Self-Assembling of Peptide α-1 of Globin Hydrolysates

Xin Qi Liu,*,† Masami Yonekura,‡ Masakazu Tsutsumi,‡ and Yoh Sano†

National Food Research Institute, Tsukuba City, Ibaraki 305, Japan, and Faculty of Agriculture, Ibaraki University, Amimachi-chuo, Ibaraki 300-03, Japan

Globin is an edible protein in a large quantity from animal blood. However, globin shows a rather low solubility at neutral pH and little advantage in comparision with other proteins applied to the food industry. To improve its functional properties and to make clear the mechanism of gel formation, the hydrolysis of globin was performed with 0.8 M citric acid. The peptide α-1 was obtained by cleavage of the peptide bond between Asp-94 and Pro-95 in the α-chain of globin with 0.8 M citric acid. Physicochemical properties and structural characterization of peptide α-1 of globin hydrolysates were studied by amino acid sequence analysis, hydrophobic interaction chromatography, and circular dichroism (CD) spectra. The peptide α-1 was very highly hydrophilic on hydrophobic chromatography. The concentration dependence of peptide α-1 indicated the dissociation and association behaviors analyzed by a light scattering method. Circular dichroism spectra showed that the content of the α-helix and *β*-sheet structures of peptide α-1 were very different from the intact α-chain. We have also found that peptide α-1 played a role in transformation of aggregates of globin hydrolysates to gel. These results suggest that peptide α-1 acts as a cross-linker between the aggregate of globin hydrolysates through the transformation process of gel.

Keywords: *Globin; globin hydrolysates; peptide α-1; CD spectra*

INTRODUCTION

Animal blood contains about 18% protein, and hemoglobin accounts for more than one-half of the blood protein. Most of the hemoglobin is not utilized in the food industry because of its unattractive color and odor. The preparation methods of decolorized globin (Tybor et al., 1975; Sato et al., 1981; Drepper et al., 1979), nutritional studies (Landmann et al., 1980; Parmer et al., 1978), and functional properties of globin such as solubility, emulsifying properties, and foaming properties were studied by several researchers (Tybor et al., 1973, 1975; Penteado et al., 1979; Crenwelge et al., 1974). Attempts have been made to incorporate globin into sausage meat (Caldironi et al., 1982), and a cheese-like emulsion was prepared using globin as an ingredient (Knapp et al., 1978). Although the abilities of proteins to form a gel and to provide a structural matrix for holding water applications are useful in the food industry (Autio et al., 1985, 1990; Hayakawa et al., 1983), very few studies have been done on the gelling properties of globin, primarily because most of the processes developed for heme and globin separation alter the proteins to such an extent that gelation does not take place. Sato et al. (1981) and Autio et al. (1985) have presented a (carboxymethyl)cellulose (CMC) column procedure for the separation of heme and globin, and the globin sample prepared by their methods formed a gel when heated. Decolorized globin prepared by acidified acetone (Tybor et al., 1975) showed acceptable water-binding properties at room temperature, and it did not form a gel after heating. On the contrary, Liu et al. (1994a) have reported that the globin made by this method formed a gel at high globin concentration (7%).

Heat-induced globin gel prepared by each of the above-mentioned methods showed a low hardness, was affected easily by the bulk factors such as pH and ions, and indicated the different functional properties (Liu et al., 1994a; Autio et al., 1985, 1990). In our previous studies (Liu et al., 1994b), it was found that the globin hydrolysates treated by citric acid showed a better solubility and ability to form a gel induced by heating than did the intact globin and formed another type of gel which was different from that of intact globin. Our results also suggested that acid hydrolysis was an efficient method of modifying and improving the new functional properties of protein by precisely controlling the hydrolysis conditions (Liu et al., 1994b).

The globin hydrolysates showed eight bands after analysis with tricine-SDS-PAGE (Liu et al., 1995, 1996), whose molecular weights ranged from 5000 to 15 000 Da. The results of gel filtration chromatography indicated that the large aggregates were formed easily in globin hydrolysates, whose aggregates were thin rods having a molecular weight of 870 000 Da and a length of 130−140 nm determined by light scattering and electron microscopic methods (Liu et al., 1995, 1996). The aggregates of globin hydrolysates existed in a monomolecular state and showed no association between aggregates of globin hydrolysates and the tendency to form a gel.

In this paper, physicochemical properties of peptide α-1 of globin hydrolysates were studied by amino acid sequence analysis, hydrophobic interaction chromatography, and circular dichroism (CD) spectra. The concentration dependence of peptide α-1 indicated the dissociation and association behaviors analyzed by a light scattering method. These results suggest that peptide α-1 acts as a cross-linker between the aggregate of globin hydrolysates through the transformation process of gel. No work on this topic has previously been published.

