CCXVIII. SOME OBSERVATIONS ON PEPTIC DIGESTION OF EGG ALBUMIN

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WITH the classical methods of investigating the enzymic hydrolysis of proteins much information has been obtained about the specificity and mode of the enzymes. By studying the split products one should also be able to gain some knowledge about the structure of the proteins themselves. For this purpose much work has been done on the isolation and identification of the end-products of complete digestion. But it would also be of great importance to find out what happens during the earlier stages of the digestion. Some investigations along these lines have previously been carried out in this laboratory. Svedberg & Eriksson [1934] and Annetts [1936] have studied the digestion products formed by the action of papain on egg albumin. In this paper some results will be given from work with pepsin on the same protein.

The egg albumin was crystallized according to the method of Hopkins [1900] modified by La Rosa [1927]. It has been found, however, by means of the improved electrophoresis methods, that this material generally contains a component with slightly lower mobility than the remainder. In four out of five preparations investigated, this component has been present to some extent. As comparative determinations have always been made this should not influence the results, but it somewhat complicates the work. On the whole, egg albumin does not seem to be such a simple and "typical" protein as is generally claimed in the literature.

The enzyme used was Northrop's crystalline pepsin. The digestion was usually carried out in 0·5 or 1*M* acetic acid at 35–40° with a pepsin concentration of 0·06 %. The degree of hydrolysis was determined by the Linderström-Lang acetone titration method.

The partially digested solution was first investigated in the electrophoresis apparatus, using the *Schlieren* method [Tiselius, 1937]. The usual method of dialysing the solution to be investigated against the electrolyte solution used as a supernatant in the electrophoresis tube cannot be applied in this case, as no doubt some low molecular weight material would dialyse out of the bag. Instead the acetic acid-digestion mixture was investigated directly, with an acetic acid solution of the same concentration as a supernatant. This deviates from the common practice of using buffered electrolyte solutions as media for protein electrophoresis experiments and makes accurate measurements of mobilities impossible. Under these conditions observations on the descending boundary (the ascending boundary is unstable) are, however, particularly well suited to give information regarding the homogeneity of the substances in the sample investigated, as the diffusion is counteracted by the automatic sharpening of

the boundaries, which therefore become exceptionally well developed. The low conductivity of the solution also permits the use of high voltage, which gives a rapid separation of the components. This procedure applied to a solution of undigested egg albumin gives one sharp boundary, which however after prolonged electrophoresis splits up into two, migrating close to each other (see above). The digestion mixtures contained one more well developed boundary of much lower mobility (see Fig. 1) evidently representing a group of substances with electrochemical properties distinctly different from those of undigested egg albumin. It is particularly interesting that in no case were any boundaries intermediate between these two observed: increasing time of digestion only changes the relative proportions between the two components. Evidently these experiments suggest that the change brought about by the digestion is a rather abrupt one, which does not give rise to any stable intermediate products, at least not as far as electrochemical properties are concerned.

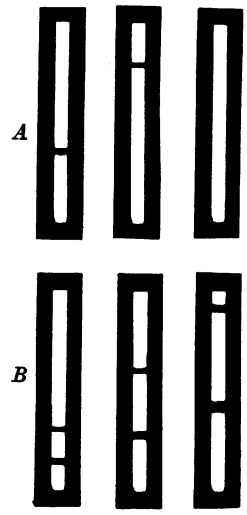

Fig. 1. Electrophoresis of egg albumin before *A* and after *B* digestion with pepsin. Potential gradient 18 V./cm. Time interval between exposures 15 min.

The slow boundary, after prolonged electrophoresis with compensation in the large type apparatus [Tiselius, 1938], splits up into at least three different components with closely similar mobilities. So far we have not made any attempts to separate these but have only attempted to characterize them as a group of split products in comparison with the undigested egg albumin.

The slow fraction is most easily isolated by electrophoretic separation in the large apparatus, the fast fraction (undigested protein) by dialysis. The electrophoretic isolation of the latter in the acetic acid medium is difficult on account of the blurring of the ascending boundaries.

Investigation of the high molecular weight fraction

The acid digestion mixture was neutralized with NaOH. The acid itself denatures the protein partly so that by the neutralization a precipitate is formed in a *p*H region around the isoelectric point. The *p*H was brought to 8, where the protein was soluble and the solution obtained was only faintly opalescent. This was then dialysed in cellophane bags to get rid of the low-molecular split products. The following four solutions were compared:

A. Protein dissolved in phosphate buffer of *p*H 8.

B. Protein treated with acetic acid and then brought to *p*H 8.

C. Protein digested with pepsin in acetic acid 1 hr. and then brought to *p*H 8. Dialysed in cellophane bag. The acetone titration shows an increase in amino-groups corresponding to 0·54 ml. 0·1*N* HCl per 0·178 g. protein. 70 % of the total nitrogen is retained by the membrane in the dialysis.

D. Protein digested with pepsin 10 hr., brought to *p*H 8, dialysed. Increase of amino-groups, 0·99 ml. 0·1*N* HCl per 0·178 g. protein. 50 % of the total nitrogen retained by the cellophane.

Electrophoresis. The electrophoretic mobility measurements were carried out in

phosphate buffer of ionic strength 0·1 and *p*H 8·0, The following results were obtained:

Solution	Mobility*
A	7·12
B	7·06
C	7·18
D	6·85

* Throughout this paper electrophoretic mobilities are expressed in units of 10^{-5}, sedimentation constants in units of 10^{-13} and diffusion constants in units of 10^{-7}.

The values found are in good agreement within the limits of experimental error.

