Tertiary Structure of α-1 Peptide of Globin Hydrolysates by the Small-Angle Solution X-ray Scattering Method

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A highly hydrophilic peptide α-1 originated from the α-chain of globin was isolated by hydrophobic chromatography. The sizes and tertiary structures of peptide α-1 monomer and aggregate were examined by the small-angle X-ray scattering (SAXS) method. The radius of gyration (R_g) of peptide α-1 in 6 M urea exhibited no concentration dependence, suggesting that peptide α-1 exists in monomeric state in this solvent. In the absence of urea the scattering patterns are composed of two components, that is, high molecular weight and low molecular weight species. The R_g of the low molecular weight component is consistent with the peptide α-1 treated with 6 M urea, indicating that this low molecular weight constituent is the monomer of peptide α-1. The R_g of the high molecular weight component increased with the peptide α-1 concentration. The weight-average molecular weight of the aggregate oligomer obtained by SAXS method was 7−9 times as large as monomer peptide α-1, which was consistent with the result by the laser light scattering study. Comparing the scattering pattern in the presence of 6 M urea with various theoretical models, the monomeric peptide α-1 took a random-coil structure and the polymer chain was distributed symmetrically. The aggregates (oligomer) of peptide α-1 also behaved as a whole in random-coil state and were formed by entanglement with the random-coil monomer.

Keywords: *Globin; globin hydrolysates; peptide α-1; small-angle solution X-ray scattering*

INTRODUCTION

Globin is a latent material that can be used as an ingredient in a variety of meat products because it can be obtained in a large quantity from animal blood and at a low price. 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). However, some disadvantageous properties, such as low solubility at the neutral pH range and unattractive color and odor, limit its application to the food industry. Several researchers have studied the decolorized globin (Tybor et al., 1975; Sato et al., 1981; Drepper et al., 1979), the nutritional aspects (Landmann et al., 1980; Parmer et al., 1978), and the functional properties of globin, such as solubility, emulsifying properties, and foaming properties (Tybor et al., 1973, 1975; Penteado et al., 1979; Crenwelge et al., 1974).

Although the abilities of proteins to form a gel and to provide a structural matrix for water-holding 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. Some studies were performed on the gel formation and the structural properties of globin. For example, Hayakawa et al. (1983) reported that globin heated at 90 °C formed the aggregates with highly entangled fibrous structures with a thickness of 8−10 nm. Decolorized globin prepared by acidified acetone (Tybor et al., 1975) showed acceptable water-binding properties at room temperature, but it could not form a gel after heating. In our previous studies, the decolorized globin prepared with this acidified acetone method successfully formed a gel at high globin concentration (7%) (Liu et al., 1994a). However, the gel made from the heat-induced globin showed a low hardness and was easily affected by the buffer conditions, such as pH and ions (Liu et al., 1994a; Autio et al., 1985, 1990).

In the further studies (Liu et al., 1994b, 1995a,b), the globin hydrolyzed with 0.8 M citric acid showed eight kinds of peptides of globin hydrolysates by tricine−SDS−PAGE, whose molecular weights ranged from 5000 to 15 000 Da. The gel filtration, transmission electron microscope, and the light scattering methods indicated that large aggregates, thin and 130−140 nm long, were formed easily in the globin hydrolysates. The aggregates were composed of two kinds of polypeptides, i.e., the β-chain (16 000 Da) originated from the native globin, and the β-1 (10 900 Da) cleaved the β-chain between 99 (Asp) and 100 (Pro). The molecular weight of the aggregate was estimated as 870 000 Da by the light scattering method (Liu et al., 1995b, 1996), indicating that the aggregate was composed of 33−34 units (each unit consisted of a ratio of 1:1 of β-chain to β-1).

