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PURIFICATION OF TRANSGLUTAMINASE AND ITS EFFECTS ON MYOSIN HEAVY CHAIN AND ACTIN OF SPENT HENS

Tsai-Fuh Tseng, Ming-Tsao Chen and Deng-Cheng Liu

Dept. of Animal Science, National Chung-Hsing University, 250 Kao-Kuang Road, Taichung, Taiwan 402, ROC.

Key Words: transglutaminase, spent hen, myosin heavy chain, actin.

Background:

Factor XIII is a Transglutaminase (TGase, EC 2.3.2.13) that occurs as a zymogen in plasma, placenta and platelets. The reaction catalyzed by Ca^{2+}-dependent Factor XIIIa involves the formation of a ε -(γ -glutamyl)-lysyl bond between an acyl donor (glutaminyl residue) and an acyl acceptor (lysyl residue). Therefore, this enzyme catalyzed conversion of soluble proteins to insoluble high molecular polymers through formation of covalent cross-links (De Backer-Royer *et al.*, 1992; Traore & Meunier, 1992). Previous research had been used with guinea pig liver where TGase was used to catalyze various proteins (α s_1-casein, k-casein, β-lactoglobulin, soybean 11S and 7S globulin) to form an intermolecular and intramolecular (ε -(γ -glutamyl)-lysyl) crosslinks (Motoki & Nio, 1983). Some experiments have used guinea pig liver TGase to induce mechanically deboned poultry meat (Akamittath & Ball, Jr., 1992) or beef (Kim *et al.*, 1993) actomyosin to polymerization. Kurth & Rogers (1984) used bovine plasma TGase to catalyze myosin and soya protein, casein or gluten to form (ε -(γ -glutamyl)-lysyl) crosslinks.

Objectives:

The purpose of this study was to purify pig plasma TGase and examine its effects on myosin heavy chain and actin of the breast muscles from spent hen at different temperatures. If successful it is expected to increase the utilization of pig plasma and spent hen.

Materials And Methods:

Purification of TGase: TGase was prepared according to procedures described by Folk & Chung (1985). **Determination of Enzyme Activity:** TGase activity was measured according to the procedure described by Folk & Cole (1966). Protein content was measured by a protein assay kit (Merck) using bovine globulin as the standard protein (Bradford, 1976). **Preparation of Myofibrillar Protein:** Six spent layer hens (approximately 60 weeks old with an average weight of 1.5 kg) were slaughtered. The breast muscles (*M pectoralis*) were immediately excised from each carcass within 10 min of slaughter (Chou *et al.*, 1997). Myofibrillar protein was prepared according to procedures described by Goll *et al.* (1974). **The Reaction of adding TGase to Myofibrillar Protein:** The reaction of adding TGase to myofibrillar protein was performed as described by Aboumahmoud & Savello (1990) and Kim *et al.* (1993)methods. Each reaction mixture contained 0.5ml myofibrillar protein solution (pH 7.0), 10mM $CaCl_2$, 20mM dithiothrietol (DDT) and 0.3 units/ml TGase. These reactions were performed at 4℃ for 0, 4, 8, 12, 24 and 48 hr; at 25℃ for 0, 1, 2, 4, 8, 12, 24 and 48 hr; at 37 ℃ for 0, 5, 10, 30 and 60 min. The control group with no TGase, had the same treatments. **SDS-PAGE performance:** The myofibril samples for SDS-PAGE were prepared by the method of Wang *et al* (1988). **Relative intensity:** The relative intensity was performed as described by Kim *et al.* (1993) .

Results and Discussions:

Purification of TGase

Purified TGase (Fig. 1, Line 4) from pig plasma (Fig. 1, Line 3) through SDS-PAGE analysis, indicated that TGase of pig plasma was composed of molecular weights of approximately 75,000 and 80,000. Guinea pig liver TGase (bought from Sigma company) has a molecular weight of 85,000 (Fig. 1, Line 2, Connellan *et al.*, 1971). Previous researches indicated human plasma (Folk & Chung, 1985; Schwartz *et al.*, 1971; Schwartz *et al.*, 1973) and pig plasma (Jiang & Lee, 1992) factor XIII that was composed as a tetramer of a_2b_2 (molecular weights of 320,000) and consists of two catalytic *a* subunits (each molecular weight was approximately 75,000) and two noncatalytic *b* subunits (each molecular weight was approximately 80,000) (Ichinose *et al.*, 1990).

