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Plastein, a Mixture of Higher-molecular Polypeptides Synthesized by Proteolytic Enzymes

ARTTURI I. VIRTANEN, HEIKKI K. KERKKONEN, TERTTU LAAKSONEN and MAIRE HAKALA

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

The investigations carried out in this laboratory on the formation of plastein have led to results which can be interpreted in two different ways depending on how large peptides compose the plasteins. (1) If the mol. wt. of plasteins is low (some hundreds), cyclopeptides must be concerned since the amount of α-amino nitrogen in preparations thoroughly extracted with water has been only 2—3 per cent of total nitrogen. The explanation of the occurrence of this small amount of α-amino nitrogen in plasteins presupposes already by-hypotheses. It may be assumed that either open peptides are absorbed in the precipitate or a part of the cyclopeptides contain a side chain with a free α-amino group. (2) If the molecular weight is several thousands, the plasteins must be open chains. All our experimental results will also suit this opinion. The presence of α-amino nitrogen in the preparations would then be easily explicable, and amino nitrogen would also express the size of peptides.

Alternative (1) seemed at first most probable because the molecular weight of plastein could be considered low both on the basis of our cryoscopic determinations [1, 3] and the ultracentrifuge determinations of Svedberg [4] and Ecker [5] recorded in the literature. Collier [6] found in his ultracentrifuge determinations particles of the size of proteins from plastein preparations in a 6.7 M urea solution. The plasteins were, however, not characterized in regard to their α-amino nitrogen content. As was shown in this laboratory [3] a large amount of peptides which are probably not synthetic products are precipitated also with raw plastein.

After dividing by electrophoresis the peptides formed in pepsin hydrolysis of zein into electrically more homogeneous fractions we were able to precipitate from them, with pepsin, plasteins not accompanied by peptides found in the hydro-

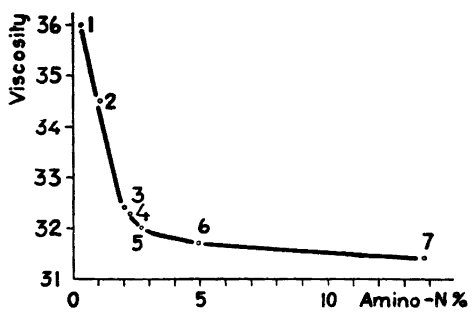

Fig. 1. Viscosity of 1 % solutions in millipoises at 18°C. Solvent 0.1 N NaOH in 60 % ethanol. (1) Zein, (2) Zein hydrolyzed with water at 100°, fraction with 1.05 % amino-N, (3) Zein plastein with 1.97 % amino-N, (4) Zein hydrolyzed with water at 100°, fraction with 2.21 % amino-N, (5) Zein plastein with 2.65 % amino-N, (6) Zein hydrolyzed with water at 100°, fraction with 4.90 % amino-N, (7) Zein hydrolyzed with water at 100°, fraction with 13.73 % amino-N.

lysate. Such plastein precipitates contained, without extraction with water, only 1.5 to 2.0 % of the total nitrogen as α-amino nitrogen. This amino nitrogen cannot be removed with water extractions, hence, it seems evident that it belongs to the plasteins. Occurrence of α-amino nitrogen in cyclopeptides is difficult to understand (*cf.* above) and therefore the low molecular weight and cyclic structure of plasteins began to seem improbable. Comparative viscosity determinations from peptides (average α-amino nitrogen 1.05 to 13.7 per cent of the total nitrogen) formed by prolonged hot water hydrolysis from zein, and from plastein preparations (α-amino nitrogen 1.97 per cent and 2.65 per cent of the total nitrogen) have yielded values which can be explained only by assuming plasteins to have an open peptide structure like the peptides formed in the hydrolysis [8] (Fig. 1).

Moreover, it was ascertained in this laboratory [8] that even rather large-sized peptides formed in the hydrolysis of zein, whose average molecular weight judged from α-amino nitrogen should be several thousands, cause both in formic acid, acetic acid, and phenol depressions of freezing point which indicate that the mol. wt. of peptides would be only a few hundreds. Therefore the values for plasteins obtained earlier by the cryoscopic method in polar solvents have no power of evidence in regard to the molecular weights of plastein. The latest determinations of mol. wt. by the cryoscopic method using a non-polar compound (lactam of 4-amino-cyclo-hexane-carboxylic acid [7]) for the solvent have yielded mol. weight values of about 4000 for plastein preparations with 1.6 % α-amino nitrogen. Judging from the amino nitrogen the average mol. wt. should be about 6000. The accuracy of the cryoscopic method for so large molecules is already poor, but the value obtained indicates, at any rate, the order of magnitude. The values obtained by the diffusion method also show that the average mol. wt. of plasteins is several thousands.

The low mol. weight would thus be supported only by the ultracentrifuge determinations mentioned above [4, 5]. The plasteins used in them have, how-

ever, been of indefinite composition and not more closely characterized. According to our present experience they have evidently contained a large number of smallsized peptides. The determinations are consequently, not convincing although the total lack of larger particles in the plastein preparations used does not agree with the idea that plastein comprises high-molecular peptides.

