COMPARATIVE DIGESTIBILITY OF LEGUME STORAGE PROTEINS

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The resistance of native legume proteins to proteolysis by mammalian digestive enzymes is an important factor contributing to the poor nutritive value of the unheated protein. Much attention in this regard has focused on phaseloin, the major storage protein of dry beans, *Phaseolus vulgaris*. While heated phaseloin is readily susceptible to proteolysis, native phaseloin has been shown to be largely resistant to complete hydrolysis by trypsin, chymotrypsin and pepsin (Romero and Ryan, 1978; Lienor and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987). Patterns of native phaseloin disappearance, and the appearance of degradation products suggest that each subunit is cleaved in a similar position near the center of the subunit. However, the enzyme cleavage sites for native or heated phaseolin have not been determined. The nucleotide sequence of phaseolin subunits is now available (Slighet et al., 1983) and allows these determinations to be made.

Legume proteins are known to differ in their nutritive value (Evans and Bandemer, 1967), but it has not been determined if these differences in nutritive value between legume proteins can be accounted for by the digestibility of the proteins. Digestion of native and heated phaseolin by various proteinases has been studied, but essentially no information is available on the digestion of vicilin from peas (*Pisum sativum*), and only tryptic hydrolysis of glycinin and β-conglycinin from soybeans (*Glycine max*) has been examined (Kamata et al., 1979, 1982).

The subunits of the 7S legume storage proteins, phaseolin, vicilin and β-conglycinin are similar in amino acid sequence. The 7S and 11S legume proteins differ in amino acid sequence but are similar in predicted secondary protein structure (Argos et al., 1985; Schuler et al., 1983). Thus, one would expect them to be broken down in similar ways by digestive enzymes. Similar regions in the proteins would be expected to be hydrophilic and positioned on the protein surface for accessibility to proteinas, but such determinations have not been made.

Information on legume protein digestibility and structure is important to plant molecular biologists to improve the nutritive value of legumes by genetic engineering. Molecular biologists must not only have information about the amino acid sequence of the protein, but also knowledge of where proteins are cleaved by mammalian digestive enzymes. Efforts might then be made to modify the protein to enhance the cleavage by proteinases, thereby improving the bioavailability of the protein.

The objectives of this investigation were to 1) compare qualitatively the degradation patterns of native and heated legume storage proteins by different proteinases and 2) to determine the cleavage sites for major proteinases in native phaseolin, then compare that region to the other legume storage proteins with regard to protein sequence and surface probability.
Legume storage proteins were isolated, then native and heat-treated proteins were digested with various proteinases (trypsin, chymotrypsin, pepsin, papain, pronase E, subtilisin, aminopeptidase) and the digests subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The N-terminal sequences of the major phaseolin breakdown products from digestion with trypsin, chymotrypsin and papain were determined by Edman degradation on a protein sequencer. The N-terminal sequences were matched to the amino acid sequence obtained from the translation of the nucleotide sequence that codes for phaseolin. Protein sequences for phasolin, vicilin, glycinin and β-conglycinin were used to calculate surface probability and hydrophilicity.

Gel electrophoresis patterns indicated that phasolin was most resistant to digestion, vicilin most susceptible, and glycinin and β-conglycinin intermediate in susceptibility to various proteinases. The native proteins were cleaved by trypsin and chymotrypsin in only limited areas of the molecule, but they were all readily digested by various proteinase upon heat treatment. The serine proteinases, trypsin and chymotrypsin, and the thiol proteinase, papain, all cleaved native phaseolin near the center of the protein molecule in a hydrophilic region predicted to be on the surface. This cleavage region in phaseolin is highly homologous in sequence with hydrophilic regions in vicilin and β-conglycinin.

Results of this investigation increase the understanding of legume protein digestibility and structure. The quantitative analysis of degradation patterns on SDS-PAGE from in vitro digestion of the proteins are consistent with tests of nutritive value conducted in vivo. A comparison of the region in native phaeolin cleaved by proteinases to homologous regions in vicilin, β-conglycinin, and glycinin suggests that legume storage proteins are at least somewhat similar in their degradation by proteinases.

Literature Cited