Degradation of Bean Proteins by Endogenous and Exogenous Proteases—A Review[1,2]

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ABSTRACT

Bean proteins are degraded by proteases when humans and insects utilize the protein for food and during germination of the seed. Whereas human digestive enzymes generally act on heat-denatured bean proteins, enzymes in the germinated seed and in the bean weevil must degrade native bean proteins. Unlike the germinated seed and the bean weevil, which utilize a cysteine proteinase along with other types of enzymes to degrade bean proteins, humans depend largely on acid and serine proteases. Comparing the breakdown of bean proteins by various proteases in these three systems leads to a better understanding of bean protein structure and nutritive value.

The proteins of *Phaseolus vulgaris* are subjected to degradation by proteases from a variety of sources. These proteins are a source of amino acids for the growing plant, for insects that infest the seeds, and for humans who consume the legumes as an economical source of protein. During germination of the seed, storage proteins are degraded and mobilized. They are broken down to amino acids, which are used for the synthesis of various enzymes, structural proteins, non-nitrogenous compounds, or for energy (Ashton 1976, Mayer and Marbach 1981, Wilson 1986, Shutov and Vaintraub 1987). The bean weevil *Acanthoscelides obtectus* is also able to degrade the proteins of *P. vulgaris* using proteolytic enzymes in its gut. This bruchid beetle can cause severe loss of stored legume seeds intended for human consumption or planting (Southgate 1979, Hariri 1981). Whereas enzymes in the germinating seed and in the bean weevil gut must degrade native bean proteins, the digestive enzymes of humans presumably have an easier task since the proteins have generally been heat denatured. The discussion to follow will consider these endogenous and exogenous proteases and their degradation of *P. vulgaris* proteins. The structure and nutritive value of bean proteins can be better understood when one compares the breakdown of bean proteins by proteases in various systems.

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GERMINATION

Degradation of Phaseolin and Other Bean Proteins

Much of the literature on the degradation of *P. vulgaris* proteins concerns the major storage protein that constitutes 50–75% of the total seed protein (Romero et al 1975). Confusion exists concerning the identity of this protein, since it has been referred to by various names, such as phaseolin, euphaseolin, glycoprotein II, G1 globulin, and vicilin (Liener and Thompson 1980). Since all of these preparations seem to share the same properties, it will be referred to as phaseolin in this paper.

Numerous studies have examined the degradation of phaseolin during germination (Table I). The degradation of this protein proceeds slowly and is nearly complete by eight to 10 days of germination (Racusen and Foote 1971, 1973; Bollini and Chrispeels 1978; Sathe et al 1983; Nielsen and Liener 1984). Reserve protein degradation is also slow during germination of other legumes including chickpea (Ganesh Kumar and Venkataraman 1978), peas (Konopska 1979, Basha and Beevers 1975), soybean (Catsimpoolas et al 1968), and mung bean (Chrispeels and Boulter 1975). Gel electrophoresis shows that germination of *P. vulgaris* for several days has little effect on phaseolin, but this is followed by a gradual degradation (Racusen and Foote 1971, 1973; Nielsen and Liener 1984; Pusztai et al 1977). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) indicates that the three subunits of phaseolin (50–52, 47–49, and 44–46 kDa) are degraded during germination into components with molecular weights (M_r) in the range of 20,000–30,000 (Bollini and Chrispeels 1978, Murray 1982, Sathe et al 1983, Nielsen and Liener 1984). The smallest phaseolin subunit is degraded more rapidly than the two larger subunits (Sathe et al

1983, Nielsen and Liener 1984). This pattern differs from that reported for the pea (Basha and Beevers 1975) and mung bean (Boulter et al 1973), where the largest subunit of the major storage protein is the first to be broken down during germination.

Little work has been reported on the breakdown during germination of *P. vulgaris* proteins other than phaseolin. The results of studies with these bean proteins listed in Table I are difficult to compare because of differences in methods of protein fractionation and other factors discussed below.

Proteolytic Activity

Proteolytic enzymes play a key role in the biochemical mechanism of germination. Protein synthesis in the developing seedling depends on the hydrolysis of seed storage proteins. This review covers the literature concerning the proteolytic enzyme activities in the cotyledons of germinating *P. vulgaris* and does not discuss proteolytic activities in other parts of the seedling.

Reports of changes in enzyme activities in the cotyledons of beans during germination are not all in agreement, and some inconsistencies can be attributed to differences in the way data are expressed. As noted by Crump and Murray (1979), to be accurate, activities must be expressed on a per organ basis and not on a parameter that changes with time, such as weight or protein. Discrepancies in the literature are also attributable to different conditions of germination such as soaking before germination, temperature, light, and seed size. Disappearance of total nitrogen or dry weight from the cotyledons more accurately determines the stage of germination than does time expressed as days of germination. Therefore, when adequate data are provided, enzyme activities reported are best compared between studies at points where equal total nitrogen (or dry weight) has been mobilized.

