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Comparison of Protein Chemical and Physicochemical Properties of Rapeseed Cruciferin with Those of Soybean Glycinin

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Rapeseeds contain cruciferin (11S globulin), napin (2S albumin), and oleosin (oil body protein) as major seed proteins. The effects of oil expression and drying conditions on the extraction of these proteins from rapeseed meal were examined. The conditions strongly affected the extraction of oleosin and only weakly affected the extraction of cruciferin and napin. The protein chemical and physicochemical properties of cruciferin, the major protein present, were compared with those of glycinin (soybean 11S globulin) under various conditions. In general, cruciferin exhibited higher surface hydrophobicity, lower thermal stability, and lower and higher solubility at $\mu = 0.5$ and $\mu = 0.08$, respectively, than did glycinin. At the pHs (6.0, 7.6, and 9.0) and ionic strengths ($\mu = 0.08$ and 0.5) examined, the emulsifying ability of cruciferin was worse than that of glycinin, except at $\mu = 0.08$ and pH 7.6. The emulsifying abilities of cruciferin and glycinin did not correlate with thermal stability and surface hydrophobicity. Higher protein concentration, higher heating temperature, higher pH, and lower ionic strength were observed to produce harder gels from cruciferin. Gel hardness partly correlated with the structural stability of cruciferin.

KEYWORDS: Rapeseed; cruciferin; napin; oleosin; glycinin; physicochemical property; emulsification; heat-induced gelation

INTRODUCTION

The increasing world population will require greater production of food proteins in the future (*1*). Putting more lands to till may not be feasible to address this problem since the land available on the Earth for cultivation is limited. Moreover, it may be difficult to produce food stably because of global warming and abnormal weather patterns. Under these limitations, one of the approaches to increase food production is to utilize effectively the underutilized or unutilized existing crops.

About 30 million tons of rapeseed are produced in the world annually, and most of them are used for oil expression. Although residues of the rapeseed after oil expression contain a high percentage of proteins (about 40−45%), most of them are used not for food production, but for feed and manure. To develop food and/or food materials from rapeseed proteins, elucidation of their protein chemical and physicochemical properties is required. Among plant proteins, soybean proteins have good physicochemical properties (*2*) and have been widely used as food materials. The comparison of the chemical and physicochemical properties of rapeseed proteins with those of soybean proteins will be useful in determining how to utilize rapeseed proteins for food. Dev and Mukherjee compared the physicochemical properties of rapeseed meal and protein isolate with those of soybean meal and protein isolate and observed that rapeseed products show emulsifying and foaming properties similar to or higher than those of soybean products at pH 7.0 (*3*). Paulson and Tung studied heat-induced gelation of rapeseed protein isolated at various pHs and ionic strengths, but no comparison with soybean protein isolate was done (*4*).

Cruciferin and napin (11S globulin and 2S albumin, respectively) are the major storage proteins of rapeseed and comprise 60 and 20%, respectively, of the total proteins in mature seeds (*5*). Soybean contains glycinin (11S globulin) as one of the two major storage proteins, but its content of 2S albumin is low. Therefore, it is desirable to compare the protein chemical and physicochemical properties of cruciferin with those of glycinin. Heat-induced gelation (*6*, *7*), film and emulsion formation (*8*), and heat stability (*9*) of cruciferin have been studied but were not compared with those of glycinin.

In this paper, we purified cruciferins from rapeseed meals, two from a commercial source, dried at low and high temper-

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atures, and the third defatted and dried at room temperature, and analyzed their protein chemical (secondary structure, thermal stability, and hydrophobicity) and physicochemical (solubility, emulsifying ability, and heat-induced association and gelation) properties in comparison with those of glycinin, except for heat-induced gelation.

