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High-Cysteine 2S Seed Storage Proteins from Quinoa (*Chenopodium quinoa*)

Keywords: *Chenopodium; quinoa; seed storage protein; 2S protein; cysteine*

INTRODUCTION

Chenopodium quinoa, commonly called quinoa or Inca rice, is indigenous to the Andes region of South America and has been cultivated as a staple food there for hundreds of years. The protein-rich seeds of quinoa were reported by Wood (1985) to be high in the essential amino acids. The 11S storage protein of quinoa, chenopodin, was recently characterized but found to be relatively low in the sulfur amino acids (Brinegar and Goundan, 1993). Another major class of quinoa storage proteins, having characteristics similar to those of the 2S storage protein family, was noted in that study but not characterized. In this paper, an isolation procedure for the 2S quinoa proteins is described along with an amino acid analysis showing them to contain high levels of cysteine and two other nutritionally significant amino acids.

MATERIALS AND METHODS

Enrichment of the 2S Protein Fraction. The seed source and extraction procedure were identical to those of the chenopodin isolation protocol (Brinegar and Goundan, 1993). The extract (from 10 g of defatted flour) was brought to pH 5.0 with 10% (v/v) glacial acetic acid with stirring. The precipitated chenopodin was removed by centrifugation at 10000*g* for 10 min at 5 °C. The pH of the supernatant was adjusted to 6.5 with 0.1 N NaOH and then brought to 90% saturation with solid ammonium sulfate at 20 °C and centrifuged as before except at 20 °C. The resulting supernatant was recovered and brought to 100% saturation with solid ammonium sulfate at 20 °C. After overnight storage at 5 °C, crystallized ammonium sulfate was redissolved by stirring at room temperature. Following centrifugation at 20 °C as before, the pellet was dissolved in 2 mL of column buffer (0.1 M NaCl, 10 mM 2-mercaptoethanol, 20 mM Tris-HCl, pH 7.5). Prior to chromatography, the sample was centrifuged briefly at 10000*g* at room temperature to remove insoluble material.

Chromatographic Purification. The entire sample (15 mg) was applied to a 1.5 × 92.5 cm column of Sephadex G-50 (fine mesh, Pharmacia, Piscataway, NJ) and eluted at 5 °C with column buffer at a flow rate of 15 mL/h. Fractions of 5 mL were collected and assayed for protein according to the method of Bradford (1976) using bovine serum albumin as a standard. Fractions containing the purified 8−9 kDa polypeptides, as determined by sodium dodecyl sulfate−polyacrylamide electrophoresis (SDS−PAGE) (Laemmli, 1970), were pooled, dialyzed against several changes of distilled water at 5 °C in 6000−8000 Da cutoff dialysis tubing (SpectraPor 1, Spectrum Medical Industries, Los Angeles, CA), and then lyophilized.

Amino Acid Analysis. Lyophilized protein (600 μg) was redissolved in 1 mL of 3 M urea/50 mM Tris-HCl, pH 7.5, and pyridylethylated according to the method of Mak and Jones (1976), dialyzed (as described above), and lyophilized. After vapor phase acid hydrolysis of the sample in 6 N HCl and 2% (v/v) phenol at 150 °C for 1 h, the phenyl isothiocyanate derivatives of the amino acids were prepared (Cohen and Strydom, 1988) and analyzed on an Applied Biosystems Model 420 amino acid analyzer.

RESULTS

Densitometry of the extractable seed proteins separated by SDS−PAGE (Figure 1, lane 2) showed that the 2S polypeptides (8−9 kDa) and the 11S polypeptides (22−23 kDa and 32−39 kDa) comprised approximately 35% and 37%, respectively, of the total protein. The 11S protein (chenopodin) was removed by precipitation at pH 5.0 (Brinegar and Goundan, 1993). The supernatant was thereby enriched in the 2S polypeptides (lane 3). Further enrichment was obtained by preparing a 90−100% ammonium sulfate cut (lane 4). This step was not quantitative, however, as more than half of the 2S protein was left in the 0−90% cut along with most of the contaminating proteins (data not shown). A final gel filtration step removed the remaining 15 kDa contaminant, resulting in a pure preparation of 2S polypeptides (lane 5). Attempts at a single-step chromatographic isolation of the 2S fraction, without the

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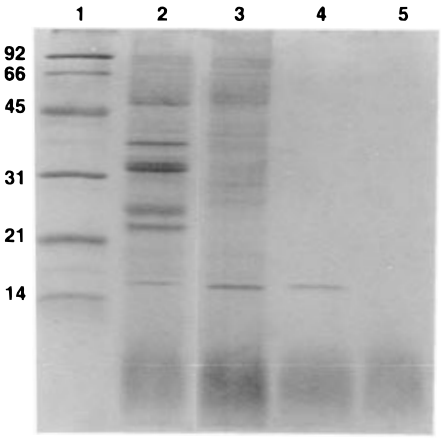