Sedimentation velocity. The sedimentation constants were determined in the ultracentrifuge using a centrifugal field of 350,000 times gravity. The solutions were made 0·2*M* in NaCl to eliminate the Donnan effect. The following sedimentation constants were found:

Solution	Sed. const.
A	3·68
B	3·69
C	3·67
D	3·64

The shapes of the sedimentation curves were very much alike and did not indicate any difference in the compositions of the four solutions.

Diffusion. The measurements were made on the same solutions as the sedimentation velocity runs. Solution B gave the diffusion constant 7·8, which is the normal one for egg albumin. For solution D the value 7·13 was obtained. The agreement is not very good. The values from different parts of the curves indicate that the solution D contains a certain amount of aggregated material. The sedimentation curves, which are better than diffusion curves in detecting non-homogeneity, show that this amount must be very slight.

Low-molecular weight fraction

The low-molecular weight component has a much lower electrophoretic mobility than the other one (see Fig. 1). The split product was isolated after different digestion times. To get some idea of its dispersity and molecular size, diffusion constant determinations were made.

Digestion time in hr.	Increase of NH_2 in ml. 0·1*N* HCl per 0·1 g. prot.	Diff. const.
3	0·79	33·7
44	1·84	32·6
63	2·11	34·5

The values of the diffusion constants for the materials isolated at different degrees of digestion agree within the limits of experimental error. They also seem to have the same mobility. Thus the same split product seems to be formed during the whole course of the digestion. It can, however, only be very incompletely characterized with these methods.

In order to get an estimate of its molecular weight a sedimentation equilibrium run was made in the oil turbine ultracentrifuge. The scale method of observation was used. The centrifuge was run at a speed of 55,000 r.p.m. The height of column of solution was 0·48 cm. and the temperature in the cell 28·4°. The equilibrium seemed to be established in 30–35 hr. The exposures used

for calculation were taken after 43, 45 and 47 hr. The values of the molecular weight obtained at different distances from the centre of rotation showed a drift from the top to the bottom of the cell, showing that the material is not homogeneous with regard to molecular weight. The weight average molecular weight was calculated by means of a formula worked out by O. Quensel (unpubl.)

$$\text{mol. wt.} = K \frac{z}{pc_0 \overline{X}},$$

where
z = scale line displacement,
p = number of intervals used,
c_0 = initial concentration,
X = mean distance from centre of rotation,

and
$$K = \frac{RT}{\omega^2(1 - V\rho)\, Gab},$$

where
R = gas constant,
T = absolute temperature,
ω = angular velocity,
V = partial specific volume,
ρ = density of solvent,
G = enlargement factor,
a = thickness of column of solution,
b = scale distance.

The value obtained in this way was from three different exposures 1080, 1082, and 1065, which gives 1080 as a mean value for the weight average molecular weight of the digestion product.

Discussion

In the aforementioned investigation of the action of papain on egg albumin it was found that the whole material underwent a slight preliminary change before it was broken down to small particles. The molecular weight was not changed, but the sedimentation constant decreased, indicating a change of shape. The electrophoretic curves showed no resemblance with those of the undigested protein. In the case of pepsin it seems that the enzyme attacks one after the other of the protein molecules and immediately breaks them down to very low-molecular weight split products. The amount of these increases as the digestion proceeds. These products have an average molecular weight of about 1000. The mean molecular weight of the amino-acid residues in egg albumin is 124, which gives eight as the average number contained in the peptides formed. The split product is naturally not homogeneous, for smaller and larger peptides may also be formed. The part of the protein which has not been broken down to small particles seems to be almost unchanged egg albumin if only the properties here investigated are considered. The electrophoretic mobility and the size and shape of the molecules are nearly the same as in the native egg albumin. Unlike the split products it gives a precipitate with anti-egg albumin rabbit serum. Part of the material becomes insoluble at the isoelectric point, but this denaturation is due to the effect of the acetic acid and not of the enzyme.

One may assume two distinctly different types of mechanism for the enzymic splitting of large molecules of which the two extremes would be: (1) all molecules are simultaneously but gradually broken down to products which are no longer attacked by the enzyme, (2) only a few molecules are attacked in each time interval but these are quite rapidly broken down to the end-products. In the former case a digestion mixture should contain a number

of products which would show a more or less continuous variation in size and other properties between those of the original and the end-products; in the latter case the mixture should contain unchanged large molecules and fully digested end-products, but no appreciable amount of intermediate substances. The results obtained in the present investigation give strong evidence for the second alternative in the case of digestion of acid-denatured egg albumin with crystalline pepsin.

It should be observed that the denaturation of proteins has been described as an "all or none" reaction of a similar character [Pedersen, 1931; Anson, 1938].

SUMMARY

A study of solutions of egg albumin partially digested with crystalline pepsin in acetic acid medium, using electrophoresis, sedimentation and diffusion methods, indicates that appreciable amounts of unchanged, acid-denatured egg albumin are present even after prolonged digestion, together with the end-products, whereas no marked amount of intermediary split products could be observed. This result suggests that the peptic digestion of egg albumin is more similar to the "all or none" type of reaction than a gradual breakdown process.

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REFERENCES

Anson (1938). In Schmidt, The chemistry of amino-acids and proteins, p. 407.
Annetts (1936). *Biochem. J.* **30**, 1807.
Hopkins (1900). *J. Physiol.* **25**, 306.
La Rosa (1927). *Chem. Anal.* **16**, 3.
Pedersen (1931). *Nature, Lond.*, **128**, 150.
Svedberg & Eriksson (1934). *J. Amer. chem. Soc.* **56**, 409.
Tiselius (1937). *Trans. Faraday Soc.* **33**, 524.
—— (1938). *Kolloidzschr.* **85**, 189.