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STUDIES ON THE MUSCLE CATHEPSIN ACTIVITY OF MACKEREL

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The pH and temperature effects on the mackerel muscle autolysis intensity were investigated. The muscle cathepsin was isolated and purified; some properties of the purified enzyme were determined.

INTRODUCTION

Various biochemical processes inducing profoud changes in the basic muscle tissue components occur during fish storage, both fresh and frozen. The most significant changes are those associated with the protein system; they are influenced by proteolytic enzymes. The protein – splitting enzymes are specific in their activity which depends on many factors, pH and temperature of the environment being some of them.

In the present study the effects of pH and temperature on the catheptic activity during mackerel muscle autolysis were followed, the muscle cathepsin was isolated and purified and some of its properties examined.

EXPERIMENTAL MATERIALS AND METHODS

Materials

Colias mackerel, *Scomber colias* (Gmelin), caught in September on the North-West African shelf and appropriately preserved on board of a commercial trawler were the object of the investigations. Fishes from a 2-hour night haul were frozen. Immediately after catching the fishes were thoroughly washed and arranged on trays in a manner eliminating any possible mechanic damage on freezing. In several minutes after preparing

the fishes, the freezing process was started in Danish freezers "Sabroe". The process lasted 170 mins, the internal temperature of the frozen block reaching ca –20°C. Having completed their freezing and taken them out of trays the fish blocks were glazed, packed in cartons and placed in ship's holds at –25°C. After 24 h the blocks were thoroughly glazed again and stored in the holds at –25°C for 18 days. Having landed and delivered fish by a refrigerated van to the department, they were stored in a freezer at –25°C. The investigations began immediately after the fishes had been brought to the laboratory, i.e., after 21 days of storage.

Methods

Fish muscles autolysis was performed in a manner described in the previous paper (Fik, 1972), using the Sörensen method (Katzenellenbogen and Mochnacka, 1969) to determine the amino nitrogen increments and expressing them as percentages of the maximum increment. The enzyme extracts were prepared by the method reported in the previous paper (Fik, 1972). Proteolytic activities fo raw extracts and partly purified preparates were determined on urea- and NaOH – denatured hemoglobin by the Anson method (Bergmayer, 1965). The purified cathepsin was incubated with synthetic substrates specific for cathepsins A, B and C of mammals in the conditions reported by Tallan et al. (1952). The cathepsin A activity against N-carbobenzoxy-1-glutamyl-L-tyrosine was determined by the ninhydrin method (Bailey, 1962), whereas cathepsin A and cathepsin B activities were determined with N-benzoyl-L-arginine amide and glycyl-L-tyrosine amide, respectively, by the Conway method (Johnston et al., 1950). The protein content was determined by the biuret method (Gornall et al., 1949), bovine serum crystalline albumin being used as a standard, and by the ultraviolet absorption method. The method described by Siebert and Schmitt (1965) was applied to the cathepsin purification.

DISCUSSION OF RESULTS

Initially the influence of pH and temperature on the mackerel muscle autolysis rate was determined, the results being presented in Figs. 1 and 2.

The autolysis proceeded with its highest intensity at pH range 4.0–4.4. After the time allowed for the autolysis had been increased from 4 to 6 hours, the course of the curve did not change and the same optimal value was maintained. Fig. 1 shows the pH shift from 4.0 toward a more acid range resulting in a rapid decrease in the autolysis rate caused by a change in catheptic activity. On the other hand, an increase in pH from 4.0 toward the alkaline range brought about a relatively slow catheptic activity decrease. At pH 5.2 after 6 hrs of proteolysis the activity was still on the 60% level of that at pH 4.0.

The optimal autoproteolysis temperature for the mackerel muscles is close to 40°C (Fig. 2). An increase from 40 to 50°C caused a considerable, by 60% fall in the catheptic activity. The enzymes of the fish species discussed, however, seem to be slightly more

