

## On the Chemical Nature of Plasteins

## Preliminary Communication

ARTTURI I. VIRTANEN and HEIKKI K. KERKKONEN

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

The chemical nature of the much discussed plasteins is illustrated by the following observations.

The egg albumen was hydrolyzed by crystalline pepsin at 37° C. The average molecular weight of the products of hydrolyzation was 233, determined by the diffusion method. The filtered clear hydrolysate was heated for half an hour in a water-bath. After cooling NaOH-solution was added to raise the pH of the hydrolysate to 4.1. After that crystalline pepsin was added to the solution which was placed at 37°. The amino groups were determined during the experiment according to Van Slyke and the carboxyl groups by the formol titration of Sørensen. The both groups decreased parallelly in about 2—4 h. Depending on the concentration of the hydrolysate and on the amount of pepsin the decrease in the amino groups was as follows (table 1).

*Table 1. The decrease in the amino groups through the influence of crystalline pepsin on the hydrolysates of egg albumen at pH 4.1.*

No. of expt.	Time of reaction	Decrease in amino groups
	h	%
1	4	about 25 (167 mg pepsin per 1620 mg hydrolysate-N in 100 ml)
2	4	» 45 (167 » » » 2940 » » » 100 » )
3	2	» 50 (167 » » » 4840 » » » 100 » )

The quantity of plastein precipitated corresponded approximately to the decrease in amino nitrogen. The molecular weights of three different preparations of separated plastein, determined cryoscopically in a phenol solution were 319, 300, and 267. Since the plastein which after determination was separated from the phenol solution was as difficultly soluble in water and equally hydrolysable in 0.1 N HCl-solution as it had been at the start it had evidently remained unchanged when dissolved in phenol. The

molecular weight sufficed to indicate that no high-molecular peptide, still less protein, could be concerned. — Svedberg observed with the plastein preparation of Folley<sup>1</sup> that no sedimentation took place in the ultracentrifuge and that accordingly, an upper limit 1 000 could be given for the molecular weight. Collier,<sup>2</sup> on the other hand, recently recorded a sedimentation in the ultracentrifuge when the plastein was dissolved in urea solution. The precipitate was inhomogeneous but contained particles of the size of protein molecules. He ascribes Svedberg's result to the decomposition of plasteins in alkaline phosphate solution.

Our plastein preparations contained about 13.6 % nitrogen. A 5 minutes' shaking after total hydrolysis yielded about 74 % amino-N of total N.

After hydrolysis with pepsin *plus* 0.1 N HCl at 30° the plastein hydrolysates were found to contain 20.9 % amino-N of total N (5 min. shaking). A 30 min. shaking yielded 38 % amino-N, copper method 37.5 %. The reason for the exceptionally high discrepancy between the results of 5 min. and 30 min. shakings is still unknown. A hydrolysis with HCl without pepsin gave 21.2 % amino-N of total N (5 min. shaking). Accordingly, plastein is decomposed at pH 1—2 as far in the absence of pepsin as in the presence of it.

The results obtained show that plasteins are substances of lower molecular weight than has been assumed. Since the substance is very difficultly soluble in water, at any rate within the region of pH 4—8, and since free amino groups are formed on dissolution at pH 1—2, it seems probable that peptides of ring structure are in question. Variations in the molecular weight of different preparations of plastein suggest a mixture of ring peptides, on the average of the size of di-tripeptides. As the formation of plastein occurs very rapidly it may be assumed that some plasteins are also formed in the digestive tract, the pH being higher when the contents of the stomach pass to the small intestine. — The investigations are continued.

#### REFERENCES

1. Folley, S. J., *Biochem. J.* **26** (1932) 99.
2. Collier, H. B., *Can. J. Research* **18 B** (1940) 305, 272.

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