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Phaseolin In Vitro Pepsin Digestibility: Role of Acids and Phenolic Compounds

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Great Northern bean (*Phaseolus vulgaris* L.) phaseolin proteolysis at 37 °C, varying HCl concentrations (10 mM to 1 M), phaseolin:pepsin ratios ranging from 5:1 to 100:1 (w/w), and incubation times up to 24 h was investigated. The results suggest that phaseolin is not resistant to in vitro pepsin hydrolysis. At a phaseolin-to-pepsin ratio of 100:1 (w/w), native phaseolin was completely digested in 24 h when incubated in 50 mM HCl, while heat-denatured phaseolin (30 min at 100 °C, boiling water bath) was digested in 1 h under similar conditions. When incubated at 37 °C for 24 h, acid alone, even at as low a concentration as 10 mM, caused a partial breakdown of native phaseolin. The degree of phaseolin hydrolysis by HCl was dependent on the acid concentration used. The rate of native phaseolin hydrolysis increased with increasing HCl concentration rather than pepsin concentration. Common food acids were able to partially hydrolyze phaseolin. Among the food acids tested, oxalic acid was the most effective in hydrolyzing phaseolin. Spectroscopic studies revealed a significant change in secondary and tertiary structures when native phaseolin was incubated in dilute HCl. None of the tested phenolic compounds adversely affected phaseolin hydrolysis by pepsin.

KEYWORDS: Phaseolin; acid; in vitro digestibility; phenolic compounds; SDS−PAGE

INTRODUCTION

Food digestion has been a topic of interest for over 2000 years. Galen, a Greek physician in the second century, suspected that food was somehow cooked in the abdominal cavity. Later, Vesalius, whom many consider to be the father of the science of human anatomy, also promoted this notion that somehow food undergoes some form of cooking in the abdominal cavity and that respiration was for cooling blood (*37*). Today, it is generally recognized that gastric acid plays an important role in food digestion. Some of the established roles of stomach acid in food digestion include providing optimum pH for pepsin activity for food protein hydrolysis, activation of pepsinogen to pepsin (by removing the N-terminal peptide), food protein denaturation, maintaining reducing conditions for improved bioavailability of certain nutrients such as vitamin C and several minerals (such as iron, copper, zinc, etc.), and killing pathogenic bacteria and thus protecting the stomach (*1*, *3*, *39*). Typically, the human stomach maintains 80−120 mM HCl in response to ingested food (*14*).

Plant foods are critical for global human nutrition supplying about 65% of food proteins (*31*). The amount of dietary proteins in foods, on a dry weight basis, varies from 5 to 15% in cereals to as much as 15−50% in certain legumes and oil seeds. Among a variety of sources of plant proteins, legumes, especially dry beans, are globally important. Dry beans are low in fat, low in sodium, high in protein, and a good source of fiber, certain minerals, and vitamins (*29*). Food protein quality differs substantially from protein to protein depending on protein digestibility, essential amino acid composition, and the availability of individual amino acids from a particular protein (*2*). Animal proteins are generally more easily digested than many plant proteins resulting in higher nutritional value for animal proteins. High-quality animal proteins used as human foods contain all of the essential amino acids when compared to the recommended amino acid pattern for humans (*10*). Plant proteins are typically deficient in one or more essential amino acids and therefore have lower nutritional value than animal proteins. The other major reason for the apparent lower nutritional value of plant proteins is their lower in vivo digestibility. In the case of legume proteins, a major plant protein source in animal and human diets, several factors such as the deficiency of sulfur amino acids; compact structure of proteins; steric hindrance by the carbohydrate moiety of glycoproteins; protein interactions with phytates, tannins, and minerals; and the presence of antiphysiological factors (proteinases inhibitors, lectins) have been proposed to explain their low protein nutritional value (*4*, *17*, *18*, *28*). Despite the proposed postulates and empirical observations, legume protein digestibility data remain largely conflicting. Consequently, it is not yet clear as to why plant proteins in general and legume proteins in particular are considered to be resistant to digestive proteinases.

Phaseolin (a major storage protein in dry beans) digestibility has been the subject of investigations for over 20 years. Many of the studies suggest resistance of phaseolin to both in vitro and in vivo proteolysis by the digestive enzymes (*5*, *12*, *17*,

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25, *30*). Most of these studies have typically used limited and specific digestion conditions in their experimental protocols such as digestion for limited time, specific phaseolin-to-pepsin ratios, and fixed HCl (or HCl−NaCl) concentrations. It was therefore not apparent whether the reported resistance of phaseolin to pepsin hydrolysis was due to restricted experimental conditions used or due to inherent resistance of phaseolin toward pepsin. We therefore investigated phaseolin in vitro pepsin hydrolysis at 37 °C under a variety of experimental conditions including incubation in different acids, variable phaseolin:pepsin ratios (5:1−100:1 w/w), 0−24 h incubation times, and in the presence of different phenolic compounds.