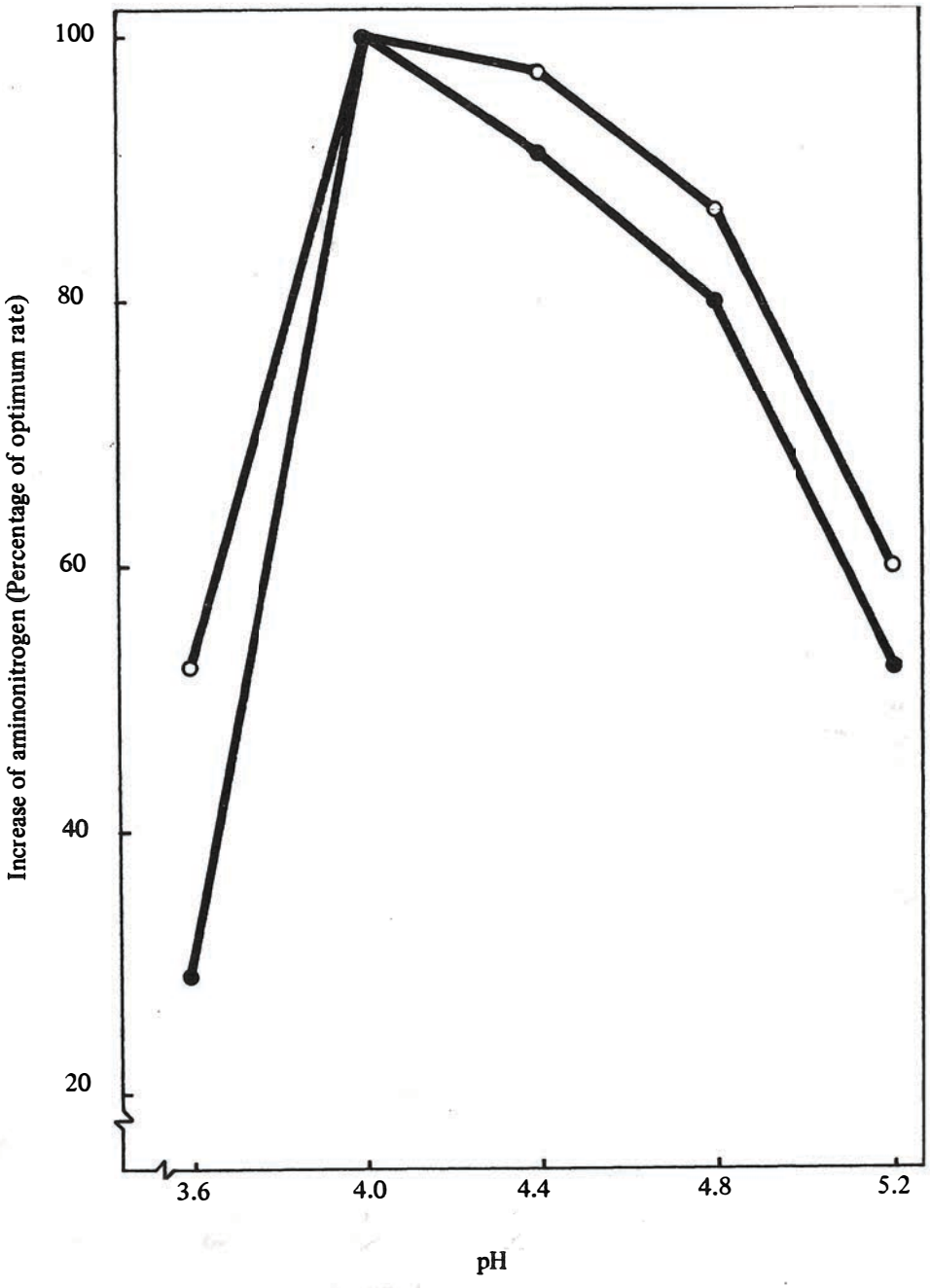

Fig. 1. Influence of pH on the autoproteolysis of mackerel muscle at 20°C. ●—●— 4 hrs incubation, ○—○— 6 hrs incubation

