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γ-GLUTAMYL TRANSPEPTIDASE FROM KIDNEY BEAN FRUIT

I. PURIFICATION AND MECHANISM OF ACTION

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SUMMARY

1. A γ-glutamyl transpeptidase from kidney bean fruit has been further purified and its properties have been studied. The purified material seems to consist of one component, as judged by its behavior in the ultracentrifuge and its elution pattern from an acrylamide-gel column. The preparation catalyzes the transfer of the γ-glutamyl moiety of glutathione, γ-glutamylaniline, and γ-glutamyl-*p*-nitroaniline to suitable acceptor molecules, usually amino acids.

2. The same preparation was found to catalyze the hydrolytic cleavage of the γ-glutamyl peptide linkage of these compounds. Evidence, based on purification, gel filtration and activation, is presented that the two activities are associated with the same enzyme.

3. From kinetic studies, it is concluded that a basic nitrogen of an imidazole group and a phenolic group of tyrosine constitute a part of the catalytic site of the enzyme molecule. On the basis of these findings a mechanism for the transfer and the hydrolytic reactions is proposed based on a nucleophilic attack of imidazole nitrogen on substrates.

4. It is suggested that the enzyme is involved in the degradation of glutathione.

INTRODUCTION

γ-Glutamyl transpeptidases from animals and microorganisms have been known for several years[1–4]. Recently, a γ-glutamyl transpeptidase was isolated from plants[5] as a result of studies on the mode of γ-glutamyl dipeptide formation. The plant enzyme is similar to the animal enzyme in catalyzing both the transfer of γ-glutamyl groups and the hydrolysis of γ-glutamyl peptides; however, it is notably different from its

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animal counterpart in several respects. The plant enzyme has no magnesium requirement, is soluble rather than particle bound, is unaffected by bromocresol green (except at high salt concentration), acts on glycylglycine very slowly, is inhibited by borate, and is activated by sodium citrate. These differences prompted an investigation of the properties of the bean transpeptidase and its mechanism of action.

MATERIALS

The following compounds were obtained from commercial sources*: γ-glutamyl-*p*-nitroaniline, α- and γ-benzyl esters of *N*-carbobenzyloxyglutamic acid, glutathione (Cyclo Chemical Company, Los Angeles, California); acetyl pyridine-DPN (Nutritional Biochemical Corporation, Cleveland, Ohio); glutamate dehydrogenase (Sigma Chemical Company, St. Louis, Missouri); and blue dextran 2000 (Pharmacia Co., Uppsala, Sweden).

γ-Glutamylaniline and α-glutamylaniline were prepared from the α-benzyl ester (or the γ-benzyl ester) of *N*-carbobenzyloxyglutamic acid and aniline by the method of SACHS AND BRAND[6]. The product was purified by two recrystallizations from water.

METHODS

Protein was determined according to the method of LOWRY *et al.*[7]. However, when a large number of samples was involved, the spectrophotometric method[8] was used, and generally, the results obtained by the two methods agreed well.

Assay of enzymic activity

a. *Assay of γ-glutamyl transpeptidase.* Transpeptidase activity was usually determined by a procedure based on that of GOLDBARG *et al.*[9] in which aniline released from γ-glutamylaniline is measured colorimetrically. A typical incubation mixture contained in 1 ml: 250 μmoles Tris buffer (pH 9.5); 1.5 mmole sodium citrate; 1 μmole γ-glutamylaniline; 10 μmoles *S*-methyl-L-cysteine; and 50–100 μg enzyme preparation from Step 4.

After incubation at 37° for 2 h, 2 ml of 40% trichloroacetic acid were added and the aniline formed was measured. Transpeptidase activity was measured by the difference between aniline formed in the presence and in the absence of a glutamyl acceptor. When more accurate determinations were needed, the amount of glutamate formed (hydrolysis) was determined and its value subtracted from the total amount of aniline formed. The determination of glutamate is described in Section b.

b. *Determination of the hydrolytic activity.* Using the synthetic compound γ-L-glutamylaniline as substrate, the hydrolytic activity of the enzyme preparation can be measured by the release of either glutamate or aniline. At pH values below 7.0, the results from both methods are identical, since no transfer occurs in this pH range. However, at pH values higher than 7.5, where γ-glutamylaniline can act both as donor and acceptor of the γ-glutamyl moiety, more aniline than glutamate is released

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because of the formation of γ-glutamyl-γ-glutamylaniline[5]. The difference is small, however, especially when a low concentration of substrate is used (see Fig. 4). Therefore, except when a very accurate determination of hydrolytic activity was needed, aniline, rather than glutamate was measured. Hydrolytic activity was measured by the same procedure described for transfer activity, except that the acceptor amino acid was omitted. Whenever the glutamate method was used, 2 μmoles of γ-glutamylaniline were used instead of 1 μmole, and at the end of the reaction, separate aliquots were taken for glutamate and aniline determinations.