MATERIALS AND METHODS

Materials. Great Northern beans (GNB), pinto beans, small red beans, walnuts, instant freeze-dried coffee (Folgers), and tea (Brooke Bond, black label) were all purchased locally. Dried almond skins were from Blue Diamond Growers, Sacramento, CA. Grape skin extract (GSKE-40), grape seed extract (Vinox Gold), and red wine concentrate were obtained from Polyphenolics, Fairport, NY. Sources of electrophoresis chemicals have been reported earlier (*32*). Sepharose S 300 HR, Con-A Sepharose 4B, ampholines (pI range 3−10), MW markers for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE), and marker proteins for isoelectric focusing were all purchased from Pharmacia Inc., Piscataway, NJ. DEAE-DE 53 cellulose anion exchange was purchased from Whatman, Hillsboro, OR. 1-Anilino-8-naphthalene-sulfonate (ANS), pepsin, ellagic acid, *p*-coumaric acid, and catechin were from Sigma Chemical Co. (St. Louis, MO). Catechol and tannin (MW 1702.1) were from Nutritional Biochemicals Corporation, Cleveland, OH. Acids and all other chemicals were of reagent or better grade and were purchased either from Sigma Chemical Co. or from Fisher Scientific Co. (Orlando, FL).

GNB Phaseolin Isolation and Purification. GNB phaseolin was purified as described earlier (*34*).

Preparation of Phenolic Compounds. Phenolic compounds from pinto bean, small red bean, almond skin, walnut, black tea, and coffee were prepared by methanol extraction. Briefly, 100 g of flour/powder was extracted with 250 mL of absolute methanol with continuous magnetic stirring for 4 h at 25 °C, the slurry was filtered under vacuum, and the residue was extracted one more time with methanol as mentioned before. Combined filtrates were concentrated (∼15-fold) by vacuum distillation at 60 °C (Rotavapor R-3000, BUCHI, Switzerland), dried in a constant temperature incubator (45 °C), finely powdered, and stored in airtight bottles at 25 °C for further use.

Phaseolin Digestions. *Preparation of Phaseolin Stock Solutions.* Phaseolin was dissolved in distilled water (containing 1 mM NaN_3) by adjusting the pH to 7.5 with 1 M NaOH. The protein solution was centrifuged (12 000*g*, 20 min, 4 °C), and the supernatant was filtered (Whatman filter paper no. 4) to remove aggregates. Soluble proteins were analyzed by the method of Lowry et al. (*20*). Stock phaseolin solution of 5 mg/mL was prepared and stored at 4 °C till further use (typically used within 5 days of preparation). When required, phaseolin that was dissolved in distilled water containing 1 mM NaN_3 was heat-denatured at 100 °C (boiling water bath) for 30 min, cooled to 25 °C, and then used for further studies.

Final Digestion Conditions. A. Phaseolin, 2 mg/mL; incubation temperature, 37°C; digestion volume, 100 μL; variable HCl concentration, 10−1000 mM; phaseolin:pepsin ratios ranging from 5:1 to 100:1 (w/w); incubation times up to 24 h.

B. In the case of digestion in food acids, 200 mM of each organic and inorganic acid (as indicated in **Figure 11**) was used in place of HCl. All other conditions were the same as in A.

C. For phaseolin digestion in the presence of phenolic compounds, a suitable amount of phenolic compound was suspended in the digestion buffer to obtain the desired phaseolin:tannin ratio. All other conditions were the same as in A.

Digestions were stopped by neutralizing the samples with suitable volumes of NaOH (0.5−1.85 M), adding SDS−PAGE sample buffer, and heating the mixture in a boiling water bath for 10 min. Appropriate blanks and controls were simultaneously included in all experiments.

Gel Electrophoresis, Glycoprotein Staining, and Isoelectric Focusing. SDS−PAGE was done according to the method of Fling and Gregerson (*9*) as described by Sathe (*32*). Gels were 1.5 mm thick with 8−25% linear monomer acrylamide gradient. Glycoprotein staining of SDS−PAGE gels was done using the GELCODE staining procedure (Pierce Chemical Co., Rockford, IL) as described by Sathe et al. (*36*). Two-dimensional gel electrophoresis (IEF in the first dimension followed by SDS−PAGE in the second dimension) was done as per the instructions in the manufacturer's handbook (Hoefer Scientific Instruments, CA; 1992). Wherever necessary, the degree of phaseolin hydrolysis was monitored by gel densitometry scanning (model GS-700; Bio-Rad Laboratories, Inc., Hercules, CA) as per the recommendations of the manufacturer. The percent phaseolin breakdown calculations were based on the disappearance of phaseolin polypeptides expressed in volume units (optical density units × area occupied by the polypeptides) and were compared with phaseolin control (no acid/no enzyme/no incubation) on the same gel.

Column Chromatography. *Anion Exchange (DEAE DE 53).* Anion exchange chromatography was done as described earlier (*36*). Protein samples prepared in 0.02 M Tris-HCl buffer (pH 8.1) were loaded onto a DEAE DE-53 anion exchange column (2.6 cm × 25 cm) previously equilibrated with sample buffer. The column was flushed with the equilibrium buffer until the absorbance (at 280 nm) of the effluent reached the baseline and then developed with 0−0.5 M NaCl linear gradient (1000 mL each) in the sample buffer.

Gel Filtration. Gel filtration chromatography was done using Sephacryl S 300 HR column (*36*). The column (1.6 cm × 85 cm) was equilibrated with 0.02 M Tris-HCl buffer (pH 8.1) containing 0.1 M NaCl and 0.001 M NaN_3 prior to loading the protein sample (prepared in the equilibrium buffer) and eluting with the equilibrium buffer.

