Purification and Characterization of the Methionine-Rich 2S Seed Proteins from the Brazil Nut Family (Lecythidaceae)

Wei-Neng Zuo[†] and Samuel S. M. Sun*

Department of Plant Molecular Physiology, University of Hawaii, Honolulu, Hawaii 96822

2S seed proteins were purified from paradise nut (*Lecythis zabucajo*) and cannonball (*Couroupita quianensis*), two members of the Brazil nut family (Lecythidaceae). The amino acid composition, subunit structure, partial protein sequence, immunoreactivity, and processing pattern of these proteins were determined. The 2S proteins from both paradise nut (PN2S) and cannonball (CB2S) are rich in the sulfur amino acids, especially methionine (Met): 14.0% Met and 4.3% cysteine (Cys) in PN2S; 19.9% Met and 4.5% Cys in CB2S. Both 2S proteins are synthesized as a precursor polypeptide of 18 kDa and processed stepwise to form the 9- and 3-kDa mature subunit polypeptides. These properties are similar to those of the Brazil nut (*Bertholletia excelsa* H.K.B.) 2S protein (BN2S). However, while the 18-kDa precursor polypeptide of PN2S, like that of BN2S, undergoes three sequential cleavages to form the two mature subunits, only two stepwise cleavages occur during the processing of CB2S.

Keywords: *Met-rich protein; 2S protein; seed proteins; Brazil nut*

INTRODUCTION

The seeds of many diverse plant species, including Brazil nut (Ampe et al., 1986; Sun et al., 1987a), rapeseed (Crouch et al., 1983; Ericson et al., 1986), cotton (Galau et al., 1992), sunflower (Kortt et al., 1991), castor bean (Sharief and Li, 1982), *Lupine* (Gayler et al., 1990), *Arabidopsis* (Krebbers et al., 1988), faba bean (Pasqualini et al., 1991), and amaranth (Segura-Nieto et al., 1992), contain 2S proteins, a class of low molecular mass (about 12 kDa) proteins. These proteins are water-soluble albumins and are abundant in the seeds, as 20−60% of the total seed protein (Yule and Huang, 1981). The 2S proteins consist of two subunits, a large and a small polypeptide, which are cleavage products of a larger precursor polypeptide (Sharief and Li, 1982; Crouch et al., 1983; Ericson et al., 1986; de Castro et al., 1987; Sun et al., 1987b). Most of the 2S proteins studied thus far are comprised of several isoforms, 2−5 in the Brassicaceae family (Monsalve and Rodriguez, 1990) and at least 10 in the Brazil nut (*Bertholletia excelsa* H.K.B.) (Ampe et al., 1986; de Castro et al., 1987; Altenbach et al., 1992b). Though occurring in diverse plant species, the 2S proteins share significant sequence identity, ranging from 21% to 44% (Sun et al., 1992; Zuo, 1993). The 2S proteins are rich in the sulfur amino acid cysteine (Cys), about 8 mol %, and the numbers and positions of the Cys residues are highly conserved in the various 2S proteins. In addition to their high Cys content, the 2S proteins of Brazil nut and sunflower are also rich in methionine (Met), 18 mol % (Altenbach et al., 1987) and 16 mol % (Kortt et al., 1991), respectively. Youle and Huang (1981) suggested that the 2S albumins are storage proteins that provide amino nitrogen as well as a source of sulfur to the germinating seeds.

Because of its rich Met content, the Brazil nut 2S (BN2S) protein has attracted much interest for its use in plant nutrition improvement and has therefore been well studied. The BN2S protein is abundant in seeds, representing 30% of the total seed protein. It consists of two polypeptide subunits of 9 and 3 kDa (Ampe et al., 1986; Sun et al., 1987a) and is synthesized as a precursor polypeptide of 18 kDa which undergoes stepwise processing from 18 to 15 to 12 kDa and finally to the 9- and 3-kDa mature subunit polypeptides (Sun et al., 1987b; de Castro et al., 1987). The cDNAs encoding the BN2S protein isoforms have been cloned and sequenced (Altenbach et al., 1987, 1992b; de Castro et al., 1987). Chimeric genes containing the BN2S coding region were stably transformed into and expressed in tobacco (Altenbach et al., 1989; Saalbach et al., 1994, 1995), rapeseed (Altenbach et al., 1992a), and *Vicia narbonensis* (Saalbach et al., 1994, 1995). Results from studies of Altenbach et al. (1989, 1992a) demonstrated that it is feasible to enhance the Met content in transgenic seeds by up to 30% through expression of the Met-rich BN2S coding region under the control of the phaseolin promoter and terminator. Data from Saalbach et al. (1995) showed that one of the R_0 transgenic *V. narbonensis* plants exhibited a 3-fold increase in the Met content over that of the wild-type seeds.