In the determination of glutamate, the enzymic method developed by KAPLAN, CIOTTI AND STOLZENBACH[10] as adapted by LEVENBERG[11] was used.

In some experiments, γ-glutamyl-*p*-nitroaniline was used as substrate[12]. In these cases, 3.5 μmoles of substrate were used in a final volume of 1 ml. Other conditions were as described earlier, except that the reaction was stopped by the addition of 2 ml of 1 M acetic acid. The *p*-nitroaniline released in the reaction was determined by measuring its absorbance at 410 mμ.

One unit of transpeptidase activity is defined as the activity that catalyzed the liberation of 1 μmole of aniline per hour under the given assay conditions.

Proteolytic digestion was carried out by mixing a solution of transpeptidase (5.2 mg/ml of 0.1 M Tris–acetate buffer (pH 8.2)) with an equal volume of trypsin or chymotrypsin (5.0 mg/ml) in the same buffer solution and incubating at 37°.

RESULTS

Purification of γ-glutamyl transpeptidase

The first two steps of the purification procedure were given in a previous work[5]. Subsequent steps include sodium nucleate precipitation of impurities, adsorption on calcium phosphate gel and chromatography on diethyl-aminoethyl cellulose (DEAE) to give an overall purification of about 360-fold (Table I). Unless otherwise stated, the purification procedure was carried out at 2–5°.

Step 1. Fresh green beans (1000 g) were ground with 1 l of 0.05 M sodium bicarbonate at 4° in a blender for 2–4 min. Large particles were removed by filtration through two layers of cheese cloth.

TABLE I

SUMMARY OF THE PURIFICATION PROCEDURE OF γ-GLUTAMYL TRANSPEPTIDASE BASED ON A BATCH OF 1000 g OF FRESH KIDNEY BEAN FRUIT

Step	*Treatment*	*Volume (ml)*	*Protein (mg/ml)*	*Total protein (mg)*	*Total units*	*Specific activity*	*Relative purity*	*Yield (%)*
1	Sodium bicarbonate extract	1170	9.5	11 115	95.50	0.0086	(1)	100
2	Ammonium sulfate dialysate	50	16.0	800	58.50	0.0730	8.5	61
3	Sodium nucleate supernatant	50	5.2	260	48.00	0.190	22.0	50
4	Calcium phosphate gel eluate	10	0.74	7.40	8.15	1.10	127.0	8.5
5	DEAE-cellulose eluate	2	0.36	0.72	2.25	3.10	360.0	2.3

Step 2: Solid ammonium sulfate was dissolved in the filtrate to make it 50% saturated. The solution was kept four hours and centrifuged at 3000 × *g* for 15 min. Solid ammonium sulfate was added to make the supernatant solution 70% saturated. After 4 h, the resultant precipitate, which contained most of the activity, was then collected by centrifugation, and the supernatant was discarded. The precipitate was dissolved in a minimum amount of water (about 50 ml), and the solution was dialyzed for 24 h against 8 l of 0.01 M potassium phosphate buffer at pH 6.8.

Step 3: The dialyzed material was diluted to a protein concentration of 15 to 20 mg per ml, and treated with one-tenth volume of a cold 2% solution of sodium ribonucleate. While stirring vigorously, the solution was then brought to pH 4.7 by the slow addition of 1 M acetic acid. The solution was kept overnight and then centrifuged at 15 000 × *g*. The supernatant (about 50 ml) contained most of the activity. Up to this stage of purification, the enzyme could be stored either frozen or lyophilized without loss of activity. After this stage, freezing or lyophilization caused immediate loss of activity (more than 50%). However, activity was completely retained in solution at 0° for at least three weeks.

