Studies on a Proteolytic Enzyme from Seeds of Peas

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The proteolytic enzymes from different plants have, in most cases, rather low hydrolytic activities. Exceptions from this rule are ficin, papain, bromelin and a few other enzymes which, however, are not present in the seeds of the plants. Very few proteolytic enzymes from seeds have been isolated probably because of the low activity of the enzymes from this source. In the seeds the protein breakdown during germination is not very rapid, *e. g.* it takes about 15 days before the reserve proteins have been broken down in seeds of peas. In the alimentary canal of animals the food must be digested in a few hours. Perhaps this is the reason why strong proteolytic enzymes are easier to isolate from the animal kingdom.

A few proteolytic enzymes with low activity from seeds have been studied. Linderstrøm-Lang and co-workers[1,2] reported a proteinase from malt. Very closely related to this enzyme is a proteinase from seeds of wheat which have been studied by Mounfield[3] and Balls and Hale[4]. Hale succeeded in purifying this enzyme by precipitation with $(NH_4)_2SO_4$ and acetone[5]. The enzyme preparations from malt and wheat contain, however, at least two proteolytic enzymes, one proteinase with its optimal activity at pH 4.1 and one dipeptidase with optimal activity at pH 8.5. Linderstrøm-Lang[2] succeeded in separating the two enzymes by storage of the enzyme solution for a short time. The dipeptidase was then inactivated. Balls and Hale[4] obtained enzyme preparations from wheat with an activity of about 1/400 of that of crystallized papain.

The protein breakdown during germination has been intensely studied from different aspects. Seeds from plants belonging to the *Leguminosae* have been used in most cases. Many attempts have also been made to isolate proteolytic enzymes from these seeds. In 1874 von Gorup-Besanez prepared a proteolytic enzyme from shoots of vetch, *Vicia sativa*[6]. He extracted the material with glycerol, filtered and precipitated the proteins with absolute ethanol. After repeated precipitations with ethanol a preparation with proteolytic activity

was obtained. A solution of the enzyme obtained in this way could dissolve a gel of fibrin. Any determination of the activity by the more usual methods was never done.

In 1919 Fischer [7] investigated the proteolytic activity in dried plant material from different *Leguminosae* plants. The enzyme preparations at each experiment contained of 10 g dried plant material. As substrate he used 3 g legumin, i. e. much more "enzyme" than substrate. No blank determinations can be found in his papers. Thus his methods and results are not very convincing.

In 1924 Blagoveschenski [8] investigated the proteolytic activity of 5 % NaCl extracts of young shoots from *Leguminosae* and other different plants. Globulins from different seeds served as substrate because Blagoveschenski believed that the globulins from the mother plant are broken down faster by the proteolytic enzyme than other substrates. Blagoveschenski and Melamed in 1934 investigated the proteolytic enzymes in seeds of *Vicia sativa* and *Phaseolus aureus* [9]. The authors used crude glycerol extracts from the seeds. No attempts to purify the extracts were made. No absolute activities, based on the content of enzyme nitrogen in the digestion solutions can be found in their paper.

In 1939 Davis [10] reported the presence of a proteolytic enzyme in lima beans, *Phaseolus lunatus*. In some experiments he used glycerol extracts from the seeds, and in some cases he extracted the pods with water. The water extract was fractionated by $(NH_4)_2SO_4$ precipitation and the fraction between 40 % and 80 % saturation was used for the digestion experiments. Davis' experiments show that a proteolytic enzyme can be extracted from seeds of lima beans.

In 1946 Labarre and Pfeffer [11] investigated the proteolytic activity in extracts from seeds of *Vicia Faba*, but never tried to isolate the enzyme. The seeds which had been germinated between 0 and 14 days were extracted with a buffer solution of pH 7.5 and the extract filtered. The extract then contained the enzyme in addition to large amounts of other proteins. Free amino groups were determined according to van Slyke immediately after extraction and after two hours at 40° C, i. e. the autolysis was measured. They found the highest rate of autolysis after 8 days of germination.

