Evaluation of the discrepancy in physiochemical and rheological properties of cross-linked myosin B proteins and biopolymers catalyzed by transglutaminase in meats

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Abstract

The objective of this study was to determine the range of protein characteristics diversity in chicken and beef as microbial transglutaminase (MTG) catalyzes their myosin B proteins. It is well-known that MTG improves the texture of meat and repairs any undesirable textural attributes (e.g. stickiness). MTG is regarded as having "super activity" which acts as combining agents, helping some amino acids "fit together" in meat proteins to generate numerous polymers, which are considered as important residual bio-products. After adding MTG to myosin B, the improvements of gel induration, ε(γ-glutamyl)lysine (G-L) content, and the viscosity measurements were higher in beef than chicken. Bands on SDS-PAGE revealed that the same proteins in various meat species vary in their size and structure. As a result, the protein aggregation in chicken and beef suggests that not all MTG-dependence biopolymers in meat proteins are created equally. As MTG reacts differently with meat proteins, the retained water capacity between proteins perhaps declines and weakens the protein bonding action. We observed that myosin B formed aggregates which associated in micelles to give the multi-structure of G-L, surprisingly more of these structures were found in beef than chicken. Data suggest that the generated mega structure of protein molecules in chicken and beef may vary greatly in size and complexity after adding MTG. We suggest that the optimal cross-links in myosin B proteins induced by MTG are heterogeneous in chicken and beef.

Background

Under certain chemical and physiochemical circumstances the catalytic agent MTG converts amino acids in meat proteins to new biopolymers, which improves the rigidity and gel elasticity of meat products. The findings of these studies showed that addition of MTG to chicken and beef myosin B generates different products (polymers) as the meats vary in morphological properties. The newly-formed polymers produced after MTG treatment, differ in terms of both rheological and physiochemical properties; the variation basically depends on the meat species form which the proteins are derived. It is necessary to evaluate the essential factors that affect the nature of the final product which varies widely between meat species even if the samples are treated under similar conditions (Ahhmed et al. 2008). In order to evaluate the discrepancies in the yield of biopolymers induced by MTG in the two meat species under the same experimental conditions, this study was designed and conducted to compare the effects of MTG on gel indurations (textural properties), ε(γ-glutamyl)lysine (ε(γ-G)L) content, surface hydrophobicity and viscosity of myosin B. This study was

also carried out to determine the diversity of protein characteristics in chicken and beef after adding MTG.

Materials and methods

The thighs of 8-week-old local Japanese broiler fowl and the biceps femoris muscles of 5–6-year-old post-breeding Japanese black cattle were sourced from a local butcher in Miyazaki, Japan. Ajinomoto Co., Japan, provided us with MTG powder, which was dissolved in 20 mM NaCl, and the concentration of MTG used in this study was 3.1 mg/ml. We studied the action of MTG on myosin B at 4 levels (1:500, 1:200, 1:100, and 1:50). The samples were divided into two groups: group 1 contained the control samples (-MTG), group 2 contained the positive samples (+MTG), both groups were incubated at 40 °C for 30 min in a shaking water-bath (Personal-11, Taitec, Tokyo, Japan).

Results and discussion

In the present study beef samples consistently showed higher values for the breaking indurations than chicken samples too (Fig. 1). This is due to the length of the myosin molecules and to the strong affinity between beef proteins and MTG in addition to the fact that chicken myosin contains other attached proteins which may play inhibitory roles. Reactive amino acids on the surface of beef myosin B are in closer proximity than those on the surface of chicken myosin B as evidenced by the results of hydrophobicity studies. In both species the more MTG that was added the greater the increase in viscosity until the samples reached a state of gelation where the stainless steel ball was no longer able to move inside the tube (Fig. 2). We found that beef proteins were more viscose than chicken, as in the case of beef the ball moved at a slower rate, and the gelation state was achieved more rapidly than in chicken samples.

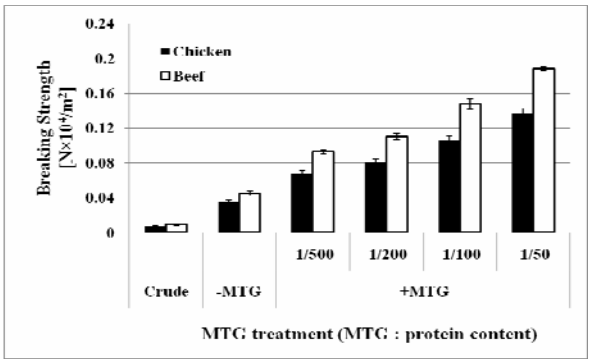

Fig. 1. Changes in breaking indurations of gel in chicken and beef myosin B as a function of MTG and temperature.

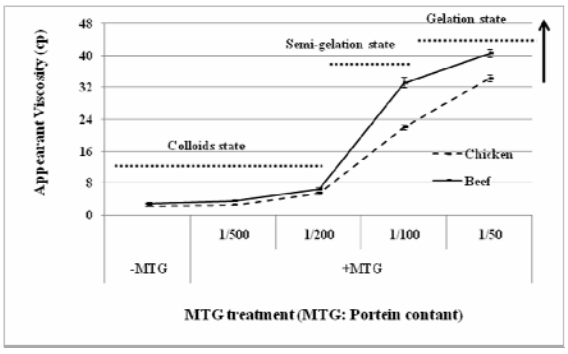

Fig. 2. The changes in viscometric properties of chicken and beef myosin B as a function of MTG.