Step 4: To the solution from Step 3, 1 ml of 85% calcium phosphate gel[13] per 15–20 mg protein was added and the mixture stirred for 2 h. The suspension was centrifuged at 5000 × *g* for 5 min and the supernatant solution was discarded. The precipitate was washed twice with 0.01 M phosphate buffer (pH 6.8), twice with 0.05 M buffer and once with 0.1 M buffer where the volume of wash equaled that of the gel suspension. The enzyme was eluted from the calcium phosphate gel by stirring twice with one volume (same as wash) of 0.5 M phosphate buffer for 1 h. After centrifugation the supernatant solution was dialyzed against 4 l of 0.01 M potassium phosphate buffer at pH 6.8 for 12 h.

Step 5: 5 ml of the enzyme preparation from Step 4 (containing about 3.5 mg protein) were put on a freshly prepared column of DEAE-cellulose (10 cm × 1 cm) and equilibrated with 0.01 M potassium phosphate buffer at pH 6.8. After adding the sample, the column was washed with 100 ml of 0.01 M potassium phosphate buffer at pH 6.8 and 50 ml of 0.01 M K_2HPO_4 at a flow rate of about 2 ml per min. Then, the enzyme was eluted with 0.05 M K_2HPO_4 at a flow rate of about 0.25 ml per min. During this phase of chromatography, 3-ml fractions were collected, and the fractions containing the activity were concentrated to a volume of about 2 ml by using dry acrylamide gel (Bio-Gel P-10). The most highly purified material behaved as a single component in the ultracentrifuge (Fig. 1 of the following paper).

Properties of the enzyme

The higher level of purification of the enzyme preparation has necessitated a redetermination and extension of some of the characteristics of the bean transpeptidase as described below.

The following reactions catalyzed by the enzyme preparation have been demonstrated in previous work[5], using the synthetic substrate, γ-glutamylaniline, as donor of the γ-glutamyl moiety:

(a) γ-Glutamylaniline + *S*-methyl-L-cysteine → γ-Glutamyl-*S*-methyl-L-cysteine + aniline

(b) 2 γ-Glutamylaniline → γ-Glutamyl-γ-glutamylaniline + aniline

(c) γ-Glutamylaniline + H_2O → Glutamic acid + aniline

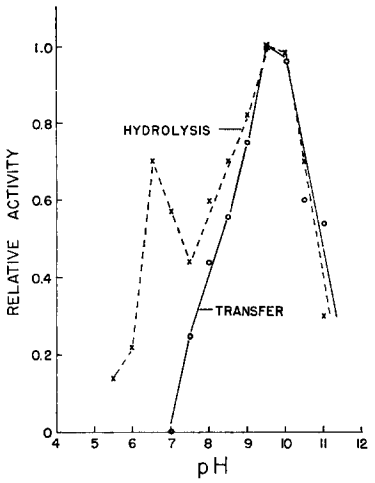


Fig. 1. Effect of pH on hydrolytic activity and transfer activity of purified kidney bean enzyme. Assay conditions are as described in METHODS.

It was also shown that the same preparation acted on glutathione in the same manner, except for the fact that glutathione could not serve as acceptor in the transpeptidation reaction (Reaction b).

Specificity studies were carried out with various γ-glutamyl donors. The results shown in Table II indicate that the enzyme in both hydrolytic and transfer reactions is much more active toward the γ-L-glutamyl moiety. The results obtained with the bean enzyme are similar to those obtained by ORLOWSKI AND MEISTER[14], working with purified hog-kidney transpeptidase.

The highly purified enzyme had the same pH optimum (9.5) as the less pure material[5] (see Fig. 1). However, further study of the two reactions revealed that at pH values below 7.5, the enzyme preparation, although active in hydrolysis, exhibited no transfer activity. Studies were undertaken to determine whether both activities are associated with the same enzyme (see later section).

The molecular weight of the enzyme preparation, as determined from gel filtration data[15], is in the neighborhood of 180 000. The value thus obtained agreed reason-

TABLE II

SPECIFICITY OF BEAN TRANSPEPTIDASE WITH RESPECT TO THE γ-GLUTAMYL MOIETY OF THE DONOR MOLECULE IN BOTH THE HYDROLYTIC AND TRANSFER REACTION, AS MEASURED BY THE RELEASE OF ANILINE OR *p*-NITROANILINE. CONDITIONS ARE AS DESCRIBED IN TEXT

Substrate	*Aniline or p-nitroaniline released*	
	Hydrolytic activity (μmoles)	*Transfer activity (μmoles)*
γ-L-glutamyl aniline	0.037	0.051
α-L-glutamyl aniline	0	0
γ-L-glutamyl *p*-nitroaniline	0.440	0.746
γ-D-glutamyl *p*-nitroaniline	0.082	0.082