Further, a highly hydrophilic peptide α-1 originated from the α-chain was isolated with hydrophobic chromatography. On the basis of N-terminal amino acid sequence and the molecular weight, it was suggested that the α-chain was cleaved between Asp (94) and Pro (95) residues (Liu et al., 1995b, 1997a). According to the light scattering and CD spectral methods, peptide α-1 showed aggregate (oligomer) formation to depend on temperature and on the concentration of peptide α-1 (Liu et al., 1997a). The aggregates of peptide α-1 (oligomer) consisted of 7−9 molecules of peptide α-1 monomer, and its secondary structure did not change even when the aggregates were formed. CD spectra showed that the content of the α-helix and β-sheet

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structures of peptide α-1 was very different from the intact α-chain (Liu et al., 1997a).

On the basis of the quasi-elastic light scattering studies of the aggregation process of globin hydrolysates and the transformation process of these aggregates to soft gel induced by peptide α-1 (Liu et al., 1997b), we observed that the gel formation of globin hydrolysate can be divided into two steps. The first step is mainly the formation process of rod-shaped aggregates of globin hydrolysate, and the following step is the formation process of early gel network structure induced by entangling peptide α-1 among rod-shaped aggregates of globin hydrolysate and then the formation of the gel, where about eight molecules of peptide α-1 combined with each other and behaved as a cross-linker entangling among the rod-shaped aggregates of globin hydrolysates to facilitate the formation of gel network (Liu et al., 1997b).

In the present study, the physicochemical properties of peptide α-1 were studied further by small-angle X-ray scattering (SAXS) in the absence and presence of 6 M urea, because SAXS is a useful method to directly investigate the size and the conformation of biological macromolecules in the solution (Sano et al., 1994, 1995, 1997). The results showed that the peptide α-1 monomers took a random-coil structure and aggregated together in a random-coil state.

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 with this method, 0.8 M citric acid was added to the powdered globin sample (Itohamu Coporation, Japan). The sample was hydrolyzed at 95 °C for 15 min, cooled to room temperature and dialyzed under flowing water with seamless cellulose tubing (molecular cutoff size 10 000 Da). The dialysate was air-dried with a spray-dryer (Pulvis minispray GA-32, Yamato Science, Japan), and the sample thus obtained is referred to as the 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 (Nacalai, Japan). The anode was 0.2 M Tris-HCl (pH 8.9) (Nacalai, Japan), the cathode was 0.1 M Tris-HCl (pH 8.25) with added 0.1 M tricine and 0.1% SDS (Nacalai, Japan). The concentration of polyacrylamide gel was 16.5% (Nacalai, Japan). The electrophoresis was done under 100 V for 10 h (Atto Corporation, Japan).

Preparation of Peptide α-1. The hydrophobic chromatography was carried out by butyl-Toyopearl 650S (Tosoh Coporation, 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. The 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 buffer pHs were 3.2); solution D, distilled water; and solution E, 0.05 N NaOH. GHP 1 fraction eluted from solution C was used as peptide α-1 sample (Liu et al., 1997a).

Small-Angle X-ray Scattering Measurements. SAXS experiments were carried out at 20 °C with the optics and detector system of SAXS (small-angle X-ray scattering equipment for solution) installed on the 2.5 GeV storage ring in the Photon Factory of the National Laboratory for High Energy Physics, Tsukuba, Japan. A wavelength, λ, of 0.149 nm was used. The X-ray scattering intensity was registered at 512 different angles by using the one-dimensional position-sensitive proportional counter with an effective length of 200 mm (Rigaku Denki, Japan). The SAXS intensity was measured for 600 s for all the solutions and buffers, and the net scattering intensities were calculated by subtracting the scattering intensities of a blank buffer solution from those of the sample solutions (Sano et al., 1995; Hiragi et al., 1988).