MATERIALS AND METHODS

Plant Material. Three samples of rapeseed (*Brassica napus*) meals were used. Two of them were commercially defatted meals dried at low (37 °C) and high (60 °C) temperatures (Honen Corp., Tokyo, Japan), which were designated as L-meal and H-meal, respectively. The third sample (N-meal) was rapeseed (var. Legend) meal defatted sequentially with *n*-hexane, acetone, and diethyl ether and then dried at 20 °C.

Extraction of Proteins. The proteins from the three samples of rapeseed residues were extracted successively with phosphate buffer, SDS buffer, and SDS−urea buffer as described below:

Defatted meals (1 g) were stirred in 20 mL of buffer A [35 mM potassium phosphate, pH 7.6, 1 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride (*p*-APMSF), 1.2 μM leupeptin, 0.2 μM pepstatin A, 0.02% (w/v) NaN_3], followed by centrifugation (28000*g*). After this step was done twice, the pellets were stirred in 20 mL of SDS buffer [62.5 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 1% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol], followed by centrifugation (28000*g*). This step was also done twice, after which the pellets were stirred in 20 mL of SDS buffer containing 7 M urea (SDS−urea buffer). This extraction was also repeated twice. The two supernatants from each step were combined and used for the protein measurement and SDS−polyacrylamide gel electrophoresis (PAGE) (*10*).

Protein Measurement. Protein concentrations of the samples were determined using a Protein Assay Rapid Kit (Wako, Osaka, Japan) with bovine serum albumin as the standard.

Purification of Cruciferin. Salt-soluble fractions were extracted from each meal using buffer A. The proteins in the salt-soluble fractions were fractionated using ammonium sulfate. The precipitate of 10−20% saturation was dissolved in and dialyzed against buffer A. The dialysate was subsequently applied to a Hi-Prep 26/60 Sephacryl S-200 HR column (Amersham Pharmacia Biotech), equilibrated with buffer A. The flow rate was 1.0 mL/min. Cruciferin was identified on the basis of its SDS−PAGE profile (*11*).

Purification of Glycinin. Glycinin-rich fraction was prepared from soybean (*Glycine max.*) cultivar Wasesuzunari meal defatted at room temperature according to the method of Nagano et al. (*12*), and then glycinin was purified by ammonium sulfate fractionation (0−65% saturation) to near homogeneity.

CD Studies. The secondary structures of cruciferin and glycinin were evaluated by circular dichroism (CD) measurement as described previously (*13*). CD spectra were recorded with Jasco model J720 spectropolarimeter. Far-UV spectra were recorded between 196 and 257 nm using a quartz demountable cell of 0.1-mm path length and a protein concentration of 0.9 mg/mL in buffer B [35 mM sodium phosphate, pH 7.6, 0.4 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 1.2 μM leupeptin, 0.2 μM pepstatin A, 0.02% (w/v) NaN_3].

Surface Hydrophobicity. The surface hydrophobicities of cruciferin and glycinin were analyzed by hydrophobic chromatography using an Octyl Sepharose 4 fast flow column (Amersham Pharmacia Biotech). Samples were dialyzed against buffer B containing 0.575 M ammonium sulfate. The dialyzed samples were applied to the column equilibrated with the same buffer. The samples were eluted with a linear gradient (0.575−0 M) of ammonium sulfate over a period of 40 min at a flow rate 0.25 mL/min.