Brazil nut belongs to an important tropical woody plant family, the Brazil nut family (Lecythidaceae), best known economically for the edible seeds of the Brazil nut and paradise nut (*Lecythis zabucajo*). Thus far, only the seed proteins of Brazil nut in this family, particularly the Met-rich 2S protein, have been studied. Most of the 2S proteins from diverse plant species, though having significant sequence identity with the BN2S protein (Altenbach et al., 1987; Sun et al., 1992), contain few Met residues. The possible biological function and molecular evolution of the Met-rich BN2S protein are thus of interest. To gain further insights into these questions and to seek possible alternative sources of Met-rich protein for nutritional improvement, we purified and analyzed the 2S proteins in the seeds of two members of the Brazil nut family, the paradise nut and cannonball. Here we report that the 2S proteins from these two plants are similar to the BN2S protein in amino acid composition, subunit structure, partial

* Author to whom correspondence should be addressed [telephone (808) 956-6893; fax (808) 956-3542; e-mail ssun@uhunix.uhcc.hawaii.edu].

[†] Present address: Department of Botany, North Carolina State University, Box 7612, Raleigh, NC 27695-7612.

S0021-8561(95)00486-9 CCC: $12.00 © 1996 American Chemical Society

amino acid sequence, immunoreactivity, processing pattern, and high methionine content. In comparison to the paradise nut and Brazil nut, the cannonball 2S protein, however, has a different protein processing pattern.

MATERIALS AND METHODS

Materials. Paradise nut (*Lecythis zabucajo*) seeds were collected from Hilo Nursery Arboretum, Hilo, HI. Cannonball (*Couroupita quianensis*) seeds were obtained from Foster Botanic Garden, Honolulu, HI. Brazil nut (*Bertholletia excelsa*) seeds were purchased from Orinda Nuts, CA. The harvested seeds were used immediately or stored at −80 °C until use.

Methods. Total protein preparation was carried out by direct extraction of the fresh mature seeds with the salt buffer as previously described (Sun et al., 1987a). The 2S protein was purified by centrifugation of the total protein in a 5−30% sucrose gradient (Youle and Huang, 1981). SDS−20% polyacrylamide slab gel electrophoresis (SDS−PAGE) of the seed proteins was performed essentially as described by Laemmli (1970).

The amino acid composition of the 2S proteins was determined with a Beckman 6300 amino acid analyzer. The 2S protein hydrolysates were prepared by acid hydrolysis in constant-boiling 5.7 N HCl containing 2% phenol and 0.01% β-mercaptoethanol for 24 h at 110 °C. The performic acid oxidation procedure was used for determination of the sulfur amino acid content (Hirs, 1967).

The amino acid sequence of the 2S protein was determined by using an Applied Biosystem Model 477A liquid pulse sequencer equipped with a Model 120 on-line PTH analyzer following the standard protocol of the vendor.

The 2S proteins, after western blotting, were detected by the goat anti-mouse alkaline phosphatase (GAM-AP) conjugate system (Bio-Rad). Monoclonal antibody specific for the 9-kDa subunit of the Brazil nut 2S or paradise nut 2S protein was used as the first antibody.

Isolation of total RNA and poly(A)+ RNA from the developing seeds was carried out by following the methods of Hall et al. (1978). *In vitro* translation was performed with a wheat germ cell-free translation system (Sun et al., 1975) using the poly(A)+ RNA isolated from the developing seeds as a template.

