Short Communication

[Agr. Biol. Chem., Vol. 36, No. 7, p. 1265~1267, 1972]

A Neutral Proteolytic System Responsible for Post-mortem Proteolysis in Rabbit Skeletal Muscle

Sir:

It is well known that proteolysis proceeds during the post-mortem storage of muscle. However, there is little knowledge about the proteolytic systems which are responsible for this process. Although it has been known that muscle contains an acidic proteolytic system which is consisted of cathepsins,[1] it has been not yet evidenced that this system is responsible for the post-mortem proteolysis. Thus, this work has been performed to identify proteolytic systems responsible for the post-mortem proteolysis in muscle.

In the initial step of this work, we have found that there is a larger increase in ninhydrin[2] positive materials (NM) than in Cu-Folin phenol reagent[3] positive materials (PM) among the nonprotein nitrogenous compounds during the post-mortem storage of the rabbit muscle (longissimus dorsi) showing a high ultimate pH, as compared with the muscle showing a low ultimate pH.[4] This finding has suggested that muscle contains a neutral proteolytic system besides the acidic one. Therefore, the following experiments have been conducted to obtain a more direct evidence.

Minced rabbit muscles (l. dorsi) were homogenized in Waring blendor with buffer solutions (pH 3.3~9.0) containing chloramphenicol, and the obtained homogenates were incubated at 37°C for 18 hr. The liberated nonprotein nitrogenous compounds (PM and NM) were determined and shown as a function of the incubation pH in Fig. 1. Figure 1 indicated that PM was most liberated at pH 3.3 while NM was most liberated at neutral regions. This result indicates that, like the acidic proteolytic system, the neutral one predicted from the previous result[4] may be preserved in the homogenized muscle. Some researchers have measured the autolysis of muscle homogenate.[5,6] In those experiments, they have used the UV absorption (280 mμ) or Folin-Ciocalteu phenol reagent[7] and not used ninhydrin reagent to determine proteolytic products, although it has been noticed that the former two tools are not available for the measurement of the autolysis in neutral regions of the

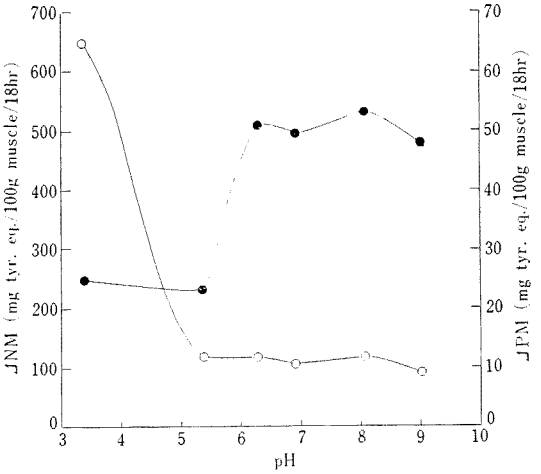

FIG. 1. Autolytic Activity of Rabbit Muscle Homogenate as a Function of pH.

One gram of minced muscle was homogenized with 4 ml of 150 ppm chloramphenicol and 1 ml of buffer solution, and the homogenate was incubated at 37°C for 18 hr. Incubation was stopped by the addition of 10% trichloroacetic acid. The liberated nonprotein nitrogeneous compounds were determined with Cu-Folin phenol reagent (○) and ninhydrin (●), and were expessed as tyrosine equivalents. Tris-maleate buffer (0.6 M) was used except McIlvaine buffer at pH 3.3.

muscle homogenate containing ribonucleases and xanthine oxidasee activity by Umaña[8] and Willemot *et al.*[9] Figure 1, is, therefore, the first information about nonprotein NM liberation from muscle homogenate as a function of pH.

Subsequently, we have attempted to separate a neutral proteolytic system from the muscle homogenate and succeeded in the following procedure.