MATERIALS AND METHODS

Globin Hydrolysates. Globin from porcine blood was precipitated with the acidified acetone method (Tybor et al., 1975; Liu et al., 1994a,b). After the globin was decolorized

* To whom correspondence should be addressed: telephone, +81-298-38-8031; FAX, +81-298-38-8082; e-mail, liuxinqi@nfri.affrc.go.jp.

† National Food Research Institute.

‡ Ibaraki University.

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with this method, 0.8 M citric acid was added to the powdered globin sample. The sample was hydrolyzed at 95 °C for 15 min, then cooled to room temperature, and was dialyzed under flowing water with Seamless Cellulose Tubing (molecular cutoff size, 10 000 Da). The dialyzate was air-dried with a spray-dryer (Pulvis minispray-GA-32, Yamato Science. Japan), and the sample thus obtained is named as a globin hydrolysate (GH) sample in this paper.

Tricine-SDS Polyacrylamide Gel Electrophoresis. Tricine-SDS-PAGE was carried out by the Schagger method (Schagger et al., 1987) by exchanging glycine with tricine. The anode was 0.2 M Tris-HCl (pH 8.9), and the cathode was 0.1 M Tris-HCl (pH 8.25) with added 0.1 M tricine and 0.1% SDS. The concentration of polyacrylamide gel was 16.5%. The electrophoresis was done under 100 V for 10 h.

Electroblotting and Amino Acid Sequence Analysis. The protein isolated by tricine-SDS-PAGE was transcribed to a polyvinylidene fluoride (PVDF) membrane with a semidry blotting apparatus. The transcription was carried out with two kinds of PVDF membranes, Immobilon (Millipore Co., U.S.) and Fluorotrans (Pvm020c-tape, Pall Co., U.S.), under 1 mA/cm^2 (constant voltage) for 45 min.

The amino acid sequence of the protein transcribed to the PVDF membranes was analyzed with a protein sequencer (Model 1494, Perkin Elmer Co., U.S.).

Hydrophobic Interaction Chromatography. The hydrophobic interaction chromatography was carried out on Butyl-Toyopearl 650S gel (Tosoh Corp., Totyo, Japan). The column (14 mm × 190 mm) was equilibrated with citrate buffer (pH 3.2) in the presence of 0.3 M NaCl. Each protein fraction of 3 mL was collected. Flow rate was 12.5 mL/h, and the protein was determined spectrophotometrically at 280 nm. The elution was done with five distinct solutions A–E, where solution A was citrate buffer containing 0.3 M NaCl; solution B, citrate buffer containing 0.15 M NaCl; solution C, citrate buffer without NaCl (all the pH of buffer were 3.2); solution D, distilled water; and solution E, 0.05 N NaOH.

Gel Preparation. The mixed solutions (total protein concentration, 5%) in various ratios of aggregates of globin hydrolysate (P_1 fraction by gel filtration) and peptide α-1 (GHP 1 fraction by hydrophobic chromatography) was heated at 90 °C for 15 min and then cooled for over 1 h to form the gel. The hardness of the gel formed was measured with a rheometer (Nrm20031, Fudo Industrial Co., Ltd., Kyoto, Japan). The gel hardness was expressed as the value of breaking stress.

CD Measurements. CD spectra of peptide α-1 were recorded with a JASCO J-600 spectropolarimeter (Japan Spectroscopic Co., Ltd., Totyo, Japan) at wavelengths of 200–250 nm. The temperature of the cell was controlled by circulating the water with a given temperature. The sample was diluted using 0.1 M citrate buffer (pH 3.2) prepared from a stock solution of the desired concentration and was made optically clean by filtering with a Millipore filter (pore size, 0.45 μm).

Determination of Molecular Weight. The apparent weight-average molecular weight was measured by the intensity of light scattered at 90° angle and at a wavelength of 436 nm with the instrument of a modified ellipsometer, an automatic light scattering analyzer AEP-100 (Shimadzu Co., Ltd., Kyoto, Japan) (Sano, 1988, 1990, 1993; Ishigami et al., 1995). The temperature was kept constant by circulating thermostatically controlled water. The apparatus constant was obtained by using a solution of bovine serum albumin of which the molecular weight was taken as 65 800 Da optically clarified through a Gelman (Germany) filter (pore size, 0.2 μm). The sample peptide α-1 solutions and the solvent for light scattering measurements were optically clarified with the same type of membrane filter.

RESULTS

Composition of Globin Hydrolysates. Tricine-SDS-PAGE was performed to analyze globin hydrolyzed by citric acid. Two well-defined bands were observed as shown in Figure 1. We named band no. 1 as peptide 1 and so on. The molecular weights of each peptide originated from globin hydrolysates were determined by comparison with standard proteins. The molecular weight of peptide 1 was 14 800 Da, that of peptide 2 was 13 700 Da, and the smallest peptide 8 was 5600 Da. The N-terminal amino acid sequences (Figure 2a,b) and molecular weights of peptides 1 and 2 were compared with the primary structure of globin β- and α-chains (Braunitzer et al., 1978). Peptide 1 can be seen as a β-chain, and another, the peptide 2, as an α-chain. Besides the β-chain and the α-chain, another six bands were also present (Figure 1). These results indicated that globin hydrolysates consisted of eight types of peptides.