Effects of TGase on Myosin heavy chain and Actin of the Breast Muscles from Spent Hen

The effects of TGase on spent hen breast muscle myosin heavy chain and actin was not changed apparently at 4℃ for 0-24 hr (Fig. 2). At a reaction time of 48 hr, the intensity of myosin heavy chain and actin decreased apparently. The relative intensity of myosin heavy chain and actin was 54% and 64% respectively. However, when the reaction was at 25℃ for 2 hr (Fig. 3), the intensity of myosin heavy chain had decreased obviously, and disappeared after 48 hr. The intensity of actin also had the same trend to decrease when the reaction time increased. A densitometer scan of the electrophoretograms indicated that the relative intensity of myosin heavy chain was 92%, 45%, 20%, 17%, 12%, 8% and 1% respectively, the relative intensity of actin was 90%, 64%, 53%, 48%, 41%, 26% and 13% respectively, at 25℃ for 1, 2, 4, 8, 12, 24 and 48 hr. The intensity of myosin heavy chain nearly disappeared at 37℃ when incubated for 5 min (Fig. 4), the intensity of actin also decreased when reaction time increased. A densitometer scan of the electrophoretograms indicated that when the reaction was at 37℃ for 5, 10, 30 and 60 min, the relative intensity of myosin heavy chain was 7%, 1%, 0% and 0% respectively, and the relative intensity of actin was 63%, 34%, 25% and 13% respectively. Therefore, the effects of TGase on the myosin heavy chain was remarkably stronger than that on actin. The effects of TGase on the breast muscles from spent hens at different temperatures, indicated a serious decrease of the intensity of myosin heavy chain and actin was obtained at the temperature of

25℃ and 37℃, and that mean higher temperature (37℃) can speed up the reaction. The same condition also was found in conclusion of Akamittath & Ball, Jr. (1992) who reported that effects of guinea pig liver TGase on the crude actomyosin from mechanically deboned poultry meat at 4℃ for 26 hr are not apparent, but at 37℃ for 10 to 40 min, myosin monomer content apparently decreased. When Kim *et al.* (1993) used guinea pig liver TGase in beef actomyosin under the temperature of 35℃ at 10 to 120 min, the myosin monomer also gradually decreased with the simultaneous appearance of myosin polymers when the reaction time increased. Cohen *et al.* (1979) and Kahn & Cohen (1981) also indicated that plasma TGase can catalyze the formation of glutamyl-lysine bonds in myosin and also between myosin and actin, myosin and fibronection, and fibrin and actin. In addition, previous researches (Kumazawa *et al.*, 1993; Kumazawa *et al.*, 1995; Kurth & Rogers, 1984; Motoki et al., 1984; Sakamoto *et al.*, 1995; Seguro *et al.*, 1995) also reported that modifications of several food proteins using TGase, and that the myosin heavy chain decreased when the reaction time increased, and this will help with the formation of crosslinks between various proteins, and that the ε - γ (glutamyl)-lysine amount of bonds will be increased. Simultaneously, this condition can help to improve or modify the function properties of food protein.

Conclusions:

In summary, purified TGase derived from the pig plasma was composed of two molecular weights of approximately 75,000 and 80,000 by the SDS-PAGE analysis. The myosin heavy chain and actin of spent hen myofibrillar proteins at temperatures of 25℃ and 37℃, treated with TGase shown that both intensities decreased when reaction time increased. In conclusion, TGase purified from pig plasma also has the same functional properties as TGase from the other sources and may be used as a food protein improver during meat processing.

Literatures:

Aboumahmoud, R. & Savello, P. 1990. Crosslinking of whey protein by transglutaminase. J. Dairy Sci. 73: 256-263.

Akamittath, J. G. & Ball, Jr., H. R. 1992. Transglutaminase mediated polymerization of crude actomyosin refined from mechanically deboned poultry meat. J. Muscle Foods 3:1-14.

Ichinose, A., Bottenus, R. E. & Davie, E. W. 1990. Structure of transglutaminases. J. Biol. Chem. 265(23): 13411-13414.

