Inhibition of Autolytic Breakdown of Muscle Proteins
by the Sarcoplasm and the Serum of Rat

In 1960, Koszalka and Miller reported the autolytic breakdown of proteins in the homogenate of rat skeletal muscle in the alkaline pH range. They purified a new alkaline protease, which was presumed to be responsible for the autolytic breakdown of muscle proteins, from the supernatant fraction of the centrifuged homogenate.

In our previous paper, however, we showed that the activity of autolytic breakdown of muscle proteins is almost exclusively localized in the precipitate of the centrifuged homogenate (myofibrillar fraction).

In the present communication, we report that the centrifuged supernatant of the muscle homogenate shows no proteolytic activity but the inhibiting activity of the autolytic breakdown of muscle proteins in the alkaline pH range.

The fact that the serum of rat shows the inhibiting activity of the autolytic breakdown of muscle proteins in the alkaline pH range, is also reported in this communication.

The following experiments were done at 4°C unless otherwise stated. The method of preparation of the myofibrillar fraction was the same as described previously, except that 50 mM KCl-10 mM KH₂PO₄-NaOH (pH 7.7) was used, instead of 50 mM KCl, as the homogenizing and washing solution. The myofibrillar fraction thus obtained was suspended in the homogenizing solution using all-glass Dounce-type homogenizer (hand operated), and the activity of the autolytic breakdown of proteins (autolytic activity) was determined.

The supernatant fluid obtained after centrifugation of the homogenate was used as the sarcoplasm. The blood was allowed to clot and the serum was obtained. The sarcoplasm or the serum was diluted, if necessary, with 50 mM KCl-10 mM KH₂PO₄-NaOH (pH 7.7) and the inhibiting activity was measured.

The previous method for the determination of the autolytic activity of the myofibrillar fraction was partly modified as follows: one milliliter of the myofibrillar fraction was mixed with 1 ml of 0.15 M glycine-NaOH buffer (pH 9.1) containing 1.8 M KCl, followed by addition of 1 ml of the homogenizing solution. In the case of measuring the inhibiting activity, the homogenizing solution was replaced by 1 ml of either the sarcoplasm or the serum. After incubation at 37°C for 30 min, the mixture was added with 5 ml of 5% (w/v) trichloroacetic acid, allowed to stand at room temperature for about 18 hr and filtered. The filtrate was diluted appropriately with water, and tyrosine equivalents of the Folin-reagent positive material released during the incubation period were determined according to the procedure of Lowry et al. The inhibiting activity, also expressed as tyrosine equivalents, was determined from the decrease of released Folin-reagent positive material.

Protein was determined by the method of Lowry et al. with the whole muscle homogenate as the standard protein. The nitrogen

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Figure 1. Inhibition of Autolytic Activity of the Myofibrillar Fraction by the Sarcoplasm.

The sarcoplasm of rat skeletal muscle was diluted with 50 mM-KCl-10 mM-KH₂PO₄-NaOH buffer (pH 7.7) and the inhibiting activity was measured.

The preparation of the boiled sarcoplasm was as follows: After boiling the sarcoplasm for 3 min, the sample was chilled in ice water and homogenized using all-glass homogenizer. The inhibiting activity of this sample was determined and indicated in the figure by the solid circle. The myofibrillar fraction used in this experiment contained 2.16 mg-N/ml.

Figure 2. Inhibition of Autolytic Activity of the Myofibrillar Fraction by the Serum.

Rat serum was diluted with 50 mM KCl-10 mM KH₂PO₄-NaOH buffer (pH 7.7) and the inhibiting activity was measured.

The myofibrillar fraction used in this experiment contained 3.35 mg-N/ml.

These results suggest that the sarcoplasm and serum contain inhibitors of the autolytic breakdown of muscle proteins.

There are many reports concerning natural protease inhibitors. However, purification and characterization of inhibitors in the skeletal muscle have not yet been done. Preliminary results of purification and characterization of the sarcoplasmic inhibitor of autolytic breakdown of muscle proteins have already been reported and will be published in the near future.

On the other hand, protease inhibitors in the serum, especially trypsin inhibitors, have been purified and characterized by many authors. Most of the trypsin inhibiting activity is attributed to the α₁-trypsin in-
hibitor\textsuperscript{9,14} which is relatively unstable to heating and to pH values below 5.\textsuperscript{8,9} Preliminary results\textsuperscript{51} of the characterization of the serum inhibitor of autolytic breakdown of muscle proteins show that the properties of the inhibitor are similar to those of the \( \alpha_1 \)-trypsin inhibitor. The fact that the serum trypsin inhibitor(s) inhibit(s) the autolytic breakdown of muscle proteins may give some suggestion for elucidating the physiological role of serum trypsin inhibitors.

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