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Hydrolysis of 7S and 11S Soy Proteins by Commercial Proteases

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蛋白分解酵素에 의한 大豆 7S 및 11S 蛋白質의 加水分解

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Abstract

Selected kinetic parameters and degree of hydrolysis(DH) were measured using commercial proteases(trypsin, alcalase and pronase) to study the affinity of these enzymes to 7S and 11S soy proteins. Electrophoretic patterns of the hydrolysates were also investigated. In general, the order of affinity between the proteins and the proteases was 11S PRF(protein-rich fraction) and 7S PRF for unheated proteins, and 7S PRF and 11S PRF for preheated proteins. Substrate inhibition was present at a substrate concentration of 1.5% or higher when preheated protein was used as the substrate. The maximum DH values of alcalase were obtained from 7S PRF(60%) and 11S PRF(80%) at 1 hr hydrolysis, respectively. Trypsin hydrolysis did not affect 11S soy protein but the acidic subunits in contrast to alcalase and pronase hydrolyses which changed almost all subunits. Alcalase hydrolysis induced distinct changes on 2S soy protein.
Key words: soy proteins, enzymatic hydrolysis, Km, DH.

Introduction

The 7S and 11S fraction are the main components of soy protein and account for about 70% of the total protein content. They are distinctly different in their functional properties[1]. Therefore, several methods have been studied to fractionate the 7S and 11S proteins[2,3]. Also, attempts were made to use the fractionated proteins in various food systems[4].

A number of authors also studied with many useful results on the functionality changes of soy proteins upon proteolysis and applications of the hydrolysate in food systems[5]. Various kinetic parameters relating to soy hydrolysate production were also estimated using pronase[6], fungal protease[7] and commercial proteases[8]. Most of these studies were carried out on soy concentrate or isolate, but not on the individual component of soy protein such as 7S and 11S soy proteins. Although the results of these studies are important for commercial applications, they seem to be insufficient to understand the role of 7S and 11S soy proteins on a soy protein hydrolysate. Futhermore, reports on the kinetic parameters of 7S and 11S soy proteins relating to proteolysis are scarce.

Therefore, the objectives of the present study were to estimate the kinetic parameters(Km and Vmax) of the 7S and 11S soy proteins using three proteases(trypsin, alcalase and pronase), and investigate the degree of hydrolysis(DH) and the electrophoretic patterns of the hydrolysates.

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Materials and Methods

Materials and protein fractionation

Soybeans(White-hilum variety) were dehulled and ground so as to pass through a 20 mesh screen. The defatted meal was ground again to pass through a 40 mesh screen before the proteins were extracted and fractionated by a method[2] with some modification as shown in Fig. 1.

Three commercial proteases, trypsin(Sigma, T-8253), alcalase(Novo, solid form, Denmark) and pronase(Calbiochem, B grade, CA), were purchused.

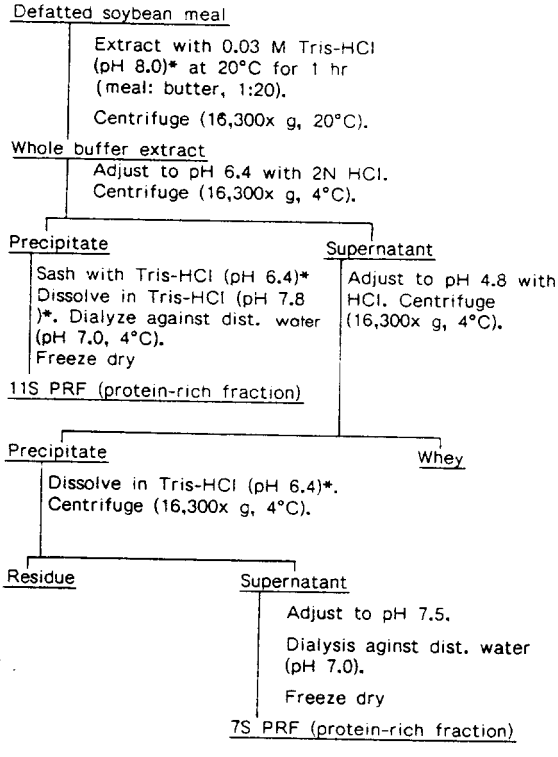

Fig. 1. Method for fractionation of 7S and 11S PRF.
* Tris-HCl (Tris hydroxymethyl amino methane-HCl) buffer containing 0.01 M 2-ME.

Measurement of kinetic parameters

The initial velocity of each enzyme was calculated from 10% trichloroacetic acid(TCA)-soluble soy protein(absorbance at 280 *nm*) using a previous method[8] under the reaction conditions summerized in Table 1. The initial velocity, increase in absorbance at 280 *nm* min was calculated from the absorbance of the solution measured after 5 min incubation(the hydrolysis curves were virtually linear in the first 5 min of the hydrolysis). Km and Vmax were calculated from the Lineweaver-Burk Plot[9]. The preheated proteins were prepared by heating at 95℃ for 5 min in the buffer before incubation.

