X-Ray Diagram of Plastein

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The investigations, which have been carried out in the Biochemical Institute in Helsinki on the structure of the so-called plastein, have shown that high-molecular polypeptides are in question. The average peptide size of different plastein preparations, calculated on the basis of amino nitrogen, has varied corresponding to about 30—100-peptides, or to mol. weights of about 3 500—12 000. The average peptide size of the protein hydrolysates used for the synthesis has usually corresponded to 5—6-peptides.

In order to be able to compare the structures of plastein and protein, from the hydrolysate of which plastein was precipitated by means of pepsin, we took X-ray photographs of pulverized plastein and zein. In the present paper these diagrams are given. The plastein used was prepared as follows.

Zein was hydrolyzed for 50 days with pepsin in a water suspension acidified with formic acid to pH 4 (150 g zein in 6 l water, + formic acid, 750 mg cryst. pepsin, 40 ml toluene, temperature 37° C). After heating for 20 min at 85° C to inactivate pepsin the insoluble residue was separated and the clear filtrate concentrated in vacuo. The solution then contained 40.0 mg N per ml. Amino-N of the solution estimated with the Cu-method (coef. 0.14) [1] was 16.3 % of total N after subtraction of ammonium and free amino acids. The average peptide size corresponded thus to about 5-peptides. 1 000 mg of cryst. pepsin was added to 400 ml of the concentrated solution (0.35 mg pepsin-N/ml), the pH of which was adjusted to 4. The precipitate was filtered with sinter 1 G2 after 20 h and washed thoroughly with water. The air-dry precipitate contained 13.0 % N. Its amino-N was 1.7 % and amide-N 12.8 % of total N. The amino-N of the initial zein was 0.25 % and amide-N 18.4 % of total N. The average peptide size of plastein thus corresponded to about 50-peptides and molecular weight to about 6 000.

For diffraction diagrams samples were prepared from zein and plastein by pressing. Their thickness varied from 1 to 2 mm. The diagrams were obtained by CuK_α-radiation filtered through Ni-filter. The ordinary transmission method (pinhole Ø = 1 mm) with the Unicam X-ray goniometer was employed. The distance between the sample and the flat film cassette was 50 mm.

The diagrams display the two rings typical of proteins and polypeptides.

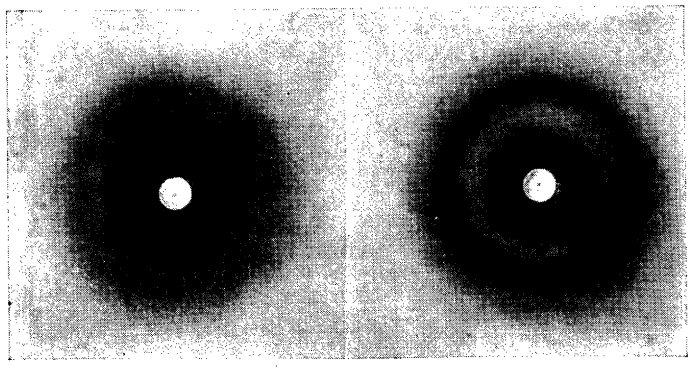

Fig. 1. X-ray diagram of zein (left) and plastein (right) precipitated by pepsin from the pepsin hydrolysate of zein. The average peptide size of plastein about 50-peptides.

Astbury interprets in general that the outer ring represents the backbone distance of a polypeptide chain and the inner ring the average side-chain distance. Both rings are much sharper in the X-ray diagram of plastein than in that of zein. The Bragg-spacings corresponding with the rings are about 4.5 Å and 10 Å in zein and about 4.7 Å and 11.1 Å in plastein in which more accurate measurements can be made. From the interpretations of Astbury regarding his X-ray studies we concluded that our X-ray diagrams are in agreement with the idea of Virtanen and his collaborators [2], based on chemical facts, that plasteins are polypeptides. One of us (V.) asked Prof. Astbury's opinion of our photograph. Prof. Astbury kindly replied (5. IV. 1950) and by his consent we publish below an excerpt from his letter.

"*Plastein from zein.* — This is the common type of diagram from a protein after it has been denatured and aggregated. It results from the unfolding or other disorganisation of the specific configuration, the liberated chains now forming more or less regular bundles in the β-configuration. The two rings are now much sharper because the chains (or portions of chains) are trying to lie as parallel as possible, linked more precisely by the sidechain and backbone linkages. The sharpness of the rings is a measure of the extent to which they succeed in doing this, the two main controlling factors being (1) the degree to which the original specific configuration has been unfolded and the chains have been freed, and (2) the average length of the chains (or portions of chains).

In my view, it follows from this line of argument that the sharpness of your plastein diagram indicates that the plastein chains must be relatively long — — certainly much longer than the 6-peptides of the pepsin hydrolysate. I am afraid it is not possible to give a more exact estimate, but I can tell you that the plastein diagram is quite as sharp as many of the diagrams I have obtained recently from synthetic polypeptides made by the Leuchs reaction. The molecular weight of these preparations is believed to be of the order of 5 000, say about 50 residues or perhaps rather fewer, which would correspond roughly to the chain-length you estimate for your plastein preparation.

To summarise, I should say that your plastein diagram is pretty good independent evidence of the presence of fairly long peptides, and is consistent both with your own estimate of the probable chain-length and with my own observations on denatured and aggregated proteins in general and on synthetic polypeptides in particular."

The X-ray diagrams thus confirm the picture obtained from plastein in the light of chemical studies as high-molecular polypeptides which are formed from comparatively low-molecular peptides (*e. g.* 4—6-peptides) by the action of proteolytic enzyme (pepsin). The result supports the reaction scheme introduced earlier [2].

1. Kerkkonen, H. K. *Acta Chem. Scand.* **2** (1948) 518.
2. Virtanen, A. I., Kerkkonen, H. K., Laaksonen, T., and Hakala, M. *Acta Chem. Scand.* **3** (1949) 520; Virtanen, A. I., Kerkkonen, H. K., Hakala, M., and Laaksonen, T. *Naturwissenschaften* **37** (1950) 139; Virtanen, A. I. *Ann. Acad. Sci. Fennicae*, Ser. A II. Chem. No. 39 (1950).

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An Improved Method for Carbethoxylations with Ethyl Carbonate

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A very important method for the preparation of β-keto esters, malonic esters, and α-cyano esters is the carbethoxylation of a ketone, an ester, or a nitrile with the aid of ethyl carbonate and sodium ethoxide, as described by Wallingford and his co-workers [1-3].

The disadvantages of this method is that the preparation of dry sodium ethoxide from sodium and alcohol is a rather cumbersome process, and the reaction