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Occurrence of low-molecular-weight and high-molecular-weight selenium compounds in fish

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Muscle from four species of fish, plaice (*Pleuronectes platessa*), mackerel (*Scomber scombrus*), cod (*Gadus morhua*), and herring (*Clupea harengus*), was analyzed for selenium in intact tissue and soluble and insoluble fractions obtained by centrifugation at 850 × *g* and 100 000 × *g*. In cod and herring muscle, 74–81% of the selenium was found in the 850*g* pellet, and the rest was mainly in the 100 000 × *g* supernatant (14–24%). For plaice and mackerel, larger proportions were found in the latter fraction (29–60%). Gel chromatography of the soluble fraction was performed to study the occurrence of low- and high-molecular-weight forms of selenium. The 100 000*g* supernatant from plaice and mackerel contained a high proportion of low-molecular-weight selenium compounds but the pattern of different peaks was not the same in the two species. The 100 000*g* supernatant from cod contained mainly high-molecular-weight selenium compounds. Storage of cod or herring in the cold for one week did not significantly affect the proportion of low-molecular-weight selenocompounds. The results indicated that the distribution of low- and high-molecular-weight selenocompounds varied in different species of fresh fish and of fish obtained frozen.

INTRODUCTION

Since the implication of selenium status as a risk factor for disease is already known, it is important to investigate the supply and uptake of selenium from different foods. For most foods, only the total content of selenium (Koivistoinen, 1980; Combs, 1988) and its regulation by soil composition, fertilization procedures, and other factors have been measured (Gissel-Nielsen *et al.*, 1984; Srikumar & Öckerman, 1991). Since animal studies indicate that the bioavailability of selenium varies with its chemical form (Mutanen, 1986), it is necessary to explore the type of selenium compounds occurring in foods. In Sweden, fish is a selenium-rich food (Thorngren & Åkesson, 1987; Svensson *et al.*, 1992); therefore in this study, the fractionation of different selenium compounds in fish was performed.

MATERIALS AND METHODS

Chemicals

Chromatographic media were obtained from Pharmacia, Uppsala, Sweden. (^{75}Se) selenite was obtained from Amersham International, Amersham, UK. Reagents for the assay of glutathione peroxidase were obtained from Boehringer-Mannheim, Mannheim, Germany.

Preparation of food samples

Samples of plaice and mackerel were obtained from a local shop. Fresh herring and cod were obtained on the day of capture from local fishermen in the most southerly part of Sweden on the Baltic Sea. The samples were immediately transported to the laboratory in an ice-box. On the same day, muscle from ten fish of each species was dissected and pooled. One part of the sample was immediately homogenized as described below, and another part was stored at +4°C for one week prior to homogenization. Samples of frozen fillets of herring, cod, mackerel (captured in the North Sea), and plaice were obtained from a local shop. They were thawed, and then ten samples were pooled, homogenized, and subjected to ultracentrifugation as described below.

The fish-muscle samples were homogenized in 4 volumes of 10mM Tris-HCl (pH 7·4) by using a Waring blendor type of homogenizer. The homogenate was centrifuged at 850 × *g* for 10 min at 4°C, and the supernatant was then centrifuged at 100 000 × *g* for 1 h. The fractions were kept frozen at −20°C until analyzed.

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Chromatography and analytical procedures

The 100 000 × *g* supernatant obtained by ultracentrifugation of fish-muscle homogenates was fractionated by gel chromatography on Sephacryl S-200 Superfine and Sephadex G-25 equilibrated in 10mM Tris–HCl (pH 7·8), 40mM ammonium acetate. The eluate was analyzed for absorbance at 280 nm and for selenium by graphite-furnace atom-absorbance spectrophotometry with Zeeman background correction (Borglund *et al.*, 1988). To the applied sample, ^3H_2O was added, and the total column volume was indicated by measuring ^3H in the eluted fractions. Selenium in particulate and soluble fractions was determined by hydride-generation atom-absorption spectrophotometry (Srikumar & Öckerman, 1991). Glutathione-peroxidase activity was measured according to Günzler *et al.* (1974). For thin-layer chromatography, cellulose plates (10 × 20 × 0·01 cm, Merck, Darmstadt, Germany) were developed in chloroform : methanol : 17·5% ammonia /41 : 41 : 18/ (Yamamoto *et al.*, 1988). After staining with iodine, spots were scraped into tubes and eluted with water followed by selenium analysis by using the graphite-furnace method.