Table II summarizes reports of protease activities for germinating *P. vulgaris* cotyledons and the conditions used to measure the activities. The cotyledons of germinating *P. vulgaris* seeds contain aminopeptidase (as measured by L-leucine-*p*-nitroanilide or leucine-tyrosine), carboxypeptidase (as measured by *N*-carbobenzoxy-L-phenylalanine-alanine), and dipeptidase (as measured by alanine-glycine) activities. They also contain enzymes capable of hydrolyzing low molecular weight substrates typically used to measure trypsinlike enzyme activity (α-*N*-benzoyl-L-

TABLE I
Studies Reporting Degradation of Reserve Proteins During Germination of *Phaseolus vulgaris*

Reference	Cultivar	Protein Studied[a]
Bollini and Chrispeels 1978	Greensleeves	*6.9 Vicilin
El-Hag et al 1978	Red Kidney	Globulin fraction E (crude globulin isolate)
Juo and Stotzky 1970	Red Kidney	Globulin proteins
		Albumin proteins
		Basic proteins
Murray 1982	Hawkesbury Wonder	*Euphaseolin
Nielsen and Liener 1984	Improved Tendergreen	*G1
Pusztai and Duncan 1971	Dwarf Bean Harvester	Globulin proteins
		Albumin proteins
Pusztai et al 1977	Processor	*Major storage protein
Racusen and Foote 1971	Pencil Pod Wax	*Glycoprotein II
Racusen and Foote 1973	Pencil Pod Wax	*Glycoprotein II
Sathe et al 1983	Great Northern	*Major storage protein and other bean proteins

[a] Those marked with an asterisk presumably refer to the major storage protein, phaseolin.

TABLE II
Studies Reporting Proteinase and Peptidase Activities in Germinating *Phaseolus vulgaris* Cotyledons

Reference	Cultivar	Substrate Utilized[a]	pH Utilized	Enzyme Activity Described[a]
Boylan and Sussex 1987	Taylor's Horticultural	^{3}H-phaseolin	5.0	Cysteine endopeptidase
Crump and Murray 1979	Hawskesbury Wonder	LPA	7.25	Aminopeptidase, AP1 and AP2
		BTEE	7.8	BTEE hydrolase
		N-CBZ-L-Tyr-*p*NP	8.0	*N*-CBZ-L-Tyr-*p*NP hydrolase
		BAPA	8.2	BAPA hydrolase
Csoma and Polgar 1984	Juliska, Cherokee	Azocasein	6.5	Cysteine endopeptidase
Feller 1979, 1981	Saxa	LPA	7.0	Aminopeptidase
		N-CBZ-L-Phe-L-Ala	5.0	Carboxypeptidase
		Azocasein	5.4, 7.5	Endopeptidase
Gepstein and Ilan 1980	Brittle Wax	Casein	5.5	Protease
		Endogenous proteins	5.5	Protease
Metivier and Paulilio 1980	Carioca	Casein	5.5	Protease
Mikkonen 1986	Processor	Leu-Tyr	9.2	Alkaline aminopeptidase
		Ala-Gly	8.5	Dipeptidase
		Leu-B-naphthyl-amide	6.4	Neutral aminopeptidase (naphthylamidase)
		N-CBZ-L-Phe-L-Ala	5.9	Carboxypeptidase
		Hemoglobin	3.7	Protease
		Casein	5.4, 7.0	Protease
Nielsen and Liener 1984	Improved Tendergreen	BAPNA	5.0–9.5	Trypsinlike enzyme
		Azocasein	5.0–9.5	Azocaseinase
		Phaseolin	5.0–6.0	Protease
Pusztai and Duncan 1971	Dwarf Bean Harvester (kidney)	LPA	7.0	Aminopeptidase
		N-CBZ-L-Tyr-*p*NP	8.05	Chymotrypsinlike enzyme
		BAPA	7.8	Trypsinlike enzyme
		Hemoglobin	4.5–8.05	Protease
		Casein	5.5	Protease
		Endogenous proteins	4.5–8.05	Autodigesting activity
Yomo and Srinivasan 1973	Kentucky Wonder	Casein	5.5	Protease
Yomo and Taylor 1973	Kentucky Wonder	Gelatin	5.5	Protease

[a] LPA, L-leucine-*p*-nitroanilide; *N*-CBZ-L-phe-L-Ala, *N*-carbobenzoxy-L-phenylalanine-L-alanine; BAPNA or BAPA, α-*N*-benzoyl-L-arginine-*p*-nitroanilide; BTEE, *N*-benzoyl-L-tyrosine ethyl ester; *N*-CBZ-L-Tyr-pNP, α-*N*-carboxy-L-tyrosine-*p*-nitrophenyl ester.

arginine-*p*-nitroanalide, BAPNA) and chymotrypsinlike enzyme activity (α-*N*-carbobenzoxy-L-tyrosine-*p*-nitrophenyl ester, *N*-CBZ-L-Tyr-*p*NP; *N*-benzoyl-L-tyrosine ethyl ester, BTEE). Protease activities have been reported using protein substrates such as casein, azocasein, or hemoglobin.