For *in vivo* pulse−chase labeling experiments, the freshly collected developing paradise nut embryos were cut into 2-mm-thick pieces. The embryo pieces were incubated with 20 μL of [^{35}S]Met (3.8 × 1014 Bq mmol^{-1}) at room temperature for 1 h. After incubation, unincorporated [^{35}S]Met was washed off with three water rinses. The embryo pieces were then incubated in 10 mM nonradioactive Met at 30 °C for specified durations. At each time point, one piece of the embryo was removed and its proteins were extracted with the salt buffer. The pulse−chase labeling procedure for cannonball was the same as described for the paradise nut, except whole embryos were used.

RESULTS

Physical and Chemical Properties. Total and 2S proteins were isolated from Brazil nut, paradise nut, and cannonball of the Brazil nut family and analyzed by SDS−PAGE (Figure 1). The profiles of these protein samples revealed that cannonball seeds contain fewer major polypeptide species with the 2S protein as their dominant component. While the 2S proteins remain abundant, the Brazil nut and paradise nut contain more species of major polypeptides, and the profile of paradise nut is more similar to that of the Brazil nut (Figure 1A).

The profiles of the purified 2S seed proteins from Brazil nut, cannonball, and paradise nut, named BN2S, CB2S, and PN2S, respectively, are shown in Figure 1B. All three 2S proteins consist of two subunit polypeptides

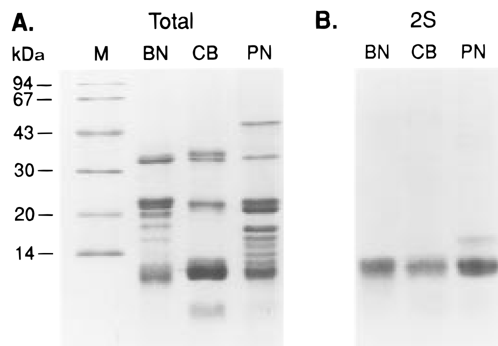

Figure 1. SDS−PAGE profiles of total (A) and sucrose gradient purified 2S proteins (B) from seeds of the Brazil nut family. Lanes: M, molecular weight markers; BN, Brazil nut; CB, cannonball; PN, paradise nut.

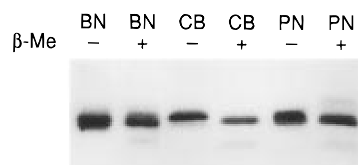

Figure 2. Migration of the purified 2S proteins in SDS−PAGE in the presence (+) or absence (−) of β-mecaptoethanol (β-Me). BN, Brazil nut; CB, cannonball; PN, paradise nut.

Table 1. Amino Acid Compositions of PN2S and CB2S

amino acid	PN2S mol %	CB2S mol %	BN2S mol %
Lys	2.8	0.3	1.6
His	1.9	1.7	1.9
Arg	10.6	15.5	12.1
Asx	4.9	2.8	4.4
Thr	3.3		0.5
Ser	6.4	6.3	5.6
Glx	23.3	27.4	23.1
Pro	7.7	8.1	6.5
Gly	7.2	4.7	7.5
Ala	4.2	1.8	1.8
Cys	4.3	4.5	9.7
Val	1.8	0.3	0.8
Met	14.0	19.9	16.1
Ile	1.1	0.2	0.7
Leu	5.7	5.6	5.7
Phe	0.9	0.8	0.4

[a] Data from Sun et al. (1987a).

with molecular masses of 9 and 3 kDa (Figure 1B). In the absence of reducing agent β-mercaptoethanol, the purified 2S proteins migrated as a single band in the SDS−PAGE gel (Figure 2, lanes 1, 3, and 5), whereas in the presence of the reducing agent, the two polypeptides, 9 and 3 kDa, were observed (Figure 2, lanes 2, 4, and 6). Note that the Coomassie-stained 3-kDa polypeptide band was not as intense as expected, due to diffusion of this low molecular mass polypeptide from the gel during staining and destaining. On the basis of the densitometric scanning of the protein gel, the 2S proteins of Brazil nut, paradise nut, and cannonball amount to 30%, 15%, and 50% of the total seed protein, respectively.

The 2S protein fractions of paradise nut and cannonball seeds were purified by sucrose gradient centrifugation and subjected to amino acid analysis. The overall amino acid profiles of PN2S, CB2S, and BN2S revealed that Arg, Glx, and the sulfur amino acids (Met + Cys) are the major components (Table 1). Both PN2S and CB2S are rich in Met, amounting to 14% and 19.9%, respectively. BN2S contains 16.1% Met (Sun et al., 1987a).