Figure 1. Electrophoretic analysis (15% SDS−PAGE) of quinoa seed protein fractions during the purification of the 2S proteins. Lane 1, molecular mass markers; lane 2, total salt-extractable proteins; lane 3, supernatant after pH 5 precipitation; lane 4, 90−100% ammonium sulfate cut of the pH 5 supernatant; lane 5, pooled 2S protein fractions after Sephadex G-50 gel filtration of the 90−100% ammonium sulfate cut. Numbers at left are sizes of the molecular mass markers (kDa). Other lower molecular mass markers (not shown) were used to estimate the molecular weight range of the 2S proteins.

Table 1. Amino Acid Composition of the Quinoa 2S Protein Fraction

amino acid	mol %	amino acid	mol %
Asx	5.3	Met	0.6
Thr[a]	0.4	Ile	1.3
Ser[a]	2.0	Leu	2.2
Glx	30.8	Tyr	2.9
Pro	4.5	Phe	1.2
Gly	7.4	Lys	0.9
Ala	1.4	His	7.6
Val	0.7	Arg	15.2
Cys[b]	15.6	Trp[c]	−

[a] Corrected for partial degradation during acid hydrolysis. [b] Determined as pyridylethylcysteine. [c] Destroyed by acid hydrolysis.

acid and ammonium sulfate precipitation steps, yielded a less pure preparation (data not shown).

After denaturation, reduction, and pyridylethylation, the amino acid composition of the purified 2S fraction was determined (Table 1). Nearly one-third of all residues in this fraction were glutamine/glutamic acid, with arginine and cysteine comprising approximately 15 mol % each. Histidine was also relatively high at 7.6 mol %.

DISCUSSION

In species for which the 2S protein structure has been characterized, such as castor bean (Sharief and Li, 1982), rapeseed (Crouch et al., 1983), and Brazil nut (Sun et al., 1987a,b), the native protein is composed of two disulfide-linked subunits with molecular masses of 3−4 and 7−9 kDa. The purified quinoa 2S protein fraction reported here is an electrophoretically heterogeneous collection of polypeptides having apparent molecular masses of 8−9 kDa under reducing conditions. It is probable that these polypeptides represent the larger class of 2S subunits. The smaller 2S subunit group, if there is one in quinoa, was not resolved under the electrophoretic conditions employed.

Quinoa seeds are known to contain high-quality protein with essential amino acid patterns meeting or exceeding the FAO standard (FAO, 1973; Wood, 1985). The 11S class of quinoa proteins (the chenopodins) contribute moderate amounts of several of the essential amino acids but are relatively low in the sulfur amino acids when compared to the amino acid composition of the total seed protein (Brinegar and Goundan, 1993). From the data reported here, it appears that the 2S class of proteins is the major contributor of sulfur amino acids in quinoa seeds.

In their study of the 2S protein fractions from 11 oilseeds, Youle and Huang (1981) reported average cysteine values of 9.0 mol % (range of 2.0−14.6 mol %) and average methionine values of 3.4 mol % (range of 0.6−15.7 mol %). By comparison, the quinoa 2S protein fraction has a higher level of cysteine at 15.6 mol % but is relatively poor in methionine at 0.6 mol %. The only other 2S seed storage protein with higher levels of combined sulfur amino acids, between 25 and 30 mol %, is the sulfur-rich Brazil nut protein (Youle and Huang, 1981; Sun et al., 1987a). (All literature values cited have been converted to mol % from the original grams per 100 g of protein values.)

Since cysteine is a nonessential amino acid, the contribution of the 2S fraction to the overall nutritional quality of quinoa proteins probably comes, in part, from the sparing effect that dietary cysteine has on methionine, an essential amino acid. When high concentrations of dietary cysteine are present, it is unnecessary to reduce the body's methionine levels by diversion of methionylsulfur into cysteine biosynthesis (Lehninger et al., 1993).

Although we describe the 2S class of quinoa proteins as "high-cysteine" proteins, the high levels of arginine (15.2 mol %) and histidine (7.6 mol %) are also nutritionally significant. Both can be synthesized by humans but are often considered to be essential amino acids because children cannot produce enough for their metabolic needs (Campbell, 1995). Therefore, the 2S protein fraction of quinoa has potential as a dietary protein supplement, especially for children, either in an enriched form or in complementation with chenopodin in quinoa grain or flour products.

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