THE DISTRIBUTION OF THE ENZYMES IN RESTING CEREALS

II. THE DISTRIBUTION OF THE PROTEOLYTIC ENZYMES IN WHEAT, RYE, AND BARLEY *

by

CHR. ENGEL AND J. HEINS
Central Institute for Nutrition Research, Utrecht (Netherlands)

In this paper the distribution of the proteinase and the dipeptidase in resting grains is discussed.

I. PROTEINASE

According to the papers of JØRGENSEN [1,2] and BALLS AND HALE [3,4] proteinase is an important factor in the baking quality of flour. The proteolytic enzyme is of the papain type and can be activated with cysteine, KCN, and H_2S (MOUNFIELD [5], BALLS AND HALE [6]). The latter authors have been able to purify the enzyme, although not to a crystalline state. Qualitatively there is no difference in the proteinase of flour and bran (HALE [7]).

So far, only PETT [8] has investigated the quantitative distribution of this enzyme from a physiological point of view in germinating wheat grains. In that investigation he used an other technique than we did, the aleurone layer was not separated and moreover autolysis was not taken into account. This autolysis cannot be neglected, especially in protein-rich parts of the grain. Therefore we repeated and extended these proteinase estimations.

According to MOUNFIELD [5] the proteinase in resting grains is only partly active. It is possible to activate the inactive enzyme by treating with cysteine hydrochloride. In the present paper the enzyme has been determined only after activation.

EXPERIMENTAL

1. *Materials.* The same samples of cereals were used as in the previous work (ENGEL [9]). An inland Dutch sample of Juliana wheat (1942 harvest) was also investigated.

2. *Sampling and histological technique.* These were as described by LINDERSTRØM-LANG AND ENGEL [10] and ENGEL [9]. The amount of proteinase being very small, it was found necessary to use more sections for each determination. We have been cutting more grains for one series of determinations. Thus, for example, for a single estimation five No. 1 sections were necessary.

3. *Determination of the proteinase activity.* In estimating the proteinase activity the method of MOUNFIELD [5], who determined by titration the amount of free amino acids formed by the enzyme activity from edestine, was applied on a micro-scale; cf. LINDERSTRØM-LANG AND HOLTER [11] and also WEIL [12].

Several slices, 25 μ thick, were transferred to a 20 μl drop of water and after two hours extraction at 20° C, 20 μl substrate-buffer solution was added. This solution con-

* For I, see CHR. ENGEL, *Biochim. Biophys. Acta.* 1 (1947) 43.

References page 196.

tained per 100 ml : 2 g edestine, 36 ml N/10 lactic acid, 54 ml N/10 sodium lactate and 10 ml 1% cysteine-HCl and had a p_H of 4.1. A lactic acid-sodium lactate buffer was used instead of the usual acetic acid-sodium acetate mixture as this latter caused difficulties in the microdetermination. To determine the autolysis, the same experiment was also carried out without edestine but the buffer solution had then to be altered a little to obtain the same p_H and contained : 31.5 ml N/10 lactic acid, 58.5 ml N/10 sodium lactate and 10 ml 1% cysteine—HCl. Substrate and buffer solutions had to be kept in the ice box ; the cysteine—HCl solution was always freshly prepared and added directly before use. The microtubes containing the enzyme-substrate mixture were always kept 24 hours at 37° C to obtain an measurable hydrolysis.

The amino acids formed were determined by a formol titration. For this to 40 µl of the enzyme-buffer-substrate solution or in the case of autolysis determination to 40 µl of the enzyme-buffer solution 110 µl formol indicator solution was added. This solution was a mixture of : 100 ml formol 37%, 100 ml water, 0.6 ml saturated solution of phenolphtaleine in ethanol and 3.4 ml 0.2% solution of thymol blue in 50% ethanol. All additions were made with a semi-automatic pipette cf. LINDERSTRØM-LANG AND HOLTER [18], the titration being carried out with N/10 NaOH to a bluish-red colour.

For each experiment four titrations had to be made : a. enzyme-buffer-substrate mixture at zero time ; b. the same after 24 hours ; c. enzyme-buffer mixture at zero time ; d. the same after 24 hours.

The proteolytic activity is calculated as the difference between total hydrolysis and autolysis and is expressed as µl N/10 NaOH per µl of tissue per 24 hours. In practice it was found possible to omit some of the titrations because those at zero time were practically the same for the different kinds of tissue.

Before starting the histological investigation the relation was determined between enzyme amount and hydrolysis under the above mentioned circumstances. To this purpose an enzyme extract was prepared from a flour rich in aleurone layer cells and the proteolytic activity was measured when different amounts of this extract acted on edestine as substrate. It was found that in the lower concentrations the hydrolysis was directly proportional to the amount of enzyme, cf. Fig. 1.

