T_3) by iodothyronine deiodinases (IDs) in the organs of the body. Two forms of ID can perform this 5′-monodeiodination of T_4 to T_3. The type I enzyme (ID-I) is present in liver and kidney and is involved in the production of plasma T_3, whereas the brain, pituitary and brown adipose tissue contain the type II enzyme (ID-II). Se deficiency inhibits the conversion of T_4 into T_3 by both ID-I and ID-II [5–8]. Loss of ID activity appears to be responsible for the impaired thyroid-hormone metabolism observed in Se-deficient animals, and we have therefore suggested that ID-I and ID-II are Se-containing or Se-dependent proteins [5,9]. Here we produce further evidence of an association of Se with hepatic ID-I.

MATERIALS AND METHODS

Reagents

^{75}Se-labelled Na^2SeO_3 (sp. radioactivity 2.67 μCi/μg), ^{125}I-labelled reverse tri-iodothyronine (rT_3; sp. radioactivity < 1200 μCi/μg) and Hyperfilm-MP were from Amersham International (Amersham, Bucks., U.K.). Amino acids for rat diets were supplied by Forum Chemicals, Reigate, Surrey, U.K. Heat-inactivated donor horse serum was obtained from Flow Laboratories, Rickmansworth, Herts., U.K. All other reagents were from Sigma Chemical Co. or BDH (both of Poole, Dorset, U.K.).

Animals and diets

Weanling male Hooded Lister rats of the Rowett Institute strain were maintained on semisynthetic diets based on amino acids containing either < 0.005 mg of Se/kg (basal; −Se diet') or supplemented with 0.1 mg of Se/kg (as Na_2SeO_3; '+Se diet') [10]. The rats were individually housed in plastic cages with stainless-steel grid tops and floors; food and distilled water were available *ad libitum*. After 6 weeks, rats were anaesthetized with diethyl ether, and blood was collected into heparinized tubes by cardiac puncture. Thereafter livers were perfused via the hepatic portal vein with 0.15 mol of KCl/l at 4 °C to remove residual blood. The livers were then homogenized in potassium phosphate (0.125 mol/l)/EDTA (1 mmol/l), pH 7.4, using four passes of a Teflon-pestle/glass-body Potter–Elvehjem homogenizer. Homogenates were centrifuged at 12000 *g* for 20 min (at 4 °C), and the resulting supernatants were further centrifuged at 105000 *g* for 60 min (at 4 °C) in an MSE 65 high-speed ultracentrifuge. The microsomal fraction was resuspended to a final protein concentration of approx. 10 mg/ml in the homogenization buffer.

For '*in vivo*' labelling studies, male Hood Lister rats (250 g body wt.) were given intraperitoneal injections of 250 μCi of ^{75}Se as Na_2SeO_3 containing 93.6 μg of Se. After 4 days the livers were removed under diethyl ether anaesthesia, and microsomal fractions were prepared as described above.

Enzyme assays

After activation with the 105000 *g* supernatant from liver homogenates, the ID-I activity in microsomal fraction was determined by using the method described by Sawada *et al.* [11], except that horse rather than human serum was used to precipitate thyroid hormones. Glutathione peroxidase activity was assayed using H_2O_2 (0.25 mmol/l) as substrate in the presence of GSH (5 mmol/l) [10].

Affinity labelling of ID-I

N-Bromoacetyl-[^{125}I]rT_3 was synthesized from [^{125}I]rT3 using the method of Nikodem *et al.* [12] for T_3. After synthesis the ^{125}I-containing affinity label was stored in ethyl acetate/methanol (7:3, v/v). The solvent was evaporated from 0.6 μCi of affinity label under a stream of dry N_2 before the addition of the ID-I-containing fraction and incubation of the mixture for 15 min at 37 °C with dithiothreitol (DTT; 3 mmol/l) and EDTA (3 mmol/l).

Abbreviations used: ID(-I), (type I) 5′-deiodinase; T_4, thyroxine; T_3, 3,3′,5-tri-iodothyronine; rT_3, reverse tri-iodothyronine (3,3′,5′-tri-iodothyronine); DTT, dithiothreitol.