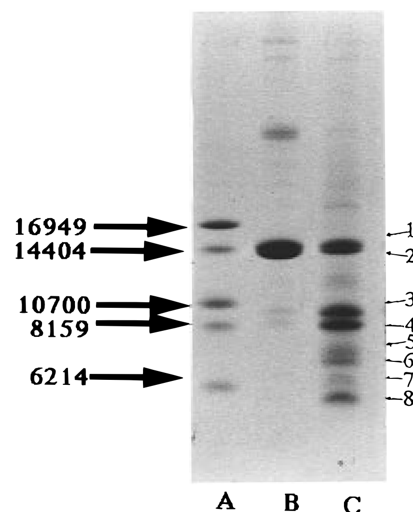
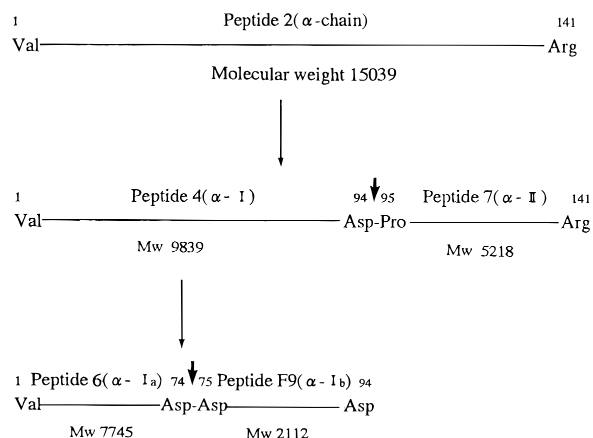


Figure 1. Tricine-SDS-PAGE patterns of globin and globin hydrolysates: (A) Molecular weight marker with myoglobin (16 949 Da), myoglobin I and II (14 404 Da), myoglobin I and III (10 700 Da), myoglobin I (8159 Da), and myoglobin II (6214 Da), which are commercial products from Pharmacia Co., Ltd. (U.S.); (B) globin; (C) globin hydrolysates.

Analysis of Peptide α-1. Solutions C, D, and E were eluted in the order of decreasing hydrophilicity on a hydrophobic chromatographic column, and they were named as GHP 1, GHP 2, and GHP 3 (Figure 3). The results showed that globin hydrolysates were mixtures consisting of at least three types of polypeptides having different hydrophobicities. The main component of the highly hydrophilic GHP 1 fraction was peptide 4 (Figure 4). GHP 2 was an α-chain, and other low molecular peptides and GHP 3 were peptide 1 and peptide 3. The amount of GHP 1 was about 18.6% of globin hydrolysates.

The N-terminal amino acid sequences and molecular weight of peptides 3–8 obtained from electrophoresis and of low molecular weight peptides obtained from reverse phase chromatography were compared with the primary structure of the globin β- and α-chains (Braunitzer et al., 1978), as is shown in Figure 2a,b. The cleavage site by citric acid was assumed in the α-chain to be located between Asp-94 and Pro-95, and between Asp-74 and Asp-75, and in the β-chain, it was located between Asp-99 and Pro-100, and between Asp-73 and Gly-74. It also suggested that peptide 4 obtained from hydrophobic chromatography was amino acid residues 1–94 of the α-1 peptide, and peptide 3 was amino acids residues 1–99 of the β-1 peptide. Those results indicated that citric acid cleaved specifically the aspartyl–peptide bond, in particular at the peptide bond between Asp and Pro residues.

Gel Formation by the Addition of Peptide α-1. The mixture of aggregates of globin hydrolysate and



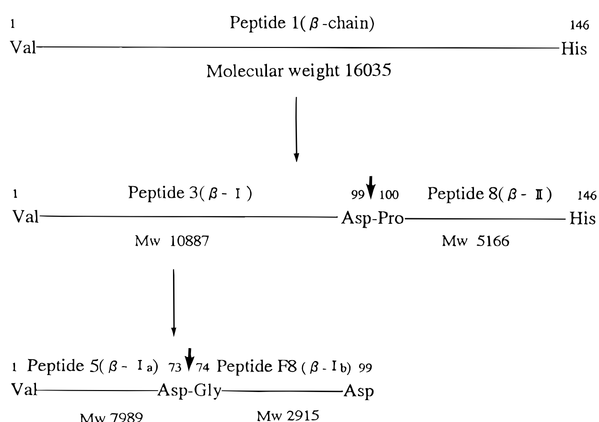


Figure 2. (a) Hydrolysis of the α-chain of globin by citric acid. (b) Hydrolysis of the β-chain of globin by citric acid. Peptides 1–8 were obtained from tricine-SDS-PAGE (see Figure 1). F 8 and F 9 were obtained through reverse phase chromatography.

peptide α-1 in various percentage ratios were used to investigate the gel formation of globin hydrolysates. As shown in Table 1, no gel formation could be observed without the addition of peptide α-1. Even when the peptide α-1 was added in a small quantity, the gel was formed. The hardness of the gel increased as the peptide α-1 concentration increased.