RESULTS

Estimation of Radius of Gyration. SAXS experiments of peptide α-1 treated with urea and without urea were carried out. The net scattering intensity $I(Q)$ is analyzed by the so-called Guinier plot

$$I(Q) = I(0) \exp(-R_g^2 Q^2/3) \quad (1)$$

where $I(0)$ and R_g denote the scattered intensity extrapolated to zero angle and the radius of gyration, respectively. $I(0)$ is proportional to the molecular weight (M) as (Hiragi et al., 1988)

$$I(0) = kMc \quad (2)$$

where c is the concentration of peptide α-1 and k is the optical constant. The magnitude of the scattering vector Q is defined in terms of the scattering angle θ and the wavelength λ of the incident beam as

$$Q = 4\pi \sin \theta/\lambda \quad (3)$$

The logarithmic plot of eq 1 gives

$$\ln I(Q) = \ln I(0) - R_g^2 Q^2/3 \quad (4)$$

the radius of gyration from the initial slope of ln $I(0)$ *vs* Q^2 plot. The Guinier plots at different peptide α-1 concentrations in the presence of 6 M urea are shown in Figure 1A. The radii of gyration calculated from Figure 1A were plotted against the concentrations of peptide α-1 in Figure 2, where the R_g showed no appreciable concentration dependence of peptide α-1, suggesting that peptide α-1 is monomeric in this solvent.

In the absence of urea, the data must be analyzed as a polydisperse system, because of the presence of monomer and aggregates (oligomer). According to Guinier's approximation for such a polydisperse system (Sano et al., 1994), the total scattering intensity can be expressed in terms of the sum of intensity scattered by the different species of components in the solution:

$$I(Q) = \sum I_i(0) \exp(-R_{g_i}^2 Q^2/3) \quad (5)$$

The Guinier plots at different peptide α-1 concentrations in the absence of 6 M urea were analyzed as a two-component system consisting oligomeric aggregate species and monomer. The experimental data shown in Figure 1B were in good agreement within errors with the calculated values of two components through the whole Q range. The radii of gyration of these two components were plotted in Figure 2 against the concentrations of peptide α-1. The radii of gyration of the monomer component were almost the same as those of the peptide α-1 in the presence of 6 M urea. On the other hand, the radii of gyration of the high molecular weight component (oligomer) increased with the peptide α-1 concentration.

Weight-Average Molecular Weight. The scattered intensity at zero angle normalized by the concentration, $I(0)/c$, is related to the weight-average molecular weight M (Sano et al., 1994). As shown in Figure 3, the weight-average molecular weight determined at the different concentrations of peptide α-1 showed the same value of

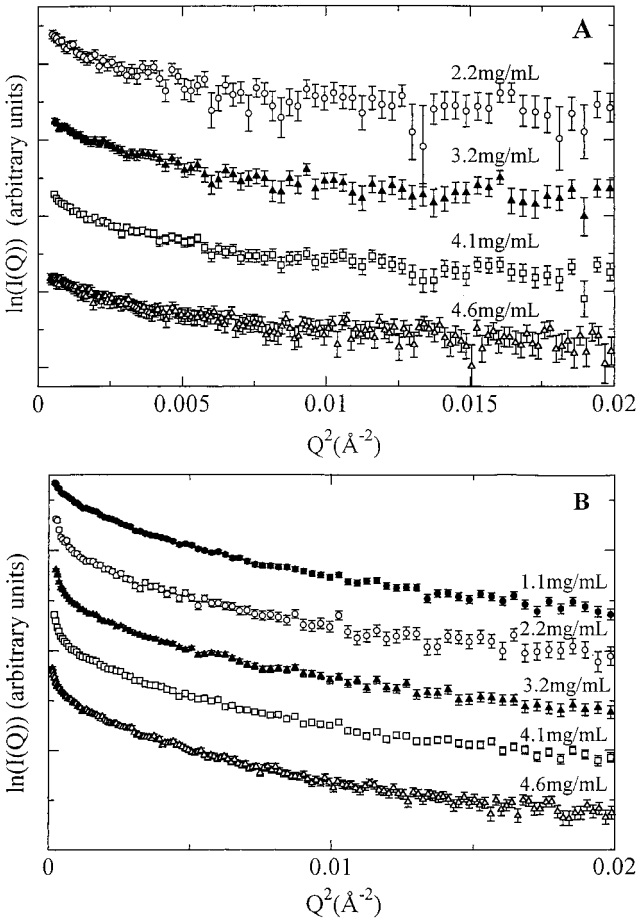

Figure 1. Guinier plots of scattering curves from the peptide α-1 treated with 6 M urea (A) and without urea (B). Protein concentrations are, from top to bottom, 2.2, 3.2, 4.1, and 4.6 mg/mL for peptide α-1 treated with 6 M urea (A) and 1.1, 2.2, 3.2, 4.1, and 4.6 mg/mL for peptide α-1 without urea (B), respectively. Proteins were dissolved in 0.1 M citric acid buffer (pH 3.2). Mean values and their standard deviations are shown with symbols and error bars.

