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subunit of the MP enzyme is enough like the CAM molecule to compete with it in such protein interactions.

ABBREVIATIONS

CAM, calmodulin (soybean calcium-binding protein); cAMP, cyclic adenosine monophosphate; DEAE, (diethylamino)ethyl; EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; HPLC, high-pressure liquid chromatography; MP, monophosphatase; PDE, phosphodiesterase; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide.

Registry No. MP, 9013-05-2; PDE, 9025-82-5.

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Comparative Digestibility of Legume Storage Proteins

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Native and heated legume storage proteins were digested with various proteinases. Gel electrophoresis patterns indicated that phaseolin (dry bean) was most resistant to digestion, vicilin (pea) was most susceptible, and glycinin and β-conglycinin (soybean) were 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 degraded upon heating. N-Terminal sequence analysis of the major breakdown products from phaseolin digestion and hydrophilicity and surface probability determinations indicated that trypsin, chymotrypsin, and papain cleave native phaseolin near the center of the protein molecule at a hydrophilic region predicted to be on the surface. This region in phaseolin is highly homologous in sequence with hydrophilic regions in vicilin and β-conglycinin.

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 (Romero and Ryan, 1978; Liener and Thompson, 1980). Considerable attention in this regard has focused on phaseolin, the major storage protein of dry beans, *Phaseolus vulgaris*. While heated phaseolin is readily susceptible to proteolysis (Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987), native phaseolin has been shown to be largely resistant to complete hydrolysis by trypsin, chymotrypsin, and pepsin (Romero and Ryan, 1978; Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987). The inaccessibility of phaseolin to enzymes has been attributed to its structural properties (Romero and Ryan, 1978), particularly its compact structure (Chang and Satterlee, 1981). Native phaseolin is much more resistant to pepsin than trypsin and is more rapidly hydrolyzed by trypsin than chymotrypsin (Romero and Ryan, 1978; Vaintraub et al., 1979; Liener and Thompson, 1980; Deshpande and Nielsen, 1987). Trypsin and chymotrypsin have been shown to cleave native phaseolin in such a way that the halves of the molecule remain intact (Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987). Previous studies have also observed that the patterns of native phaseolin disappearance and the appearance of degradation products suggest each subunit is cleaved in a similar position near the center of the subunit (Romero and Ryan, 1978; Deshpande and Nielsen, 1987). However, enzyme cleavage sites for native or heated phaseolin have not been determined. Information on the nucleotide sequence of phaseolin subunits (Slightom et al., 1983) now allows these determinations to be made.

Legume proteins are known to differ in their nutritive value. When the nutritive value of unheated dry beans, peas, and soybeans is compared, that of dry beans is lowest, that of peas highest, while that of soybeans intermediate (Evans and Bandemer, 1967). While the nutritive value of dry beans and soybeans is greatly increased by heat treatment, that of peas is not appreciably increased (Evans and Bandemer, 1967). The described differences in nutritive value exist between the three legumes despite the fact that they contain nearly equal quantities of methionine and/or cystine (Kakade, 1974). It has not been determined whether 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 (Deshpande and Nielsen, 1987), but essentially no information is available on the digestion of vicilin (*Pisum sativum*). While tryptic hydrolysis of glycinin and β-conglycinin (*Glycine max*) has been examined (Lynch et al., 1977; Kamata and Shibasaki, 1978; Kamata et al., 1979a,b, 1982), little is known about the degradation of these proteins by other proteinases. One research group (Vaintraub et al., 1976, 1979) observed

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that native glycinin is more readily hydrolyzed by trypsin, chymotrypsin, or pepsin than is native phaseolin. However, results from studies that report the degradation of legume storage proteins by proteinases generally cannot be compared since digestion conditions differ.

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 protein structure preference (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 proteinases, 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 have not only information about the amino acid sequence of the protein but also knowledge of where proteins are cleaved by mammalian proteinases. Efforts might then be made to modify the protein to enhance the cleavage by proteinases, thereby improving the bioavailability of the protein.

The purpose of this investigation was to compare qualitatively the degradation patterns of native and heated legume storage proteins by different proteinases. Cleavage sites in native phaseolin for major proteinases were determined, and that region was compared to the other legume storage proteins with regard to protein sequence and surface probability.