The most successful attempts so far to isolate proteolytic enzymes from leguminosae seeds were made in 1946 by Mergentime and Wiegand [12]. They extracted green peas with water and then the pH was brought to pH 4.0, by which procedure part of the globulins (legumin) is precipitated and discarded. The supernatant solution was used as enzyme solution. They found the pH optimum of this proteolytic enzyme to be pH 5.5 when acting on casein. Only

a few values (here called enzyme-N) of the nitrogen content of the enzyme solutions are published. These values show, however, that the enzyme solutions were very concentrated and the ratio $\dfrac{\text{enzyme-N}}{\text{substrate-N}}$ during the digestions was about 1/4, which is very high. Their method of purification is unsatisfactory because all globulins and low-molecular nitrogen products are not removed by the method used. Another striking thing is that they did not succeed in isolating any protein fraction from peas which was free of proteolytic activity and which could be digested by fresh pea enzyme.

It can be seen that a great number of attempts to isolate proteolytic enzymes from seeds or shoots of *Leguminosae* plants have been made. However, no satisfactory determinations of absolute activities have been carried out. The experiments which are described in this paper have been performed in order to obtain more information about these enzymes, *i. e.* their absolute activities and their possible purification.

EXPERIMENTAL

I. Glycerol extracts of germinated seeds

A. Extraction of the enzyme

Seeds of yellow peas, *Pisum sativum*, (Field variety, "Torsdagsärt II") were used. Previous investigations at this Institute [13] have shown that the protein breakdown is most intense 6—10 days after the beginning of the germination. This is in agreement with the results of Labarre and Pfeffer [10]. Thus the seeds were allowed to germinate for 7—8 days before the enzyme was extracted.

The seeds were placed in water overnight in order to swell and then allowed to germinate between wet filter papers in Petri dishes. After 8 days they were washed several times with water and then ground for 6 minutes in a Waring blendor with 50 % glycerol. The weight of the extraction solution was twice that of the wet peas. The extraction was carried out at + 4° C for 20 hours. The extract was filtered through a piece of linen cloth and then centrifuged for 30 minutes at 3 500 r.p.m. (radius 5 cm). The first measurements were performed with enzyme solutions prepared in this way.

B. Preliminary determinations of the proteolytic activity

Commercial edestin (La Roche) was used as substrate because it can be obtained in large amounts and it is a rather homogeneous protein. The edestin was dissolved to 2.5 % in a buffer containing 0.038 M HAc and 0.011 M NaAc.

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The pH of the substrate solution was 4.6. This pH value was chosen because edestin has a high solubility at this pH but is precipitated when the pH is increased. Later experiments have shown that the pH optimum of the proteolytic enzyme isolated from seeds of peas is above pH 6, where edestin is insoluble. It is, however, very difficult to pipette solutions containing precipitated edestin and therefore a pH value was chosen where clear solutions of the substrate can be obtained, and the activity determinations can be performed with satisfactory accuracy.

The breakdown of the substrate was followed by formol titration according to Sørensen. The method used was that indicated by Mounfield [3]. The titration was carried out with $N/20$ NaOH. Previous potentiometric titrations on the substrate solution showed no buffer capacity in the pH-range 8—9.

In all experiments described here 15.0 ml substrate solution were mixed with 2.0 ml enzyme solution. 5.0 ml of this mixture were pipetted into three 25 ml Erlenmeyer flasks and 2 drops of toluene were added to each flask. Two flasks were placed in a thermostate at $+40°$ C and the third one was titrated immediately. In later experiments the digestion was carried out in one flask only, and 5.0 ml were withdrawn after different times of digestion. The results from the activity determinations of an enzyme solution prepared in the way described above is shown in Table 1.

Table 1. Digestion experiment using 2.5 % edestin at pH 4.6 as substrate solution. Temperature $+40°$ C. Enzyme solution: 50 % glycerol extract from seeds of peas, germinated for 8 days. The enzyme solution contained 2.52 mg N/ml. 2 ml enzyme solution and 15 ml substrate solution were digested. Formol titrations were carried out on 5 ml digestion solution.

