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THE EFFECT OF OVALBUMIN ON THE PROTEASE ACTIVITY

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ABSTRACT

Chicken egg white is generally used in the industry because of its excellent effect to improve physical property of surimi products. The effects of egg white are believed to be derived from its inhibitory activity on surimi endogenous proteases which reduce gel forming ability. However, there are no detailed investigations about inhibitory effects of egg white on the proteases. The effect of ovalbumin (OVA), which is the main protein of egg white, on trypsin activity was investigated in the present study. N-OVA was purified from fresh chicken egg white by ammonium sulfate fractionation and anion-exchange chromatography (Q-sepharose). S-OVA and I-OVA were prepared by heating N-OVA solution at pH10, 55°C for 24h and at 97°C for 30min (pH was not controlled) respectively. To investigate the effect of OVA on the trypsin activity, casein-trypsin mixture solution was heated with or without OVAs at 40°C and the content of peptide generated from casein was measured. SDS-PAGE of casein was also conducted. The amount of peptide from casein was decreased in the presence of OVAs, regardless of OVA types. SDS-PAGE showed all types of OVA inhibited casein degradation. Those suggest that all types of OVA have inhibitory effect on the trypsin activity.

Keywords : Surimi, Ovalbumin, Proteases, Egg white

INTRODUCTION

Chicken egg white is generally used as additive of surimi products because of its excellent effect to improve the physical properties. Effects of egg white are believed to be derived from its inhibitory activity on surimi endogenous proteases which reduce gel forming ability. However, there are no detailed investigations about inhibitory effects of egg white on the proteases. OVA is main protein of egg white and considered to have no inhibitory effect against proteases due to its structure[1]. However, there are few study which actually investigate about inhibitory effect of OVA. Furthermore, protease is very important factor for the physical properties of surimi products. Therefore effect of OVA on protease activity was investigated and the detail of my presentation was summarized in the proceeding.

MATERIAL AND METHODS

1. Material

Albumin from chicken egg white Grade VII (Sigma-Aldrich) was purchased as commercial N-OVA. Fresh egg was purchased from poultry farm. Trypsin(porcine pancreas) and casein were purchased from Wako Pure Chemical Industries.

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2. Purification of N-OVA

2.1 Preparationof mucinfree egg

OVA was purified from egg white according to the method of Croguennec et al.[2] with minor modifications. Egg white was separated from whole egg and homogenized. Homogenized egg white was diluted with 20mM Tris-HCl buffer (pH8.2). Mixture was adjusted pH to 6.0 by 1M HCl, stirred for 3h and centrifuged at 2000×*g* for 3min. Supernatant was adjusted pH to 8.2 by 1M NaOH.

2.2 Ammonium sulfate fraction

Unexpected material was precipitated and removed by 21.5% saturated ammonium sulfate and centrifugation at 10000×*g* for 15min. Supernatant was dialyzed.

2.3 Anion exchange chromatography

Dialyzed supernatant was diluted with equal volume of 20mM Tris-HCl buffer (pH8.2) and run into column (Q sepharose Fast Flow, GE Healthcare). Unexpected material was removed by 0.5M NaCl-20mM Tris-HCl buffer (pH8.2). OVA fraction was eluted by 1.0M NaCl-20mM Tris-HCl buffer (pH8.2) and dialyzed. Dialyzed solution was freeze-dried.

3. Preparation of OVA derivatives

3.1 S-OVA

S-OVA was prepared by heating 50mg/mL N-OVA solution at 55°C, pH10.0 (pH was adjusted by 0.1M NaOH) for 24h [3]. After heating, solution was cooled by ice water, adjusted pH to 7.0 and freeze-dried.

3.2 I-OVA

I-OVA was prepared by heating 0.3mg/mL N-OVA solution at 97°C for 30min (pH was not controlled) [4]. After heating, solution was cooled by ice water and freeze-dried.

4. SDS-PAGE of N-OVA

Protein pattern was checked by SDS-PAGE. SDS-PAGE was conducted according to the method of Laemmli [5]. 10% gel was used and gel was stained by Oriole Fluorescent Gel Stain (Bio-Rad).

5. Differential scanning calorimetry (DSC)

N-, S- and I-OVA were dissolved with20mM phosphate buffer (pH 7.4). Protein concentration was 10mg/mL and heating rate was 5°C/min.

6. Effect of OVA on trypsin activity

Effect of OVA on trypsin activity was investigated according to the method of Bougatefet al. [6] with minor modifications. Trypsin was used as a protease and casein was used as a substrate. 250μL trypsin solution (final concentration was 0.001mg/mL) was mixed with equal volume of OVA solution (final concentration was 0.05mg/mL) and then incubated at room temperature for 15 min and pre-heated at 40°C. Pre-heated (40°C) 100mM Tris-HCl buffer (pH8.0) containing 1% casein (w/v) was added. Reaction was conducted by heating trypsin-

casein mixture at 40°C for 0, 10, 30 min. After heating, 15%TCA(w/v) was added to stop the reaction and mixture was centrifuged at 10,000×*g* for 15min. Peptide content of supernatant was measured by Lowry method. SDS-PAGE was conducted at the same time. Casein before adding TCA was used as sample.

RESULTS AND DISCUSSION

1. SDS-PAGE of N-OVA

The result of SDS-PAGE showed that N-OVA was purified well.

2. DSC

N-OVA had a peak near 78°C, S-OVA had a peak near 86°C and I-OVA had no peak. The result of DSC showed that OVA derivatives (S-OVA and I-OVA) were prepared correctly.

3. Effect of OVA on trypsin activity

Peptide content was decreased in the presence of OVA regardless of OVA types. SDS-PAGE showed all types of OVA inhibited casein degradation. Those suggested all types of OVA have inhibitory effect on trypsin activity.

CONCLUSION

It is considered that OVA has no inhibitory effect against proteases due to its structure. However, in this study, OVA has inhibitory effect against trypsin. There is a possibility that OVA inhibit trypsin activity due to not reaction center loop insertion but another mechanism.

REFERENCES

J.A. Huntington, P. E. Stein: *Journal of Chromatography B* 756 (2001): 189-198
T. Croguennec, F. Nau, S. Pezennec, and G. Brule: *Journal of Agricultural and Food Chemistry* 48 (2000): 4883-4889
M. B. Smith, J. F. Back: *Australian Journal of Biological Sciences*18 (1965): 365-377
P. Mellet, B. Michels and J. G. Bieth: *The Journal of Biological Chemistry* 271 (1996): 30311-30314
U. K. Laemmli: *Nature* 227 (1970): 680-685
A. Bougatef, N. Souissi, N. Fakhfakh, Y. E. Triki and M. Nasri: *Food Chemistry* 102 (2007): 343–350