BN2S: NH-**Pro Arg Arg** Gly Met **Glu** Pro **His** Met **Ser** Glu...

CB2S: NH-**Pro Arg Arg** Pro Glu **Glu** Ser **His** Leu **Ser** Gln...

Figure 3. N-Terminal amino acid sequences of the 9-kDa subunits of the 2S proteins from Brazil nut and cannonball. Identical amino acids are in bold.

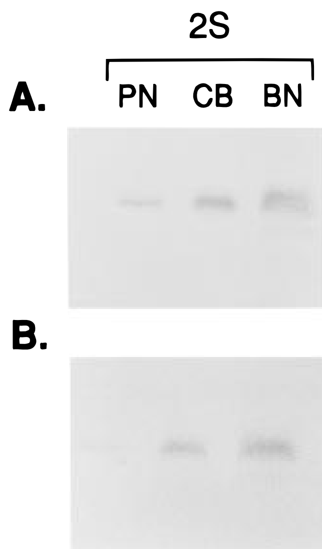

Figure 4. Western blot and immunodetection of 2S proteins from the Brazil nut family. The 2S proteins from Brazil nut (BN), paradise nut (PN), and cannonball (CB) were electrophoretically separated, transferred to membrane, reacted with antibody, and then detected by enzyme-linked reaction. (A) Treatment with monoclonal antibody specific for the 9-kDa subunit of BN2S; (B) treatment with monoclonal antibody specific for the 9-kDa subunit of CB2S.

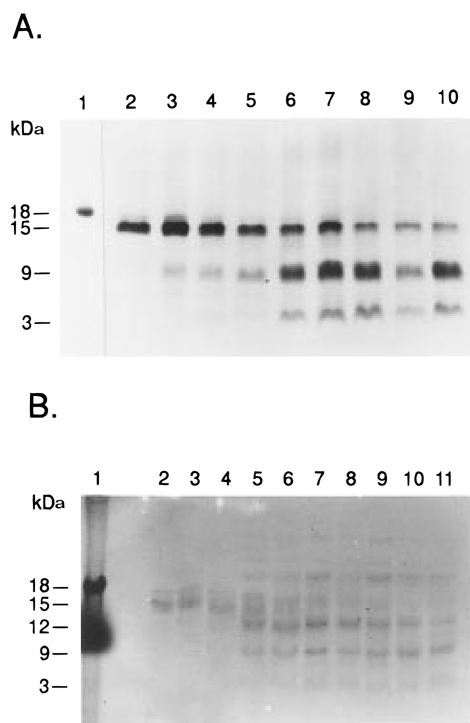

Figure 5. Processing of the precursor polypeptides of 2S proteins from cannonball (A) and paradise nut (B). Polypeptides synthesized *in vitro* in the wheat germ system containing [^{35}S]Met by RNAs from developing seeds of cannonball (A, lane 1) and paradise nut (B, lane 1) were analyzed by SDS−polyacrylamide gel and visualized by autoradiography. Embryo slices, pulse labeled with [^{35}S]Met for 1 h at 30 °C, were incubated with unlabeled Met for 0−15 h. The labeled polypeptides were then analyzed with the translation products. (A) lanes 2−10, products after chasing with unlabeled Met for 0 and 15 min and 0.5, 1, 3, 5, 8, 10, and 12 h, respectively; (B) lanes 2−11, chasing for 0, 0.5, 1, 2, 3, 5, 7, 9, 12, and 15 h, respectively. The sizes of the major labeled polypeptides are as indicated by bars in Figure 6.

The N-terminal amino acid sequence of CB2S was determined. As shown in Figure 3, the initial 11-amino-acid sequence of the CB2S 9-kDa polypeptide shares 54.5% identity with that of BN2S (Altenbach et al., 1987). Two Met residues appeared in the 11 N-terminal amino acids of BN2S, whereas no Met residues were found in this region of CB2S.

The 2S proteins from paradise nut, cannonball, and Brazil nut were analyzed by western blot using the monoclonal antibody specific for the 9-kDa polypeptide of BN2S or CB2S as the first antibody. Results revealed that the BN or CB antibody cross-reacted with the 9-kDa subunit polypeptides of all three 2S proteins (Figure 4).