MATERIALS AND METHODS

Preparation of Legume Storage Proteins. Phaseolin was isolated from *P. vulgaris* L. var. Improved Tendergreen by the method of Hall et al. (1977), who referred to their preparation as the G1 fraction. The isolation procedure was repeated to precipitate the phaseolin three times before it was dialyzed against distilled water and lyophilized. Vicilin was isolated from *P. sativum* var. Blue Bantam according to the procedure of Gatehouse et al. (1981), which uses precipitation with ammonium sulfate and fractionation on a column of Ultrogel AcA 22. The β-conglycinin and glycinin were isolated from *G. max* var. Century as described by Coates et al. (1985). The glycinin obtained by this procedure was further purified on a Sepharose CL-6B column and the 11S peak collected, dialyzed against distilled water, and lyophilized.

Digestion of Proteins for SDS-PAGE. The following enzyme preparations were obtained from Sigma Chemical Co., St. Louis, MO: trypsin (from bovine pancreas, Type III-S, 13000 units/mg of protein on BAEE, Lot 65.5-8190), chymotrypsin (from bovine pancreas, Type II, 48 units/mg of protein on BAEE, Lot 15F-8160), bacterial protease (subtilisin Carlsberg, from *Bacillus subtilis*, Type VIII, 11.9 units/mg of casein, Lot 104F-0099), pronase E (from *Streptomyces gresius*, Type XIV, 5.8 units/mg on BAEE, Lot 113F-8135), aminopeptidase (from porcine intestinal mucosa, 100 units/g on L-leucine β-naphthylamide, Lot 80F-80101), and pepsin (from porcine stomach mucosa, 3200 units/mg of protein, Lot 64F-8080). Unless mentioned otherwise, all chemicals used were of reagent grade.

The legume storage proteins were dissolved in the appropriate buffer at 2.5 mg/mL, and an aliquot (500 μg of Lowry protein) was digested with the proteinases. The buffer systems selected for the different proteinases used were 50 mM Tris-HCl, pH 8.1, containing 20 mM $CaCl_2$ for trypsin and chymotrypsin; 50 mM phosphate buffer, pH 7.5, for subtilisin and pronase E; 50 mM phosphate, pH 7.1, for aminopeptidase; 50 mM phosphate, pH 6.2, for papain; and 50 mM HCl for pepsin. Heated protein samples were prepared by dissolving them in the appropriate buffer and heating the stoppered test tubes in a boiling water bath at 99 °C for 15 min. All native (unheated) digestion assays were conducted at 37 °C with a 10:1 or 100:1 protein to enzyme ratio, while only a 100:1 ratio was used for the heated proteins. The digestion reaction (30 min, except for the time course studies) was stopped by adding the sample buffer for SDS-PAGE (0.05 M Tris-HCl, pH 6.8; 1% SDS, 30% glycerol, 2% β-mercaptoethanol, and 0.01% bromophenol blue) and immediately heating the tubes at 99 °C for 3 min prior to electrophoresis. For each enzyme, all assays were carried out at least in duplicate, and the gels shown are for a typical run.

SDS-PAGE. SDS-PAGE was performed by the method of Fling and Gregerson (1986) with a 8–25% linear gradient and no urea present. The molecular weight marker proteins phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase 30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa) were from Pharmacia Inc., Piscataway, NJ, and the low molecular weight kit containing myoglobin polypeptide backbone (16.95 kDa), myoglobin fragments I + II (14.4 kDa), fragment I (8.16 kDa), fragment II (6.21 kDa), and myoglobin fragment III (2.51 kDa) was from Sigma.

Digestion of Phaseolin for Sequence Analysis. Native phaseolin was dissolved in 0.1 M ammonium bicarbonate at pH 7.8 (for trypsin and chymotrypsin digestion) or 50 mM phosphate at pH 6.2 (for papain) and reacted wtih trypsin, chymotrypsin, or papain for 4 h at 37 °C, using a 100:1 protein to enzyme ratio. The reaction was stopped by adding formic acid to a final concentration of 9%. Samples were then dialyzed against 9% formic acid, with 12000–14000 molecular weight cutoff tubing. Dialysis was utilized to remove any small peptides that might interfere with the determination of the N-terminal sequence of the major breakdown products.

N-Terminal Sequence Analysis. The N-terminal sequences of the major phaseolin breakdown products from digestion with trypsin, chymotrypsin, and papain were determined by Edman degradation on a Beckman Model 890C protein sequencer. The N-terminal sequences were matched to the amino acid sequence obtained from the translation of the nucleotide sequence coding for phaseolin (Slightom et al., 1983). The molecular weights of the major breakdown products were calculated. Native phaseolin was assumed to contain 399 amino acid residues, after a signal peptide of 21 residues is cleaved from the total sequence of 420 residues (Slightom et al., 1983).

