**α- and β-Setarins: Methionine-Rich Proteins of Italian Millet**  
(*Setaria italica* (L.) Beauv.)

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**ABSTRACT**

Differential cryoprecipitation was employed for the purification of two methionine-rich proteins, α-setarin and β-setarin from the alcohol-soluble prolamin fraction (setarin II) of Italian millet flour. Both α- and β-setarins were low molecular weight polypeptides rich in sulfur amino acids. Methionine accounted for eight residues each out of a total of 63 and 71 amino acid residues in α- and β-setarin, respectively. The peptide map pattern of cyanogen bromide cleaved α-setarin suggests that the methionine residues were randomly distributed throughout the polypeptide chain.

Isolation of Setarins

Three setarin fractions were isolated from the prolamin fraction of Italian millet by a modified cryoprecipitation procedure of Melcher and Fraij (1980) as shown in Figure 1. All extractions were done at ambient temperature for 30 min, and supernants were recovered by centrifugation at 2,000 × g for 10 min. Samples were frozen overnight at −20°C and cryoprecipitates were recovered by centrifugation at −10°C at 2,000 × g for 10 min.

**Analytical Methods**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 15% acrylamide gels was performed according to Laemmli (1970). Bovine serum albumin (66K), ovalbumin (43K), soybean trypsin inhibitor (20.1K), lysozyme (14.3K), and insulin β-chain (3.5K) were used as standard marker proteins.

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**Fig. 1.** Scheme of isolation of methionine-rich proteins. EtOH = ethanol, NaOAC = sodium acetate, 2-ME = 2-mercaptoethanol.
methionine than in zein I polypeptide. The amino acid composition of the fraction isolated by cryoprecipitation was 6.4-fold higher in setarin II; the levels of methionine in a- and B-setarin were 69.2% and 55.7%, respectively.

Both a- and B-setarin contained considerably higher amounts of the sulfur amino acids methionine and cysteine. Melcher and Fraij (1980) reported the enrichment of methionine in setarin II, a higher degree of purification of one of the sulfur-rich polypeptides of zein II protein fraction by cryoprecipitation. Modification of their cryoprecipitation procedure gave three protein preparations designated as y-, B-, and a-setarin, based on their increasing solubility in the solvent system of 70% ethanol (v/v), 0.6% sodium acetate (w/v), and 0.5% 2-mercaptoethanol.

Sequential extraction of the defatted millet flour according to the scheme shown in Figure 1 resulted in three major protein fractions, namely, albumin-globulin, setarin I (true prolamin), and setarin II (prolamin-like). Yields by weight, protein, and sulfur content of various protein fractions are summarized in Table I. Based on their amino acid compositions, the sulfur-rich polypeptide fraction of setarin II revealed several polypeptides in the molecular weight range of 43.6–7.9K. a-Setarin showed a single electrophoretic band of low molecular weight (7.9K). B-Setarin also gave a major band corresponding to a molecular weight of 9.1K. On the other hand, γ-setarin consisted of three polypeptides of 7.9K, 21.8K, and 36.3K, of which the low molecular weight polypeptide was the most prominent. a-Setarin appeared to be electrophoretically homogeneous in SDS-PAGE.

RESULTS AND DISCUSSION

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Estimates of the minimum molecular weights of a- and B-setarin were analyzed by SDS-PAGE under reducing conditions (Fig. 2). The electrophoretic patterns of setarin II revealed several polypeptides in the molecular weight range of 43.6–7.9K. a-Setarin showed a single electrophoretic band of low molecular weight (7.9K). B-Setarin also gave a major band corresponding to a molecular weight of 9.1K. On the other hand, γ-setarin consisted of three polypeptides of 7.9K, 21.8K, and 36.3K, of which the low molecular weight polypeptide was the most prominent. a-Setarin appeared to be electrophoretically homogeneous in SDS-PAGE.

Estimates of the minimum molecular weights of a- and B-setarin could be made from the amino acid compositions (number of residues) of these polypeptides. These estimates of molecular weights, 7.4K for a-setarin and 8.3K for B-setarin, are in close agreement with the molecular weight estimates arrived at by SDS-PAGE. Compared to other methionine-rich proteins, a- and B-setarin are low molecular weight polypeptides. Paulis and Wall (1971) obtained methionine-enriched polypeptides of 17.5K from glutamic acid, proline, and lysine and higher amounts of leucine and phenylalanine. Setarin II was distinct from both a- and B-setarin in possessing negligible amounts of serine, tyrosine, and cysteine but had much higher levels of alanine, leucine, and glutamic acid. The amino acid composition of polypeptides present in the cryosupernatant after recovery of a-setarin was not examined.

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alcohol-soluble glutelin by gel filtration. A-secalin of rye was reported to have a polypeptide of molecular weight 10K that is rich in methionine (Charbonnier et al 1980). In sorghum prolamin, Beckwith and Jones (1972) showed that a 17K polypeptide is present that has 10% of its amino acid residues as methionine.

N-terminal analysis of α-setarin showed that methionine was present at the amino terminal end of the polypeptide. However, a very small amount of leucine was also detected as N-terminal residue of α-setarin, which was perhaps contributed by a contaminating polypeptide that coprecipitated with α-setarin during cryoprecipitation. Thus, α-setarin also appeared to be a fairly homogeneous polypeptide by the criteria of N-terminal analysis.

Amino acid analysis of α-setarin revealed that it had eight methionine residues out of a total of 63 amino acid residues and that β-setarin had eight residues of methionine out of 71 amino acid residues. Nearly 60% of the methionine in the setarin II fraction can be accounted for by the methionine in α and β-setarin (Table I). It was of interest to gain an insight into the distribution of these methionine residues in the polypeptide chain. Separation of the CNBr-cleaved, performic acid oxidized α-setarin by two-dimensional peptide mapping showed at least nine ninhydrin positive spots of varying intensities on the peptide map. It appears from these results that the large number of methionine residues present in α-setarin are randomly distributed along the polypeptide chain and not clustered.

CONCLUSIONS

α- and β-setarin constitute the major sulfur-rich proteins of the Italian millet prolamin fraction. They have rather similar amino acid profiles and molecular sizes. Molecular biology of setarin biosynthesis, and especially the synthesis of methionine-rich proteins of Italian millet, needs to be further investigated now that a simple procedure for isolation of the sulfur-rich proteins of Italian millet has been worked out.

LITERATURE CITED


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