Differences in myosin heavy chain mRNA expression levels among chicken muscles reflect differences in protein polymerization by transglutaminase

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Abstract

In this study we investigated the differences in microbial transglutaminase (MTG)-induced protein polymerization levels of the major muscle protein myosin heavy chain (MHC), in relation to their mRNA ratios, among different chicken muscles, and quantified their mRNA expression levels. SDS-PAGE demonstrated that MHC from these muscles differed in terms of its response to MTG. The expression levels of seven different MHC genes were investigated in four different muscles (pectoralis major, biceps femoris, gizzard and heart), using real-time-polymerase chain reaction analysis. The results indicated variations in expression of these genes, in terms of both specificity and quantity. The dominant genes in the pectoralis and biceps muscles were MyHC-6 and MyHC-1, respectively. Gizzard muscle expressed only MyHC-11, but this was expressed at high levels, and was also the most highly expressed gene among all the muscles. MyHC-7 was expressed only in cardiac muscle, though this muscle also expressed other MHC genes. The relationship between MHC mRNA expression and protein specificity suggests that the same proteins are responsible for different biological and biochemical reactions in different muscles. These results also indicate that variations in MHC mRNA expression parallel to the MHC protein specificity in different chicken muscles, and may account for the different MTG reactivities demonstrated by SDS-PAGE. We conclude that variations in gelation properties induced by transglutaminase between muscles reflect the different MHC protein levels, which in turn reflect the differences in MHC mRNA ratios among the different muscles.

I. Introduction

All types of chicken skeletal and smooth muscles express MHC proteins during growth, but with different and specific ratios of MHC genes. The MHC proteins present in the muscles in adult birds reflect their mRNA expression levels. MTG (EC 2.3.2.13) is used as a biocatalytic meat product-improvement additive, which is widely used to improve the gelation properties of proteins, thus avoiding some undesirable attributes such as stickiness and excessive meat adhesiveness (Ahhmed, et al., 2007). We examined the rheological properties of whole chicken muscles (skeletal muscle, smooth and cardiac muscles) and we found that MTG significantly improved the texture of whole chicken muscles. The results of a previous study (Ahhmed, et al., 2009b) support the suggestion that the abilities of MTG to catalyze the crosslinking of muscle proteins is muscle-type specific. The differences in protein extractability among chicken muscles extracted with Guba-Straub-ATP (GS-ATP) solution could be related to the reaction mechanisms between MTG and the major meat-muscle protein, MHC, as suggested by SDS-PAGE analysis. We hypothesized that differences in MHC structure or isoforms would therefore be reflected by different reactivities with MTG, which would consequently improve the texture and gelation states of meat from different muscles differentially. It is of great interest to suggest that variation in gelation states induced by MTG among chicken muscles related to the isoforms in major proteins (MHC). In this study, we therefore evaluated the major differences in MHC mRNA expression patterns (isoforms) among skeletal, smooth and cardiac muscles in chicken (sourced from the same bird), in order to identify the factors influencing MTG activity.

II Materials and Methods

Broiler chickens (*G. gallus*) at 8 weeks old were sourced from a local butcher in Japan. After slaughter, four muscle types were isolated (thigh, breast, gizzard, heart) and their pHs were determined to be 5.5. The

concentration of MTG stock solution used in this study was 3.2 mg/ml. The meat was minced in a meat grinder, and formed into sausages by mixing 50 g ground meat with 30 ml distilled water, 1.4 g NaCl, and 0.21 g sodium pyrophosphate in a meat blender. Subsequently, 1 ml MTG solution (or 1 ml water in the control) was added. Samples from each group were incubated at 40°C for 30 min. Proteins of samples were extracted in GS-ATP solution (0.09 M KH_2PO_4, 0.06 M K_2HPO_4, 0.3 M KCl, and 1 mM ATP (pH 6.5)). The extracted proteins were subjected to SDS-PAGE experiment, followed by Coomassie Brilliant Blue staining, as described previously (Ahhmed et al., 2008; Ahhmed et al., 2009a). We also extracted RNA from other fresh muscles, and the concentrations were then measured using a spectrophotometer (Jasco, V-550, spectrophotometer, Nihon Bunko, Japan). The remainder of the samples were stored at -80°C for cDNA synthesis. cDNA synthesis was carried out with slight modification of the method described by Ozaki, et al., (2010),

In the current study, we analyzed MHC mRNA expression using real-time-polymerase chain reaction (PCR) techniques to determine the expression ratios of the different MHC genes in different muscles. The MYH primers and ribosomal protein large P1 (RPLP1) primers used as a reference gene for real-time PCR analysis were purchased from Takara Bio and their specificities were determined using BLAST at NCBI (1990). Finally, samples were placed in a PCR cycler (Applied Biosystems, GeneAMP-PCR system 9700, USA) and the reactions were carried out as follows: 95°C for 30 seconds, followed by 45 cycles of 95°C for 5 seconds, and 60°C for 20 seconds. Each run was completed with a melting curve analysis to confirm the specificity of amplification and the absence of primer dimers. The amplification of genomic DNA was prevented by DNase treatment of extracted RNA.

III. Results and Discussion

3.1. SDS-PAGE*:*

The sizes and densities of the MHC bands differed among the different muscles, as shown by SDS-PAGE (Fig. 1). This indicated differences in protein structure, which could explain the differential effects of MTG. Differences in the locations of Gln and Lys could account for the different reactivities with MTG, which connects to proteins through these amino acids. The differences in MHC protein structure may reflect differences in mRNA expression, and mRNA expression levels were therefore investigated in four different chicken muscle types.