Chrispeels and co-workers (1979) hypothesized a mechanism to control the catabolism of reserve proteins in legumes, based on their work with mung bean seedlings. They suggested that the protein bodies of the resting bean contain reserve proteins and enzymes such as carboxypeptidases, but not endopeptidases required for reserve protein degradation. However, such endopeptidases are synthesized during germination in the cytoplasm and transported into the protein bodies, where they catalyze the degradation of reserve proteins in cooperation with the carboxypeptidases. Mikkonen (1986) reported a low carboxypeptidase activity in resting seeds of *P. vulgaris*, but both Mikkonen (1986) and Chrispeels and Boulter (1975) found that carboxypeptidase activity increases during germination. This implies that, like the endopeptidases, carboxypeptidases are synthesized during germination and transported into the protein bodies. The carboxypeptidase activity peaked at day 6 of germination in a study by Feller (1979) and at day 10 in a study by Mikkonen (1986). However, the peak carboxypeptidase activity in both experiments occurred when approximately half of the total nitrogen had been mobilized.

Crump and Murray (1979) found two different aminopeptidase activities in dry beans that peaked between days 2 and 4 during germination. However, aminopeptidase activity has most often been reported to be highest in the nongerminated seed and to decrease during germination (Pusztai and Duncan 1971, Feller 1979, Mikkonen 1986). The activities of an alkaline aminopeptidase, an alkaline dipeptidase, and a neutral aminopeptidase (naphthylamidase) have been shown to be highest in the nongerminated cotyledons, then decrease during germination (Mikkonen 1986, Mikkonen and Mikola 1986, Mikkonen et al 1986).

Pusztai and Duncan (1971) found the activity on *N*-CBZ-L-Tyr-*p*NP to be stable to day 7, then increase to a maximum at day 14 (approximately 50% nitrogen mobilization), when expressed on a dry weight basis. Crump and Murray (1979), who expressed data on a cotyledon basis, reported a fourfold increase in activity on *N*-CBZ-L-Tyr-*p*NP from day 4 to its maximum at day 10 (approximately 55% nitrogen mobilization) and maximum activity on BTEE at day 6. However, Crump and Murray (1979) suggested the activity against these two substrates is due to a peptide hydrolase or esterase and not to a chymotrypsinlike enzyme.

Activity on BAPNA during germination was reported to remain constant for 16 days when calculated on a dry weight basis, by which time approximately 70% of the nitrogen had been mobilized (Pusztai and Duncan 1971). When calculated on a cotyledon basis, it was reported to remain at a maximum level from day 2 to 10 (approximately 55% nitrogen mobilization) before decreasing (Crump and Murray 1979). Nielsen and Liener (1984) showed a small increase in activity on BAPNA during 10 days of germination when calculated on a dry weight or protein basis and a decrease in activity on a cotyledon basis. The optimum pH for hydrolysis of BAPNA by bean extract is 8–9, and cysteine proteinase enhancers or inhibitors have no effect on this activity (Nielsen and Liener 1984).

Numerous research groups have found that the activity (on a seed or cotyledon basis) on casein or azocasein is optimized at pH 5–6 and peaks at days 5–7 of germination (Pusztai and Duncan 1971, Yomo and Srinivasan 1973, Gepstein and Ilan 1980, Metivier and Paulilo 1980, Feller 1979, Nielsen 1982, Nielsen and Liener 1984). Nitrogen mobilization for this time period of germination ranged from approximately 15% to 90% in these studies. Mikkonen (1986) reported the activity on casein at pH 5.4 on a seed basis peaked at day 10 of germination (approximately 50% nitrogen mobilization). However, when calculated on a dry weight or protein basis, Nielsen and Liener (1984) reported that this activity on azocasein at pH 5.5 increased through day 10 of germination, at which time 90% of the nitrogen had been mobilized (Nielsen 1982). Proteolytic activity on casein or azocasein at pH 5.5 has been shown by several research groups to be enhanced at least twofold by mercaptoethanol (Yomo and Srinivasan 1973, Feller 1979, Nielsen and Liener 1984) and inhibited by *p*-chloromerouribenzoate (*p*-CMB) (Yomo and Srinivasan 1973) or *N*-ethylmaleimide (Nielsen and Liener 1984). Extracts of the germinated beans break down phaseolin at pH 5–6 (Nielsen and Liener 1984). This activity is enhanced by mercaptoethanol and completely eliminated by the cysteine proteinase inhibitors, *N*-ethylmaleimide, leupeptin, and E-64 (L-*trans*-epoxysuccinylleucylamide [4-guanidino] butane). These observations led to the conclusion that a cysteine proteinase with an acid pH optimum is largely responsible for the breakdown of the major storage protein during germination.