Surface Probability and Hydrophilicity Plots. Calculations of surface probability for legume storage proteins were made with software described by Devereux et al. (1984), and plots from these calculations were drawn by the program LOTUS 1,2,3. Amino acid surface probabilities were calculated by a procedure similar to that reported by Emini et al. (1985), which is based on atomic coordinates, side-chain geometry and energy calculations, and solvent accessibility for 28 proteins (Janin et al., 1978). Protein sequences used were reported by Slightom et al. (1983) for phaseolin (β-subunit), Lycette et al. (1984) for vicilin, Doyle et al. (1986) for β-conglycinin (α′-subunit), and Marco et al. (1984) for the glycinin (Gy2 subunit). These same sequences and a procedure similar to that described by Kyte and Doolittle (1982) were used to prepare hydrophilicity plots for the legume storage proteins.

RESULTS

Trypsin Digests. The three subunits of native pha-

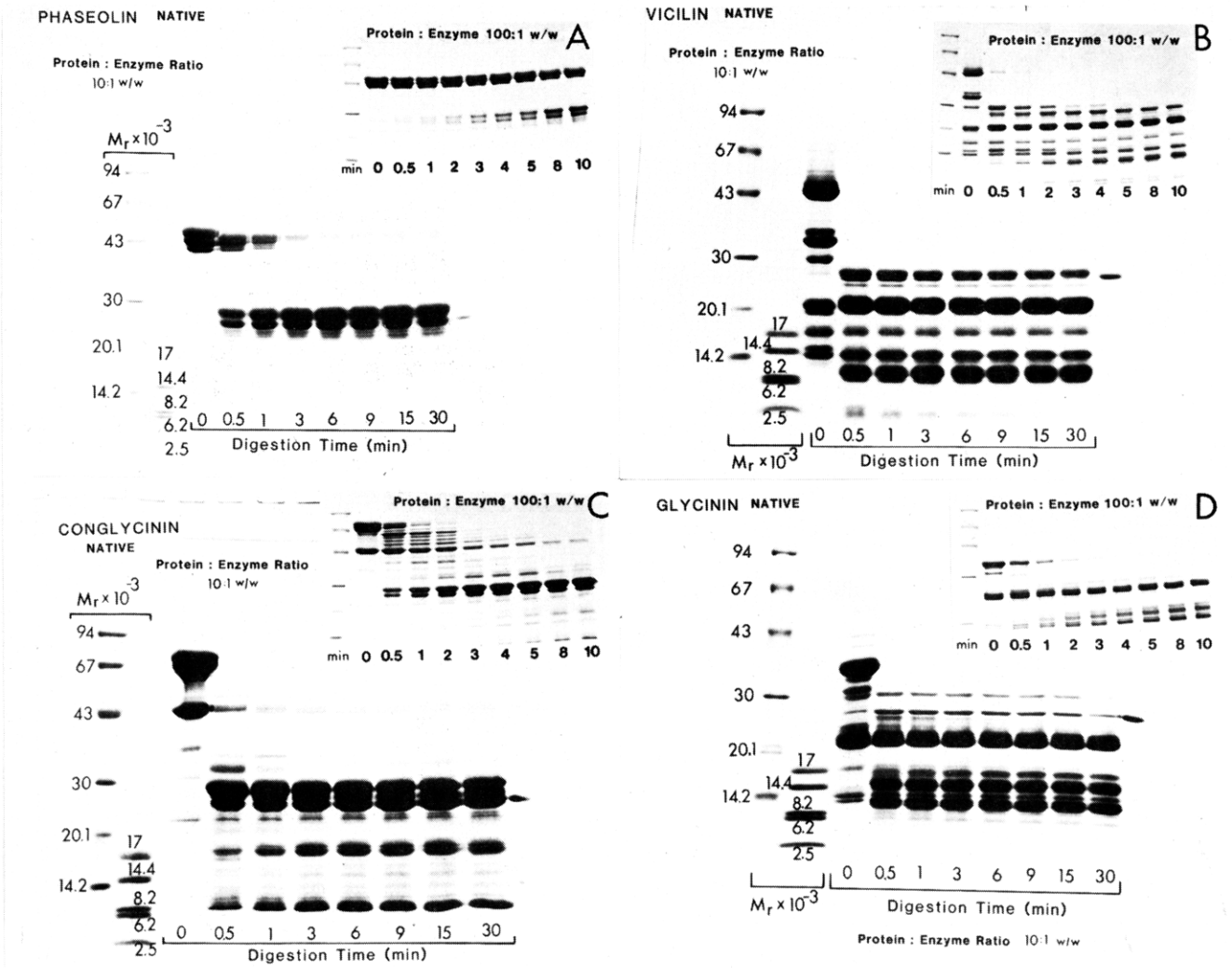

Figure 1. SDS-PAGE patterns of native legume storage proteins subjected to trypsin in time course digestion. The numbers at the bottom of each lane denote the time of digestion (min), with a 10:1 or a 100:1 protein to trypsin ratio (w/w). The molecular weight markers are those described under Materials and Methods: A, phaseolin; B, vicilin; C, β-conglycinin; D, glycinin.