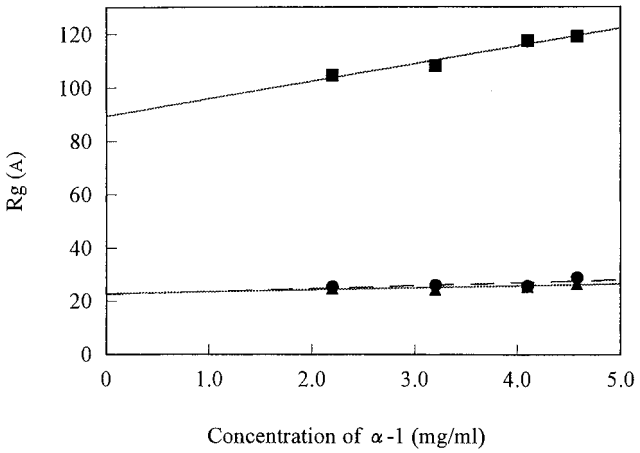

Figure 2. Concentration dependence of radius of gyration (R_g) obtained from Guinier plots of peptide α-1 in the presence of 6 M of urea (●) and in the absence urea (■, ▲), respectively.

10 000 Da in the presence of 6 M urea. In the absence of urea the weight-average molecular weight for monomer was also 10 000 Da and gave a good agreement with the value treated with urea. The weight-average molecular weight of high molecular weight component (oligomer) increased with the concentration of peptide α-1, which was 7−9 times as large as that of monomer peptide α-1. This is a good agreement with the results

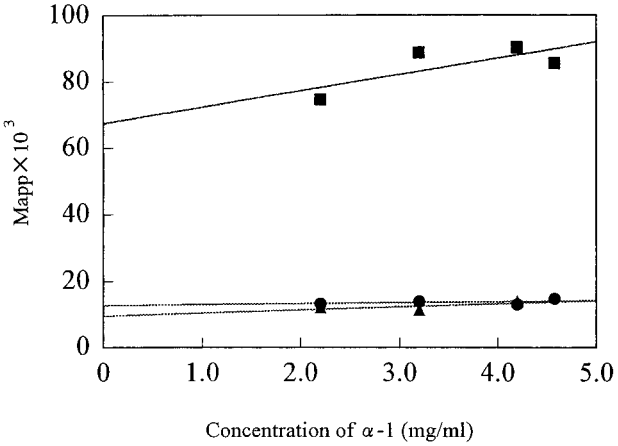

Figure 3. Concentration dependence of weight-average molecular weight of peptide α-1 in the presence of 6 M urea (●) and in the absence of urea (■, ▲), respectively.