Solubilization of ID-I

Microsomal suspension (2 ml) was mixed with 1 ml of potassium phosphate (0.125 mol/l), pH 7.4, containing CHAPS (18 g/l), EDTA (1 mmol/l) and DTT (3 mmol/l). Thereafter the mixture was centrifuged at 105000 *g* for 1 h at 4 °C and the supernatant, which contained 70% of the original microsomal ID-I activity, was retained for subsequent fractionation.

Fractionation of ID-I

The solubilized microsomal fraction (2 ml) was applied to an 85 cm × 2 cm column packed with Sepharose CL-6B. The column was eluted with potassium phosphate (125 mmol/l)/DTT (1 mmol/l)/CHAPS (1.0 g/l)/EDTA (1 mmol/l), pH 7.4; 2 ml fractions were collected. Each fraction was assayed for ID-I activity, glutathione peroxidase activity, total protein and ^{75}Se radioactivity. Two 0.5 ml subsamples were taken from the column fraction containing maximum ID activity (tube 26; see Fig. 1 below). One subsample was allowed to react with label *N*-bromoacetyl-[^{125}I]rT_3 for 15 min then both subsamples were dialysed for 18 h twice against 500 ml of Tris (50 mmol/l)/EDTA (0.5 mmol/l)/DTT (1.0 mmol/l), pH 7.4. Thereafter the samples were concentrated by using Centricon filters (10000-M_r cut-off; Amicon) and treated with glycerol, SDS and DTT to give final concentrations of 20% (v/v), 4% (w/v) and 50 mmol/l respectively. The solution was then boiled for 2 min and subjected to electrophoresis [13] (1.5 mm; 12% acrylamide gel/4% stacking gel) using a Bio-Rad Miniprotean II system (Bio-Rad, Watford, Herts., U.K.). Gels were fixed, stained with Coomassie Blue and dried under vacuum. Proteins labelled with ^{75}Se or ^{125}I were detected by autoradiography, with exposure being carried out at −70 °C for 72 h. Further quantitative information on the distribution of radioactivity was obtained by cutting gels after autoradiography into 1 mm bands and counting their ^{75}Se or ^{125}I activity using a Packard Cobra γ-radiation counter with channel settings to allow dual-isotope counting.

RESULTS

Microsomal fraction ID-I activity

After 6 weeks of experiment, Se-containing glutathione peroxidase activity was 1.28±0.09 units/mg of protein (means± S.E.M.) in liver from the Se-supplemented group compared with 0.007±0.001 unit/mg of protein in the rats consuming an Se-deficient diet, confirming that the latter animals were Se-deficient.

ID-I activity in liver microsomal fractions from rats which had consumed Se-deficient diet for 6 weeks from weaning was approx. 10% of that in fractions from Se-supplemented control rats with or without activation by liver supernatant (Table 1). The addition of the 105000 *g* liver supernatants from +Se or −Se rats were equally effective in activating the ID-I activity in microsomes from +Se or −Se rats (Table 1). In Hooded Lister rats, maintained on a standard laboratory diet (Labsure, Cambridge, U.K.) and the same age as the Se-supplemented animals, hepatic-microsomal-fraction ID-I activity was 323±8 fmol of iodine liberated/min per mg of protein (mean±S.E.M.) from activation with cytosol. This is similar to the activity in Se-supplemented rats, which is, therefore, not abnormally elevated (Table 1).

Solubilization and chromatography of microsomal-fraction ID-I activity

When the microsomal fraction was solubilized with CHAPS (6 mg/ml) in the presence of DTT (2 mmol/l) and centrifuged, 70% of the initial ID-I activity was recovered in the supernatant. It was established in preliminary studies that this was the optimal concentration of CHAPS for solubilization of ID-I activity. When the solubilized hepatic microsomal fraction from ^{75}Se-treated rats was subjected to exclusion chromatography on an agarose-gel column, two peaks of ^{75}Se radioactivity were obtained

Table 1. ID-I activity in microsomal fractions from Se-deficient and Se-supplemented rats

Hepatic microsomal and 105000 *g* supernatant fractions were prepared from +Se and −Se rats as described in the Materials and methods section. The Table shows microsomal ID-I activity in the presence and absence of supernatant. Results are means±S.E.M. for three rats/group. Significant differences from the +Se activity are shown: * $P < 0.001$.

