

Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food

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Abstract

Soy hydrolysate and the soy-fermented foods, natto and tempeh, were dephosphorylated, deglycosylated and digested with a variety of endoproteases (pronase, trypsin, Glu C protease, plasma proteases and kidney membrane proteases) to generate oligopeptides. The peptides were purified and characterized. They demonstrated a range of biological activities – angiotensin converting enzyme (ACE) inhibitory, anti-thrombotic, surface tension and antioxidant properties. The biologically active peptides were mostly derived from glycinin, a highly expressed soy protein, and were found mainly in the pronase, kidney membrane proteases and plasma proteases digests of the fermented foods. The proteases of lower specificity produced more oligopeptides and a higher percentage of bioactive peptides than the proteases of higher specificity, namely trypsin and Glu C. A peptide with ACE inhibitory activity was found in the pronase digest of natto, which also produced a peptide with surface active properties. The kidney membrane hydrolysate of natto contained an ACE inhibitor, in addition to a peptide with anti-thrombotic activity bearing homology to hirutinin, a previously described synthetic thrombin inhibitor [DiMaio, Gibbs, Lefebvre, & Munn, 1992, *J. Med. Chem.* 35, 3331]. Synthetic analogs were also evaluated as substrates for some inhibitors.

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1. Introduction

Soybean, from the most cultivated plant in the world, is rich in proteins (40–50%), lipids (20–30%) and carbohydrates (26–30%), and consequently the subject of extensive scientific research. Like most legume seeds, this bean contains several proteins, most of which contribute to nutrition and good health. Recently, interest in the composition of soy and its fermented products has grown since potential anti-carcinogens and other therapeutic agents have been reported (Messina, Persky, Setchell, & Barnes, 1994). Several groups have previously shown that the peptide content of soy-fermented products is greater than that of unfermented soybeans (Okamoto, Hanagata, Kawamura, & Yanagida, 1995).

They are produced partly by the hydrolytic action of microbial enzymes (Hesseltine, 1983).

The major soy proteins (greater than 85%) are β -conglycinin and glycinin. Other minor proteins/glycoproteins include lipoxygenases, lectins, trypsin inhibitors and α -amylases (Liu, 1997). Glycinin does not contain any carbohydrate, whereas conglycinin is a glycoprotein containing approximately 4% carbohydrate (mainly mannose moieties). Some of the minor soy proteins may also be phosphorylated and/or glycosylated.

In soy hydrolysate and soy-fermented foods, the proteins are only partly hydrolyzed because of the inability of most proteases to cleave glycoproteins, phosphoproteins, other post-translationally modified species, or domains that contain a higher number of disulfide bridges. The fermented foods, natto and tempeh, and soy hydrolysate were treated with alkaline phosphatase and endoglycosidases. The intermediate products were

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subsequently hydrolyzed by various proteases to produce oligopeptides. The peptides were purified, characterized and evaluated for biological activity. The digests of the pronase, kidney membrane and plasma proteases contained peptides with the greatest biological activity.

2. Materials and methods

Porcine pancreatic trypsin was obtained from Promega (Obispo, CA). Bovine plasma, Glu C protease, pronase and alkaline phosphatase were purchased from Sigma (St. Louis, MO). Bz–Gly–His–Leu and ACE were also purchased from Sigma Chemical Co. (St. Louis, MO). *N*-Glycanase, *O*-glycanase, α -mannosidase and endoglycosidase H were obtained from Genzyme Diagnostics (Cambridge, MA). BioRad Protein Assay Kit was purchased from BioRad (Montreal, Canada). Bovine serum albumin and lysozyme were obtained from Calbiochem (San Diego, CA).

The analytical butyl, octyl, phenyl, pH stable and octyldecylsilyl columns were purchased from Vydac (San Jose, CA). The amino-bonded columns were obtained from Supelco Canada. The protected amino acids (9-fluorenylmethyloxycarbonyl and *tert*-butyloxycarbonyl) were obtained from Applied Biosystems (Foster City, CA).

2.1. Preparation of natto

Bacillus subtilis was obtained from the American Type Culture Collection ATCC No.41332. Natto was produced in petri dishes in triplicate according to the method of Steinkraus, Van Buren, Hackler, and Hand (1965). Fifty grams of soybeans was cleaned and soaked overnight until they doubled in weight. The soaked beans were steamed at 130 °C for 32 min, cooled to 40 °C before inoculation with *B. subtilis* approximately 1 g/ml and incubated at 38 °C for 24 h. The mixture was sampled every 4 h with a final sample taken at 30 h. Samples were left at 5 °C for 24 h to complete maturation prior to analysis.