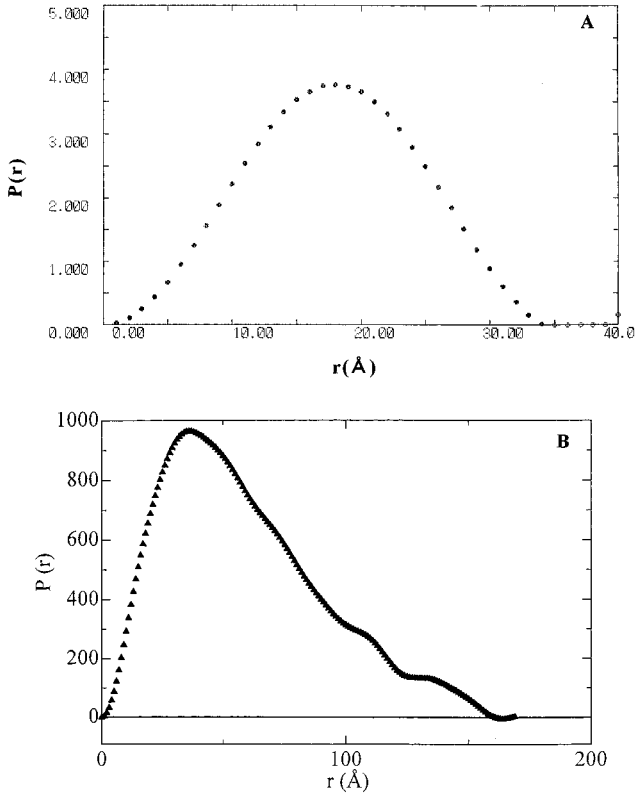

Figure 4. Distance distribution function, $p(r)$ of peptide α-1 (concentration: 4.6 mg/mL) in the presence of 6 M urea (A) and in the absence of urea (B).

obtained by the laser light scattering method in the previous paper (Liu et al., 1997a).

Distance Distribution Function. The distance distribution function is useful for the interpretation of the longest linear dimension (d_{max}) of the molecule and its overall shape and conformation (Sano et al., 1995). It is computed through a conventional Fourier transformation:

$$P(r) = (^1/_2\pi^2)\int I(Q)(Qr)\sin(Qr)\, dQ \qquad (6)$$

The distance distribution functions obtained in the presence of 6 M urea showed a symmetric and unimodal pattern as presented in Figure 4, leading us to propose that the polymer chain of the peptide α-1 monomer distributed spherically in urea solution. The largest

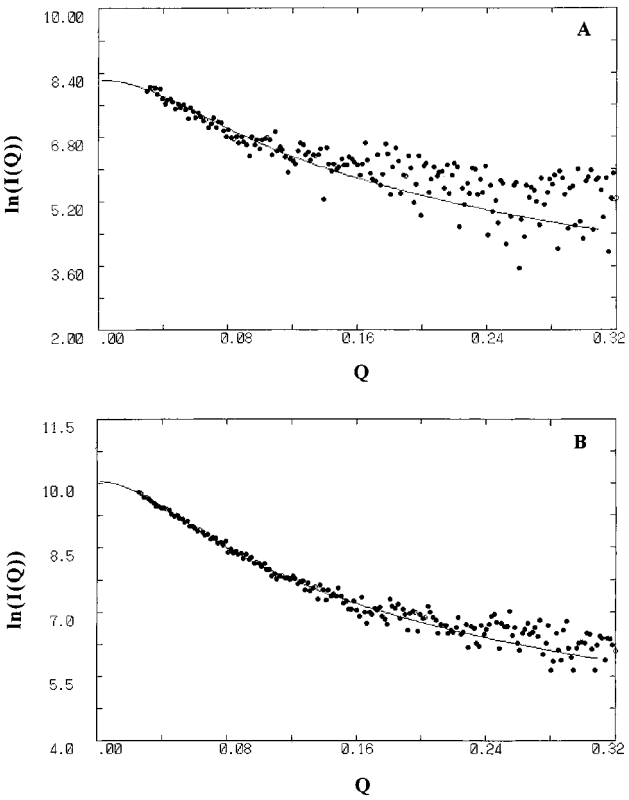

Figure 5. Angular dependence of the intensities of the scattered light in 4.6 mg/mL peptide α-1 treated with 6 M urea (A) and without urea (B). Closed circles show the experimental values. The theoretical curve calculated with eqs 7 and 8 as the random-coil model is shown with the solid line.

dimension of the scatterer is estimated from the point at which the function approaches zero. The maximum distance of monomeric peptide α-1 in the presence of urea is estimated as 33 Å. The distance distribution functions of oligomeric peptide α-1 in the absence of urea showed the longest dimension 102 Å. The unsymmetric profile of the distribution function may be due to the polydispersion effects.

