Sulfur Amino Acid-Rich Proteins in Acha (*Digitaria exilis*), a Promising Underutilized African Cereal

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We reported previously that acha (*Digitaria exilis*), a promising but underutilized African cereal, is rich in methionine and will make an excellent complement for legumes. Two sulfur amino acid-rich proteins that contribute to this property with the following properties have now been identified: M1, 19 000 MW, 21.6% of extractable protein, 6.0% Met, 6.4% Cys; M2, 17 500 MW, 12.9% of extractable protein, 7.8% Met, 5.3% Cys. The N-terminal sequences of the two proteins had 64% similarities with each other. M2 was 72% homologous with maize zein 10 000 methionine-rich protein and its precursor, while M1 had 53% homology with a bovine transcription factor and 46% homology with *Brassica napus* Napin1 precursor and two eukaryotic transcription factors.

INTRODUCTION

Acha, also called fonio and "hungry rice", grows wild in Nigeria and other West African countries but has not been cultivated or rigorously investigated. Acha has high methionine content (FAO, 1970), although its protein content is typical of cereals. In a previous work, we confirmed its high methionine content of 4.8% and a cysteine content of 2.5% (de Lumen et al., 1986), making it an excellent complement for legumes which are widely eaten in Africa. Acha could also provide a cheap source of methionine needed to detoxify the cyanides in cassava, a staple food and major carbohydrate source in developing countries of Africa.

We hypothesize that the proteins contributing to the unique amino acid profile of acha have methionine and cysteine contents higher than 4.8% and 2.5%, respectively. We report here the identification, amino acid composition, and N-terminal sequences of two methionine-rich and cysteine-rich proteins in acha and their similarities with other proteins.

MATERIALS AND METHODS

Source of Acha Samples. The samples were provided by Dr. Steven Adewusi of the Department of Chemistry, Obafemi Awolowolo University, Ile-Ife, Nigeria.

Sample Preparation and Protein Extraction. Seeds were ground to 80-mesh size flour and extracted with buffer [0.5 M NaCl, 0.02 M TES (pH 7.8), 0.001 M PMSF, 2% SDS, 0.05 M β-mercaptoethanol] at a ratio of 10 mL of buffer to 400 mg of acha flour by stirring for 45 min at room temperature. The supernatant solution obtained by centrifugation at 1500*g* for 20 min was freeze-dried and redissolved in water (200 mg of freeze-dried extract in 2 mL). Due to interference in commonly used protein assays by mercaptoethanol in the extracting buffer, a modified Kjeldahl method (Fukumoto and Chang, 1982) was employed to determine protein in the freeze-dried material using a conversion factor of 6.25.

Identification of Methionine-Rich Proteins, Amino Acid Analysis, and Sequencing of N Terminus. The identification of methionine-rich proteins (MRP) was carried out according to the procedure of de Lumen and Kho (1987). Briefly, the proteins were separated by SDS–PAGE (15% gel), electroblotted onto Immobilon membrane (Millipore Corp.), stained with Coomassie Blue, and reacted with [1-^{14}C]iodoacetic acid at pH 2, which favors alkylation of the thioether moiety of methionine. For each protein band, the ratio of the densitometric area in the autoradiograph (i.e., methionine signal) to that in protein-stained Immobilon membrane (i.e., protein signal) is an index of the methionine concentration. The putative MRP bands were cut out from the membrane and submitted for micro amino acid analysis and N-terminal sequencing to the Protein Structure Laboratory, University of California at Davis (George and de Lumen, 1991). The sequences were compared with proteins in Intelligenetics' Protein Identification Resource Data Base (PIR).

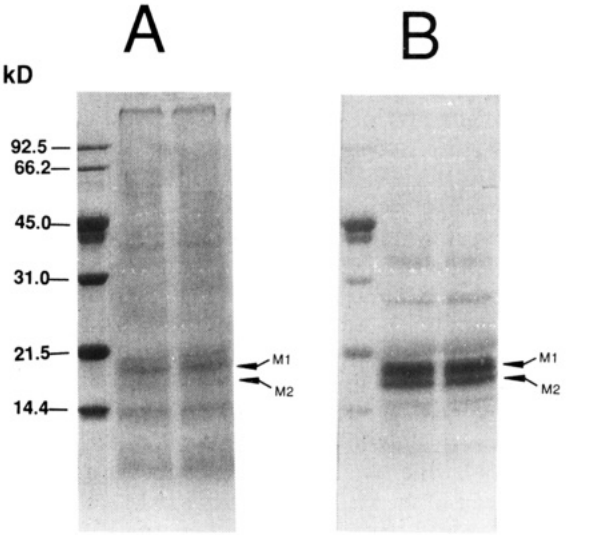

Figure 1. (A) Total extractable proteins from acha separated on SDS–PAGE (15% gel), electroblotted onto Immobilon membrane, and stained with Coomassie Blue. Each of the duplicate lanes contained 215 μg of total protein. (B) Autoradiograph of electroblotted proteins after reaction with [1-^{14}C]iodoacetic acid at pH 2 that favors alkylation of methionine residues. For details, please see George and de Lumen (1991).

Chemical Score Calculation. The chemical score was calculated using the amino acid patterns for preschool children (2–5 years old) (FAO/WHO, 1985) as suggested by the joint FAO/WHO (1990) report.