2.2. Preparation of tempeh

The initial preparation for tempeh consisted of boiling soybeans (approximately 100 g) for 30 min, draining, splitting and dehulling. The dehulled soybeans were acidified at 120 °C for 10 min in lactic acid (0.1 M) and surface dried on a perforated metal tray. Tempeh was produced in petri dishes in triplicate according to the method of Steinkraus et al. (1965). The spores were prepared by *Rhizopus oligosporus* NRRL 2710 fungus culture grown on dextrose agar at 30 °C and harvested after approximately 5 days with sterile distilled water. The mycelium was broken by vortexing, retained by

passing the suspension through a 100 μ m filter and washed three times with distilled water. The spores were removed from the filter with distilled water resulting in a final concentration of 10^6 spores/ml.

The soybean preparation was added to 10 ml of the spore suspension and 50 g of the mixture was added to each 90-mm diameter vented petri dish. The dishes were placed in humidity-controlled incubators at 30 °C. Uninoculated dishes containing the soybean preparation were used as controls. Samples were taken at 0, 4, 8, 12, 16, 20, 24, 27 and 30 h for analysis. Natto, tempeh and soy protein hydrolysate were purchased locally and used to compare against experimental batches. They were kept at 4 °C when not in use. Solid samples were pulverized with a mortar and pestle and thoroughly dried before use.

2.3. Peptide synthesis

Peptides were synthesized by the solid phase method on an Applied Biosystems 430A Peptide Synthesizer. *tert*-Butyloxycarbonyl-Gln phenylacetaminomethyl resin (Applied Biosystems, 0.64 mmol/g) was used as the solid-phase support. Amino acid coupling was mediated by dicyclohexylcarbodiimide/*N*-hydroxybenzotriazole, and deprotection was carried out with 50% trifluoroacetic acid in methylene chloride for 3 min followed by an additional 20-min cycle. Side-chain protecting groups were as follows: Asp (cyclohexyl), Glu (benzyl), His (benzyloxymethyl), Arg (Tos), Tyr (*N*²-benzyloxycarbonyl-*p*-bromobenzyl) and Ser (benzyl). The fully protected peptide resin was treated with liquid hydrogen fluoride (HF) containing anisole and dimethyl sulfide (10% by volume) at –5 °C for 60 min. Excess HF was removed under a stream of nitrogen, and the residual solid was extracted with ether and filtered. The resin was extracted three times with glacial acetic acid and water followed by lyophilization. The lyophilized crude peptides were purified to homogeneity by reverse-phase chromatography on a Vydac octadecyl silica (15 Å) glass column (1.5 $\times$ 30 cm, 40 psi) using a linear gradient of a solvent system gradient from 5% to 75% acetonitrile in 70 min. The collected fractions were further purified using a Vydac C₁₈ analytical column.

2.4. Protein assay

Protein content (before incubation) of samples was assessed by the Bradford method on a Varian–Cary 2000 double-beam spectrophotometer. Bovine serum albumin was used as a standard. One to one hundred micrograms of albumin was mixed with the dye reagent and read, after a 5-min incubation period, at 595 nm. Samples at different stages of purification were diluted appropriately and the absorbance was read and compared to the standard.

2.5. Dephosphorylation

Samples were incubated with 3%(w/w) alkaline phosphatase in 10 mM Tris–HCl buffer, pH 7.5, at 37 °C for 8 h to hydrolyze protein phosphate groups. Additional amounts of alkaline phosphatase were sometimes used to ensure complete dephosphorylation. This stage was attained when the chromatographic retention remained constant with the addition of phosphatase and incubation time. Samples were lyophilized before proceeding.

2.6. Deglycosylation

Quantities and experimental conditions used were essentially those recommended by the manufacturer. Appropriate amounts of endoglycosidases were incubated with known quantities of the mature natto, tempeh soy hydrolysate. Aliquots were evaluated to ensure complete deglycosylation. One percent of the samples was removed and applied to the HPLC system described. Deglycosylated species are more hydrophobic and are retained longer on an ODS column.

2.7. Proteolysis

The lyophilized samples were redissolved in 1 ml of 100 mM ammonium bicarbonate and aliquoted into Eppendorf tubes for digestion with commercial proteases and other proteolytic preparations.

2.8. Commercial proteases

Ammonium bicarbonate (1%) was used for all digestions. The ratio of enzyme to substrate to obtain complete digestion was between 2 and 10: 100 (w/w) as per the suppliers' recommendations.

Aliquots were removed and chromatographed to determine the state of hydrolysis. Hydrolysis was deemed complete (approximately 50 min for pronase and 12 h for chymotrypsin) when no increase in total peptides was observed upon addition of protease with an additional incubation period.