Results concerning the involvement of an apparent cysteine proteinase in germination have been confirmed by two research groups. Csoma and Polgar (1984) purified a cysteine proteinase synthesized during germination of *P. vulgaris* seeds. The purified enzyme had a M_r of 23,400 and resembled papain in that its thiol group is part of an interactive system where its nucleophilic reactivity is enhanced. However, it differs from papain in the pK_a values for acylations and in the magnitudes of the pH-independent rate constants for alkylations with iodoacetate. Boylan and Sussex (1987) purified and characterized an endopeptidase containing cysteine from germinated seeds of *P. vulgaris* cv. Taylor's Horticultural. The enzyme had a M_r of 30,000, an optimum activity on ^{3}H-phaseolin at pH 5, and showed maximum activity at five days of germination, when approximately 60% of the total nitrogen had been mobilized. No endopeptidase activity was detected in the cotyledons until after the first day of seedling growth, when nitrogen mobilization was yet unapparent. The endopeptidase cleaved the three subunits of phaseolin into three clusters of fragments having M_r of 27,000, 25,000, and 23,000. The cleavage site was not determined, but the size distribution of the fragments suggested that each of the subunits is cleaved at sites near the middle of the polypeptide chain. Boylan and Sussex (1987) suggested that the endopeptidase catalyzes the initial endoproteolytic cleavages of phaseolin during germination, then other proteases can further degrade the protein. It should be noted that Vavreinova and Turkova (1975) reported the isolation of a sulfhydryl endopeptidase from *P. vulgaris*, but it is unclear if this enzyme was obtained from the seed or the leaves.

The enzyme purified from germinating *P. vulgaris* cotyledons is closely related to other plant endopeptidases that are generally monomeric, cysteine enzymes with M_r of 23,000–37,000 (Ryan and Walker-Simmons 1981). The best known cysteine enzymes from plants are papain (*Carica papaya*), ficin (*Ficus glabrata*), bromelain (*Ananas comosus*), and actinidin (*Actinidia chinensis*). Of leguminous species, the cotyledons of peas (*Pisum sativum*) (Basha and Beevers 1975), castor beans (*Ricinus communis* L.) (Tully and Beevers 1978; Alpi and Beevers 1981 a,b), black gram (*Vigna mungo*) (Mitsuhashi et al 1986), and mung beans (*Vigna radiata*) (Chrispeels and Boulter 1975, Baumgartner and Chrispeels 1977) contain cysteine enzymes, as suggested by enhancement and inhibition patterns. The best characterized of these proteinases is vicilin peptidohydrolase, which was purified from germinating mung bean cotyledons (Baumgartner and Chrispeels 1977). The enzyme consists of a single polypeptide chain with a M_r of 23,000. This cysteine proteinase, with optimum activity at pH 5.1, accounts for 95% of the endopeptidase activity in the cotyledons and digests vicilin, the major storage protein of *V. radiata* seeds.

In summary, the literature reports indicate that phaseolin and other bean proteins are degraded slowly during germination. Several types of enzyme activity have been detected in the germinating cotyledon. The literature on changes in enzyme activities contains numerous apparent discrepancies, some of which can be accounted for by differences in the conditions of germination and the method of reporting data. The enzyme in germinating bean cotyledons most thoroughly studied is a cysteine endopeptidase. This proteinase appears to cleave at a site near the middle of phaseolin subunits, but the exact cleavage site has not

been determined. Other enzymes must be present in the germinating cotyledon to further degrade phaseolin to peptides and amino acids that can be utilized by the growing plant. Wilson (1986) has suggested that degradation of the storage protein in dicots is initiated by an endopeptidase that inflicts a limited number of specific proteolytic cleavages to make the protein susceptible to further attack by the same enzyme or other proteases. It is likely that the cysteine endopeptidase is capable of making this initial cleavage in phaseolin. It remains to be determined how this cysteine proteinase acts on other bean proteins, which other proteases are involved in the further attack of bean proteins, and at what point during germination these proteases act.

INSECT GUT PROTEASES

Species of the insect family Bruchidae are most closely associated with the damage to legume seeds caused by insects. Within this family, some 20 species belonging to six genera attack grain legumes (Southgate 1979). Infestation by bruchids starts in the field and continues in storage, leading to severe losses. The bruchids of most concern in the storage of *P. vulgaris* are the bean weevil, *A. obtectus* (Say), and the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Schoonhoven and Cardona 1982). While insects have been used to evaluate the nutritional quality of *P. vulgaris* proteins (Shariff et al 1981), very little information is available in the literature explaining how insects digest the protein of legumes.