RESULTS

Selenium in particulate and soluble fractions from fish

The samples of fish obtained from different sources contained 0·2–0·4 μg selenium/g, except for one higher value for a sample of plaice (Table 1). The main fractions obtained at centrifugation were the 850 × *g* pellet and the 100 000 × *g* supernatant. Between 12 and 60% of the recovered selenium was found in the latter fraction, and the lowest values were found for cod and herring.

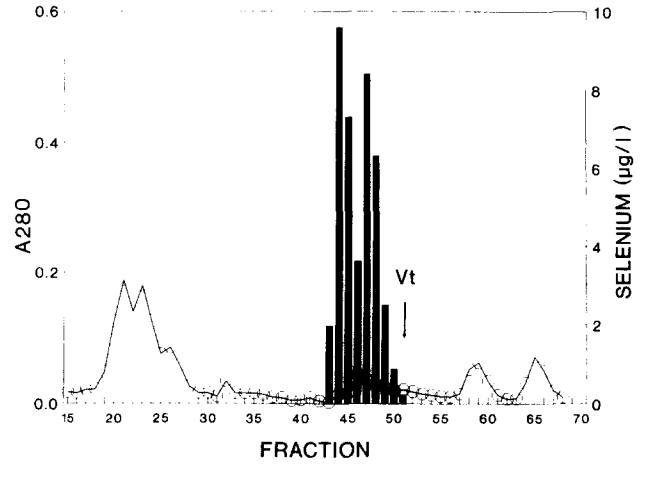
Table 1. Selenium content in fractions prepared from different fish[a]

Fish	Whole food μg Se/g (w/w)	Fraction (% of recovered Se)		
		850*g* Pellet	100 000*g* Pellet	100 000*g* Supernatant
Fresh fish				
Plaice	1·76	nd	nd	>50
Mackerel	0·42	40	nd	60
Herring				
fresh	0·31	77	6	18
stored[b]	0·33	73	5	22
Cod				
fresh	0·40	81	5	14
stored[b]	0·41	85	3	12
Frozen fish				
Plaice	0·29	69	2	29
Mackerel	0·34	54	1	45
Herring	0·23	74	1	24
Cod	0·22	78	3	19

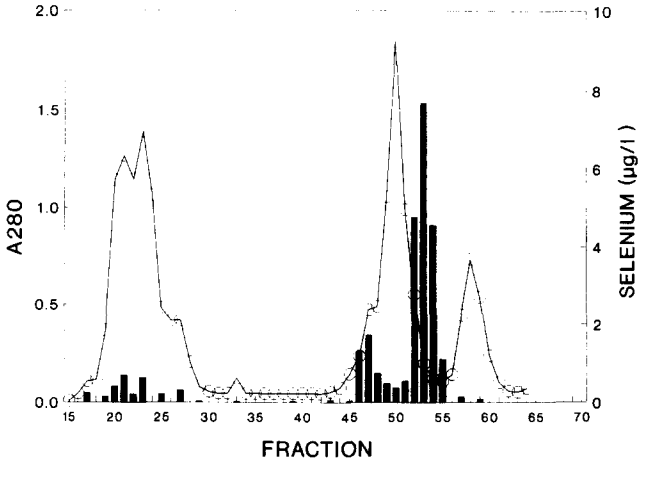
[a] Homogenized fish was subjected to centrifugation at 850*g* and then at 100 000*g*. The analyses were done on pooled samples of ten fish, except for fresh plaice and mackerel, which were single samples.
[b] Stored in the cold for one week.