Affinity Chromatography (Con-A Sepharose 4B). Con-A Sepharose 4B chromatography was done as described by Sathe (*33*) to determine if acid exposure caused sugar removal from phaseolin polypeptides. All chromatography steps were done at 4°C. Protein elution from all columns was monitored by measuring absorbance of each fraction at 280 nm and SDS−PAGE gel electrophoresis of selected fractions.

UV and Circular Dichroic (CD) Spectroscopy. UV spectroscopic analysis of proteins was done at 25 °C in sodium phosphate buffer (50 mM, pH 7.5) with a single beam Beckman spectrophotometer (model DU640, Beckman Instruments Inc., Fullerton, CA). The spectra were recorded over a wavelength range of 250−350 nm at a scan speed of 120 nm/s. The average of five scans per sample was used for analysis. CD measurements were taken in the far-ultraviolet region (190−260 nm) using a CD spectrometer (model 202, Aviv Instruments, Inc., NJ), a 0.1 cm quartz cell, and a final protein concentration of 0.2 mg/mL in sodium phosphate buffer (50 mM, pH 7.5). The scan rate, time constant, and sensitivity of the equipment was appropriately set to get the best signal-to-noise ratio. An average of three scans was used to calculate the mean residue ellipticity (θ), expressed as the degree cm^2 $dmol^{-1}$ using mean residue molecular weight of the phaseolin as 115 (*6*). The secondary structure parameters were calculated using CDPro (http://lamar.colostate.edu/∼sreeram/CDPro/index.html).

Fluorescence Emission Spectra and Quenching Studies. Fluorescence emission spectra were recorded at 25 °C using a Perkin-Elmer Fluorometer (model LS 50B) (Perkin Elmer Corp., Atlanta, GA). The excitation wavelength was set at 295 nm (for tryptophan fluorescence), and the emission spectra were recorded over a wavelength range of 280−400 nm. Quenching studies with a neutral quencher (acrylamide) were done as described by Sze-Tao and Sathe (*40*). The final sample volume was 1 mL, and the final buffer concentration was 50 mM sodium phosphate buffer (pH 7.5). Appropriate solvent blanks including the correct amount of additive were used for spectral analysis. Excitation and emission slits were set at 5 nm each for both spectral and quenching studies. The scan speed for spectral studies was set at 100 nm/min.

Quenching calculations were done using the Stern−Volmer equation (*15*):

$$F_o/F = 1 + K_{sv}[Q]$$

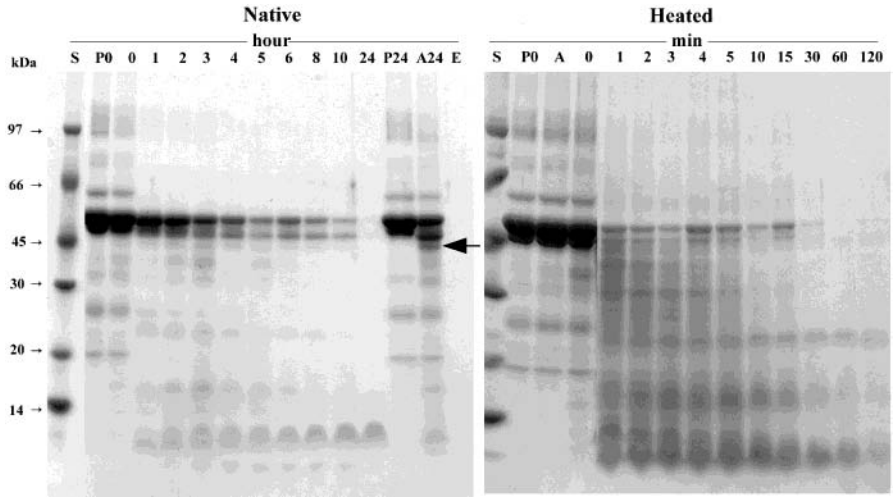

Figure 1. Digestion of native and heat-denatured phaseolin by pepsin. The protein load was 30 μg each, and the pepsin control load was 0.30 μg. The phaseolin:pepsin ratio was 100:1 (w/w); HCl concentration, 50 mM; time (min/h) indicated at top of the lane; S, MW markers; P0, phaseolin no acid control (0 h); P24, phaseolin no acid control (24 h); A24, phaseolin acid control (24 h); E, enzyme control (24 h).

where F_o and F are the emission intensities in the absence and presence of the quencher, respectively, [Q] is the molar concentration of the quencher, and K_{sv} is the Stern−Volmer quenching constant (*38*).

Surface Hydrophobicity. Surface hydrophobicity (S_o) was determined using a hydrophobic fluorescence probe, ANS, according to the method of Nakai and Li-Chan (*23*). Relative fluorescence intensity (RFI) was measured using Perkin-Elmer Fluorometer (model LS 50B) (Perkin Elmer Corp.) with the excitation and emission wavelengths set at 390 and 470 nm, respectively. The initial slope (S_o) was calculated from the plot of fluorescence intensity vs protein concentration using least-squares regression analysis and taken as an index of surface hydrophobicity of the protein (*13*).

