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Separation of soluble selenium compounds in different fish species

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Abstract

Sephacryl S-100 was used for systematic studies on soluble selenocompounds in fish. Commonly-eaten species (mackerel, herring, salmon, rainbow trout, eel, cod, plaice, turbot, flounder and dab) were investigated. The mackerel, herring and the flat fish species contained 0.262–0.498 μg Se/g wet weight whereof 23–34% was soluble. Gel chromatography showed that cod, salmon, rainbow trout and eel had most of the soluble selenium (76–88%) in the high-molecular-weight range (> 10 kDa). For mackerel and herring, about half of the selenium eluted in the high-molecular-weight range and half in the low-molecular-weight range. All flat fish species contained large amounts of low-molecular-weight selenocompounds with an average apparent molecular weight of 2 kDa. This study shows that there are large differences in selenium distribution among fish species.

1. Introduction

Selenium is an essential element and over eleven selenoproteins have been identified in mammals (Johnsson, Åkesson & Alexander, 1997). The functional role of several selenoproteins is still unknown but selenium is essential for at least two groups of enzymes — glutathione peroxidases and iodothyronine deiodinases.

Selenium can be ingested in different forms. The inorganic forms selenite and selenate are found in water or diet supplements. Selenomethionine and selenocysteine in selenoproteins mainly come from plant and animal foods. The insertion of selenocysteine into proteins is genetically encoded and in mammals all proteins with selenium in a functional role contain the element in this form (Thomson, 1998). Selenomethionine is, on the other hand, randomly incorporated into methionine-containing proteins.

In Sweden the recommended daily intake of selenium is 40 μg for females and 50 μg for men (SNR, 1997). A majority of the population have an intake below these values (Becker, 1994), but compared to other European countries the selenium status for Swedish adults is in the middle of the observed range (van Dokkum, 1995). A high selenium intake is believed to reduce the risk for some forms of cancer and for cardiovascular disease. For example, selenium supplementation to patients with a history of carcinomas of the skin gave a reduced incidence of other kinds of cancer (Clark et al., 1996).

In Sweden, fish contain higher amounts of selenium than most other foods and an increased intake of fish could be one way to raise the selenium intake. However, several studies indicate a low availability of selenium from fish. In one study (Thorngren & Åkesson, 1987), human subjects increased the fish intake by 150–200 g/day and this gave a raised mean plasma selenium level but only with about 13%. In another study, plasma selenium was found to increase slightly with increasing fish consumption but no increase in glutathione peroxidase or selenoprotein P was observed (Huang, Åkesson, Svensson, Schütz, Burk & Skerfving, 1995). Meltzer, Bibow, Paulsen, Mundal, Norheim and Holm (1993) compared supplementation of selenium in the form of wheat or fish and found that wheat increased the plasma selenium whereas no effect was observed after increased fish intake. Further evaluation of the use of increased fish intake to increase the selenium status of the consumer requires knowledge of both the amount, the chemical form (speciation) and the bioavailability of selenium.

Several chromatographic methods can be used for speciation of selenium compounds in fish. Since previous data (Åkesson & Srikumar, 1994) indicate that

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some fish (e.g. flat fish) may contain significant amounts of unknown low-molecular-weight selenocompounds, one purpose of this study was to explore gel chromatography systems suitable for the separation of small molecules. A series of chromatographic supports, based on highly cross-linked porous agarose beads to which dextran has been covalently bonded (Superdex), are commercially available and to our knowledge they have not previously been investigated with respect to separation of selenocompounds. The second purpose of the study was to investigate selenium forms in different fish species with focus on commonly-eaten species and on different flat fish species, which have not been studied previously.

2. Materials and methods

2.1. Materials

Frozen homogenates of fish fillets were obtained from DLO Netherlands Institute for Fisheries Research, IJmuiden, The Netherlands [plaice (*Pleuronectes platessa*), eel (*Anguilla anguilla*), cod (*Gadus morhua*)] and from the Institute of Nutrition, Bergen, Norway [rainbow trout (*Oncorhynchus mykiss*), salmon (*Salmo salar*)]. Herring (*Clupea harengus*) and mackerel (*Scamber scombrus*) were bought fresh in a local fish shop (mackerel had been collected outside the western coast of Sweden and herring outside the southern coast). Fresh plaice, turbot (*Psetta maxiama*), flounder (*Platichthys flesus*) and dab (*Limanda limanda*), caught outside the western coast of Sweden, were obtained by arrangement with local fishermen. The fresh fish were immediately frozen and some days later thawed and the fillets removed, homogenized individually, and then frozen again.