Distribution of selenium in 100 000 × *g* supernatants

Gel chromatography of the soluble fraction from plaice showed that most of the protein eluted in fractions 20–25, but no selenium was detected in these fractions (Fig. 1(a)). Most of the recovered selenium was located in two peaks in fractions 43 and 46, eluting before the total column volume. Similarly, the soluble fraction from mackerel contained only a little selenium in the high-molecular-weight fraction (Fig. 1(b)), but it showed another pattern for the low-molecular-weight compounds with peaks in fractions 46, 47, and 53. The resolution of different selenium peaks from the mackerel sample was not improved by using Sephadex G-25. When the soluble fractions were treated with cold 6% TCA, 92% of the selenium recovered from plaice supernatant was still soluble, as was 59% of that from mackerel supernatant, which is compatible with the data in Fig. 1.



(a)



(b)

Fig. 1. Gel chromatography of 100 000 × *g* supernatant from plaice (a) and mackerel (b) on Sephacryl S-200. Symbols: Circles, A280; bars, selenium. The arrow indicates the total column volume.

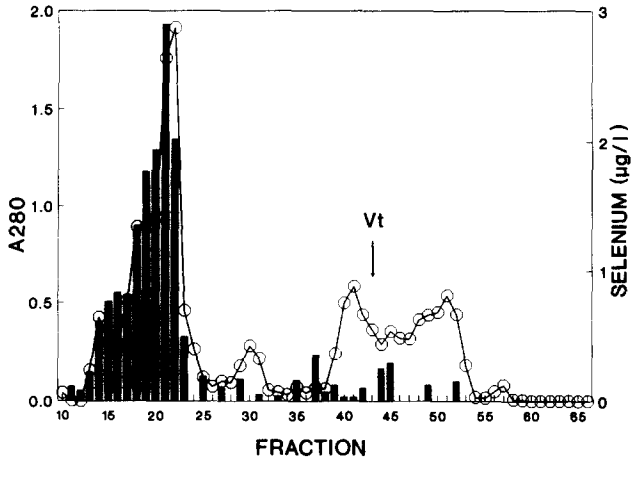
Analysis of the soluble fraction from fresh cod showed a major selenium peak in the protein fraction and only trace amounts of selenium in the low-molecular-weight fraction (Fig. 2(a)). Herring-muscle supernatant yielded one major selenium-containing peak eluting among proteins and another among low-molecular-weight compounds (Fig. 2(b)).

To investigate the possibility that the low-molecular-weight selenium compounds found in fish were formed by degradation of selenoproteins or other high-molecular-weight compounds during storage, the distribution of selenium in these samples was compared with that in samples from the same batch stored for one week in the refrigerator prior to homogenization and centrifugation. Chromatography of supernatant from stored cod and herring by means of Sephadex G-25 did not show any significant increase in low-molecular-weight selenium compounds as compared with the fresh samples, which indicated that any changes occurring during storage in the cold was not the main cause for the differences in selenium distribution in different fish.

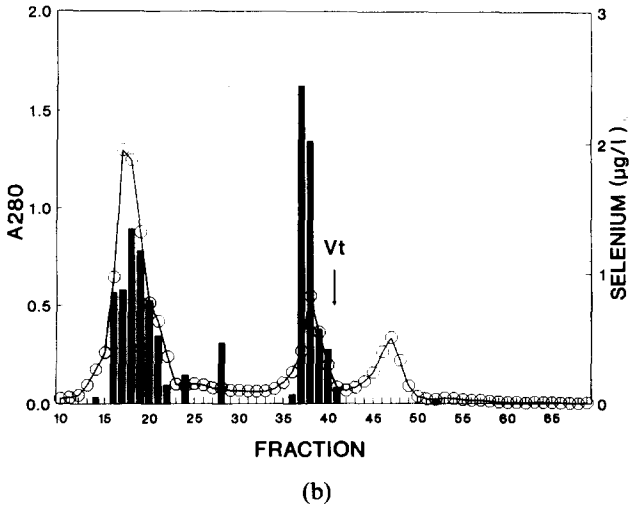
Distribution of soluble selenium compounds in frozen fish