2.9. Kidney membrane proteases

Kidney membrane proteases were isolated by the method of Maeda, Balakishnan, and Medhi (1983) with modifications. Briefly 1 g of tissue was minced finely and added to a 3-fold excess of 10 mM ammonium acetate, pH 7.4, containing 1 mM MgCl and 1 mM dithiothreitol. The preparation was homogenized using a Polytron homogenizer in bursts of 5 s, separated by 2 min of cooling. This procedure resulted in efficient cell disruption. After layering over 1 ml of a 40% sucrose solution, the membranes were pelleted by centrifugation at 95,000g for 10 min.

An aliquot of membrane, 10 µg protein content as determined by the BioRad method (Bradford, 1976), and 100 µg of sample were incubated at 37 °C in a total volume of 500 µl. One hundred-microliter portions were removed at zero (control), 30, 60 and 120 min. The reaction was terminated with boiling for 2 min before centrifuging at 6000g for 2 min. Aliquots of the supernatant were injected into the HPLC to verify the extent of hydrolysis.

2.10. Plasma proteases

Experiments were carried out in 50 mM Tris–HCl, pH 7.8. Sample (100 µg) was incubated at 37 °C with the plasma proteases (10 µg protein content as determined by the BioRad method on reconstituted plasma to a total volume of 500 µl). One hundred-microliter portions were taken at 0, 30 and 60 min. The reactions were arrested by freezing at –80 °C. Mixing with an equal volume of 20% trichloroacetic acid precipitated the endogenous proteins and the supernatant was lyophilized.

2.11. Peptide extraction

The samples were extracted with a mixture of 0.5 ml water, 0.5 ml acetonitrile and 10 µl trifluoroacetic acid (TFA). The mixture was sonicated for 5 min, vortexed for 2 min, then centrifuged at 27,000g for 10 min. The supernatant was retained. Two more extractions were performed, the supernatants pooled, filtered through a 0.45-µm filter and lyophilized.

2.12. Liquid chromatography

The peptide extracts were chromatographed on a Hewlett Packard Model 1090 LC equipped with a diode array detector and ChemStation software. Peptides were separated on a Vydac ODS 300 Å RP column at a flow rate of 1 ml/min and a gradient from 5% to 75% acetonitrile in 70 min. The column was equilibrated with 5% acetonitrile and tested with 500 ng of 99.9% pure hen egg white lysozyme to verify system performance (i.e., resolution, signal intensity, bleeding, tailing, etc.). The eluent was monitored at 206 and 280 nm with a bandwidth of 2 nm and a reference wavelength of 550 nm.

2.13. Mass spectrometry

Mass spectra were obtained in the positive mode on a triple stage mass spectrometer Model API-III (Sciex), calibrated with the ammonium adduct ions of polypropylene glycol (PPG) of known mass to charge ratios in the range (0–2470 amu). Instrument tuning, data acquisition and processing were controlled by a MacIntosh G4 workstation with Programs Tune 2.5, Rad 2.6

and MacQuan 1.6. Additional data were collected on a Dell Precision Workstation 220 from a QSTAR Pulsari (Sciex, Toronto, Canada) with Analyst QS 1.0 software. The instrument was calibrated with an octapeptide (MW 829.5398) and cesium iodide (MW 132.9054) for accurate mass determination. Briefly the samples and standards were dissolved in a solution (a mixture of 50% methanol, 45% water and 5% formic acid) and infused through a stainless steel capillary (100 μ M ID). A stream of air (pneumatic nebulization) was introduced to assist in the formation of submicron droplets. These droplets were evaporated at the interface by nitrogen gas producing highly charged ions, which were detected by the analyzer. In some experiments the chromatographically separated peptides were infused directly into the mass spectrometer.

2.14. Biological activities

The biological activities were assessed on peptides with greater than 95% purity. Anti-thrombotic activity was determined according to the method of Szewczuk, Gibbs, Yue, and Purisima (1992). The method of Mulligan and Gibbs (1990) was utilized to measure surface tension. The ferric thiocyanate method was used to determine antioxidant activity (Mitsuda, Yasumoto, & Iwami, 1966).

The ACE inhibitory activity was measured according to the principles of Cushman and Cheung (1971). ACE solution (50 μ l, 4 mU) was preincubated with each sample (50 μ l) for 15 min at 37 °C. The enzymatic reaction was initiated by adding 150 μ l of 8.33 mM of the substrate Bz-Gly-His-Leu in 125 mM Tris-HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37 °C, the reaction was arrested by adding 250 μ l of 1 N HCl. The Gly-His bond was cleaved and the Bz-Gly produced by the reaction was extracted with 1.5 ml ethyl acetate. The mixture was centrifuged to separate the ethyl acetate layer; 1 ml of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm against a series of standards. The ACE inhibitory activities were expressed as IC_{50} , the sample concentration inhibiting 50% of the ACE activity.