seolin (MW 50–52K, 47–49K, 44–46K) were degraded by trypsin to products with approximate molecular weights of 22–25K (Figure 1A). Each native phaseolin subunit appeared to be cleaved near the center by trypsin. Like phaseolin, native vicilin, glycinin, and β-conglycinin were quite resistant to complete hydrolysis by trypsin (Figure 1A–D). Of the four native proteins subjected to trypsin digestion, phaseolin degradation was the slowest and vicilin breakdown was the fastest, based on visual observation of SDS gels. Glycinin breakdown under the conditions used was faster than that of β-conglycinin. The major breakdown products for all the native proteins appeared to be approximately half the molecular weight of the original subunits. Upon heat treatment, all the storage proteins were readily digested by trypsin (Figure 2A–D). Visual observation of gels indicated that heated phaseolin and glycinin were degraded by trypsin at a somewhat faster rate than vicilin and β-conglycinin.

Digestion by Various Proteinases. In cases with a large amount of degradation by proteinases, the major breakdown products were of similar size irrespective of the type of proteinase used (approximately 21–27 kDa) (Figure 3A). The molecular weights of breakdown products were more variable for the other native proteins digested with various proteinases for 30 min (Figure 3B–D). The subunits of glycinin, β-conglycinin, and vicilin are not as similar in size as are those of phaseolin, so it is more difficult to visually analyze the digestion breakdown products. Among the serine proteinases, subtilisin and pronase E hydrolyzed the native legume proteins more completely than did trypsin or chymotrypsin, which have a more restricted specificity. Breakdown by subtilisin and pronase E was much less complete for native phaseolin than for the other native proteins. Chymotrypsin was less effective than trypsin on all native proteins except vicilin.

Native phaseolin was markedly resistant to a carboxyl proteinase (pepsin) and somewhat resistant to a thiol proteinase (papain). Pepsin and papain were somewhat more effective on native β-conglycinin and readily degraded native glycinin and vicilin. Aminopeptidase was ineffective in causing apparent molecular weight changes in native or heated proteins digested for 30 min.

Phaseolin digestion by most proteinases was much more complete on heated compared to native protein. This difference between native and heated protein was not as great for the other legume storage proteins. In fact, for these other proteins, native proteins were more completely degraded by subtilisin or pronase E than were the heated proteins. This pattern was also true for pepsin digestion of vicilin or glycinin. However, the heated seed proteins may have had lower solubility and therefore reduced accessibility to some proteinases.

Enzymatic Cleavage of Native Phaseolin. Sequence data indicated that trypsin, chymotrypsin, and papain cleave native phaseolin in the same region of the molecule (Figure 4). Such cleavage with trypsin would produce