Determination of degree of hydrolysis(DH)

The hydrolysis was carried out on unheated proteins in a batch. A 2-liter flask containing 1 liter of 1% protein solution in 0.01M sodium phosphate-citric acid(pH 7.0) containing 0.01% sodium azide was maintained at 40℃ with agitation. After equilibration for 10 min, each enzyme was added at an enzyme to substrate ratio of 1/100(w/w). The trinitrobenzene sulfonic acid(TNBS) method(10.11) was used to determined the DH on the 2 m*l* aliquots taken at 10 min intervals during the course of the hydrolysis. The DH is defined as the follows: [(total free amino group in sample minus free amino group in control)/total free amino group] × 100.

Electrophoresis of hydrolysates

The 1 hr hydrolysates remaining after the determination of DH were used for electrophoresis by a previous method[8].

Results and Discussion

Protein fractionation

7S and 11S soy proteins were fractionated with 0.03M Tris-HCl buffer(pH 8.0) containing 0.01M 2-mercaptoethanol(ME), based on the continuous

isolation procedure of 7S and 11S soy proteins. Although the procedure included a cryo-precipitationg step for 11S protein, the step was omitted from this study since it was impractical on a large-scale and continuous fractionation[12]. Also, dialysis in this study was carried out against distilled water(pH 7.0), rather than against Tris -HCl buffer in order to exclude the effects of Tris -HCl and 2-ME in the fractionated proteins. Thus the fractions of 7S and 11S soy proteins in this study were termed 7S and 11S PRF(protein-rich fraction), respectively.

Table 1. Enzyme reaction conditions on the soy proteins for kinetic constants

Substrate concentration (%,w/v): 0.5, 0.75, 1.0, 1.5, 2.0, 2.5
Enzyme concentration (mg/ml): 0.5
[E] / [S]: 1/50, 1/75, 1/100, 1/150, 1/200, 1/250
Reaction time (min): 0, 1, 3, 5, 8, 10, 15, 20
Incubation temperature (°C): 40
Buffer: pH 7.0, sodium phosphate-citric acid

Kinetic parameters

Prior to determining Km and Vmax, it is necessary to measure initial velocity of the hydrolysis. Fig. 2 and 3 show the plots of initial velocities against initial substrate concentrate at 40℃ and pH 7.0. For unheated soy proteins, initial velocities increased almost in proportion to the increase in substrate concentrations under 2.5%. For all proteases, 7S PRF were more sensitive than 11S PRF. For preheated soy proteins, the initial velocities were also dependent on substrate concentrations to some degree(below 1.5%, w/v), but at relatively high concentrations(above 1.5%, w/v), they were generally decreased except in the hydrolysis of 11S protein by trypsin. Unlike unheated proteins, the initial velocities of a preheated 11S protein had higher values than those of 7S protein on alcalase and pronase hydrolyses.

Preheating soy isolate had two significant effects in pronase hydrolysis: improved initial velocity and substrate inhibition on the initial velocity[6]. The improved initial velocities from this study were achieved by preheating 11S protein, especially for alcalase and pronase hydrolyses. But the initial velocity of 7S protein was remarkably decreased by preheating. The substrate inhibition on initial velocity was observed on the hydrolyses of preheated soy proteins. Several mechanisms are consistent with these

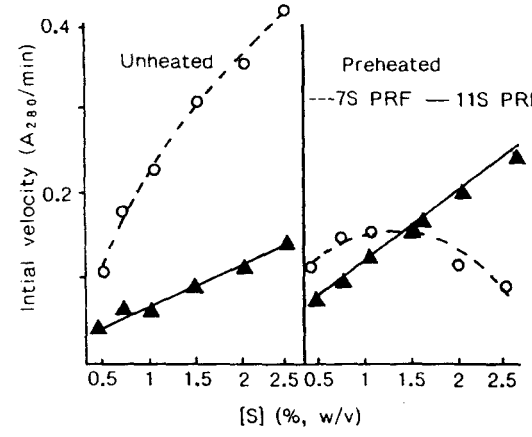

Fig. 2. Initial velocities of trypsin hydrolysis on preheated and unheated soy proteins.