TABLE III

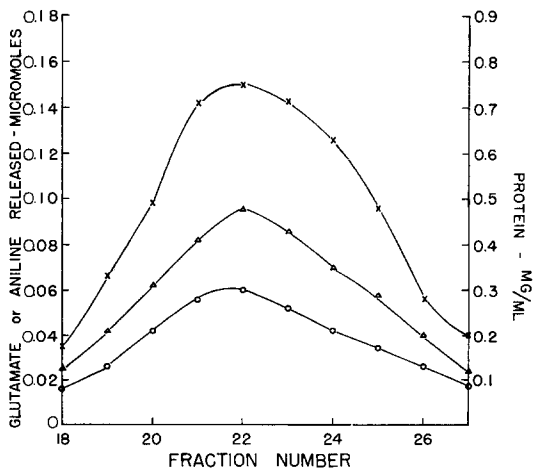
RATIOS OF TRANSFER TO HYDROLYTIC ACTIVITY OF BEAN TRANSPEPTIDASE THROUGHOUT THE PURIFCATION PROCEDURE, AS MEASURED BY THE RELEASE OF ANILINE AND GLUTAMATE, RESPECTIVELY

Step	*Treatment*	*Transfer activity / Hydrolytic activity*
1	Sodium bicarbonate extract (crude)	1.50
2	Ammonium sulfate dialysate	1.58
3	Sodium nucleate supernatant	1.35
4	Calcium phosphate gel	1.68
5	Column chromatography with DEAE-cellulose	1.50

ably well with values obtained by sedimentation where the preparation appeared to consist of one component as judged from the schlieren pattern (see Fig. 1 of the following paper). The sedimentation coefficient, $s_{20,w}$, of the preparation as calculated from the movement of the maximum ordinate is 4.82. An estimate based only on this sedimentation coefficient gives a molecular weight of approx. 150 000, a value which may be misleading[16] because it assumes a spherical molecule.

Hydrolytic and transfer activity as functions of the same enzyme protein

BINKLEY[17] showed that purification of renal glutathionase resulted in the loss of hydrolytic activity. However, ORLOWSKI AND MEISTER[14] in a more highly purified preparation found significant hydrolysis. The following evidence strongly indicates that the extensively purified bean transpeptidase catalyzes both hydrolysis and transfer.



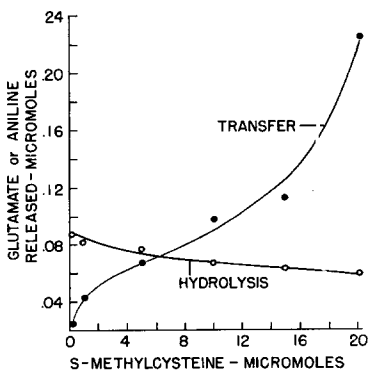


Fig. 2. Elution of kidney bean fruit enzyme from a column of Bio-Gel P-300. For activity test, 0.25 ml was taken. Other conditions as described in METHODS. (○—○), proteins. (△—△), hydrolytic activity. (×—×), transfer activity.

Fig. 3. Inhibition of the hydrolytic activity upon addition of acceptor amino acid. Assay conditions are as described in METHODS. Solid circles refer to transfer activity. Open circles refer to hydrolytic activity.

1. Evidence from the purification procedure. Throughout the various steps of purification, the ratio of the specific activities of transfer to hydrolysis remained unchanged, as shown in Table III.

2. Acrylamide gel filtration. The passage of highly purified enzymic preparation (Step 5 of the purification procedure) through a column of Bio-Gel P-300 (50 cm × 0.9 cm) gave one peak. The results illustrated in Fig. 2 indicate that the two activities have similar effluent profiles. Further, the ratio of the two activities is the same in the various parts of the peak.

3. Activation of the enzyme preparation by carboxylic acids. It has been reported previously[5] that the addition of sodium citrate or ethylenediaminetetraacetate (EDTA) to the reaction mixture increased markedly the activity of the enzyme. During the progress of this work, it has been found that this effect is also shown by the sodium salts of other carboxylic acids (see Table I of the following paper). Since both hydrolytic and transfer activities were increased to the same extent by many salts, the most reasonable explanation of this finding is that both processes are associated with the same enzyme.

