CCXVII. THE PROTEOLYTIC ENZYMES OF SPROUTED WHEAT. III

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IN two previous communications the author [1936, 1, 2] has described the chief properties of the proteinase and dipeptidase found in aqueous extracts of sprouted wheat. The present paper is a continuation of these studies and deals primarily with the action of the proteinase on gelatin, ovalbumin and on the principal proteins of wheat, namely, gliadin and glutenin, both separately and in the form of gluten.

EXPERIMENTAL

The Sørensen method of formaldehyde titration in two stages, (a) and (b), again formed the basis of investigation, enzyme activity being recorded in terms of $N/20$ NaOH increase.

Substrates. The gluten, gliadin and glutenin used as substrates were prepared from a sample of untreated Canadian flour. The gluten was obtained from a dough made by mixing 100 g. flour with 55 ml. tap water. The dough was first immersed in tap water at 18° for 1 hr. and was then kneaded under a stream of tap water until all starch, etc. had been removed. After a final washing in distilled water and the removal of surplus water the gluten mass was cut into small pieces and mixed with sufficient $N/5$ acetic acid to yield a final gluten concentration of 10 %, allowance being made for the water retained by the gluten. This water content was determined by drying a sample for 24 hr. at 100°. Gluten, of course, consists almost entirely of gliadin, glutenin and water, the two proteins together forming about 34 % and the water about 66 % of the mass, although small amounts of lipins, mineral matter etc., remain firmly adherent.

The gliadin and glutenin were prepared simultaneously by a modification of methods proposed by Troensegaard [1931] for gliadin and by Damodaran [1931] for glutenin. Wet gluten, obtained as described above, was cut into small pieces and the gliadin extracted by several changes of 70 % ethyl alcohol at 28°. The dissolved protein was precipitated by concentration of its solution under reduced pressure at 30° and purified, firstly by treatment with ether to remove fats, lipins etc., secondly with distilled water to remove mineral matter etc., and finally by re-solution in 70 % alcohol and precipitation by concentration again. Dehydration with alcohol in gradually increasing concentration up to absolute alcohol completed the process.

Treatment of the air-dried residue from the gliadin extraction with 0.2 % NaOH yielded a solution of glutenin which was precipitated by adjusting the pH to 6.8–7.0 (the isoelectric point of glutenin) by addition of acetic acid. The precipitate was purified by repeated dissolution in 0.2 % NaOH and subsequent precipitation with 0.4 % acetic acid at pH 6.8–7.0. The protein precipitate was washed with distilled water by decantation until free from acid, then with absolute alcohol and finally with ether. Both preparations were thereafter kept *in vacuo* over P_2O_5 .

RESULTS

Determination of pH optimum for gelatin. A freshly prepared 10% aqueous solution of Gold Leaf gelatin was used as substrate. The corrected curve shown in Fig. 1 has an optimum at pH 5.1. In the same figure are included the experimental test and control curves from which the corrected curve was calculated by difference. The control curve was determined on mixtures containing buffers and enzyme extract only.

Fig. 1.

Fig. 2.

Fig. 1. Effect of variation in pH on gelatin hydrolysis. Conc. of gelatin in reaction mixtures: 2.0%. Enzyme dilution 2:7. $M/7$ acetate, $M/7$ KH_2PO_4 -NaOH and $M/7$ K_2HPO_4 -NaOH buffers. Titres as ml. $N/20$ NaOH per 5 ml. sample. Reaction 4 hr. at 40° . Δ Acetate buffers. $\square$ KH_2PO_4 -NaOH buffers. $\odot$ K_2HPO_4 -NaOH. + Control curve. $\times$ Corrected curve.

Fig. 2. Effect of variation in pH on gluten hydrolysis. Conc. of gluten in reaction mixture: 3.0%. Enzyme dilution 2:7. $M/7$ potassium phosphate-NaOH buffers. $\square$ Test curve. $\odot$ Corrected curve. $\times$ Control curve.

Action of proteinase on ovalbumin. Over the range covered by acetic acid—sodium acetate buffers (pH 3.75–5.7) no increase in titre above that of the control value was observed after 4 hr. at 40° . This result was confirmed after 4 hr. and 24 hr. respectively, at 40° by Harris's [1923] method of titration in 85% alcohol using thymolphthalein as indicator. As substrate, a filtered 5% solution of dried egg albumin (Merck) in $N/5$ acetic acid was used.