Molecular Weight of Peptide α-1 and Self-Assembling. The molecular weight of peptide α-1 was determined by a light scattering method. The molecular weight of α-1 peptide treated with 8 M urea showed no concentration dependence of the peptide and was 10 000 Da in comparison to 9839 Da from the amino acid sequencing method. Both values are in agreement with each other, suggesting that α-1 peptide exists in the monomolecular state in 8 M urea solution.

The concentration dependence of molecular weight of the peptide without treatment with urea was also shown

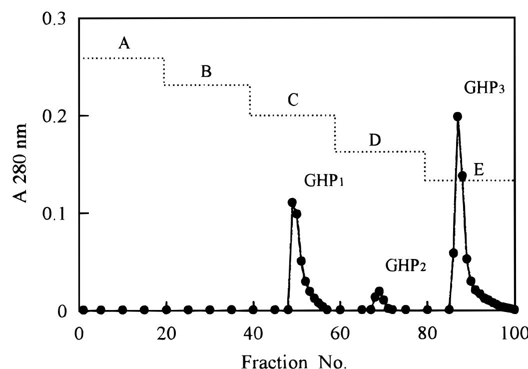


Figure 3. Hydrophobic chromatography of globin and globin hydrolysates. Eluates: (A) 0.1 M citric acid buffer + 0.3 M NaCl (pH 3.2); (B) 0.1 M citric acid buffer + 0.15 M NaCl (pH 3.2); (C) 0.1 M citric acid buffer (pH 3.2); (D) distilled water; (E) 0.05 N NaOH.

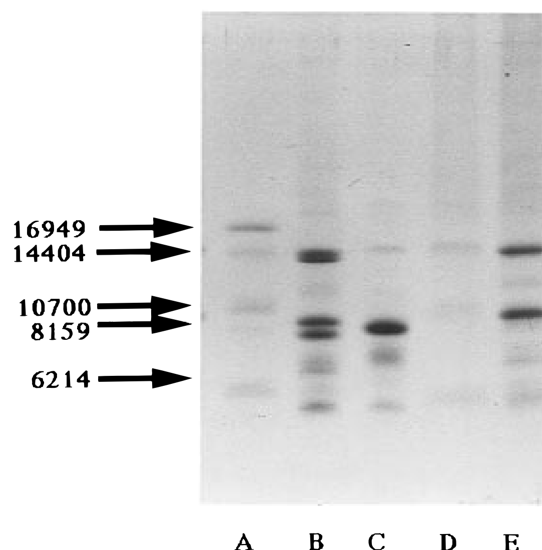


Figure 4. Tricine-SDS-PAGE patterns of the eluates from hydrophobic chromatography: (A) molecular weight marker proteins shown in Figure 1; (B) globin hydrolysates; (C) fraction GHP 1; (D) fraction GHP 2; (E) fraction GHP 3.

Table 1. Gel Formation by the Addition of Peptide α-1[a]

aggregates (%)[b]	5.0	4.5	4.0	3.5	3.0
peptide α-1 (%)[c]	0.0	0.5	1.0	1.5	2.0
gel formation	−	+	+	+	+
breaking stress (g)[d]		0.22	0.35	0.40	0.57

[a](+) Gel was formed. (−) Gel was not formed. [b] Aggregates: P_1 fraction of gel filtration (Liu et al., 1996). [c]Peptide α-1: GHP 1 fraction of hydrophobic interaction chromatography. [d] Measured with a rheometer after heating at 90 °C for 15 min and then cooling for over 1 h.

in Figure 5. There was a tendency of molecular weight to increase, depending on the peptide concentration. The molecular weight indicated was a 7–9-fold larger result than that of α-1 peptide monomer at either concentration. This revealed that α-1 peptides had a common property of association and the degree of association depended on its concentration.