An early study suggested that the larvae of *A. obtectus* and the related bruchid *Callosobruchus maculatus* (cowpea weevil), possess only very low levels of protease activity (Applebaum 1964). These assays were performed at neutral-alkaline pH and were primarily designed to detect serine endopeptidase activity. Recently there have been reports of a cysteine proteinase with an acidic pH optimum in the gut of the bruchid, *C. maculatus* (Gatehouse et al 1985, Kitch and Murdock 1986, Murdock et al 1987).

The literature on *A. obtectus* midgut enzymes reports α- and β-glucosidases, α- and β-galactosidases, and high activity against starch and pectin (Leroi et al 1984). Applebaum and Guez (1972) found that *A. obtectus* was able to digest a toxic bioactive heteropolysaccharide containing arabinose, xylose, rhamnose, glucose, and galactose. Only recently has the proteolytic activity of the *A. obtectus* gut been examined. The proteolytic enzymes in the guts of *A. obtectus* are of interest, not only to compare to proteases in the germinating bean and human digestive system but also as a vulnerable site in this major storage pest. Characterizing the gut protease of *A. obtectus* and evaluating its importance in the life of the insect could reveal a new target for directed plant breeding work to confer bruchid resistance upon *P. vulgaris*.

Wieman and Nielsen (1988) isolated and partially characterized a major digestive protease from the gut of *A. obtectus*. The enzyme appears to have a cysteine group and an acidic group at or near the active site. The thiol enhancers, cysteine and dithiothreitol, enhanced activity, whereas the thiol inhibitors *p*-CMB and E-64 and the acidic protease inhibitor Pepstatin A eliminated all activity. The molecular weight of the protease was estimated to be 23,600 by gel filtration and 25,300 by SDS-PAGE. The pH range for optimum activity of this protease was pH 4.5–7.0 using the substrate benzoyl-DL-arginine-2-naphthylamide (BANA). Native phaseolin was somewhat resistant to degradation by a crude gut extract of *A. obtectus* and the purified protease. When native phaseolin was subjected to the purified insect protease, the molecular weights of breakdown products were in the M_r range of 21,000–24,000. Heated phaseolin was readily degraded by the purified protease to small molecular weight polypeptides. Whole bean extract and the globulin fraction of *P. vulgaris* appeared to be somewhat degraded by a crude gut extract and by the purified protease, while the albumin fraction was more resistant. Some evidence was obtained suggesting the presence of other proteolytic enzymes in the crude gut extract (Wieman 1986), but they have not been identified or characterized. Such proteinases and peptidases would be required to complete the degradation of phaseolin to amino acids or peptides small enough to be utilized by the insect, but these have not been identified.

MAMMALIAN DIGESTIVE PROTEASES

Degradation of Phaseolin

Most studies designed to answer questions related to the nutritional quality of *P. vulgaris* proteins have focused on proteins in the globulin fraction (Table III). In particular, considerable attention has been directed toward phaseolin. Heated phaseolin is highly susceptible to digestion by mammalian digestive enzymes (Liener and Thompson 1980, Bradbear and Boulter 1984, Deshpande and Nielsen 1987a). However, the resistance of native phaseolin to proteolytic attack by mammalian digestive enzymes contributes to the poor nutritive value of the unheated bean (Waterman and Johns 1921, Vaintraub et al 1976, Romero and Ryan 1978, Vaintraub et al 1979, Liener and Thompson 1980, Chang and Satterlee 1981, Bradbear and Boulter 1984, Deshpande and Nielsen 1987a). The inaccessibility of phaseolin to enzymes has been attributed to certain of its structural properties (Romero and Ryan 1978; Vaintraub et al 1976, 1979; Bradbear and Boulter 1984), in particular its compact structure (Chang and Satterlee 1982) and the stability and stearic hinderances imparted by its carbohydrate moieties (Chang and Satterlee 1981, Semino et al 1985).

The degradation of native and heated phaseolin has been studied in vitro by examining the protein banding patterns on SDS-PAGE, and by treating the protein digest with trichloroacetic acid then analyzing the supernatant for free amino groups using trinitrobenzene sulfonic acid. Native phaseolin has been shown to be largely resistant to in vitro hydrolysis by pepsin, trypsin, and chymotrypsin (Vaintraub et al 1976, 1979; Romero and Ryan 1978; Liener and Thompson 1980; Bradbear and Boulter 1984; Deshpande and Nielsen 1987a). Digestion with a combination of these enzymes does little or nothing to overcome the resistance of native phaseolin to hydrolysis (Romero and Ryan 1978, Vaintraub et al 1979, Liener and Thompson 1980). Trypsin more readily degrades native phaseolin than does pepsin or chymotrypsin (Romero and Ryan 1978, Vaintraub et al 1979, Liener and Thompson 1980, Deshpande and Nielsen 1987a). Pepsin hydrolysis of native phaseolin reportedly stops after only 2.4% of the peptide bonds have been cleaved (Vaintraub et al 1979) and causes no change in electrophoretic mobility of the protein (Liener and Thompson 1980, Deshpande and Nielsen 1987a). The low in vitro digestibility of phaseolin by trypsin as compared to trypsin digestion of bovine serum albumin appears to be partially related to the lower concentration of trypsin-susceptible bonds (Romero and Ryan 1978). Vaintraub et al (1976) found that trypsin cleaved only 15% of all the trypsin-hydrolyzable bonds in native phaseolin, whereas this value would be 21% using the data of Romero and Ryan (1978) and the known lysine plus arginine content of phaseolin.