RESULTS AND DISCUSSION

Role of Gastric Acid (HCl) in Phaseolin Digestion. Preliminary studies comparing native and heat-denatured phaseolin digestibility, as expected, indicated that heat-denatured phaseolin was completely digested in 1 h (**Figure 1**). A closer look at this figure, in contrast to various reports (mentioned earlier in the Introduction) on the inherent resistance of native phaseolin to digestive proteinases, revealed that even the native phaseolin was completely digested by pepsin in 24 h (see lane marked 24 h). What was even more interesting in this experiment was the observation that native phaseolin was partially hydrolyzed by HCl (50 mM) alone (note the lane A24 marked with the arrow indicating a shift in the mobility of one of the phaseolin polypeptides). Subsequent experiments using varying HCl concentrations clearly showed that HCl, even at 10 mM concentration, caused a partial breakdown of native phaseolin at 37 °C (**Figure 2**). At higher HCl concentrations (200−500 mM), native phaseolin was substantially degraded and at 1 M HCl concentration phaseolin was essentially completely degraded in 24 h at 37 °C (**Figure 2**). Increasing pepsin concentration, at fixed phaseolin concentration and constant HCl (50 mM), did not increase the rate of native phaseolin proteolysis (**Figure 3**) even though pepsin remained active after 12 h under the experimental conditions (data not shown). When native phaseolin was hydrolyzed using different pepsin and HCl concentrations, it was apparent that phaseolin breakdown was dependent more on HCl concentration than pepsin amount (**Figure 4**). These results suggested a significant role for HCl in pepsin in vitro digestion of phaseolin.

Effect of Gastric Acid (HCl) on Phaseolin Biochemical and Structural Properties. That HCl causes peptide bond

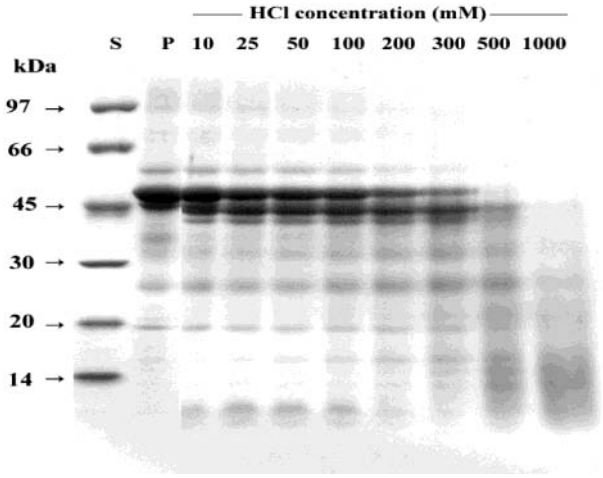

Figure 2. Effect of acid on native phaseolin hydrolysis. Protein load in each lane was 30 μg. HCl concentration (mM) indicated at top of the lane; incubation time, 24 h; incubation temperature, 37 °C; S, MW markers; P, phaseolin control (no acid).

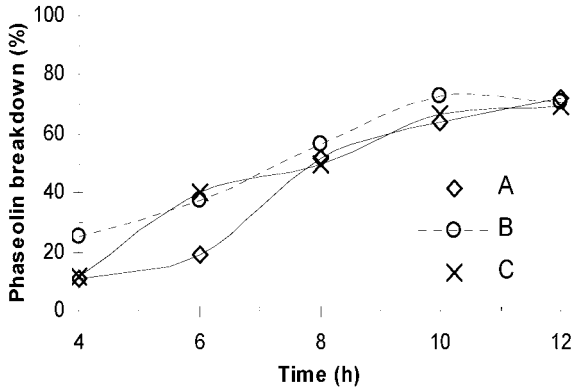

Figure 3. Effect of pepsin concentration on native phaseolin digestibility. **A**–**C**, respectively, phaseolin:pepsin ratios of 5:1, 50:1, and 100:1 (w/w).

hydrolysis under severe thermal stress (as in HCl digestion when proteins are analyzed for amino acid composition) or that HCl deamidates proteins under acid conditions thereby altering protein structure (*19*, *21*, *27*) is well-known. However, to the best of our knowledge, we do not know of any studies that demonstrate the acid-mediated structural changes in proteins at

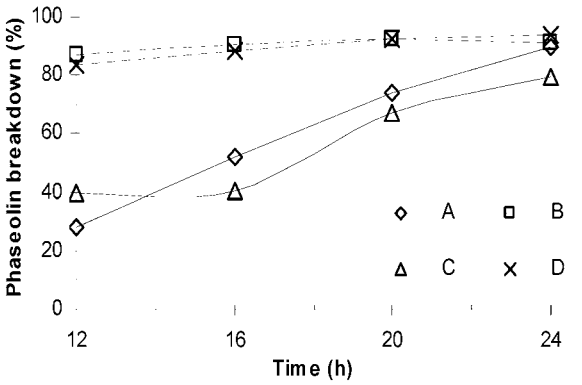

Figure 4. Combined effect of pepsin and acid on native phaseolin digestibility. (**A**) 50 mM HCl, phaseolin to pepsin = 100:1; (**B**) 200 mM HCl, phaseolin to pepsin = 100:1; (**C**) 50 mM HCl, phaseolin to pepsin = 5:1; (**D**) 200 mM HCl, phaseolin to pepsin = 5:1.