The molecular weight of the α-1 peptide treated with heat was also measured. As shown in Figure 5, it had a tendency to decrease the molecular weight, to increase with heating. This behavior was the same as the untreated α-1 peptide. However, the molecular weights were smaller than that of untreated α-1 peptide at each concentration. Therefore, it can be concluded that the

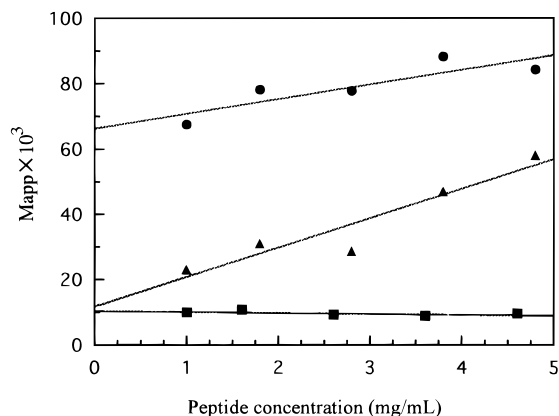


Figure 5. Concentration dependence of apparent molecular weight of peptide α-1. Symbols in this figure show untreated (●), heated at 50 for 15 min (▲), and treated with 8 M urea (■), respectively.

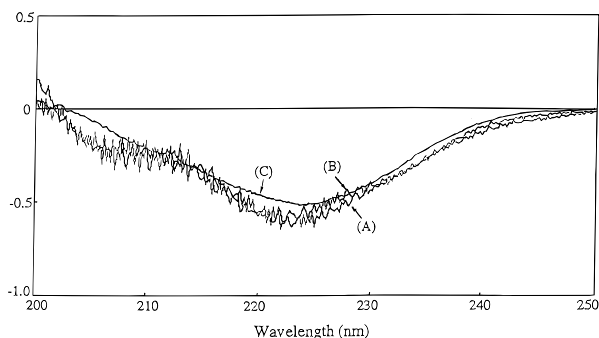


Figure 6. Circular dichroism spectra of α-1 peptide. The measurement was carried out in 0.1 M citric acid buffer (pH 3.3) at 20 °C (A) and 50 °C (B) and the α-chain itself (C).

Table 2. Estimation (%) of Secondary Structure of α-1 Peptide and α-Chain[a]

	α-1		α-chain
	20 °C	50 °C	20 °C
α-helix	23.2	22.3	13.3
β-sheet	0.0	0.0	5.7
turn	41.0	40.4	45.5
random coil	35.8	37.3	35.4

[a]Values calculated by the Yang model (1986).

association and dissociation of the α-1 peptide depends not only on concentration but also on temperature.

Secondary Structure of Peptide α-1. Circular dichroism (CD) spectra were measured to investigate the secondary structure of α-1 peptide. The CD spectra of α-1 and α-chain itself were measured at 20 °C (Figure 6). The curve fitting analysis with using the method of Yang et al. (1986) was shown in Table 2. From the table, the content of α-helix in peptide α-1 was 23% and virtually no β-sheet was observed, but in the α-chain, the content of α-helix was 13%, which was 10% less than that of the α-1 peptide, and contained an appreciable amount of β-sheet and a higher turn structure in α-chain.

We also determined the CD spectra of peptide α-1 at 50 °C. The result showed that the content of the α-helix decreased only 1% by comparison with that determined at 20 °C.

DISCUSSION

In previous studies, we observed that gel formed by heating at 90 °C for 15 min a solution of intact globin (7 mg/mL), and the gel formed showed very low hardness (Liu et al., 1994a). To improve the functional properties of the gel formed by globin, we hydrolyzed the globin by citric acid at 95 °C, which are named as globin hydrolysates. The globin hydrolysates showed higher solubility and better gel-formation properties than intact globin (Liu et al., 1994b). Under our experimental conditions, it was observed that six types of new peptides were produced (Figure 1) and each constituent content can be changed by controlling the experimental conditions.

We have found that thin rod-shaped aggregates of globin hydrolysates were formed by both the gel filtration and the light scattering method of globin hydrolysates (Liu et al., 1996). The rod-shaped aggregates of globin hydrolysates had molecular weights of 872 000 Da and lengths of 130−140 nm. The aggregates of globin hydrolysates existed in a monomolecular state. It is necessary that the cross-combination should take place between aggregates of globin hydrolysates to form a network of the gel. Table 1 showed that the gel did not form without adding the peptide α-1, and the gel was formed whenever the peptide α-1 was added. Furthermore, the gel hardness increased with an increase in peptide α-1 concentration, indicating that peptide α-1 plays a role in transformation of aggregates of globin hydrolysates to gel. In other words, peptide α-1 can be seen as cross-linker between aggregates of globin hydrolysates.

Matsudomi et al. (1992) have reported that the disulfide bond is important for the formation of gels from the mixture of α-lactalbumin and β-lactoglobulin. Furthermore, Zheng et al. (1993) found that the breaking of hydrophobic interactions and disulfide bonds severely decreases the hardness of the gel. They considered that the covalent cross-linking (disulfide bonds) plays a role in stabilization and promotes the elasticity of legumin gel networks, while noncovalent forces (hydrogen bonds and hydrophobic interactions) predominate in maintaining the structure and increasing the viscosity. These covalent and noncovalent bonds are probably formed by subsequentially heating after a self-supporting gel has been established. This might relate to textural properties of the gel. But there were no Cys residues in peptide α-1, which may play a part in cross-linking through the disulfide bond, so it must be explained how the physicochemical properties of peptide α-1 itself take an important role in the formation of cross-links. Therefore the characterization of the physicochemical properties of peptide α-1 was necessary to make clear the mechanism of gel formation.

