THE ENZYMATIC SYNTHESIS OF PROTEIN. V.

A NOTE ON THE SYNTHESIZING ACTION OF TRYPSIN.

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In extending our investigation of the enzymatic synthesis of protein to the synthesizing action of commercial trypsin (1) the findings of Henriques and Gjaldbäk (2) were reviewed. In their experiments on plastein formation by trypsin, these authors observed a curious simultaneous hydrolysis and synthesis. This observation is confirmed.

It was first necessary to determine the optimum pH for tryptic synthesis.

Solutions were made up as recorded in Table I, using a peptic hydrolysate of albumin as substrate, and adding the amounts of acid, alkali, or water necessary to obtain the required pH and a constant concentration of digest.

The trypsin employed was prepared from "Difco" trypsin. A 10 per cent solution of the commercial product was adjusted to pH 6.5, centrifuged, filtered, and then precipitated with 9 volumes of alcohol. The precipitate was dried, first with alcohol and ether, and finally in a vacuum desiccator. A fine, white product was obtained. The solution was adjusted to pH 6.5, because at that hydrogen ion concentration, as Northrop showed (3), the autodestruction of trypsin is least rapid. Out of each of the above mixtures two 10 cc. portions were pipetted into 50 cc. Erlenmeyer flasks containing 0.10 gm. of the prepared trypsin, dissolved in 1 cc. of water. The solutions were thoroughly stirred and set away with chloroform in the incubator at 37°C.

Of the sixteen flasks, eight were analyzed for protein at the end of 3 days, and the other eight at the end of 5 days.

At the end of 1 hour, the contents of Flasks 1, 2, 3, and 4 showed precipitates and were almost stiff. Flasks 6 and 7 were immovable transparent jellies. Flask 5 contained some precipitate, but was also somewhat gelatinous.

At the end of 2 days, Flask 7 (pH 9.0) contained a large number of white particles dispersed through the jelly. These, under the microscope, were seen to be composed of crystals resembling those

TABLE I.

No.	Amount of digest.	2.0 N HCl.	2.0 N NaOH.	H_2O	pH
	cc.	*cc.*	*cc.*	*cc.*	
1	20	1.5		5.5	5.4
2	20	0.9		6.1	5.8
3	20			7.0	6.5
4	20		0.6	6.4	7.1
5	20		2.0	5.0	7.7
6	20		3.3	3.7	8.3
7	20		5.0	2.0	9.0
8	20		6.4	0.6	9.9

TABLE II.

Effect of C_H on Tryptic Synthesis.

pH	Protein N in per cent of total N.	
	3 days.	5 days.
5.4	12.8	14.0
5.8	13.1	15.4
6.5	11.5	13.2
7.1	9.1	7.9
7.7	7.6	
8.3	8.1	6.6
9.0	6.4	6.2
9.9	5.1	6.7

of tyrosine and cystine, and further evidence that they consisted of these amino acids was obtained from their solubilities, Millon's and sulfur tests. They failed to give the biuret reaction. The particles were present in all the alkaline mixtures, diminishing in number with decrease of alkalinity. The acid solutions contained the typical protein precipitates found in peptic synthesis, and no precipitated amino acids.

At the end of the period of incubation the mixtures were diluted to 100 cc. and 40 cc. of each of the resulting suspensions were pipetted into 10 cc. of 2 per cent trichloroacetic acid. In this concentration of acid, tyrosine and cystine are quite soluble. The mixtures of diluted trichloroacetic acid were allowed to stand for 24 hours in order to allow the precipitated amino acids to redis-

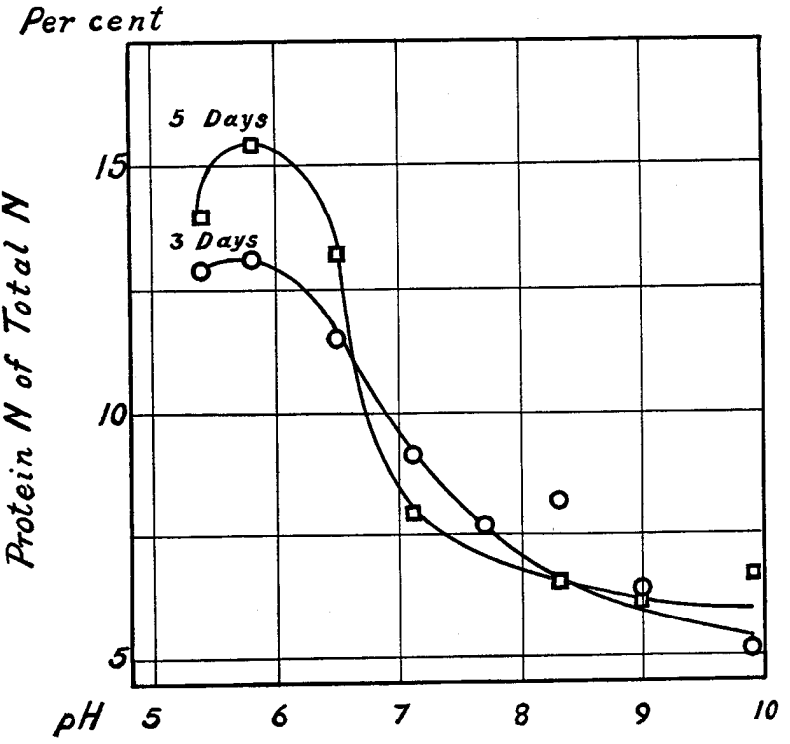

FIG. 1. Relation between C_H and the synthesizing action of trypsin.

solve, and the resolution was facilitated by frequent rubbing and shaking. The precipitated protein was then filtered off and the total nitrogen of the filtrate determined by the macro Kjeldahl method.

The difference between this and the total nitrogen of the original, diluted digest was a measure of the protein synthesized. The results of the analyses of the 3 and 4 day series are stated in Table II and Fig. 1.

The substance synthesized has been called protein because it possesses the general properties of protein. It resembles the protein synthesized by pepsin from a similar peptic hydrolysate of egg albumin. It is very soluble in dilute alkali, less so, but still fairly soluble, in dilute acid. It gives the biuret reaction and is precipitated from its solution in acid by trichloroacetic acid.

The optimum pH for tryptic synthesis, as Fig. 1 shows, is in the neighborhood of 5.7. From the location of the optimum the synthesizing enzyme is clearly not pepsin. In all the alkaline solutions, as Henriques and Gjaldbäk found, a simultaneous hydrolysis ensues. Whether or not both these reactions are promoted by the same enzyme cannot be decided by these experiments. The hydrolysis is strikingly brought out by the greater steepness of the 5 day curve than that of the 3 day curve. Whereas all the flasks on the acid side of pH 7.0 contained larger amounts of protein at the end of 5 days than at the end of 3, on the alkaline side they were found to contain consistently less. The synthesis is a more rapid reaction than the hydrolysis, which is to be expected in solutions of such high concentrations. The hydrolysis following later, and continuing for a longer time, to some extent reverses the synthesis that has occurred.

SUMMARY.

1. The optimum hydrogen ion concentration for tryptic synthesis of protein in a peptic digest of egg albumin is shown to be in the neighborhood of pH 5.7.

2. As had been observed previously by Henriques and Gjaldbäk, in neutral and alkaline reactions, an hydrolysis also occurs simultaneously with the synthesis.

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