γ -Glutamyl Transfer Reactions in Bacteria

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SUMMARY

γ -Glutamyl transfer activity was found to be widely distributed in different bacterial species. The γ -glutamyl transfer from glutathione to water and acceptors other than water was studied with cell-free preparations of *Proteus morganii*. In the absence of added acceptor, the γ -glutamyl residue was predominantly transferred to water; however, some transfer to the substrate, resulting in the formation of γ -glutamylglutathione, was detected. In the presence of acceptors (amino acids or peptides) all the γ -glutamyl residue was transferred to the added acceptor. The different reaction products were isolated and identified. Kinetics and properties of the γ -glutamyl transfer reaction were studied.

INTRODUCTION

γ -Glutamyl transfer from glutathione (GSH) to amino acids, peptides or water has been studied with enzymes of animal origin, mainly kidney (Hanes, Hird & Isherwood, 1950, 1952; Kinoshita & Ball, 1953; Fodor, Miller & Waelsch, 1953; Hird & Springell, 1954*a, b*; Ball, Revel & Cooper, 1956; Revel & Ball, 1959; Avi Dor, 1960; Binkley, 1961). Data on the γ -glutamyl transfer in bacteria are rather scarce. Only qualitative data on transfer of the γ -glutamyl moiety by washed suspensions and cell-free extracts of *Proteus vulgaris* have been reported (Samuels Talalay, 1954). The occurrence of γ -glutamyl transfer from glutathione in washed suspensions of different bacterial species was studied in order to gain information about the distribution and the activity of the enzyme. Since *P. morganii* showed pronounced transfer activity, cell-free extracts of this micro-organism were used extensively.

METHODS

Materials. Glutathione and all amino acids and peptides used were commercial preparations, most of them obtained from Nutritional Biochemicals Corp., Cleveland, U.S.A.

Preparation of washed bacterial suspensions. Bacteria were grown for 18 hr at 37° in Roux bottles containing nutrient agar. The bacteria were collected and washed 3 times with distilled water. Their optical density at 550 m μ was determined with a Coleman Junior Spectrophotometer. Optical density readings were converted to dry weight, by using a standard curve prepared for *Escherichia coli* B.

Determination of γ -glutamyl transfer with washed suspensions. The reaction mixture, 1.0 ml. total volume, contained: GSH, 10 μ mole; phosphate buffer (pH 7.5), 60 μ mole; and suspensions of washed bacteria equiv. to 2–4 mg. dry weight. Acceptor,

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when added, was 20 μ mole. The reaction was run at 37° for 45 min. in a gas phase containing more than 99 % (v/v) nitrogen. The acceptors used were glycylglycine and L-methionine.

Preparation of cell-free extracts. *Proteus morganii* was grown in nutrient broth with aeration for 18 hr at 37°. The organisms were collected in a Sharples Centrifuge, washed 3 times with distilled water and disintegrated for 10 min. in a 10 KC Raytheon Sonic Oscillator. The unbroken organisms and cell debris were removed at 40,000 rev./min. in a Spinco Model L Ultracentrifuge and the supernatant thus obtained served as the enzyme source. The enzyme was dialysed overnight, in the cold, against distilled water. Storage at -20° preserved the enzymic activity for several months. Protein was estimated by the biuret method according to Mehl (1945).

Determination of γ -glutamyl transfer with cell-free extracts. Unless otherwise stated, the reaction mixture, 1.0 ml. total volume, contained: GSH, 20 μ mole; tris buffer (pH 8.5), 400 μ mole; and 1.6 mg. protein. Acceptor, when added, 80 μ mole of the L form or 160 μ mole of the racemic compound. The reaction was run at 37° for 45 min. in a gas phase containing more than 99 % (v/v) N₂.

Estimation of glutathione. The reaction was stopped and proteins were precipitated by diluting the reaction mixture with 20 vol. of 5 % (w/v) metaphosphoric acid. The glutathione content of the filtrate was determined by the alloxan method of Patterson & Lazarow (1955). A glutathione standard curve and appropriate blanks were run concurrently.

Quantitative paper chromatography. The reaction mixture was treated with 2 vol. of hot ethanol containing 10 mM-N-ethylmaleimide. The protein precipitate was filtered off and 0.01 or 0.02 ml. samples were applied to Whatman 3 MM paper (for chromatography). The solvents used were propanol + water (80 + 20 by vol.), or tertiary butanol + formic acid + water (70 + 15 + 15 by vol.). Known amounts of the different markers were run simultaneously. The chromatograms (descending) were run for 16–18 hr at room temperature, developed with buffered ninhydrin (Haschen, 1956), sprayed with ethanolic Cu(NO₃)₂ reagent (Kawerau & Wieland, 1951) for colour stabilization and eluted with methanol (Hanson, Blech, Hermann & Kleine, 1959).

Methods for the isolation and identification of peptides. The reaction mixture, containing the peptide to be tested, was applied in a straight line 10 cm. from the bottom of the paper (Whatman 3 MM); usually 1 ml. reaction mixture was applied to each sheet (46 × 57 cm.). The chromatograms were run for 16–18 hr. The position of the peptides was determined by spraying small strips on both sides of the chromatogram. The areas containing the peptides were cut out and the compounds were concentrated and eluted according to Reith (1957). After two repeated isolation procedures, using propanol + water in the first and tertiary butanol + formic acid + water in the second, the peptide solution in 6 N-HCl was heated in a sealed ampoule for 10 hr at 105°. The hydrolysate was dried over Ca₃(PO₄)₂ and the products determined by paper chromatography. The actual values were obtained by converting the optical density readings (at 505 m μ) into μ mole according to appropriate standards. Similarly treated filter paper was used as a blank.