Protein Processing. Both *in vitro* cell-free translation and *in vivo* pulse−chase labeling techniques were used to elucidate the maturation process of PN2S and CB2S proteins. These techniques have been previously used to study the processing of BN2S (Sun et al., 1987b). To identify the possible precursor polypeptides for PN2S and CB2S, poly(A)+ RNAs from the developing seeds of paradise nut and cannonball were translated in a wheat germ cell-free translation system using [^{35}S]Met as the labeled amino acid. A polypeptide of 18 kDa, as observed earlier for the Brazil nut 2S protein (Sun et al., 1987b), was heavily labeled in the translation products of both the cannonball (Figure 5A, lane 1) and paradise nut (Figure 5B, lane 1).

In vivo labeling of the developing paradise nut and cannonball seeds with [^{35}S]Met for 1 h resulted in incorporation of nearly all of the label into a 15-kDa polypeptide (parts A and B of Figure 5, lanes 2) rather than the 18-kDa species observed in the *in vitro* translation products. Subsequent incubation of the [^{35}S]-Met-labeled seeds with unlabeled Met at 30 °C for various durations revealed further transitions of the major labeled polypeptides. In cannonball seeds, after a 3 h chase, the radioactivity in the 15-kDa polypeptide was seen to decrease while the amount of radioactivity in the 9- and 3-kDa polypeptides began to increase (Figure 5A, lane 5). After a 12-h chase, most of the radioactivity was in the 9- and 3-kDa polypeptides; only trace amounts remained in the 15-kDa species (Figure 5A, lane 10).

In paradise nut, similar transitions of radioactivity between polypeptides occurred during the chase incubation except that a 12-kDa polypeptide was observed between the 15-kDa and the 9- and 3-kDa polypeptides. During early incubation, the amount of radioactivity in the 15-kDa polypeptide decreased while that in the 12-kDa polypeptide increased (Figure 5B, lanes 5−8). Further chasing resulted in declining radioactivity in the 12-kDa polypeptide with concomitant increases in the radioactivity of the 9- and 3-kDa polypeptides (Figure 5B, lanes 8−11). The *in vivo* polypeptide labeling pattern of paradise nut is thus similar to that observed for the Brazil nut (Altenbach et al., 1986).

DISCUSSION

The 2S proteins are the major seed proteins in Brazil nut, paradise nut, and cannonball. In cannonball, the 2S protein represents more than half of the total seed protein. PN2S, BN2S, and CB2S all contain high levels

of arginine/aspartic acid and glutamine/glutamic acid, characteristic of seed storage proteins. Like BN2S and most of the other 2S seed proteins, PN2S and CB2S consist of two subunits linked by disulfide bond(s) (Figure 2). Only in the later stages of seed development are the 2S proteins and their mRNAs detectable (data not shown), suggesting that the 2S protein genes are expressed temporally in seeds. The results of immunological reaction (Figure 4) indicated that BN2S, PN2S, and CB2S share antigenic similarities. The data from this study strongly suggest that PN2S and CB2S, like BN2S (Altenbach et al., 1989), are seed storage proteins.

The 2S proteins of paradise nut, cannonball, and Brazil nut share much similarity in their physical, chemical, and biological properties, including subunit structure, amino acid profile, N-terminal sequence, immunoreactivity, and precursor polypeptide processing, indicating a close taxonomic relationship among these three members of the Brazil nut family. Several of these properties, however, are also shared with the 2S seed albumins of diverse plant species including rapeseeds, *Arabidopsis*, sunflower, castor bean, cotton, and *Lupine*, suggesting that these 2S seed proteins are probably derived from a common ancestral gene.