Kahn, D. R. & Cohen, I. 1981. Factor XIIIa-catalyzed coupling of structural proteins. Biochim. Biophys. Acta 668: 490-494.

Kim, S. H., Carpenter, J. A., Lanier, T. C. & Wicker, L. 1993. Polymerization of beef actomyosin induced by transglutaminase. J. Food Sci. 58(3): 473- 474, 491.

Kurth, L. & Rogers, P. J. 1984. Transglutaminase catalyzed cross-linking of myosin to soya protein, casein and gluten. J. Food Sci. 49: 573-576, 589.

Motoki, M. & Nio, N. 1983. Crosslinking between different food proteins by transglutaminase. J. Food Sci. 48: 561-566.

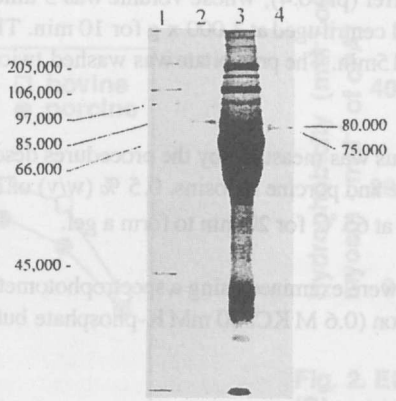

Fig. 1. Electrophoretic pattern of purified pig plasma TGase. Lane 1: Standard proteins; Lane 2: TGase of guinea pig liver (Sigma, T5398); Lane 3: Pig plasma; Lane 4: Purified TGase from pig plasma.

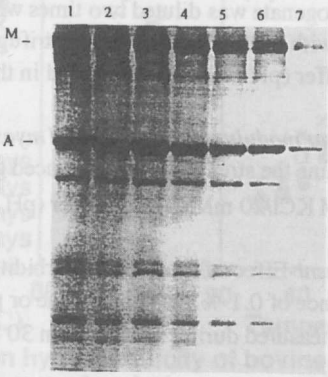

Fig. 2. Effect of TGase on myosin heavy chain and actin of the breast muscles from spent hen at 4 ℃ for 4(lane 3), 8(lane 4), 12(lane 5), 24(lane 6) and 48(lane 7) hour. Lane (1) control(or TGase) sample at 4 ℃ for 0 hour; (2) control sample at 4 ℃ for 48 hour. Abbreviations: M= Myosin heavy chain; A= Actin.

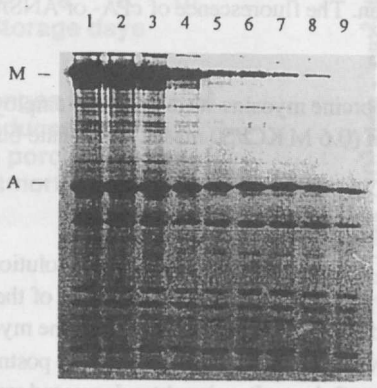

Fig. 3. Effect of TGase on myosin heavy chain and actin of the breast muscles from spent hen at 25 ℃ for 1(lane 3), 2(lane 4), 4(lane 5), 8(lane 6), 12(lane 7), 24(lane 8) and 48(lane 9) hour. Lane (1) control(or TGase) sample at 25 ℃ for 0 hour; (2) control sample at 25 ℃ for 48 hour. Abbreviations: M= Myosin heavy chain; A= Actin.

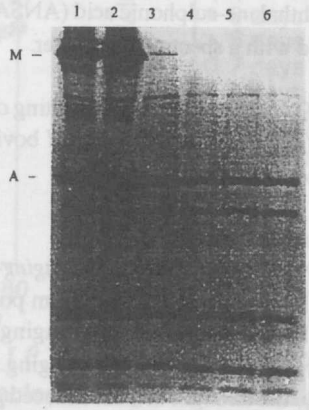

Fig 4. Effect of TGase on myosin heavy chain and actin of the breast muscles from spent hen at 37 ℃ for 5(lane 3), 10(lane 4), 30(lane 5) and 60(lane 6) min. Lane (1) control(or TGase) sample at 37 ℃ for 0 min.; (2) control sample at 37 ℃ for 60 min. Abbreviations: M= Myosin heavy chain; A= Actin.