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Speciation and subcellular location of Se-containing proteins in human liver studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and hydride generation-atomic fluorescence spectrometric detection

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Abstract Speciation of Se-containing proteins in the subcellular fractions of human liver was studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by hydride generation-atomic fluorescence spectrometric (HG-AFS) detection. It was found that about 24 kinds of Se-containing proteins existed in subcellular fractions of normal human liver. The molecular weights (MW) of the subunits were mostly in the range 20–30 kDa and 50–80 kDa. Major Se-containing protein fractions at 61 kDa and 21 kDa are probably selenoprotein P and glutathione peroxidase, respectively. The 54 kDa protein is probably a thioredoxin reductase, which is presented in nuclei, mitochondria, lysosome, microsome and cytosol. We noticed that the Se-containing protein with the lowest MW of 9.3 kDa only existed in lysosome. Most of the proteins have not been identified and would require further investigation to characterize them. The specific subcellular distributions of different Se-containing proteins suggest that they could play important biological roles in each organelle.

Keywords Se-containing proteins · Gel electrophoresis · Speciation · HG-AFS · Human liver

Introduction

Selenium is an essential element in living systems. It may play an important role in many kinds of human diseases, such as cardiovascular diseases [1], certain types of cancer [2], HIV infection [3, 4], Alzheimer's diseases [5], etc. Since bioactivity of selenium is a consequence of individual chemical species, there is considerable interest in the speciation of selenium in the mammalian organism. Inasmuch as selenium was primarily present in the form of selenoproteins in mammalian systems, most studies were focused on the speciation of Se-containing proteins.

Selenocysteine, the 21st amino acid, is specifically inserted by the UGA codon into the active centres of true selenoproteins [6]. Apart from selenoproteins, non-specific Se-binding proteins or Se-containing proteins also exist. The knowledge of the known selenoproteins could not explain all biological functions of selenium and there is an increasing interest in the purification of new selenoproteins in different biological species.

Until now, about 16 selenoproteins with specific functions have been identified [5], including the glutathione peroxidase (GPX) family (EC1.11.1.9) (cellular or classic, extracellular or plasma, phosphorlipid hydroperoxide and gastrointestinal), selenoprotein P, selenoprotein W, iodothyronine deiodinase (5'-ID, EC3.8.1.4) type I, II and III, thioredoxin reductase (TrxR, EC 1.6.4.5), selenophosphate synthetase, prostate epithelial selenoprotein (15 kDaa), DNA-bound spermatid selenoprotein (34 kDa), 18 kDa selenoprotein, and sperm mitochondrial capsule selenoprotein. Beside these, there are still more than 10 selenoproteins to be identified though their physiological roles cannot yet be explained clearly [7].

The tissue distribution of many kinds of selenoproteins varies in different biological species. For example there is a high level of selenoprotein W in sheep heart tissue, but almost none in rat heart tissue [8]. Moreover, different distributions of specific selenoproteins in various tissues of the same species have also been mentioned previously. Even in the same tissues or organs, varying distributions of selenoproteins also existed in rat liver organelles as reported by Behne et al. [9, 10]. Thus, it is necessary to study speciation of selenoproteins in human liver components and to obtain more information for the understanding of the biological function of selenium in human be-

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ings. The purpose of this study is to investigate the subcellular distribution of Se-containing proteins in human liver.

Materials and methods

Chemicals

Frozen human liver samples were obtained from subjects within 24 h of death and were confirmed to be normal specimens by pathological examination and hepatitis B and C antibody tests. SDS-PAGE materials: SDS, acrylamide, *N,N'*-methylene bisacrylamide, TRIS base, glycine, *N,N',N,N'*-tetramethylethylenediamine (TEMED) and ammonium persulfate were purchased from Pharmacia Biotech, Uppsala, Sweden. Beckman L7 centrifuge (Beckman Co.) was used in separating the subcellular fractions. All glassware were immersed into 4 mol L^{-1} nitric acid and precleaned with ultra-pure water.

Preparation of subcellular fractions

About 80 g of liver sample were washed and cut into small pieces with titanium knives. They were homogenized with 3 volumes of 0.01 mol L^{-1} HEPES buffer containing 0.25 mol L^{-1} sucrose at pH 7.4 in a glass-homogenizer. The homogenates were separated into nuclear, mitochondrial, lysosomal, microsomal and cytosolic fractions by differential centrifugation as described previously [11, 12]. All these operations were carried out at 4 °C.