The 2S proteins of the Brazil nut family, however, differ from most other 2S proteins in that they are exceptionally rich in Met (14−19.9 mol %) and are abundant (15−50%) in the seeds. Considering, for example, that 50% of the total protein in cannonball is comprised of the 2S protein, the seeds of these plants must contain very high amounts of Met. The majority of known plant proteins contain relatively low levels of Met (1−2%) as predicted by the theory of molecular evolution (Ohta and Kimura, 1971). The high content of Met residues in the 2S protein of the Brazil nut family is thus very distinctive and in contrast to this theory, suggesting that a specific function of the 2S protein might have evolved in the Brazil nut family. The soil in the Amazon region is rather poor in sulfur (Sanchez et al., 1982), which prompted us to suggest earlier that the high levels of Met in the Brazil nut might be required to provide an adequate supply of Met to the germinating seeds (Altenbach et al., 1987). While the finding in this study that the 2S seed proteins of paradise nut and cannonball of the Brazil nut family are also very rich in Met appears to support this possibility, the exact function of the Met-rich 2S proteins awaits further elucidation. Nevertheless, this finding provides two alternative sources of Met-rich protein genes. One of them, PN2S, is from an edible source, the paradise nut.

The *in vitro* translation and *in vivo* pulse−chase experiments revealed that the 2S proteins of paradise nut, cannonball, and Brazil nut are synthesized as an 18-kDa precursor polypeptide, which is subsequently processed into a 15-kDa intermediate polypeptide. The cleaved 3-kDa peptide most likely represents the signal sequence that directs the deposition of the 2S protein into protein bodies (Altenbach et al., 1987). In the paradise nut and Brazil nut, two additional cleavages occur sequentially and result in the formation of a 12-kDa intermediate and then the 9- and 3-kDa mature polypeptides (Figure 5B). In cannonball, however, the pulse−chase experiments showed a direct transition of the 15-kDa CB2S intermediate into the 9- and 3-kDa subunits (Figure 5B); a 12-kDa intermediate polypeptide was not observed. Figure 6 illustrates the two processing patterns of the 2S proteins in the Brazil nut family. It appears that the two cleavages involved in processing the 15-kDa intermediate polypeptide into the 9- and 3-kDa subunit polypeptides occur sequentially in PN2S and BN2S but simultaneously in CB2S. The 12-kDa precursor polypeptide was observed in the developing Brazil nut for about 1 month (Sun et al., 1987), suggesting a possible function for this polypepetide or an absence at this particular development stage of a required protease to further cleave the 12-kDa polypeptide. Amino acid sequence data of the first 11 amino acids of BN2S and CB2S (Figure 3) indicated that both BN2S and CB2S start with the same sequence, suggesting that their processing sites are likely to be the same. The similarity of PN2S and BN2S in their protein processing patterns and total protein profiles (Figure 1A) indicates that the paradise nut may be taxonomically more closely related to the Brazil nut than to the cannonball. The morphology and structure of the flowers, fruits, and seeds of these three plants (Prance and Mori, 1979) appear to support this observation.

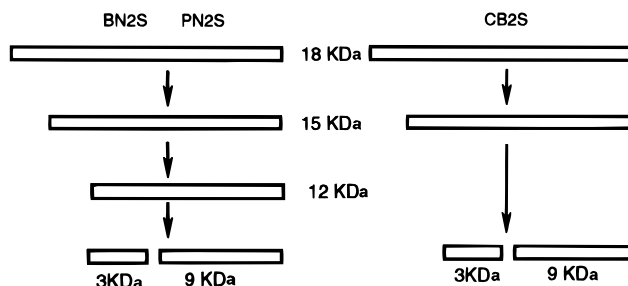

Figure 6. Proposed processing schemes of the 18-kDa precursors of the 2S protein in Brazil nut family. Three sequential cleavages are involved in the maturation of the Met-rich 2S proteins from Brazil nut and paradise nut, while only two stepwise cleavages of the cannonball 2S protein precursor occur in the formation of the 9-and 3-kDa mature subunit polypeptides.

ABBREVIATIONS USED

BN2S, Brazil nut 2S protein; PN2S, paradise nut 2S protein; CB2S, cannonball 2S protein; Met, methionine; Cys, cysteine.

ACKNOWLEDGMENT

We gratefully thank Dr. Niel Reimer for help with amino acid composition analysis and protein sequencing, Dr. Albert Benedict for raising the antibody, the Hilo Nursery Arboretum for paradise nuts, and the Foster Garden in Honolulu for cannonball fruits.

LITERATURE CITED

Altenbach, S. B.; Pearson, K. W.; Leung, F. W.; Sun, S. S. M. The stepwise processing of sulphur-rich seed protein from Brazil nut (*Bertholletia excelsa* H.B.K.). In *Molecular Biology of Seed Storage Proteins and Lectins*; Shannon, L. M., Chrispeels, M. J., Eds.; Waverly Press: Baltimore, MD, 1986; pp 137−146.

