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Comparative Study of Soybean Plasteins Synthesized with Soluble and Immobilized α-Chymotrypsin

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Plasteins were prepared from low molecular weight peptides of a peptic digest of soybean protein. The plasteins were fingerprinted on silica gel by a combination of electrophoresis and TLC, eluted, hydrolyzed, and subjected to amino acid analysis. The results indicated that the plasteins from soluble α-chymotrypsin were richer in glycine, valine, leucine, and serine than the plastein prepared from immobilized α-chymotrypsin. Plasteins prepared from immobilized α-chymotrypsin were rich in glutamic acid, lysine, alanine, and threonine, suggesting a much more hydrophilic plastein than prepared from the soluble form of the enzyme.

In a previous study, Pallavicini et al. (1980) reported that plasteins prepared from several sources exhibited slightly different patterns when subjected to isoelectric focusing. The most significant differences were between plasteins prepared from soluble and immobilized α-chymotrypsin. The differences occurred in plasteins prepared form hydrolysates of soybean, alfalfa, and wild grass leaf protein. The differences in mobility were interpreted to mean that there were slight differences in the isoelectric points of plasteins prepared from soluble and immobilized α-chymotrypsin. Plasteins offer considerable potential for control of functional and nutritional characteristics and for this reason they are of great interest to the food industry. Several reports emphasize the interest and potential of plasteins (Pallavicini et al., 1980; Yamashita et al., 1970a,b; Onoue and Riddle, 1973; Savangikar and Joshi, 1979; Hofsten and Lalasidis, 1976; Eriksen and Fagerson, 1976).

In this study, plasteins prepared from peptic digests of soy protein are compared when the plastein are made by using either soluble or immobilized α-chymotrypsin. Comparisons are made by fingerprinting the plasteins on TLC and by amino acid analysis of the new peptides recovered from the fingerprinting.

METHODS AND MATERIALS

Protein Extraction. Soybean flour (12.5% moisture) was purchased from a local supplier and was stored at −20 °C until used. The extraction of the proteins from soy flour and the hydrolysis with pepsin (pH 1.6–1.8, 40 °C for 40 h) was done as previously described by Pallavicini et al. (1980). The low molecular weight peptide fraction of the hydrolysate was prepared by dialysis of the peptic digest against water. Spectrapor membrane tubing with a molecular exclusion of 3500 was used for the separation. The diffusate was concentrated in vacuo at 40 °C. The concentrate was then freeze-dried. The freeze-dried material was used at a 30% (w/v) concentration as the substrate for plastein synthesis with either soluble or immobilized α-chymotrypsin.

Plastein Preparation with Soluble α-Chymotrypsin. The 30% low molecular weight peptide fraction was filtered through Whatman No. 4 paper and the solution was incubated with α-chymotrypsin (salt free, type II, from Sigma Chemical Co., St. Louis, MO). The following conditions were used: substrate concentration 30% (w/v); enzyme/substrate ratio 1/100; pH 5.0; incubation temperature 38 °C for 6 h. The water-insoluble plastein products were purified by dialysis for 3 days at 5 °C according to Noguchi et al. (1975). The dialysis was carried out in a Spectrapor membrane tubing with an exclusion limit of 8000. Dialysis was carried out against four changes of distilled water. At the end of dialysis the contents of the bag were freeze-dried and used for the fingerprinting.

Plastein Preparation with α-Chymotrypsin Immobilized on Chitin. The enzyme immobilization procedure was as previously described by Pallavicini et al. (1980). Briefly the α-chymotrypsin was immobilized on 20–30-mesh chitin with glutaraldehyde and packed in a jacketed column. Column operation conditions were as previously described. The reaction product containing fractions from the column were pooled and dialyzed to retain only the water-insoluble fractions. Dialysis conditions were the same as for the plasteins prepared by soluble α-chymotrypsin. At the end of dialysis the contents of the bag were freeze-dried and fingerprinted.

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Table I. Amino Acid Composition of Plastein Peptides Prepared with Soluble or with Immobilized α-Chymotrypsin from the Low Molecular Weight Peptide Fraction of Soybean Protein Hydrolysates. The Peptides Are Numbered as in the Maps (Figures 1 and 2)[a]