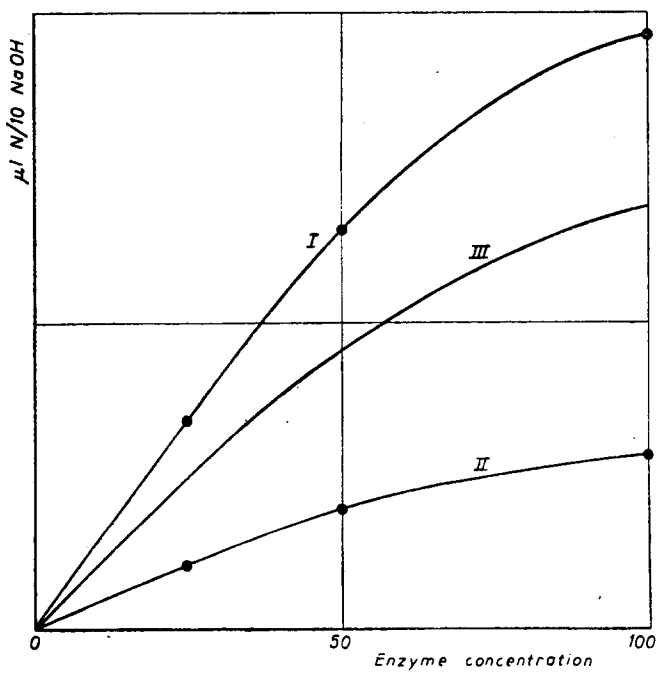

Fig. 1.
Curve I total hydrolysis after 24 hours.
Curve II autolysis after 24 hours.
Curve III the enzyme activity on edestine.

References page 196.

RESULTS AND DISCUSSION

In Fig. 2 are given the results for a series of determinations on the outer layers of the wheat grain. Eight slices were used in the determination of each point of the curves I and II.

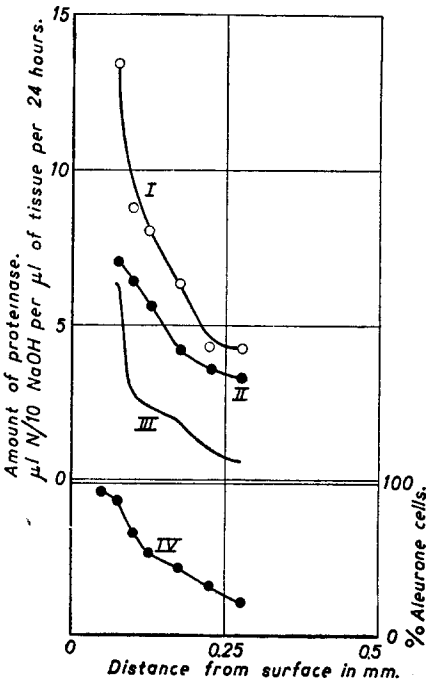

Fig. 2. The distribution of proteinase in the outer layers of a grain of Juliana wheat
Curve I total hydrolysis.
Curve II autolysis.
Curve III enzyme activity.
Curve IV percentage of aleurone cells in the sections.

Curve I is a measure of the total hydrolysis; curve II of the autolysis and curve III the difference between I and II, the real enzymatic activity towards edestine.

Curve IV gives the percentage of aleurone tissue in the sections.

From these experiments it appeared that the proteinase is principally located in the aleurone cells. The subaleurone layer, which had a high amylase content as we could show in a previous paper [9] did not show a particularly high proteinase content. The bulk of the endosperm was almost proteinase free.

In rye and barley we have estimated the proteinase only in the aleurone layer and in the inner endosperm. For the determinations in the aleurone cells we used several (5 to 8) slices containing only epidermal and aleurone tissue. The epidermis was found to be free of all enzymatic activity. For the purpose of calculation of activity per µl of tissue it was not taken into account. To estimate the proteinase in the inner endosperm a piece was taken which had been freed from aleurone and subaleurone. To calculate the enzyme activity per µl of tissue, it was necessary to weight these samples and to estimate their specific gravities.

In Table I total hydrolytic, autolytic and enzymatic activity is given.

TABLE I

PROTEINASE CONTENT OF THE ALEURONE AND ENDOSPERM OF WHEAT, RYE AND BARLEY, EXPRESSED AS µl N/10 NaOH PER µl OF TISSUE PER 24 HOURS

Tissue		Wheat Manitoba	Wheat Juliana	Rye Petkus	Barley Abed Kenia
Aleurone	total hydrolysis . .	8.0	15.7	29.2	5.4
	autolysis	3.2	8.8	12.0	4.7
	enzyme activity . .	4.8	6.9	17.2	0.7
Endosperm	total hydrolysis . .	0.75	0.65	1.1	0.0
	autolysis	0.7	0.55	0.8	0.0
	enzyme activity . .	< 0.1	0.1	0.3	0.0

References page 196.