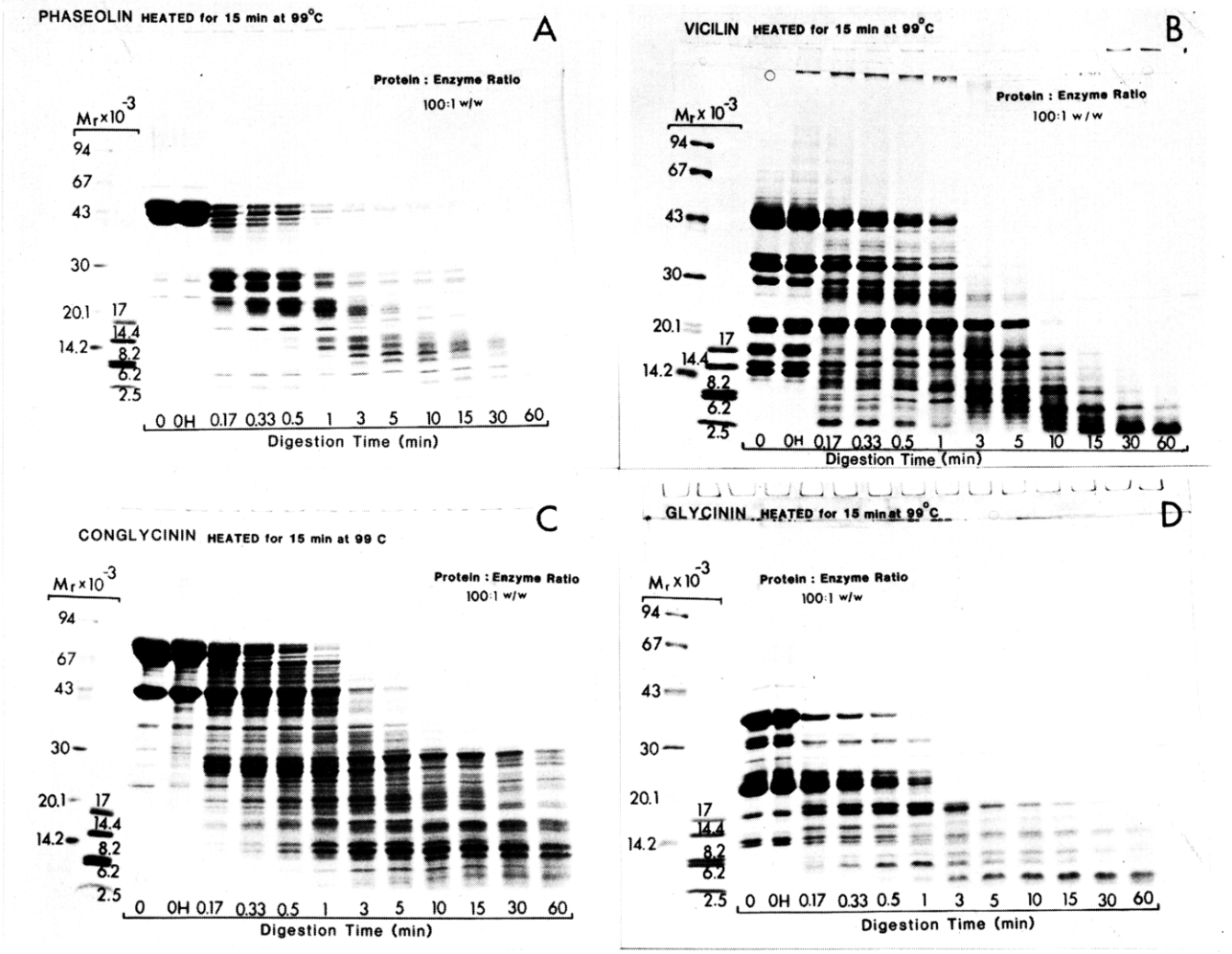

Figure 2. SDS-PAGE patterns of heated legume storage proteins subjected to trypsin in time course digestion. The number at the bottom of each lane denotes the time of digestion (min), with a 100:1 protein to trypsin ratio (w/w). O and OH indicate the native control and the heated control, respectively. The molecular weight markers are those described under Materials and Methods: A, phaseolin; B, vicilin; C, β-conglycinin, D, glycinin.

breakdown products of approximately 24 700 and 21 300 Da, which is consistent with the size of digestion products observed by SDS-PAGE.

Surface Probability and Hydrophilicity Plots. The major cleavage sites in native phaseolin for trypsin, chymotrypsin, and papain occurred in an extended region of hydrophilic amino acid residues. This region was predicted to be positioned on the surface of the protein molecule (Figure 5A) and would likely be accessible to proteinase action. The phaseolin and vicilin hydrophilicity plots were very similar, with the exception of one large hydrophilic region unique to vicilin (residues 187–202 on Figure 5B) just prior to the cleavage site region in phaseolin and a hydrophilic carboxy terminus in phaseolin (residues 413–439 on Figure 5A) (hydrophilicity plots are not shown). This region, unique to vicilin, had a high probability of occurring on the protein surface (Figure 5B). Unlike phaseolin, vicilin also had a region with high surface probability at residues 320–340. The average hydrophilicity of vicilin was higher (0.6057) than that of the phaseolin (0.3872), which was consistent with the fact that vicilin was more susceptible to proteolysis than phaseolin. However, it must be remembered that many factors other than hydrophilicity determine the susceptibility of a protein to proteolysis. One-third of the β-conglycinin sequence at the N-terminus was very hydrophilic and had a high surface probability while the latter two-thirds was more variable and has only three regions with high surface probability (Figure 5C). The glycinin sequence was quite variable in hydrophilicity and had regions scattered throughout the sequence with high surface probability (Figure 5D).

DISCUSSION

Patterns of phaseolin degradation by various proteinases are comparable to those reported previously. The new knowledge of the major proteinase cleavage region in phaseolin gives increased understanding not only of phaseolin but also of the other legume storage proteins. Several factors made it possible to conduct N-terminal sequence analysis of phaseolin using a mixture of polypeptides resulting from digestion, rather than using individual isolated polypeptides. It is known that phaseolin subunits are highly homologous in sequence (Slightom et al., 1985) and that the N-terminus of phaseolin is blocked (Hall, T., personal communications). Since degradation of native phaseolin by trypsin, chymotrypsin, or papain is so limited, two major polypeptides from a phaseolin subunit apparently remain after dialysis, only one of which can be sequenced.