4. Inhibition of the hydrolytic activity by the presence of acceptor molecules. If the hydrolysis and transfer are catalyzed by the same protein, it would be expected that

TABLE IV

EFFECT OF TRYPSIN AND CHYMOTRYPSIN ON KIDNEY BEAN TRANSPEPTIDASE*

Time of incubation with trypsin or chymotrypsin (h)	*Transfer activity / Hydrolytic activity*	
	Trypsin treated	*Chymotrypsin treated*
0	1.1	1.0
1	0.95	1.03
2	0.95	1.03
4	0.95	1.15
6	0.97	1.1
8	0.97	0.77
24	0.74	0.64

* Samples of the enzyme preparation taken from Step 4 of the purification procedure were incubated with trypsin or chymotrypsin as indicated. Assay conditions are as described in METHODS.

the acceptor molecule (amino acid) would reduce hydrolysis. Fig. 3 shows that high concentrations of methylcysteine reduce hydrolysis about 30%.

5. The effect of proteolytic digestion on enzyme activity. The effect of proteolytic digestion on the transfer and hydrolytic activities of bean transpeptidase is shown in Table IV. These results show that digestion of the bean transpeptidase has produced a significant change in the ratio of transfer to hydrolytic activity. A change in ratio of transfer to hydrolytic activities could well be interpreted as a difference in sensitivity of two separate proteins to proteolysis. However, in view of the strong evidence for one protein, already presented, it is highly probable that proteolytic digestion has preferentially removed the site or sites necessary for the transfer catalysis. This implies that at least two sites must be present in the enzyme molecule—one that binds

the γ-glutamyl substrate and is the only site necessary for hydrolysis, and one site that binds an amino acceptor. Kinetic data presented below lend support to this view. Further, according to this hypothesis, one would expect transfer activity to be lost faster than hydrolytic activity, as was observed.

Kinetic studies and mechanism of action

The above results indicate that hydrolysis and transfer are catalyzed by one protein which has one site for the γ-glutamyl peptide and a second binding site for the acceptor amine. On this premise, an enzymic mechanism has been developed from the kinetic data.

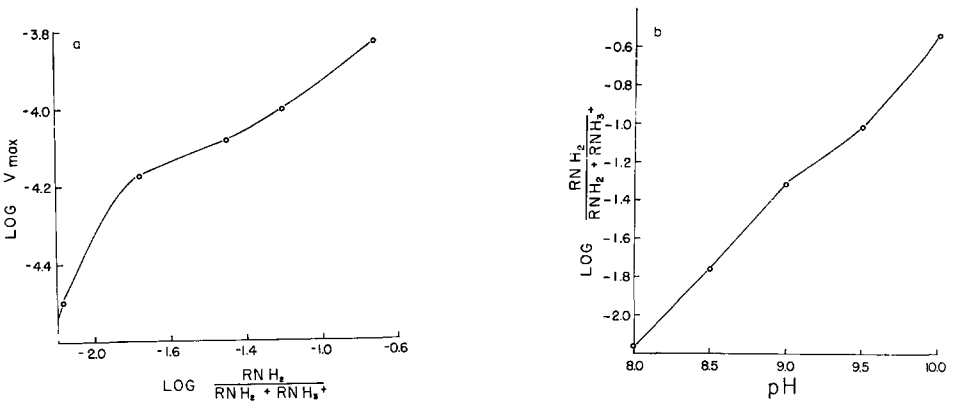


Fig. 4. The active form of the α-amino group in the transfer catalyzed reaction. a. Log v_{max} of the transfer reaction as function of log of the fraction of the uncharged form of the α-amino group of *S*-methyl cysteine present at a given pH. v_{max} is expressed as moles/h of aniline released under the standard assay conditions described in METHODS. b. Log of the fraction of the uncharged form ($R—NH_2$) of *S*-methyl-L-cysteine as function of the pH.

