THE ACTION OF TRYPsin ON NATIVE AND DENATURED PROTEINS

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It has been demonstrated by several authors that ovalbumin (1), pseudoglobulin (2), serum albumin (2, 3), lactoglobulin (4), and other proteins are attacked by trypsin less readily in their native state than when they are denatured. Experiments carried out in our own laboratory revealed, however, that the tryptic hydrolysis of fibrinogen and of myosin proceeds at the same rate before and after their denaturation (5). In these as well as in most of the previously mentioned experiments, commercial trypsin or insufficiently purified preparations of the enzyme have been used. On account of the low efficiency of such preparations rather large quantities of enzyme were required; their amount varied from 5 per cent (4) to 80 per cent (2) of the proteins examined. The commercial trypsin (Merek) used in our previous experiments (5) contained approximately 0.06 trypsin unit (6) [T. U.] ÷ Kest. F. per gm. of nitrogen. The possibility could not be excluded that the opposite results, obtained with fibrinogen and myosin on the one hand and with albumins and globulins on the other hand, might be due to the effect of inhibitors or other impurities present in the trypsin preparations. We have repeated therefore our experiments with trypsin, prepared according to Northrop (7). Owing to the greater efficiency of the purified enzyme, we were able to reduce the amount of the enzyme to 0.5 to 2.0 per cent of the proteins examined; at the same time the duration of our previous experiments was reduced substantially, e.g. from 24 hours to 30 minutes. The possibility that labile proteins are denatured by the buffer solution or by toluene at 38° is reduced in such short experiments.

EXPERIMENTAL

Preparation of Substrates—Crystalline ovalbumin was prepared from hen's eggs (8), pseudoglobulin and euglobulin from sheep serum (9), collagen from ox bones (10), myosin from fresh rabbit muscle (11); fibrinogen was precipitated from blood plasma of the ox by NaCl (12). The precipitate was dissolved in water and reprecipitated by mixing with 2 volumes of a saturated solution of NaCl, containing 0.3 per cent of potassium oxalate. Ovalbumin and pseudoglobulin were purified by dialysis against distilled water.
Preparation of Trypsin—Trypsinogen was extracted from fresh ox pancreas with sulfuric acid according to the method of Kunitz and Northrop (7). Chymotrypsinogen was removed by the addition of ammonium sulfate; trypsinogen, precipitated by further addition of ammonium sulfate, was reprecipitated, washed with a saturated solution of magnesium sulfate in 0.02 N sulfuric acid (7), and dissolved in a small quantity of water. This solution was activated by the addition of borate buffer solution and by keeping in the ice box at pH 8 for several days (7). The Trypsin I solution used in Experiments 1 to 6 (Table I) contained 5.9 mg. of protein N per ml. with an activity of 0.17 [T. U.]/mg. (6); Trypsin II used in Experiments 7 to 12 contained 1.8 mg. of protein N and 0.08 [T. U.]/mg. per ml. respectively.

Determination of Rate of Hydrolysis—5 ml. of a solution containing 2.5 per cent of the native protein and 1 per cent of NaCl were put in each of eight small flasks. Since euglobulin and collagen do not dissolve in saline solution, 2.5 per cent suspensions of these proteins were used. 2 ml. of a 0.2 M solution of phosphate buffer, pH 8.0, and 1.0 ml. of water were added to each flask. Four of these flasks were weighed and then kept in a boiling water bath for 30 minutes. The coagulated protein was finely ground and water was added to its suspension in order to replace the water lost by evaporation. All flasks were then warmed to 38°; 1 drop of toluene and 0.05 ml. of the solution containing Trypsin I were added in Experiments 1 to 6; 0.17 ml. of Trypsin II was added in Experiments 9 to 11; and 0.05 ml. in Experiments 7, 8, and 12. At the times $t$ (recorded in Table I) 2 ml. of a neutralized 30 per cent solution of formaldehyde and 0.1 ml. of 1 per cent phenolphthalein were added to each of the protein samples and the rate of hydrolysis was determined by titration with 0.1 N NaOH. The results obtained from formal titrations are shown in Table I.

Results

The experiments recorded in Table I reveal the different behavior of globular proteins (ovalbumin, serum globulin) on the one hand and fibrous proteins (fibrinogen, myosin) on the other. Fibrous proteins were hydrolyzed by trypsin at the same velocity before and after denaturation of the protein, while globular proteins were attacked by trypsin much more rapidly after denaturation, although only the surface of the coagulated particles could be accessible to the enzyme. No hydrolysis or a trace only took place in the experiments with native globular proteins in the course of the first 30 minutes. But even after 4 or 24 hours the degree of hydrolysis of native globular protein remained far below that of denatured protein. Collagen, although a fibrous protein, is resistant to trypsin. We attribute
this to the insolubility of collagen in water and to the fact that coarse particles of the protein were used in our experiments. Heating in the water bath brought about a swelling of these particles and partial dissolution, i.e. formation of gelatin.