Trypsin and chymotrypsin cleave native phaseolin in such a way that the main part of the molecule remains intact. The major degradation products from trypsin and chymotrypsin digestion of native phaseolin subunits are in the range of M_r 22,000–30,000 (Liener and Thompson 1980, Bradbear and Boulter 1984, Deshpande and Nielsen 1987a). The major degradation products are also of similar size when digested by subtilisin, pronase E, or papain (Deshpande and Nielsen 1987a). The patterns of native phaseolin disappearance and the appearance of degradation products suggest that each subunit is cleaved in a similar position near the center of the subunit (Romero and Ryan 1978, Deshpande and Nielsen 1987a). The hydrolysis of phaseolin by enzymes is similar between cultivars of *P. vulgaris* (Deshpande and Nielsen 1987a). *N*-terminal sequence analysis of the major breakdown products from phaseolin digestion indicates that trypsin, chymotrypsin, and papain all cleave native phaseolin in the same region of the molecule (Fig. 1) (Nielsen et al, *in press*). Such cleavage would produce breakdown products of approximately 24,700 and 21,300 Da, which is consistent with those observed by

SDS-PAGE. The major cleavage sites in native phaseolin for these proteases occur in an extended region of hydrophilic amino acid residues that are predicted to occur on the protein surface and would be accessible to protease action.

While native phaseolin has been shown to be largely resistant to breakdown by pure endopeptidases from the stomach or small intestine (e.g. trypsin, chymotrypsin, pepsin), one report from the literature (Sgarbieri et al 1982) suggests that these pure endopeptidases, either alone or in combination, are poor models for studying the in vivo digestibility of this protein. Nearly 90% of native phaseolin was digested in vitro to small, trichloroacetic acid soluble segments by sequential treatments with stomach and small intestine extracts from rats and with pepsin and pancreatin. Heating this protein for 10 min at 100° C had no effect on the rate or extent of in vitro proteolysis. Additional experiments with rat or human stomach and small intestine extracts are needed to confirm

TABLE III
Studies Reporting Digestion of Phaseolin and Other *Phaseolus vulgaris* Protein Fractions by Various Proteinases and Peptidases

Reference	Cultivar	Protein Studied[a]	Enzymes Tested In Vitro	Parameters Measured In Vivo
Antunes and Sgarbieri 1980	Rosinha G2	Whole bean flour, Six water-soluble or salt-soluble fractions		Protein efficiency ratio, Digestibility
Bradbear and Boulter 1984	Dwarf White	Phaseolin	Aminopeptidase Papain Prolidase Pronase Proteinase-K Trypsin	
Chang and Satterlee 1981	Great Northern	*Major storage protein	Combination of bacterial protease, chymotrypsin, peptidase, and trypsin	
Deshpande and Nielsen 1987a	17 Cultivars	Phaseolin	Aminopeptidase Chymotrypsin Papain Pepsin Pronase Subtilisin Trypsin	
Deshpande and Nielsen 1987b	11 Cultivars	Whole bean flour, Water-soluble fraction, Salt-soluble fraction	Trypsin	
Liener and Thompson 1980	Sanilac	*G1	Chymotrypsin Pepsin Trypsin Chymotrypsin-pepsin-trypsin combination	True digestibility, Weight of pancreas
Marquez and Lajolo 1981	Carioca	Albumins *Globulin G1 Globulin G2 Glutelin	Trypsin Pancreatin Pepsin-pancreatin	
Nielsen 1982	Improved Tendergreen	Phaseolin	Combination of bacterial protease, chymotrypsin, peptidase, and trypsin	
Romero and Ryan 1978	Improved Tendergreen	*G1	Chymotrypsin Pepsin Trypsin Pepsin-chymotrypsin Pepsin-trypsin	
Sathe et al 1982	Great Northern	Whole bean flour Albumins Globulins Protein conc. Protein isolate	Combination of chymotrypsin, peptidase, and trypsin	
Seidl et al 1969	Cubagua	One globulin fraction	Chymotrypsin Ficin Hurain Pepsin Subtilisin Trypsin	
Sgarbieri et al 1982	Processor	*Glycoprotein II	Pepsin-pancreatin, or rat stomach extract then rat intestinal contents	Digestibility for whole digestive tract of for stomach plus small intestine
Vaintraub et al 1976		*7S protein	Chymotrypsin Trypsin	
Vaintraub et al 1979		*7S protein	Pepsin Pepsin-trypsin	
Waterman and Johns 1921	Navy	Phaseolin	Pepsin Trypsin	

[a] Proteins marked with an asterisk presumably refer to the major storage protein, phaseolin.

these results, because they are not in agreement with other in vitro studies using native phaseolin.