3. Results and discussion

Fig. 1 shows the peptide profile of the pronase digest of natto. As shown, several peaks were not resolved in the first chromatographic run. The approximate region of the peptides of interest is shown. They were collected and re-chromatographed with a slower gradient on the same or a different column. Several unresolved peptides became fully resolved by this procedure.

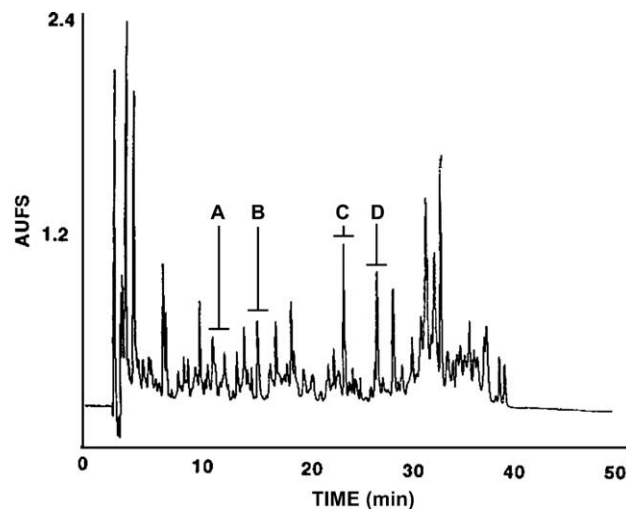


Fig. 1. HPLC peptide profile of the pronase digest of natto. The approximate region of the peptides of interest and their designation are indicated.

The peptide profile of the kidney membrane digestion of tempeh is shown in Fig. 2. Multiple column chromatography was also used to improve resolution.

Fig. 3 shows the HPLC profile of the plasma proteases' digest of soy hydrolysate. Peptides J and K were purified, assessed and were also found to be biologically active. Their intrinsic activities and characterization will be reported elsewhere. This preparation was difficult to hydrolyze and produced fewer peptides than the fermented products. The significance of pretreatment with the strains used in fermentation is quite evident.

Fig. 4 shows the mass spectrum of the extracted peptide mixture from the kidney membrane digest of natto. Peptides ranging from 6 to 13 residues were identified by LC-MS in the mixture. Several peptides

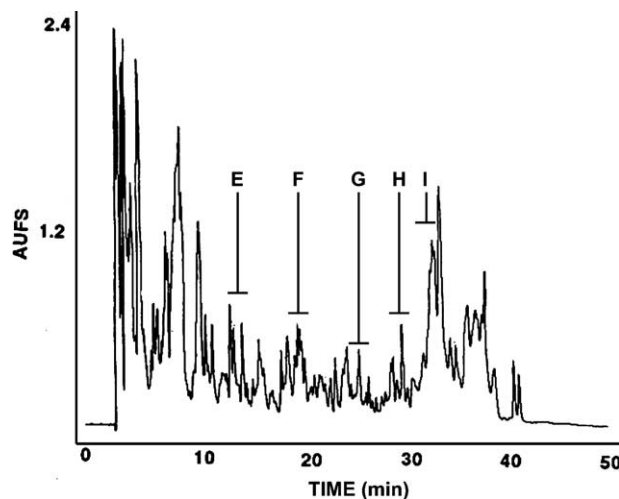


Fig. 2. HPLC peptide profile of the kidney membrane digest of tempeh. Multiple column chromatography was also used to improve resolution.

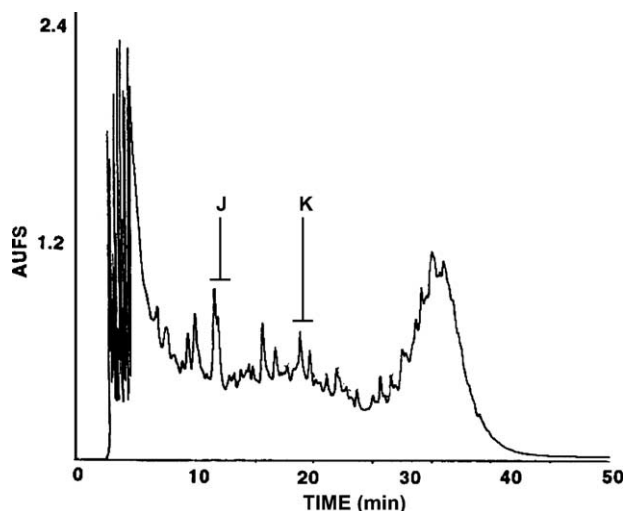


Fig. 3. HPLC peptide profile of the plasma proteases' digest of soy hydrolysate. Fewer peptides were produced than the fermented foods' digests.

were doubly or triply charged. They are indicated in the figure with the source from which they were derived. Each peptide was fragmented to produce its product ion spectrum. These spectra were submitted to commercial protein/peptide databases to positively confirm their precursor proteins.