3.2. Real-time-PCR:

In general, skeletal and smooth muscles differed in terms of the relative expression levels of HMC genes. Cardiac muscle was similar to skeletal muscle, but with some variations in the types and relative proportions of MHC genes expressed. MyHC-1 was expressed in skeletal (and cardiac) muscles, but not in gizzard (smooth) muscle; however, its ratio relative to other MHC genes varied dramatically, and it was most highly expressed in the biceps muscle. MyHC-2 was also detected in skeletal (and cardiac) muscle, but not in gizzard muscle. MyHC-4 was similarly expressed in pectoralis and cardiac muscles, but its expression level was slightly higher in biceps muscle. The results of this study suggest that the MyHC6 gene product plays a crucial role in regulating the reaction between MTG and glutamine and lysine in MHC. Biceps muscles showed the greatest improvement in texture following MTG treatment, and the ratio of MyHC6 expression between pectoralis and biceps muscles was 3:1. MyHC-7 could play a role in the morphological uniqueness of cardiac muscle, where it was expressed at a low level. Gizzard and cardiac muscles both expressed MyHC-11, which was not expressed in pectoralis or biceps muscles; the expression level in gizzard muscles was much higher than that in cardiac muscles (340:1),(fig. 2-5). This suggests that gizzard and cardiac muscles might share some attribute, possibly related to their continuous physiological and biological functions, and their mechanical actions in digesting food or pushing blood into the blood vessels. However, they remain distinct in many of their characteristics and biochemical mechanisms.

These results suggest that the biological and biochemical parameters of the muscles should be taken into consideration when using MTG to improve meat texture, because the structural properties of the MHC proteins present in different muscle types will influence their response to MTG.

IV. Conclusions

This study confirmed that the mRNA expression levels of different MHC genes in chickens varied among different muscles, and that these variations in gene expression levels reflected the muscle-specific protein expression levels. The results support our

hypothesis that the improvement of meat texture by MTG treatment is not only species-specific, but also muscle-specific. The muscle-specific ratio of MHC proteins paralleled MHC gene expression, resulting in equivalent, muscle-specific responses to MTG. Inter-muscle variations in protein-protein interactions with MTG reflected the muscle-specific responses to MTG crosslinking.

In conclusion, the different ratios of expressed MHC mRNAs in chicken muscles leads to inter-muscle differences in many biological and biochemical reactions, including the reaction between MHC proteins and transglutaminase. Muscles from different species and different muscles within the same species differ greatly in terms of their MHC mRNA contents. This leads to diversity in rheological, physicochemical and structural properties among different chicken muscle types. The results of this study contribute to our understanding of the use of MTG in the chicken-product industry, by demonstrating the role of MHC isoforms in controlling the textural changes induced in different muscles by MTG.

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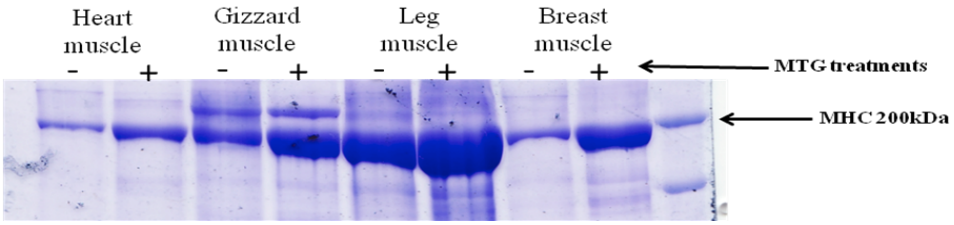

Fig. 1. SDS-PAGE pattern of MHC proteins bands in chicken muscles treated with MTG.

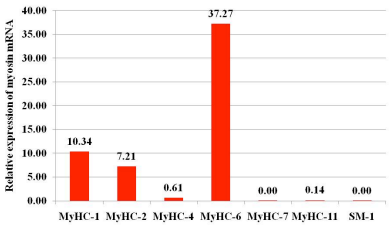

Fig. 2. MHC mRNA expression in skeletal muscles (pectoralis).

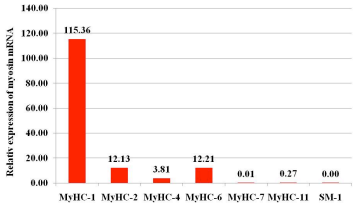

Fig. 3. MHC mRNA expression in skeletal muscles (biceps).

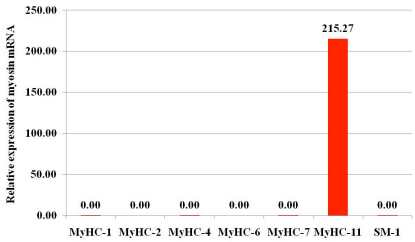

Fig. 4. MHC mRNA expression in smooth muscles (gizzard).

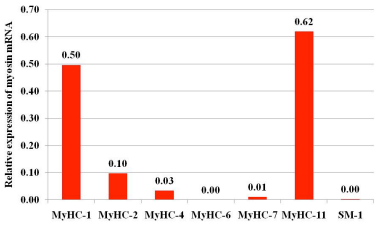

Fig. 5. MHC mRNA expression in cardiac muscles (heart).