ACTA CHEMICA SCANDINAVICA 2 (1948) 933—937

Short Communications

Observations on the Formation and Structure of Plastein

(3rd Preliminary Communication)

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Butler *et al.*[1] have recently advanced the idea that in the process of plastein formation new peptide bonds do not arise in the protein hydrolysates. Precipitation would likewise not be caused by enzymatic reaction but by denaturation of some high-molecular products of proteolysis. This concept is based on the findings made with peptic digests of insulin.

The precipitation of the said authors does not correspond to the plastein formation examined in this laboratory during the recent years.

When zein is hydrolyzed with crystalline pepsin at about pH 1 at 37° C, the solution obtained contains 15—21 % α-amino-N estimated by van Slyke's method (5 min. shaking) and approximately the same amount by the Cu method by using the factor 0.14[2]. Taking into account the formation of free amino acids it can be concluded from the free amino groups that the average size of peptides in pepsin hydrolysate varies between hexa and tetrapeptides, depending on the reaction time and the quantity of pepsin. It is probable that at first octapeptides and still higher peptides are formed and from these tetrapeptides. In old pepsin hydrolysates there are still smaller peptides [3].

When pepsin is destroyed by keeping the solution for half an hour at 80—85°, the clear hydrolysate evaporated in a vacuum until the solution contains about 30 mg N per ml, and the pH of the solution adjusted to 4 with sodium hydroxide, the solution remains clear at room's temperature *. Crystalline pepsin admixed to the solution causes a very rapid formation of white precipitate. After standing overnight at 37° C the separated precipitate (»raw plastein») contains about 30 % of the nitrogen of the total hydrolysate. The amount of the precipitate varies greatly depending on the concentration of the hydrolysate as will be seen from Table 1.

During the precipitation of plastein amino N decreases. In our recent experiments the amount of amino N in *raw plastein + filtrate* has been 13.5—18 % lower than in the initial hydrolysate.** Hence, with the formation of plastein new peptide bonds are formed. Hydrazine which prevents the protein hydrolysis with pepsin [5] does not affect plastein formation.

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* From a highly concentrated hydrolysate a brown sticky mass may be precipitated without pepsin when the pH of the solution is changed to 4. On the basis of the free NH_2 groups this substance is a mixture of open peptides whose average size corresponds to about hexapeptides, consequently, to somewhat larger peptides than those of the hydrolysate on an average. No decrease of amino groups does thereby occur, hence the question is about »denaturation» and precipitation reactions. Such a precipitate perhaps corresponds to the »plastein» of Butler *et al* [1].

** The values regarding the plastein formation from egg albumen given in the first communication [4] signify the decrease of the amino groups in the solution during precipitation of raw plastein and not the loss of the amino groups in the whole system.

Table 1. Dependence of the formation of raw plastein on the concentration of the pepsin hydrolysate of zein.

N mg/ml hydrolysate	N in pepsin mg/ml hydrolysate	Yield of raw plastein mg/40 ml hydrolysate	N of raw plastein, % of N in the hydrolysate
10	0.35	458	13.9
20	»	1658	25.2
30	»	3102	31.5
40	»	4561	34.7
56.5	»	6613	35.7

Table 2. Dependence of the formation of raw plastein on the concentration of pepsin.

N, mg/ml hydrolysate	N in cryst. pepsin, mg/ml hydrolysate	Yield of raw plastein mg/10 ml hydrolysate	N of raw plastein, % of N in the hydrolysate
28.35	0	0	0
»	0.1	859	32.7
»	0.3	996	36.5*
»	0.7	1012	37.2
»	1.0	1034	37.4

* The decrease of amino groups was abt. 17 % of the amino groups in the hydrolysate.

