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Dependence of microbial transglutaminase on meat type in myofibrillar proteins cross-linking

Abdulatef Mrghni Ahhmed [a,b], Rumiko Kuroda [a,c], Satoshi Kawahara [a], Kazuyoshi Ohta [a,b], Koji Nakade [a,b,d], Takayoshi Aoki [b], Michio Muguruma [a,b,*]

[a] Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan
[b] The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065, Japan
[c] Junwa Medical Center for Rehabilitations, Miyazaki 880-2112, Japan
[d] Central Research Institute, Ito Ham Food, Inc., Moriya 302-0104, Ibaragi, Japan

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ABSTRACT

The objectives of this study were to determine the factors that cause differences in the improvements of gel strength and ε(γ-glutamyl)lysine (G-L) content in chicken and beef (Japanese black cattle) myofibrillar proteins after adding microbial transglutaminase (MTG). As the amount of MTG added increased, the breaking strength increased progressively ($p < 0.01$) in chicken and beef samples, with the exception of chicken samples treated at 40 °C. The values of elasticity in the chicken samples were lower than those of the beef samples ($p < 0.01$). Surprisingly, the elasticity level, ε(γ-glutamyl)lysine contents and myosin heavy chain (MHC) band sizes of chicken and beef at all levels of MTG were significantly different ($p < 0.01$). The results of this study suggest that MTG activity was affected by MTG inhibitors; that MTG develops the texture of myofibrils differently in different species. However, the activity is limited and inconstant among meat proteins, as suggested by the data collected from the chicken samples. As a result, when the transferable amino acid residues are depleted (cross-linked) by MTG activity, the function of MTG will be insignificant. The correlation between MTG and different sources of meat protein is quite unstable but it is strong, which was observed when chicken and beef responded differently to MTG because their chemical and physiological properties were different. The remarkable rate of formation of cross-linked proteins and the discrepancy between the expected and observed amount of dipeptide raises the possibility that there are enzymes capable of reversing the reaction induced by transglutaminase in chicken and beef myofibrils. In summary, our results suggest that access of MTG to chicken and beef myofibrils is different because it depends on physiological (muscles and their fibre types), biological (substrates) and biochemical (inhibitors and amino acids) variables.

1. Introduction

Treatment with microbial transglutaminase (MTG) enhances the texture and gel strength of meat and meat proteins in many products by forming a bond between glutamine and lysine, which improves the rigidity and gel elasticity of meat products, avoiding some undesirable attributes such as stickiness, high viscosity and excessive meat adhesiveness.

Muguruma and his associates conducted many studies on the functionality of MTG and its effects on the gelation properties of meat products. The results of a study on the improvement in chicken sausage texture induced by transglutaminase at a low level of phosphate suggested that the texture was improved by the formation of a network structure, which contributed to the hardness of meat gels with added biopolymers (Muguruma et al., 2003). There have been many studies of the gelation of chicken myofibrillar proteins: Lesiow and Xing (2001) reported that under dynamic condition aggregation has a major role in producing differences of gel elasticity between myofibrillar proteins in both white and red meat. A study of the inconsistency in the improvement of gel strength in chicken and pork sausage induced by MTG was conducted by Kawahara, Ahhmed, Ohta, Nakade, and Muguruma (2007), who suggested that the binding between myofibrillar proteins and MTG is strong. Furthermore, MTG achieves different levels of improvement in chicken and pork products that are treated mechanically, such as sausages.

It is necessary to understand the protein reactions induced by MTG binding and the effect on ATPase activity in meat proteins because of the important economic benefits of using MTG to improve the textural quality of meat products. MTG catalyses the formation

* Corresponding author. Address: Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan. Tel./fax: +81 985 58 7203.
E-mail address: muguruma@cc.miyazaki-u.ac.jp (M. Muguruma).

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of an ε-(γ-glutamyl)lysine bond, and therefore depends on the availability of glutamic acid and lysine residues, as well as other factors that are discussed below. El Alaoui, Legastelois, Roch, Chantepie, and Quash (2006) reported that the amount of N isopeptides follows transglutaminase activity closely during the lag phase of growth of both chicken embryo cells and human carcinoma of the larynx cells. Some details of the mechanism of MTG activity are not known and the substrates of tissue transglutaminase have not been identified in cells or tissues (Fesus & Tracsa, 1989).

MTG aggregates proteins by catalysing the formation of bonds between amino acid residues by the transfer of the active radicals of certain amino acids (free or aggregated) of isopeptides to other amino acids. It is well-known that each amino acid has at least one carboxyl (COOH) group, which is acidic, and one amino (NH_2) group, which is basic. Preferably, amino acids join together in long or short chains, the amino group of one amino acid linking with the carboxyl group of another. The linkage is known as a peptide bond, and a chain of amino acids is known as a polypeptide. However, peptide bonds sometimes occur by the action of enzymes, such as MTG, which was used in this study. It plays a functional role in coupling glutamine with lysine, which eventually forms a polymer with ε(γ-glutamyl)lysine structure. The potential catalytic functions of MTG as well as other residues cause the side chains of glutamine (C-terminal) to interact with the side chains of lysine (N-terminal). So MTG catalyses the interconnections of myofibrils and giant polymers can be created. This final stage of protein aggregation and accumulation improves the gel elasticity of meat protein and forms a protein–protein network (PPN). The gelation that is induced by adding MTG to meat products has been observed by many researchers, and gel strength is further enhanced by heat treatment subsequent to the action of MTG.