Altenbach, S. B.; Pearson, K. W.; Leung, F. W.; Sun, S. S. M. Cloning and sequence analysis of a cDNA encoding a Brazil nut protein exceptionally rich in methionine. *Plant Mol. Biol.* **1987,** *8*, 239−250.

Altenbach, S. B.; Pearson, K. W.; Meeker, G.; Staraci, L. C.; Sun, S. S. M. Enhancement of the methionine content of seed proteins by the expression of a chimeric gene encoding a methionine-rich protein in transgenic plants. *Plant Mol. Biol.* **1989,** *13*, 513−522.

Altenbach, S. B.; Kuo, C-C.; Staraci, L. C.; Pearson, K. W.; Wainwright, C.; Georgescu, A.; Townsend, J. Accumulation of a Brazil nut albumin in seeds of transgenic canola results in enhanced levels of seed protein methionine. *Plant Mol. Biol.* **1992a**, *18*, 235–245.

Altenbach, S. B.; Pearson, K. W.; Sun, S. S. M. Nucleotide sequences of cDNAs encoding two members of the Brazil nut methionine-rich 2S albumin family. *Plant Physiol.* **1992b,** *98*, 1520–1522.

Ampe, C.; Van Damme, J.; de Castro, L. A. B.; Sampaio, M. A. M.; Van Montagu, M.; Vandekehove, J. The amino-acid sequence of the 2S sulphur-rich proteins from seeds of Brazil nut (*Bertholletia excelsa* H.B.K.). *Eur. J. Biochem.* **1986**, *159*, 597–600.

Crouch, M. L.; Tenbarge, K. M.; Simon, A. M.; Ferl, R. cDNA clones for *Brassica napus* seed storage proteins: evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide. *J. Mol. Appl. Genet.* **1983**, *2*, 273–283.

de Castro, L. A. B.; Lacerda, Z.; Aramoyo, R. A.; Sampaio, A. M.; Gander, E. S. Evidence for a precursor molecule of Brazil nut 2S seed proteins from biosynthesis and cDNA analysis. *Mol. Gen. Genet.* **1987**, *206*, 338–343.

Ericson, M. L.; Rodin, J.; Leenman, M.; Glimelius, K.; Josefsson, L. G.; Rask, L. Structure of the rapeseed 1.7 S storage protein, napin, and its precursor. *J. Biol. Chem.* **1986**, *261*, 14576–14581.

Galau, G. A.; Wang, H. Y. C.; Hughes, D. W. Cotton *Mat*5-A (C164) gene and *Mat*5-D cDNAs encoding methionine-rich 2S albumin storage proteins. *Plant Physiol.* **1992**, *99*, 779–782.

Gayler, K. R.; Kolivas, S. K.; Macfarlane, A. J.; Lilley, G. G.; Baldi, M.; Blagrove, J.; Johnson, E. D. Biosynthesis, cDNA and amino acid sequence of a precursor of conglutin δ, a sulfur- rich protein from *Lupinus angustifolius*. *Plant Mol. Biol.* **1990**, *15*, 879–893.

Hall, T. C.; Buchbinder, B. U.; Pyne, J. W.; Sun, S. M.; Bliss, F. A. Messenger RNA for G1 protein of French bean seeds: cell-free translation and product characterization. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3196–3200.

Hirs, C. H. W. Performic acid oxidation. *Methods Enzymol.* **1967**, *11*, 197–199.

Kortt, A. A.; Caldwell, J. B.; Lilley, G. G.; Higgins, T. J. V. Amino acid and cDNA sequences of a methionine-rich 2S protein from sunflower seed (*Helianthus annuus* L.). *Eur. J. Biochem.* **1991**, *195*, 329–334.

Krebbers, E.; Herdies, L.; De Clercq, A.; Seurink, J.; Leemans, J.; Vandamm, J.; Segura, M.; Gheysen, G.; Van Montagu, M.; Vandekerckhove, J. Determination of the processing sites of an *Arabidopsis* 2S albumin and characterization of the complete gene family. *Plant Physiol.* **1988**, *87*, 859–866.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Monsalve, R. I.; Rodriguez, R. Purification and characterization of proteins from the 2S fraction from seeds of the *Brassicaceae* family. *J. Exp. Bot.* **1990**, *41*, 89–94.