Determination of pH optimum for gluten. The effect of variation in pH on gluten hydrolysis by wheat proteinase was determined after 4 hr. at 40° . The results in Fig. 2 show that the corrected curve has an optimum range of pH 5.6–6.4, with a mean value of pH 6. All reaction mixtures contained a considerable amount of precipitated gluten proteins.

Effect of NaCl on gluten hydrolysis. In view of the inhibitory effect of certain concentrations of NaCl on edestin hydrolysis by wheat proteinase [Mounfield, 1936, 1, Fig. 7], parallel experiments were conducted at pH 6 with gluten (final conc. 2.4%) as substrate. With increasing concentrations of NaCl up to 0.25 M the difference from the control value without salt did not exceed 0.04 ml. $N/20$ NaOH which is below the limit of experimental error. As in the preceding experiment all reaction mixtures contained a curdy white precipitate of gluten proteins, the amount of which was not, however, increased by increasing salt concentration. Sodium chloride up to 0.25 M is therefore without influence on gluten hydrolysis by wheat proteinase.

Relation between initial reaction velocity and gluten concentration. Variation in gluten concentration produced the results indicated by the upper and lower lines in Fig. 3. The increases for total titre ($a+b$) and titre (b) did not bear a constant ratio to each other as expected and both are therefore reproduced in the figure after deduction of control values without gluten. With increased concentration of gluten there was a corresponding increase in the amount of precipitate in the reaction mixtures.

Fig. 3.

Fig. 3. *Effect of gluten concentration.* Enzyme dilution 2 : 7. $M/7$ potassium phosphate-NaOH buffer at pH 6. Reaction 4 hr. at 40° . $\odot$ Total titre without cyanide. $\times$ Titre (b) without cyanide. $\square$ Titre (b) with cyanide.

Fig. 4.

Fig. 4. *Effect of edestin concentration.* Enzyme dilution 2 : 7. $M/7$ acetate buffers at pH 4.1. $\odot$ Total titre without cyanide. $\times$ Titre (b) without cyanide. $\square$ Total titre with cyanide. $\triangle$ Titre (b) with cyanide.

The curve for titre (b) reaches a maximum at a gluten concentration of approximately 1.5 % whereas the curve for total titre continues to rise over the whole range of substrate concentration examined.

Relation between initial reaction velocity and edestin concentration. The effect on proteinase activity of variation in edestin concentration differs to some extent from that obtained with gluten as substrate. The ratio between the increases in total titre and titre (b) (see Fig. 4), is almost constant throughout at 2 : 1.

Effect of NaCN on hydrolysis of edestin at varying concentrations of the latter. Increases in titre in the presence of cyanide may conceivably be due to a more complete hydrolysis of the cleavage products rather than to a simple acceleration of reaction velocity. Data which appear to throw some light upon this problem were obtained by redetermining the curves showing the effect of edestin concentration on proteinase activity in the presence of a constant concentration of cyanide, i.e. 0.01 M (see Fig. 4). The curves for total titre and for titre (b) in the presence of cyanide are proportionately higher in both cases than are the corresponding curves in the absence of cyanide.

Influence of cyanide upon proteinase activity using gluten as substrate. The addition of cyanide to mixtures containing gluten as substrate caused no activation of the wheat proteinase as measured by increase in total titre. There was, however, some alteration in the ratio of titre (a) to titre (b): the latter was increased slightly at the expense of the former, total titre remaining the same as in the absence of cyanide. The middle line in Fig. 3 represents the effect upon titre (b) of a constant concentration (0.01 *M*) of cyanide when the concentration of gluten is varied.

Effect of cyanide on control values. During the determination of control values (without added substrate) in connexion with the foregoing cyanide experiments it became evident that at certain pH values the cyanide caused not only a small but definite increase in total titre over and above the corresponding control value without cyanide but also an appreciable alteration in the ratio titre (a) to titre (b). The normal control mixtures consisted of enzyme extract + buffer, with water in place of substrate solution: the cyanide controls contained NaCN to a final concentration of 0.01 *M*. In Table I are presented a few typical values for titres (a) and (b) at different pH values with and without cyanide.