Peptide α-1 was a highly hydrophilic polypeptide isolated from hydrophobic chromatography, which had a molecular weight of 9839 Da. Furthermore, with using peptide α-1 treated with urea and/or heated at 50 °C, the molecular weights in solution were measured by a light scattering method as shown in Figure 5. Peptide α-1 was a monomer in the presence of urea and showed no concentration dependence. The estimated molecular weight was 10 000 Da, which was in agreement with the amino acid sequence data. However, the molecular weight in the absence of urea was 7−9-fold more than that of the monomer, which can be considered that peptide α-1 can self-associate and form aggregates. The association depends on the concentration of peptide α-1. On the basis of the observation that the aggregates of peptide α-1 dissociated to monomer when urea was present, we regarded that aggregates of peptide α-1

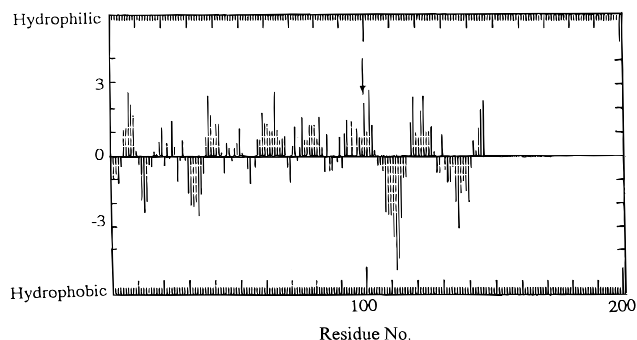


Figure 7. Hydrophilic and hydrophobic profiles of the α-chain calculated by the method of Kyte et al. (1982).

could be formed by noncovalent bonds. Moreover, when the molecular weight was measured at 50 °C, the aggregates of peptide α-1 formed were smaller than those formed in untreated peptide α-1, although the molecular weight depends on the concentration of peptide α-1. From these results, it can be concluded that the association of peptide α-1 depends on the concentration of peptide α-1 and temperature. The evidence that the aggregates of peptide α-1 began to dissociate on heating suggested that the force maintaining the stability of tertiary structure was not the increase of hydrophobic interaction but mainly the breakage of the hydrogen bonds between the aggregates of peptide α-1, and that the surface properties of peptide α-1 may be important in its stability. The distribution of hydrophilic and hydrophobic residues in the α-chain was counted with the Kyte and Doolittle method (1982) as was shown in Figure 7. The hydrophobic domain is located in residues 100−140 of the α-chain, and its hydrophilic domain is located in peptide α-1 (residues 1−94). We concluded that the functional properties of proteins had a close relationship with the primary structure from the function of peptide α-1. Mulvihill et al. (1979) have reported that the ability of emulsification showed a great change when the hydrophobic domain of αs1-casein was removed by restricting hydrolysis with chymosin.

On the basis of the N-terminal amino acid sequence and molecular weight, it was suggested that the α-chain was cleaved between Asp-94 and Pro-95 residues, and between Asp-74 and Asp-75 residues (Figure 2a, arrow), and the β-chain was cleaved between Asp-99 and Pro-100 residues, and between Asp-73 and Gly-74 residues (Figure 2b, arrow). Those results indicated that citric acid cleaved specifically the aspartyl−peptide bond, in particular at the peptide bond between Asp and Pro residues. Piszkiewicz et al. (1970), Landon (1977), and Inglis (1983) have reported that aspartyl−peptide bonds are selectively hydrolyzed under mildly acidic conditions and at relatively high temperature. The mechanism of this hydrolytic reaction is concluded to proceed via intramolecular catalysis by carboxylate anion displacement of the protonated nitrogen of the peptide bond. The enhanced rate with proline as compared to other amino acids is undoubtedly due to the greater basicity of the proline nitrogen. Our results were in agreement with these previous findings.

