

Dependence of the Peptic Hydrolysis of Zein at Different pH on the Protein Concentration

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In examining the so-called plastein formation we have paid attention to the degree and velocity of hydrolysis of zein at different pH while pepsin acts as a hydrolyzing enzyme. This question has gained more importance since the formation of plastein was shown to be an enzymatic synthesis of polypeptides and hence, a reverse to hydrolysis¹⁻³. The chemical nature of the insoluble fraction remaining of the hydrolysis of zein has also been a subject of our research.

EXPERIMENTAL

Zein was hydrolyzed at pH about 1, 2, 3, and 4 with cryst. pepsin. Parallel experiments were made at each pH with 2 g of zein in 80 ml, 800 ml, and 1 200 ml of water acidified with HCl. The amount of pepsin was always 5 mg (0.7 mg N) per 1 g zein. In order to avoid zein becoming lumpy and thus disturbing the reaction velocity, the finely ground zein was mixed with quartz sand before putting it in the solution. In this way consistent results were obtained in parallel experiments. The quantity of hydrochloric acid at pH 3 and especially at pH 4 was so small that the pH rose rapidly during the hydrolysis. The acidity of the solutions was followed with repeated determinations of pH by means of a glass electrode and hydrochloric acid was added when necessary. pH could thus be maintained with an accuracy of ± 0.4 . A slight rise in pH occurred also at pH 1 and pH 2, but no acid was then added.

The hydrolysis of zein was followed by determining the nitrogen brought to the solution.

The chemical nature of the solid substance remaining in the solution at the end of the experiment was examined

1) by determining the total N and the amino N of the substance. The latter was determined as a rule by means of the Cu method of Pope using the coefficient 0.14. The agreement between this and van Slyke method is satisfactory at least in this particular case.

2) by determining the solubility of the precipitate in 60 % alcohol. Zein dissolves in it whereas the polypeptides which are formed in the plastein synthesis are soluble only to a smaller extent.

3) by comparing the x-ray diagrams of zein, plastein, and the precipitate insoluble in the hydrolysis solution. The x-ray diagrams were taken, as described in the previous paper⁴, from 1–2 mm thick samples pressed from dry powder.

The dependence of the plastein synthesis on the pH was determined by using a peptic hydrolysate of zein for the starting material. It was prepared by letting cryst. pepsin act for 28 days on a zein suspension acidified with formic acid. pH was at the start 1.78, at the end 2.15. 96.1 % of N was brought to the solution and amino-N was 10.1 % of total N, thus the average size of peptides corresponded to 9.7-peptides.

The clear solution was evaporated to a small volume and by adding alkali (NaOH) or acid (formic acid) a pH series was arranged with pH 1, 2, 3, 4, 5, and 6. Each solution contained 40.0 mg N per 1 ml. The results appear from Table 1.

Table 1. Dependence of plastein precipitation on pH. Experimental time 1 h.

pH	Volume ml	Total N mg	N mg/ml	Cryst. pepsin mg	Precipitate			Loss of amino- N mg	Loss of amino-N, % of initial amino-N
					Dry matter mg	N mg	N % of total N		
1	11	440	40	30	—	—	—	0	0
2	11	440	40	30	42.5	5.44	1.23	1.3	1.4
3	11	440	40	30	317.8	40.67	9.24	6.67	7.2
4	11	440	40	30	637.0	80.9	18.38	14.03	15.1
5	11	440	40	30	389.8	49.9	11.34	6.69	7.3
6	11	440	40	30	126.5	16.10	3.65	4.17	4.5

The peptide size of the hydrolysate was so large that a prolonged hydrolysis would have caused a rise in the amino-N. But within 1 h the synthesis is distinct.

In another experiment where zein was hydrolyzed at pH 1 for 60 days and the average peptide-size of the hydrolysate corresponded to 4.2-peptides the drop in amino nitrogen was 2.2 % at pH 1.5 within 1 h and 4.6 % within 2 and 20 h. The N-concentration of the hydrolysate was in this experiment 53.4 mg per ml.

The dependence of the plastein precipitation on the N-concentration of the hydrolysate is illustrated by the curve in Fig. 1. It does not give a right picture of the corresponding dependence of the plastein synthesis because zein was hydrolyzed in a hydrochloric acid solution at pH 1.2–1.5 and plastein was precipitated from the salt-containing hydrolysate at pH 4. A great amount of small peptides is then precipitated at least in higher concentrations in addition to the synthesized polypeptides⁵. The amount of the plastein precipitate is thus greater than that corresponding the synthesis. We shall deal with this question in another connection at a greater length. The interrupted line represents the synthesis calculated from the decrease of amino nitrogen. The values have been obtained from experiments made in another connection and not from parallel experiments, hence they are approximate.