Fig. 5 shows the product ion spectrum of the doubly charged peptide at 632.2780 amu. Several characteristic fragmentation ions can be seen in the spectrum. The

mass spectrum of the peptides from the plasma proteases' digest of tempeh is shown in Fig. 6. Several precursor sequences are shown in the figure.

Tables 1 and 2 show the amino acid composition of soybean before, during and after inoculation by *B. subtilis* and *R. oligosporus*, respectively. All analyses were performed in triplicate. In the natto, the composition was fairly constant. In the tempeh, however, some residues were changed significantly whilst other residues remained unchanged. The peptidase activity is clearly different in the two strains.

Table 3 shows the bioactivities of peptides purified from the pronase digest of mature natto. An ACE inhibitory peptide, peptide A of glycinin, was found in the digest. Several peptides from the digest contain a proline residue at the C-terminus. This is probably due to the presence of a specific post-proline cleaving enzyme in the enzyme preparation. Enzymes with similar characteristics have previously been reported (Park, Gibbs, & Lee, 1996). A proline residue might be located at or near the active site of ACE, a property that can be exploited in the design of anti-hypertensive drugs. Another peptide in the natto pronase digest, peptide B, was able to decrease the surface tension of water and is termed a biosurfactant. A compound is considered a surfactant if it can lower the surface tension of water from 72 mN/m. This peptide is very amphiphilic with a hydrophobic tail and is in agreement with the general design of most surface-active agents (Mulligan & Gibbs, 1990). It also had antioxidative properties.

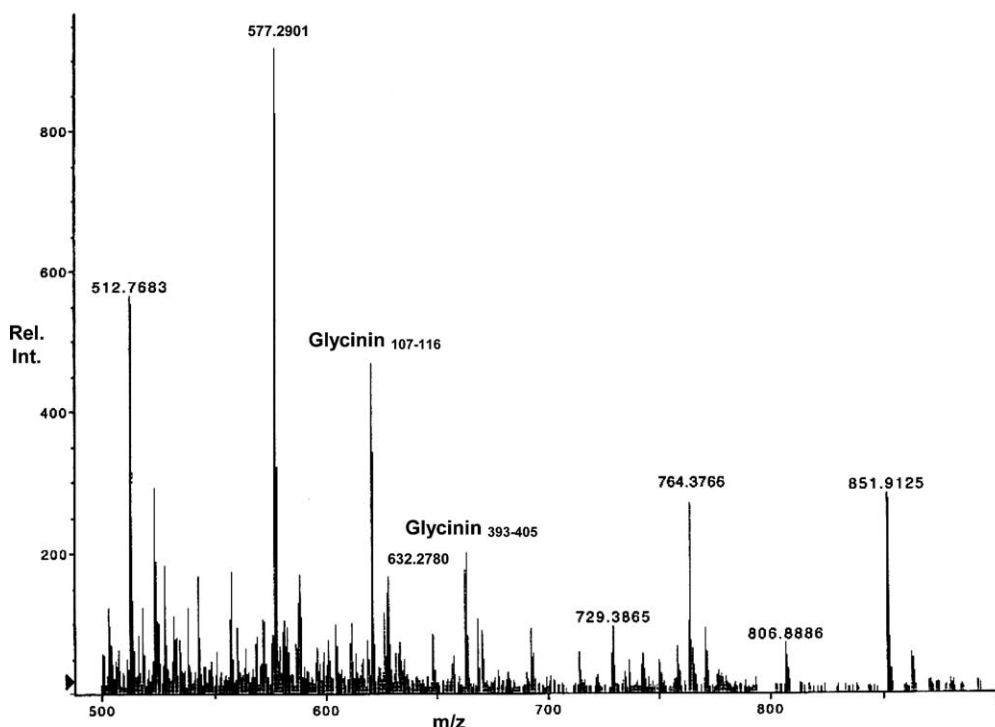


Fig. 4. Mass spectrum of the kidney membrane digest of natto. The precursor sequences are indicated.