The sequence data obtained indicate that 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. This in-

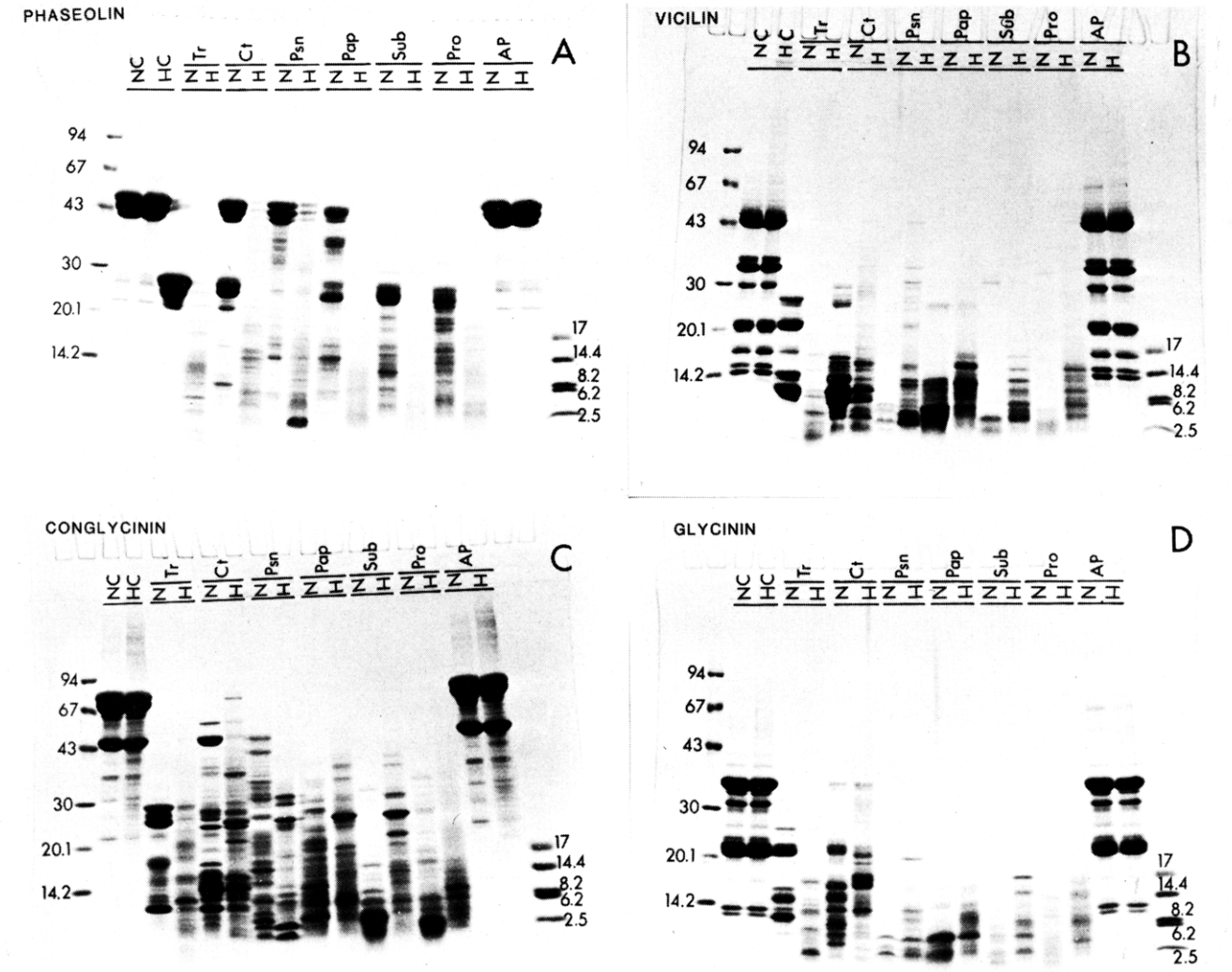

Figure 3. SDS-PAGE patterns of legume storage proteins subjected to 30-min digestion with different proteinases. N and H indicate the native and heated proteins, respectively. Tr, Ct, Psn, Pap, Sub, Pro, and AP denote digestions carried out with trypsin, chymotrypsin, pepsin, papain, subtilisin, pronase E, and aminopeptidase, respectively. The molecular weight markers are those described under Materials and Methods: A, phaseolin; B, vicilin; C, β-conglycinin; D, glycinin.