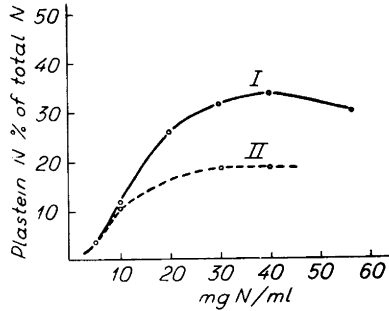


Fig. 1. Dependence of plastein formation at pH 4 on the N-concentration of zein hydrolysate.

I Precipitated N % of total N of the hydrolysate.

II Decrease of amino-N.

RESULTS

The results of the pepsin hydrolysis of zein in different concentrations and at different pH are presented by curves in Figs. 2. It can be seen from them that at pH 1 and pH 2 the zein concentrations of 375 mg N/100 ml, 37.5 mg N/100 ml, and 25 mg N/100 ml have allowed an almost complete (97 %) hydrolysis in the highest concentration and a complete hydrolysis in a 10- and 15-fold dilution. At pH 3 and 4, on the other hand, zein was only partly brought to the solution in the highest concentration. At pH 4 only about 40 % of the zein was brought to the solution during 13 days and the direction of the curve shows that the percentage rises then very slowly with prolonged time. The degree of hydrolysis, again, rises distinctly while the dilution in-

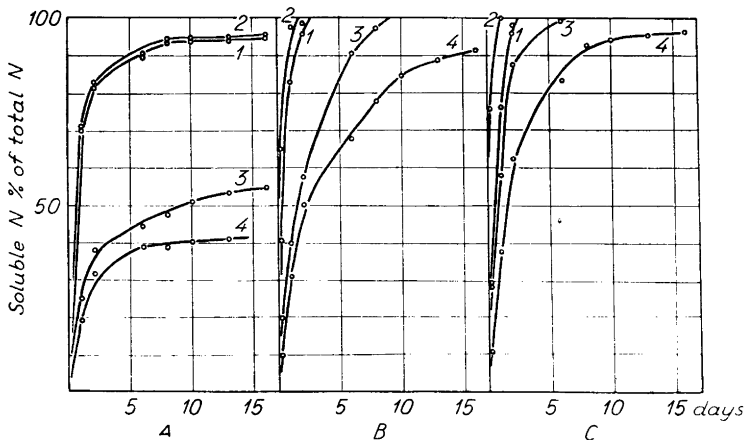


Fig. 2. Hydrolysis of zein at different pH and different concentrations. The numbers indicate the pH of the solution during hydrolysis.

A = Zein concentration 3.75 mg N/ml
 B = » » 0.375 » »
 C = » » 0.25 » »

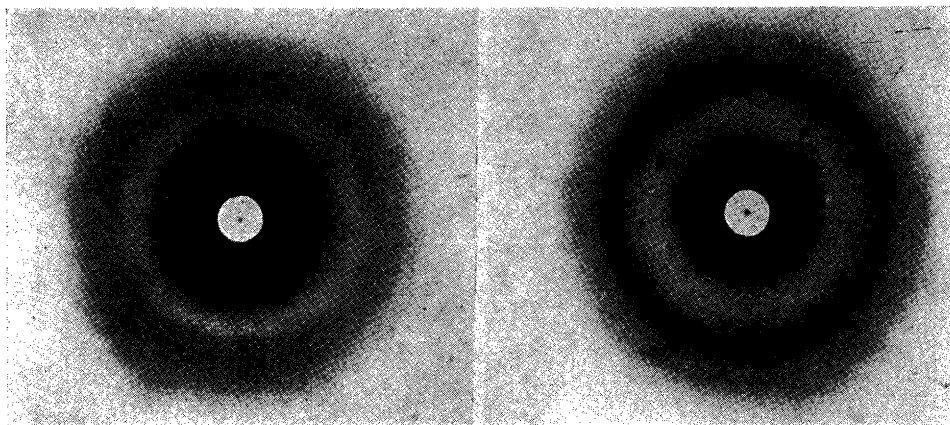


Fig. 3. X-ray diagram of zein (left) and plastein (right) precipitated from the pepsin hydrolysate of zein. The average peptide size of plastein about 50-peptides.

creases. In a 15-fold dilution the zein is almost entirely brought to the solution in 16 days.