	peptides obtained with soluble chymotrypsin					peptides obtained with immobilized chymotrypsin				
amino acid	1	2	3	4	5	1	2	3	4	5
aspartic acid[b]	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)
threonine	0.3	0.4	0.3	0.7 (1)	0.3	0.5 (1)	0.2	0.2	4.3 (4)	0.4
serine	1.3 (1)	0.9 (1)	1.7 (2)	2.8 (3)	2.0 (2)	3.0 (3)	0.6 (1)	0.4	1.3 (1)	2.0 (2)
glutamic acid[c]	1.3 (1)	1.3 (1)	0.6 (1)	1.7 (2)	1.5 (2)	1.0 (1)	2.3 (2)	1.3 (1)	3.2 (3)	0.9 (1)
proline										
glycine	2.2 (2)	2.0 (2)	2.3 (2)	3.7 (4)	2.7 (3)	3.3 (3)	1.3 (1)	1.2 (1)	1.1 (1)	2.2 (2)
alanine	0.8 (1)		0.6 (1)	1.0 (1)	0.8 (1)	1.1 (1)	0.4	0.5 (1)	1.7 (2)	0.9 (1)
valine	0.5 (1)	0.5 (1)	0.6 (1)	0.7 (1)		0.4	0.3	0.4	1.0 (1)	
cysteine										
methionine										
isoleucine	0.3	0.4	0.4	0.3		0.3	0.2	0.2		
leucine	0.6 (1)	0.7 (1)	0.6 (1)	1.0 (1)	0.5 (1)	0.5 (1)	0.4	0.4	0.9 (1)	0.5 (1)
tyrosine	0.2					0.2	0.1			
phenylalanine	0.2			0.5 (1)		0.3	0.2	0.2	0.4	1.2 (1)
lysine	0.4	0.3	0.4	0.7 (1)	0.3	0.5 (1)	0.4	0.2	0.7 (1)	0.6 (1)
histidine	0.1			0.3	1.5 (2)	0.5 (1)	0.1	0.1		
arginine						0.3		0.2	0.3	
tryptophan	0.3						0.4			
total residues	(8)	(7)	(9)	(16)	(12)	(13)	(5)	(4)	(15)	(10)

[a] Values shown are molar ratios with respect to aspartic acid (which includes deaminated asparagine), and the assumed number of residues is given in parentheses. All amino acids were calculated without correction for losses during acid hydrolysis or for the presence of impurities. Values for residues present of less than 0.01 mol were omitted. [b] Sum of aspartic acid and asparagine. [c] Sum of glutamic acid and glutamine.

Thin-Layer Fingerprint. Both types of plasteins were mapped on silica gel plates (20 × 20 cm, 0.25 cm thick, Merck Kieselgel 60 F 254). Each sample (10 mg, dry basis) was dissolved in 1 mL of pyridine/acetone/acetic acid/water (2/15/8/75), and 60 μL of this solution was applied to a previously activated plate. The same solvent was used to saturate the plate which was subjected to electrophoresis (200 V, 24 mA, 36-h running time, room temperature). After electrophoresis the plate was allowed to dry and the plate was then subjected to ascending TLC developed with 1-butanol/pyridine/acetic acid/water (75/50/15/60) for 8 h, followed by electrophoresis for 8 h in the first dimension under the same conditions described above. The plates were visualized by spraying with fluorescamine spray (Felix and Jimenez, 1974) and viewed under ultraviolet light at 366 nm.

Spot Elution and Amino Acid Analysis. After the spots were located on the plates they were circled, carefully removed from the plate, and eluted by the procedure of Schiltz et al. (1977). The eluents were hydrolyzed under nitrogen with 6 N HCl for 22 h at 105 °C. On parallel plates the eluted peptides were hydrolyzed with 14% barium hydroxide at 105 °C under nitrogen for 40 h to determine tryptophan, with an LKB amino acid analyzer according to the manufactures' suggested methods for amino acid hydrolysates.

RESULTS AND DISCUSSION

The fingerprint obtained for the plasteins formed by soluble α-chymotrypsin appeared to contain five components as shown in Figure 1. The plasteins prepared with immobilized α-chymotrypsin also appeared to contain five components (Figure 2) but the mobilities were slightly different than the plasteins from the soluble enzyme. These results agree with the previous results reported by Pallavicini et al. (1980) where it was shown that the peptides differed slightly when subjected to isoelectric focusing in polyacrylamide gels. In all samples examined, after the fluorescamine treatment, weakly fluorescent zones were observed near the origin and scattered among the other major components. However, no amino acids were detected in these spots. It is assumed that these spots are

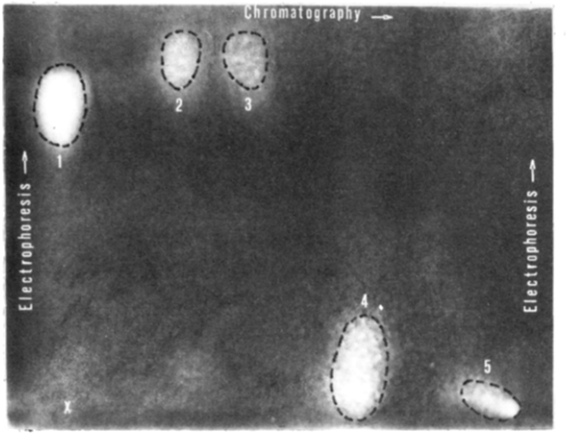

Figure 1. Peptide map of the plastein prepared with soluble α-chymotrypsin from the low molecular weight peptide fraction of soybean protein hydrolysates.

due to impurities or minor aggregation products. In order to check homogeneity of the peptides the recovered peptides were rechromatographed under different conditions. The resulting chromatograms all showed a single spot with the same mobility as in the original chromatogram.