Flask	Digestion solution	Time h	Titer ml $N/20$ NaOH	Time h	Titer ml $N/20$ NaOH	Increase in formol titration ml $N/20$ NaOH	Time h	Titer ml $N/20$ NaOH	Increase in formol titration ml $N/20$ NaOH
A	Edestin + 50 % glycerol	0	3.65	6.2	3.64	$-$ 0.01	23.2	3.62	$-$ 0.03
B	Buffer pH 4.6 + enzyme	0	3.46	6.2	3.51	0.05	23.2	3.54	0.08
C	Edestin + enzyme	0	4.05	6.2	4.25	0.20	23.2	4.64	0.59

According to Table 1 the breakdown in flask C can easily be measured after about 6 hours at + 40° C. It can also be seen that the rate of breakdown decreases with time. In flask B, containing enzyme and buffer only, a small increase in the titer was observed, probably due to the fact that the enzyme solution contained large amounts of protein other than enzyme which are broken down. In flask |A the titer decreased with time because fresh edestin solution was used. In later experiments the substrate solution was placed at 40° C over night before it was used for digestion experiments. After that no change could be observed in the titer of the substrate. In the activity determinations the changes in the titers of the flasks A and B must be taken into consideration. Thus, the real change in flask C after 6.2 hours digestion was 0.16 ml $N/20$ NaOH. These corrections have been made in all experiments described in this paper. They are, however, very small when purified enzyme solutions are used.

C. *The unit of proteolytic activity*

It is very difficult to choose a suitable activity unit for an enzyme of this kind which has a very low activity and also is very impure. Preliminary experiments with crude extracts and also with purified enzyme solutions have shown that the rate of breakdown is proportional to enzyme concentration. During the first stage of the digestion experiment the curve of breakdown is practically linear. The results are shown in Figure 1 and Figure 2. If these facts are taken into consideration, an activity unit defined by Northrop (14) but somewhat modified can be used. A correction has been made for the dilution of the enzyme. The formula is:

$$\begin{bmatrix} A \end{bmatrix}^{40° \quad pH\ 4.6}_{\substack{Edestin \\ 2.5\ \%}} = \frac{\dfrac{\text{milliequivalents NaOH}}{\text{ml. digestion solution}}}{\text{hours} \times \dfrac{\text{mg. enzyme-N}}{\text{ml. digestion solution}}}$$

From Table 1 the following value of the activity of the enzyme used is obtained:

$$A = \frac{\dfrac{0.16}{5} \times \dfrac{1}{20}}{6.2 \dfrac{2 \times 2.52}{17}} = 0.87 \times 10^{-3}$$

The activity unit used here does not take into consideration the fact that the breakdown decreases with time. Thus the measurements must be made as soon as possible after the start of the experiment. But Table 1 shows that the
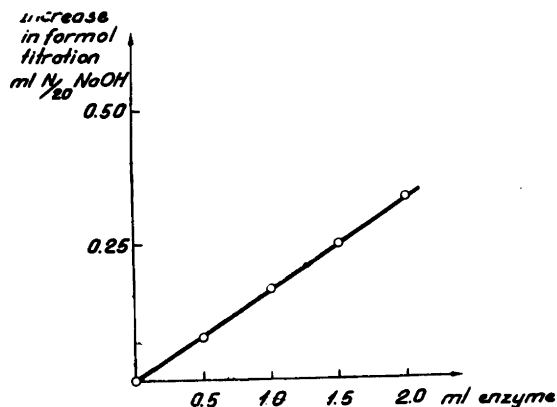
Fig. 1. The dependence of edestin break-down on enzyme concentration. 15 ml 2.5 % edestin, pH 4.6, x ml enzyme solu-tion and (2—x) ml H_2O were digested at + 40° C for 4 hours. The enzyme solution contained 0.760 mg N/ml. Formol titrations were carried out on 5.0 ml.

breakdown cannot be easily measured before about four hours if crude glycerol extracts are used. It is thus difficult to obtain the initial rate due to the low activity of the enzyme. Preliminary experiments have shown, however, that the error is not very large if the measurements are made within 6 hours from the start.

D. *Purification experiments on the glycerol extracts*

The glycerol extracts contain large amounts of proteins with no enzyme activity. Some methods to purify the enzyme were tried. The activities of the different fractions are found in Table 2.