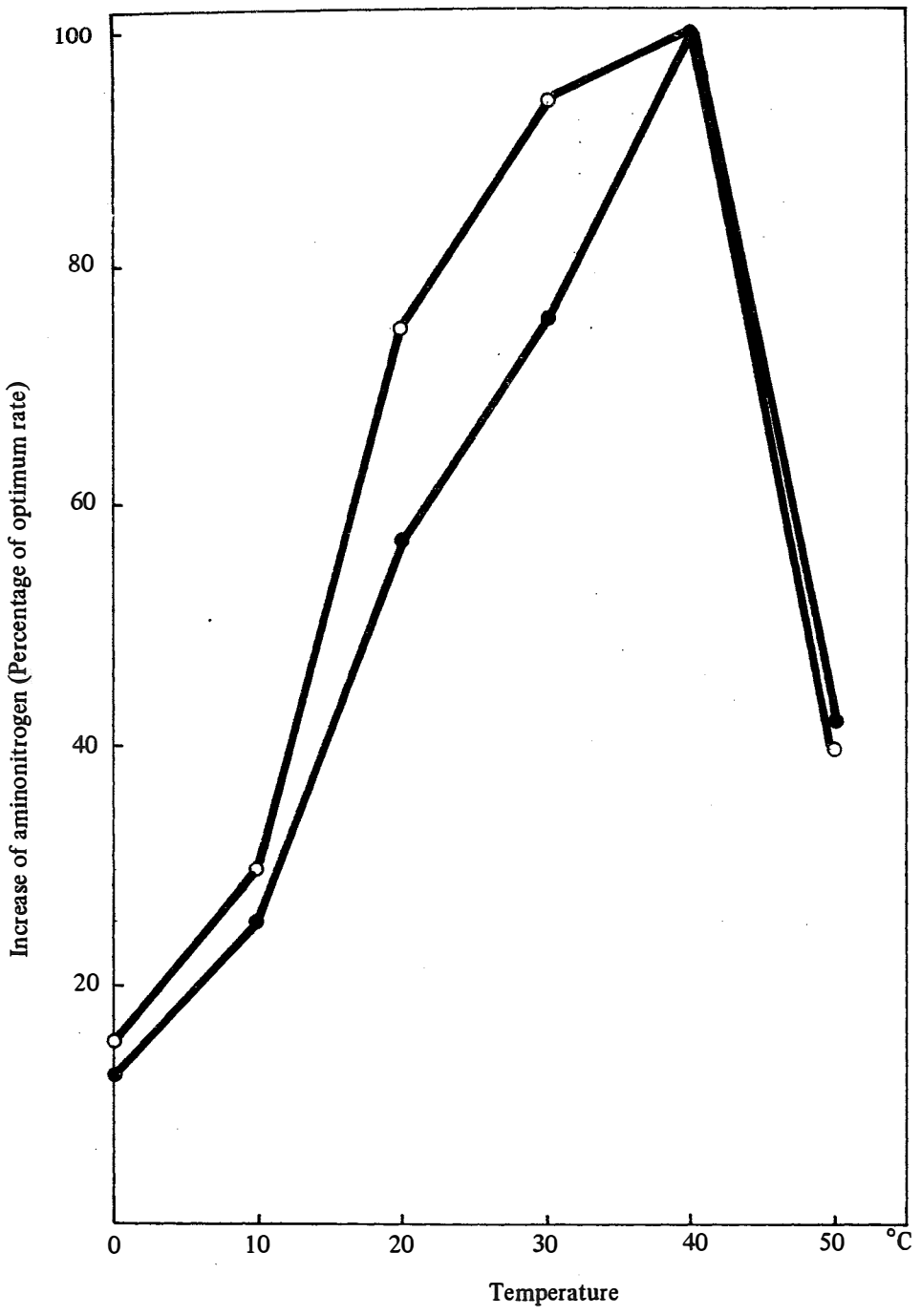

Fig. 2. Influence of temperature on the autoproteolysis of mackerel muscle at pH 4.0. ●—●— 4 hrs incubation, ○—○— 6 hrs incubation