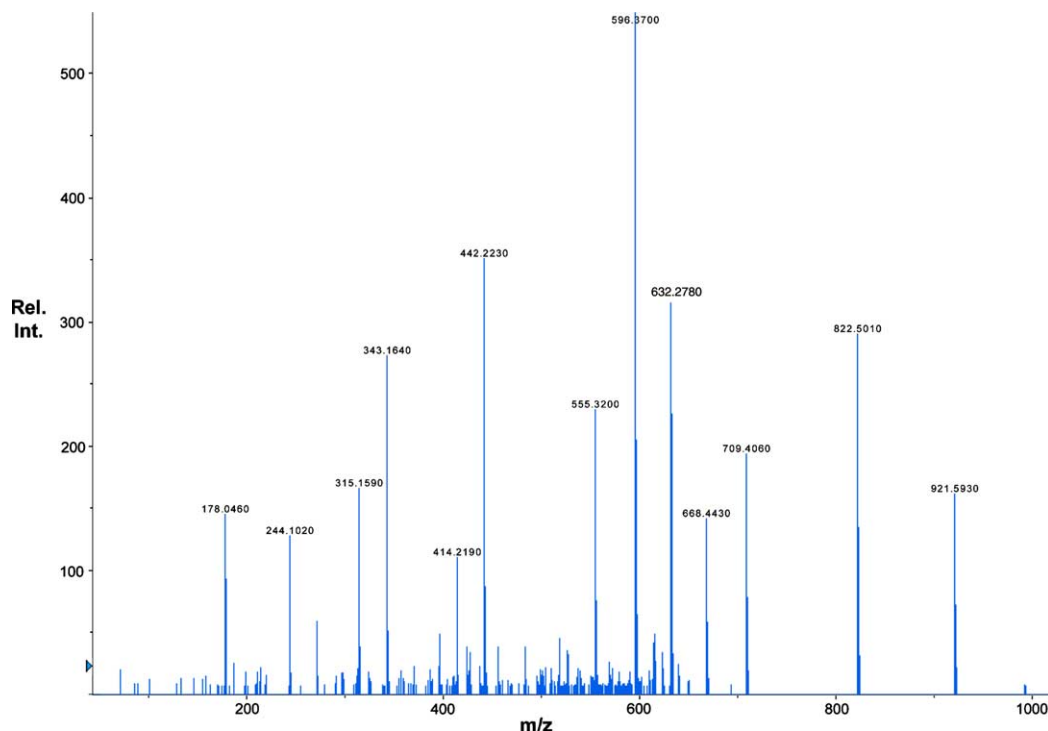


Fig. 5. Product ion spectrum of the doubly charged peptide at 632.2780 amu. Several characteristic fragmentation ions can be seen in the spectrum.

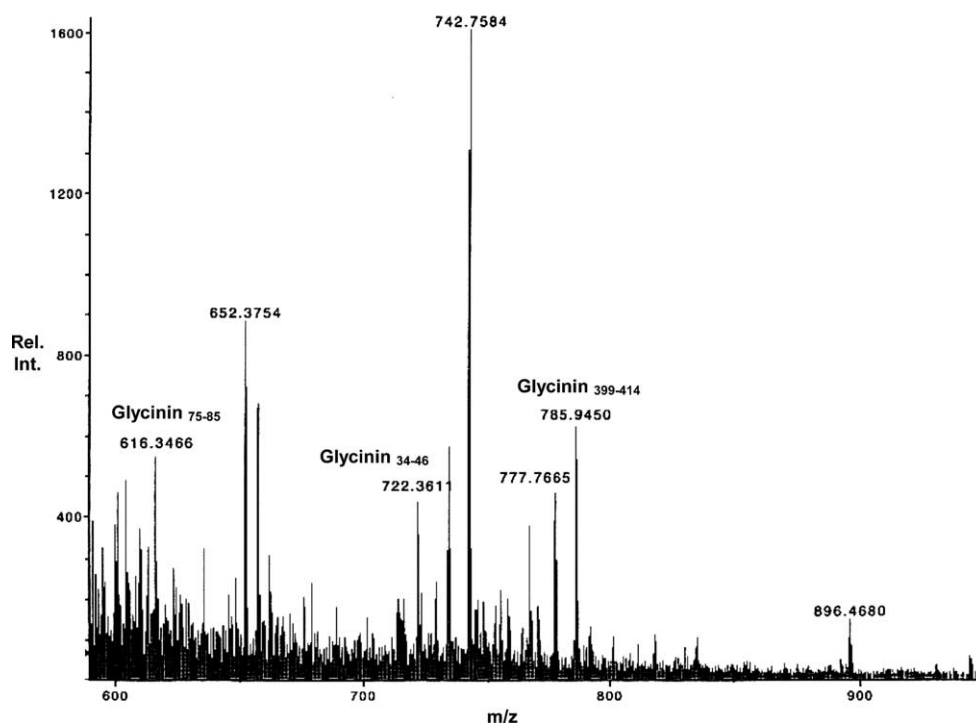


Fig. 6. Mass spectrum of the plasma protease digest of tempeh.

Table 4 shows the kidney membrane digest of mature tempeh. One of the peptides, peptide E, had both ACE inhibitory and antioxidant properties. It was produced by a tryptic-like cleavage with a C-terminal arginine.