2.2. Extraction of fish

The homogenate was allowed to thaw at room temperature on a plate covered by a plastic film. The thawing period varied from 2 to 4 h, depending upon the different fish samples. The homogenate was distributed in centrifuge tubes (50 ml) and one volume of buffer (20 mM Tris–HCl (pH 7.5), 0.15 M ammonium acetate) was added. A Polytron homogeniser (speeds 4,5,6; 30 s each) was used to homogenise the samples to a uniform consistency (Shen, Hoek van Nieuwenhuizen & Luten, 1997). The sample was centrifuged (48 000 *g* for 30 min, BECKMAN model J2-21) at 4°C and the supernatant and residue were collected and weighed. The protein content was measured in the homogenates, the residues and the supernatants according to the Kjeldahl method (Kjeltec Auto 1030 Analyser, Höganäs, Sweden). Selenium was determined in the samples after digestion with perchloric acid and nitric acid followed by hydride generation atomic absorption spectrophotometry (Srikumar, 1993).

2.3. Gel chromatography

Two prepacked columns from Pharmacia were used: Hi Prep 16/60 Sephacryl S-100 (fractionation MW range 1–100 kDa) and Hi Load 16/60 Superdex 30 pg (range 1–10 kDa). The total column volume was 120 ml and the eluent was the same buffer as was used for the fish extraction. Calibration of the columns was done with a molecular weight marker kit from Sigma plus cyanocobalamin. Seleno-L-methionine and seleno-DL-cystine (Sigma) were also applied separately. To be able to detect selenium in the collected fractions, a relatively large amount of fish supernatant (2.35 ml) was applied to the column, in a cold room. The supernatant obtained from eel was very viscous and had to be diluted with buffer 1:1 before application. To determine the total volume of the column ^3H_2O (50 μl, 70 000 cpm) was added to all samples. A flow rate of 18 ml/h was used and 60×3-ml fractions were collected. The eluate was analysed for absorbance at 280 nm.

The selenium content in the chromatographic eluates was analysed with a graphite furnace atomic absorption spectrophotomety with Zeeman background correction (Borglund, Åkesson & Åkesson, 1988). The advantage of this method is that a predigestion procedure is not needed and it is also possible to analyse small volumes. To be able to detect the selenium in the collected fractions for cod, salmon, rainbow trout and eel it was necessary to concentrate the fractions by lyophilization before the selenium analysis.

3. Results

3.1. Content of selenium and protein in soluble and insoluble fractions of different fish species

The homogenates from herring, mackerel, plaice, turbot, flounder and dab contained between 16.7 and 22.1 g protein/100 g wet weight (Table 1). The supernatant contained 2.2–4.5 and the residue 19.2–23.0 g protein/100 g. The proportion of protein recovered in the supernatant was from 20 to 23% except for turbot that had 16%. Total recovery of protein was above 90% for all fish species.

The total selenium content in the homogenate was between 0.262 and 0.498 μg/g wet weight in herring, mackerel and different flatfish (Table 2). The insoluble residue contained from 0.268 to 0.498 μg/g whereas it was 0.068 to 0.117 μg/g in the supernatant, mackerel having the highest and dab and herring the lowest amount of selenium in the latter fraction. The recoveries in residue plus supernatant were >90%. The proportion

Table 1
The contents of total, soluble and insoluble protein in different fish species (g/100 g wet weight[a])

Fish species	Total protein (homogenate)	Soluble protein (supernatant)	Proportion soluble protein (%)	Insoluble protein (residue)	Recovery (%)
Herring	17.8	3.1	23	19.5	106
Mackerel	22.1	4.5	21	23.0	97
Plaice	19.2	3.1	20	19.2	91
Turbot	17.2	2.2	16	20.1	98
Flounder	18.1	3.0	20	20.6	96
Dab	16.7	2.6	20	20.0	102

[a] The analysis were done in duplicate on 1–2 individual samples of fish.