The proteinase activity towards edestine is considerable in the aleurone tissue, but very small in the endosperm and within the experimental error in most of the cases.

From the autolysis determinations it appeared that the endosperm showed some proteinase activity towards its own proteins.

Because of its low activity it was not possible to estimate the proteinase in the anatomically different parts of the germ of the various cereal grains studied. Therefore we have taken only the total germ without other parts of the grain. For each experiment 3—5 germs were prepared free from other tissue. From the specific gravity and the weight we have calculated the activity per µl. The results are given in Table II.

TABLE II

PROTEINASE CONTENT OF THE TOTAL GERM OF WHEAT, RYE AND BARLEY, EXPRESSED AS µl N/10 NaOH PER µl OF TISSUE PER 24 HOURS

	Wheat Manitoba	Wheat Juliana	Rye Petkus	Barley Abed Kenia
Total hydrolysis	4.4	6.4	4.1	3.6
Autolysis	3.6	5.1	2.7	2.4
Enzyme activity	0.8	1.3	1.4	1.2

In contradiction to PETT [8] we found only relatively small amounts of proteinase in the germ part of the grain.

2. DIPEPTIDASE

The distribution of cereal dipeptidase in relation to germination has been investigated by PETT [8], who used an other technique as we mentioned above. It seemed of interest to extend our investigations to this enzyme, especially as PETT could not investigate the aleurone layer seperately.

EXPERIMENTAL

1. *Materials and histological technique.* They were the same as in the preceeding section.

2. *Determination of the dipeptidase activity.* The dipeptidase activity was determined by titration of the amount of free amino acids formed by the enzyme activity from dl-alanyl-glycine.

Slices, 25 µ thick were placed in a 7 µl drop of water and after half and hour extraction, 7 µl substrate-buffer solution was added with a semi automatic pipette. This solution contained per 100 ml: 13 g dl-alanyl-glycine, 4 ml N/2 NH_4OH and 4.6 ml M/3 KH_2PO_4 solution, and had a p_H of 7.65. To determine the autolysis the experiment was also carried out without substrate. The microtubes containing the enzyme-substrate mixture were kept at 40° C, one hour in the case of germ tissue and six hours in the case of aleurone and endosperm tissue.

The hydrolysis was stopped by the addition of 40 µl of the formol-indicator solution. Titration of the samples was carried out by exactly the same procedure as in the proteinase determination. The enzyme activity is calculated as the difference between total hydrolysis and autolysis and is expressed as µl N/10 NaOH per µl of tissue per hour.

References page 196.

RESULTS AND DISCUSSION

In Fig. 3 are given the results of the determinations on the outer layers of the grains. The epidermal layer did not show any activity of this enzyme as in the case of proteinase and amylase. Except in rye the autolysis is within the experimental error zero. From the curves I (total hydrolysis) and II (autolysis) it appears that the aleurone cells contained a large amount of dipeptidase. From curves III, the percentage of aleurone tissue, it may be derived that the subaleurone and endosperm did not contain dipeptidase in an appreciable amount.

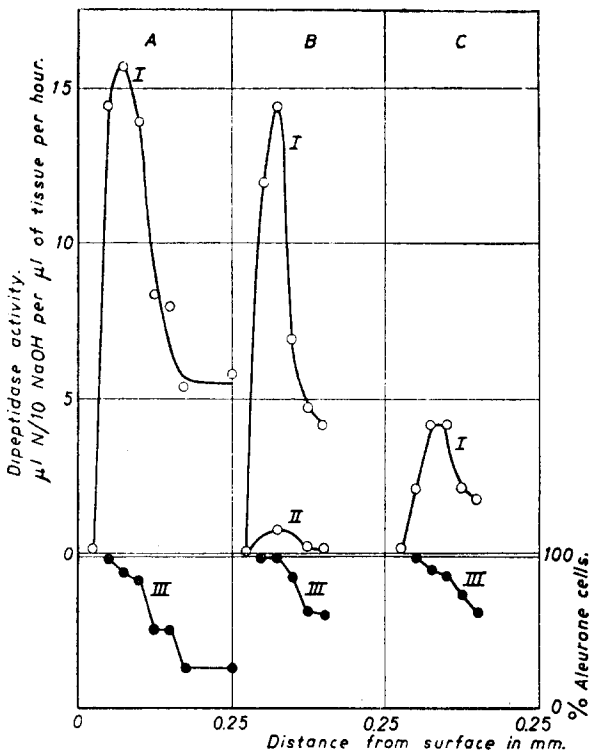

Fig. 3. The distribution of dipeptidase in the outer layers of a grain.
A Manitoba wheat.
B Petkus rye.
C Abed Kenia barley.
Curve I total hydrolysis.
Curve II autolysis.
Curve III percentage of aleurone cells.