DSC Measurement. The differential scanning calorimetry (DSC) measurements of cruciferin and glycinin were carried out on a Microcal MC-2 ultrasensitive microcalorimeter (Micro Cal Inc., Northampton, MA) as described previously (*13*). All DSC measurements were performed at a protein concentration of 0.5 mg/mL. Protein samples in the following solutions were used for the measurement: (a) 20.4 mM H_3BO_3, 24.7 mM KCl, 14.1 mM NaOH, 0.42 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 1.2 μM leupeptin, 0.2 μM pepstatin A, 0.02% (w/v) NaN_3 for pH 9.0 and $\mu = 0.5$; (b) 20.4 mM H_3BO_3, 24.7 mM KCl, 14.1 mM NaOH, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 1.2 μM leupeptin, 0.2 μM pepstatin A, 0.02% (w/v) NaN_3 for pH 9.0 and $\mu = 0.08$; (c) 4.6 mM NaH_2PO_4, 30.4 mM Na_2HPO_4, 0.4 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 1.2 μM leupeptin, 0.2 μM pepstatin A, 0.02% (w/v) NaN_3 for pH 7.6 and $\mu = 0.5$; (d) 1.3 mM NaH_2PO_4, 8.7 mM Na_2HPO_4, 0.05 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 1.2 μM leupeptin, 0.2 μM pepstatin A, 0.02% (w/v) NaN_3 for pH 7.6 and $\mu = 0.08$; (e) 16.9 mM Na_2HPO_4, 4.9 mM $C_6H_8O_7$, 0.42 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 0.02% (w/v) NaN_3 for pH 6.0 and $\mu = 0.5$; (f) 16.9 mM Na_2HPO_4, 4.9 mM $C_6H_8O_7$, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 0.02% (w/v) NaN_3 for pH 6.0 and $\mu = 0.08$.

Solubility as a Function of pH. The solubilities of cruciferin and glycinin as a function of pH were measured as described previously (*14*). Protein solutions (0.8 mg/mL) were kept at 4 °C for 18 h at various pHs at $\mu = 0.5$ and 0.08. After centrifugation, protein concentrations in the supernatant were determined using a Protein Assay Rapid Kit (Wako). Solubility is expressed as a percentage of the total protein content in the sample.

Analysis of Emulsifying Ability. The emulsifying abilities of cruciferin and glycinin were measured as described previously (*14*). To prepare emulsions, 0.25 mL of soybean oil and 1.5 mL of protein solutions (0.5 mg/mL) (pH 6.0, 7.6, or 9.0 and $\mu = 0.08$ or 0.5), prepared as described in the section on DSC Measurement, were homogenized for 30 s with a high-speed homogenizer (model NS-50, Nichion Irikakikai Ltd., Chiba, Japan) and further sonicated using an ultrasonic homogenizer (model US-150, Nihonseiki Kaisha Ltd., Tokyo, Japan) for 1 min. The particle size distribution of the emulsions was measured using a laser diffraction instrument (model LA 500, Horiba Seisakusho Ltd., Kyoto, Japan). Each sample was analyzed several times, and the typical pattern was presented.

Analysis of Heat-Induced Association. The heat-induced associations of cruciferin and glycinin were examined as described previously (*14*). Protein (1 mg/mL) in buffer B without 10 mM 2-mercaptoethanol was heated at 70, 80, 90, or 100 °C for 5 min. The solutions were passed through a membrane filter (0.22 μm) after heating and then fractionated via gel filtration chromatography using KW804 and SB806M columns (Showa Denko, Tokyo, Japan) and subjected to the multiangle laser light-scattering experiments. Light scattering was measured on a Dawn DSP-F MALLS (Wyatt Technology, Santa Barbara, CA).

Heat-Induced Gelation. Protein solutions (10 μL, 5 or 7.5%) (pH 6.0, 7.6, or 9.0 and $\mu = 0.08$ or 0.5), prepared as described in the section on DSC Measurement, except for the absence of 2-mercaptoethanol, were pipetted into 0.2 mL tubes, and mineral oil (20 μL) was layered over them. After centrifugation (12100*g* × 5 min) to remove bubbles, the samples were heated for 5 min at 80, 90, or 100 °C, followed by rapid cooling to 15 °C, and kept for 30 min using Gene Amp 2400 thermal cycler (PE Applied Biosystems). Hardness of gels was measured by a Reoner RE-3305 (Yamaden, Japan) at 20 °C. The diameter of the plunger was 1 mm. The forces required to compress 0.75 mm into gels were recorded as hardness.