Ohta, T.; Kimura, M. Amino acid composition of protein as a product of molecular evolution. *Science* **1971**, *174*, 150–153.

Pasqualini, S.; Lluch, C.; Antonielli, M. Seed storage proteins in several genetic lines of *Vicia faba*. *Plant Physiol. Biochem.* **1991**, *29*, 507–515.

Prance, G. T.; Mori, S. A. The Actinomorphic-flowered New World *Lecythidaceae*. In *Flora Neotropics*; Monograph 21, *Lecythidaceae*, Part I; The New York Botanical Garden: New York, 1979; pp 1, 39–71, 107.

Saalbach, I.; Pickardt, T.; Machemehl, F.; Saalbch, G.; Schieder, O.; Muntz, K. A chimeric gene encoding the methionine-rich 2S albumin of the Brazil nut (*Bertholletia excelsa* H.B.K.) is stably expressed and inherited in transgenic grain legumes. *Mol. Gen. Genet.* **1994**, *242*, 226–236.

Saalbach, I.; Waddell, D.; Pickardt, T.; Schieder, O.; Muntz, K. Stable expression of the sulfur-rich 2S albumin gene in transgenic *Vicia narbonensis* increases the methionine content of seeds. *J. Plant Physiol.* **1995**, *145*, 674–681.

Sanchez, P. A.; Bandy, D. E.; Villachica, J. H.; Nicholaides, J. J. Amazon basin: management for continuous crop production. *Science* **1982**, *216*, 821–827.

Segura-Nieto, M.; Vazquez-Sanchez, N.; Rubio-Velazuqez, H.; Olguin-Martinez, L. E.; Rodriguez-Nester, C. E.; Herrara-Estrella, L. Characterization of amaranth (*Amaranthus hypochondriacus* L.) seed proteins. *J. Agric. Food Chem.* **1992**, *40*, 1553–1558.

Sharief, F. S.; Li, S. S. L. Amino acid sequence of small and large subunits of seed storage protein from *Ricinus communis. J. Biol. Chem.* **1982**, *257*, 14753–14759.

Sun, S. M.; Buchbinder, B. U.; Hall, T. C. Cell-free synthesis of the major storage protein of the bean, *Phasoelus vulgaris* L. *Plant Physiol.* **1975**, *56*, 780–785.

Sun, S. S. M.; Leung, F. W.; Tomic, J. C. Brazil nut (*Bertholletia excelsa* H.B.K.) protein: fractionation, composition, and identification of a sulfur-rich protein. *J. Agric. Food Chem.* **1987a**, *35*, 232–235.

Sun, S. S. M.; Altenbach, S. B.; Leung, F. W. Properties, biosynthesis and processing of a sulphur-rich protein in Brazil nut (*Bertholletia excelsa* H.B.K.). *Eur. J. Biochem.* **1987b**, *162*, 477–483.

Sun, S. S. M.; Zuo, W. N.; Tu, H. M. Molecular approaches for enhancing amino acid quality of plant proteins. In *Biosynthesis and Molecular Regulation of Amino Acids in Plants*; Singh, B. K., Flores, H. E., Shannon, J. C., Eds.; American Society of Plant Physiologists: Rockville, MD, 1992; pp 208–216.

Youle, R. J.; Huang, A. H. Occurrence of low molecular weight and high cysteine containing albumin storage proteins in oilseeds of diverse species. *Am. J. Bot.* **1981**, *68*, 44–48.

Zuo, W-N. Sulfur-rich 2S proteins in *Lecythidaceae* and their methionine-enriched forms in transgenic plants. Ph.D. Disertation, University of Hawaii, 1993.

Received for review July 27, 1995. Accepted January 18, 1996.[⊗] This project was supported by the U.S. Department of Agriculture under CSRS Special Research Grant 88-34135-3592 managed by the Pacific Basin Advisory Group (PBAG), and published as Journal Series 4154 of the Hawaii Institute of Tropical Agriculture and Human Resources. W.-N.Z. was a fellowship recipient of the Food and Agriculture Organization of the United Nations.

JF950486G

[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1996.