Table I

pH value		4.11	5.23	6.02	7.15	8.08
Without cyanide	Titre (a)	0.02	0.02	0.01	0.02	0.02
	Titre (b)	0.08	0.10	0.20	0.29	0.27
	Total	0.10	0.12	0.21	0.31	0.29
With cyanide	Titre (a)	0.00	0.00	-0.04	-0.08	-0.09
	Titre (b)	0.11	0.14	0.28	0.44	0.46
	Total	0.11	0.14	0.24	0.36	0.35

The influence of cyanide is first to produce a slight increase in total titre and secondly to enlarge titre (b) at the expense of titre (a). Both these changes are more pronounced at the more alkaline reactions.

Action of wheat proteinase on gliadin and glutenin. Gliadin and glutenin prepared in the manner already outlined do not appear to be affected to any great extent by wheat proteinase. Over the range pH 3.75–8.2 no increases in titre greater than those of the control could be detected except at about pH 5. At this point a slight optimum was discernible in the case of gliadin. Substrate concentrations of 2.0 % in *N*/5 acetic acid were employed. Doubling the concentration of protein produced no further effect upon the titre increase. These results were confirmed on four occasions with separate preparations of gliadin and glutenin.

In view of any possible modification of the glutenin by the NaOH used in its preparation the action of wheat proteinase was determined on a sample of glutenin prepared as already described with the exception that solution in 0.2 % NaOH and precipitation with 0.4 % acetic acid were omitted, the air-dried residue being simply dehydrated with increasing concentrations of alcohol. The proteinase was found capable of attacking this substrate and although the extent of scission was by no means as great as in the case of gluten it was sufficiently great to reveal a slight optimum at approximately pH 5.5.

DISCUSSION

The investigation of the effect of wheat proteinase on proteins other than edestin reveals a somewhat unexpected range of specificity. Gelatin and gluten are readily attacked whereas ovalbumin and the constituent proteins of gluten—

gliadin and glutenin—are not. Gelatin shows an optimum at $pH\ 5.1$ (see Fig. 1), a value which is close to the isoelectric point of the protein, $pH\ 4.9$ [Pauli & Modern, 1925]. Papain [Willstätter *et al.* 1926], yeast proteinase [Willstätter & Grassmann, 1926] and malt proteinase [Lüers & Malsch, 1929] also attack gelatin optimally at $pH\ 5$. These facts suggest that plant proteases, generally, hydrolyse the unionized form of gelatin.

It will be observed from Fig. 1 that phosphate used as buffer has a slight retarding influence on the rate of proteolysis. The phosphate curve is, however, parallel over its whole range with the acetate one. The importance of the control curve correction is made particularly manifest in Fig. 1, an apparent optimum of $pH\ 6$ being reduced thereby to one of $pH\ 5.1$. It should be borne in mind, however, that the use of the correction curve (obtained from mixtures of buffer and fresh enzyme extract only) is based on the assumption that on the addition to the control mixture of another substrate such as gelatin the latter will not be preferentially attacked by the proteinase, an assumption which may not be entirely justified.

The behaviour of wheat proteinase towards ovalbumin finds again a parallel in the behaviour of papain [Willstätter & Grassmann, 1924], yeast proteinase [Grassmann & Dyckerhoff, 1928] and malted barley proteinase [Linderstrøm-Lang & Mill, 1929] towards this substrate; in no instance is the native protein hydrolysed. The albumin is however attacked by yeast proteinase which has been activated by cyanide while the denatured protein is degraded by papain without cyanide activation [Willstätter *et al.* 1926]. The above evidence of absence of hydrolysis of ovalbumin was secured either by alcohol or formaldehyde titration of the end products of hydrolysis. On the other hand, by measuring the disappearance of substrate, as indicated by the loss of coagulability of the ovalbumin, Hopkins & Kelly [1931] were able to demonstrate an optimum of $pH\ 3.3-3.6$ for the action of malted barley proteinase on the native protein. It is conceivable that the chemical changes accompanying the loss of coagulability are not of sufficient magnitude to influence a formaldehyde or alcohol titre. Northrop [1922], for example, obtained different results on measuring the effect of substrate concentration on the tryptic digestion of casein according to whether he measured the rate of disappearance of casein or that of amino-nitrogen liberation.

