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γ-Glutamylpeptidase in Sprouting Onion Bulbs

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Our observations on the function of glutaminase in the germinating seeds of chive [1] have prompted further study on the decomposition of γ-glutamylpeptides in onion bulbs. In these experiments in which γ-DL-glutamyl-*p*-nitroaniline (GPNA) was used as substrate, we have at last been able to show that an enzyme which splits the γ-glutamyl bond hydrolytically is to be found in sprouting onion bulbs. The enzyme seems to be taken up with difficulty by M/15 phosphate buffer pH 7.4, whereas it is obtained from the germinating seeds with this buffer. Hydrolysis is best achieved using crushed onion bulbs at the sprouting stage as the enzyme preparation. An onion (71.5 g) which had been kept in moistened sand for a month and which had green leaves of about 1 cm length (the sprouting was very slow in winter), was frozen, ground finely and dialyzed in M/15 phosphate buffer pH 7.4 for 22 h against the same phosphate buffer. Dialysis was then continued for four hours against M/15 phosphate buffer pH 7.8, after which the outer solution was renewed and dialysis continued a further 16.5 h. 1 ml of dialyzed suspension and the same volume of M/15 phosphate-buffer pH 7.8 were used in the experiment. The amount of GPNA in the test solution was 0.8 mg. The test solutions were kept at 37°C for 3 h, after which 7 ml absolute ethanol was added to each of them. The solution could not be clarified by centrifugation or filtration. The composition of the test solutions and optical densities (4100 Å) are presented in Table 1 (P = phosphate buffer).

The results showed that onion crush had a γ-glutamyl peptidase effect. Because of the turbidity of the solution the experiment could not yet be regarded as satisfactory. Therefore another experiment was performed with the same dialyzed onion crush, which had been stored at −20° for 2 days. In each experiment there were 3 ml of onion crush and 3 ml of M/15 phosphate buffer pH 7.8. The amount of GPNA in the test solution was again 2.4 mg. After 3 h at 37°C the test solutions were centrifuged, without prior precipitation of proteins with ethanol. When the solutions were turbid, they were kept overnight at about −20°, after which centrifugation gave clear solutions which were then filtered through paper. The results are shown in Table 2. On the basis of this experiment it has been definitely proved that active γ-glutamyl-peptidase is found in sprouting onion.

The same crushed onion was used in a further experiment, which was similar to that shown in Table 1 except that the turbid solutions, centrifuged after adding ethanol, were kept overnight at −20°C, after which they were centrifuged and filtered through paper. Clear solutions were then obtained. Also determinations made with heated crushed onion were included in the experiment. The results are shown in Table 3. This experiment confirmed the results given in Table 1.

Table 1. Composition of the test solutions, and optical density at 4100 Å.

1.	P	0	
2.	P + GPNA	0.037	
3.	P + »	0.029	0.034
4.	P + »	0.037	
5.	P + onion (turbid solution)	0.268	
6.	P + » » »	0.223	0.206
7.	P + » » »	0.204	
8.	P + »	0.124	
9.	P + » + GPNA (turbid solution)	0.428	
10.	P + » + » » »	0.403	0.396
11.	P + » + » » »	0.358	

Table 2. Composition of the test solutions, and optical density at 4100 Å.

1.	P	0	
2.	P + GPNA	0.188	
3.	P + »	0.178	0.185
4.	P + »	0.190	
5.	P + onion	0.970	
6.	P + »	0.885	
7.	P + »	0.815	0.909
8.	P + »	0.970	
9.	P + »	0.930	
10.	P + »	0.885	
11.	P + » + GPNA	2.010	
12.	P + » + »	1.980	2.052
13.	P + » + »	2.165	

Table 3. Composition of the test solutions, and optical density at 4100 Å.

1.	P	0	
2.	P + GPNA	0.037	
3.	P + »	0.034	0.036
4.	onion + P	0.129	0.114
5.	» + P	0.099	
6.	» + P + GPNA	0.424	0.399
7.	» + P + »	0.373	
8.	» + heating + P	0.092	
9.	» + » + P	0.108	0.100
10.	» + » + P + GPNA	0.123	
11.	» + » + P + »	0.102	0.113

The pH of crushed onion is about 6 and at such acidity the γ-glutamylpeptidase found in *Allium* species still functions fairly well. The cleavage of γ-glutamylpeptides in sprouting onion is relatively slow and the activity of the γ-glutamylpeptidase can be thought to be sufficient to explain the hydrolysis of the peptides mentioned.

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