The form of the α-amino group taking part in the transfer reaction

A rather high pH optimum of the γ-glutamyl-transpeptidase has been observed for both the bean enzyme (see Fig. 1) and the kidney enzyme[18,19]. The high pH optimum suggests that the active form of the α-amino acid taking part in the transfer reaction has an uncharged α-amino group. Following this assumption, HIRD AND SPRINGELL[19] attempted to explain the relative acceptor activities of the various amino acids in the animal transpeptidase system in terms of their pK values. This approach proved to be unsatisfactory because other factors, such as the length and the chemical nature of the side chain of the amino acid, determine the fitness of an amino acid to serve as acceptor in the transpeptidase system. More conclusive results concerning the active form of the amino group taking part in the transpeptidation process were obtained by comparing the rate of enzymic activity with protonation of the amino acid acceptor at various pH's. In Fig. 4a, the logarithm of the maximum velocity of γ-glutamyl transpeptidase reaction calculated at various pH's is plotted against the logarithm of the fraction of the uncharged form of *S*-methyl-L-cysteine present at the corresponding pH. In Fig. 4b, the logarithm of the fraction of the uncharged form at a given pH is plotted against that pH for comparison. Inspection of these figures shows that there is good correlation between the v_{max}'s of the transfer catalyzed reac-

tion and the concentration of the non-protonated form of the α-amino group of the amino acid, which in itself is a function of pH.

The poor correlation at low pH's, where the concentration of the non-protonated amino form of the acceptor molecule is very low, may be explained as due in part to departure from Michaelis–Menten conditions and in part to the enzyme hydrolytic activity. Thus, there is good reason to believe that the non-protonated form of the α-amino group of the acceptor molecule is the active form in the transfer catalyzed reaction, and this fact could explain the high pH optimum of γ-glutamyl transpeptidase and the absence of transfer below pH 7.0. An alternative possibility is that the high pH affects some group or groups in the enzyme molecule, but subsequent data show that the effects of pH on the substrate rather than on the enzyme influence enzymatic activity.

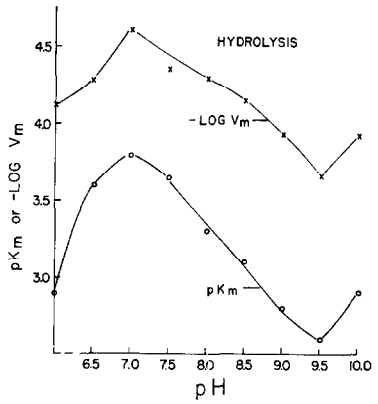
Effect of pH on K_m and v_{max} of transfer and hydrolytic activities

The kinetic constants, K_m and v_{max}, were measured over a range of pH values for both the hydrolytic and transfer reactions and these results are shown in Figs. 5 and 6. The inflection points shown by these curves imply that the pK_1 and pK_2 values for hydrolytic activity are 7.0 and 9.5 respectively, and that the pK_1 and pK_2 values for transfer activity are 7.8–8.2 and 9.6–9.7 respectively. The pK values of 7.0 and 7.8–8.2 suggest that an imidazole ring and an α-amino group, respectively[21], are involved in the catalytic process. However, in the case of pK 9.5–9.7, there are several possible ionizing groups which may be responsible for this pK value, and these are the thiol of cysteine, the ε-amino group of lysine and the phenolic group of tyrosine. Previous studies[5] showed that no sulfhydryl groups are involved in the catalytic process. In addition, the fact that the enzymic activity is more affected by chymotrypsin than by trypsin (see Table IV) suggests that a phenolic group of tyrosine rather than an ε-amino group of lysine is involved. Further, the nature of the dissociation of these groups around pH 10, and the type of the catalysis involved makes it more likely that a phenolic group is responsible for the inflection point of the curve at pH 9.5 than an amino group.

Enzymic mechanism

On the basis of the data presented, conclusions concerning the groups involved in the enzymic catalysis of the hydrolytic and the transfer reactions may be summarized as follows: An imidazole and a phenolic group are involved in the hydrolytic catalyzed reaction. Following DIXON's analysis[22] of the effect of pH on enzymic catalysis, the curves in Figs. 5 and 6 suggest that the two groups are present in both the free enzyme and in the enzyme–substrate complex. (Rule 10 of DIXON[22].)

In the case of the transfer reactions, the two groups involved in the catalytic process are an α-amino group and a phenolic group. As judged from the curves presented in Fig. 5, both groups belong the to enzyme–substrate complex. The conclusion was already drawn that the phenolic group actually is present in the enzyme molecule. Since in the transfer reaction, the acceptor amino acid is part of the enzyme–substrate complex, the α-amino group involved in the transfer reaction (and absent from the hydrolytic reaction) belongs to the substrate and not to the enzyme molecule. This is consistent with the suggestion previously made that the high pH must affect the



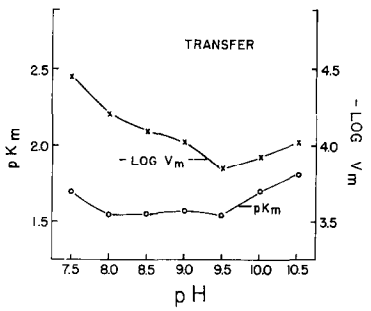


Fig. 5. The effect of pH on the maximum velocity and the Michaelis constant of the hydrolytic activity of the kidney bean enzyme. v_{max} is expressed as moles/h of glutamate produced under the standard assay conditions, described in METHODS, and K_m as moles. The graphical method of LINEWEAVER–BURK[20] was used for the calculation of v_{max} and K_m.