From the curves in Fig. 2 it is clear that wheat gluten is acted upon by the proteinase present in aqueous extracts of sprouted wheat, the reaction having an optimal range of $pH\ 5.6-6.4$ with a mean value of 6.0 . This figure is slightly to the acid side of the isoelectric points of gliadin and glutenin as reported by Tague [1925], who found a value for gliadin of $pH\ 6.5$ and for glutenin of $pH\ 6.8-7.0$. The choice of gluten as a substrate is of course open to criticism: gluten is neither a pure protein nor simply a group of proteins. It contains in addition to gliadin and glutenin small quantities of fats and lipins, carbohydrate, mineral matter etc. A typical analysis of dried gluten is given below [Norton, 1906]:

	%
Fat or ether extract	4.20
Carbohydrate	9.44
Fibre	2.02
Mineral matter	2.48
Gliadin	39.09
Glutenin	35.07
Globulin (10% NaCl extract)	6.75
	<hr/> 99.05

Gluten was nevertheless employed as such, firstly on account of its unique position among proteins, secondly, because it is presumably the natural substrate for wheat proteinase, and thirdly, on account of its considerable technical importance. That the results shown in Fig. 2 are due to hydrolysis of the principal gluten proteins and not to the decomposition of concomitant substances such as fats and lipins is proved by the method of their determination: increases in titre (*b*) by formaldehyde titration can be due only to the formation of amino-acids or closely related compounds. Further, although there arises the possibility of the hydrolysis of other proteins such as wheat globulin or leucosin which remain in the mass of gluten, nevertheless the extent of this hydrolysis could have but little influence upon the final result on account of the extreme dilution of these proteins in the final mixtures. For example, assuming the amount of globulin in the sample of dry gluten used in the present experiments to have been 7%, the concentration of the globulin in the final reaction mixtures could not at any time have exceeded 0.21%. This corresponds to a final crude gluten percentage of 3. If then the curves in Fig. 3 are assumed to be the outcome of, say, wheat globulin hydrolysis, then not only are titre increases of considerable magnitude obtained at substrate concentrations of 0.21% but appreciable increases still accrue at concentrations as low as or even lower than 0.007% (corresponding to a gluten percentage of 0.1). Judging from the data obtained with edestin and gelatin such an interpretation is highly improbable.

Gluten hydrolysis by wheat proteinase does not appear to be affected by sodium chloride in concentrations up to 0.25 *M*, nor is the amount of substrate precipitate present in each mixture increased by the salt addition. Edestin, on the other hand, is precipitated by sodium chloride in acid solution and the rate of hydrolysis of edestin in the presence of this salt has been shown to depend upon the amount of protein remaining unprecipitated [Mounfield, 1936, 1]. The apparently obvious explanation that by precipitation the edestin is removed from the sphere of activity of the enzyme cannot, however, be accepted as the sole reason for the inhibitory effect, since precipitation of edestin can occur in certain mixtures containing acetate buffers only, particularly at about *pH* 5, without an equivalent reduction in rate of hydrolysis [Mounfield, 1936, 1, Fig. 1]. The edestin precipitated in sodium chloride solutions does not therefore appear to be identical with that formed by simple adjustment of an edestin solution to its isoelectric point. This suggests the formation of an edestin-sodium chloride complex which is not attacked by the proteinase. That the latter enzyme is capable of directly hydrolysing precipitated substrate is proved still further by the experiments on the relation between initial reaction velocity and gluten concentration. At all concentrations from 0.05 to 3.0% the gluten appeared to be completely precipitated, yet with increase in gluten concentration there was continued increase in total titre (see Fig. 3).

Figs. 3 and 4 depict the results of parallel experiments on the variation in initial reaction velocity with substrate concentration using gluten and edestin, respectively. In the case of gluten the titre (*b*) curve reaches a limiting value at a substrate concentration of approximately 1.5%, while for edestin the curve becomes almost horizontal at approximately 2% substrate. The latter curve was published in an earlier communication [Mounfield, 1936, 1] but is here reproduced for convenience in reference. The substrate concentration at which half the limiting velocity is reached should be numerically equal to the Michaelis constant, K_m , a characteristic of the enzyme. For edestin this has already been reported as occurring at about 0.05%. From Fig. 3 the value for gluten is seen to be approximately 0.25% although in view of the complex nature of this

substrate and the probable interference of dipeptidase activity (see below) the precise meaning to be attached to this information is rendered even more obscure than in the case of edestin.

The curve in Fig. 3 showing increase in total titre ($a+b$) with variation in substrate concentration does not run parallel with that for titre (b), the ratio titre (a) : titre (b) increasing with increasing substrate concentration. In Fig. 4 however the curves for total titre and titre (b) approximate more closely in shape, the ratio total titre : titre (b) being almost constant at 2 : 1. In neither case is a limiting value reached within the limits of the experiment.