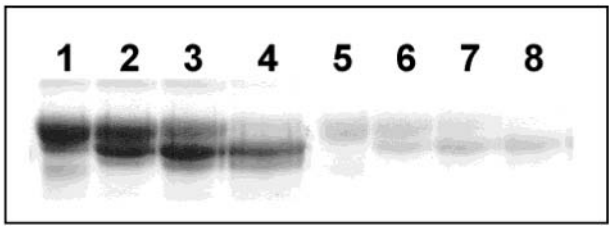

Figure 5. Glycoprotein staining of HCl-incubated phaseolin. Lanes: 1–4, Coomassie Brilliant Blue R250 staining of phaseolin incubated with 0, 50, 200, and 500 mM HCl for 12 h, respectively; 5–8, glycoprotein staining of phaseolin incubated with 0, 50, 200, and 500 mM HCl for 12 h. Protein load in each lane was 30 μg. Note that all phaseolin polypeptides stain positive for glycoprotein staining.

37 °C that subsequently lead to enhanced proteolysis observed in our current study. It was therefore important to investigate selected molecular characteristics of phaseolin that were altered as a result of its exposure to HCl incubation.

Electrophoresis and Chromatography Studies. SDS−PAGE analysis of HCl-incubated phaseolin resulted in the formation of phaseolin polypeptide(s) with increased mobility (indicated by arrow in **Figure 1**) with simultaneous production of several smaller peptides in ∼8−16 kDa range (**Figures 1** and **2**). SDS−PAGE electrophoresis of HCl-treated phaseolin with subsequent glycoprotein staining of the gels did not reveal any deglycosylation of phaseolin polypeptides (**Figure 5**). Lack of deglycosylation of phaseolin polypeptides upon HCl incubation was further corroborated by the lack of presence of deglycosylated polypeptides in the flow through fractions when HCl-incubated phaseolin was passed through a Con-A Sepharose 4B column (data not shown). Sephacryl S-300 HR gel filtration chromatography indicated no significant change in the hydrodynamic radius of HCl-incubated phaseolin as the elution volume for HCl-treated and the control phaseolins remained the same (**Figure 6**). The elution volume for both control and acid-treated phaseolin was 143 mL (tube number 29). Despite the shift in isoelectric pH of the acid-treated phaseolin, DEAE-DE 53 anion exchange chromatography of HCl-incubated phaseolin failed to demonstrate any change in the ionic properties (data not shown) indicating that the change in electrical charge of acid-treated phaseolin was not of sufficient magnitude to cause a significant change in elution off the DEAE DE 53. Collectively, column chromatography data did not reveal any major changes in phaseolin structure upon HCl incubation.

Two-dimensional gel electrophoresis of HCl-treated phaseolin indicated a shift in phaseolin polypeptides toward an acidic pH

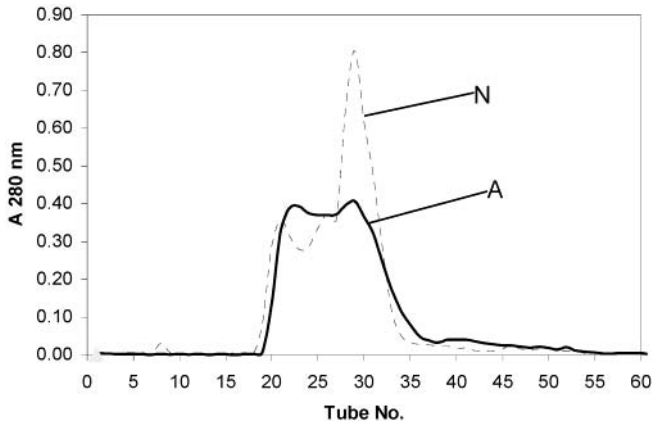

Figure 6. Elution profile of native (N) and HCl (A) (200 mM, 12 h) treated phaseolin off Sephacryl S-300 HR (1.6 cm × 85 cm) gel filtration column. The column flow rate was 15 mL/h, and three fractions were collected per hour. Note that the elution volume for N and A is the same (V_e = 143 mL) (second peak represents phaseolin). Identical protein loads for N and A were used.

(**Figure 7**). Asparagine and glutamine amino acids are known to undergo deamidation under mild acid conditions (*27*). Because asparagine and glutamine through their participation in hydrogen bonding help stabilize protein structure, deamidation of asparagine and glutamine may result in destabilization (unfolding) of the phaseolin structure, which in turn may improve phaseolin susceptibility to proteolysis by pepsin. It was therefore of interest to investigate the conformational changes (caused by HCl incubation) in phaseolin structure.

Spectroscopic Studies. UV absorption spectra for both acid-treated and heat-denatured phaseolin showed a slight blue shift (λ_{max} 277 nm for native phaseolin to ∼275−276 nm for acid and heat-denatured phaseolin) with no major changes in the absorption intensity of the spectra (data not shown). The fluorescence emission spectra of HCl-incubated and heat-denatured phaseolins are shown in **Figure 8I**. The tryptophan emission maximum observed at 333 nm for the native phaseolin showed a red shift to 338 nm upon HCl incubation, suggesting transfer of tryptophan residues to a more polar environment (*15*). Furthermore, a significant increase in Stern−Volmer constants (K_{sv}), indicative of increased accessibility of tryptophan residues to acrylamide quencher, confirmed the change in conformation of phaseolin upon HCl treatment (**Figure 8II**).

