4-P14

SOLUBILIZATION OF MYOFIBRILLAR PROTEINS OF CHICKEN SKELETAL MUSCLE IN WATER

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BACKGROUND

There have been many attempts to change the types of food and ways in which food can be utilized. For example, soybean and milk proteins have all been examined in detail. Although meat, or skeletal muscle of livestock and poultry are essential protein supplies for human beings and rich in essential amino acids, their availability has been limited because of their properties. The main limiting factor is the solubility of meat proteins. As some meat proteins are insoluble in water or solution of low ionic strength, high concentration of sodium chloride is needed to solubilize them.

Muscle proteins have been generally grouped into one of three categories based on their solubility. The salcoplasmic proteins are soluble in water or solutions of low ionic strength. The myofibrillar proteins are soluble in relatively high concentrations of salt(>0.3 M). The storoma proteins are insoluble in high concentrations of salt, consisting of connective tissue proteins and myofibrillar proteins unextracted. The myofibrillar proteins are the major portion of meat protein and occupy about 50% of total proteins of muscle tissues. So, if it is possible to solubilize myofibrillar proteins in water or solutions of low salt concentration, they could be used in many ways, for example, as liquid nutrition for patients who couldn't eat and as a source of nutritional supplement in case of emergency.

In 1949, A.Szent-Györgyi described in his writings that rabbit skeletal muscle myosin was soluble in water. Hultin and his collaborators have performed the solubilization of cod, chicken breast and mackerel light muscles in solutions of low ionic strength. However, we have not been able to succeed in the solubilization of chicken breast muscle using their method.

OBJECTIVES

For the purpose of making meat available for animal protein food, we have tried to make skeletal muscle proteins soluble in water or solutions of low ionic strength. In this paper, we have established a method to solubilize myofibrillar proteins of chicken skeletal muscle in water by improving Hultin's method.

MATERIALS AND METHODS

Materials

Chicken breast and leg muscles and pork *longissimus dorsi* muscle were used.

Preparation of Water-Soluble Myofibrillar proteins

The procedure is shown in Figure 1.

〈washing procedure〉

Comminuted chicken breast muscle was homogenized with a cold solution containing 25 mM NaCl and 5 mM L-histidine, then centrifuged for 20 min at 18,000 g. The supernatant was removed and the same procedure was repeated twice against precipitate. The precipitate was washed with a cold solution containing 2.5 mM NaCl and 5 mM L-histidine and then spun down.

〈water-extraction procedure〉

The final precipitate obtained by washing step was added to 20 volume of distilled water, followed by sonication for 5 min. The suspension was centrifuged for 20 min at 37,000 g. The supernatant obtained was defined as a fraction of water-soluble myofibrillar proteins.

Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed according to the method of Fairbanks *et al.* Gels were stained with Coomassie Brilliant Blue R-250.

RESULTS AND DISCUSSIONS

The SDS-PAGE patterns of the chicken breast muscle proteins at the various washing and extraction steps are shown in Figure 2. Lanes 1and 2 are whole chicken breast muscle and supernatant removed by 4th wash, respectively. Lane 2 contains several kinds of proteins and one of these was identified with α-actinin. Lanes 3 and 4 show the suspensions before and after sonication, respectively. Connectin/titin observed in lane 3 was not found in lane 4. This fact suggests that connectin/titin was destructed by sonication. Lane 5 shows water soluble myofibrillar proteins. This fraction contained major myofibrillar proteins and comprised about 45% of the total muscle proteins (80% of total myofibrillar proteins). α-Actinin and connectin/titin are major components of myofibrils of skeletal muscle, the former constitutes the Z-disk and the latter connects thick filament to the Z-disk. It is suggested that removal of α-actinin and destruction of titin are concerned with solubility of miofibrillar proteins.

Figure 3 shows water-soluble myofibrillar proteins of chicken breast and leg muscles and pork *longissiumus dorsi* muscle. These three proteins also comprise about 45% of the total muscle protein. It shows that myofibrillar proteins chicken leg and pork *longissimus dorsi* muscles prepared according to procedure shown in Figure 1 are also soluble in water .

