ELSEVIER

Meat Science 60 (2002) 267–270

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Purification of transglutaminase and its effects on myosin heavy chain and actin of spent hens

Tsai-Fuh Tseng[a], Ming-Tsao Chen[b,*], Deng-Cheng Liu[b]

[a]*Department of Animal Science, National Chia-Yi University, Chia-Yi, Taiwan 60083*
[b]*Department of Animal Science, National Chung-Hsing University, Taichung, Taiwan 40227*

Received 14 January 1999; received in revised form 21 May 1999; accepted 24 May 1999

Abstract

The purpose of this study was to purify pig plasma transglutaminase (TGase) and examine its effects on the myosin heavy chain and actin of the breast muscles from spent hens at different temperatures. TGase (0.3 units/mg) was added to myofibrillar proteins solution (0.5 ml) at 4°C for 0, 4, 8, 12, 24 and 48 h; at 25°C for 0, 1, 2, 4, 8, 12, 24 and 48 h; at 37°C for 0, 5, 10, 30 and 60 min. The results of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) showed that plasma TGase was composed of units of molecular weights approximately 75,000 and 80,000. TGase added to the myofibrillar proteins solution indicated that the concentration of the myosin heavy chain and actin decreased when incubated at 4°C for 48 h and when incubated at 25°C for 2 h. Moreover, the relative intensity determined by scanning densitometry of the SDS–PAGE gel indicated that the myosin heavy chain and actin concentration decreased to 45 and 64%, respectively. In addition, the relative intensity of the myosin heavy chain and actin declined to 7 and 63%, respectively, when incubated at 37°C for 5 min. The relative intensity of both the myosin heavy chain and actin decreased with time when incubated at 25 and 37°C.

Keywords: Actin; Myosin; Transglutaminase

1. Introduction

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 catalyzes the conversion of soluble proteins to insoluble high molecular polymers through formation of covalent cross-links (De Backer-Royer, Traore, & Meunier, 1992; Traore & Meunier, 1992). Cohen, Young-Bandala, Blankenberg, Siefring, and Bruner-Lorand (1979) and Kahn and Cohen (1981) have shown that plasma TGase can catalyze the formation of glutamyl-lysine bonds in myosin, myosin and actin, myosin and fibronection, and fibrin and actin. Therefore, TGase can be used to improve or modify the functional and rheological properties of food proteins (De Backer-Royer et al., 1992; Kim, Carpenter, Lanier, & Wicker, 1993; Traore & Meunier, 1992).

Previous research has used guinea pig liver TGase with various proteins (αs_1-casein, k-casein, β-lactoglobulin, soybean 11S and 7S globulin) to form intermolecular and intramolecular [ε-(γ-glutamyl)-lysyl] crosslinks (Motoki & Nio, 1983). Some workers have used guinea pig liver TGase with mechanically deboned poultry meat (Akamittath & Ball Jr., 1992) or beef (Kim et al., 1993) to polymerisation automyosin. Kurth and Rogers (1984) used bovine plasma TGase with myosin and soya protein, casein or gluten to form [ε-(γ-glutamyl)-lysyl] crosslinks. The purpose of this study was to purify pig plasma TGase and examine its effects on the myosin heavy chain and actin of the breast muscles from spent hens. If successful, it would increase the utilization of pig plasma and spent hens.

2. Materials and methods

2.1. Purification and enzyme activity of TGase

Purification of TGase was performed according to procedures described by Folk and Chung (1985), and its activity was measured according to the procedure described by Folk and Cole (1966).

* Corresponding author. Tel.: +886-4-22850788; fax: +886-4-22850505.
E-mail address: michen@dragon.nchu.edu.tw (M.-T. Chen).

0309-1740/02/$ - see front matter
PII: S0309-1740(01)00132-2

2.2. Reaction of TGase with myofibrillar protein

Myofibrillar protein was prepared according to procedures described by Goll, Young and Stromer (1974). Reaction of TGase with myofibrillar protein was performed as described by Aboumahmoud and Savello (1990) and Kim et al. (1993). SDS–PAGE was used to assess the reaction between the proteins as described by Laemmli (1970) and Wang, Gresser, Shultz, Bulinski, Lin, and Lessard (1988). The relative intensity of protein components was determined as described by Kim et al. (1993).