1. *Dialysis.* The glycerol extracts were dialysed against water through a cellophan bag. A dense precipitate was obtained which after dissolving in 50 % glycerol had no measurable activity. The supernatant solution was still active.
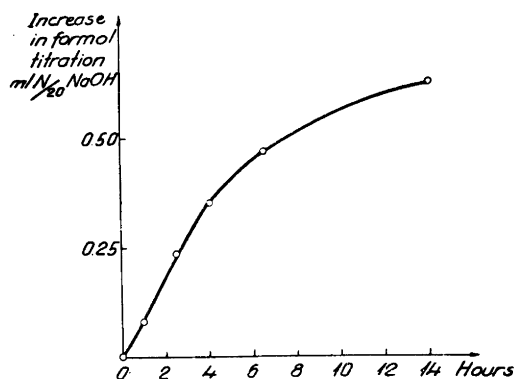

Fig. 2. The breakdown of edestin by pea proteinase as a function of time. 33 ml 2.5 % edestin, pH 4.6, and 4 ml enzyme solution, containing 0.760 mg N/ml were digested at + 40° C. After different times of digestion 5.0 ml were withdrawn with a pipette and titrated with N/20 NaOH and 4 ml 35 % formaldehyde.

2. *Ultrafiltration.* The dialysed solution was ultrafiltrated through a collodion membrane in order to concentrate the enzyme [15]. The solution which passed through the membrane had no measurable activity but the remaining fraction was still active. In most cases the solutions were concentrated so that the volume left after filtration was about 1/5 of the initial volume.

3. *Precipitation with* $(NH_4)_2SO_4$. Dialysed glycerol extracts were precipitated at pH 5 at + 4°C with solid $(NH_4)_2SO_4$ at different concentrations. The precipitates were centrifuged down and dissolved in water. The solutions obtained were dialysed against water in order to remove all ammonium sulphate, since traces of this salt precipitate edestin. Upon dialysis to the salt-free state dense precipitates were obtained. These precipitates were dissolved in 50 % glycerol and the proteolytic activity of the solutions measured. It was, however, very low. The supernatant solution from the dialysis showed high activity.

The results from the purification experiments described above are found in Table 2. All the enzyme solutions were investigated in the electron microscope in order to see if any bacteria were present. This was never the case.

Table 2. Determination of the proteolytic activity of enzyme preparations from seeds of peas at pH 4.6. Temperature + 40° C. Substrate 2.5 % edestin. The experiments were performed in the way described in legend of Table 1.

Enzyme preparation	Nitrogen content of the enzyme solutions mg N/ml	Time h	Increase in the titer ml. $N/20$ NaOH	Activity × 10^3
A. Glycerol extract no.1	2.52	6.2	0.16	0.87
B. » » » 2	2.24	4.8	0.19	1.5
C. » » » 3	6.12	4.3	0.19	0.61
D. Solution B, dialysed	0.408	7.0	0.15	4.5
E. Solution D, ultra-filtrated	3.13	7.0	0.59	2.3
F. Solution C, dialysed Precipitated with $(NH_4)_2SO_4 \cdot 0-80$ % fraction	1.17	6.3	0.67	7.7
G. Glycerol extract no. 6, treated as solution F. 0—30 % fraction	0.141	6.2	0.09	8.7
H. As solution G. 30—60 % fraction	1.22	6.2	0.76	8.5
I. As solution G. 60—80 % fraction	0.760	6.2	0.54	9.7

The values of the activities from Table 2 show that the crude glycerol extracts have a proteolytic activity of the magnitude 1×10^{-3}. The dialysed glycerol extract no. 2 has an activity about 3 times higher than the undialysed extract. Thus a great deal of protein with no proteolytic activity is removed by dialysis. Ultrafiltration of the dialysed extract gives a solution with lower activity, *i. e.* inactivation occurs by ultrafiltration. It is, however, possible to concentrate the enzyme with this method. Precipitation with $(NH_4)_2SO_4$ of the dialysed extracts gives an enzyme preparation with high activity. In the experiments described here about the same activity was obtained independently of the amount of $(NH_4)_2SO_4$ used. Probably the enzyme is precipitated together with those proteins which have no proteolytic activity. In the last experiment in Table 2, the enzyme was distributed in the following way; solution G contained 5 %, solution H 65 % and solution I 30 % of the enzyme. In many cases the enzyme solutions were dried in vacuum at low temperature over $CaSO_4$. The white powder obtained in this way had the same proteolytic acitivity as before the drying procedure.