As was reported already in our previous communication [3] the only quantitative criterion for the formation of plastein in protein hydrolysate — the hydrolysis of zein performed with pepsin at pH 1—2 and the plastein formation with pepsin at pH 4 — is the decrease of α-amino nitrogen. But this can be noted only in such concentrated hydrolysates in which the enzymatic hydrolysis has proceeded so far that amino nitrogen does no longer essentially increase (free amino acids are split from the pepsin hydrolysate to some extent, though slowly even after cessation of the hydrolysis proper). If plastein is precipitated before the hydrolysis has ceased decrease of amino nitrogen cannot often be noted because the larger peptides are simultaneously hydrolyzed. In completely hydrolyzed concentrated pepsin hydrolysates the decrease of amino nitrogen through the effect of pepsin can be shown also at pH 1—2, though no precipitate is then formed, at least not rapidly. At a higher pH, precipitate is formed almost momentaneously. The optimum for precipitation is pH 4. Decrease of amino nitrogen is also greatest in this acidity.

We have also hydrolyzed zein with pepsin at pH 4 and after concentration of the clear solution precipitated plastein with pepsin at the same pH. The yield of raw plastein was then much smaller than when zein was hydrolyzed at pH 1—2. This seems to be largely due to the fact that raw plastein which is obtained from zein hydrolyzed at pH 4 does not contain small-molecular products of hydrolysis, at any rate not appreciably, whereas the zein hydrolysate concentrated at pH 1—2 yields at pH 4 raw plastein the nitrogen of which may be composed up to the half of small-sized peptides removable with water. In the first experiment, when zein was hydrolyzed at pH 4, 92.2 per cent of the total nitrogen in zein was brought into solution in 26 days. α-Amino nitrogen in the hydrolysate was 16.4 % (NH_3-N, amide-N, and N of free amino acids subtracted) corresponding on the average to 5—6 peptides. In the concentrated hydrolysate (38.4 mg N per ml) precipitation of plastein was 8.8 % (N % of the total N of the solution). The decrease of amino N was 9.7 %, thus the precipitate evidently contained synthesized peptides only. The amino N of the precipitate was 2.0 % of the total N. The part of zein which was not brought into solution by peptic hydrolysis (7.8 % N of total N) was dissolved in 60 % alcohol up to 45.9 % N of total N. This can

be taken for zein. The fraction insoluble in alcohol contained 1.6 per cent α-amino N, hence it corresponded in size to »plasteins».

In the second experiment in which the hydrolysis of zein was also performed at pH 4, 19.2 per cent of the nitrogen brought into solution was α-amino nitrogen (NH_3-N, amide-N, and N of free amino acids subtracted) corresponding on the average to 5-peptides. Precipitation of plastein from the concentrated solution (38.6 mg N per ml) was in this case 12.9 % (N % of the total N of the solution). The decrease of amino N was 12.2 %. The plastein precipitate contained 1.6 % of the total nitrogen as amino nitrogen.

After the small molecular size of plasteins was proved invalid all the observations so far can be interpreted in the following way. The formation of plasteins by pepsin is a reaction reversed to hydrolysis. In dilute solutions (in our experiments the amount of zein corresponded to 2—3 mg N per ml) the equilibrium lies very far on the side of hydrolysis. When the hydrolysate is concentrated (in our plastein experiments the concentrated solution usually contained 30—45 mg N per ml) the reaction reversible to hydrolysis comes forth. At pH 4 this reaction proceeds farthest because the reaction product precipitates optimally and is thus removed from the system. The following scheme presents our concept of the action of pepsin.

Zein ⟶ High-molecular peptides (very different mol. size, highest peptide demonstrated by us in zein hydrolysis abt. 40-peptides, in plastein synthesis abt. 50-peptides) ⟶⟵ Low-molecular peptides (average tetra-penta-peptides)

The plasteins which have been precipitated from electrophoretically divided peptide fractions and contain least amino nitrogen (1.5 % of total N) correspond, on the basis of amino nitrogen, to the average mol. weight of abt. 6000, those containing most amino nitrogen (4.4 %) to abt. 2500. All the precipitates are unhomogeneous and contain obviously larger and smaller peptides.

The most abrupt decrease of α-amino nitrogen effected by pepsin in concentrated pepsin hydrolysate at pH 4 (zein hydrolyzed at pH 1) was in our experiments about 19 %. If, after removal of the precipitate, the filtrate is concentrated again, pepsin does no longer cause precipitation of plastein. This suggests that all peptides are not suitable for the synthesis.

SUMMARY

The former idea of the low molecular weight of plasteins (less than 1 000) does not agree with the more recent findings of this laboratory. The parallel viscosimetric determinations with plastein preparations and with polypeptide fractions formed in the hydrolysis of zein as well as the cryoscopic determinations using a non-polar solvent (lactam of 4-amino-cyclo-hexane-carboxylic acid) support the concept that the average molecular weight of plasteins is several thousands. The diffusion experiments also lead to similar results. Accordingly, the plasteins must be regarded as open polypeptides whose α-amino nitrogen results from the end groups. Thus pepsin causes both the hydrolysis and synthesis of polypeptides.

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