EFFECT OF ULTRASONICATION ON SOLUBILIZATION OF SKELETAL MUSCLE MYOFIBRILLAR PROTEINS IN NEUTRAL PH AND LOW IONIC STRENGTH SOLUTION

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Background

Meat, skeletal muscle of livestock and poultry, is a very concentrated source of protein that has a high biological value because its amino acid composition resembles that of human proteins containing all essential amino acid. Although meat contains high quality proteins for human, utilization of meat protein has been limited. One of the factors limiting utilization is the limited solubility of meat proteins. Although the myofibrillar proteins are major protein fraction of meat and represent about 50% of total proteins of muscle tissue, they are considered insoluble in solutions of low ionic strength and relatively high concentration of salt are required to solubilize them. If myofibrillar proteins could be solubilized in water or low salt solutions, they could be used in many ways. For Example, they could be used as liquid diet for elderly. Low salt solubilization will also advance the study of myofibrillar proteins.
We established a method to solubilize more than 80% of chicken breast muscle myofibrillar proteins in water. To accomplish solubilization, it is essential to maintain myofibrillar suspension at neutral pH and low ionic strength and disrupt some myofibrillar proteins by ultrasonication. However, we haven't clarified water-solubilization mechanism of myofibrillar proteins under this condition.

Objectives

The objective of this study is to examine the effect of ultrasonication on solubilization of myofibrillar proteins at neutral pH and low ionic strength solution in order to clarify the water-solubilization mechanism of myofibrillar proteins.

Methods

Water-soluble myofibrillar proteins were prepared according to the procedure shown in Fig.1.This procedure is divided into two processes, washing procedure and water-solubilization procedure.
Washing Procedure; Comminuted chicken breast muscle was homogenized with ten volumes of a cold solution containing 25 mM NaCl, 5 mM L-His (pH 7.5) in a Warling Blender. After filtering through four-layers gauze, the suspension was centrifuged for 20 min at 18,000 G. The supernatant was discarded, the precipitate was homogenized with 5 volumes of a cold solution containing 25 mM NaCl, 5 mM L-His (pH 7.5) followed by centrifugation for 20 min at 18,000 G. The supernatant was again discarded and the same procedure was repeated against precipitate. The precipitate obtained by third wash was homogenized with five volumes of a cold solution containing 2.5 mM NaCl, 5 mM L-His (pH 7.5), then spun down.
Water-Solubilization Procedure; Cold distilled water was added to the precipitate obtained by washing step adjusting final protein concentration of 6 mg/ml and homogenized. This myofibrillar suspension was sonicated for 0-7 min. After ultrasonication, the suspension was centrifuged for 20 min at 37,000 G and the obtained supernatant was defined as water-soluble myofibrillar proteins.
Preparation procedure for water-soluble myosin is shown in Fig.2. Myosin prepared from chicken breast muscle was diluted to protein concentration of 6 mg/ml. Diluted myosin was dialyzed against neutral pH and low ionic strength solution containing 1 mM NaCl, 5 mM L-His (pH 7.5), and sonicated for 0-7 min. After ultrasonication, myosin was centrifuged for 120 min at 100,000 G and the obtained supernatant was defined as water-soluble myosin.

Results and Discussion

As shown in Table 1, solubility of myofibrillar proteins was increased with ultrasonication time and ultrasonication at least 7 min was required to solubilize more than 80% of myofibrillar proteins in neutral pH and low ionic strength solution. Although additional ultrasonication had no effect on solubility, the amount of solubilized myosin heavy chain was reduced with ultrasonication time (data not shown). Without ultrasonic treatment, myofibrillar suspension at neutral pH and low ionic strength shows low solubility. Myofibrils under such a condition might be still retained in some high-ordered structures and swells excessively because they capture some solubilized myofibrillar proteins and water in the structures. On the other hand, ultrasonication for only 1 min was required to solubilize more than 80% of prepared myosin. Although additional ultrasonication was no effect on myosin solubility, myosin heavy chains were somewhat damaged by the excess ultrasonic treatment (data not shown). In the case without ultrasonication, myosin wasn't separated into supernatant and precipitate after centrifugation because myosin under the pH and ionic condition swells excessively, suggesting that myosin molecules under the condition also construct high-ordered structures.
All these facts suggest that physical force applied by ultrasonication would act on high-ordered structure of myofibrils rather than each single molecule of myofibrillar proteins, resulting in water solubilization.

Pertinent literature

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(washing procedure)
Chicken breast muscle: homogenized in 10 volumes of 25 mM NaCl, 5 mM L-His (pH 7.5),
centrifuged at 18,000 G for 20 min
↓
precipitate: homogenized in 5 volumes of 25 mM NaCl, 5 mM L-His (pH 7.5),
centrifuged at 18,000 G for 20 min
↓
precipitate: homogenized in 5 volumes of 25 mM NaCl, 5 mM L-His (pH 7.5),
centrifuged at 18,000 G for 20 min
↓
precipitate: homogenized in 5 volumes of 2.5 mM NaCl, 5 mM L-His (pH 7.5),
centrifuged at 18,000 G for 20 min
↓
precipitate: washed myofibrils

(water-solubilization procedure)
washed myofibrils: homogenized in distilled water
↓
sonicated for 0, 1, 3, 5, 7 min.
↓
centrifuged at 37,000 G for 20 min.
↓
supernatant; water-soluble myofibrillar proteins

Fig.1 Preparation Procedure of Water-Soluble Myofibrillar Proteins

Myosin: prepared from chicken breast muscle
↓
dialyzed against 1.0 mM NaCl, 5 mM L-His(pH 7.5)
↓
sonicated for 0, 1, 3, 5, 7 min.
↓
centrifuged at 100,000 G for 120 min.
↓
supernatant; water-soluble myosin

Fig.2 Preparation Procedure of Water-Soluble Myosin

Table 1. Effect of Ultrasonication Time on Solubilization of Myofibrillar Proteins

Sonication time (min)	Solubility (%)
0	11
1	19
3	25
5	68
7	90

Table 2. Effect of Ultrasonication Time on Solubilization of Myosin

Sonication time (min)	Solubility (%)
0	----
1	85
3	84
5	84
7	84

Protein solubility was expressed as the percentage of the amount of supernatant protein after the centrifugation to the total amount of protein before centrifugation.