CONCLUSIONS

More than 80% of myofiblillar proteins of chicken breast and leg muscles and pork *longissimus dorsi* muscle were soluble in solutions of low ionic strength. To accomplish this solubilization, it might be necessary to remove α-actinin in washing step and destruct titin by sonication.

〈**washing procedure**〉
chicken breast muscle: homogenized in
10 volume of 25 mM NaCl and 5 mM L-histidine(pH 7.5),
centrifuged at 18,000g for 20 min 《1st wash》
→ supernatant
↓
precipitate: homogenized in
5 volume of 25 mM NaCl and 5 mM L-histidine(pH 7.5),
centrifuged at 18,000g for 20 min 《2nd wash》
→ supernatant
↓
precipitate: homogenized in
5 volume of 25 mM NaCl and 5 mM L-histidine(pH 7.5),
centrifuged at 18,000g for 20 min 《3rd wash》
→ supernatant
↓
precipitate: homogenized in
5 volume of 2.5 mM NaCl and 5 mM L-histidine(pH 7.5),
centrifuged at 18,000g for 20 min 《4th wash》
→ supernatant
↓
precipitate 《washed myofibrils》

〈**water-extraction procedure**〉
washed myofibrils: homogenized in
20 volume of distilled water.
↓
sonicated for 5 min.
↓
centrifuged at 37,000g for 20 min.
→ supernatant
《water-soluble myofiblillar proteins》
↓
precipitate

Figure1. Preparation Procedure of Water-Soluble Myofiblillar Proteins

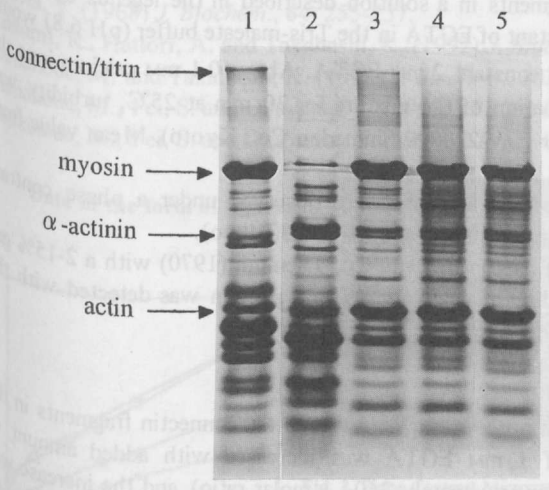

Figure 2. Chicken breast muscle proteins at various steps of preparation of water-soluble myofibrillar proteins analyzed by 2-12% SDS-polyacrylamide gradient gel electrophoresis.
lane 1, whole muscle; lane 2, supernatant removed by 4th wash; lane 3, suspension before sonication; lane 4, suspension after sonication; lane 5, water-soluble myofibrillar proteins

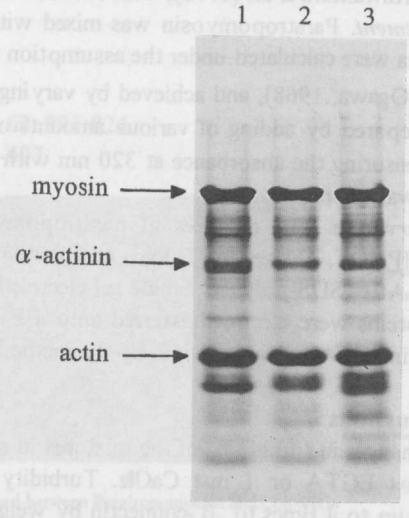

Figure 3. Water-soluble myofibrillar proteins of chicken breast and leg muscles and pork *longissimus dorsi* muscle.
lane 1, chicken breast muscle
lane 2, chicken leg muscle
lane 3, pork *longissimus dorsi* muscle

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