RESULTS AND DISCUSSION

Extraction of Proteins. To investigate the effects of the desolvation conditions on rapeseed protein solubility, proteins were sequentially extracted from three defatted meals using potassium phosphate buffer, followed by SDS buffer, and finally SDS−urea buffer. The largest amount of protein extracted by phosphate buffer was from the N-meal (110.1 mg), followed by the L-meal (93.5 mg) and H-meal (62.0 mg) (**Table 1**). The amounts of protein extracted by SDS buffer were similar among the three meals, while with SDS−urea buffer, the order was H-meal (67.0 mg), L-meal (39.2 mg), and N-meal (20.4 mg).

Table 1. Amounts of Proteins in the Extracts

sample	amount of proteins in extracts (mg) potassium phosphate buffer	SDS buffer	SDS-urea buffer	total amount (mg)
N-meal	110.1	53.1	20.4	183.6
L-meal	93.5	49.7	39.2	182.4
H-meal	62.0	55.3	67.0	184.3

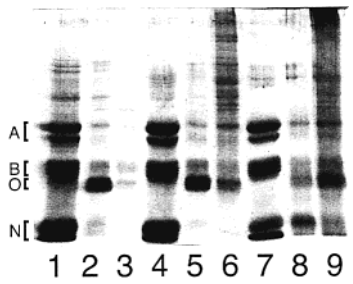

Figure 1. SDS–PAGE of the extracts from N-, L-, and H-meals. Proteins extracted by potassium phosphate (lanes 1, 4, 7), SDS buffer (lanes 2, 5, 8), and SDS–urea buffer (lanes 3, 6, 9) from N- (lanes 1–3), L-(lanes 4–6), and H-meals (lanes 7–9). A, B, O, and N indicate acidic and basic polypeptides of cruciferin, oleosin, and napin, respectively.

The total amount of proteins extracted from each meal was very similar among three meals (182.4−184.3 mg). These results indicate that commercial expression of oil causes denaturation of proteins in the meal, especially in the case of drying at high temperature.

To determine the protein composition of each extract, we analyzed the extracts by SDS−PAGE. It is known that under reducing conditions, cruciferin gives two major components which correspond to acidic (MW ∼30 000−37 000) and basic (MW 22 000−24 000) polypeptides (*11*, *15*), and napin also gives two components of light (MW 4000) and heavy (MW 10 000) chains (*16*). Bands corresponding to cruciferin and napin polypeptides were detected in the extracts from the three meals by potassium phosphate buffer (**Figure 1**, lanes 1, 4, and 7). Since the volume of each of the extracts subjected to SDS−PAGE was the same, it is apparent from **Figure 1** that the extraction of cruciferin and napin is lower in the case of H-meal than in the cases of N- and L-meals. In the latters, the ratio of cruciferin and napin, estimated densitometrically, was around 1:1. This ratio was similar to that reported previously (*17*). These results indicate that N- and L-meals are suitable for extracting cruciferin and napin by potassium phosphate buffer.

The extracts from N- and L-meals by SDS buffer showed primarily the bands corresponding to oleosin (MW ∼19 000) (*18*) and minor bands corresponding to cruciferin and napin polypeptides. However, the extract from H-meal by SDS buffer gave a fairly clear band corresponding to napin and faint bands corresponding to oleosin and cruciferin.

The extract from N-meal by SDS−urea buffer gave very faint bands corresponding to cruciferin and oleosin. The extract from L-meal gave faint bands of polymerized proteins, seen as smears, in addition to those corresponding to cruciferin and oleosin. On the other hand, the extract from H-meal gave intense bands corresponding to oleosin and polymerized proteins and faint ones corresponding to napin and cruciferin. These results indicate that oil expression and drying at high temperature cause denaturation and polymerization of cruciferin, napin, and oleosin, and that the effect on oleosin is very severe.