RESULTS AND DISCUSSION

The high methionine content of acha suggests that there are MRP that contribute to this property. We have now

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Table I. Amino Acid Composition (Grams per 100 g) of Acha and Proteins M1 and M2

amino acid	acha	M1	M2
His	2.3	2.9	0.6
Ile	3.8	2.2	2.7
Leu	9.8	6.3	6.6
Lys	3.1	1.1	2.6
Met	4.8	6.0	7.8
Cys	2.5	6.4	5.3
Phe	5.2	2.7	4.5
Tyr	3.1	4.1	4.0
Thr	3.9	4.2	4.5
Val	5.5	4.5	3.7
Ser	5.0	4.4	5.4
Arg	4.7	4.9	5.7
Pro	6.4	7.9	5.8
Gly	3.8	8.2	10.3
Asp	7.2	4.5	6.6
Glu	18.8	24.3	18.2
Ala	8.4	5.4	5.7
Met + Cys	7.3	12.4	13.1
first limiting amino acid	Lys	Lys	His
chemical score,[a] %	53	19	32

[a] Based on the pattern of requirement for preschool children, 2–5 years old (FAO/WHO, 1985).

identified two sulfur amino acid-rich proteins that make up approximately 35% of the total extractable proteins in acha. Figure 1A shows the extractable proteins of acha separated on SDS–PAGE, electroblotted onto Immobilon membrane, and stained with Coomassie Blue. The corresponding autoradiograph of the electroblotted proteins after reaction with [1-^{14}C]iodoacetic acid is shown in Figure 1B. Proteins M1 (19 000) and M2 (17 500) appeared as intense bands on the autoradiograph, indicating that they were methionine-rich. This was confirmed by micro amino acid analysis of the bands cut out of the membrane. The methionine and cysteine contents of M1 were 6.0% and 6.4%, respectively, while those of M2 were 7.8% and 5.3%, respectively, which were higher than those in acha (Table I). The sums of the sulfur amino acids in M1 and M2 were at least 3-fold higher than corresponding values in the amino acid pattern of infants and preschool children recommended as references for comparison by FAO/WHO (1985, 1990). Lysine, typically limiting in cereals, was the first limiting essential amino acid in M1, while that of M2 was histidine. The chemical score of M1 was lower than that of M2, which was lower than that of acha. Proteins M1 and M2 made up 21.6% and 12.9% of the total extractable protein, respectively, as determined by densitometric scan of the Coomassie Blue stained membrane.

The N-terminal sequences of M1 and M2 showed 64% similarities (Table II). A doublet of methionine residues in positions 10 and 11 indicated the methionine-richness of M2. Doublet and triplet methionine residues are found in other methionine-rich proteins (Kortt et al., 1991; Pedersen et al., 1986; Kirihara et al., 1988a,b). Homologies with other proteins are presented in Table II. Most interesting was M2's 72% homology with a 10 000 MW methionine-rich maize zein (Kirihara et al., 1988a) and its precursor (Kirihara et al., 1988b). M1 was 53% homologous with a bovine transcription factor GHF-1 and 46% homologous with *Brassica napus* Napin1 precursor, transcription factors pit-1, and antennapedia homeotic domain.

A number of MRP have been identified in seed proteins and some of their genes subsequently cloned. These include a Brazil nut albumin (Altenbach et al., 1987), a 10 800 MW soybean albumin (George and de Lumen, 1991), 10 000 and 15 000 MW zeins from maize (Kirihara et al., 1988a,b; Pedersen et al., 1986), a 10 000 MW rice prolamin (Musumura et al., 1989), a 10 000 MW sunflower seed albumin (Kortt et al., 1991), and 7900 and 9100 MW proteins from millet (Ponnappanaren and Virupaksha, 1990). Other than serving as sulfur reserves, it is not known if the sulfur amino acid-rich proteins have other biological roles. It is interesting to note that M1 was at least 46% homologous with three eukaryotic transcription factors (Table II). The conserved methionine-rich region of a nuclear protein from *Drosophila* and rat is proposed to play an important indirect role in its DNA-binding property as a transcription factor that controls terminal differentiation in *Drosophila* embryo (Weigel and Jackle, 1990). The relatively high abundance of MRP in the cotyledon and endosperm precludes their possible role as transcription factors, but it would be interesting to establish if certain MRP are nuclear proteins, particularly if they are found in the embryonic axis.

Identifying underutilized plants and promoting their cultivation and use is one way to meet the need to diversify the sources of foods for humans. The high total sulfur amino acid content of acha makes this cereal an excellent complement for legumes which are widely consumed in Africa. As this cereal is targeted for study and cultivation, the two MRP offer an opportunity for use as indicators of methionine/cysteine contents in selecting varieties with high sulfur amino acid contents and for further nutritional improvement through genetic engineering once the genes for the MRP are cloned.

ABBREVIATIONS USED

FAO, Food and Agriculture Organization; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; PMSF, phenylmethanesulfonyl fluoride; MRP, methionine-rich proteins; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Table II. N-Terminal Amino Acid Sequences of Acha Proteins M1 and M2 and Homologies with Other Proteins

	1	2	3	4	5	6	7	8	9	10	11	12	13
M1	Thr	His	Thr	Pro	Gly	Gln	Gln	Ala	Pro	Pro	Met	His	Gln
M2	Thr	His	Leu	Pro	Gly	Gln	Leu	Pro	Pro	Met	Met		

protein	homology, % M1	homology, % M2	protein	homology, % M1	homology, % M2
M1	100	64	M2	64	100
maize 10 000 MW methionine-rich zein		72	bovine transcription factor GHF-1	53	
rabbit cytochrome P450 IIC-3		54	*B. napus* Napin1 precursor	46	
sheep lutropin beta chain		54	transcription factor pit-1	46	
			antennapedia homeotic domain	46	

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