3. Results and discussion

3.1. 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 proteins of molecular weight 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, Chung, Whetzel, Bradley, & Folk, 1971). Previous research has indicated that human plasma (Folk & Chung, 1985; Schwartz, Pizzo, Hill, & McKee, 1971, 1973) and pig plasma (Jiang & Lee, 1992) factor XIII is a tetramer of a_2b_2 (molecular weight 320,000) and consists of two catalytic *a* subunits (each of molecular weight approximately 75,000) and two noncatalytic *b* subunits (each of molecular weight approximately 80,000; Ichinose, Bottenus, & Davie, 1990). Therefore, our results are as expected.

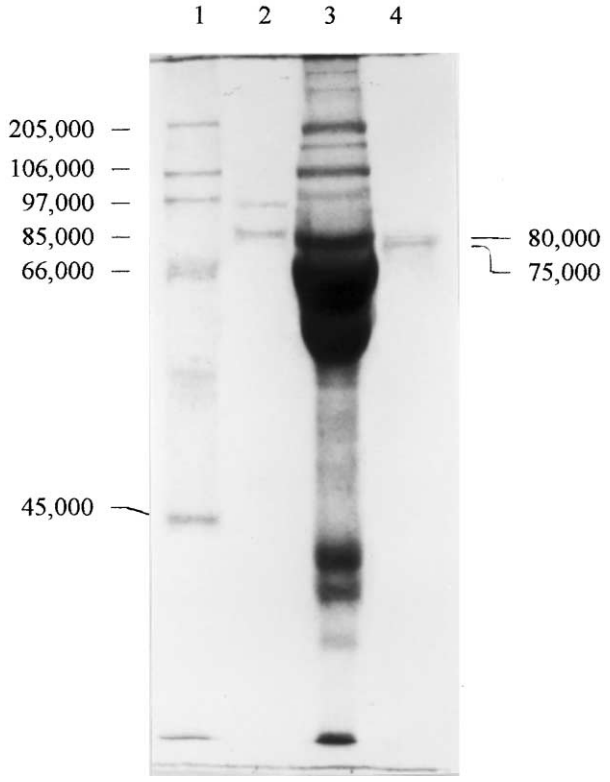

Fig. 1. Electrophoretic pattern of purified pig plasma TGase. Lane 1: standard proteins; Lane 2: TGase of guinea pig liver (Sigma, T5398); Lane 3: pig plasma; Lane 4: purified TGase from pig plasma.

3.2. Effects of TGase on the myosin heavy chain and actin of the breast muscles from spent hens

Little effect of TGase on spent hen breast muscle myosin heavy chain and actin was seen at 47°C for 0–24 h. At 48 h, the intensity of the myosin heavy chain and actin decreased to about 54 and 64%, respectively of the initial values. However, after 2 h at 25°C (Fig. 2), the intensity of myosin heavy chain had decreased and was not seen after 48 h. The intensity of the actin band also decreased as the reaction time increased. A densitometer scan of the electrophoretograms indicated that the relative intensity of the myosin heavy chain was 92, 45, 20, 17, 12, 8 and 1% and that of the actin was 90, 64, 53, 48, 41, 26 and 13%, after 1, 2, 4, 8, 12, 24 and 48 h at 25°C. The intensity of myosin heavy chain was barely visible when incubated for 5 min at 37°C (Fig. 3), the intensity of the actin band also decreased with reaction time. A densitometer scan of the electrophoretograms indicated that after 5, 10, 30 and 60 min, the relative intensities of myosin heavy chain bands were 7, 1, 0 and 0%, respectively, and those of the actin were 63, 34, 25 and 13%, respectively.

The effects of TGase on the breast muscles from spent hens at the different temperatures, indicated a marked decrease in the intensity of myosin heavy chain and