When the α-chain of globin was hydrolyzed by citric acid, the hydrophobic domain of C-terminal was removed, and then the peptide α-1 showed a stronger hydrophilicity. The change in primary structure of α-1 peptide affects the higher-order structure and different functions from the α-chain. Moreover, the α-chain of globin contains a Cys residue, whereas peptide α-1

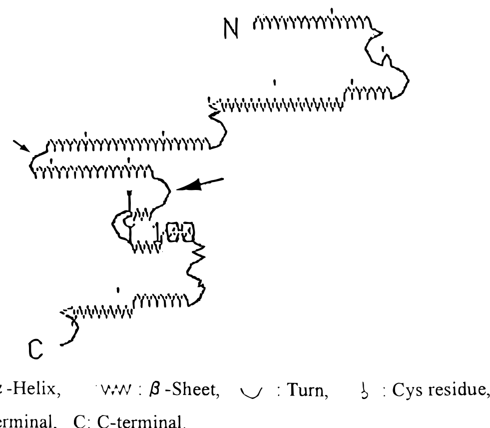


Figure 8. Prediction of the secondary structure of globin α-chain calculated from amino acid sequence data by the method of Chou-Fasman (1974a,b).

contains no Cys residue, and therefore peptide α-1 cannot form a cross-link by a disulfide bond. The ability of the association and dissociation behavior of peptide α-1 is also related to its secondary structure. The ellipticity of the α-chain at 222 nm increased greatly compared with that of peptide α-1, and in the case of peptide α-1, the content of the α-helix was 10% more than that of the α-chain, whereas those of the β-sheet and turn structure were about 5% less than that of the α-chain, respectively. These results were almost the same as the secondary structure of the α-chain conjectured by Chou and Fasman (1974a,b) (see Figure 8). The content of α-helix and β-sheet of peptide α-1 was different from that of α-chain, which causes the different spatial structure. Furthermore, the functional difference of the gel formation also may be induced from the different secondary structure and the presence of Cys residue.

The molecular weight of peptide α-1 was determined at 50 °C, as shown in Figure 5. The result indicated that the association and dissociation of peptide α-1 depended upon the temperature. To investigate the effect of temperature on the conformation change, the circular dichroism spectrum of peptide α-1 was measured at 50 °C. As was shown in Figure 6, the ellipticity at 50 °C was almost the same as at 20 °C, and the content of α-helix reduced about 1% by the analytical method of Yang (1986). From these results, it is concluded that the effect of heating only reduced the molecular weight of the aggregate of peptide α-1, but had little effect on the secondary structure of peptide α-1 monomer. The aggregates of peptide α-1 containing 7−9 molecules of peptide α-1 were produced while the secondary structure was held invariant.

Peptide β-1 originated from the β-chain combined with the β-chain by a noncovalent bond to form a rod-shaped aggregate, whose length was 130−140 nm and molecular weight was 870 000 Da (Liu et al., 1996). Peptide α-1 originated from the α-chain had a high hydrophilicity and an α-helix, showing the properties of association and dissociation. Peptide α-1 dissociated upon heating and associated while cooling. We suggest that about eight molecules of peptide α-1 combine with each other as a cross-linker between the rod-shaped aggregates of globin hydrolysates to form the network of gel, producing the final gel.

ABBREVIATIONS USED

CMC, (carboxymethyl)cellulose; SDS, sodium dodecyl sulfate; CD, circular dichroism; PAGE, polyacrylamide

gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; PVDF, polyvinglidene fluoride.

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LITERATURE CITED

Autio, K.; Lyytikainen, H.; Malkki, Y.; Kanko, S. Penetration studies of blood globin gels. *J. Food Sci.* **1985**, *50*, 615–617.

Autio, K.; Saito, M.; Kohyama, K.; Nishinari, K. Globin protein gelation: the effect of pH and temperature. *Food Hydrocolloids* **1990**, *4* (2), 87–93.

Braunitzer, G.; Schrank, B.; Stangl, A.; Scheithauer, U. Hemoglobins, XXI: Sequence analysis of porcine hemoglobin. *Hoppe-Seylers Z. Physiol. Chem.* **1978**, *359*, 137–146.

Caldironi, H. A.; Ockerman, H. W. Incorporation of blood protein into sausage. *J. Food Sci.* **1982**, *47*, 405–408.

Chou, P. Y.; Fasman, D. G. Conformational parameters for amino acids in helical β-sheet and random coil regions calculated from proteins. *Biochemistry* **1974a**, *13*, 211–222.

Chou, P. Y.; Fasman, D. G. Prediction of protein conformation. *Biochemistry* **1974b**, *13*, 222–245.

Crenwelge, D. D.; Dill, C. D.; Tybor, P. T.; Landmann, W. A. A comparison of the emulsification capacities of some protein concentrates. *J. Food Sci.* **1974**, *39*, 175–177.

Drepper, G.; Drepper, K. Verfahren zur herstellung neuer eiweiBprodukte aus schlachttierblut fur nahrungsmittel. *Fleischwirtschart* **1979**, *59*, 1252–1257.

Hayakawa, S.; Suzuki, Y.; Nakamura, R.; Sato, Y. Physicochemical characterization of heat-induced soluble aggregates of bovine globin. *Agric. Biol. Chem.* **1983**, *47* (2), 395–402.

