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PRESENCE OF GLYCOPROTEINS IN CHICKEN MYOFIBRILLAR FRACTIONS

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Introduction

Protein glycosylation is an effective method for improving the functional properties of food proteins. Non-enzymatic glycosylation is a complex series of reactions between amino groups of proteins in living systems. Recently, Saeki et al. (1997) and Sato et al. (2000) noted that fish and shellfish myosin in myofibrils became water-soluble in a physiological condition. Syrovy and Hodny (1993) revealed that in adult rat skeletal muscle heavy chains of myosin, actin, and tropomyosins were glycated i*n vitro*. There are not yet known glycoprotein or glycosylated myofibrillar proteins in skeletal muscle of domestic animals.

Objectives

The aim of this work is to investigate the presence of the glycoprotein in chicken myofibrillar fractions.

Methodology

Preparation of myofibril fractions: Myofibrils were prepared from the fresh chicken pectral muscles as described by Tatsumi et al. (1993), suspended in a basal solution containing 0.1 M KCl, 5 mM EDTA, 1 mM DTT, 1 mM NaN_3 and 10 mM Tris-maleate buffer, pH 7.0, and spun down for 10 min at 1000 x g_{max} to eliminate mitochondria, fragmented sarcoplasmic reticula and cell membranes. The washing of myofibrils was continued 5 times. The resulting sediment was collected, and dialyzed against the distilled water, four times, for at least 6 hours each. The dialysate was concentrated using the collodion bags and then lyphilized (Mf fraction).

Deglycosylation from Mf fraction: Glycoproteins (50 µg) were hydrolyzed in 2.5 M trifluoroacetic acid (TFA) at 100°C for 6 h in order to liberate neutral and amino sugars. TFA was removed from the hydrolysate by rotary evaporation. The hydrolysate was lyophilized and dissolved in water. This solution was passed through an anion-exchange coloum (AG 1-X8 Resin hydrogen form, Bio-Rad Laboratories, USA), and the elution was done with distilled water. The eluted fraction was then passed through a cation-exchange coloum (AG 50W-X8 Resin hydrogen form, Bio-Rad Laboratories, USA), was

eluted with distilled water, the fraction was collected, and freeze-drying. The ovalbumin (OA) was used as a positive control.

SDS-PAGE and Glycoprotein blots: Myofibril fractions were suspended with a solution containing 5 mM EDTA and 5 mM Tris-HCl buffer, pH 8.0, and solubilized in a solution containing 1 % SDS, 1% 2-mercaptoethanol, 10% glycerol, 5 mM EDTA and 5 mM Tris-HCl buffer, pH 8.0, then heated at 100°C for 2 min. Samples were subjected to SDS-PAGE by the method of Laemmli (1970) using 7.5 to 17% polyacrylamide and 1.75 to 3.3% glycerol gradient gels (Ahn et al., 2003), and 15% polyacrylamide gels. In the method of Weed (1976), the myofibril samples containing 10 mM Tris-Bicine (pH 8.3), 1% SDS, 2% 2-mercaptethanol, 10% glycerol, and bromphenol blue tracking dye, were heated at 100°C for 5 min and then applied to 7.5% polyacrylamide gels. For the electrophoretic transfer of proteins to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, USA), myofibrillar proteins separated by SDS-PAGE were electrophoretically transferred on to PVDF membranes by the method of Towbin et al. (1979), and were visualized by methods reported previously (Kondo et al., 1991) using a kit (GP Sensor, Seikagaku Corp., Japan).

Protein and Sugar determination: Concentrations of proteins were measured by the biuret reaction (Gornall et al., 1949) standardized by the micro-Kjeldahl process. Sugar concentration was measured by the phenol-sulfuric acid method (Dubois et al., 1956).

Results & Discussion

To confirm the glycoprotein in Mf fraction, we would try to visualize the glycoprotein using the Periodic-Acid Shiff based reactions between acid-Schiff base with biotin hydrazide and, streptavidin–HRP on the PVDF membrane. Figure 1 shows the exsistence of sugar chain on the dot-blot. The protein concentrations in OA and Mf fractions were 15 μg and 45 μg, two images were the protein staining (Fig.1A) and the sugar staining (Fig.1B). Two kinds of images showed the protein staining (Fig.1A) and the sugar staining (Fig.1B). Figure 1B indicates the positive, so the myofibrillar protein may conjugate the sugar chain, thus, may be glycoprotein. Table .1 showed the sugar concentration in Mf farction. Nuetral and acid sugar fraction prepared from Mf, and the total sugar content in Mf was 2.52 ± 0.37 μg/mg freeze-dry weight, 3.47 ± 0.52 μg/mg myofibrillar protein. OA derived fraction, positive control, have 31.43 ± 3.22 μg/mg freeze-dry weight, 34.93 ± 3.57 μg/mg protein.

To estimate the glycoprotein in Mf, we carried out the protein staining and the sugar staining in 7.5-17% polyacrylamide gradient gels (Fig.2), 7.5% gel (Fig.3), and 15% gels (Fig.4). Figure 2 showed several positive bands, two bands were high molecular weight more than myosin heavy chain, and four bands were low molecular weight less than α-actinin. In other buffered electrophoresis system (Fig.3), there were two positive bands around myosin heavy chain, and one lower molecular weight-band than α-actinin. In Fig.4, we estimated one lower molecular weight-band than α-actinin. Compared with the immunostaining image using anti-α-actinin antibody, there was not a band in same position. It is assumed that this band is the 85 kDa band. We suggested that there were several glycoproteins among the myofibrillar proteins.