Table 2
The contents of total, soluble and insoluble selenium in different fish species (μg/g wet weight[a])

Fish species	Total selenium (homogenate)	Soluble selenium (supernatant)	Proportion soluble selenium (%)	Insoluble selenium (residue)	Recovery (%)
Herring	0.347	0.068	23	0.343	98
Mackerel	0.498	0.117	29	0.498	102
Plaice	0.322	0.088	33	0.307	101
Turbot	0.473	0.093	24	0.495	97
Flounder	0.371	0.101	34	0.380	105
Dab	0.262	0.068	32	0.268	102

[a] The analysis were done in duplicate on 1–2 individual samples of fish.

of soluble selenium was similar in all species, around 30% for mackerel, plaice, flounder and dab and somewhat lower (23–24%) for herring and turbot.

3.2. Comparison of selenium patterns analysed on Sephacryl S-100 and Superdex 30 columns

For Sephacryl S-100, the high-molecular-weight compounds (MW > 10 kDa) eluted in fraction 11–28 while the corresponding fraction interval for Superdex 30 was more narrow, 14–24. Cyanocobalamin (1.35 kDa) unexpectedly eluted after the total column volume on Sephacryl S-100, probably due to some unexplained interaction with the gel.

The selenium standard compounds, selenomethionine and selenocystine, eluted just before the total column volume (fraction 41) on both columns. The peak maximum for Se-methionine was in fraction 39 for both columns while Se-cystine eluted in fraction 38 for Sephacryl S-100 and in fraction 40 for Superdex 30. To evaluate the recovery of selenocompounds using different detection methods, two different amounts of the seleno amino acids were applied, one high (2–3 mg) that could be detected with A280 nm and one low (2 μg) that was analysed for selenium with GFAAS-Zeeman. No difference in the elution was seen when high or low amounts were applied on any column and the amount of selenium corresponded well with the absorbance at 280 nm. On both columns the recovery of selenium was > 90%.

A plaice extract was used to further evaluate the columns, since it contains a high amount of soluble selenium compounds in the low-molecular-weight range. Its elution profile was similar on both columns (Fig. 1). Some selenium was detected in fractions 12–30 but most of the selenium eluted in fractions 32–40 with a selenium peak in fraction 36.

Although the Superdex 30 column may represent an advantage with respect to chromatographic resolution, the difference from that obtained by Sephacryl S-100 was not very marked. Moreover, the Superdex system tended to be more sensitive to repeated runs of samples containing high amounts of protein and lipids resulting in increasing back pressure which, only partially, could be eliminated by column cleaning procedures. It should also be noted that a Superdex column is more expensive than a Sephacryl column. Thus, acceptable resolution of soluble selenocompounds from fish, especially with samples containing much protein and lipids, could be obtained using Sephacryl S-100. For selected samples Superdex 30 may give superior separation.

3.3. Gel chromatography of soluble selenium compounds from fish

To compare the results obtained from different Sephacryl S-100 runs, three different groups of compounds were considered: the compounds of high-molecular-weight (above 10 kDa), the compounds of low-molecular-weight eluted before the total column volume (below 10 kDa)