Tertiary Structure of Peptide α-1 as a Random Coil. Though the radius of gyration indicates size of a solute molecule, its exact conformation cannot be known from this one parameter. The detailed conformation of a solute molecule may be speculated by comparing the observed scattering curve with the calculated one for various models, such as sphere, rod, and random-coil. The theoretical scattering curve for random-coil model is calculated with the Debye equation:

$$P(\theta) = 2(Z - 1 + e^{-Z})/Z^2 \quad (7)$$

$$Z = R_g^2 Q^2 \quad (8)$$

We tried many models to explain the agreement between the experimental data and the theoretical values. The random-coil model was much more suitable in this case. As shown in Figure 5A for peptide α-1 in urea solution, the experimental data were in good agreement with the calculated curve as a random-coil through the whole ranges. We also analyzed the three-dimensional structure of the aggregates (oligomer) with the model fitting. The result was also good with a random-coil model as monomer peptide α-1 (see Figure 5B). This suggests that the peptide α-1 monomer takes a random coil structure and aggregates together in random-coil state.

DISCUSSION

In the previous study, we have succeeded in preparing a gel with better functional properties using the hydrolysates of globin (Liu et al., 1997a). An aggregate consisting of two kinds of globin hydrolysates was observed with gel filtration chromatography. One of the hydrolysates is the β-chain (16 000 Da) originated from the native globin, the other is the β-1 (10 900 Da) originated from β-chain by cleavage of the β-chain between 99 (Asp) and 100 (Pro). Light scattering measurement and transmission electron microscope results showed that the aggregate of globin hydrolysates is a thin rod-shaped structure with a length of 130−140 nm (Liu et al., 1996).

Using hydrophobic chromatography, we also isolated a highly hydrophilic polypeptide α-1 originated from the α-chain. By adding the peptide α-1 to rod-shaped aggregates of globin hydrolysates, a gel with more excellent functional properties was formed. The hardness of a gel increases with the peptide α-1 concentration implying that the peptide α-1 plays a role in the transformation of aggregates of globin hydrolysates to gel (Liu et al., 1997a).

On the basis of observations of the gel formation process of globin hydrolysates mediated by peptide α-1 and using quasi-elastic light scattering technique, we proposed that the following two steps were concerned in the process of gel formation. The first one was mainly the formation process of rod-shaped aggregates of globin hydrolysates, and the second was the formation process of early gel network structure induced by entangling peptide α-1 among rod-shaped aggregates of globin hydrolysates, with the gel forming last (Liu et al., 1997b). From CD spectra we speculated that peptide α-1 was in a random coil state which made it easy to bind with thin rod-shaped aggregates (Liu et al., 1997b).

In the present work, the direct structural characteristics of the monomer and aggregate (oligomer) of peptide α-1 were studied by SAXS in the presence and absence of 6 M urea. As shown in Figure 2, the concentration dependence of R_g was not observed and the peptide α-1 behaved as a monomer in 6 M urea solution, which was in good agreement with the previous study of the peptide α-1 with the light scattering method (Liu et al., 1997a). The weight-average molecular weight of peptide α-1 monomer obtained by SAXS was 10 000 Da (see Figure 3), whose value was the same as the light scattering method and also the amino acid sequencing method (9839 Da) (Liu et al., 1997a). The profile of distance distribution functions in Figure 4A gives us the information about the size and the conformation of the peptide α-1 monomer. The longest dimension of the monomer was 33 Å. The symmetric and unimodal scattering curve demonstrated that the chain of the peptide α-1 monomer distributed symmetrically in urea solution (Figure 4A). The model fitting result led us to propose that the monomer exists in a random-coil structure (Figure 5A), which agreed well with the speculation of the CD data in the previous paper (Liu et al., 1997a).

