

Studies on Tryptase from a Whale (*Balaenoptera borealis* L.)

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In order to investigate the presence of protein decomposing enzyme in the pancreas of a whale (*Balaenoptera borealis* Lesson), the pancreas was dehydrated and degreased with acetone and other, dried and prepared into a powder. Following results were obtained.

1) The powder obtained by grinding the dried powder in the mortar and putting it through No. 5 sieve can pass the test for pancreatin described in Par. 6 of Japanese Pharmacopoeia.

2) The most suitable pH value for gelatine is in the range of pH 8—9 (Fig. 1) and the most suitable temperature (for 1 hour duration of activity is 50°C.)

3) When the amount of base material is constant and the amount of enzymes varied, the activity curve (Fig. 2) shows that the amount of enzyme and activity are proportionate.

4) The activity curve (Fig. 3) obtained by holding the amount of enzyme, material, pH and the temperature of activity at a constant and varying the time of activity, shows it increasing up to 4—hour period.

5) Distribution of tryptase in various parts of pancreas did not vary greatly between those in tail, body and head parts, but the amount in the tail part was 110% of the total of other two parts.

The purification of pancreatic tryptase is to follow the preparative methods for chymotrypsin and trypsin crystals from bovine pancreas as reported by Northrop. Preparations are now being made for carrying out the experiments. Although no definite report can yet be made, the authors are convinced that if bovine pancreas is used, crystals of chymotrypsinogen and trypsinogen can be prepared comparatively easily by the Northrop method.

EXPERIMENTAL

1) Preparation of enzyme powder

Pancreas obtained from a whale (*Balaenoptera borealis* Lesson) dissected at Ayukawa in Miyagi Prefecture was immersed in twice its volume of

acetone for 10 days, taken out, finely cut with scissors and put through a mincing machine. To this was added 2 to 3 volumes of acetone, drained, and the process repeated 3 to 4 times to dehydrate and degrease. Finally, the residue was shaken with an equal mixture of acetone and ether, filtered, in the open and then dried in a vacuum desiccator over calcium chloride. From 2500 g of pancreas, 300 g of dried substance were obtained. This substance was ground in a mortar and put through a No. 5 sieve (J.P.). Yield of the powder to the dried substance, ca 40%.

2) Pancreatin Test, Par. 6, Japan Pharmacopoeia

0.1 g Enzyme powder prepared as in the above (1) was taken in a 500 cc measuring bottle distilled water added to make the total volume 500 cc and filtered. 2 cc of the filtrate was dissolved in 1 cc N/10 KOH and distilled water added to make the total volume 50 cc, 3 cc of this solution was added to 2 cc distilled water, reacted for 1 hour at 40°C, after which 3 drops of acetic acid alcohol solution (1 parts glacial acetic acid, 9 parts water, 10 parts alcohol were added. Only slight opalescence could be seen.

3) Determination of suitable pH of activity

3 g Enzyme powder as described in (1) was added to 30 cc 80% glycerine and left standing over-night at room temperature. A clear upper solution was separated centrifugally, 9 times its volume of distilled water was added and 1 cc of this solution was used as enzyme sample. For the base material, 0.5 g of gelatine was added to 10 cc each of glycocoll-NaOH buffer solution (Sørensen's) of varying pH values, and melted at 50°C. To this was added 1 cc enzyme sample at 40°C, held there for 2 hours and the amino acids thus formed titrated, in Willstätter's alcohol solution, with N/5 alcoholic KOH solution with thymo!-phthalein as an indicator. On the other hand, the base with 1 cc of distilled water instead of the enzyme powder was titrated and the difference between the two values were taken as the degree of digestion at that pH value. Results are shown in Fig. 1.

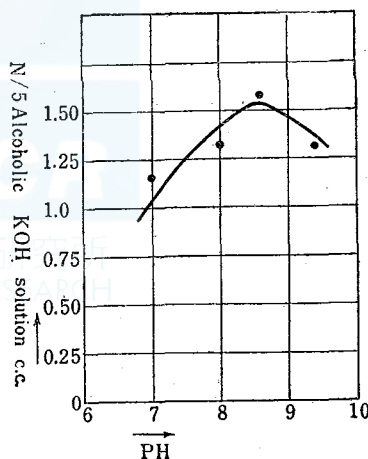


Fig. 1

4) Activity curve by the change in the amount of enzyme

0.5 cc, 1 cc, 2 cc, 3 cc, 4 cc and 5 cc of the glycerine extract of pancreas, as described in (3), were added to solutions in which 0.5 g gelatine was dissolved in 10 cc NaOH-glycocol buffer solution of pH 8.1 and water added to make the total volume 15 cc. This was reacted for 2 hours at 40°C and finally titrated as in (3). Results are shown in Fig. 2.

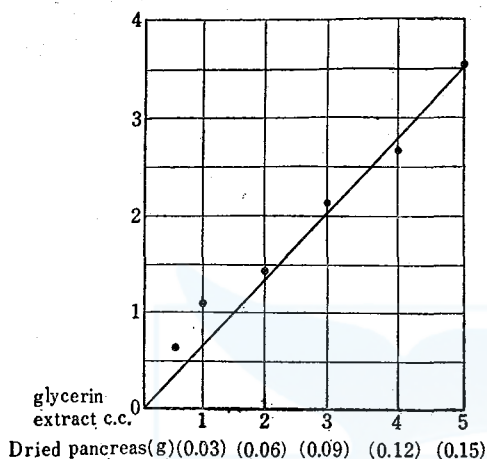


Fig. 2

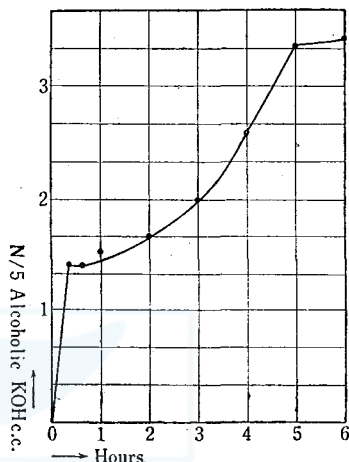


Fig. 3

5) Activity curve for varied action time

2 cc glycerine extract of pancreas as described in (3) was dissolved in 10 cc solution of NaOH-glycocol buffer solution of pH 8.1 in which a base of 0.5 g gelatine was dissolved. The reaction temperature was kept constant at 40°C and time varied at 15 minutes, 30 minutes, 1, 2, 3, 4, 5 and 6 hours. Titration was performed as in (3). Results are shown in Fig. 3.

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