EFFECT OF PROTAMINE AND ITS HYDROLYSATES ON THE RHEOLOGICAL PROPERTIES OF PORCINE MYOFIBRIL GELS

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I. INTRODUCTION

Protamine (PRO) is a small arginine (Arg)-rich peptide present in the sperm head in animals [1]. It has been reported that the addition of PRO enhances the functional properties such as foaming activity and viscosity of bovine serum albumin [2]. Arginine also has some functional properties such as refolding and solubilization of proteins from insoluble pellets, and suppression of protein aggregation. In this study, we investigated that the relationship between the tryptic digestion of PRO and the effect of the hydrolysates on the physicochemical properties of porcine myofibril gels.

II. MATERIALS AND METHODS

Porcine myofibril (Mf) was prepared from commercial pork loin. PRO was digested with trypsin at the weight ratio of substance (S: PRO) and enzyme (E: trypsin) of 1000 and 5000. After the dialysis of the hydrolysates, the resultant permeable substances (PEs) and impermeable substances (IMs) were recovered, respectively. PRO and its hydrolysates (PEs and IMs) were subjected to the qualitative and quantitative analysis of free amino acids.

Various physicochemical properties of Mf added with PRO or its hydrolysates were measured using rheometry, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and scanning electron microscopy (SEM).

III. RESULTS AND DISCUSSION

PH1 and PH2 whose degree of hydrolysis were 25.9% and 12.8% prepared at the S/E ratios of 1000 and 5000, respectively. The amino acid compositions of PH1 and PH2 were similar to that of PRO which consisted mainly of Arg. Several expected amino acids were also detected in them. Though intact PRO contained small amount of free amino acids, the volume of free Arg increased after the tryptic digestion in both IMs and PEs which were recovered by dialysis of PH1 and PH2 against distilled water, respectively. Unexpectedly large number of free Arg was detected in IMs of PH1.

Intact PRO strongly inhibited the gelation of Mf though PEs of the hydrolysates did not decrease the gel hardness (Fig. 1). IMs of them also inhibited the gel hardness. IMs of PH1 decreased the gel hardness more than intact PRO and IMs of PH2, suggesting that IMs of the hydrolysates would have certain peptides which play role on the gelling inhibition of Mf. On the other hand, PE of PH2 increased the gel hardness, suggesting IMs of the hydrolysates may have certain compounds which could facilitate the gelation of Mf.

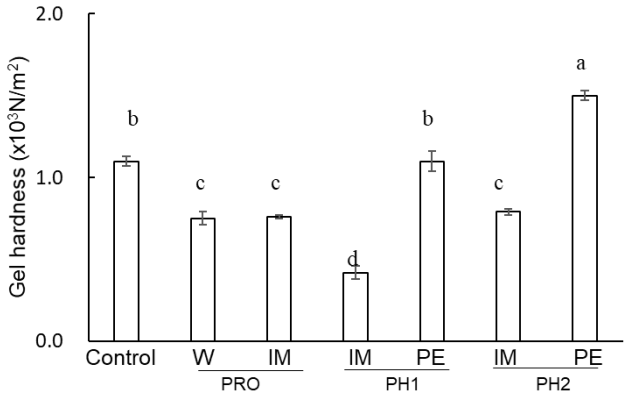

Figure 1 Hardness of porcine myofibril gels in the presence of impermeable and permeable fractions from tryptic digested PRO after dialysis. Control, myofibril alone; W, whole (no dialysis); IM and PE, impermeable and permeable substances after dialysis, respectively; Dara bearing similar superscripts are not significantly different (p>0.05).

SDS-PAGE analysis demonstrated that the addition of PRO facilitated the solubilization of myosin-binding protein C (MBP-C) and myosin light chain (MLC) 1 and the insolubilization of α-actinin in Mf. However, these actions were shown in the presence of neither IMs nor PEs of the hydrolysates. After heating, myosin heavy chain (MHC) and actin bands were detected in Mf with PRO and PEs. However, the MHC band was not visible in the presence of IMs of the hydrolysates. Both the MHC and actin bands disappeared after the addition of the IM of PH1 (Fig. 2). SDS is an anion detergent to be able to bind to positive-charged amino acids [3]. IMs of PH1 may inhibit the binding of SDS to Mf. SEM showed a fragile gel network structure with Mf in the presence of PRO. Protein aggregation was observed by the addition of the IMs of the hydrolysates. PEs of PH2 gave a fine gel network structure with Mf, also suggesting both the gelation inhibiting and facilitating peptides may coexist in tryptic PRO hydrolysates.

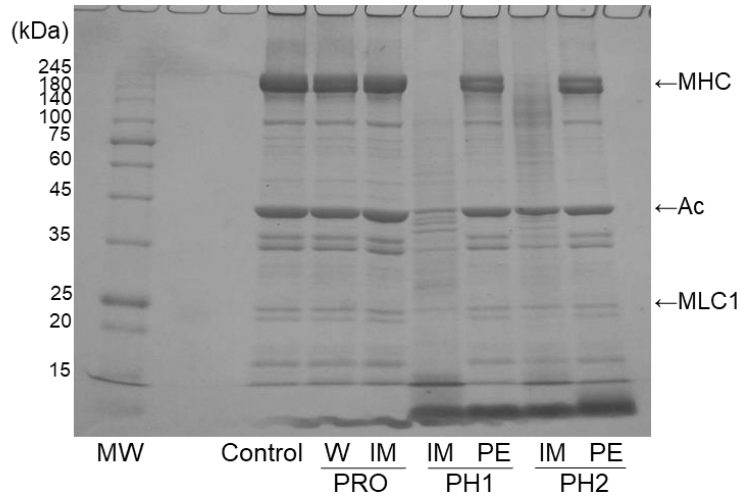

Figure 2 SDS-PAGE pattern of the heat-treated myofibril in the presence of IM and PE fractions from tryptic PRO hydrolysates. 12.5% acrylamide of separation gel; MW, molecular weight marker; Control, myofibril alone; W, whole (no dialysis); IM and PE, impermeable and permeable substances after dialysis, respectively; PRO, PH1, and PH2, Refer to Figure 1. MHC, myosin heavy chain; Ac, actin; MLC1, myosin light chain 1

IV. CONCLUSION

Our study demonstrated that PRO and its tryptic hydrolysates inhibited the thermal gel formation of Mf. Furthermore, it was suggested that the inhibition mechanism would be different intact PRO and its hydrolysates. These results provide some information on the novel physicochemical properties of PRO and the potential for development of a new meat texture modifier.

ACKNOWLEDGEMENTS

We appreciate Dr. Nitta at Fukushima University for providing technical assistance with SEM analysis. This work was supported by JSPS KAKENHI Grant Number JP26660102.

REFERENCES

1. Balhorn, R. (2007). The protamine family of sperm nuclear proteins. Genome Biology 8, 227 (doi:10.1186/gb-2007-8-9-227)
2. Rousseau, D., Glaser, L. A., Paulson, A. T., Speers, R. A. & Yada, R. Y. (2007). Foaming behavior of mixed bovine serum albumin-protamine systems. Food Hydrocolloids 21, 495-506.
3. Pitt-Rivers, R. & Impiombato, F. S. (1968). The binding of sodium dodecyl sulphate to various proteins. Biochemical Journal 109, 825-830.