Although native phaseolin is very resistant to hydrolysis by individual treatment with trypsin, chymotrypsin, or pepsin, the hydrolysis is greatly increased by heat treatment (Romero and Ryan 1978, Liener and Thompson 1980, Deshpande and Nielsen 1987a). Vaintraub et al (1979) found that phaseolin is completely digested by pepsin only after denaturation by heat treatment or the action of guanidine hydrochloride. Heated phaseolin is more or less completely digested by trypsin (Bradbear and Boulter 1984, Deshpande and Nielsen 1987a). After heating, the degree of digestion by trypsin and chymotrypsin has been shown to approach that of casein, and actually exceed it for pepsin (Liener and Thompson 1980). The increases in hydrolysis by each enzyme caused by heat treatment of phaseolin have been shown to be substantially larger in than the increases seen after heat treatment of bovine serum albumin (Romero and Ryan 1978).

The digestibilities of native and heated phaseolin can be compared between various in vitro and in vivo studies. Bradbear and Boulter (1984) compared the yield of amino acids from phaseolin preparations after complete acid hydrolysis to that after enzymatic hydrolysis with a combination of papain, prolidase, and aminopeptidase M. The mean recovery of amino acids was 91 and 13% after enzymatic hydrolysis of heated phaseolin and native phaseolin, respectively. The in vitro digestibility of native and heated phaseolin also has been determined by two groups according to the four-enzyme procedure of Satterlee et al (1979), which utilizes trypsin, chymotrypsin, peptidase, and bacterial protease. Chang and Satterlee (1981) reported values of 74 and 91% for native and heated protein, respectively, which are very similar to the values of 79 and 92% reported by Nielsen (1982). Liener and Thompson (1980) found the true digestibility in rats in a four-week study to be 57% for native phaseolin and 92% after heat treatment (diet not supplemented with methionine). When Sgarbieri et al (1982) fed native phaseolin to previously fasted rats in a single dose by intubation, the digestibility was between 80 and 90%. However there was also increased production of insoluble intestinal secretions containing relatively large amounts of nitrogen. Because this nitrogen passed into the caecum, it reduced the apparent digestibility of native phaseolin to about 35%.

The in vivo and in vitro digestibility values reported above are similar for heated phaseolin, but results are not in agreement for native phaseolin. The large differences in in vitro digestibility values reported for native phaseolin are likely attributed to the method of measuring protein breakdown and the assay conditions. While Bradbear and Boulter (1984) apparently used the ninhydrin reagent to measure free amino groups after enzymatic hydrolysis, the four-enzyme procedure of Satterlee et al (1979) uses the change in pH due to enzymatic hydrolysis to calculate the digestibility. Both in vitro methods utilize not only endopeptidases, but also exopeptidases, which should more closely simulate the conditions required for complete digestion to the point at which they can be utilized by humans. However, the proteases used and time and temperature conditions differ between the two assays. The inconsistencies between in vivo digestibility results for native phaseolin can be explained at least partially by the differences between a four-week study and a single-dose treatment. While many in vitro and in vivo tests work well to estimate the nutritive value of heat-treated and high-quality proteins, all tests have their disadvantages when testing unheated and low-quality proteins.

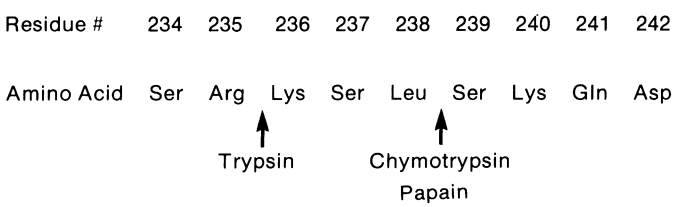

Fig. 1. Region of the phaseolin amino acid sequence cleaved when trypsin, chymotrypsin, or papain are reacted with native phaseolin. Phaseolin sequence used is that derived from the nucleotide sequence reported by Slightom et al (1983).