Since a large proportion of the fish provided to the consumer is supplied frozen, commercially obtained frozen-muscle samples of the same four species were studied. On gel chromatography of the soluble fraction from plaice, small amounts of selenium were observed in the major protein fraction, and most of the selenium was in more than one peak, migrating close to the total column volume (Fig. 3(a)). The location of the latter peak was similar to that found in the sample shown in Fig. 1(a). Moreover, for frozen mackerel, most of the soluble selenium was located in two peaks close to the total volume (Fig. 3(b)), which was analogous to the two low-molecular-weight peaks in the sample shown in Fig. 1(b). The distributions of selenium in soluble fractions from frozen cod and herring were similar to those found for fresh fish (Fig. 2).

In the experiment involving the use of frozen fish, the activity of glutathione peroxidase was measured in the soluble fractions (Table 2). Mackerel supernatant had the highest values of selenium and enzyme activity,

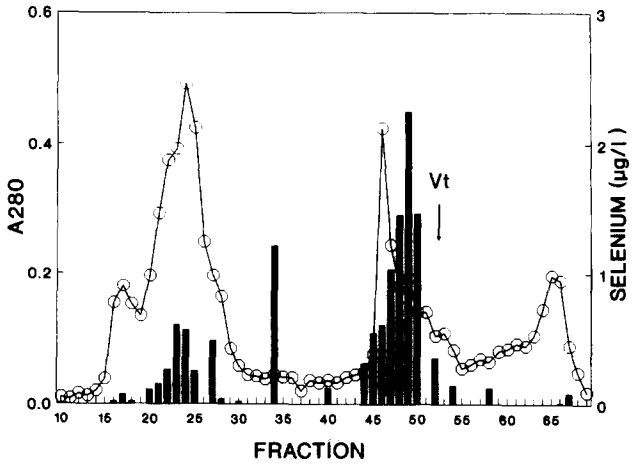


(a)

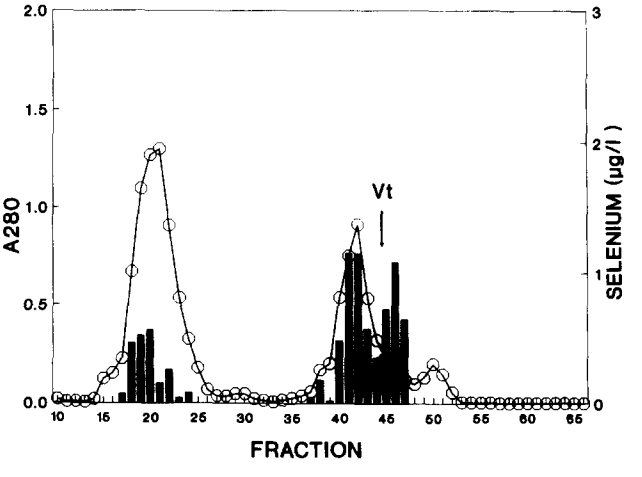


(b)

Fig. 2. Gel chromatography of 100 000 × *g* supernatant from fresh cod (a) and herring (b) on Sephacryl S-200. Symbols as in Fig. 1.



(a)



(b)

Fig. 3. Gel chromatography of 100 000 × *g* supernatant obtained from frozen plaice (a) and mackerel (b) on Sephacryl S-200. Symbols as in Fig. 1.

Table 2. Activity of glutathione peroxidase in 100 000 g supernatants from fish muscle

	Plaice	Mackerel	Cod	Herring
Glutathione peroxidase (μmol/min.litre)	9	22	<2	14

but otherwise there was no clear relation between enzyme activity and selenium concentration.