CD measurements of HCl-incubated phaseolin indicated ∼2-fold increase in the random structure of phaseolin at the expense of the α-helix rather than β-sheets and β-turns (**Figure 9**). Deshpande and Damodaran (*7*) reported a 3 and 5.5% decrease in the α-helix and β-sheet, respectively, and an 8% increase in random coils when phaseolin was heated (moist heat, 99 °C, 30 min). Similarly, we find a 5 and 3% decrease in the α-helix and β-sheet, respectively, with a concomitant increase of 10.4% in random coils (**Figure 9**) as a result of heat denaturation. However, phaseolin incubation in HCl resulted in 14.2 and 1.3% decreases in the α-helix and β-sheet, respectively, with a 21.1% increase in random coils (**Figure 9**). The increase in phaseolin surface hydrophobicity (**Figure 10**) upon heat denaturation was slightly less (5.1-fold increase) than that induced by HCl treatment (5.4-fold increase). Collectively, CD and surface hydrophobicity data suggest that phaseolin denaturation by HCl is different, both qualitatively and quantitatively, than that induced by heat treatment. Thus, although both HCl and heat denaturation treatments improved in vitro pepsin hydrolysis of

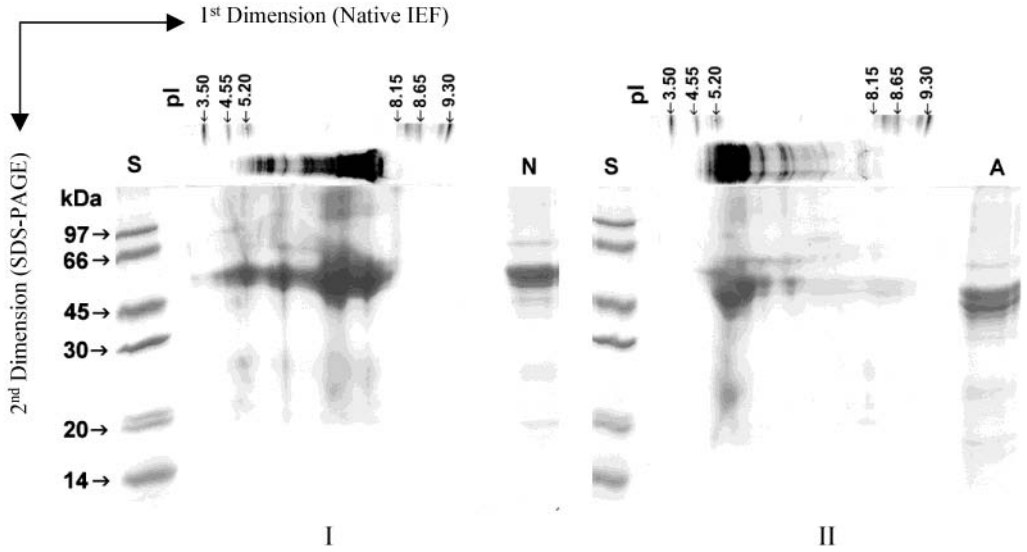

Figure 7. Two-dimensional gel electrophoresis of native (I) and HCl (II) (200 mM, 12 h) treated phaseolin. *S*, MW markers; N, native phaseolin; A, HCl-treated phaseolin. Note the shift in isoelectric pH of acid-treated phaseolin.

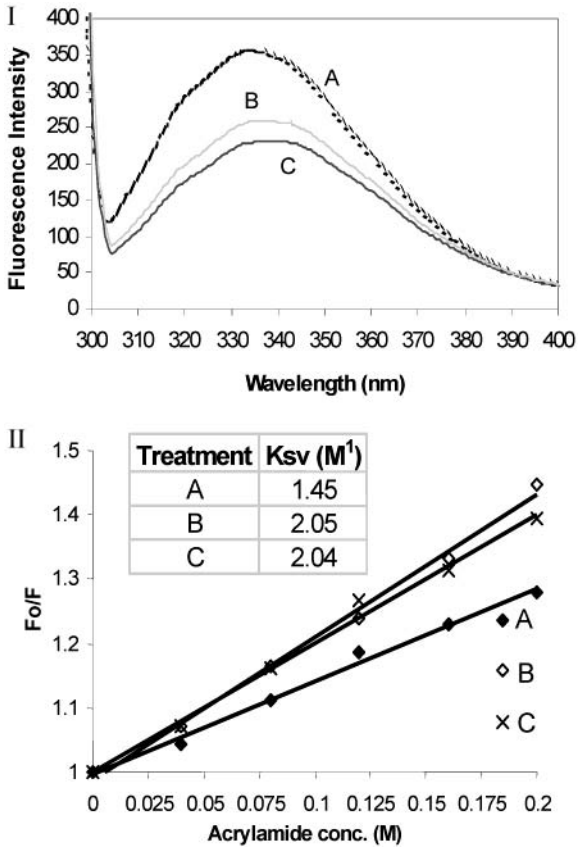

Figure 8. Fluorescence spectra (**I**) and Stern–Volmer plots and quenching constant (K_{sv}) for acrylamide (neutral quencher) quenching (**II**) of native (**A**), heat-denatured (**B**) (100 °C, 30 min), and HCl-incubated (**C**) (200 mM, 12 h) phaseolin. Tryptophan fluorescence was monitored following excitation at 295 nm. The emission wavelength for quenching measurements was set at 333 nm (λ_{max} for native phaseolin).