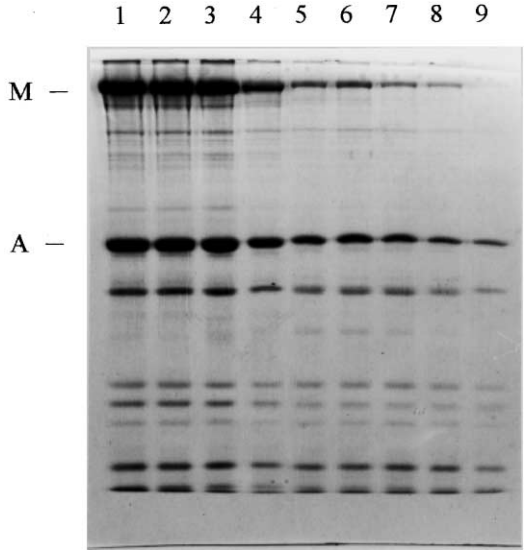

Fig. 2. Effect of TGase on myosin heavy chain and actin of the breast muscles from spent hens at 25°C for 1 (lane 3), 2 (lane 4), 4 (lane 5), 8 (lane 6), 12 (lane 7), 24 (lane 8) and 48 (lane 9) h. Control (or TGase) sample at 25°C for 0 (lane 1) and 48 (lane 2) h. Abbreviations: M = myosin heavy chain; A = actin.

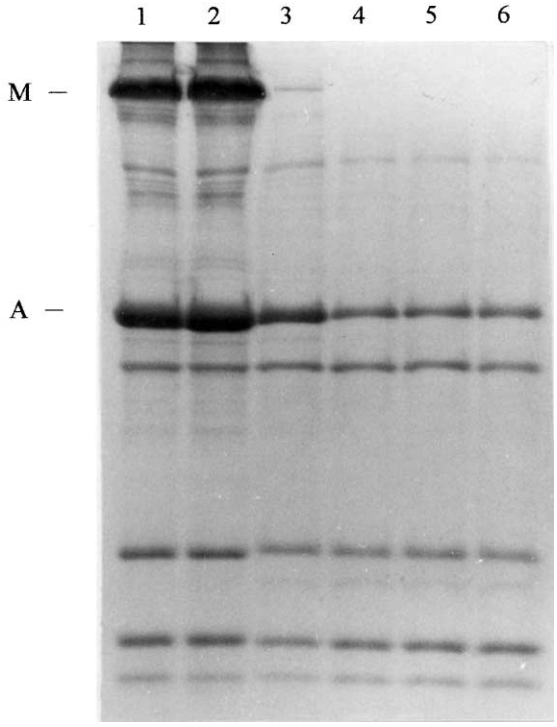

Fig. 3. Effect of TGase on myosin heavy chain and actin of the breast muscles from spent hens at 37°C for 5 (lane 3), 10 (lane 4), 30 (lane 5) and 60 (lane 6) min. Control (or TGase) sample at 37°C for 0 (lane 1) and 60 (lane 2) min. Abbreviations: M = myosin heavy chain; A = actin.

actin bands at 25 and 37°C. Akamittath and Ball (1992) also reported that little reaction of guinea pig liver TGase on crude actomyosin extracted from mechanically deboned poultry meat was seen at 4°C for 26 h, but at 37°C, for 10–40 min, myosin monomer content decreased. Kim et al. (1993) reacted guinea pig liver TGase with beef actomyosin at 35°C for 10–120 min and found the myosin monomer concentration gradually decreased, with the simultaneous appearance of myosin polymers, as the reaction time increased. Cohen et al. (1979) and Kahn and Cohen (1981) also found that plasma TGase can catalyze the formation of glutamyl-lysine bonds in myosin and between myosin and actin, myosin and fibronection, and fibrin and actin. Many workers (Kumazawa, Seguro, Takamuro, & Motoki, 1993; Kumazawa, Numazawa, Seguro, & Motoki, 1995; Kurth & Rogers, 1984; Motoki, Nio, & Takinami, 1984; Sakamoto et al., 1995; Seguro, Kumazawa, Ohtsuka, Toiguchi, & Motoki, 1995) have also reported polymerisation of several food proteins using TGase.

4. Conclusions

In summary, purified TGase derived from the pig plasma is composed of two proteins of molecular weight approximately 75,000 and 80,000. Myosin heavy chain and actin from spent hen myofibrillar proteins when treated with TGase at 25 and 37°C reacted rapidly. Thus, TGase purified from pig plasma has the same functional properties as TGase from other sources and may be used as a protein modifier in meat processing.

References

Aboumahmoud, R., & Savello, P. (1990). Crosslinking of whey protein by transglutaminase. *Journal of Dairy Science*, *73*, 256–263.

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

Cohen, I., Young-Bandala, L., Blankenberg, T. A., Siefring, G. E., & Bruner-Lorand, J. (1979). Fibrinoligase-catalyzed cross-linking of myosin from platelet and skeletal muscle. *Archives of Biochemistry and Biophysics*, *192*, 100–111.

