Short Paper

Combined Action of Carp Muscle Cathepsins A and D on Proteins*¹

Combined action of cathepsins A and D on hemoglobin at pH 4.5 was originally reported using partially purified chicken muscle enzymes by IODICE *et al.*¹⁾ Later, MAKINODAN and IKEDA²⁾ recognized the action on carp muscle cathepsins A and D. It is generally guessed that various kinds of proteases act synergistically for the physiological digestion of body proteins or the autolysis after the death of animals. The above reports^{1,2)} are important as the data that a combined proteolytic action for protein digestion was manifested, although they are on the exogenous protein, hemoglobin. However, the enzyme preparation used contained still contaminating protein, so reinvestigation by pure enzyme preparation was expected. Recently we^{3,4)} have purified cathepsins A and D from carp muscle electrophoretically homogeneously. Accordingly, we reexamined the combined action of the both enzymes on the hydrolysis of proteins.

Carp muscle cathepsins A and D were purified as reported previously:^{3,4)} acid treatment (pH 4.2) of 0.1 M NaCl extract, ammonium sulfate fractionation (0–60%), acetone fractionation (30–60%), DEAE-Sephadex A-50 chromatography, electrofocusing, and Sepharose 6B gel filtration. The obtained enzyme preparations were electrophoretically homogeneous. Carp muscle myofibrils and sarcoplasmic proteins were obtained by the method of TOKIWA and MATSUMIYA⁵⁾ and the previous report,⁴⁾ respectively. The reaction against endogenous proteins was as follows: the reaction mixture of 2.5 ml containing 0.12 M acetate buffer, pH 4.5, enzyme solution and endogenous protein was incubated at 37°C. As a blank the mixture of buffer and enzyme solution and the mixture of buffer and substrate were incubated separately, then mixed (2.5 ml).

Fig. 1 shows the action of carp muscle cathepsins A and D on hemoglobin at pH 4.5. Cathepsin A did not act on hemoglobin, but cathepsin D hydrolyzed it as recognized previously.²⁾ However, the increase of ninhydrin positive matters observed when the both enzymes were added simultaneously was much greater than that under the addition of cathepsin D alone. The results show that peptides produced by cathepsin D were further hydrolyzed by cathepsin A. Thus, the combined action of carp muscle cathepsins A and D on hemoglobin was confirmed. So, we examined the action of cathepsin A against the condensate of 5% trichloroacetic acid (TCA) filtrate after the mixture of cathepsin D and hemoglobin being incubated (TCA was excluded from the TCA filtrate by shaking with ethyl ether). But any hydrolytic product was not observed by the ninhydrin method. For the combined action the coexistence of cathepsins A and D and hemoglobin in the reaction mixture may be absolutely necessary, or the substrate for cathepsin A may exist in 5% TCA precipitate fraction.

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*¹ タンパク質に対するコイ筋肉カタレプシン A および D の協同作用.

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Fig. 1. The action of cathepsins A and D on hemoglobin at pH 4.5. Reaction mixture (2.5 ml) contained 25 mg hemoglobin, cathepsin(s) (A, 5 μ g; D, 11 μ g) and 100–140 mM acetate buffer (pH 4.5). After incubation at 37°C for the indicated periods, the reaction was stopped by adding 2.5 ml of 5% TCA. In the measurement of blank value, enzyme and hemoglobin were incubated separately, then combined after adding TCA. Ninhydrin color value (ΔE_{570}) was determined on 1 ml of TCA filtrate.

Next, we examined the similar combined action on carp muscle myofibrils and sarcoplasmic proteins. Cathepsin D brought the increase of hydrolytic products with the increase of incubation time against each protein, but cathepsin A showed hardly detectable activity on them. The combined action on both proteins was not observed, too. We²⁾ previously reported that carp muscle cathepsin A preparation acted on sarcoplasmic proteins. But we discussed in the report that the activity might depend on contaminating proteolytic activity other than cathepsin D. From the present view, however, the activity was probably attributed to the autolysis of the prepared sarcoplasmic fraction (centrifugal supernatant at 13,000 $\times$ g of muscle homogenate) rather than contaminating proteolytic activity. Accordingly, on the basis of the present results, it may be concluded that carp muscle cathepsin A does not act on not only actomyosin and myofibrils but sarcoplasmic proteins at least under the present experimental condition. This work was supported by a research grant from the Ministry of Education of Japan.

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