3-P8

AGGREGATION AND GELATION MODEL OF PORCINE BLOOD GLOBIN TREATED WITH PEPSIN

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Background:

Slaughtered animal blood is rich in protein, with hemoglobin accounting for more than half of it. Hemoglobin has limited appeal as a food ingredient because of its disagreeable color due to heme. Acidified acetone effectively removes heme from hemoglobin to produce globin with a low solubility in an aqueous system. Nakamura *et al.* (1984) improved the functional properties of globin by enzymatic and chemical modification. We reported succinylation (Miyaguchi *et al.*, 1989) or hydrochloric hydrolysis (Miyaguchi *et al.*, 1995) to be useful for improvement of functional properties such as solubility, emulsification and thermal gelation. Gelation is one of the most important properties of protein from the structural aspect of food products. For effective utilization of blood globin, thermal gelation of globin has been studied (Autio *et al.*, 1985, Hayakawa et al., 1983). We found for the first time that gelation occurs during peptic proteolysis of globin (Miyaguchi *et al.*, 1996), and we have partially studied the gelling properties of peptic globin hydrolysates (Miyaguchi *et al.*, 1997, 1998a) and their aggregate formation (Miyaguchi *et al.*, 1998b). As the protease-induced gelation mechanism of globin is poorly understood, obtaining more information concerning its gelling properties are of scientific and practical interest.

Objectives:

The aim of this study has been as follows: first, a gelling test of globin was done using various proteases. The gel structure of globin was observed with scanning electron microscopy (SEM). Next, to clarify the presence of aggregates in peptic globin hydrolysates, gel filtration and hydrophobic chromatography were conducted. Resulting N-terminal amino acid sequences of peptides were determined. Further, a protease-induced aggregation and gelation model of globin is included in this study.

Methods:

1) Preparation of porcine blood globin: Porcine red blood cells were diluted twice with water for hemolysis. Hemoglobin was obtained by centrifugation at 8000×g for 30 min. Globin was prepared from hemoglobin by the acidified acetone method of Tybor *et al.* (1975), slightly modified by Miyaguchi et al. (1989).

2) Gelation characteristics of globin treated by protease: Protease-induced globin gel was prepared according to the method of Miyaguchi *et al.* (1998a). Pepsin (1:10000, lot M2t5081, Nakalai Tesque Inc., Kyoto, Japan), chymosin (R-7751, Sigma, Mo., USA), papain (1:350, Wako Pure, Osaka, Japan), ficin (F-3266, Sigma, Mo., USA) and trypsin (2000E/g Merck KgaA, Darmastadt, Germany) were used in the protease treatment. Gel strength (breaking stress) was obtained based on various rheological data using a rheometer (NRM-2003J, Fudoh, Tokyo, Japan) equipped with a rheoplotter (FR-801, Fudoh, Tokyo, Japan). The gel specimens were freeze-dried at −50℃ for 3 days to prepare it for SEM observation. Gel structures were observed with a scanning electron microscope (JST-300, JEOL, Tokyo, Japan).

3) Characterization of peptides from aggregate fractions: Peptides in aggregate fractions of peptic globin hydrolysates were separated as in the previous paper (Miyaguchi *et al.*, 1998b). Gel filtration was conducted on a TSK-gel Toyopearl HW-50F column (20mm×300mm, Tosoh, Tokyo, Japan) using 50mM borate buffer containing 0.1M NaCl, pH9.4 as the eluent. Hydrophobic chromatography was carried out using five eluents: 50mM borate buffer containing 100 mM ammonium sulfate, pH9.4 (eluent A), 10mM borate buffer containing 10 mM ammonium sulfate, pH9.4 (eluent B), 10mM borate buffer, pH9.4 (eluent C), 10mM sodium carbonate, pH11.0 (eluent D), and 0.01N NaOH, pH12.0 (eluent E). High-performance liquid chromatography (HPLC) analysis was conducted with a TSK G2000SWXL column (7.8mm×300mm, Tosoh, Tokyo, Japan). Sodium dodesyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) was carried out using tricine. The bands corresponding to the desired peptides were applied to an automatic protein sequencer (Model 494, Applied

Biosystems, CA, USA) for N-terminal amino acid sequence determination.

Results and discussions:

Globin gel was formed by pepsin in the region of pH 2.0-4.0. On the other hand, the gels were not formed by the other proteases (chymosin, papain, ficin and trypsin). Hemoglobin did not form a gel from any of all proteases used. It was confirmed that gelation occurs in cases of peptic proteolysis of globin.

Addition of sodium chloride increased the gel strength of peptic globin, and the maximum gel strength was noted at 50mM. The gel strength was not influenced by an addition of calcium chloride but was reduced as additions of sodium thiocyanate increased. There is only one thiol group per monomer and no disulfide bonds (Braunitzer *et al.*, 1978). Therefore, this data suggested that the gelation of globin would be enhanced through non-covalent (hydrophobic) interactions between constituents. Fine filaments constructing the gel network were observed by SEM in globin treated with pepsin. SEM observations also show these filaments of globin in the presence of 50mM sodium chloride were thicker than those without sodium chloride.

Gel filtration demonstrated the presence of a fraction with a high molecular mass (Mm) above 15kDa (globin monomer) in peptic globin hydrolysates. This aggregate fraction (F1) was applied to hydrophobic chromatography, being divided into five fractions (F1a-F1e) with each eluent. Moreover, HPLC analysis showed that the F1c fraction, which was eluted with eluent C, had not only F1c-L (globin hydrolysates with Mm under 12.4kDa), but also F1c-H (an aggregate with Mm of 60-300kDa) with a high rate. A peptide with Mm of about 6.5kDa showed strongly in the F1c-H fraction by SDS-PAGE analysis, and the N-terminus sequence agreed with those of f 86-f 91 of β -globin by amino acid sequence analysis. It was clarified that the 6.5k-peptide would be a f 86-f 141 residue (Mm 6117 Da) of β -globin based on the specificity of the pepsin. This peptide may have a sequence of hydrophobic amino acids of f 109- f 114 residue (Val-Ile-Val-Val-Val-Leu).

In view of these results, the pepsin induced gelation mechanism of globin would be as follows: pepsin yields several peptides from globin. Hydrophobic aggregation of the 6117Da peptides occurs firstly. Next, the other peptides from α and β -globin partly participate in the aggregation. At last, the aggregate develops a formation of a three-dimensional network.

The information obtained from these studies may contribute to increased utilization of porcine globin as a food ingredient. In particular, globin could be used as a new gellant.

Conclusions:

1) Non-thermal gelation occurs in cases of peptic proteolysis of globin.
2) Addition of sodium chloride increased the gel strength of peptic globin hydrolysates. Fine filaments constructing the gel network were observed by SEM in globin treated with pepsin, becoming thicker from addition of sodium chloride.
3) An aggregate with Mm of 60-300kDa was separated from peptic globin hydrolysates by gel filtration and hydrophobic chromatography, having the hydrophobic peptide (Mm 6117 Da) of β -globin.
4) It was suggested that pepsin-induced gelation of globin would occur through an aggregation of the 6117Da peptide followed by the developing formation of a three-dimensional gel network.

Pertinent literature:

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