Connellan, J. M., Chung, S. I., Whetzel, N. K., Bradley, L. M., & Folk, J. E. (1971). Structural properties of guinea pig liver transglutaminase. *Journal of Biological Chemistry*, *246*, 1093–1098.

De Backer-Royer, C., Traore, F., & Meunier, J. C. (1992). Polymerization of meat and soybean proteins by human placental calcium-activated Factor XIII. *Journal of Agricultural and Food Chemistry*, *40*, 2052–2056.

Folk, J. E., & Chung, S. I. (1985). Transglutaminases. *Methods in Enzymology*, *113*, 358–375.

Folk, J. E., & Cole, P. W. (1966). Mechanism of action of guinea pig liver transglutaminase. *Journal of Biological Chemistry*, *241*, 5518–5525.

Goll, D. E., Young, R. B., & Stromer, M. H. (1974). Separation of subcellular organelles by differential and density gradient centrifugation. *Proceedings of Reciprocal Meat Conference*, *27*, 250–257.

Ichinose, A., Bottenus, R. E., & Davie, E. W. (1990). Structure of transglutaminases. *Journal of Biological Chemistry*, *265*, 13411–13414.

Jiang, S. T., & Lee, J. J. (1992). Purification, Characterization and utilization of pig plasma Factor XIIIa. *Journal of Agricultural and Food Chemistry*, *40*, 1101–1107.

Kahn, D. R., & Cohen, I. (1981). Factor XIIIa-catalyzed coupling of structural proteins. *Biochimica et Biophysics Acta*, *668*, 490–494.

Kim, S. H., Carpenter, J. A., Lanier, T. C., & Wicker, L. (1993). Polymerization of beef actomyosin induced by transglutaminase. *Journal of Food Science*, *58*, 473–474, 491.

Kurth, L., & Rogers, P. J. (1984). Transglutaminase catalyzed cross-linking of myosin to soya protein, casein and gluten. *Journal of Food Science*, *49*, 573–576, 589.

Kumazawa, Y., Numazawa, T., Seguro, K., & Motoki, M. (1995). Suppression of surimi gel setting by transglutaminase inhibitors. *Journal of Food Science*, *60*, 715–717, 726.

Kumazawa, Y., Seguro, K., Takamuro, M., & Motoki, M. (1993). Formation of ε-(γ-glutamyl)lysine cross-link in cured horse mackerel meat induced by drying. *Journal of Food Science*, *58*, 1062–1064, 1083.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, *227*, 680–685.

Motoki, M., & Nio, N. (1983). Crosslinking between different food proteins by transglutaminase. *Journal of Food Science*, *48*, 561–566.

Motoki, M., Nio, N., & Takinami, K. (1984). Functional properties of food proteins polymerized by transglutaminase. *Agricultural and Biological Chemistry Journal*, *48*, 1257–1261.

Sakamoto, H., Kumazawa, Y., Toiguchi, S., Seguro, K., Soeda, T., & Motoki, M. (1995). Gel strength enhancement by addition of microbial transglutaminase during onshore surimi manufacture. *Journal of Food Science*, *60*, 300–304.

Schwartz, M. L., Pizzo, S. V., Hill, R. L., & McKee, P. A. (1971). The subunit structures of human plasma and platelet Factor XIII (fibrin stabilizing factor). *Journal of Biological Chemistry*, *246*, 5851–5854.

Schwartz, M. L., Pizzo, S. V., Hill, R. L., & McKee, P. A. (1973). Human Factor XIII from plasma and platelets. *Journal of Biological Chemistry*, *248*, 1395–1407.

Seguro, K., Kumazawa, Y., Ohtsuka, T., Toiguchi, S., & Motoki, M. (1995). Microbial transglutaminase and ε-(r-Glutamyl) Lysine crosslink effects on elastic properties of kamaboko gels. *Journal of Food Science*, *60*, 305–311.

Traore, F., & Meunier, J. C. (1992). Cross-linking activity of placental FXIIIa on whey proteins and caseins. *Journal of Agricultural and Food Chemistry*, *40*, 399–402.

Wang, S. M., Greaser, M. L., Schultz, E., Bulinski, J. C., Lin, J. J. C., & Lessard, J. L. (1988). Studies on cardiac myofibrillogenesis with antibodies to titin, actin, tropomyosin, and myosin. *Journal of Cell Biological*, *107*, 1075–1083.