In Fig. 4 are given the results of a series of determinations on the germ of Manitoba wheat. The germ was cut in sections 25 µ thick from the root tip to the scutellum and was prepared free from surrounding tissue. In the scheme below the graph the approximate location of the parts of the germ is given ; the position of the root tip and of the epithelial layer of the scutellum is, however, not exact.

It appeared that autolysis in the germ slices was zero.

Curve I represents total hydrolysis and is irregular. This irregularity was not caused by experimental difficulties but by the differing enzyme content of the parts of the germ. From the curve it appears that the rootlet and also the epithelial layer of the scutellum are particularly rich in dipeptidase.

References page 196.

The determinations on rye and barley gave similar results to those obtained on wheat and are therefore not presented here.

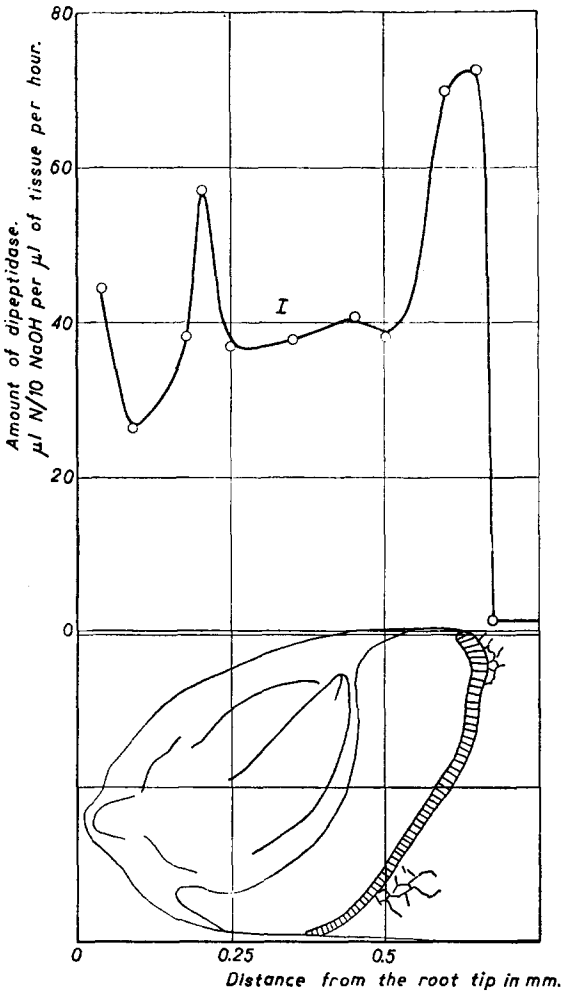

Fig. 4. The distribution of the dipeptidase in the isolated germ sections of Manitoba wheat.

SUMMARY

The aleurone cells of wheat, rye and barley are rich in proteinase and dipeptidase. The endosperm contains only very small or negligible amounts of both enzymes.

In the germ part of the grain only a moderate quantity of proteinase was found, but it was too small to estimate it in the various parts of the germ.

The dipeptidase activity is high in the whole germ, especially in the rootlet and in the epithelial layer of the scutellum.

RÉSUMÉ

L'assise protéique des grains du blé, du seigle et de l'orge est riche en protéinase et en dipeptidase. L'endosperme ne contient que de très petites quantités ou des quantités négligeables des deux enzymes. Dans la partie germinale du grain, on n'a trouvé qu'une quantité modérée de protéinase ; elle a été considérée trop faible pour qu'on la détermine

References page 196.

dans les diverses parties du germe. L'activité de la dipeptidase est élevée dans tout le germe, spécialement dans la radicule et dans la couche épithéliale du scutellum.

ZUSAMMENFASSUNG

Die Weizen-, Roggen-, und Gersten-Aleuronzellen sind reich an Proteinase und an Dipeptidase. Das Endosperm enthält nur sehr geringe oder zu vernachlässigende Mengen von beiden Enzymen. Im Keim des Kornes wurde nur eine mässige Menge von Proteinasen gefunden ; diese Menge ist zu gering, um in den verschiedenen Teilen des Keimes festgestellt zu werden. Die Aktivität der Dipeptidase ist sehr gross im ganzen Keim, besonders im Keimwürzelchen und in der Epitheliumschicht des Scutellum.

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