Treatment	% secondary structure parameter			
	a-helix	b-sheets	b-turns	random
A	32.7 ± 4.8	20.5 ± 3.0	21.2 ± 0.6	25.7 ± 4.5
B	27.7 ± 4.2	17.5 ± 3.4	19.0 ± 1.6	36.1 ± 7.1
C	18.5 ± 0.6	19.2 ± 4.3	16.7 ± 1.3	47.0 ± 4.0
LSD	8.5	10.3	3.2	15.3

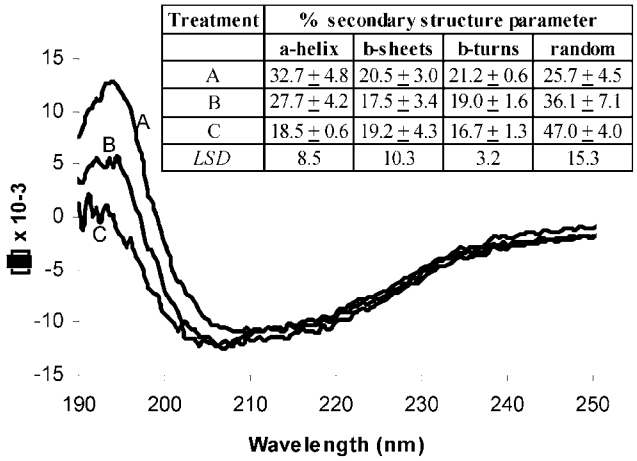

Figure 9. Far-UV circular dichroic (CD) spectra for native (**A**), heat-denatured (**B**) (H, 100 °C, 30 min), and HCl-incubated (**C**) (200 mM, 12 h) phaseolin. [θ] represents ellipticity in deg cm^2 dmol^{-1}. Data are expressed as mean ± SEM; Fisher's least significant difference (LSD). Differences between two means within the same column exceeding this value are significant ($p = 0.05$).

phaseolin, the structural motifs available for pepsin attack in each case may differ significantly and warrant further investigations.

Role of Food Acids on Phaseolin Digestion. A variety of naturally occurring food acids may serve different roles in foods including taste, pH balance, and food preservation, to name a few. However, the role of food acids in improving food protein digestibility remains largely underexplored. Encouraged by our observations that even dilute HCl (10 mM) could hydrolyze native phaseolin at 37 °C, we included a number of acids (both organic and inorganic) commonly encountered in the food supply in our experiments (**Figure 11**). As can be seen from **Figure 11A**, acids other than HCl also caused partial hydrolysis of phaseolin. Among the food acids tested, oxalic acid was the most effective in facilitating phaseolin hydrolysis (**Figure 11B**, lanes 9 and 10). These results indicate that not only the mere presence of acid but also the type of acid has a profound effect on phaseolin digestion in vitro. Whether our data are unique to phaseolin or whether this is a common mechanism for several plant proteins remains to be determined. Further studies are underway in our laboratory to determine whether exposing plant proteins to HCl and several common food acids improve in vitro pepsin digestion of those proteins. Our planned long-term studies also include in vivo animal studies to determine if the acid exposure of a plant protein results in improved in vivo protein digestibility.

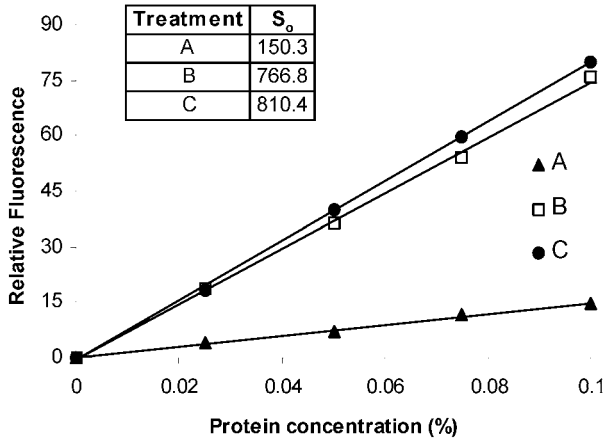

Figure 10. Surface hydrophobicity (S_0) of native (**A**), heat-denatured (**B**) (100 °C, 30 min), and HCl-incubated (**C**) (200 mM, 12 h) phaseolin. The hydrophobic fluorescence probe used was ANS. Excitation and emission wavelengths were set at 390 and 470 nm, respectively.

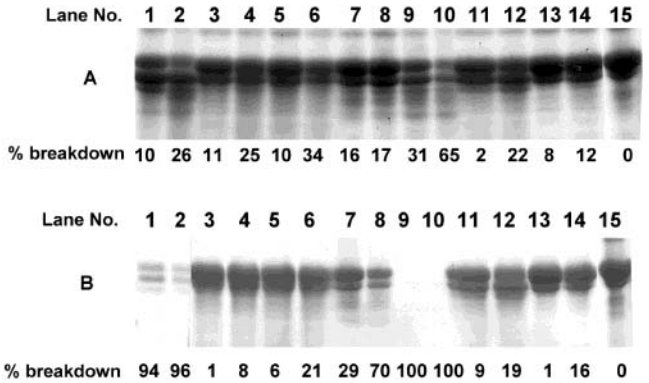

Figure 11. Effect of organic/inorganic acids on native phaseolin hydrolysis. (**A**) Acid alone; (**B**) acid and pepsin. The protein load in each lane was 30 μg. The acid concentration was 200 mM. Lanes: 1–2, HCl; 3–4, formic acid; 5–6, acetic acid; 7–8, citric acid; 9–10, oxalic acid; 11–12, phosphoric acid; 13–14, lactic acid; 15, phaseolin control (no acid incubation). The two successive lanes for each acid treatment represent 12 and 24 h incubation at 37 °C, respectively. Note the dramatic effects of HCl and oxalic acid on phaseolin susceptibility to pepsin.