Inglis, A. S. Cleavage at Aspartic Acid. *Methods Enzymol.* **1983**, *91*, 324–332.

Ishigami, Y.; Osman, M.; Nakahara, H.; Sano, Y.; Ishiguro, R.; Matsumoto, M. Significance of β-sheet formation for micellization and surface adsorption of surfactin. *Colloids Surf. B* **1995**, *4*, 341–348.

Knapp, F. W.; Schmidt, R. H.; Mauldin, W. J.; Ahmed, E. M. Evaluating cheese-like emulsions from animal blood proteins and whey solids. *J. Food Prot.* **1978**, *41*, 257–258.

Kyte, J.; Doolittle, R. F. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **1982**, *157*, 105–132.

Landmann, W. A.; Dill, C. W.; Young, C. R. Nutritive value of globin-amino acid and complementary globin -cereal mixtures. *J. Nutr.* **1980**, *110*, 2254–2262.

Landon, M. Cleavage at Aspartyl-Prolyl Bonds. *Methods Enzymol.* **1977**, *47*, 145–149.

Liu, X. Q. (Shinki Ryu); Yonekura, M.; Tsutsumi, M. Gel formation of globin prepared by acid-acetone method and globin hydrolyzates. *Nippon Shokuhin Kogyo Gakkaishi* (in Japanese). **1994a**, *41* (3), 178–183.

Liu, X. Q. (Shinki Ryu); Yonekura, M.; Tsutsumi, M. Gel properties of globin hydrolyzates. *Nippon Shokuhin Kogyo Gakkaishi* (in Japanese). **1994b**, *41* (3), 196–201.

Liu, X. Q. (Shinki Ryu); Yonekura, M.; Tsutsumi, M. Aggregates formation of globin hydrolyzates by their association. *Nippon Shokuhin Kogyo Gakkaishi* (in Japanese). **1995**, *42* (8), 562–568.

Liu, X. Q.; Yonekura, M.; Tsutsumi, M.; Sano, Y. Physicochemical Properties of Aggregates of Globin Hydrolyzates. *J. Agric. Food Chem.* **1996**, *44* (10), 2957–2961.

Matsudomi, N.; Oshita, T.; Sasaki, E.; Kobayashi, K. Enhanced heat-induced gelation of β-lactoglobulin by α-lactalbumin. *Biosci. Biotech. Biochem.* **1992**, *56* (11), 1697–1700.

Mulvihill, D. M.; Fox, P. F. Proteolytic of chymosin on bovine αs-casein. *J. Dairy Res.* **1979**, *46*, 641–651.

Parmer, E. L.; Surak, J. G.; Knapp, F. W. Nutritional evaluation of porcine globin using tetrahymena pyriformis strain E. *J. Food Sci.* **1978**, *43*, 499–501.

Penteado, M. D. V. C.; Lajolo, F. M.; Nilton Pereira dos Santos, N. P. Functional and nutritional properties of isolated bovine proteins. *J. Sci. Food Agric.* **1979**, *30*, 809–815.

Piszkiewicz, D.; Landon, M.; Smith, E. L. Anomalous cleavage of aspartyl-proline peptide bonds during amino acid sequence determinations. *Biochem. Biophys. Res. Cammun.* **1970**, *40* (5), 1173–1178.

Sano, Y. Optical anisotropy of bovine serum albumin. *J. Colloid Interface Sci.* **1988**, *124*, 403–406.

Sano, Y. Intrinsic optical anisotropy of southern bean mosaic virus. *J. Colloid Interface Sci.* **1990**, *139*, 14–19.

Sano, Y. Degree of linear polarization method for determination of intrinsic optical anisotropy of southern bean mosaic virus. *Biopolymers* **1993**, *33*, 69–74.

Sato, Y.; Hayakawa, S.; Hayakawa, M. Preparation of blood globin through carboxymethyl cellulose chromatography. *J. Food Technol.* **1981**, *16*, 81–91.

Schagger, H.; Jagow, G. V. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **1987**, *166*, 368–379.

Tybor, P. T.; Dill, C. W.; Landmann, W. A. Effect of decolorization and lactose incorporation on the emulsification capacity of spray-dried blood protein concentrates. *J. Food Sci.* **1973**, *38*, 4–6.

Tybor, P. T.; Dill, C. W.; Landmann, W. A. Functional properties of proteins isolated from bovine blood by a continuous pilot process. *J. Food Sci.* **1975**, *40*, 155–159.

Yang, J. T.; Wu, C.-S. C.; Martinez, H. M. Calculation of protein conformation from circular dichroism. *Methods Enzymol.* **1986**, *130*, 208–269.

Zheng, B. A.; Matsumura, Y.; Mori, T. Relationships of molecular forces to rheological and structural properties of legumin gels from broad beans. *Biosci. Biotech. Biochem.* **1993**, *57*, 1257–1260.

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