Distribution and effects of natural selenium in soybean proteins and its protective role in soybean β-conglycinin (7S globulins) under AAPH-induced oxidative stress

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ABSTRACT

The effects of selenium (Se) on the protein content, amino acid profile, secondary structure and subunit composition of soy proteins and its distribution were evaluated, as was the effect of peroxyl radicals produced by thermal decomposition of AAPH on the conformational changes of Se-enriched β-conglycinin (S-7S). The Se biofortification ability of soy was very strong, 7S had strongest ability to incorporate Se, and lower amounts of inorganic Se existed in Se-enriched beans. Se could promote protein synthesis and thus improve the protein content, increase the total amino acid content with a decrease in cysteine, combine into low-molecular-weight proteins, and influence the secondary structure of soybean proteins. Se was involved in the relevant protein changes in surface hydrophobicity, intrinsic fluorescence, infrared absorption and solubility and played an antioxidant role as an effectual "protector" to reduce the influence of peroxyl radical oxidation on S-7S, thereby maintaining the structural rearrangement between aggregation and protein unfolding.

1. Introduction

Selenium (Se) is one of the most important micronutrients for humans and animals, required for the activity of a number of selenium-dependent enzymes, such as glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases, and selenophosphate synthetases (Allan, Lacourciere, & Stadtman, 1999; Letavayova, Vlckova, & Brozmanova, 2006). Se deficiency can decrease GPx activity, reduce antioxidant capacity and directly affect cell division, reproduction, genetics and growth, thereby interfering with the metabolism of proteins, polysaccharides and nucleic acids so that heart disease, muscular dystrophy and disorder in human reproduction occur (Oropeza-Moe, Wisløff, & Bernhoft, 2015). Therefore, adequate daily Se intake is required to maintain human health. According to the US Food and Nutrition Board, the recommended dietary allowance for both sexes is 55 μg Se/day, and 400 μg Se/day is advised as the tolerable upper intake amount in the USA; this limit is 300 μg Se/day in Europe (Thiry, Ruttens, De Temmerman, Schneider, & Pussemier, 2012). For human beings, the amount of Se in the diet largely depends on the Se content of the local soil, as Se is taken up from the soil and enters the food chain through plants. Unfortunately, 70% of the regions in the world are lacking Se, and the number for China is 72% (Gao et al., 2011). Thus, discovering and studying Se-enriched foods and other Se supplements

has great significance.

Currently, selenomethionine (SeMet) and Se-enriched yeast are consumed widely as Se supplements, whereas selenocysteine (SeCys) is typically found as the main form of Se in mammalian proteins in foods of animal origin (Rayman, 2008). SeMet cannot be synthesized in higher animals and humans but represents the major nutritional source of Se, and Se-enriched yeast that essentially consists of SeMet has gradually taken the place of inorganic Se salts in animal nutrition. However, they are used as over-the-counter nutritional Se supplements based on SeMet being classified as a very toxic amino acid and the existence of food safety risks for synthetic SeMet and selenium-enriched yeast, although both could be employed as feed additives (Schrauzer, 2003). As we all know plants can naturally accumulate Se from seleniferous soils and convert inorganic Se into organic forms. However, the particularity of Se is that the range between toxicity and deficiency is very narrow. Se-enriched food produced from agronomic biofortification of crops with Se through foliar or soil application, which are the most commonly used practices, could also contain exceptionally high amounts of Se. This may vary depending on the Se biofortification process and accumulation ability of the plant itself for this element; for example, two Brazil nuts can provide approximately 100 μg of Se (Thiry et al., 2012). Meanwhile, it is worth noting that the benefit of Se is based on its chemical forms besides the ingested concentration.

Researchers have generally believed that organic forms of Se have lower toxicity, higher absorption and better antioxidant properties than inorganic forms (Yang et al., 2012). Inorganic forms such as selenate (SeVI) and selenite (SeIV) are common sources of Se that have been limited in the diet universally because of their toxicity and potential environmental pollution, while the organic species of Se such as SeMet, SeCys and Se-methylselenocysteine (SeMC) are amino acid forms of Se that benefit health and have high bioavailability (Thiry et al., 2012). Although organic and inorganic forms of Se seem to have similar efficacy in the body to produce selenoproteins (Shiobara, Yoshida, & Suzuki, 1998), the chemical transformation of Se in metabolic processes (Letavayova et al., 2006) and even immunomodulatory effects (Schrauzer, 2003) depend on the Se chemical form in the diet. Although inorganic Se is present in foods either as sodium selenate or sodium selenite, it occurs much less frequently and in very low amounts; for example, the selenium species in Se-enriched pakchoi were found to be SeMC, SeMet, inorganic selenium and several unknown species (Thosaikham et al., 2014); however, infant formulas and parenteral feeding mixtures still contain them—even so, SeMet or Se yeast are not the normal nutritional forms of Se (Schrauzer, 2003).