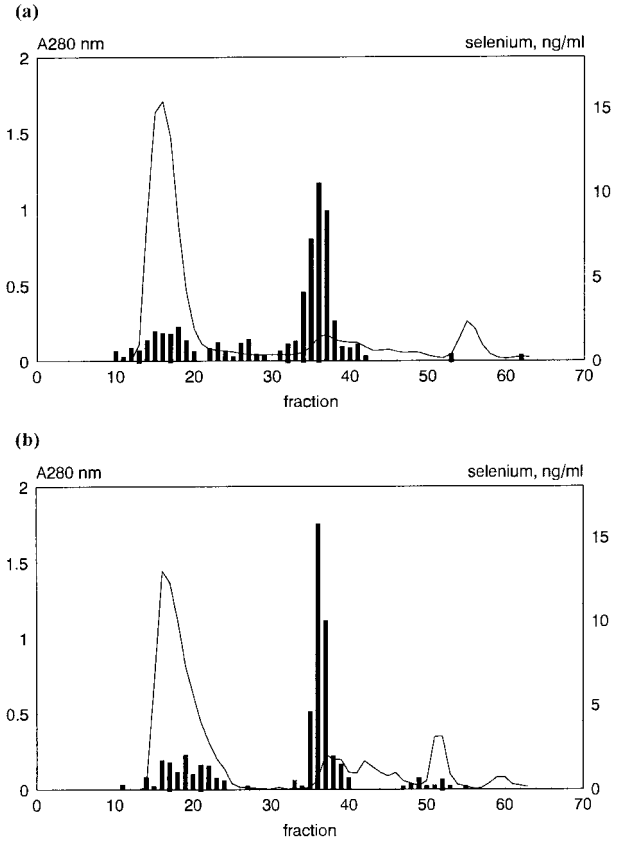


Fig. 1. Gel chromatography of plaice supernatant on (a) Sephacryl S-100 and (b) Superdex 30. Total column volume (V_t): fraction 41, line: A280, bars: selenium.

and the compounds eluted after the total column volume.

The protein distribution profiles were similar for all fish species — one major peak eluting in fractions 12–20 and flatter peaks in the low-molecular-weight region and in the region after the total column volume (Figs. 2–6).

The selenium distributions are presented in Fig. 2–6 and in Table 3. In mackerel, the same amount of selenocompounds was eluted in the high-molecular-weight region as after the total column volume (Fig. 2). Herring contained 61% of its total soluble selenium in high-molecular-weight compounds and almost a quarter of its selenocompounds were eluted after the total column volume (Fig. 2).

For cod, salmon and rainbow trout, about 88% of the selenium was found in fractions containing compounds with a MW > 10 kDa and the remaining 12% was found in the low-molecular-weight fractions (Figs. 3 and 4). Eel also had most of the soluble selenium in high-molecular-weight components (76%), 10% was eluted in low-molecular-weight compounds and 14% eluted after the total column volume (Fig. 4).

Another type of selenium distribution was observed for the flat fish species (Figs. 5 and 6). In plaice, half of the soluble selenium was in high-molecular-weight

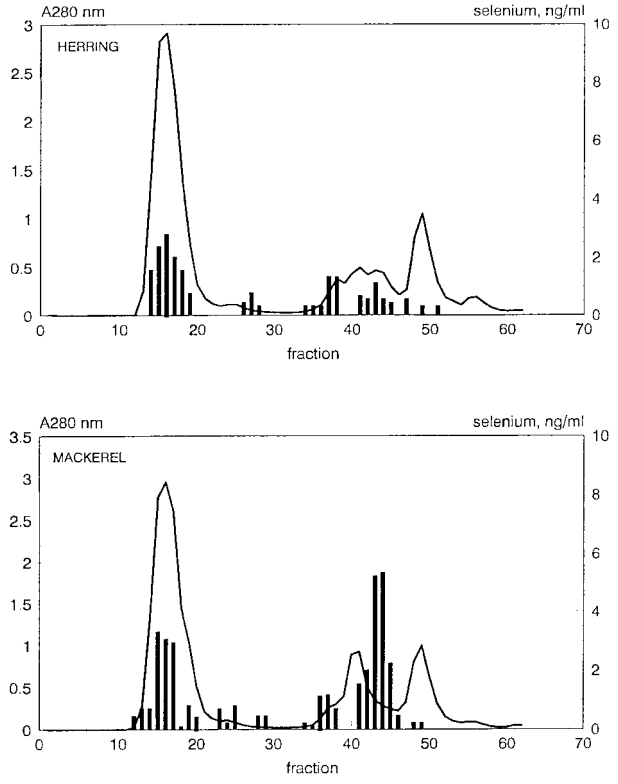


Fig. 2. Gel chromatography of herring and mackerel supernatant on Sephacryl S-100. Total column volume and symbols as in Fig. 1.