Fig. 6. The effect of pH on the maximum velocity and the Michaelis constant of the transfer activity of the kidney bean enzyme. Conditions are the same as for Fig. 5.

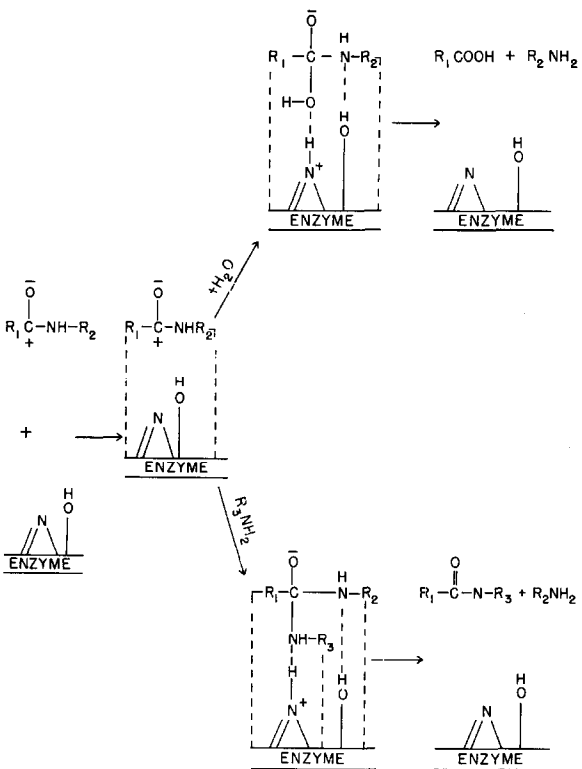


Fig. 7. Proposed mechanism for the hydrolysis and transfer mediated by bean transpeptidase.

protonation state of the α-amino group of the substrate (the acceptor) and not some functional group in the enzyme molecule.

Thus, assuming that the groups mentioned above are involved in the catalytic process, it would appear to be consistent with the data to suggest the following scheme as a possible mechanism for the hydrolytic and the transfer reactions (Fig. 7). As shown in this scheme, the same mechanism is proposed for both the hydrolytic and the transfer processes. In the sequence of reactions proposed for hydrolysis, it is postulated that the activated complex (Fig. 7) is produced as a result of a nucleophilic attack by the basic nitrogen of the imidazole ring on the carbonyl carbon atom of the γ-glutamyl peptide linkage through a molecule of water, concomitant with an electrophilic attack by the acidic hydrogen of the phenolic group on the nitrogen of the peptide bond.

In the transfer catalyzed reaction, the same sequence and type of reactions are proposed, with the exception that the acceptor amino acid is substituted for water (Fig. 7). It was emphasized above that only the uncharged form of the α-amino group takes part in the transfer reaction. The mechanism proposed is consistent with this behavior. Further, it is suggested that only one enzyme is responsible for both reactions as indicated earlier. Comparison of the two reactions indicates that under conditions where the transfer reaction takes place, the hydrolytic process should be inhibited, and this is what happens (Fig. 3).

It should be emphasized that the mechanism proposed in Fig. 7 must be considered speculative until more direct evidence for the involvement of histidine and tyrosine in the active site is available. However, the mechanism is consistent with present information and provides a reasonable working hypothesis for further investigation.