CD Studies. No significant difference in patterns of CD spectra was observed among the cruciferins from the three rapeseed meals (**Figure 2**). Therefore, cruciferins from the L- and H-meals have a secondary structure similar to that of cruciferin from N-meal, indicating that oil expression and the drying process do not affect the secondary structures of cruciferins.

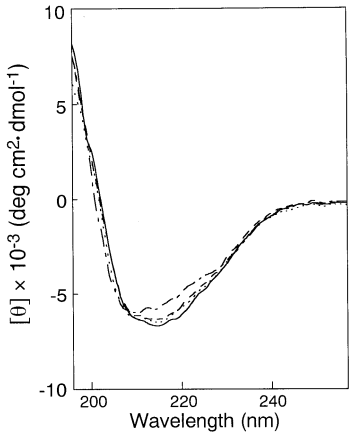

Figure 2. CD spectra of glycinin and cruciferin. Glycinin, dashed and single-dotted lines; cruciferin from N-meal, solid line; cruciferin from L-meal, dashed line; cruciferin from H-meal, dotted line.

The CD spectra of cruciferins were somewhat different from those of glycinin (**Figure 2**). X-ray crystallography of 7S globulin (*19*−*21*) and 11S globulin precursor (*22*) indicate that the two globulins share a very similar three-dimensional structure, and that the number and the length of α-helices are variable, although those of β-strands are fairly conservative. Wright aligned amino acid sequences in order to maximize the homology among legumins from various legume and nonlegume seeds and suggested five variable regions (*23*). The primary structures and lengths of these five variable regions are quite different between glycinin and cruciferin. These differences in α-helix and random structures may, therefore, have an effect on the difference in the CD spectra of glycinin and cruciferin.

Surface Hydrophobicity. Surface hydrophobicity is an important factor for physicochemical properties such as emulsifying and foaming abilities (*24*, *25*). We assessed the surface hydrophobicities of the three cruciferins and glycinin by measuring their elution time when subjected to hydrophobic chromatography at pH 7.6 and $\mu = 0.5$ (the longer the elution time, the higher the surface hydrophobicity). Glycinin did not bind to the column equilibrated by the starting buffer. On the other hand, the three cruciferins bound to the column equilibrated by the starting buffer and were eluted at the same elution time, 32−34 min (data not shown), indicating that the three cruciferins have similar hydrophobicity which is higher than that of glycinin. Therefore, the oil expression and drying process do not cause a significant difference in the hydrophobicity of cruciferin.

DSC Measurement. Structural stability is an important factor for emulsifying and gel-forming abilities. The thermal denaturation midpoint temperature (T_m) values of cruciferins (85.0 and 84.6 °C) from L- and H-rapeseed meals were a little lower than that (86.6 °C) of cruciferin from the N-meal at $\mu = 0.5$ and pH 7.6. Therefore, the oil expression and drying process have a small effect on the thermal stability of cruciferin.

The T_m values of cruciferin from L-meal were compared with that of glycinin at $\mu = 0.5$ and 0.8 at pH 6.0, 7.6, and 9.0 (**Figure 3**). Glycinin could not be subjected to measurement at $\mu = 0.08$ and pH 6.0 because of its insolubility. The T_m of cruciferin exhibited ionic strength dependence but not pH dependence. Glycinin exhibited higher T_m values than cruciferin

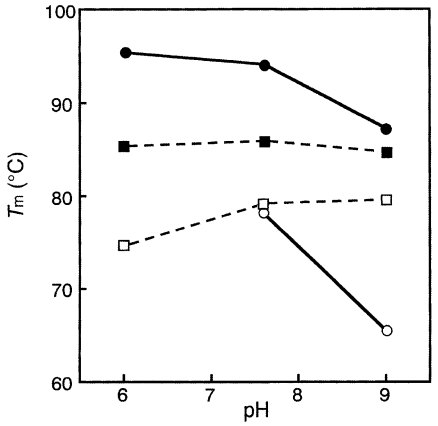

Figure 3. T_m values of glycinin and cruciferin from L-meal. Open and closed circle (glycinin) and square (cruciferin from L-meal) indicate T_m values at $\mu = 0.08$ and 0.5, respectively.