Conclusions

We prepared myofibril fraction from chicken pectral muscle. This fraction showed the positive spot against sugar staining and this sugar concentration had 3.47 μg/mg myofibrillar protein. Two types of the buffered system SDS-PAGE, there were several positive bands against sugar staining. Among the different concentration gels, there were common positive bands containing 85-kDa band. We suggested that there were glycoproteins in myofibril fraction.

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Tables and Figures

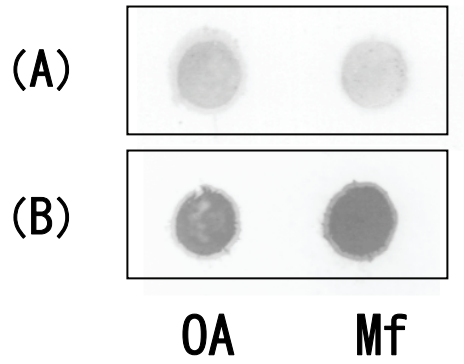

Figure 1 Sugar and protein staining images
Sugar staining; (B) CBB staining OA, ovalbumin; Mf, myofibril fraction

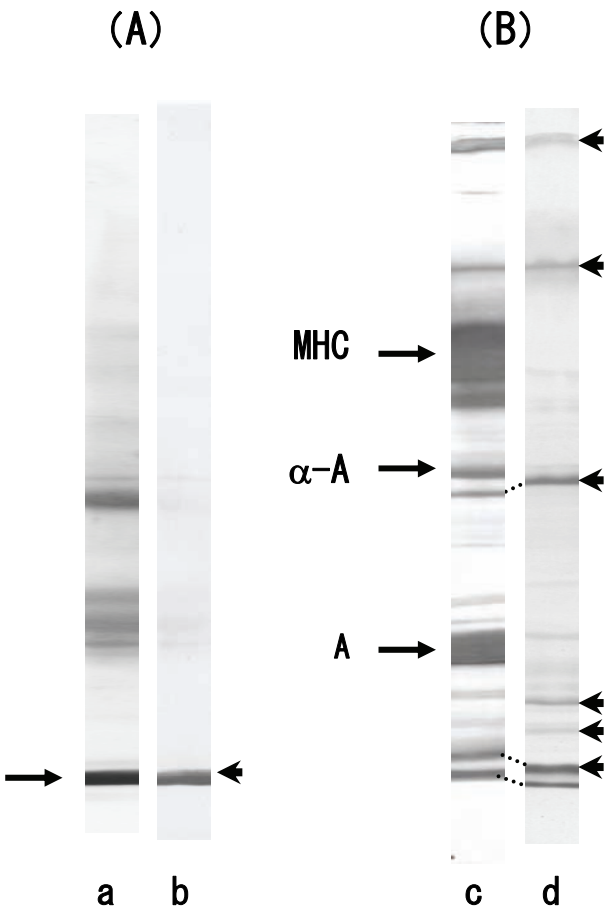

Figure 2 7.5-17% SDS-PAGE image
CBB staining (a,c); Sugar staining (b,d) A, ovalbumin; B, myofibril fraction

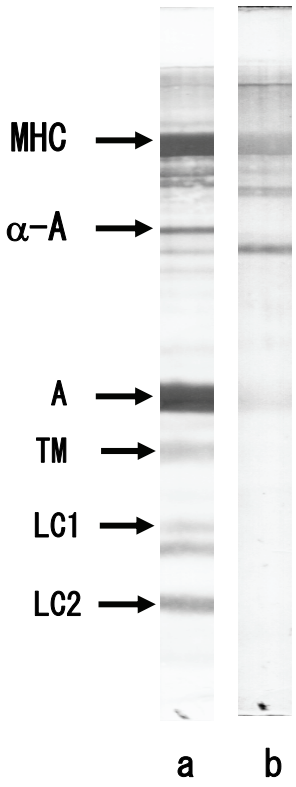

Figure 3 7.5% SDS-PAGE image
CBB staining (a); Sugar staining (b)

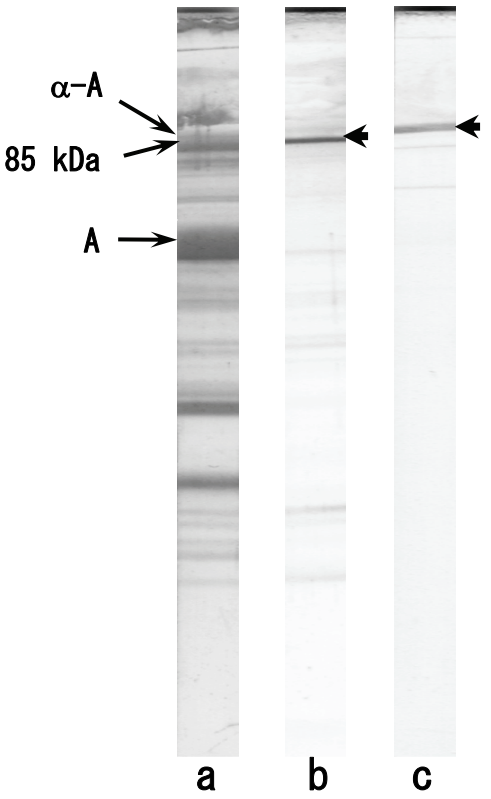

Figure 4 15% SDS-PAGE image
CBB staining (a); Sugar staining (b) ;Anti-α-actinin antibody staining (c)