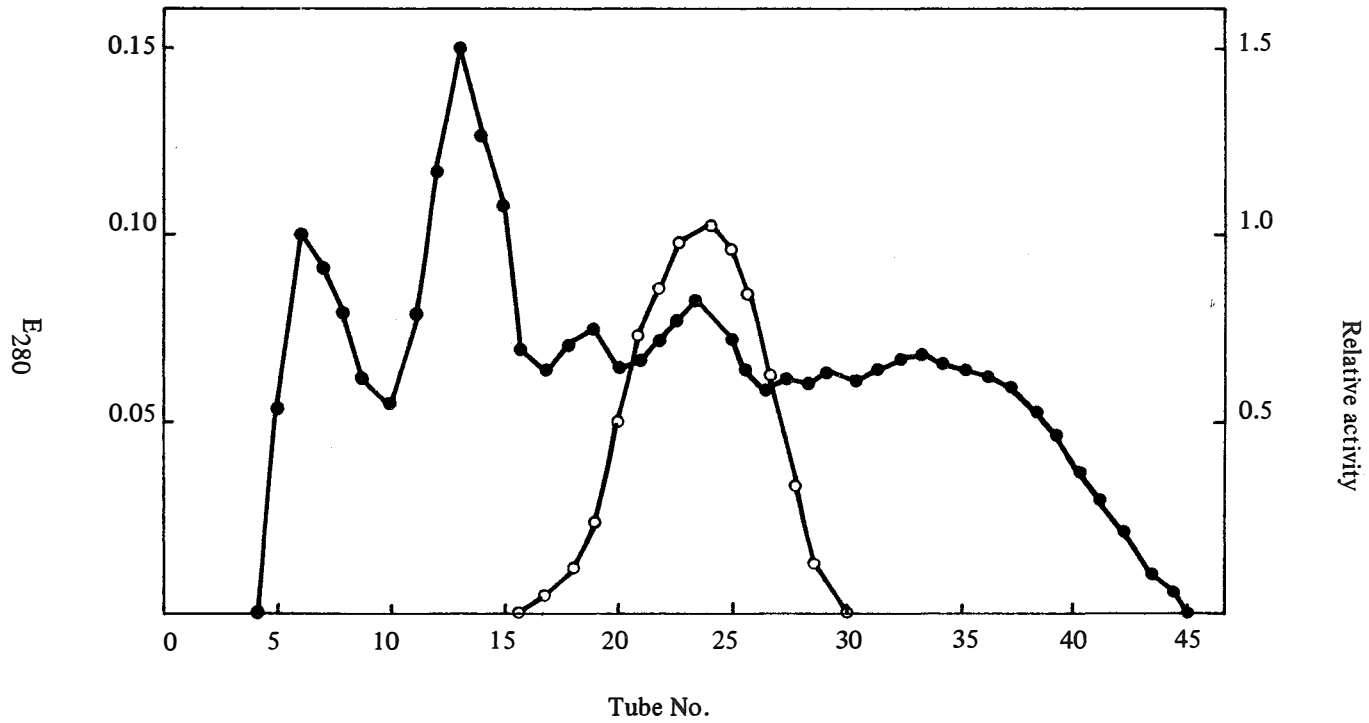

Fig. 3. Elution profile of fraction 5 from Sephadex G–200 column. Column, 1.5 by 30 cm. Volume per tube, 4.0 ml. ●—●— E_{280}. ○—○— Relative activity, μM tyrosine/h/ml