We have conducted studies on the effects of MTG on cross-linking proteins in different minced meats (Ahhmed et al., 2007a; Kawahara et al., 2007), and we have observed that MTG catalyses protein–protein interaction differently depending on the temperature. This study was designed to compare the effects of MTG on the gel properties of different myofibrillar proteins. We investigated the action of MTG on chicken and beef myofibrillar proteins at two different temperatures. Ramirez and Xiong (2003) studied the effects of transglutaminase on the gelation of a myofibrillar and soy protein mixture, and reported that transglutaminase is an excellent agent for producing an adhesive mixed protein gel structure with a reduced requirement for myofibrillar proteins. Consumers in many countries have become more demanding about food quality (Ahhmed et al., 2007a), and consumer acceptance of processed meat is determined by the product quality, particularly flavour, texture, and storage stability (Ramirez & Xiong, 2003). Earlier studies showed that the proteins in chicken and beef respond differently to the activity of MTG. There is a need to determine the reason why MTG improves the texture of meat differently and the mechanisms underlying its actions with myofibrillar proteins of different species. The objectives of this study were to determine reactive MTG levels for chicken and beef myofibrillar proteins as substrates and to focus specifically on the factors affecting the reaction of MTG with meat proteins.

2. Materials and methods

2.1. Materials

The thighs of 8-week-old chickens were sourced from a butcher in Miyazaki, Japan, and stored for 1 day at 4 °C; the pH upon arrival in the laboratory was 5.5. The *biceps femoris* muscles of 5–6-year-old post-breeding Japanese black cattle were obtained from Minami Kyushu Chikusan Kogyo Ltd., Kasgoshima, Japan. The beef was vacuum-packed and stored for 4–5 days at 4 °C; the pH was 5.6 and the meat was grade A-3. The visible fatty tissue was removed from both types of meat.

2.2. Methods

The samples were divided into two groups: group 1 contained the control and positive samples and was incubated at 40 °C for 30 min in a water-bath (Thermo-minder, Sm-05, Taitec, Tokyo, Japan); group 2 contained negative and positive samples and was heated at 78 °C for 30 min in a shaking water-bath (Personal-11, Taitec, Tokyo, Japan). Group 1 samples were incubated at 40 °C to avoid heat-denaturation of proteins. The functional properties of myofibrillar proteins are related to their thermal stability and interactions (Foegeding & Liu, 1995). The samples were subjected to a test of textural properties with a creep metre, and other measurements were made, such as protein extractability and evaluation of the degree of protein cross-linking by SDS-PAGE. Protein concentrations were determined by the biuret method (Gornall, Baradawill, & David, 1949). The ε(γ-glutamyl)lysine (G-L) content was determined by HPLC after enzymatic digestion as described (Ahhmed, Kawahara, Soeda, & Muguruma, 2005; Ahhmed et al., 2007a; Kawahara et al., 2007).

2.3. Preparation of MTG solution

Ando et al. (1989) reported the purification of MTG from the culture filtrate of strain S8112, which was assumed to belong to the genus *Streptoverticillium*. The transglutaminase secreted by *S. mobaraense* is used in the food industry (Kikuchi, Date, Yokoyama, Umezawa, & Matsui, 2003). In this study, MTG was obtained from Ajinomoto Co., Japan, and dissolved in 20 mM NaCl as described previously (Ahhmed et al., 2007a; Erwanto et al., 2005; Kawahara et al., 2007). The concentration of MTG used in this study was 3.4 mg/ml.

2.4. Preparation of myofibril samples

The chicken and beef were minced separately in a meat grinder (MK-GL 20-W National), placed into a borate/KCl solution, homogenised in a laboratory knife mill (Grindomix GM 100, Retsch Kurt Retsch GmbH & Co. KG, Germany), and centrifuged (Himac CR 20E, Hitachi, Tokyo, Japan) at 12,000 rpm for 20 min at 3 °C. The homogenates were centrifuged three times; after each centrifugation, the homogenates were mixed gently with borate/KCl buffer solution and centrifuged again. The supernatant, which contained water-soluble proteins, mitochondrial enzymes, haemoglobin, myoglobin, and inorganic substances, was discarded. The upper layer of the precipitant was removed and used as a myofibrillar protein pellet as described (Ahhmed et al., 2007b). The final samples were made from 50 g of myofibrils, 30 ml of distilled water, 1.4 g of NaCl, and 0.21 g of sodium pyrophosphate; MTG (3.4 mg/ml) was added in 0.1 ml portions to different levels; zero, 0.1 ml, 0.2 ml, 0.3 ml up to1.0 ml (we studied the action of MTG at 10 levels).

2.5. Textural properties test

The texture of the chicken and beef myofibrillar protein preparations was measured to determine the effect of adding MTG to meat products. Shear force was evaluated with a knife fixed on a creep metre (Rheoner II, Yamaden Co. Ltd., Tokyo, Japan). The samples were subjected to a puncture test as described previously (Ahhmed et al., 2007a). The samples were prepared as a 1 cm × 1 cm × 1 cm cube and the knife speed was 1 mm/s. Five