Fluorescence intensity measurements indicated a decreased surface hydrophobicity as a function of MTG treatment on myosin B (Fig. 3). Such a decrease in surface hydrophobicity of highly hydrophilic proteins (myosin B) generally indicates that the polarity of the amino acids has been reduced, which means that a considerable number of amino acids have interacted with each other as a result of the action of MTG. The more structures created by the action of MTG the greater the reduction in hydrophobicity. Moreover, the differences in control samples were probably due to the amino acids being encased inside the strands of proteins in myosin B as there appeared to be fewer differences in chicken samples than in beef. The physical properties (intensity, Rf and relative quantity, etc.) of MHC bands visualized by SDS-PAGE revealed that the

same proteins in various meat species vary in their size and structure (Fig. 4). The G-L contents in the myosin B of both chicken and beef increased significantly ($P< 0.05$) and ($P< 0.01$), respectively in samples treated with MTG (Fig. 5). It is not only the multi-structure of G-L that contributed to the differences in the data, but we suggest that an additional reaction also occurred between the carboxyl group and amino groups of other protein at certain amino acids ends.

MTG is a biopolymer-generating engine that couples amino acids to produce a mega complex of molecules that enhance the rigidity and textural properties of meat. We observed that the cross linked molecules of myosin B in both species were aggregated and fitted together in a micelle design. Those multi-structures have many projections which are thought to be myosin B tail.

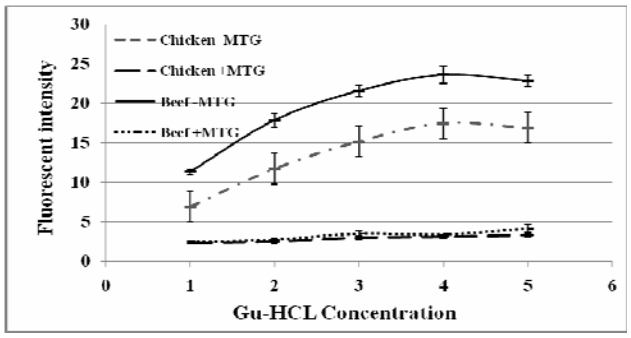

Fig. 3. Effect of Gu-HCl on fluorescence intensity (surface hydrophobicity) of cross linked proteins in myosin B.

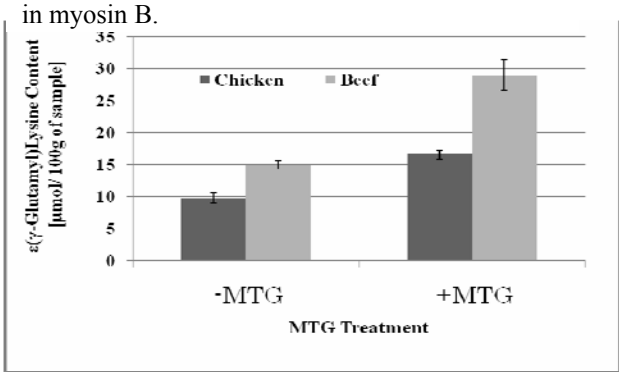

Fig. 5. Changes in ε-(γ-glutamyl) lysine content (µmol/100g of sample).

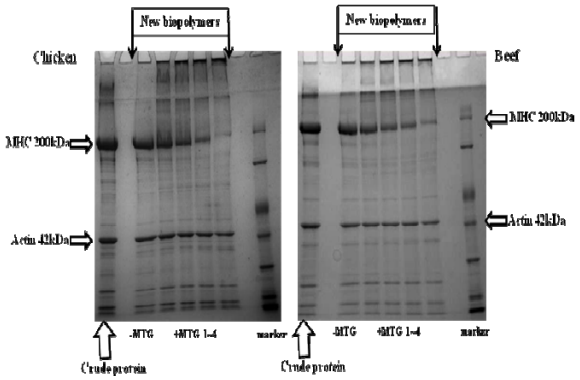

Fig. 4. SDS-PAGE pattern illustrates how MTG influences MHC in chicken and beef myosin B.

Conclusions

After examining the rheological and the physicochemical properties as well as the microscope images, we hypothesized that the structure of certain proteins in chicken and beef differ from one another; however the variance in the myosin B is a consequence of the amino acids sequences.

If there is a large number of Gln and Lys residue encased in the strands of protein, they will be unable to react with MTG, and this will lead to greater diversity in the rheological and physico-chemical properties of different meat species. This was substantiated by the hydrophobicity and scanning electron microscope data. Another possibility is that the variation in the total data also provides a strong indication that some proteins in chicken samples play an inhibitory role which leads to a reduction in MTG activity.

References

Ahhmed, A.M., Kuroda, R., Kawahara, S., Ohta, K., Nakade, K., Takayoshi, A., Muguruma, M. (2008). Dependence of microbial transglutaminase on meat type in myofibrillar proteins cross-linking. Food Chem. *In Press*.