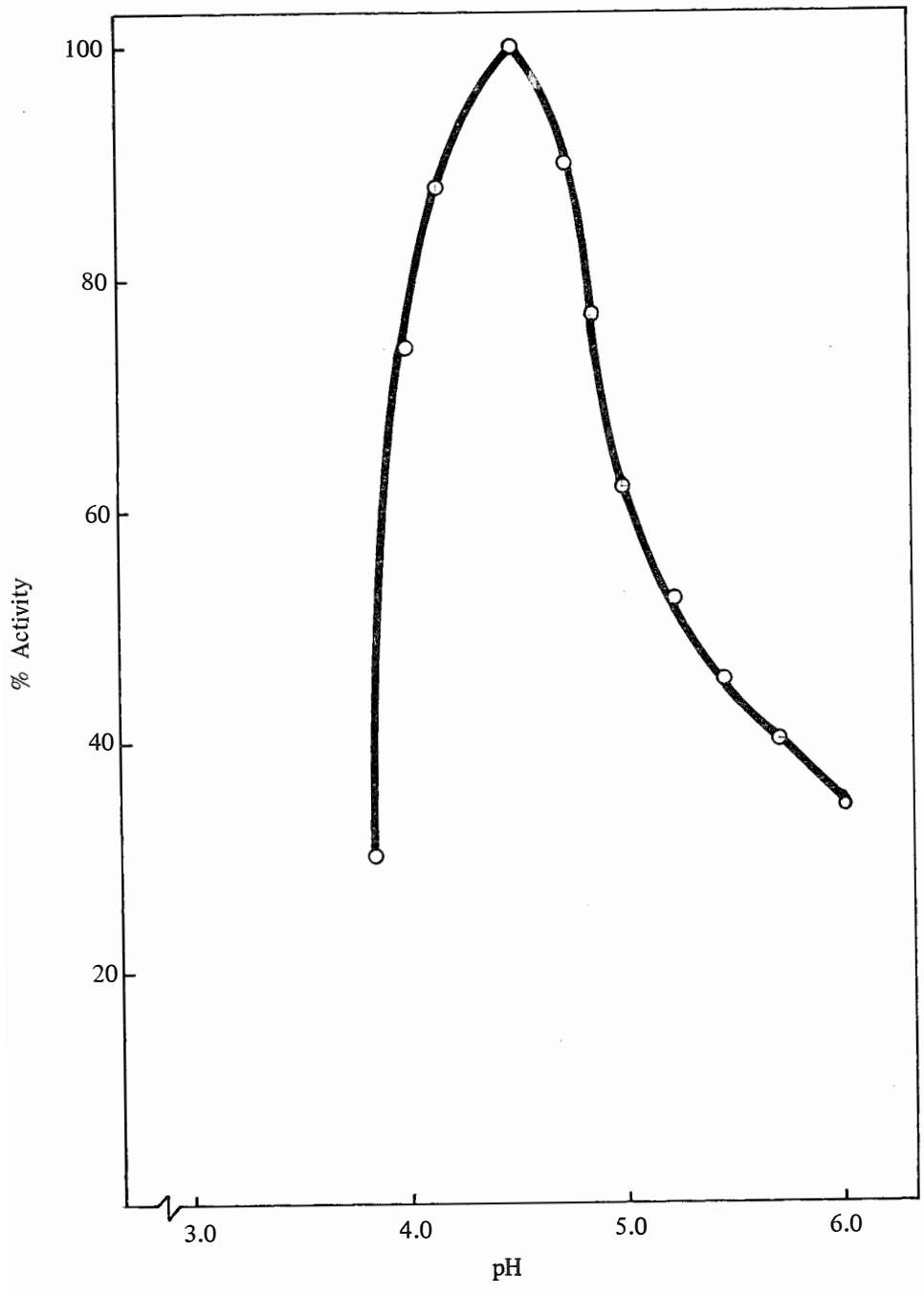

Fig. 4. Influence of pH on the activity of fraction 6, at 40°C, with denatured hemoglobin as the substrate

Table 1

Purification of mackerel muscle cathepsin

Fraction	Volume (ml)	Protein		Activity		Purification	Yield (%)
		mg/ml	mg/fraction	μM tyrosine/h/fraction	μM tyrosine/h/mg protein		
1. Crude extract (500 g of the muscle)	1260	26.0	32760	2653	0.081	1	100
2. First acid-heat treatment	1180	7.0	8260	2023	0.245	3.4	76
3. Ammonium sulfate precipitation	200	10.0	2000	1260	0.630	7.8	47
4. Second acid-heat treatment	210	2.0	420	1075	2.56	31.6	40
5. Acetone precipitation	15	3.2	48	451	9.4	116	17
6. Eluate from Sephadex G–200	30	0.32	9.6	269	28.0	345	10

resistant to denaturation changes occurring with temperature than the muscle cathepsins of horse mackerel (Fik, 1972).

Carrying on the studies, the mackerel muscle cathepsin was isolated and purified, and some of its properties examined. The enzyme was purified 345 fold with 10% yield (Table 1). The first acid treatment using 2N acetic acid at 35°C resulted in 3.4-fold enrichment of the enzyme. The enzyme thus obtained could be stored overnight at 0°C with no noticeable changes in its activity. Ammonium sulphate fractionation increased the enzyme activity 8-fold over the non-purified extract. Lyophilized enzyme preparate of the 4 th fraction maintained its activity for several months at 0°C. Acetone fractionation yielded a 3.4-fold increase in the enzyme activity over its initial value. Pooling the highest activity fractions after the Sephadex G-200 filtration (Fig. 3), the main peak of catheptic activity was obtained. This fraction was freeze-stored for several weeks, no change in its enzymatic activity being detected. Obviously, durability of the lyophilized enzyme is unlimited.