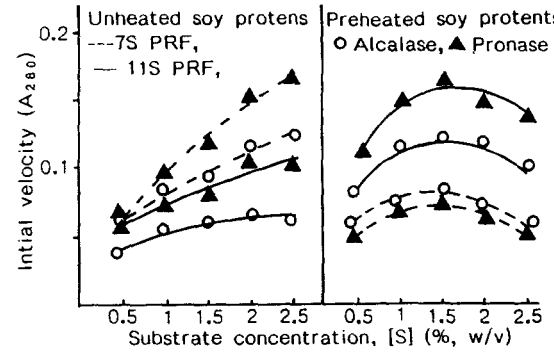

Fig. 3. Initial velocities of enzymatic hydrolysis on preheated and unheated soy proteins.

substrate inhibitions. Fox exemple, high substrate concentration in denatured soy proteins could have caused the removal of an essential cofactor, or resulted in a proportion of the enzyme molecules engaged in multiple binding patterns of substrate[13]. Similar preheating effcts on soy isolate have also been reported[8]. Consequently, the substrate inhibition seems to be a common phenomenon occuring in soy protein hydrolysis regardless of the source of proteases used.

Kinetic constants(Km and Vmax) of the hydrolysis of soy proteins were calculated from the Lineweaver-Burk Plot of the initial velocities as shown in Table 2. For unheated soy proteins, the Km values of 11S protein were lower than 7S, while for preheated soy proteins, the values were higher than 7S. The highest value was obtained from the trypsin hydrolysis of unheated 7S protein. The difference in Km between unheated and preheated proteins also was about 27 times for 7S protein in trypsin hydrolysis. Apparently this results are caused by trypsin inhibitor presented in unheated 7S PRF. From the Michaelis-Menten equation, a low value of Km indicates a high affinity of the enzyme for its substrate, since Vmax will already be attained at low substrate concentrations[14]. Therefore, the results of Km indicate that all proteases appear to have greater affinity toward the preheated soy proteins than the unheated ones. In general, native globular proteins are more easily hydrolyzed in their denatured forms than their native ones[15]. Also, the orders of affinity were 11S PRF > 7S PRF in unheated proteins, and 7S PRF > 11S PRF in preheated proteins.

Table 2. Kinetic constants for proteinase reaction on the hydrolysis of 7S and 11S PRF at pH 7.0, 40℃, [E]=0.5mg/ml[a]

Enzyme	Constant[b]	Unheated		Preheated[c,b]	
		7S PRF	11S PRF	7S PRF	11S PRF
Trypsin	Vmax	3.80	0.31	0.37	0.49
	Km	16.03	2.77	0.59	1.62
Alcalase	Vmax	0.14	0.08	0.09	0.16
	Km	0.67	0.49	0.21	0.43
Pronase	Vmax	0.22	0.11	0.09	0.21
	Km	1.21	0.48	0.29	0.41

a: Calculated from Lineweaver-Burk Plot(r>0.95)
b: Unit; Vmax=O.D. increased at 280 nm/min, Km=%. w/v
c: Preheated at 95℃ for 5 min in buffer (pH 7.0) before incubation
d: Data from linear portion on Lineweaver Burk Plot was considered

Degree of hydrolysis(DH)

The DH values of unheated protein hydrolysis by three proteases were determined by the TNBS method[10,11] as shown in Fig. 4. Although the 10% TCA-soluble protein method[16] has been used for the same purpose as the TNBS method, the TNBS method seems to be more suitable to determine DH of proteins under the reaction conditions used in this study.

The DH values of trypsin were considerably lower than those of alcalase and pronase, indication that trypsin could not effectively hydrolyze these proteins. The maximum DH values obtained at 1 hr for alcalase was 60% for 7S PRF and 80% for 11S PRF, respectively. The results indicate that alcalase is more sensitive to 7S protein than 11S protein. That is, several inhibitors existed in 7S PRF could not affect the alcalase hydrolysis.

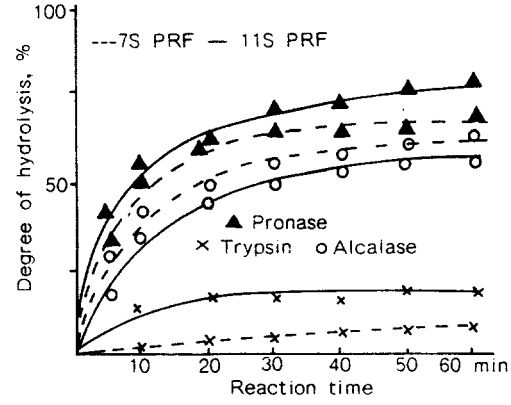

Fig. 4. Degree of hydrolysis(DH) of soy proteins with proteases measured by TNBS method.