Okamoto et al. (1995) fractionated the viscous material from beans in a natto preparation and found that the low molecular weight fractions contained potent ACE inhibitory activity. Another peptide in that digest, pep-

Table 1
Amino acid composition (mol%) of natto

Amino acid	Cleaned soybean	Natto (after 12 h)	Mature natto (24 h)
Gly	3.3	3.4	3.3
Ala	4.4	4.5	4.5
Val	5.6	5.6	5.5
Ile	5.2	5.2	5.3
Lue	8.6	8.6	8.4
Asx	11.0	11.0	11.1
Glx	18.0	18.0	18.1
Lys	6.8	6.8	6.8
Arg	5.6	5.3	5.2
His	3.3	3.2	3.1
Phe	5.1	5.2	5.2
Tyr	2.8	2.8	2.8
Pro	8.3	8.2	8.3
Try	1.0	1.0	1.0
Met	1.1	1.2	1.1
Cys	0.7	0.7	0.7
Ser	6.0	6.3	6.2
Thr	3.5	3.6	3.6

Table 2
Amino acid composition (mol%) of tempeh

Amino acid	Cleaned soybean	Tempeh (after 12 h)	Mature tempeh (24 h)
Gly	3.3	4.1	4.3
Ala	4.4	3.2	4.4
Val	3.0	3.0	3.1
Ile	5.2	5.6	6.6
Lue	8.6	7.9	12
Asx	11.0	10.1	6.1
Glx	18.0	16.3	12.3
Lys	6.8	7.6	4.3
Arg	5.6	5.8	5.7
His	3.1	1.6	3.4
Phe	5.1	4.3	5.6
Tyr	5.4	4.2	3.6
Pro	8.0	8.8	12.4
Try	1.0	1.0	1.1
Met	1.4	1.4	1.4
Cys	0.9	1.1	1.1
Ser	5.0	10.1	4.6
Thr	4.5	7.2	10.3

peptide G, possesses moderate anti-thrombotic properties. The peptide has 63% homology to a previously reported thrombin inhibitor, hirutinin (DiMaio, Gibbs, Lefebvre, & Munn, 1992). It contains an Arg–Pro bond that can be modified into a non-cleavable moiety to confer stability and become an unhydrolyzable inhibitor. Peptide I from glycinin was a weak ACE inhibitor with antioxidant properties.

Several synthetic peptides were evaluated as substrates for ACE and thrombin inhibition (DiMaio, Gibbs, Munn, Lefebvre, & Ni, 1990). Some were shown to be competitive substrates. The most potent ACE inhibitor and a peptide with anti-thrombotic activity were synthe-

sized as described in Materials and methods. Their biochemical activities were similar to the purified sequences.

The kinetics of peptide A, an ACE inhibitor, is shown in Fig. 7. The data were analyzed using a nonlinear regression program RNLIN in the IMSL library (IMSL, 1987) to estimate the kinetic parameters. ACE activity was measured with varying substrate (0.1, 0.3, 0.5 and 0.7 mM) and inhibitor concentrations. The association (K_{on}) and dissociation (K_{off}) constants were $8.4 \pm 0.2 \times 10^5 \text{ M}^{-1}$ and $1.7 \times 10^{-1} \text{ s}^{-1}$, respectively, with a binding constant (K_i) of $0.2 \times 10^{-6} \text{ M}$. Similarly, the K_i of the most potent anti-thrombotic peptide (peptide G from glycinin) was estimated as $1.1 \times 10^{-6} \text{ M}$.

Table 3
Peptides from pronase digest of mature natto

Peptide name	ACE inhibitory activity IC_{50} (μM)	Antithrombotic activity IC_{50} (μM)	Surface tension (mN/m)	Anti-oxidant activity
A	0.2 ± 0.1	162.1 ± 1.1	39.2 ± 1.0	–
B	40.0 ± 0.1	76.6 ± 1.1	31.6 ± 0.9	+
C	8.7 ± 0.6	36.2 ± 1.6	62.4 ± 0.8	–
D	14.2 ± 1.5	85.4 ± 0.9	71.3 ± 0.6	+

–, inactive sequence; +, denotes activity.

Table 4
Peptides from kidney membrane digest of mature tempeh

Peptide name	ACE inhibitory activity IC_{50} (μM)	Antithrombotic activity IC_{50} (μM)	Surface tension (mN/m)	Anti-oxidant activity
E	0.6 ± 0.1	39.3 ± 1.8	59.7 ± 1.4	+
F	555.5 ± 17.2	148.2 ± 2.1	64.3 ± 1.2	–
G	126.4 ± 4.2	9.8 ± 0.9	68.2 ± 1.1	–
H	70.3 ± 2.6	44.6 ± 2.1	69.3 ± 0.8	–
I	3.7 ± 0.2	136.2 ± 4.6	33.2 ± 1.0	+

–, inactive sequence; +, denotes some activity.