PHASEOLIN

Residue # in phaseolin	232	233	234	235	236	237	238	239	240
Amino Acid (β-subunit)	Ser	Ser	Ser	Arg ↑ Trypsin	Lys	Ser	Leu ↑ Chymotrypsin Papain	Ser	Lys
VICILIN	Ser	Ser	Ser	Lys	Lys	Ser	Val	Ser	Ser
β-CONGLYCININ (Cgy α' subunit)	Ser	Ser	Ser	Arg	Lys	Thr	Ile	Ser	Ser
GLYCININ (Gy 2 subunit)	Asn ↑	Gly	Ile	Asp	Glu	Thr	Ile	Cys	Thr

Figure 4. Region of phaseolin amino acid sequence cleaved when trypsin, chymotrypsin, or papain is reacted with native phaseolin (β-subunit). Amino acid sequences for the same region, based on sequence alignment as determined by Argos et al. (1985), are also given for vicilin, β-conglycinin (α'-subunit), and glycinin (Gy 2 subunit). Arrow below glycinin sequence indicates the in vivo cleavage site (Moreira et al., 1979; Staswick et al., 1981).

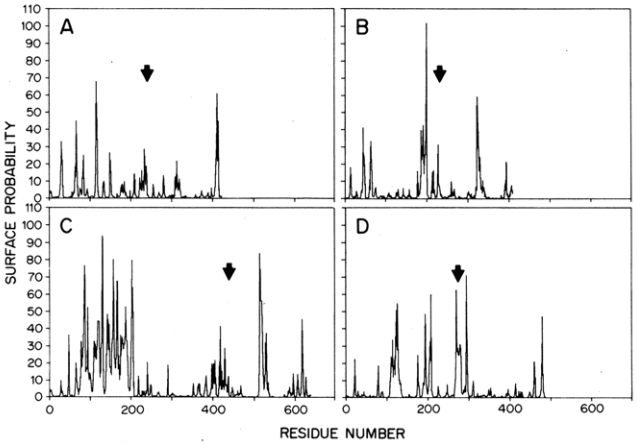

Figure 5. Surface probability plots for phaseolin (β-subunit) (A), vicilin (B), β-conglycinin (α'-subunit) (C), and glycinin (Gy2 subunit) (D). Arrow indicates enzyme cleavage region determined for phaseolin and hydrophilic regions in other proteins at same relative position.

formation on papain agrees with reports of cysteine proteinases in the germinating bean seed (Boylan and Sussex, 1987) and in the gut of larval bean weevils (Wieman and Nielsen, 1988), which appear to cleave native phaseolin subunits at a site near the middle of the polypeptide chain. Unlike the germinating seed and the bean weevil, humans do not utilize a thiol proteinase in their digestive system. However, digestion in humans is usually on heat-denatured protein rather than native protein.

Our results concerning proteolytic breakdown of glycinin and β-conglycinin give a clearer understanding of the digestion and structure of these two soy proteins. The breakdown products from trypsin hydrolysis of native

glycinin as observed by SDS-PAGE in our study are generally smaller than the 29 000 and 32 000 molecular weight fragments reported by Kamata and Shibasaki (1978). However, in that study, glycinin was digested under conditions of high ionic strength (0.01 M Tris-HCl, pH 8.0, with 0.5 M NaCl) and gels were run in the absence of a reducing agent. It has since been reported that tryptic digestion of glycinin (Kamata et al., 1979a), but not β-conglycinin (Kamata et al., 1982), is affected by ionic strength. Our study and that of Kamata et al. (1982) showed that trypsin hydrolysis of native β-conglycinin resulted in the generation of five stable fragments. Kamata et al. (1982) reported similar molecular weights for fragments from trypsin hydrolysis of glycinin and β-conglycinin to suggest conformational similarity between the two globulins. However, gels showing major breakdown products in our study indicate the molecular weights to be quite different between the two soy proteins. Protein bands on SDS-PAGE have been observed previously to appear between the α- and β-subunits of β-conglycinin. These were thought by some authors to be different subunits (Thanh and Shibasaki, 1977) but suggested by others to be simply a result of proteolysis (Barton et al., 1982). Figure 1C clearly shows that these bands appear in the very early stages of hydrolysis by trypsin.