Digestibility of Protein Fractions

In addition to the studies with pure phaseolin, several studies have examined the in vitro and in vivo digestibilities of various *P. vulgaris* fractions (Table III). Antunes and Sgarbieri (1980) found that unheated bean flour and six isolated fractions were toxic when fed to weanling rats, but heat treatment improved the nutritive value of all samples, including albumin and globulin fractions. In a study by Sathe et al (1982), the in vitro digestibility of unheated bean flour, albumins, globulins, protein concentrates, and protein isolates was 9, 3, 10, 14, and 29%, respectively. Dry heat increased the digestibility to 69, 24, 30, 24, and 34%, respectively, but the values were raised even higher by moist heat. Marquez and Lajolo (1981) found that heating improved the in vitro digestibilities of globulin and glutelin fractions. However, unlike the albumin results of the two previously cited studies, Marquez and Lajolo (1981) found that heat treatment reduced the digestibility of the albumin fraction. They found that peptides with M_r of 14,000 and 20,000 remained in the residue after digestion of autoclaved albumin and heating caused the appearance of high molecular weight aggregates. These researchers reported evidence for a relatively heat-stable trypsin inhibitor in the albumin fraction, which may have affected its digestibility.

Deshpande and Nielsen (1987b) also obtained evidence that protein-protein interactions and heat-stable inhibitors influence the digestibility of proteins in the albumin fraction, especially in certain bean varieties. The salt-soluble protein fractions of 11 *P. vulgaris* cultivars tested were more readily and completely hydrolyzed by trypsin than were the water-soluble proteins. Phaseolin was highly susceptible to trypsin when present in the salt-soluble fractions but was less readily hydrolyzed in the presence of certain water-soluble proteins. Phaseolin, present as a contaminant in the water-soluble fraction of Light Red Kidney, Pinto, Black Beauty, Small White, Small Red, and Great Northern beans, was much less hydrolyzed by trypsin compared to the remaining varieties investigated. Further work with the Great Northern bean albumin fraction showed that it contained heat-stable trypsin and chymotrypsin inhibitory activity. Like Marquez and Lajolo (1981), Deshpande and Nielsen (1987b) observed that some proteins in the heat-treated, water-soluble fraction failed to enter the gel, suggesting that protein aggregates of very high molecular weight were formed during heating and were not degraded by trypsin.

To summarize the literature reports examining the susceptibility of phaseolin and other bean protein fractions to mammalian digestive enzymes, numerous studies show that native phaseolin is quite resistant to proteolysis. The size of the degradation products from native phaseolin suggests that, with the likely exception of some potential cleavage sites for endopeptidases near the amino or carboxy terminal regions, only a small segment near the center of each subunit is accessible to proteases such as trypsin and chymotrypsin. Discrepancies in digestibility values exist between and among various in vitro and in vivo studies, but assay conditions vary considerably between many of these studies. However, upon heat treatment of phaseolin most in vitro and in vivo studies have shown drastic improvements in digestibility. The in vitro digestibility of the bean globulin fraction is increased by heat treatment, but data are conflicting for the effect of heat treatment on the digestibility of the albumin fraction. The high molecular weight aggregates formed when the albumin fraction is heated may not be readily degraded by mammalian digestive proteases. Further in vitro and in vivo studies are necessary to better understand the digestion of albumin proteins and their influence on the breakdown of other bean proteins. Heat-stable trypsin-chymotrypsin inhibitory activity has been detected in the albumin fraction of some *P. vulgaris* cultivars. It seems unlikely that such inhibitors are present in large enough quantities to cause any negative nutritional effects to humans under normal dietary conditions, but their effects have not yet been tested in any in vivo studies.

SUMMARY

The proteins of *P. vulgaris* are subjected to degradation by proteases found in the germinating cotyledons, the insect gut, and the human digestive system. The germinating bean cotyledon and the bean weevil gut both contain a cysteine proteinase with an acidic pH optimum. These proteinases seem to cleave native phaseolin at a site near the middle of the polypeptide chain. In both systems, other enzymes must be present to further degrade this major storage protein to peptides and amino acids that can be utilized for growth and development of the plant or insect. These other enzymes have not been clearly identified or characterized with regard to their role in protein degradation. Unlike germinating cotyledons and insects, humans do not utilize a cysteine proteinase in their digestive system. Humans also have the easier task of degrading heat-denatured protein rather than native protein. The serine proteinases, trypsin and chymotrypsin, cleave native phaseolin subunits in the same region of the amino acid sequence as does the thiol proteinase papain. Breakdown patterns of native phaseolin reacted with the cysteine proteinases of the germinated bean cotyledon or the gut of larval bean weevil suggest that they cleave phaseolin in the same region near the center of each protein subunit. Heated phaseolin is readily hydrolyzed by mammalian digestive proteases to small molecular weight peptides. While the salt-soluble fraction of bean proteins appears to be readily digested upon heat treatment, there are still some questions about the effect of heat denaturation on the digestibility of the water-soluble fraction. The albumin proteins require further study to determine how they affect the digestibility of other bean proteins. Changes in the structure of proteins in the albumin fraction upon heat treatment and their susceptibility to mammalian digestive proteases also need further investigation.

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