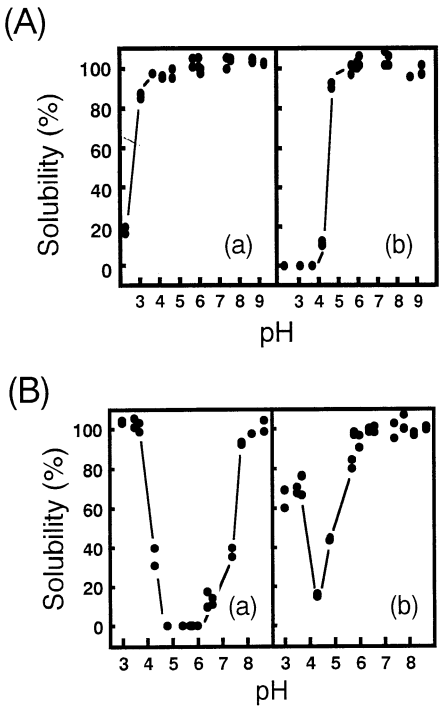

Figure 4. pH dependency of the solubilities of glycinin and cruciferin at $\mu = 0.5$ (A) and 0.08 (B): (a) glycinin and (b) cruciferin from N-meal.

at all pHs examined at $\mu = 0.5$. On the other hand, the T_m value of glycinin was similar to that of cruciferin at $\mu = 0.08$ and pH 7.6 and significantly lower than that of cruciferin at $\mu = 0.08$ and pH 9.0. ΔH of glycinin at $\mu = 0.08$ and pH 9.0 was significantly lower than that of cruciferin, but they were similar to each other under the other conditions. These results indicate that cruciferin and glycinin have different thermal stabilities and that environmental factors (pH and ionic strength) influence cruciferin and glycinin differently.

Solubility. Solubility is a fundamental property for expression of physicochemical properties. Solubilities as a function of pH of glycinin and cruciferin were measured at $\mu = 0.5$ and 0.08. Since the solubility profiles of three cruciferins were found to be very similar to each other, only the profile of cruciferin from the N-meal is shown (**Figure 4**). At $\mu = 0.5$, glycinin and cruciferin were soluble at pH >3.5 and >5.0, respectively (**Figure 4A**). On the other hand, at $\mu = 0.08$, glycinin and cruciferin were insoluble at pH 4.0−7.5 and 3.5−5.7, respectively (**Figure 4B**). These phenomena were probably isoelectric

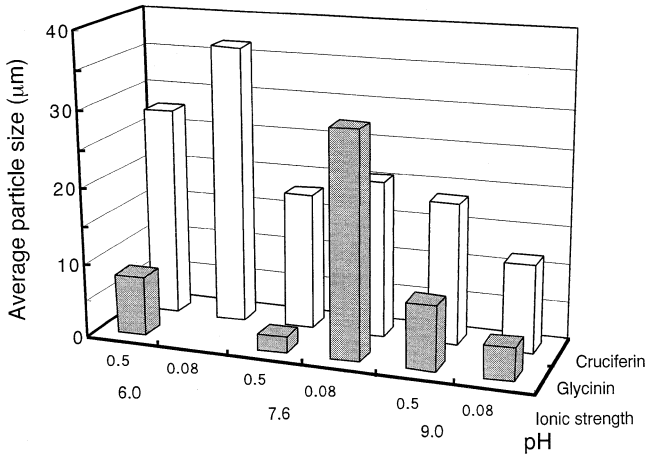

Figure 5. Average particle sizes of emulsions using glycinin and cruciferin from L-meal. Open and stippled bars indicate the values of cruciferin from L-meal and glycinin, respectively.

precipitations. Consequently, cruciferin is more insoluble at $\mu = 0.5$ and more soluble at $\mu = 0.08$ than glycinin. However, it is noteworthy that cruciferin exhibited low solubility (~60%) even at pH 3, although glycinin was completely soluble. The variable regions of cruciferin and glycinin are rich in glutamine and acidic amino acids, respectively (*19*). This is probably one of the reasons why cruciferin and glycinin exhibit different solubility profiles.

