65. Purified Proteases.

V. Gastric protease or pepsin:
Its purification, properties, composition and constitution.

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The gastric protease (pepsin) has been studied and the entire data so far obtained are described in the following lines:

Although gastric protease has some typical properties, the components are quite similar to other proteases. All the proteases have similar constitutions, having dipeptides formed from tyrosine and oxyproline as the central skeleton, and sulphur and ammonia.

The so-called crystals of trypsin and pepsin described by Northrop and Sumner must be regarded as impurities, not proteases, because of their protein nature.

The purification method of gastric protease differs somewhat from that of other proteases and it is described in the following scheme:

Crude pig gastric protease (Pepsin J.P.H.) [2 Kg]

| Dissolved in water, filtered and acetic acid added |

Clear acidic solution

| Insoluble precipitates produced by adding a sufficient amount of lead acetate collected on a filter paper |

Precipitates

| Suspended in water, added with hydrochloric acid and the insoluble residue removed |

Filtrate

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

Precipitates

| Dissolved in water and insoluble residue removed |

Solution

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

Precipitates

| Dissolved in water, 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

| Dissolved in water and the treatment of the solution with alcohol repeated |
Precipitates
Dissolved in water, dialysed through an animal membrane and the protease then precipitated by adding alcohol. The precipitates collected and washed with alcohol and ether

Purified gastric protease (4 g)

The preparation thus purified is a yellowish white amorphous powder, which is easily soluble in cold water, and its aqueous solution exhibits very strong proteolytic power, but no reaction of other enzymes.

By analysis, it has been shown that the preparation contains

\[
\begin{align*}
N &: 13.6\%, \\
C &: 51.7\%, \\
H &: 6.4\%, \\
S &: 2.3-2.7\%.
\end{align*}
\]

The purified gastric protease indicates a higher destruction temperature than previously reported, as shown in the following table:

<table>
<thead>
<tr>
<th>Protease</th>
<th>Destruction temperature</th>
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<tbody>
<tr>
<td>Gastric protease</td>
<td>70°C or slightly over</td>
</tr>
<tr>
<td>Pancreatic protease</td>
<td>70°C</td>
</tr>
<tr>
<td>Mould protease</td>
<td>70°C</td>
</tr>
<tr>
<td>Papain protease</td>
<td>85°C</td>
</tr>
</tbody>
</table>

The destruction temperature of gastric protease, however, falls down more and more with the increased quantities of acids, i.e., acetic acid and hydrochloric acid, as the following chart shows:

The gastric protease is destroyed more easily in acidic solution with acetic acid than in neutral solution. It is also easily destroyed in acidic solution with hydrochloric acid.
The optimum conditions for the action of gastric protease are also obtained anywhere within the limits of the destruction curves as in the case of other proteases, and the strong proteolytic power is shown in the chart.

We see, thus, that there exist remarkable differences among proteases in the behavior toward acids.

The purified preparation of gastric protease contains only a little ash when burned in crucibles.

On decomposing the gastric protease with alkali, ammonia gas is evolved and easily detected. Furthermore, organic sulphur reaction is detected when lead acetate and acetic acid are added to the alkaline solution, producing brownish black precipitates of lead sulphide.

The pure preparation of gastric protease gives no reduction of Fehling's solution and no α-naphtol reaction, exhibiting non-saccharide nature.

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

All these experimental results are quite the same as with other proteases, hence the author becomes to believe that all the proteases have similar components and constitutions.

The studies on the decomposition products of the gastric protease with 25% sulphuric acid according to the author's method, as already mentioned in the preceding paper of this series, also demonstrate that tyrosine, oxyproline but no other amino acid are separated and indentified and that the components of gastric protease are also the same with other proteases.

Tyrosine: C:58.6%, H:6.8%, N:7.8% (Cal. C:59.7%, H:6.1%, N:7.7%)
Copper salt of oxyproline, N:9.0% (Cal. N:8.7%)
The purified gastric protease gives a quite similar spectrogram with other proteases, yielding typical and simple absorption bands as shown in the following figure.

It is one of the most important facts that all the proteases give quite same spectrograms which belong to the simplest and clearest ones. From the spectrograms, above shown, it is clear that the place of tyrosine in the molecules of proteases are also the same, because of non-divergency of absorption bands and that one molecule of tyrosine is contained in each protease molecule to judge from the intensities of the absorption.
It may be supposed at present that proteases from different sources may have the following structural formula in order to satisfy the experimental facts, as already mentioned in the papers of this series, i.e.,

1. From the spectrographic studies, it is supposed that proteases may be constituted with one molecule each of tyrosine and oxyproline and since all the proteases give biuret reactions, the dipeptides of tyrosine and oxyproline have to form the central skeletons of proteases.

2. Proteases evolve ammonia gas by treating with alkali, even
at low temperature, and 1/3 of total nitrogen contained in protease molecules can be easily estimated when heated with alkali, therefrom the dipeptides of the central skeleton have to be oxyprolinyl-tyrosine and the other I molecule of nitrogen has to be ammonia.

(3) Proteases never form any thiohydantoins according to the method of P. Schpack and W. Kumpf, therefore it is clear that proteases have no free carboxyl group.

(4) Proteases evolve hydrogen sulphide gas when heated with concentric mineral acids and contain very unstable sulphur which cannot be estimated at constant value by ordinary analytical method, therefrom it is supposed to be thionylamide at present.

Accordingly the following structure may be proposed for these proteases:

![Structure Diagram]

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