Determination of protein

A small amount of each fraction was diluted and the protein content was analyzed by Coomassie Brilliant Blue as described by Bradford [13] using bovine albumin as standard.

Electrophoresis

SDS-PAGE was run in 12% acrylamide for separation gel and 5% acrylamide for stacking gel and stained with Coomassie brilliant blue R-250 [14]. Each subcellular fraction was homogenized again by ultrasonication. To the samples were added two volumes of sample buffer (0.01 mol L^{-1} pH 8.0 Tris-HCl buffer containing 4% SDS, 10% β-mercaptoethanol, 80% sucrose and 0.04% bromophenol blue) and immersed in a boiling water bath for 3 min. 50 μL of heated sample was immediately applied to a single slot before electrophoresis. Each gel slab contained 12 slots with the same sample. Subsequently, the gel slab was fixed with 50% trichloroacetic acid, stained with Coomassie Brilliant Blue R-250 solution for 3–6 h, followed by destaining for 6–48 h with 7% acetic acid. The molecular weight of each protein band could then be calculated according to the standard curve of purified marker proteins including rabbit phorsphorase (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43.6 kDa), bovine carbonic anhydrase (31.1 kDa), trypsin inhibitor (20.1 kDa) and egg lysozyme (14.4 kDa).

Determination of selenium concentration

The determination of selenium concentration in stained bands from SDS-PAGE gel was performed by HG-AFS using a WPY-2 model atomic fluorescence spectrometer made in China. Relevant protein bands and blank bands from the upper to bottom sides were carefully cut out from the gel. The strips with the same protein band were put together and then digested with a mixture of super-pure nitric acid (3 mL) and perchloric acid (1 mL) at 100 °C for 2–3 h. 1 mL of 5 mol L^{-1} HCl was then added and the mixture was left for an additional 10 min to reduce the existing Se^{VI} to Se^{IV}. Finally, the digested samples were diluted to 10 mL and run on a $NaBH_4$-acid system to detect the H_2Se content. This procedure can decrease the interference from other elements. Quantitative determination of selenium in each subcellular fraction was also performed in the same way. The detection limit was 0.05 ng.

Unfortunately, (until now) there are no SRM and methods suitable for speciation separation. Therefore, the quality assurance was performed by analysing the standard reference material of bovine liver NIST-1577a (National Institute for standard and technology, USA).

Results

The HG-AFS method for selenium detection is of high sensitivity and only needs a small amount of sample. Thus, it is convenient for the determination of Se occurrences in tiny fractions after biological separation. For obtaining accurate and reliable analytical results, the analytical quality assurance was evaluated using SRM NIST 1577a. The average value of five independent determinations was 0.707±0.029 mg kg^{-1}, which was coincident with the certified value (0.71±0.03 mg kg^{-1}) [15]. Thus, during the controlled low temperature digestion procedure, only negligable amounts of Se may be lost.

In Table 1, the data for the volume and selenium content of each fraction used for electrophoresis are listed. It can be seen that the selenium concentrations differed on the basis of dry weight with protein content. The results indicated that much more protein were likely to be bound with selenium in the cytosolic fraction than in the nuclear and mitochondrial fraction.

After staining, more than 20 protein bands were observed in each liver component. Each band was cut down carefully and analysed for selenium content. The blank bands or the blank gel were analysed at the same time. The selenium content in each protein band was calculated after subtracting the selenium values of the blank gel with the same size. Then, the Se-data for different protein bands are expressed as a percentage of the total selenium present in the original samples. The distributions of the

Table 1 The selenium content in various subcellular fractions of human liver[a]

	Nuclei	Mitochondria	Lysosome	Microsome	Cytosol
Protein (mg mL^{-1})	55.5	27.5	16.4	24.1	19.1
Total Se (ng) [a]	29.8	36.9	27.8	56.0	61.7
Total Se concentration (ng g^{-1} dry weight)	0.92	1.05	0.20	0.54	0.46
Se concentration (ng mg^{-1})protein	0.54	1.15	1.20	1.21	2.69