Emulsifying Ability. The emulsifying abilities of three cruciferins and glycinin were assessed by measuring the particle sizes of the emulsions at $\mu = 0.5$ and pH 7.6 (the smaller the size, the better the emulsifying ability). The three cruciferins exhibited similar particle sizes, which were larger than those of glycinin (data not shown). Therefore, glycinin has better emulsifying ability than cruciferin, although glycinin has higher thermal stability and lower surface hydrophobicity than does cruciferin. We observed that the thermal stability and surface hydrophobicity of individual subunits of β-conglycinin (7S globulin of soybean) are related to the emulsifying ability (*14*, *26*, *27*). Therefore, the factor accounting for emulsifying ability may be different between 11S and 7S globulins.

Furthermore, we examined the emulsifying abilities of cruciferin from L-meal and glycinin at pH 6.0, 7.6, and 9.0 at $\mu = 0.08$ and 0.5 (**Figure 5**). The emulsifying ability of cruciferin was worse than that of glycinin, except at $\mu = 0.08$ and pH 7.6. At $\mu = 0.5$, those of cruciferin at pH 7.6 and 9.0 were better than that at pH 6.0. On the other hand, glycinin exhibited better emulsifying ability at pH 7.6 than at pH 6.0 and 9.0 at $\mu = 0.5$. These phenomena are not related to their thermal stability.

Heat-Induced Association. Heat-induced association is related to heat-induced gelation. To examine heat-induced association, heated samples (1 mg/mL) were fractionated by using gel filtration chromatography (**Figure 6**). Since the three cruciferins exhibited similar behavior, only the results of cruciferin from the N-meal are shown with those of glycinin. The intact species of three cruciferins dramatically decreased with heating at >90 °C, although that of glycinin decreased with heating only at 100 °C. This is due to the difference in their denaturation temperatures (cruciferin, 86.6 °C; glycinin, 93.9 °C; see **Figure 3**).

Glycinin formed large soluble aggregates upon heating at 90 °C, with a concomitant decrease of the intact species. However, the heat-induced soluble aggregates of glycinin were not observed upon heating at 100 °C, despite the dramatic decrease

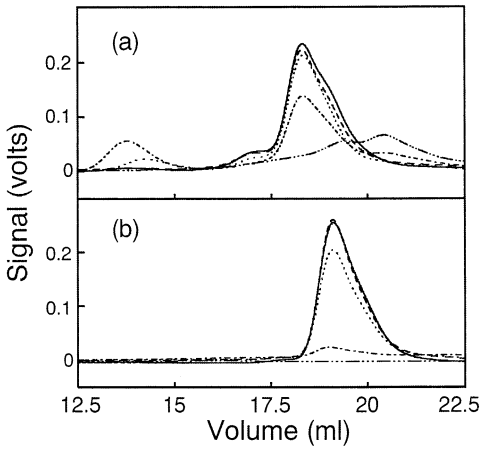

Figure 6. Elution patterns of glycinin (a) and cruciferin from N-meal (b) heated at various temperatures. The samples were heated at 70 (dashed line), 80 (dotted line), 90 (dashed and single-dotted line), and 100 °C (dashed and triple-dotted line). Nonheated samples were shown by solid line.