In order to further characterize the plasteins the eluted peptides were subjected to amino acid analysis. Table I contains the results of the amino acid analysis of the various plasteins eluted from the chromatograms. The values in Table I represent means of three determinations on each of three separate plastein preparations. All of the peptides found were free of proline, cysteine, and methionine. Collectively, the peptides prepared by using soluble α-chymotrypsin contained higher levels of alanine, lysine, threonine, and glutamic acid. These differences may be responsible for the differences in migration observed here and previously by Pallavicini et al. (1980). These differences would easily account for great variation in the isoelectric point of the peptide. These results clearly indicate that immobilization of the α-chymotrypsin changes the specificity of the enzyme for plastein formation. The nature of this change in specificity will be studied in future

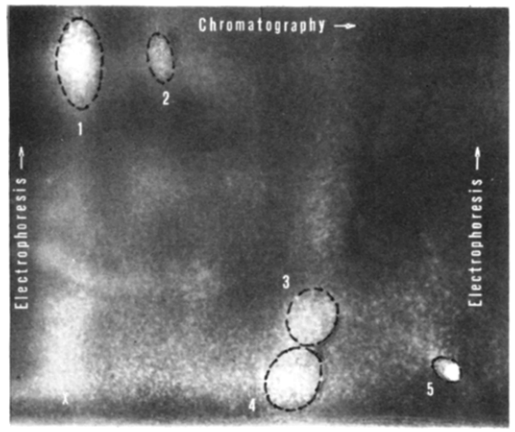

Figure 2. Peptide map of the plastein prepared with immobilized α-chymotrypsin from the low molecular weight peptide fraction of soybean protein hydrolysates.

work. The importance of this in preparation of plasteins for food use is not clear.

Peptides synthesized by using the immobilized enzyme were smaller than the smaller peptide prepared by using the soluble enzyme. The plasteins prepared with the immobilized enzyme (peptides 2 and 3) were found to be penta- and tetrapeptides, respectively. The average molecular weight was 1248 (10.4 residues) for the peptides from soluble α-chymotrypsin and 1084 (9.04 residues) for the peptides prepared from the immobilized enzyme. These results compared favorably with the results obtain previously (Pallavicini et al., 1980), which indicated an average molecular weight found for the soy plasteins in the current study is slightly higher than that obtained from soy hydrolysate and ^{14}C-labeled methionine ethyl ester substrate reported by Monti and Jost (1979) and lower than that of other plasteins prepared from soy by using soluble α-chymotrypsin (Yamashita et al., 1970b, 1974) and other free proteases (Yamashita et al., 1972, 1975; Tsai et al., 1974).

From the data presented above, we cannot completely exclude the possibility that small quantities of peptide material remain unresolved by the fingerprint technique. The immobilized enzyme system has great advantages in the preparation of plasteins in that the enzyme can be recovered and recycled, simplifying plastein purification. The plasteins prepared by using the immobilized enzyme are different than those from soluble enzyme.

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On the Electrophoretical Differentiation and Classification of Proteins. 12. Comparative Investigation of Yeast Proteins of Different *Saccharomyces* Species and Various Strains Belonging to the Species *Saccharomyces cerevisiae* Hansen, by Means of Isoelectric Focusing in Polyacrylamide Gels

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Isoelectric focusing in cylindrical polyacrylamide gels was used to resolve the water-soluble proteins of *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Saccharomyces bayanus*, *Saccharomyces rouxii*, *Saccharomyces aceti*, and seven strains of the species *Saccharomyces cerevisiae* Hansen. The cell disruption was carried out with freeze–pressing. About 30 protein bands were found with isoelectric points of 4.5–9.7. The trend in the pH gradient was determined by measuring the pH of the gel slices with a special one-rod electrode. The results were reproducible under constant working conditions. The protein patterns of the seven *S. cerevisiae* strains are found to be very similar, although those of the species are partly variable; hence, the question of correct systematic classification arises.

In recent years, attention has been focused on physicochemical methods for the identification of microorganisms which may provide alternatives to the conventional techniques. These new approaches include the gas chromatography (Drucker, 1981; Jantzen and Hofstad, 1981; Mitruka, 1975; Meuzelaar et al., 1975; Dees and Moss, 1978; Wasserfallen and Rinderknecht, 1978), mass spectrometry (Wieten et al., 1981; Kistemaker et al., 1975; Mitchell et al., 1978), impedance measurement (Ur and

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