[a]used for SDS-PAGE separation

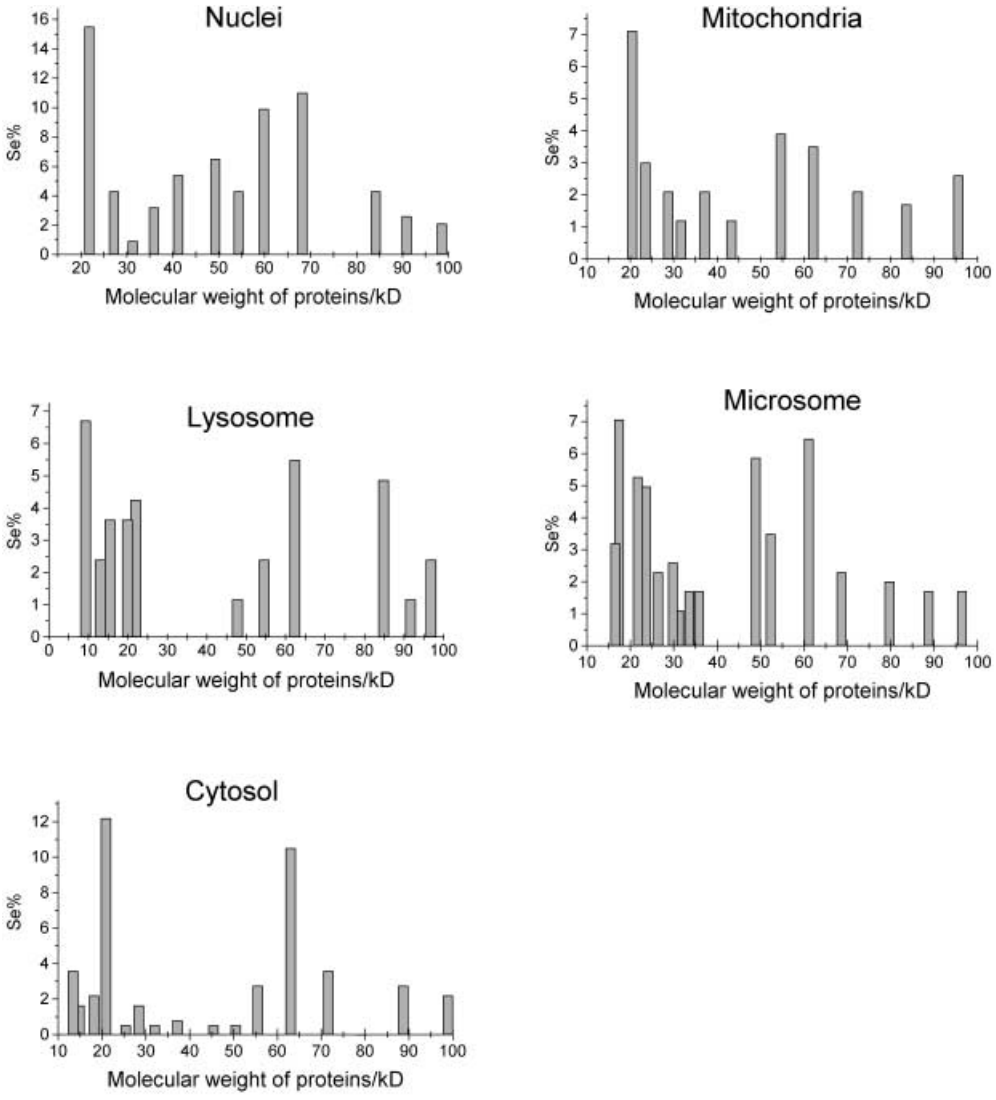

Fig. 1 Percentage of Se in Se-containing proteins or protein subunits of various subcellular fractions in human liver specimens determined by SDS-PAGE and HG-AFS. The data for different Se-containing proteins are expressed as a percentage of the total selenium present in samples

Se-containing proteins in each subcellular fraction of human liver are shown in Fig. 1.

It was defined that the Se-content of the protein(s) subunits called main Se-containing proteins had to be 2 times higher than in the blank gel of the same gel site, whereas the Se-content in Se-containing proteins of each corresponding subcellular fraction is less than 2%. The main Se-containing proteins or subunits and their possible attributes in various subcellular fractions are shown in Table 2.

It was found that about 24 Se-containing proteins existed in subcellular fractions. The molecular weights (MW) of the subunits were mostly in the range 10–30 kDa and 50–80 kDa. Among them, major Se-containing protein fractions at about 61 kDa and 21 kDa existed in each fraction and were probably selenoprotein P and glutathione peroxidase, respectively. The 21 kDa Se-containing protein was mostly concentrated in cytosol, mitochondria and nuclei. The 54 kDa protein was probably a thioredoxin reductase, which was present in each liver component with higher abundance. We noticed that the lowest MW Se-containing protein of 9.3 kDa existed in lysosome. The highest MW Se-containing proteins of 96 kDa was distributed in mitochondria, microsome and cytosol.