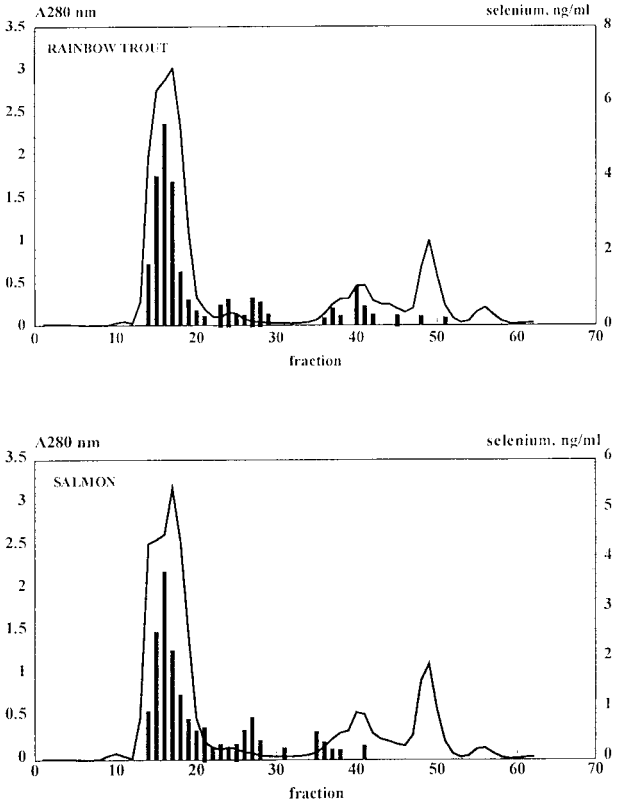
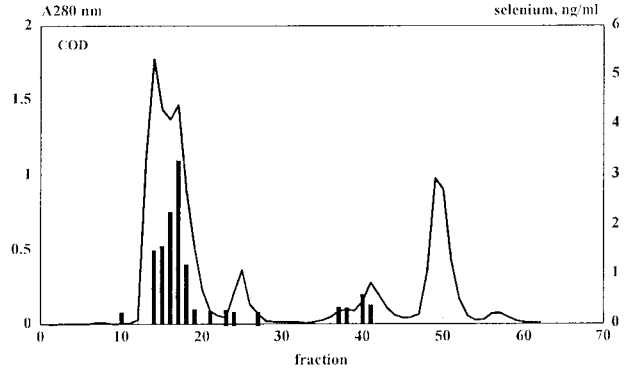


Fig. 3. Gel chromatography of rainbow trout and salmon supernatant on Sephacryl S-100. Total column volume and symbols as in Fig. 1.



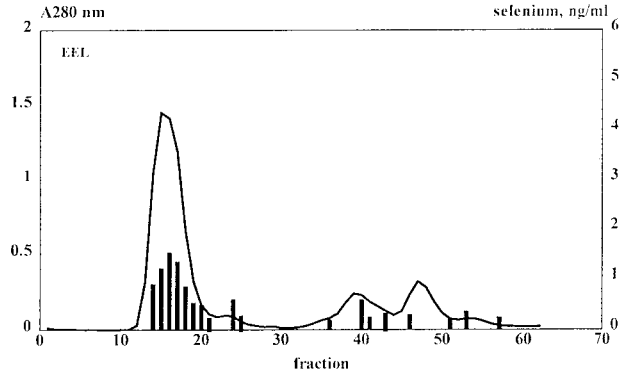
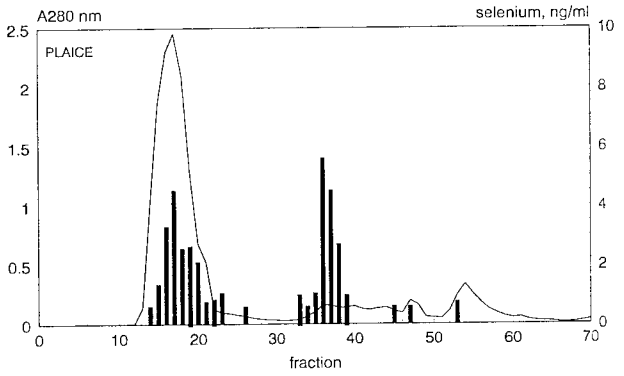


Fig. 4. Gel chromatography of cod and eel supernatant on Sephacryl S-100. Total column volume and symbols as in Fig. 1.