of the intact species. Under this condition, we did not observe insoluble aggregates after heating. Therefore, these results indicate that glycinin formed large soluble aggregates which could not pass through the filter before gel filtration. In contrast, cruciferin formed insoluble aggregates, depending on its T_m value, with a concomitant decrease or disappearance of the intact species. These results indicate that cruciferin and glycinin exhibit different behavior upon heating. Nakamura et al. found that the number of free cysteine residues influences the transparency of heat-induced gel (*28*). The number and topology of cysteine residues are different between glycinin and cruciferin (*19*). Therefore, these may affect the difference in their behaviors upon heating. This is consistent with the report that disulfide interchange reactions occur easily between the disulfide bond linking the acidic and basic polypeptides and an additional cysteine residue (position 11 from the N-terminal of the basic polypeptide) near the cysteine residue (position 7) participating in the interchain disulfide bond (*29*).

Heat-Induced Gelation. At first, we compared heat-induced gelation of cruciferin with that of glycinin at pH 7.6 and $\mu = 0.5$. Under these conditions, heat-induced gels from cruciferin were opaque and not elastic, although those from glycinin were transparent and elastic. This phenomenon shows that cruciferin forms insoluble aggregates due to SH/S−S exchange upon heating, as described above. Paulson and Tung (*4*) observed that opaque gels from rapeseed protein isolate and transparent gels from succinylated isolate responded differently to rheological tests. Thus, comparison of heat-induced gelation of cruciferin with that of glycinin is meaningless. So, we examined gelation of only cruciferin from the L-meal.

The cruciferin solutions (5% or 7.5%) were heated at 80, 90, or 100 °C at pH 6.0, 7.6, or 9.0 and at $\mu = 0.08$ or 0.5. Under any conditions, cruciferin formed opaque and not elastic gels. Their hardness was measured (**Figure 7**). Heating at 80 °C induced gelation of cruciferin at $\mu = 0.08$, but not at $\mu = 0.5$. This is due to the fact that the T_m value at $\mu = 0.08$ is lower than 80 °C but higher at $\mu = 0.5$. The overall results of gelation indicate that higher protein concentration, higher heating temperature, higher pH, and lower ionic strength result in higher gel hardness. These results are approximately consistent with the results of Léger and Arntfield (*7*). The effect of ionic strength and the fact that the differences in gel hardness between 80 and 90 °C and between 90 and 100 °C are quite large at $\mu =$ 0.08 and at $\mu = 0.5$, respectively, are probably due to the difference in the T_m values at the two ionic strengths. In other words, the gel hardness is likely to correlate somewhat with structural stability. However, it does not correlate with pH dependence of structural stability. Therefore, some structural characteristics that are dependent on pH, such as electrostatic situation in addition to structural stability, probably contribute to the gel hardness.

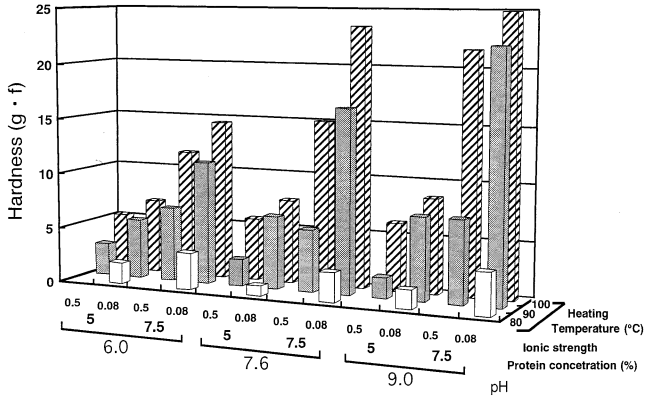

Figure 7. Hardness of gels using cruciferin from L-meal at various conditions. Open, stippled, and hatched bars indicate the values heated at 80, 90, and 100 °C, respectively.

ABBREVIATIONS USED

p-APMSF, (*p*-amidinophenyl)methanesulfonyl fluoride; CD, circular dichroism; DSC, differential scanning calorimetry; PAGE, polyacrylamide gel electrophoresis.

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