Soybean as an economical and quality protein source is extensively used in infant formula and food supplements because of its high protein levels (~40%) and high nutritional value (Fukushima, 1991). Typically, high protein-containing food materials tend to be particularly rich in Se, which means that soybean would be more effective for Se biofortification than other plant materials. In soybeans grown hydroponically using intrinsically labeled ^{75}Se and analyzed for radioactivity and protein composition by SDS-PAGE, over 80% of the ^{75}Se is bound with protein, and the Se bioavailability of the soybean protein isolate is 86–96% (Sathe, Mason, Rodibaugh, & Weaver, 1992). In relatively high Se-enriched soybean plants grown in soil supplemented with sodium selenite, high-molecular-weight selenospecies (82% of total Se, remainder unknown) and low-molecular-weight selenium species (approximately 74% of total Se, with selenocysteine and SeMet as the major selenium compounds) were contained in Se-enriched beans, with lower amounts of inorganic Se (Chan, Afton, & Caruso, 2010). While for soybean grown in natural environments from a different seleniferous region, Se was found mainly in water-soluble proteins and chitin or polysaccharides of soybean; Se was incorporated into these three components in proportion to the Se content, but the disposition of Se in β-conglycinin (7S) and glycinin (11S) under excessive Se environments was mostly nonspecific and could be easily removed from proteins during SDS-PAGE separation (Wang, Xie, & Peng, 1996). Soy protein, in accordance with the sedimentation coefficients, can be divided into 2S, 7S, 11S and 15S, four major globulins, the percentage contents of which were 15%, 34%, 41.9% and 9.1% (Fukushima, 1991), respectively. However, to the best of our knowledge, the selenium content in soybean protein isolate has been intensively studied but few publications have discussed the distribution of Se in soybean protein fractions (Sathe et al., 1992; Wang et al., 1996), and the effects of selenium on proteins with regard to the separation of protein globulins and the Se supplement application of Se-enriched protein components, especially for soybean planted in natural Se-enriched soils.

A number of studies have shown that Se can significantly improve the antioxidant activity of Se-enriched proteins from mushrooms (Zhao, Zhao, Hui et al., 2004), rice (Liu, Zhao, Chen, Gu, & Bu, 2012), soybean (Hu et al., 2014) and *Spirulina platensis* (Chen & Wong, 2008); although the antioxidant mechanism of Se-rich proteins was unclear, their activities were closely related to Se. On the other hand, proteins are major targets for oxidants because of their high abundance in biological systems and high rate constants for reaction (Davies, 2005). Free radicals and reactive oxidation products of lipid peroxidation could trigger a number of changes in the side chains and polypeptide backbone of soy protein, resulting in fragmentation, cross-linking, aggregation and conformational changes, which ultimately could affect the functional properties of soybean protein (Wu, Hua, & Lin, 2014). However, as such

an important functional product may be used as a Se supplement, and our previous study found that the antioxidant activity of soybean selenoprotein was approximately 4-fold compared to the control (Hu et al., 2014); however, there is very little information on the status of Se-enriched soybean proteins upon exposure to free radicals or reactive oxidation products during processing and storage. In addition, the antioxidant effects of Se-enriched soybean protein components are rarely reported, and the related antioxidative mechanisms by which such proteins inhibit protein oxidation have yet to be clearly elucidated.

Therefore, the main objectives of this study were to evaluate the distribution and effects of natural selenium in proteins from ordinary and Se-enriched soybean and to clarify the effect of oxidation on the structures and properties of Se-enriched soybean proteins, as well as whether selenium would reduce protein oxidation and reflect the protective effects of selenoproteins under 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) induced oxidative stress, thus providing useful information regarding their potential commercial applications.

2. Materials and methods

2.1. Materials

Se-enriched soybeans (variety of "Dong Nong 42") were obtained from Natural Selenium Base of Fengcheng (Se content of natural seleniferous soil in the range of 0.4–0.99 μg/g and an average of about 0.538 μg/g), Jiangxi, China. The soybeans were planted in the spring, harvest in the summer of 2016 and left to dry at room temperature. Ordinary soybean as the same variety as Se-enriched soybean was purchased from the local market (Nanchang, Jiangxi, China). The soybean was grounded into a fine powder and defatted with *n*-hexane/ethanol (10:1, v/v) twice to obtain the defatted flours. The defatted soybean flour was then passed through a 100-mesh sieve (0.15 mm) before protein extraction. 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and 1-anilino-8-naphthalene-sulphonate (ANS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Preparation of soybean protein isolate and fractions

Soybean protein isolate (SPI) was prepared as previously described (Jiang, Chen, & Xiong, 2009) with some modifications. Defatted soybean flour was dispersed in distilled water (1:10, w/v), adjusted to pH 8.0 with 1 mol/L NaOH, stirred for 3 h at room temperature and then centrifuged at 5530 g for 20 min. The supernatant was precipitated at pH 4.5 using 1 mol/L HCl at 4 °C for 1 h and separated by centrifugation at 5530 g for 20 min, and then the protein isolate was dissolved in deionized water and adjusted to pH 7.5 before dialyzing with deionized water for 24 h at 4 °C. The protein isolates from ordinary soybean and selenium-enriched soybean were freeze-dried, stored at 4 °C and named O-SPI and S-SPI (subsequent samples were also named in the same way).

The β-conglycinin (7S) and glycinin (11S) protein fractions were isolated by the modified method (Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992). The defatted soybean flour was mixed with a 15-fold volume of distilled water, and then the pH of the dispersion was adjusted to 7.5 with 1 mol/L NaOH and continuous stirring at room temperature for 4 h. The soybean protein was obtained by centrifugation at 9000*g* for 30 min at room temperature. Dry sodium bisulfite was then added to the supernatant (0.98 g/L), and the pH was adjusted to pH 6.4 using 1 mol/L HCl. The mixture was kept at 4 °C overnight and centrifuged at 9000*g* for 20 min, and the precipitate was the insoluble 11S fraction. The resultant supernatant was adjusted to pH 5.0 with 1 mol/L HCl, contained 0.25 mol/L NaCl, and was kept at 4 °C for 1 h, and the insoluble fraction was removed by centrifugation at 9000*g* for 20 min. The last supernatant was diluted 2-fold with ice water, adjusted to pH 4.8 with 1 mol/L HCl, kept at 4 °C for another 1 h, and then