In this connexion it should be borne in mind that titre (b) represents principally the increase during hydrolysis in amino-acid-N together with a portion of the polypeptide amino-N while total titre includes also that portion of the polypeptide amino-N not measured in titre (b) and also any carboxyl groups liberated, for example, by ester scission [Richardson, 1934; 1935; Grünhut, 1919]. Ester scission is unlikely in the present instance and in any case would be very largely eliminated by the deduction of control values so that increases in titre (a) or titre (b) may safely be assumed to result from hydrolysis of protein only.

Now the mean pK' of most peptides is about 8.3 while that of amino-acids is about 9.6 [Richardson, 1934] so that peptide formation alone (if such should occur) would lead to increases in both titre (a) and titre (b). Amino-acid production on the other hand would result in increase in titre (b) without appreciable rise in titre (a). The distinction is of course by no means quantitative but merely indicates a tendency. The hydrolysis therefore of, say, glycylglycine ($pK'=7.75$) to glycine ($pK'=9.75$) or of leucylglycine ($pK'=7.83$) to glycine and leucine ($pK'=9.6$) should cause a decrease in titre (a), a comparatively large increase in titre (b) and a small increase in total titre. These changes were observed experimentally and are discussed below.

Thus the continued rise in total titre with increasing substrate concentration in Fig. 3 after titre (b) has attained an almost constant value suggests at first sight a continuance of polypeptide formation after amino-acid production has reached its maximum. Whether or not this be due to the activities of more than one enzyme it is difficult to state with certainty. In the gluten experiment with reaction conditions at pH 6 it is however probable that the proteinase and dipeptidase are active simultaneously, in which case a constant ratio between total titre and titre (b) cannot be expected. It is possible also for the dipeptidase to reach saturation point at a lower percentage of substrate than does the proteinase. In the case of edestin the reaction conditions (pH 4.1) would almost immediately cause the destruction of any dipeptidase.

It is therefore highly suggestive to discover that in this connexion there is no disturbance of the balance between polypeptide and amino-acid productions when edestin concentration is increased. There is an almost constant ratio between total titre and titre (b) (see Fig. 4), so that if produced the two curves should become horizontal at the same point. The activities of a single enzyme, namely, wheat proteinase, thus appear to account for the relevant facts.

The activation of wheat proteinase by cyanide using edestin as substrate has already been described [Mounfield, 1936, 2]. According to the hypothesis of Bersin [1933] and co-workers cyanide activates by reducing the S—S linkage of the inactive oxidized form of the enzyme to the SH form. This merely has the effect of increasing the quantity of active enzyme so that the course of any given proteolysis should not thereby be altered except in velocity. This reasoning is supported by the results of the experiments on the effect of cyanide on the relations between edestin concentration and initial reaction velocity (see Fig. 4).

The ratio total titre : titre (*b*) with cyanide is almost identical with the corresponding ratio without cyanide, namely, 2 : 1 in each case. This indicates quite clearly that cyanide caused no disturbance of the equilibrium between polypeptide and amino-acid production. The cyanide merely elevates both total titre and titre (*b*) curves to proportionate extents and does not induce a more complete hydrolysis of the split products.

The inability of cyanide to effect an increase in the rate of hydrolysis of gluten as measured by increase in total titre (see Fig. 3) is somewhat surprising since in most other respects this substrate behaves similarly to edestin. It is unusual to find that activation by cyanide depends upon the substrate. It is possible that the mechanism of attack by the enzyme is different in the case of gluten or it may be that the method of preparation of the substrate permits the introduction into the reaction mixture of reducing substances which completely activate the proteinase and render the cyanide ineffective.

Another explanation however presents itself. Since proteins most probably contain the S—S and SH type of linkages it is conceivable that they also are affected by cyanide in much the same manner as papain and related enzymes are considered to be affected. Such an assumption would explain not only the dependence of activation upon choice of substrate (gluten for example might be regarded as a fully "activated" protein, the addition of cyanide causing no further modification or increase in susceptibility) but it would also form the basis of a rational explanation of the extension of the range of specificity exhibited by cyanide-activated papain, cathepsin and yeast proteinase. According to Willstätter & Grassmann [1924] untreated papain attacks only gelatin, denatured albumin and possibly peptones whereas after activation native albumin, histones, protamines and even certain tripeptides are included within its range of substrates. An almost identical widening of substrate range occurs with cathepsin [Waldschmidt-Leitz *et al.* 1929] and yeast proteinase [Grassmann & Dyckerhoff, 1928]. Instead, therefore, of supposing that the enzyme is the activated component it is equally reasonable to consider the protein as being "activated" or rendered more susceptible to attack. Indeed, both enzyme and substrate may be affected in this way.