On the other hand, the Guinier plots at different peptide α-1 concentrations in the absence of 6 M urea showed that there were two components in the solution (shown in Figure 1B), one of which was a high molecular weight (oligomer) and the other was a low molecular weight one. The R_g value of the low molecular weight component was the same as monomeric peptide α-1 treated with 6 M urea, suggesting that the low molec-

ular weight constituent is the monomer of peptide α-1. The R_g of the high molecular weight constituent showed the concentration dependence of peptide α-1, implying that this component is an aggregate (oligomer) of peptide α-1 (Figure 2). The weight-average molecular weight of the low molecular weight component was 10 000, giving good agreement with the result obtained from 6 M urea solution. While the weight-average molecular weight of the high molecular weight component increased with the concentration of peptide α-1 and was 7−9 times as large as that of the monomer, which strongly supported by our previous studies (Liu et al., 1997a,b). In Figure 4B, the aggregate had a longest dimension of 102 Å, existing in a random-coil state (Figure 5B). On the basis of SAXS results described above, we suggest as a model for peptide α-1 aggregate (oligomer) that about eight molecules of each peptide α-1 in random-coil structure form the aggregate existing as a whole in random-coil state without changing the random-coil structure.

In our previous study (Liu et al., 1994b), we found that the globin hydrolysates treated by citric acid showed better solubility and more excellent ability on gel formation induced by heating than intact globin and formed another type of gel which was different from that of intact globin. The 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). In the case of globin hydrolysates, the peptide α-1, which originated from globin α-chain, had a high hydrophilicity and showed the properties of association and dissociation, depending on concentration of peptide α-1 and temperature. The presence of hydrophobic peptide α-1 induced the ability of gel formation of hydrolysate globin and was easy to form the matrix structure included the rich water in places. It is very useful to exploit the so-called soft gel in the food industry to control the physical texture. Using a spiral structural peptide such as peptide α-1 as a gel junction, improvement of the functional gel in ham and sausage may be expected.

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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.

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

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 Eiweissprodukte aus Schlachttierblut für Nahrungsmittel (A method of manufacturing new protein products from animal blood for use in food and feed). *Fleischwirtschaft* **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.

Hiragi, Y.; Inoue, H.; Sano, Y.; Kajiwara, K.; Ueki, T.; Kataoka, M.; Tagawa, H.; Izumi, Y.; Muroga, Y.; Amemiya, Y. Temperature dependence of the structure of aggregates of tobacco mosaic virus protein at pH 7.2. static synchrotron small-angle X-ray scattering. *J. Mol. Biol.* **1988**, *204*, 129−140.

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.

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.

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* **1994a**, *41* (3), 178−183 (in Japanese).

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

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

Liu, X. Q. (Shinki Ryu); Yonekura, M.; Tsutsumi, M. Restrictive hydrolysis of globin by citric acid. *Nippon Shokuhin Kogyo Gakkaishi* **1995b**, *43* (2), 141−145 (in Japanese).

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.

Liu, X. Q.; Yonekura, M.; Tsutsumi, M.; Sano, Y. Self-assembling of peptide α-1 of globin hydrolyzates. *J. Agric. Food Chem.* **1997a**, *45* (2), 328−333.

Liu, X. Q.; Yonekura, M.; Tsutsumi, M.; Sano, Y. Quasi-elastic light scattering study on globin hydrolyzates gel formation process. *J. Agric. Food Chem.* **1997b**, *45* (5), 1574−1578.

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.

Sano, Y.; Inoue, H.; Kajiwara, K.; Urakawa, H.; Hiragi, Y. Self-assembling process of cylindrical virus coat protein as observed by synchrotron small-angle X-ray scattering. *J. Biochem.* **1994**, *115*, 1058−1063.

Sano, Y.; Inoue, H.; Hiragi, Y.; Urakawa, H.; Kajiwara, K. Solution X-ray scattering study of reconstitution process of tobacco mosaic virus particle using low-temperature quenching. *Biophys. Chem.* **1995**, *55*, 239−245.

Sano, Y.; Inoue, H.; Kajiwara, K.; Hiragi, Y.; Isoda, S. Structural analysis of A-protein of cucumber green mottle mosaic virus and tobacco mosaic virus by synchrotron small-angle X-ray scattering. *J. Protein Chem.* **1997**, *16*, 151−159.

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.

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