Electrophoresis of hydrolysates

Electrophoretic patterns of protein hydrolysates are shown in Fig. 5(by trypsin) and in Fig. 6(by alcalase and pronase). The patterns of 7S PRF were almost similar to those of the control(native protein) as to the location and concentrations of bands. However, the pattern of 11S PRF was changed by trypsin hydrolysis. That is, the trypsin treated 11S PRF formed a wide band in the PAGE gel and a new band(arrow in Fig. 5) with a high Rm(relative mobility) under the basic subunits of 11S protein in the SDS-PAGE gel, instead of disappeared acidic subunits of 11S protein. The molecular weight of the new band in the SDS -PAGE gel was estimated as approximately equal to ribonuclease(13,700 daltons), a marker protein[12]. The apparent preferential hydrolysis of the acidic over the basic subunits by pepsin and trypsin was probably due to the greater hydrophobic character and compactness of the basic subunits of 11S protein[17].

According to the results of PAGE(Fig. 6), the 7S PRF band gradually disappeared by alcalase treatment without forming any new bands, while 11S PRF formed a relatively wide band located at the halfway between the two bands of unhydrolyzed 11S protein(control protein). Alcalase hydrolysis distinctly changed the location of 2S band(arrow in Fig. 6) as compared with trypsin and pronase hydrolyses, i.e., the position of 2S band shifted more toward the bottom of the PAGE gel, which indirectly indicates that alcalase can change the conformation or peptide chains of the 2S protein fraction. Several trypsin inhibitors and cytochrome C exist in the 2S fraction, as well as other minor protein[18]. Therefore, it is possible that certain changes have been induced by the alcalase hydrolysis of these compounds. This hypothesis should, of course, be proved by additional experiments.

In pronase hydrolysis of soy proteins, a distinct change in the electrophoretic pattern of 11S PRF hydrolysate occured, forming two bands at the lower portions of the PAGE gel in contrast to the two bands of the native 11S protein(Fig. 6). However, SDS-PAGE patterns of alcalase and pronase hydrolysis were similiar each other, represented by a long smeared band without separation into distinct bands.

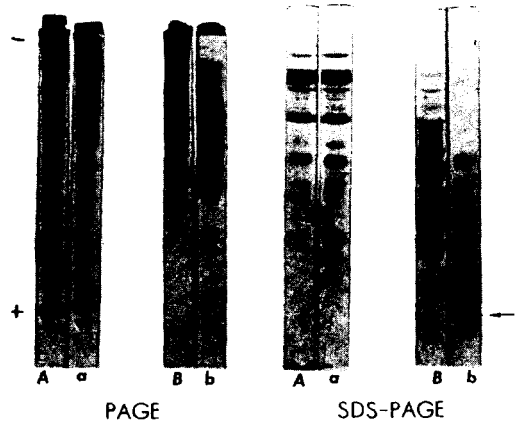

Fig. 5. PAGE and SDS-PAGE of soy proteins and their trysin hydrolysates.
A, a; 7S PRF and its hydrolysates, B, b; 11S PRF and its hydrolysates

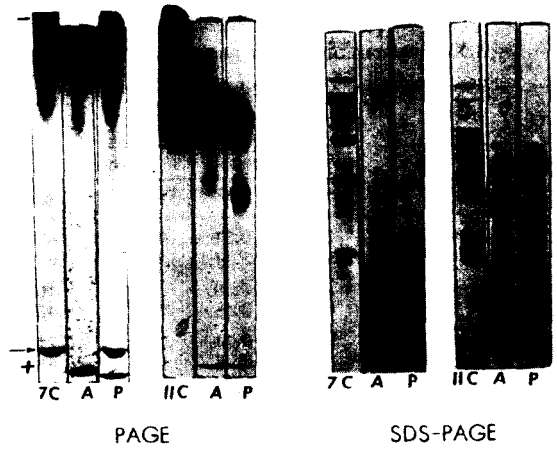

Fig. 6. PAGE and SDS-PAGE of soy proteins and their enzyme hydrolysates.
7S ; 7S PRF control, 11C; 11S PRF control, A, alcalase hydrolysate, P, pronase hydrolysate.

요 약

分劃된 大豆 7S 및 11S 蛋白質의 蛋白質分解酵素(trypsin, alcalase 및 pronase)에 대한 反應性을 動力學變數인 Km과 Vmax 및 加水分解度(DH)를 測定하여 檢討하였으며 가수분해물의 電氣泳動分析을 실시하였다.

酵素의 大豆蛋白質에 대한 親化力은 대체로 加熱處理에 의하여 상당히 改善되었으나 加熱處理된 蛋白質은 基質濃度 1.5%(w/w) 以上에서 加水分解에 대하여 基質阻害作用을 보였다.

1時間 加水分解에 따라 alcalase에 의하여 가장 큰 加水分解度가 얻어지으며 7S 蛋白質에 대하여 60% 및 11S 蛋白質에 대하여 80%였다. Trypsin은 加熱處理되지 않은 蛋白質에는 11S 蛋白質의 acidic subunit 이외에는 거의 作用하지 못했으며 alcalase는 특이적으로 2S 蛋白質에 變化를 가져오는 것으로 電氣泳動分析에서 나타났다.

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