Although legume seeds are generally always heated before consumption, results of this study suggest there are differences between legumes in the ability of proteinases to digest the native proteins. Native vicilin, glycinin, or β-conglycinin may be degraded by pepsin in the stomach sufficiently to allow further breakdown by trypsin or chymotrypsin in the intestine. However, native phaseolin is extremely resistant to pepsin digestion. Pepsin pretreatment does not increase digestion of native phaseolin by trypsin (Vaintraub et al., 1979), nor can a combination of trypsin, chymotrypsin, and pepsin overcome its resistance to proteolysis (Liener and Thompson, 1980). The order in digestibility of the major storage proteins agrees with the comparative results of nutritive value for the unheated legume seeds. The observed ease by which vicilin is degraded prior to heat treatment is consistent with its relatively high nutritive value and its lack of improvement upon heating.

The amino acid alignment of seed storage proteins described by Argos et al. (1985) indicated that the cleavage region in phaseolin is highly homologous in sequence with the other 7S proteins such as vicilin and β-conglycinin (Figure 4). Argos et al. (1985) noted that the greatest predicted structural preference difference between the 11S and 7S seed storage proteins occurred just prior to the junction between the acidic and basic polypeptides of the 11S proteins. The 11S molecules apparently have large insertions of variable size in this region. These insertions were predicted to have helical and turn conformations, located on the protein surface, and therefore would be accessible to hydrolytic enzymes. Glycinin subunits are likely to be readily cleaved by proteinases in that region. When amino acid sequences of the proteins are aligned on the basis of homology of secondary structural preference (Argos et al., 1985), the major trypsin cleavage site for native phaseolin lies three amino acid residues to the right of the end of this predicted exposed loop in glycinin. The acidic and basic peptide components of glycinin are cleaved in vivo at that site (indicated by arrow in glycinin sequence in Figure 4). Surface probability plots indicate that this general region in both the 7S and 11S seed storage proteins is hydrophilic and is predicted to occur on the surface of the protein (indicated by arrows in Figure 5). Cleavage by proteinases likely occurs in that region for all the native protein molecules, and any additional cleavages depend on unique characteristics of the protein.

In summary, results of this investigation increase the understanding of legume protein digestibility and structure. The qualitative analyses of degradation patterns from in vitro digestion of the proteins are consistent with tests of nutritive value conducted in vivo. A comparison of the region in native phaseolin cleaved by proteinases to homologous regions in vicilin, β-conglycinin, and glycinin suggests that legume proteins are at least somewhat similar in their degradation by proteinases.

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Registry No. Tr, 9002-07-7; Ct, 9004-07-3; Psn, 9001-75-6; Pap, 9001-73-4; Sub, 9014-01-1; Pro, 9036-06-0; AP, 9031-94-1.

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Reinvestigation of the Sapogenins and Prosapogenins from Alfalfa (*Medicago sativa*)

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The structures of the hydrolysis products of the alfalfa saponins have been reinvestigated as preliminary studies of the native saponins. Identified known products are the soyasapogenols A, B, C, and E, hederagenin, and medicagenic acid. Bayogenin and zanhic acid known from other sources have now been characterized in alfalfa. The elusive lucernic acid is presumed to be a lactone artefact derived from zanhic acid, i.e. 16-hydroxymedicagenic acid. Five prosapogenins containing medicagenic acid and zanhic acid (plus glucose, glucuronic acid, or sophorose) have been identified in the saponins hydrolysates; their structures have been established without degradations and mainly rest on NMR and MS measurements. An HPLC method is proposed for the determination of medicagenic acid content in alfalfa.

Alfalfa (lucerne, *Medicago sativa*, Papillionaceae) is one of the richest sources of vegetable proteins in temperate climates (Carlsson, 1983). The aerial parts of the plant are used as a forage or are industrially processed to yield leaf protein concentrates. These protein concentrates are well balanced in amino acids and are rich in vitamins, carotenoids, and xanthophylls (Gastineau and De Mathan, 1981). At the present time, they are one of the main pigmentation sources in poultry rations (Livingston et al., 1980). Their development however is somehow hampered by their high content in antifeeding substances, which, at high doses, bring loss of weight (Heywang and Bird, 1954) and a decrease in egg production (Anderson, 1957). Previous work has shown that these properties are linked to saponins and especially to a triterpene, medicagenic acid (1) (Gestetner et al., 1971), which was a subject of intense study in the 1950s. It has also been shown that these saponins strongly interact with cholesterol; they may show promise in the treatment of hypercholesteremia and atherosclerosis (Malinow et al., 1982). Despite all this work, little is known today about their structure–activity relationship (Morris et al., 1961; Gestetner, 1971; Massiot et al., 1986). As part of a cooperative program aiming at introducing new varieties of alfalfa devoid of these undesired properties, the structures of the saponins of all parts of the plant are being reinvestigated. This first article describes the extraction of the saponin mixture and their hydrolysis into triterpenes. The following paper in the series will report on the structures of the alfalfa root sa-

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