In spite of its inability to affect the increases in total titre in the gluten experiment cyanide does however cause a small but definite increase in titre (*b*) and a corresponding reduction in titre (*a*) (see Fig. 3). In accordance with the argument outlined above this disturbance in the equilibrium suggests that the quantity of amino-acid is augmented by a more complete hydrolysis of peptide linkages brought about in all probability by cyanide activation of the dipeptidase. That such is still possible to a small extent at *pH* 6 has already been demonstrated [Mounfield, 1936, 2]. Support is gained for this view from the data cited in Table I. In the absence of all substrate except that naturally present in the sprouted wheat extract by virtue of its method of preparation (probably wheat globulin and leucosin together with peptides etc.) slight activation by cyanide as measured by increase in total titre is again observed. Further, the same depression of titre (*a*) and increase in titre (*b*) is evident. The phenomenon is absent below about *pH* 5.5 but is prominent between *pH* 7 and 8 a range which covers the optimum for dipeptidase activity, to which the effect is no doubt due. The middle line in Fig. 3 is of course plotted from values obtained after deduction of such controls carried out in the presence of cyanide. Reference has already been made to the fact that precisely similar results were recorded during the measurement of the hydrolysis of glycylglycine and leucylglycine by wheat dipeptidase.

An unusual feature of this work has been the almost complete inability of the enzymes of sprouted wheat extract to attack either glutenin or gliadin as prepared by the methods outlined in the introduction to this paper. In the case of gliadin it is true there is evidence of a slight optimum at or near pH 5 but the extent of the hydrolysis in no way compares with that secured with other substrates. Doubling the concentration of substrate was without influence upon the titre increase, a fact which disposes of any error which might have arisen through the use of substrate concentrations below saturation point. The device of using a mixed substrate of glutenin and gliadin in equal proportions was also without avail—there appears to be no complementary relationship between the two proteins in this connexion.

It has been pointed out by Dakin & Dudley [1915] that contact with alkali, which causes racemization of proteins can render the protein molecule resistant to attack by proteolytic enzymes. This might conceivably be the explanation for the slight increase in the extent of hydrolysis of glutenin when prepared without contact with alkali, but it is certainly not responsible for the resistance shown by the gliadin used in these experiments.

Several possibilities suggest themselves but none appears to offer a satisfactory explanation of the phenomena. In the first place it is conceivable that by prolonged contact with reagents such as ethyl alcohol and acetic acid, necessary for their preparation, the proteins have been modified in such a way as to obstruct proteolysis. This however is contrary to commonly observed facts, since denaturation, coagulation and allied changes tend rather to render the protein structure more susceptible to attack. Secondly, the method of preparation may have introduced substances possessing power to inhibit proteolysis or, on the other hand, may have removed certain necessary activators. The latter two suggestions offer most promise, but so far work along these lines has proved unfruitful. Attempts to activate with cyanide, for example, have been unavailing.

SUMMARY

1. Wheat proteinase hydrolyses gelatin optimally at pH 5.1 and wheat gluten at pH 6.0 but does not attack ovalbumin, and affects glutenin and gliadin only slightly.
2. Amino-acid production from gluten reaches a maximum at about 1.5% substrate concentration while half the maximum reaction velocity is reached at about 0.25% gluten. Sodium chloride is without effect upon gluten hydrolysis.
3. The effect on initial reaction velocity at pH 4.1 of variation in edestin concentration is explicable on the assumption that only wheat proteinase is active. With gluten as substrate and reaction conditions at pH 6 the end products appear to be capable of further modification by the participation of wheat dipeptidase in the reaction.
4. Activation of wheat proteinase by cyanide using edestin as substrate merely affects reaction velocity and does not bring about a more complete hydrolysis of the cleavage products.
5. No activation with cyanide is observed when gluten is employed as the substrate for the proteinase. The decrease in the ratio titre (a) : titre (b) may be attributed to a slight activation of the concomitant dipeptidase.

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