Effect of Phenolic Compounds on Phaseolin Digestion by Pepsin. Interactions of protein with phenolic compounds and implications of such interactions on protein nutritional quality have been discussed (*8*, *26*). When beans are prepared and/or consumed with a variety of other foods containing phenolic compounds, it is possible that the phenolic compounds may interact with phaseolin and subsequently negatively influence phaseolin digestibility. When phenolic compounds from diverse food sources were added to GNB phaseolin prior to pepsin digestion, they had no adverse effect on phaseolin digestion (**Figure 12**). Increasing the amount of phenolic compound did not adversely affect phaseolin proteolysis either (**Figure 13**). These results are consistent with similar observations reported earlier (*22*, *24*, *35*).

CONCLUSIONS

Results of the present investigations suggest that type and concentration of acid and incubation time affect the extent of phaseolin hydrolysis by pepsin in vitro. Acid alone causes significant hydrolysis of phaseolin, and such acid hydrolysis enhances susceptibility of phaseolin to pepsin attack in vitro. However, whether acid hydrolysis of phaseolin can improve in

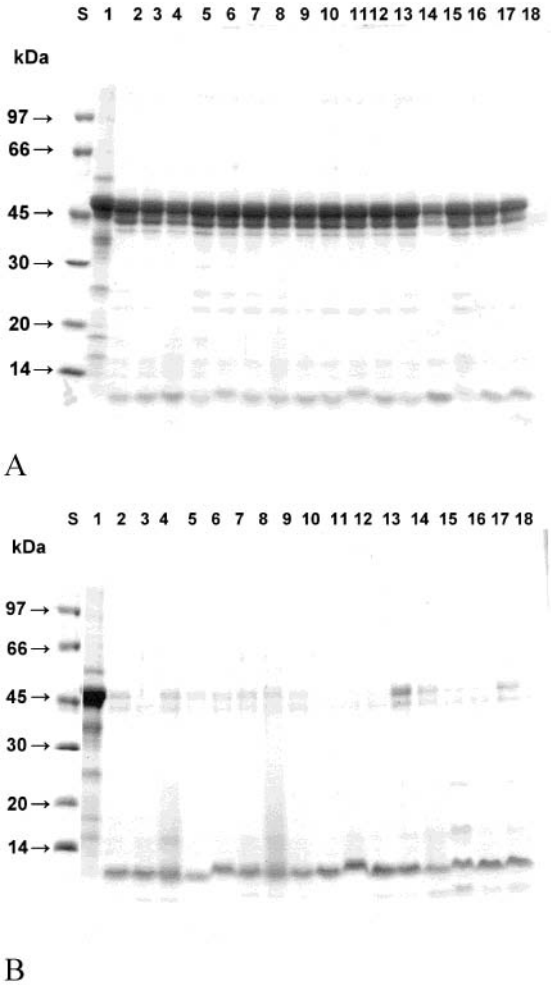

Figure 12. Role of phenolic compounds on breakdown of native (**A**) and heat-denatured (**B**) phaseolin by pepsin. Lanes: S, MW markers; 1, phaseolin control (no tannin, no enzyme); and 18, pepsin control (0.3 μg load). Lanes 2–17 represent phaseolin digestion by pepsin for 4 h at 37 °C in the presence of phenolic compounds from 2, no tannin; 3, grape skin; 4, grape seed; 5, wine concentrate; 6, almond skin; 7, coffee; 8, tea; 9, walnut; 10, small red beans; 11, pinto bean; 12, catechin; 13, ellagic acid; 14, tannin; 15, polyvinylpyrrolidone; 16, catechol; 17, vanillin. The protein load in each lane was 30 μg. The phaseolin:phenolic compound(s) ratio was 1:1 (w/w), and the phaseolin:pepsin ratio was 10:1 (w/w). HCl concentration was 50 mM.

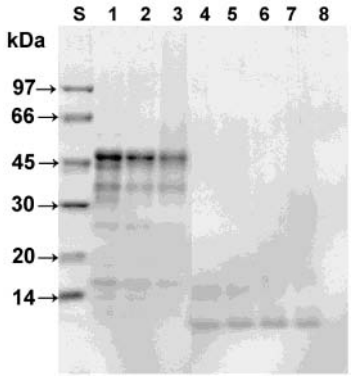

Figure 13. Effect of tannin on phaseolin digestion by pepsin. The protein load in each lane was 30 μg. Lanes: S, MW markers; 1, phaseolin control (no heating, no tannin, no enzyme); 2, phaseolin control (heat-denatured phaseolin:tannin::1:1, no enzyme); 3, phaseolin control (native phaseolin: tannin::1:1, no enzyme); 4–7, heat-denatured phaseolin digested for 30 min, respectively, at a phaseolin:tannin ratio of 1:0, 10:1, 5:1, and 1:1; 8, pepsin control (0.3 μg load). The phaseolin:pepsin ratio was 100:1. All ratios used were w/w. HCl concentration was 50 mM.

vivo digestibility of phaseolin remains to be determined. Our investigations also demonstrated that tested phenolic compounds did not interfere with phaseolin in vitro proteolysis by pepsin.

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