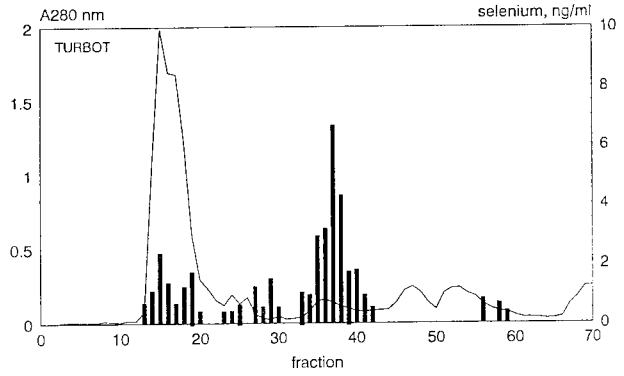


Fig. 5. Gel chromatography of plaice and turbot supernatant on Sephacryl S-100. Total column volume and symbols as in Fig. 1.

compounds and half in low-molecular-weight compounds. The other flat fishes had the majority of their selenium incorporated in low-molecular-weight compounds, for turbot 63%, for flounder 69% and for dab 72%. Almost no selenocompounds were eluted after the total column volume for flounder and dab. All flat fish species had the highest selenium concentration in fraction 36 or 37, indicating that they contain similar selenocompounds with an average apparent MW of about 2 kDa. This peak did not correspond to selenomethionine or selenocystine in the chromatography system and was not detected in other fish species.

4. Discussion

In comparison with our data, a Finnish study (Nuurtamo, Varo, Saari & Koivistoinen, 1980) found lower selenium contents in herring and flounder (0.16–0.24 μg/g) while a German study (Oehlenschläger, 1990) found higher contents in herring and plaice (0.48–0.51 μg/g). A Norwegian study (Lie, Lied, Maage, Njaa & Sandnes, 1994) detected a higher amount of selenium in herring, a similar content in plaice, and lower contents in mackerel and turbot compared with our results. Eurola, Ekholm, Ylinen, Koivistoinen and Varo (1991) found lower selenium levels compared with ours, 0.13–0.242 μg/g, in 20 samples of Baltic herring obtained in different seasons. A large variation in selenium content, 0.15–2.01 μg/g, was found in 31 common fish species sampled in south-eastern Spain but 87% of the fish species had a content below 0.5 μg/g (Diaz-Alarcón, Navarro-Alarcón, López-Martínez & López-García del la Serrana, 1994). A similar value was obtained for fish caught outside Australia; 86% of the fish analysed had a selenium content below 0.5 μg/g (Maher, Baldwin, Deaker & Irving, 1992). The selenium levels found in fish do not seem to correlate with the age of the fish (Frøslie, Norheim & Sandlund, 1985) and no significant seasonal variation in selenium content has been found (Vos & Hovens, 1986). Thus, the differences in selenium levels in studies are probably mostly due to different geographical areas of fish capture.

Only a few other investigations have been done with extraction of soluble selenocompounds from fish. Cappon and Smith (1982) extracted 55% of the selenium from mackerel flesh with water, but only 29% was extracted with the buffer used in the present experiments. Shen et al. (1997) extracted plaice and 33% of the protein and 44% of the selenium was water-soluble.

