Biochemical Studies on the Soya Bean, II
Action of the Enzymes in Soya Bean Seedlings on Glycinin

By

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In the first part of this article, the writer and S. Komatsu stated that the proteins in the soya bean are transformed partly into simpler nitrogenous compounds during the process of germination, which is evidently the result of the action of enzymes occurring in the seedlings. J. P. Street and E. M. Baily[1] mentioned in their study of soya beans that a protease of the peptoclastic type occurs in it, but no further information was given.

A. Wurtz and E. Bouchut[2] have observed an enzyme in the papaya, which acts on protein; Chittenden[3] and his collaboraters have been engaged for many years in the study of the propeolytic enzymes of vegetable origin, and the papain from papaya has been investigated by them with regard to its hydrolytic action on egg albumin, which it converts into albumoses and peptone.

Other proteolytic enzymes in plant organs have been studied by A. F. Blood[4] (the erepsin of the cabbage) and by H. Lundin[5] (some enzymes of malt). Urease has been observed by many investigators to occur in soya beans and especially in the germinated seeds, and according to N. Onodera[6] the activity of the urease of soya bean extract is decreased by dialysis, but increased in the presence of a co-enzyme.

1. Jour. Indus. and Engin. Chem., **7**, 853 (1915).
2. Compt. Rend. Acad. Sci., **89**, 425; **91**, 787.
3. Chittenden: Studies in physiological Chem., 160.
4. J. Biol. Chem., **8**, 215 (1910).
5. Bioch. Zs., **131**, 193 (1922).
6. Biochem. Jour., **32**, 290 (1915).

The chemical changes of the proteins in soya beans during germination will be explained by the study of the proteolytic action of enzymes isolated from the germinated seeds on the soya proteins. Glycinin from the seeds was, therefore, treated with a water extract of the seedlings, which was supposed to contain some proteolytic enzymes.

In the experiments, the extract was prepared from 50 soya bean seedlings, crushed to powder, treated with 500 c.c. of distilled water at room temperature for 3 hrs., and filtered. The following experiments were performed:

50 c.c. of the seedling extract, which contained 44 mg. of nitrogen, were put into an Erlenmeyer flask. To this were added 0.5 gm. of pure glycinin which was isolated by Dr. Hibino from soya beans, containing 85 mg. N, and dissolved in 10 c.c. of 1 % NaCl solution, 40 c.c. water and 10 c.c. toluene. The flask was then stoppered with cotton and incubated at 40°C. The pH of this mixture is 6.46.

For comparison, an Erlenmeyer flask containing 50 c.c. of the extract covered with toluene was incubated at 40°C. The pH of this solution is 6.4.

Both the sample from the seedlings and the control after having been incubated at 40°C for 24 hrs., 48 hrs., 72 hrs., and 96 hrs., were heated on a water bath for about 30 mins. and then filtered. The total nitrogen in the filtrates was determined by Kjeldahl's method, and the free amino-N by van Slyke's method; the proteose, peptone and polypeptide, and pH value were also determined, with the following results:—

Table I

I

The Extract and Glycinin

Time for digestion	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Total volume	90 c.c.	88 c.c.	90 c.c.	89 c.c.
Total-N	40.1 mg.	40.5 mg.	47.3 mg.	51.9 mg.
Free amino-N	7.7 „	10.8 „	12.6 „	12.6 „
Proteose-N	8.6 „	7.7 „	8.5 „	10.1 „
Peptone & polypeptid-N	31.5 „	32.8 „	38.8 „	41.8 „
pH	6.2	6.2	5.8	5.8

II

The Extract

Time for digestion	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Total volume	92 c.c.	90	90	90
Total-N	24.4 mg.	24.8 mg.	28.8 mg.	32.9 mg.
Free amino-N	6.1	6.5	8.2	9.0
Proteose-N	3.8	4.2	7.3	9.0
Peptone & polypeptid-N	20.6	20.6	21.5	23.9
pH	6.2	6.0	6.0	5.8

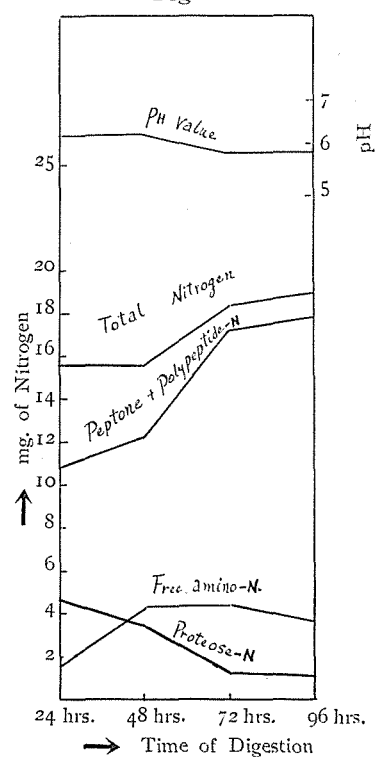
Table II.

Time for digestion	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Total-N	15.7 mg.	15.7 mg.	18.5 mg.	19.0 mg.
Free amino-N	1.6	4.3	4.4	3.6
Proteose-N	4.8	3.5	1.2	1.1
Peptone & polypeptid-N	10.9	12.2	17.3	17.9

The chemical changes which actually occur in glycinin owing to the proteolytic enzymes in the extract are expressed by the results shown in Table II and Fig. 1, which were calculated from experimental results I and II in Table I.

The chemical mechanism for the conversion of glycinin by enzymes into simple nitrogenous compounds will be learned from the change in the nitrogen distribution of the reaction product with time. As a matter of fact, the nitrogen of the peptones and polypeptides increased and the proteose nitrogen decreased with progress of time, and the chemical changes of the protein is very like, as a whole, those of the nitrogenous matter in the soya bean during germination. The change in the free amino-N with time, as will be seen in the Fig. 1,

Fig. 1



which does not run parallel with that of the pH-value, is mostly due to the liberation of an acidic amino acid, such as aspartic or glutaminic acid, from the protein molecule in the course of digestion by the enzyme.

The enzymatic action of urease in seedlings is interesting in connection with the proteolytic action of other enzymes in them. 50 c.c. of the extract were, therefore, mixed with 5 c.c. of 1 % urea solution (23 mg. N), and kept for $1\frac{1}{2}$ hrs. at 40°C and the ammonia resulting from the enzymatic reaction was determined[1] by absorbing with $\frac{1}{14}$ N H_2SO_4 solution.

The experimental results show that 15.5 mg. N in urea were converted into ammonia in the seed extract, and 20.2 mg. N in the seedling extracts; in other words, the urease of the seedlings is more active on urea than that of the seeds.

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1. Zeit. f. Physiol. Chem., **37**, 161 (1902).