By allowing the raw plastein precipitate to stand for some days in water at room's temperature (toluene as a bactericide) large amount of peptides are brought into the solution. Their average size, on the basis of amino nitrogen, is about that of penta-hexapeptides. After this abundantly peptides still pass to boiling water. These correspond approximately to octa-decapeptides on the basis of amino nitrogen. The higher the concentration of the hydrolysate from which raw plastein is precipitated, the more water-soluble parts the precipitate contains. The yield of raw plastein diminishes after extraction with cold and hot water, often with 40—50 % and more.

The peptides extracted from the raw plastein with cold and hot water do not give any precipitate with trichloracetic acid. It seems that they are not synthetical products but peptides formed during peptic hydrolysis.

The plastein left over after a thorough treatment with cold and hot water (»real plastein») still contains some amino nitrogen. The lowest percentage of amino N in our plastein preparations has been 2.2 % of total N. In the previous communication it has been held possible that the small amount of NH_2-N found in van Slyke's estimation may arise from amide groups and from the guanidine group of arginine. Since, however, the Cu-method yields approximately the same result as does van Slyke's method, evidently α-amino nitrogen is in question.

If the molecular weight of the insoluble plastein is less than 1000, as has been supposed, the substance must be a mixture of cyclopeptides [6]. The small amount of α-amino nitrogen may then result either from open peptides still present in the plastein preparation or from side chains attached to the ring. The low molecular weight of raw plastein (abt. 300) found cryoscopically in several estimations in phenol solution is, however, obviously too

low (cf. the corresponding estimations with gramicidin [7] in phenol giving values abt. 900 instead of the probable value abt. 3000). New estimations with thoroughly extracted plastein in formic acid have given values for mol. weight from 600 to 750 (Miettinen). In the peptic digest of plastein about 17—20 % amino nitrogen are formed, accordingly, the average size of cyclopeptides would correspond to tetra-pentapeptides [6], provided that amino nitrogen arises at the opening of the ring. The average mol. weight found cryoscopically in formic acid is in agreement with this opinion. The size of the individual cyclopeptides is naturally unknown.

The observations of Svedberg [8] and Ecker [9] that no sedimentation takes place in alkaline plastein solutions in the ultracentrifuge give evidence for a low molecular weight of plastein. Unfortunately, the preparations used for the estimations have been raw plasteins the amino N of which was unknown.

The observed »denaturation» of plastein during the hot water treatment adds for its part to the difficulties met in the plastein examination. Pepsin hydrolyzes rapidly only cold-water-treated plastein at pH 1—2, whereas after a thorough hot-water-treatment plastein is very slowly and incompletely hydrolyzed. The pepsin-resistant plastein dissolves also very difficultly in an alkaline solution at pH 9 to 13 where raw plastein dissolves very easily without decomposition (no rise in NH_2-N). Clear solution is obtained from the hot-water-treated plastein only by adding alcohol to the alkaline solution up to about 50—60 per cent. In the course of the hot water treatment of long duration amide nitrogen splits almost entirely from the plastein. This may, however, not be the cause to the rise of the pepsin-resistance of plastein and to the lowering of its solubility. We have discussed the possibility that the free carboxyls of the aminodicarboxylic acids would participate in the intramolecular ester formation and that the »denaturation» of plastein would result from such a lactone formation.

In view of the above observations, only the decrease in the amino nitrogen can be taken for a criterion of the formation of plastein. The amount of the precipitate and its nitrogen content do not give a real picture of it.

By using the membraneless electrophoresis apparatus of Spies [10] we are attempting to divide the pepsin hydrolysate of zein into different peptide fractions and to examine the plastein formation from peptides of different size.*

* *Addition to proof.*

In the first experiment on this line Virtanen and Maire Hakala formed plastein precipitates in different peptide fractions the average molecular size of which ranged from 4 to 6 peptides. The amino nitrogen of the plastein preparations formed was 2—3 % of the total nitrogen without cold and hot water extraction. The preparations were soluble in alkaline solution (pH 9). Cryoscopical determination of the molecular weight of a plastein with 2.1 % amino N gave in absolute formic acid a value of 445 which corresponds fairly well to cyclotetrapeptide.

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Received December 20, 1948.