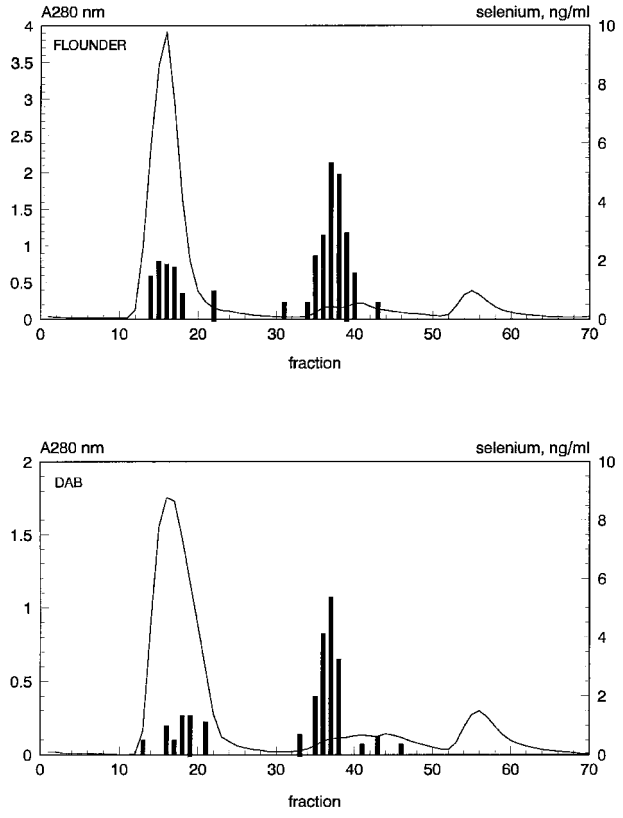


Fig. 6. Gel chromatography of flounder and dab supernatant on Sephacryl S-100. Total column volume and symbols as in Fig. 1.

Corresponding values for herring were 32 and 49%. We again obtained somewhat lower values, for plaice 20 and 35% and for herring 23 and 23% of the protein and selenium, respectively, being soluble. Explanations for the lower extraction rate in the present study could be that buffer was used instead of water and the ratio of fish and buffer was higher (1:1) compared with the other studies that used ratios of 1:2 (Cappon & Smith, 1982) and 1:4 (Shen et al., 1997).

Some of the species studied here have been fractionated by gel chromatography previously (Åkesson and Srikumar, 1994; Shen et al., 1997). In the study by Åkesson and Srikumar (1994), a Sephacryl S-200 column was used (fractionation range: 5–250 kDa). For cod and herring, similar proportions of selenocompounds in high-molecular-weight compounds and in low-molecular-weight compounds as well as in compounds eluted after the total column volume were found. For plaice, this experiment showed that 42% of the selenium was in low-molecular-weight components while, in the previous study, a much higher amount (>70%) was incorporated in these compounds. However, the distribution for the plaice used in Fig. 1 was similar to that in the study by Åkesson and Srikumar (1994). Shen et al. (1997) also analysed cod, herring and plaice using a Sephacryl S-300 column (fractionation range: 10–1500 kDa). They found a higher amount of selenium in the low-molecular-weight region and in compounds eluted after the total column volume for these fish species examined in our study.

Table 3
Distribution of soluble selenium compounds (%)

Fish species	hmw[a] (>10 kDa)	lmw[b] (<10 kDa)	atv[c]
Mackerel	44	12	43
Herring	61	17	22
Salmon	89	11	–
Rainbow trout	87	10	3
Eel	76	10	14
Cod	87	13	–
Plaice	52	42	5
Turbot	29	63	8
Flounder	29	69	2
Dab	26	72	2

[a] hmw: High molecular weight compounds.
[b] lmw: Low molecular weight compounds.
[c] atv: Compounds eluted after the total volume of the column.

The differences in selenium distribution between fish species probably have a number of explanations. One may be difference in muscle composition. For example, active species differ from passive ones in having a higher proportion of dark muscle. It could also be influenced by the variability of the feed (Maher, 1987) but also by differences in selenium metabolism among species. The variation in selenium distribution for the same fish species in different studies can be due to different methodology.

In a recent study on Latvian fish consumers (Hagmar, Persson-Moschos, Åkesson & Schütz, 1998), a positive correlation between the number of fish meals per month and plasma selenium was found, the level being 81% higher for high fish consumers. Thus, this study indicated that fish intake may have a considerable impact on the selenium status. On the other hand, minor or no effects of fish on selenium status have been found in other studies (Huang et al., 1995; Meltzer et al., 1993; Thorngren & Åkesson, 1987). The variable results could, for example, be due to differences in the population groups studied and in the fish species consumed. To further evaluate fish as a source for selenium, more bioavailability studies and speciation studies are needed. One problem with speciation studies is that the level of selenium in chromatographic effluents is often low. In this study we used GFAAS to detect selenium in gel chromatography fractions. It is a sensitive technique but in some samples the amounts of selenium were close to the detection limit, resulting in increased imprecision. In the future we plan to evaluate the use of ICP-MS detection to increase the analytical precision for low selenium concentrations.

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