II.   W a t e r   e x t r a c t s   o f   g e r m i n a t e d   s e e d s

The experiments above show clearly that a proteolytic enzyme from germinated seeds of peas can be extracted with 50 % glycerol, and the enzyme can be concentrated about ten times. It is, however, difficult to purify the enzyme more because glycerol extracts hold too many nitrogen products other than enzyme which contaminate the enzyme even after fractionation with $(NH_4)_2SO_4$. Some experiments with water extracts of the germinated seeds were performed which showed that the enzyme could be extracted with water, and about the same activities as by glycerol extraction were obtained. After precipitation with $(NH_4)_2SO_4$ of the dialysed extract between 0—80 % saturation and subsequent dialysis of the dissolved precipitate, the activity obtained was about $15—17 \times 10^{-3}$, *i. e.* somewhat higher than before. Further purification by precipitation with $(NH_4)_2SO_4$ to different concentrations was tried but with no success.

III.   W a t e r   e x t r a c t s   o f   u n r i p e   s e e d s

In order to get more active enzyme solutions, some experiments with unripe seeds were carried out. Earlier unpublished investigations by the present author have shown that during the first stage of ripening about 60 % of the total nitrogen is of lowmolecular nature in the seeds. The ripe seeds contain only 17 % lowmolecular nitrogen, and during germination this value

never is higher than 45 %. Thus, if the unripe seeds contain the same proteolytic enzyme as that which can be extracted from the germinated seeds, then the unripe seeds must be the best material for the isolation of the enzyme, because most of the nitrogen can be removed by dialysis from the extracts. As a working hypothesis it was considered that the proteolytic enzymes from seeds can be used by the plant both for hydrolytic and synthetic purposes. In the unripe seeds, where 60 % of the total nitrogen is of lowmolecular nature, the protein synthesis is very intense, and thus the proteolytic enzymes work as synthetic enzymes. If these enzymes are extracted and used in digestion experiments of the kind described above, they should have hydrolytic activity, because water is in excess.

Seeds of peas of the same variety as used in earlier experiments were harvested three weeks before the usual time of harvesting when the seeds are ripe. The seeds were separated from the pods and allowed to dry. After two months they were placed in water overnight and then macerated in a Waring blendor for 6 minutes, frozen down to $-16°$ C and dried in vacuum over $CaSO_4$. The dry powder was then extracted with water. 1.5 g dried seed material and 25 ml H_2O were used at each experiment. As can be seen from Table 3, enzyme preparations from unripe seeds are more active than those from germinated seeds. This fact, however, probably is due to the low concentration of high molecular nitrogen products other than enzyme in the water extracts of the unripe seeds i. e. probably very little of the enzyme is adsorbed on nitrogen products from the seeds. When using the enzymes with the highest activity the ratio $\dfrac{\text{enzyme-N}}{\text{substrate-N}}$ was about $\dfrac{1}{300}$.

It is clear from the results above that for further purification of the proteolytic enzyme from pea seeds dialysed water extracts from unripe peas should be used. Such experiments were not carried out in the present study because of insufficient amounts of unripe seeds.

IV. Determination of the pH optimum

For further characterization of the enzyme the pH optimum for a few substrates was determined. The substrates used were gelatin, legumin, and vicilin, two of which, gelatin and vicilin, are soluble in the whole pH range studied. It is important not to use buffer systems with buffer capacity in the pH range 8—9 if the breakdown is measured by formol titration. This difficulty was avoided by using unbuffered solutions. The pH of the substrate solutions was adjusted with N HCl and N NaOH. The solution was then heated for a few hours to 40° C, and the pH measured with a glass electrode

Table 3. Proteolytic activities of enzyme preparations from unripe seeds of peas. The digestion conditions are the same as in Table 2.