The purified cathepsin showed a maximum activity against the denatured hemoglobin at pH 4.4 (Fig. 4). This result is close to the pH optimum for purified muscle cathepsins of cod (Siebert, 1962) and hake (Fik, 1973), differing slightly from the optimum for

purified cod spleen cathepsin (Siebert et al., 1963) and greatly deviating from the optimum for purified tuna muscle proteinase (Groninger, 1964). The pH optimum for the purified cathepsin is almost identical with that for the mackerel muscle autolysis (Fig. 1). The enzyme purified during the studies presented showed good hemoglobin – splitting properties but remained inactive against synthetic substrates specific for the mammalian cathepsins A, B and C. Similar results were obtained by Siebert and Schmitt (1965) and Groninger (1964) for the cod muscle cathepsin and tuna muscle proteinase, respectively. Thus the mackerel muscle cathepsin is different from the mammalian cathepsins which can be classified according to their synthetic substrates – splitting properties (Tallan et al., 1952). The classification of the cathepsin discussed could only be made basing on an appropriate standard protein decomposition. Siebert and Schmitt (1965) made an attempt to classify the cod cathepsins of muscles and spleen against insuline. However, the insuline B chain decomposition showed no different properties when compared to the cathepsins tested so far against this protein and did not allow an exact enzyme specificity classification to be made. Therefore, the intracellular occurrence and optimum activity within the acid range are still retained as classification characters of the fish proteinases catheptic nature.

The temperature coefficients, Q_{10}, of the purified mackerel muscle cathepsin were comprised within 1.6–2.3 when measured at 10–40°C. Regarding the thermal lability, usually the temperature the 30-minute action of which results in a 50% enzyme inactivation, is determined. According to Siebert and Schmitt (1965) this temperature is alike for various enzymes of fish and mammalian muscles and is comprised within 42–48°C.

CONCLUSIONS

1. The mackerel muscle cathepsin showed the optimal activity at pH 4–4.4 and ca 30°C.

2. The purified mackerel muscle cathepsin did not split the synthetic substrates specific for the mammalian cathepsins A, B and C.

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BADANIA NAD AKTYWNOŚCIĄ KATEPSYN MIĘŚNIOWYCH MAKRELI

Streszczenie

Zbadano wpływ pH i temperatury na aktywność katepsyn w procesie autoproteolizy mięśni makreli. Stwierdzono, że autoproteoliza przebiegała najintensywniej w pH 4–4,4 i temperaturze około 40°C. Zmiana pH od optimum w kierunku kwaśnym powodowała gwałtowniejszy spadek szybkości proteolizy niż w kierunku zasadowym. Podwyższenie temperatury z 40 do 50°C spowodowało 60% zmniejszenie aktywności katepsyn.

W dalszej kolejności wyizolowano i 345-krotnie oczyszczono katepsynę mięśniową makreli. Enzym oczyszczony wykazał optymalną aktywność w stosunku do zdenaturowanej hemoglobiny w pH 4,4. Katepsyna dobrze rozkładała zdenaturowaną hemoglobinę lecz nie rozkładała substratów syntetycznych właściwych dla katepsyn A, B i C ssaków. Różni się więc ona pod względem specyficzności od katepsyn ssaków, które można klasyfikować poprzez rozkład odpowiednich substratów syntetycznych.

М. Фик

ИССЛЕДОВАНИЯ АКТИВНОСТИ МЫШЕЧНЫХ КАТЕПСИНОВ СКУМБРИИ

Р е з ю м е

Исследовано влияние pH и температуры на активность катепсинов в процессе автопротеолиза мышц скумбрии. Установлено, что наиболее интенсивно автопротеолиз протекал при pH 4–4,4 при температуре около 40°С. Отклонение pH от оптимума в кислотном направлении вызывало более резкое снижение скорости протеолиза, чем в щелочном направлении. Повышение температуры с 40° до 50° вызвало 60%-ное снижение активности катепсинов.

Затем был выделен и 245 раз очищен мышечный катепсин скумбрии. Очищенный энзим характеризуется оптимальной активностью по отношению к денатурированному гемоглобину при pH = 4,4. Катепсин хорошо расщеплял денатурированный гемоглобин, но не расщеплял синтетических субстратов, характерных для катепсинов A, B и C млекопитающих. Таким образом, он отличается по своей специфике от катепсинов млекопитающих, которые можно классифицировать путём расщепления соответствующих синтетических субстратов.

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