**Table I**

*Hydrolysis of Native and of Denatured Proteins by Trypsin at pH 8*

The third column of the table indicates the quantity of 0.1 N NaOH, required for formol titration at the beginning of the experiment; the figures in the last three columns indicate the excess of 0.1 N NaOH required.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Protein</th>
<th>Quantity of 0.1 N NaOH: t = 0</th>
<th>Excess 0.1 N NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ml.</td>
<td>ml.</td>
</tr>
<tr>
<td>1</td>
<td>Ovalbumin, native</td>
<td>1.31</td>
<td>0.17 0.22 0.32</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>1.11</td>
<td>0.28 0.48 1.00</td>
</tr>
<tr>
<td>2</td>
<td>Pseudoglobulin, native</td>
<td>2.16</td>
<td>0.04 0.11 0.28</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>1.70</td>
<td>1.07 1.16 2.04</td>
</tr>
<tr>
<td>3</td>
<td>Collagen, native</td>
<td>0.73</td>
<td>0.07 0.19 0.50</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>0.80</td>
<td>0.18 0.68 3.16</td>
</tr>
<tr>
<td>4</td>
<td>Fibrinogen, native</td>
<td>1.26</td>
<td>0.81 1.39 2.13</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>0.87</td>
<td>0.78 1.67 2.42</td>
</tr>
<tr>
<td>5</td>
<td>Myosin, native</td>
<td>1.94</td>
<td>1.28 1.72 2.74</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>1.58</td>
<td>1.80 1.78 2.79</td>
</tr>
<tr>
<td>6</td>
<td>Casein (Merck)</td>
<td>1.03</td>
<td>1.83 2.19 3.10</td>
</tr>
<tr>
<td>7</td>
<td>Pseudoglobulin, native</td>
<td>1.50</td>
<td>0.10 0.15 0.30</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>1.25</td>
<td>0.40 1.05 1.55</td>
</tr>
<tr>
<td>8</td>
<td>Euglobulin, native</td>
<td>1.32</td>
<td>0.10 0.28 0.93</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>1.19</td>
<td>0.25 0.96 1.59</td>
</tr>
<tr>
<td>9</td>
<td>Ovalbumin, native</td>
<td>2.17</td>
<td>0.0 0.10 0.30</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>2.17</td>
<td>0.23 0.63 1.15</td>
</tr>
<tr>
<td>10</td>
<td>Collagen, native</td>
<td>1.60</td>
<td>0.10 0.18 0.29</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>1.73</td>
<td>0.29 0.79 1.02</td>
</tr>
<tr>
<td>11</td>
<td>Fibrinogen, native</td>
<td>1.96</td>
<td>0.46 0.84 1.18</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>2.05</td>
<td>0.50 0.95 1.31</td>
</tr>
<tr>
<td>12</td>
<td>Myosin, native</td>
<td>3.56</td>
<td>0.10 0.54 1.76</td>
</tr>
<tr>
<td></td>
<td>&quot; denatured</td>
<td>3.52</td>
<td>0.09 0.66 1.63</td>
</tr>
</tbody>
</table>

**Discussion**

The resistance of native proteins to trypsin had been ascribed originally to the presence of an antitryptic factor in the blood serum (13) or in raw egg white (14). It is, however, extremely improbable that such a factor would be present in all fractions of the blood serum (i.e. serum albumin, pseudoglobulin, and euglobulin) at the same time (5).

Linderström-Lang (15) and Lundgren (16) attribute the resistance of
native globular proteins to the fact that the atomic groups serving as points of attack for the enzyme are inside the globular molecule and inaccessible to the proteinase. Denaturation, which apparently involves an unfolding of the closely packed peptide chains (17, 18), renders these groups accessible to the enzyme. The view of Linderström-Lang (15) is corroborated by our own experiments, for no steric hindrance can be expected in fibrous proteins such as fibrinogen or myosin, whose peptide chains are either expanded or only slightly folded.

These results are probably of some importance for the nutrition of man. It is well known that the proteins of foodstuffs are attacked at first by pepsin at pH 1 to 2; the old experience that native and denatured proteins are hydrolyzed by pepsin at the same rate was confirmed by experiments carried out in our laboratory. But in patients suffering from gastric achyia, when no digestion occurs in the stomach, the raw proteins of the food enter the intestines and undergo there the action of trypsin. According to our experiments one would expect that raw globular proteins resist the action of trypsin, while fibrous proteins ought to be rapidly hydrolyzed. Actually Talarico (19) showed some time ago that raw eggs are resistant to trypsin, while boiled eggs are rapidly hydrolyzed; raw meat, however, is digested just as rapidly as boiled meat. Results of similar experiments carried out in our laboratory agree with those of Talarico (19), and so details of these experiments may be omitted. We attribute the digestion of raw meat by trypsin to its high content of fibrous proteins.

Text-books of biochemistry (20) or of therapeutics (21) used to recommend raw protein food, e.g. raw meat, which is more easily digested than boiled food. This is contradictory to the results of our experiments. According to these experiments boiling may raise the digestibility of raw protein food, but it never reduces it.

SUMMARY

1. While globular proteins such as ovalbumin or serum globulin are hydrolyzed by trypsin very slowly in their native state, fibrous proteins such as fibrinogen or myosin are hydrolyzed by trypsin at the same rate before and after denaturation. The susceptibility of native fibrous proteins to the attack of trypsin is attributed to the expanded configuration of their peptide chains, which renders their peptide bonds accessible to the enzyme.

2. The digestibility of raw protein food (meat, eggs, milk) by trypsin is not superior to that of boiled food.

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