Enzyme preparation	Nitrogen content of the enzyme solution mg N/ml	Time h	Increase in formol titer ml $N/20$ NaOH	Activity $\times$ 10^3
No. 1				
a. 0.2 M NaCl extract	1.54	3	0.70	13
b. 1 a dialysed, supernatant	0.077	3.3	0.22	74
c. 1 b, precipitated with $(NH_4)_2SO_4$ 0—80 % fraction	0.099	3	0.33	94
No. 2				
a. 0.2 M NaCl extract	1.23	19.7	1.60	5.6
b. 2 b dialysed, supernatant	0.082	19.7	0.60	31
c. 2 b, precipitated with $(NH_4)_2SO_4$, 0—80 % fraction	0.064	19.7	0.63	42
No. 3				
a. Water extract	1.11	4.1	0.26	4.8
b. 3 a, dialysed supernatant	0.092	5.5	0.22	37
c. 3 a, dialysed precipitate dissolved in water	0.164	4.1	0.01	1.3
No. 4				
a. Water extract	2.07	3.6	0.38	4.3
b. 4 a, dialysed	0.100	3.6	0.30	71
No. 5				
Water extract, dialysed	0.130	3.3	0.32	63
No. 6				
Water extract, dialysed	0.159	3.3	0.32	52
No. 7				
Water extract, dialysed	0.142	5.6	0.37	40

before and after the digestion experiment. The difference never exceeded 0.1 pH unit. Blank determinations were performed only at pH 3, pH 6.5 and pH 8. No change in the titer of the blanks were observed. The substrate solutions used are described below.

1. *Vicilin.* A dried preparation of vicilin from peas was used. It was homogeneous by ultracentrifugation and electrophoresis. The concentration used was 2.5 % in 0.2 M NaCl 5 ml of the vicilin solution were pipetted into 25 ml. Erlenmeyer flasks and the pH adjusted to the desired value as described above. 0.4 ml of dialysed enzyme solution from unripe seeds was pipetted into each flask. 0.4 ml H_2O was pipetted into each blank. The enzyme solution contained 0.077 mg N/ml. Formol titrations were carried out on 2.0 ml of the digestion solution after 0 and 18.7 hours digestion at + 40° C. In Fig. 3 the increases in formol titration are recalculated to 5 ml of digestion solution.

2. *Legumin.* 8 g commercial legumin (Merck) probably inhomogeneous, were suspended in 300 ml 0.2 M NaCl. The legumin solution was then treated as in the preceding experiment, but 15 ml were pipetted into each flask. 2 ml of enzyme preparation containing 0.856 mg N/ml, from germinated seeds were pipetted to each flask. Titrations were carried out on 5 ml after 0 and 7 hours digestion at + 40° C.

3. *Gelatin.* A stock solution of granular gelatin (Eimer and Amend, N.Y. U.S.A.) in water was made up. The gelatin concentration was 4 %, and a little crystal of thymol was added in order to avoid infection. Two experiments were carried out in the same way as described for legumin. In the first experiment the enzyme used was from germinated seeds, containing 0.856 mg N/ml. The digestion time was 5 hours. In the second experiment an enzyme preparation from unripe seeds, containing 0.077 mg N/ml, was used. The digestion time was 17.5 hours.

The results of these experiments are shown in Fig. 3. The pH optimum 6.7 is the same for the enzyme from germinated and unripe seeds when digesting gelatin. When vicilin is used as substrate the optimum is more acid, or about pH 6.0—6.3, which has been checked by several experiments. When digesting legumin, the optimum is close to pH 7. It is, however, important to remember that the legumin used was not homogeneous.

DISCUSSION

The experiments described above show that it is possible to extract a protein fraction with proteolytic activity from seeds of peas. It is possible to purify the enzyme by dialysis, ultrafiltration and precipitation with $(NH_4)_2SO_4$,
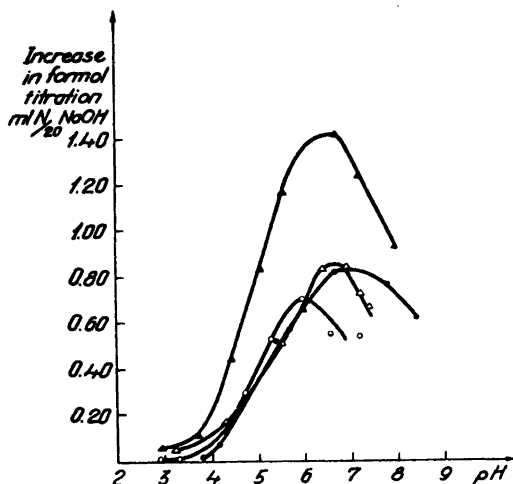
Fig. 3. Determination of pH optimum for pea proteinase.