Discussions

The present data should reflect the real state and metabolism of the selenoprotein distribution in human liver, because the samples were taken from normal subjects who lived in a ‘adequate-selenium’ area of China.

About 21 and 61 kDa Se-containing proteins were dispersed in each fraction with higher abundance, which were likely to be selenoprotein P and glutathione peroxidase, respectively. Selenoprotein P and glutathione peroxidase are selenoproteins that are synthesized by hepatocyte [16]. The 20–24 kDa Se-containing proteins observed in this study may be subunits of GPX, since GPX is recognized to contain 20–24 kDa subunits varying with biological species. The GPX was the most predominant selenoprotein in all the human liver components except

Table 2 Distribution of main Se-containing protein in subcellular fractions of human liver

Average MW (kDa)	Nuclei	Mitochondria	Lysosome	Microsome	Cytosol	Possible attribute
99				+	+	
96		+		+		
89					+	
84	+		+			
80				+		
72					+	
68	+++			+		
61	+++	+	++	++	+++	Selenoprotein P
54	+	+	+	+	+	TrxR
49	++			++		
41	+					
36	+			+		
34				+		
30				+		ID
27	+			+	+	GPx or PHGPx
24		+	+	++		
21	+++	++	+	++	+++	
20			+			
18					+	
17				++		
16			+	+		
15					+	
13					+	
9			++			

+ (2–5%), ++ (5–10%) and +++ (>10%) represent the percentage of Se content in Se-containing proteins of each corresponding subcellular fraction. Selenium content in each protein band was 2 times higher than that in a blank gel with same gel size.

lysosome, which was in agreement with our previous report [11].

The biological function of iodothyronine deiodinase is concerns the formation and regulation of active thyroid hormone. Studies have shown that the nuclei of rat liver and kidney have acceptors that bind with triiodothyronine (T3) and the binding-T3 constituted 12.9% of the total T3 [17]. We reported the highest iodine concentration and most of the iodine was found in its nuclear fraction in human liver [18]. Furthermore, a 28 kDa selenoprotein was identified as a subunit of iodothyronine deiodinase in rat thyroid, liver and kidney, which was the main selenoprotein in the thyroid [19]. These previous findings supported the idea that the 27 kDa Se-containing protein in human liver nuclei should be iodothyronine deiodinase.

In our previous study, eight kinds of Se-containing proteins were found in the subcellular fractions of human liver by size exclusion chromatography (SEC) [11]. The more accurate molecular weight and better resolution were obtained by SDS-PAGE than SEC. Some proteins can be dissociated to subunits under the influence of SDS. One of the important advantages of SDS-PAGE was that selenium of weakly bound or non-specific incorporation into proteins would be removed after electrophoresis.

In the literature, 12 Se-containing proteins or subunits with MW between 12.1 and 75.4 kDa were found in the subcellular fractions of rat liver by means of ^{75}Se tracer and SDS-PAGE separation [9]. GPx was concentrated in the cytosol and mitochondria. Subsequently, an additional six Se-containing proteins were found in rat liver, but only a few of them have been sequenced and identified [10]. In comparing with rat liver, species of Se-containing proteins in human liver were similar except for high MW parts, in spite of the fact that their abundances were quite different. The differences in Se-containing protein distribution patterns between rat and human liver could be explained by the differences in selenium metabolism among different biological species.

Selenium was found to regulate the function and structure of liver mitochondria, selectively inhibit the energy metabolism and hexose kinase activity of hepatoma-derived cell lines in vitro [20]. Selenite supplementation in vivo also inhibited the liver cancer incidence of local residents in the Qidong area of China. Previously, we also found that the deficient or excess concentration of selenium inhibited the metabolic activity of mitochondria [21]. Thus, selenium status bears a close relationship to the normal function of human liver [22].

Our present studies provided the basic data for selenoproteins in normal human liver exposed to prolonged a generally adequate Se-level. Further study of the Se distribution in human hepatoma specimens is needed to get more detailed information, to find new Se species and to elucidate their physiological role in the liver. This will contribute to the biochemistry of selenium.

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