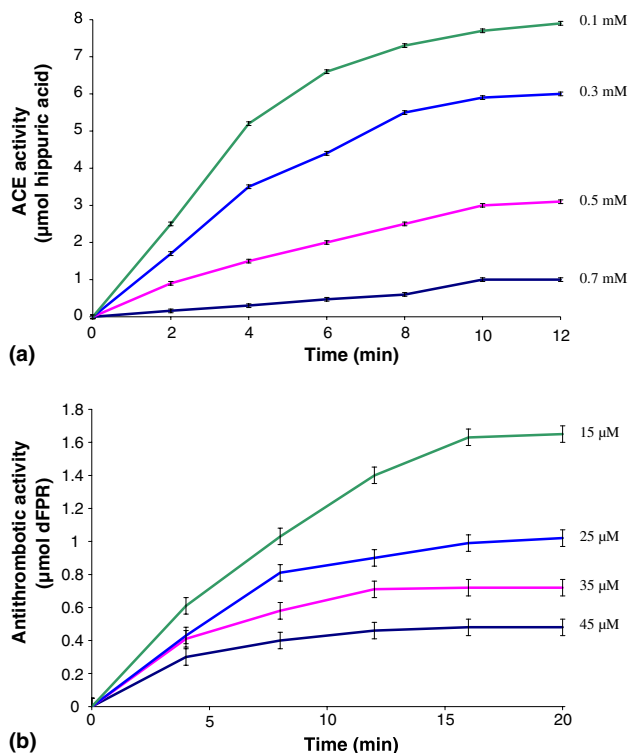


Fig. 7. Kinetics of inhibition (a) from the most potent ACE inhibitor and (b) for the most active anti-thrombotic inhibitor.

Soy proteins consist mainly of glycinin and β -conglycinin, but also contain several minor proteins. Glycinin was the precursor for 95% of the peptides isolated in these experiments. The other main protein, β -conglycinin, was found to be resistant to proteolytic attack even by the multi-enzyme systems, plasma proteases and kidney membrane proteins. Astwood, Leach, and Fuchs (1996) showed that β -conglycinin was more stable to acid hydrolysis than other non-allergenic foods. In another study, a homolog of β -conglycinin was found to be resistant to tryptic proteolysis (Deshpande & Nielsen, 1987).

Dephosphorylation and deglycosylation were performed prior to endoproteolytic digestion to facilitate complete hydrolysis, particularly of β -conglycinin. The glycosidases used (*N*-glycanase, *O*-glycanase, α -mannosidase and endoglycosidase H) will release all common classes of linked oligosaccharides found in proteins. In the deglycosylation experiments, HPLC analyses were used to establish glycosylation state. Deglycosylated moieties are more hydrophobic than their glycosylated analogues and are retained for a longer period on a ODS column (data not shown).

Plasma proteases were chosen as an enzyme system since plasma may contain every enzyme associated with living tissues (Fishman & Dvelling, 1985). If a compound is difficult to hydrolyze in physiological amounts of these enzymes, it has an excellent chance of reaching and acting on a given target organ. The

plasma proteases preparation was in the form of a dehydrated powder and required reactivated by hydration. Kidney homogenate membrane enzyme preparation also contains a battery of proteolytic enzymes, some of which are known to degrade peptides and proteins.

4. Conclusions

Several bioactive peptides were found in soy-fermented foods digested with proteolytic enzymes. The activities range from ACE inhibitory, anti-thrombotic, surface-active and antioxidative properties. Glycinin, one of the main proteins in soy, was the precursor of most of the isolated peptides. β -conglycinin, the other main protein in soy, was found to be partly resistant to hydrolysis even after removal of its few glycosylation sites (approximately 4% sugars). It would appear that the various proteases in *Bacillus* and *Rhizopus* strains hydrolyzed the main soy proteins into large peptides, with subsequent degradation to oligopeptides primarily by peptidase activity in the preparation. Since the soy hydrolysate was only exposed to the exogenous proteolytic enzymes and not to the *Bacillus* and *Rhizopus* strains, it produced less peptides than the fermented foods.

ACE inhibitors and peptides with antioxidant properties were first described in milk products (Berthou et al., 1987) and subsequently were studied extensively. However, a systematic study of bioactive peptides in soy has not been previously undertaken.

The inhibitory activities of the peptides mentioned in this paper can be improved. Their sequences provide a starting point for molecular modeling and thermodynamic studies. The residues critical for binding can then be derived, and homologues can be synthesized to produce compounds of more potent inhibitory activity.

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