Identity of low-molecular-weight selenium compounds

Several simple selenium compounds were run in the Sephacryl S-200 system to obtain information on the identity of the compounds in fish samples. Sodium selenate and ^{75}Se-labelled sodium selenite eluted well after the total column volume, which indicated that they were not identical to the low-molecular-weight selenium compounds in fish supernatant. The same finding was made when (^{75}Se) selenite was added to the 100 000 × *g* supernatant of mackerel before chromatography. Selenocystine eluted just before the total volume and when chromatographed in buffer containing 1mM mercaptoethanol, it eluted later than the selenium peaks from mackerel supernatant (Fig. 1(b)). When mackerel supernatant was chromatographed in the presence or absence of mercaptoethanol, no difference in the retention volume of selenium compounds was observed. Selenomethionine eluted just before the total volume, and some of the selenium from fish eluting at this position may be identical to selenomethionine. The fractions containing the two low-molecular-weight selenium peaks from plaice supernatant (Fig. 1(a)) were pooled, lyophilized, and analyzed by thin-layer chromatography. The first peak chromatographed with selenocystine and the second one with selenomethionine.

DISCUSSION

In mammalian tissue, several specific selenoproteins, mainly from the glutathione peroxidase family, have been found (Burk & Hill, 1993). Of the selenium not located in such proteins, the major part seems to be bound to proteins (Borglund *et al.*, 1988), probably as unspecifically incorporated selenoamino acids. Such selenium-containing proteins may be regarded as biologically inactive storage forms, which may be selenium carriers in foods. Low-molecular-weight selenium compounds function as intermediaries in the synthesis of selenoproteins (Ganther, 1984), but they have not been unequivocally demonstrated in chemical amounts in living tissue.

Only a small amount has previously been published about the molecular forms of selenium in fish. Cappon and Smith (1981, 1982) reported that 15—35% of fish selenium was in the form of selenate after acid digestion, the rest being SeII and SeIV. It is, however, uncertain if the valencies found after digestion represent those in undigested material. More interestingly, these authors found that 55–60% of total selenium was water-extractable and that only a small proportion of the extracted selenium was precipitated with TCA. Our findings for plaice and mackerel are consistent with these observations, but the data for cod and herring (not studied by Cappon and Smith) showed a lower proportion of soluble selenium. The present chromatographic experiments gave no indication that fish contained free selenate taken up from surrounding water, but the possible existence of bound selenate cannot be excluded. Braddon-Galloway and Sumpter (1986) reported that tuna liver contained a low-molecular-weight selenoprotein (approximately 2000), but this protein has not been demonstrated in other tissues.

The present study demonstrated glutathione-peroxidase activity in the soluble fraction from fish, confirming data from measurements in crude extracts from other fish species (Smith & Shrift, 1979), but the proportion of fish selenium accounted for by this and other selenoenzymes is not known. Glutathione-peroxidase activity may be one of the factors protecting fish tissue against oxidative deterioration both *in vivo* and *post mortem*, as hypothesized from studies in yeast (Galiazzo *et al.*, 1987). The present experiments suggest that selenite, selenate, and selenocystine are not dominating components among the low-molecular-weight selenium compounds of fish. Maybe selenomethionine is a major component, but this will require further verification. The identification is complicated by the lability of some low-molecular-weight selenium compounds and their possible binding to other components. Previously, selenomethionine has only been identified after hydrolysis of proteins in a few foods by using mass spectrometry (Yasumoto *et al.*, 1988) or chromatography (Olson *et al.*, 1970; Beilstein *et al.*, 1991).

Studies in several animal species have indicated that the bioavailability of selenium from fish is ≤ 50% of that in selenite (Miller *et al.*, 1972; Cantor *et al.*, 1975; Alexander *et al.*, 1983), but, in other studies, values between 50 and 100% were obtained (Ringdal *et al.*, 1985; Mutanen *et al.*, 1986; Hassan *et al.*, 1987). A low bioavailability was also observed for selenium in soluble fish preparations (Miller *et al.*, 1972; Cantor *et al.*, 1975). The divergent results probably reflect the use of different animals and variations in the methods of assessment of bioavailability (Mutanen, 1986). The bioavailability of the selenium fractions observed in the present study needs to be studied.

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