64. Purified Proteases.

IV. Mould protease or Mould tryptase : *Its purification, properties, composition and constitution.*

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(Comm. by R. MAJIMA, M.I.A., April 12, 1944.)

In this paper the results of further studies on mould protease will be described briefly.

The nature of mould protease is quite similar to those of papain and pancreatic proteases. All these three proteases are of non-protein nature, contrary to the generally accepted idea.

In purifying mould protease it has been most difficult to remove diastase which adhered to it in large quantities.

The purification method of mould protease from Takadiastase and Koji is described in the following scheme:—

Crude mould protease (Takadiastase) [2 Kg]

Dissolved in water, filtered and acetic acid added

Clear acidic solution

Insoluble residue produced by adding a sufficient amount of lead acetate removed

Filtrate

Precipitates obtained by saturating the solution with ammonium sulphate collected on a filter paper

Precipitates

Dissolved in water and insoluble lead sulphate removed

Solution

Precipitates obtained by adding again ammonium sulphate to saturation collected and washed with the saturated ammonium sulphate solution

Precipitates

Dissolved in water and alcohol added up to 50~55 % and the alcoholic layer separated after settling a while

Alcoholic solution

A sufficient amount of strong alcohol added, whereby precipitation taking place

Precipitates

Dried in vacuum desiccator and extracted quickly with a little cold water to remove diastase, the insoluble residue then dissolved in water and the treatment of the solution with alcohol repeated

Precipitates

Dried in vacuum and quickly extracted again with a little cold water, the insoluble residue further dissolved in water

Aqueous solution

Dialysed through animal membrane and the protease precipitated by adding alcohol to the solution contained in the animal membrane. The precipitates collected and washed with alcohol and ether

Purified mould protease (5g)

The preparation thus purified is a colorless amorphous powder which is easily soluble in water and its aqueous solution has very strong proteolytic power as shown in the following chart, but has no action of amylolytic and other enzymes.

Chart 1. Purified mould protease (10 % solution) [at 40°C]

By analysis the preparation was shown to contain

N: 13.2 %, C: 52.5 %, H: 6.5 %, S: 0.8~1.2 %

The purified mould protease has shown many similar properties with those of papain protease as described in the following lines:—

The pure preparation of mould protease indicates a higher destruction temperature than previously reported.

Protease	Destruction temperature
Mould protease	70°C or slightly over
Pancreatic protease	70°C or slightly over
Papain protease	85°C or slightly over

The destruction temperature, however, falls down more and more with the addition of acetic acid, whereby the destruction temperature curve falling down practically along the curve of that of papain protease as shown in the chart.

The optimum conditions for the action of mould protease are obtained in acidic solutions acidified with acetic acid, quite similar to those of papain protease.

It is clear that there exists no remarkable difference between the mould protease and papain protease in the behavior toward acetic acid.

Like other proteases, the purified preparation of mould protease is also destroyed by alkali, i. e., NH_4OH , NaOH , KOH , etc.

The pure preparation contains almost no ash, when burned in crucibles, and gives no reaction of phosphorous.

② Destruction temperature.

① Destruction curve of purified mould protease.

Chart 2. Destruction curve of mould protease.

Fig. 1. Spectrogram of mould protease.

The mould protease preparation, however, gives a typical sulphur reaction, when boiled with alkali and afterward added with lead acetate. It gives also ammonia gas, when boiled with alkali as detected clearly with Litmus paper.

All these reactions, are quite similar to those of papain protease.

The purified preparation of mould protease gives no reduction of

Fehling's solution and no α -naphtol reaction, exhibiting the non-saccharide nature.

It contains 13 % nitrogen, which gives biuret, ninhydrine and Liebermann's pirrole reactions.

Next, the decomposition products of the pure preparation of mould protease with 25 % sulphuric acid were examined, and tyrosine, oxypoline but no other amino acid were separated and identified.

Tyrosine: C:58.5 %, H:6.6 %, N:7.7 % [Cal. C:59.7 %, H:6.1 %, N:7.7 %]

Copper salt of oxypoline: N:9.2 % [Cal. N:8.7 %]

It is most important to note that all the three proteases, which originated from either animal or vegetable source have similar components.

The purified preparation of mould protease gave also quite simple and typical spectrogram at ultraviolet region, quite similar to those of other proteases as shown in the following Figure.

As may be seen in the above experiments, mould protease has also quite similar constitution to those of other proteases, i. e., tyrosine and oxypoline form the central skeleton of mould protease as described in the preceding papers of this series.

The author wishes to express his cordial appreciation to Prof. Dr. B. Suzuki, for his guidance and also wishes to express his sincere thanks to Dr. S. Kato for her kind counsel.