▲ = gelatin + enzyme from unripe seeds.

△ = gelatin + enzyme from germinated seeds.

● = legumin + enzyme from germinated seeds.

○ = vicilin + enzyme from unripe seeds.

i. e. removing nitrogen products other than enzyme. By all the purification experiments, however, part of the activity is lost, probably depending on inactivation. By dialysis of an extract against water, about 30 % of the total activity is lost, and it seems as if still more is lost by precipitation with $(NH_4)_2SO_4$. As has been said above, part of the enzyme probably is adsorbed to other proteins in the solutions. The possibility that an activating substance is removed during the purification experiments has not been investigated. From Table 3 it can be seen that the precipitate after water dialysis is still active. If the precipitate is dissolved in 0.2M NaCl and dialysed once more, the globulin fraction is then inactive. Mergentime and Weigand [12] did not succeed in preparing a pea protein which was free of proteolytic enzymes and which could be digested by fresh pea enzyme. The present author has shown that the two pea globulins vicilin and legumin are digested by pea proteinase. Mergentime and Weigand [12] found the pH optimum for pea proteinase to be pH 5.5 for casein splitting, *i. e.* one pH unit lower than the value determined in this paper for gelatin splitting. This difference is probably due to the different substrates used.

The pea proteinase has a low proteolytic activity. It is very difficult to compare the activity of this weak enzyme with activities of stronger enzymes. However, a comparison with the activity of crystalline pepsin will be made. From Northrop's determinations of the activity of crystalline pepsin [14] the activity value $A = 11$ can be calculated when edestin was used as substrate and the same activity unit as in the present paper is used. Thus pepsin is about 100—200 times more active than the best pea proteinase preparations

from unripe seeds. It is, however, important to remember that the determinations of the activity of pea proteinase were performed at pH 4.6, *i.e.* not at the pH-optimum of the enzyme. From the values in Fig. 3 and Table 3 it can be seen that the activity of pea proteinase when splitting gelatin at pH 6.7 is about the same as when splitting edestin at pH 4.6 ($A = 89 \times 10^{-3}$ compared with $A = 74 \times 10^{-3}$ for the same enzyme preparation). Northrop's values give $A = 100 \times 10^{-3}$ for pepsin when splitting gelatin at its pH optimum. Thus pea proteinase and pepsin have about the same activity when digesting gelatin, but pepsin is much stronger when edestin is used as substrate. It is clear that we are here dealing with two different types of proteolytic enzymes. A comparison with the activity of wheat proteinase can also be done. Unpublished investigations by the present author have shown that a water extract of ground seeds from wheat, germinated for 6 days, had a proteolytic activity $A = 4.6 \times 10^{-3}$ when splitting edestin at pH 4.1, the pH optimum of this enzyme. The enzyme was purified by dialysis against water and precipitated with $(NH_4)_2SO_4$. The fraction between 40—80 % saturation had the activity $A = 47 \times 10^{-3}$, *i. e.* the activity was of the same order of magnitude as the activity of pea proteinase from unripe seeds.

The nature of the pea proteinase is not yet clear. No determinations of the dipeptidase activity have been carried out. The possibility that the proteinase is accompanied by a dipeptidase in the same way as in seeds of wheat and malted barley is under investigation.

SUMMARY

1. A protein fraction with proteolytic activity has been isolated from seeds of peas. The highest activity is obtained when unripe seeds are used.

2. The pH optimum of the pea proteinase was determined to be pH 6.7 when splitting gelatin.

3. Crystalline pepsin is 100—200 more active than the best pea proteinase obtained when edestin is used as substrate. The activity of pea proteinase is of the same order of magnitude as the activity of wheat proteinase.

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