These results indicate that a reaction reverse to hydrolysis prevents hydrolysis at pH 3 and especially at pH 4 which is the optimum of the plastein synthesis. At pH 1—2 the synthesis is very weak and experimentally detectable only in much more concentrated solutions than the highest one used in this

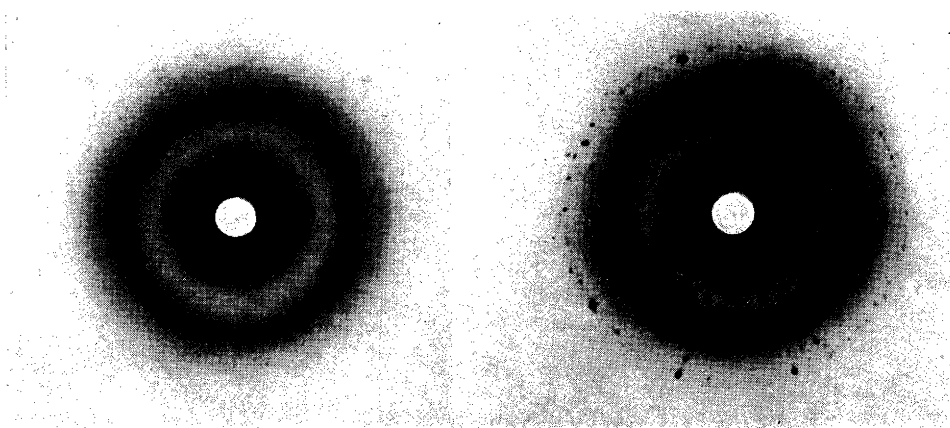
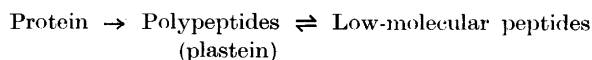


Fig. 4. X-ray diagram of a mixture of zein and plastein (1 : 1) (left) and insoluble residue, which was left over on hydrolysis of zein at pH 4 (right). The spots in the figure are caused by quartz sand particles among the preparation.

work. A complete or nearly complete hydrolysis of zein is then natural. At pH 4, again, the plastein synthesis is experimentally detectable even in such a peptide concentration which is brought about by hydrolysis in the highest zein concentration used in this work. As 40 % of zein nitrogen has been brought to the solution the hydrolysis contains 1.5 mg N per 1 ml. In a zein hydrolysate which contained 1.55 mg N per 1 ml, pepsin produced during a longer time small precipitate whose amino-N was 3.7 % of total N. Plastein synthesis proceeds thus weakly in this peptide concentration.

The insoluble substance which remains over of the hydrolysis at pH 4 dissolves only partly in 60 % alcohol at room temperature. Since zein dissolves quantitatively in this solvent the insoluble substance cannot be exclusively zein. Amino nitrogen of this substance is also higher (in different preparations 0.6—1.6 % of total N) than in zein (about 0.25 % of total N). It is evident, therefore, that the insoluble fraction contains considerably — in experiments of longer duration perhaps chiefly — polypeptides. These may be either products of synthesis or partly high-molecular products of zein hydrolysis, which have not decomposed further. Our concept of the course of protein hydrolysis and plastein synthesis shows the formation possibilities of the insoluble substance in the pepsin hydrolysis:



The x-ray diagrams of zein, plastein and the insoluble substance in pepsin hydrolysis as well as of the mixture of plastein and zein (1 : 1) are given in Figs. 3 and 4.

The rings are sharper in the diagram of the insoluble substance than in zein, but nevertheless slightly more diffuse than in plastein. The x-ray research thus leads to the same result as the determinations of amino nitrogen and solubility in regard to the chemical nature of the precipitate.

The ratio of equilibria in the pepsin hydrolysis of zein is very complicated. The system contains at first only insoluble zein, then growing amounts of soluble peptides and later increasing amounts of polypeptides, which are precipitated. The precipitate then contains simultaneously zein and polypeptides.

SUMMARY

Comparative experiments on the hydrolysis of zein by the action of pepsin at different pH and in different concentrations of zein have shown that at pH 1 and 2 the nitrogen of zein is almost or entirely brought to the solution in concentrations of 3.75 mg N, 0.375 mg N and 0.25 mg N per 1 ml. On the

other hand, at pH 3 and especially 4 a considerable or the major part of nitrogen is in the insoluble residue.

The amino nitrogen of the insoluble residue is considerably higher than that of zein and the residue dissolves only partly in 60 % alcohol, in which zein dissolves completely. Accordingly, the residue contains considerably polypeptides (plastein). The x-ray diagrams lead to the same result in regard to the nature of the residue as determinations of amino nitrogen and solubility.

The results suggest that the insoluble residue in the pepsin hydrolysis of proteins at pH 3 and most distinctly at pH 4, which is optimum to the plastein synthesis, is due to the formation of insoluble polypeptides.

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