After minced muscle was homogenized in Waring blendor with 5 volumes of 0.12 M tris-HCl buffer solution (pH 7.2), the homogenate was centrifuged at 6000 × *g* for 15 min. The supernatant (crude extract) showed about 70% of the autolytic activity (determined by PM and NM) of the homogenate. A half volume of acetone was added to the extract and the precipitate was collected by the centrifugation. The precipitate was dissolved in a portion of 0.01 M tris-HCl buffer solution (pH 7.2) and the solution was dialyzed against the same buffer solution. After dialysis, the solution was centrifuged and the supernatant (F-1) was obtained.

The proteolytic activity (determined by PM and NM) of F-1 was demonstrated by the incubation with Hammarsten casein (Merck, Co.) in 0.06 M tris-HCl buffer solution (pH 7.2) at 37°C for 18 hr. F-1 showed about 2 times as great as the specific activity of the crude extract and recovered about 40% of the activity of the crude extract. F-1 also hydrolyzed horse myoglobin (crystalized, Miles-Seravac Ltd.) and undenatured bovine hemoglobin (exhaustively dialyzed against water, Difco Lab.) at similar rate as casein at neutral regions. The proteolytic activity of F-1 on hemoglobin was shown as a function of pH in Fig. 2. The decrease of the activity of F-1 at acidic regions indicated the removal of cathepsin from the crude extract by the extraction and the fractionation with acetone. Figure 2 shows that there is a higher increase in NM than in PM among nonprotein nitrogeneous compounds at neutral regions. Since

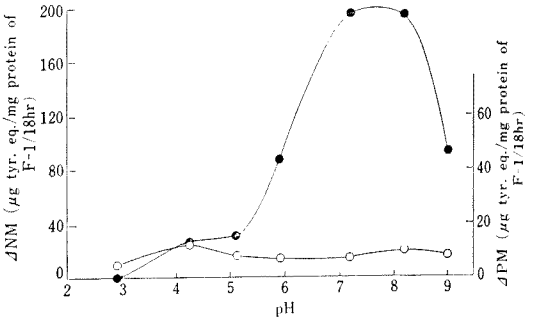

FIG. 2. Proteolytic Activity of F-1 as a Function of pH.

Two ml of F-1 was mixed with 2 ml of buffer solution containing 1.8% hemoglobin and 200 ppm chloramphenicol, and the mixture was incubated at 37°C for 18 hr. Protein content of F-1 was 5.73 mg/ml. The following procedures and the symbols were the same as in Fig. 1. McIlvaine buffer was used below pH 6 and 0.6 M tris-HCl buffer was used above pH 7.

this pattern is similar to that of the autolysis at neutral regions shown in Fig. 1, F-1 is assumed to exhibit the main proteolytic system responsible for muscle autolysis at neutral regions.

It is, consequently, concluded that there is a neutral proteolytic system responsible for post-mortem proteolysis in rabbit muscle besides a well-known acidic one. This work is the first demonstration of the presence in skeletal muscle of a neutral proteolytic system active on both endogeneous and exogeneous substrate. The property of the neutral proteolytic system and its function in living muscle are being studied.

We are deeply indepted to Dr. Tadashi Noguchi of the University of Tokyo for his valuable advice.

REFERENCES

1) A. L. Tappel, *Lysosomes in Biol. Pathol.*, vol. 2, N. Holland, Amsterdam, 1969, p. 167.
2) E. W. Yemm and E. C. Cocking, *Analyst*, **80**, 209 (1955).
3) O. H. Lowry, N. J. Rosenbrough, A. L. Farr and

R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
4) A. Okitani, K. Shinohara, M. Sugitani and M. Fujimaki, in preparation.
5) R. J. Pennington, *Biochem. J.*, **88**, 64 (1963).
6) L. Berlinguet and U. Srivastava, *Canad. J. Biochem.*, **44**, 613 (1966).
7) M. L. Anson, *J. Gen. Physiol.*, **22**, 79 (1938).
8) R. Umaña, *Anal. Biochem.*, **26**, 430 (1968).
9) J. Willemot, M. Lalanne and L. Berlinguet, *Arch. Biochem. Biophys.*, **133**, 359 (1969).

Akihiro OKITANI
Masao FUJIMAKI

Department of Agricultural Chemistry,
Faculty of Agriculture,
The University of Tokyo,
Tokyo

Received May 4, 1972