Activator	Microsomal fraction...	ID-I activity (fmol of iodine liberated/min per mg of protein) +Se	−Se
None		18±2.1	2.1±0.6*
+Se supernatant		342±33	33±27*
−Se supernatant		342±40	44±24*

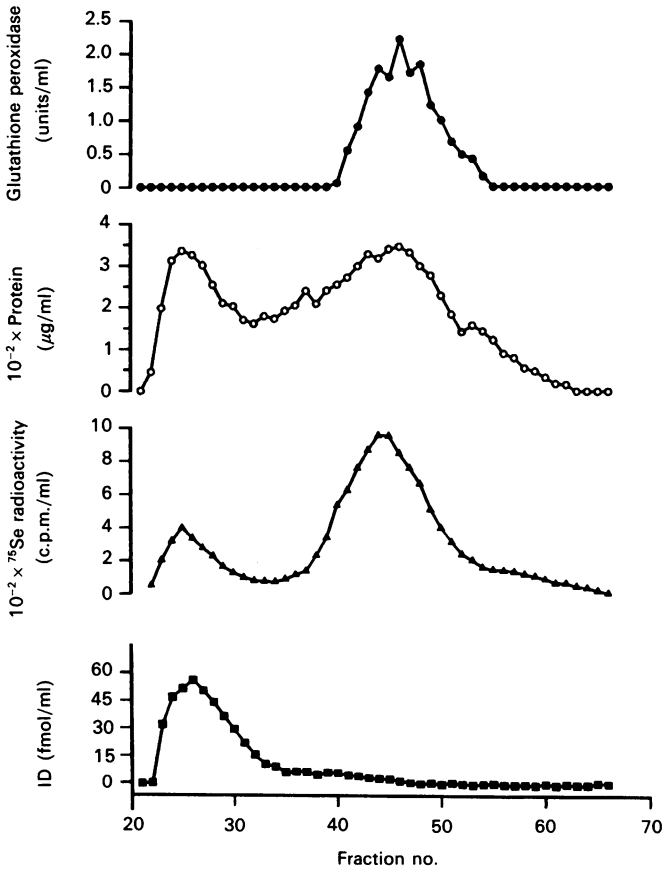

Fig. 1. Chromatography of solubilized ^{75}Se-labelled hepatic microsomes (microsomal fraction) on Sepharose 6B-CL

Hepatic microsomes were prepared from rats injected 4 days previously with ^{75}Se-labelled Na_2SeO_3. The microsomes were solubilized and chromatographed on Sepharose 6B-Cl; thereafter ID-I and glutathione peroxidase activity were determined in 2 ml column fractions, as described in the Materials and methods section.

(Fig. 1). One was eluted at the void volume and corresponded to the single peak of ID-I activity. The second peak coincided with the single peak of glutathione peroxidase activity. Two samples were taken from the central fraction of the deiodinase peak, and one of these was allowed to react with *N*-bromoacetyl-[^{125}I]rT$_3$ to label the enzyme. The samples were separated by SDS/PAGE and were shown by autoradiography to contain a radioactive band corresponding to a protein with M_r 27400±300 (mean± S.E.M., five determinations). The intensity of this band was considerably increased in the subsample that was treated with the ^{125}I-containing affinity label (Fig. 2). Determination of the radioactivity in subsections of the gels showed that the ^{125}I from the affinity label co-migrated with the ^{75}Se resulting from treatment of the rats *in vivo* (Fig. 3).

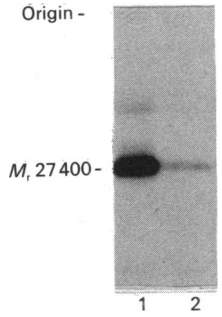

Fig. 2. Autoradiograph of ID-I-containing column fractions after separation of proteins by SDS/PAGE

After treatment of rats with ^{75}Se-labelled Na_2SeO_3 and chromatography of solubilized hepatic microsomal fraction (Fig. 1), the column fraction with the greatest ID-I activity was separated by SDS/PAGE. Lane 2, fraction before reaction with *N*-bromoacctyl-[^{125}I]rT$_3$; lane 1, fraction after reaction with *N*-bromoacetyl-[^{125}I]rT$_3$.