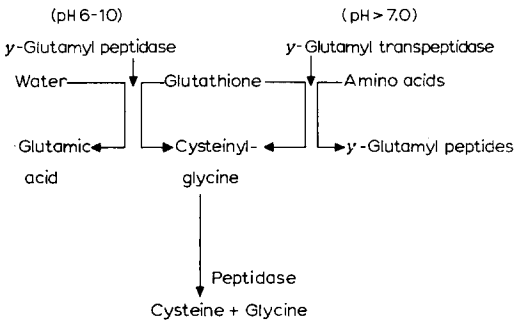
DISCUSSION

There is no indication that the highly purified preparation of bean transpeptidase differs in its catalytic properties from the crude extract or the ammonium sulfate fraction[5]. This was apparently not the case with renal glutathionase in which purification brought about the separation of the transfer from the hydrolytic activity[17], although there are differences of opinion[14]. On the basis of evidence derived indirectly from kinetic data, failure to separate the two activities by purification should be anticipated.

The finding that the enzyme preparation from beans exhibits little transfer activity at physiological pH's, while still retaining its hydrolytic activity, indicates that the primary function of the bean enzyme is hydrolysis, as suggested for the animal system[19]. Since this hypothesis implies that the breakdown of glutathione in plants proceeds *via* the same sequence of reactions suggested for its breakdown in animal tissues[23–26], investigations along this line should reveal cysteinylglycine dipeptidase activity in plant tissues, but tests of such activity have not been reported.

As already stated, these conclusions are consistent with a previous suggestion[5] that the plant transpeptidase may account for the biosynthesis of a number of γ-glutamyl dipeptides. According to the hypothesis presented in this work, these dipeptides arise as byproducts of the first reaction in the sequence leading to the complete degradation of glutathione. This is illustrated by the following scheme, which is

similar to that proposed by HIRD AND SPRINGELL[19] for the breakdown of glutathione in animal tissues.



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REFERENCES

1 L. R. HAC, E. E. SNELL AND R. J. WILLIAMS, *J. Biol. Chem.*, 159 (1945) 273.
2 E. S. HANES, F. J. R. HIRD AND E. A. ISHERWOOD, *Nature*, 166 (1950) 288.
3 E. S. HANES, F. J. R. HIRD AND E. A. ISHERWOOD, *Biochem. J.*, 51 (1952) 25.
4 P. S. TALALAY, *Nature*, 147 (1954) 516.
5 J. F. THOMPSON, D. H. TURNER AND R. K. GERING, *Phytochemistry*, 3 (1963) 33.
6 H. SACHS AND E. BRAND, *J. Am. Chem. Soc.*, 75 (1953) 4608.
7 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
8 O. WARBURG AND W. CHRISTIAN, *Biochem. Z.*, 310 (1942) 384.
9 J. A. GOLDBARG, O. M. FRIEDMAN, E. P. PINEDA, E. E. SMITH, R. CHATTERJI, E. H. STEIN AND A. M. RUTENBURG, *Arch. Biochem. Biophys.*, 91 (1960) 61.
10 N. O. KAPLAN, M. M. CIOTTI AND F. E. STOLZENBACH, *J. Biol. Chem.*, 221 (1956) 833.
11 B. LEVENBERG, *J. Biol. Chem.*, 239 (1964) 2267.
12 M. ORLOWSKI AND A. MEISTER, *Biochim. Biophys. Acta*, 73 (1963) 679.
13 O. LEVIN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. V, Academic Press, New York, 1962, p. 27.
14 M. ORLOWSKI AND A. MEISTER, *J. Biol. Chem.*, 240 (1965) 338.
15 J. R. WHITAKER, *Anal. Chem.*, 35 (1963) 1950.
16 H. K. SCHACHMAN, *Ultracentrifugation in Biochemistry*, Academic Press, New York, 1959, p. 236.
17 F. BINKLEY, *J. Biol. Chem.*, 236 (1961) 1075.
18 E. G. BALL, J. P. REVEL AND O. COOPER, *J. Biol. Chem.*, 221 (1956) 895.
19 F. J. R. HIRD AND P. H. SPRINGELL, *Biochem. J.*, 56 (1954) 417.
20 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
21 E. J. COHN AND J. T. EDSALL, *Proteins, Amino Acids and Peptides*, Reinhold, New York, 1943, p. 445.
22 M. DIXON, *Biochem. J.*, 55 (1953) 161.
23 E. F. SCHROEDER AND G. E. WOODWARD, *J. Biol. Chem.*, 120 (1937) 209.
24 G. E. WOODWARD AND F. E. REINHART, *J. Biol. Chem.*, 145 (1942) 471.
25 F. BINKLEY AND K. NAKAMURA, *J. Biol. Chem.*, 186 (1950) 731.
26 F. BINKLEY AND C. K. OLSON, *J. Biol. Chem.*, 188 (1951) 451.