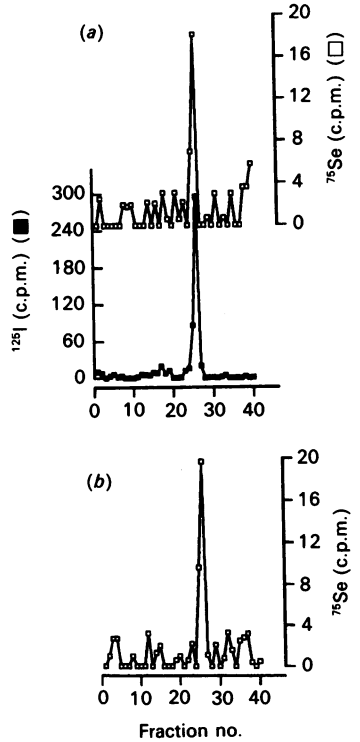

Fig. 3. ^{75}Se and ^{125}I radioactivity in fractions of the polyacrylamide gel used to separate ID-I-containing column fractions

The results were obtained from the gel used to produce the autoradiograph shown in Fig. 2. (*a*) Lane 1; sample from column after reaction with *N*-bromoacetyl-[^{125}I]rT$_3$; (*b*) lane 2; sample before reaction with *N*-bromoacetyl-[^{125}I]rT$_3$.

DISCUSSION

Se deficiency adversely affects thyroid-hormone metabolism and decreases 5'-deiodination of T_4 in tissue homogenates from rats [5–9,14,15]. These changes are consistent with an essential role for Se in the IDs, and we have suggested that these enzymes may be selenoproteins [5,6]. Decreased tissue ID activities in Se deficiency could in theory result from a defect in either the microsomal ID or the cytoplasmic factors [11] necessary for activation of the enzyme. However, the equal abilities of liver supernatants from Se-deficient or Se-supplemented rats to activate ID-I activity in hepatic microsomal fractions (Table 1) shows clearly that the effect of Se deficiency on ID activity occurs in the particulate fraction. This conclusion is supported by decreased ID-I activity in hepatic microsomes from Se-deficient rats (Table 1).

More direct evidence for an association between Se and ID-I was obtained on exclusion chromatography of solubilized hepatic microsomes from ^{75}Se-treated rats, since both ^{75}Se and ID-I activity were present in the fraction eluted at the void volume (Fig. 1). The M_r of this ^{75}Se-labelled protein as established by SDS/PAGE was 27400±300 and therefore similar to that of hepatic ID-I [16]. Since hepatic ID-I represents only 0.01% of the total protein in the microsomal fraction [16] and is, moreover, very labile, it is difficult to purify by conventional techniques [17]. However, it was possible to overcome these problems by use of an ^{125}I-containing affinity label to determine directly the relationships between ID-I and Se in the partially purified fraction. The occurrence of ^{75}Se, from '*in vivo*' labelling of the enzyme, and of ^{125}I, from '*in vitro*' affinity labelling, in the same fraction separated by electrophoresis provides convincing evidence that ID-I is indeed an Se-containing enzyme. This therefore extends the previous indirect evident based on greatly decreased ID activity in Se-deficient rats [5–7]. The stoichiometry of Se incorporation into hepatic ID-I and elucidation of its role in the expression of enzyme activity will have to await purification of the protein in sufficient quantities for Se analysis and mechanistic studies.

By use of a ^{75}Se-labelling procedure, Behne and co-workers [3,4] have identified up to 13 selenoproteins in homogenates of rat tissues. One of these, selenoprotein 7, had M_r 27800±400 and was found in liver, kidney and thyroid gland. It is therefore similar in M_r to the Se-containing protein which bound the ID affinity label, bromoacetyl-rT$_3$ (Fig. 2), and also has similar tissue distribution to ID-I [18].

The present results support the conclusion that hepatic ID-I is an Se-containing protein. The loss of hepatic and other ID-I activities in Se deficiency explains the associated adverse effects on thyroid-hormone metabolism. Since the activities of the two Se-containing enzymes glutathione peroxidase and ID-I are lost at similar stages of Se depletion [7], changes in thyroid-hormone metabolism as well as impairment of peroxide metabolism should be considered as the origins of the biochemical, metabolic and pathological consequences of Se deficiency.

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