Dinitrophenyl (DNP) derivatives of the isolated peptides were prepared according to Rosenthal & Tabor (1956). The purity of each derivative was tested by paper

chromatography. Hydrolysis and determination of the products was carried out as above.

Pyrrrolidone carboxylic acid was tested for by the chlorine starch iodine method (Rydon & Smith, 1952) according to Ellfolk & Synge (1955).

RESULTS

Distribution of γ-glutamyl transfer activity in washed suspensions of various bacterial species

The search for *γ*-glutamyl transfer in the different bacteria used (Table 1) was done under conditions which have been found to be optimal for the transfer in washed suspensions of *Proteus vulgaris*. The acceptors used were L-methionine or glycylglycine and the disappearance of glutathione was measured by the alloxan method. Out of 16 different bacterial species tested 13 were found capable of hydrolysing glutathione, while the remaining three (*Escherichia coli*, *E. freundii* and *Staphylococcus aureus*) showed weak activity. The most active organisms were *Achromobacter delicatulum*, *P. morganii* and *P. vulgaris*. Addition of either of the acceptors increased the cleavage of the tripeptide by most of the bacterial species tested. *Proteus morganii*, because of its high activity, was chosen for a more extensive examination.

Table 1. *Distribution of γ-glutamyl transfer activity in washed suspensions of various bacterial species*

The different reaction mixtures, total volume 1.0 ml., contained: GSH, 10 μmole, or GSH, 10 μmole, and acceptor, 20 μmole; phosphate buffer (pH 7.5), 66 μmole; and washed bacterial suspensions 2–4 mg. dry weight. Incubation: 45 min. at 37°, under nitrogen. GSH cleavage was determined by the alloxan method.

Organism	Glutathione cleaved (μmole/mg. dry weight)		
	Without acceptor	Acceptor glycylglycine	Acceptor L-methionine
<i>Aerobacter aerogenes</i>	0.25	0.24	0.32
<i>A. aerogenes</i> 8303	0.13	0.11	0.17
<i>Achromobacter delicatulum</i>	3.20	3.70	3.20
<i>Alcaligenes bookeri</i>	0.11	0.40	0.40
<i>A. faecalis</i>	0.10	0.60	0.45
<i>Bacillus mycoides</i>	0.32	0.25	0.25
<i>B. subtilis</i>	0.72	0.76	0.92
<i>Escherichia coli</i> B	0.02	0	0.07
<i>E. freundii</i>	0.07	0.05	0.02
<i>Klebsiella ozaenae</i>	0.38	0.37	0.47
<i>Proteus vulgaris</i> (HX19)	1.10	1.42	1.42
<i>P. morganii</i>	1.78	2.48	2.48
<i>Pseudomonas</i> sp.	0.45	0.40	0.52
<i>P. fluorescens</i>	0.40	0.40	0.30
<i>P. non liquefaciens</i>	0.40	0.40	0.32
<i>Staphylococcus aureus</i>	0.02	0.15	0.17

Quantitative determination of reactants and products in γ-glutamyl transfer reactions

Quantitative data on the cleavage of glutathione and on the appearance of the breakdown products, as measured by the alloxan method and quantitative paper

chromatography, are given in Table 2. In the absence of added acceptor most of the glutathione was split into glutamic acid (Glu) and cysteinylglycine (CySH-Gly), according to the reaction

A new product (R_f value lower than glutathione) was detected by paper chromatography; acid hydrolysis of this compound yielded glutamic acid, cysteic acid and glycine at a ratio of 2:1:1. This product was identified as glutamylglutathione, probably γ -glutamylglutathione. The formation of this tetrapeptide may be through a transfer reaction in which glutathione served both as donor and acceptor of the γ -glutamyl moiety

The alloxan method does not distinguish between glutathione and γ -glutamylglutathione (Revel & Ball, 1959). Therefore the discrepancy in the amounts of glutathione cleaved as measured by the two methods (Table 2) can be explained. Glutamic acid, which is formed only in reaction I, can be taken as an index of the amount of γ -glutamyl transfer to water. The chromatographic method of analysis showed that 4 μ mole of GSH disappeared; of this 2.7 μ mole could be accounted for by hydrolytic cleavage (reaction I), while the rest (1.3 μ mole) was used to form 0.65 μ mole of γ -glutamylglutathione (reaction II). Lack of a γ -glutamylglutathione marker prevented the quantitative determination of this product. However, the amount of cysteinylglycine formed (3.3 μ mole) verified the accuracy of the calculation, according to which 3.35 μ mole cysteinylglycine (2.7 + 0.65) should have been formed. The γ -glutamyl transfer to glutathione (reaction II) amounted, therefore, to 19.4% of the glutathione cleaved.

Table 2. *Glutathione breakdown in the presence and absence of acceptor*

The reaction mixtures, total volume 1.0 ml., contained: GSH, 20 μ mole, or GSH, 20 μ mole, and acceptor, 80 μ mole; tris buffer (pH 8.5), 400 μ mole; and 1.6 mg. *Proteus morganii* extract. Incubation under nitrogen for 45 min. at 37°. The disappearance of GSH was measured by the alloxan method and quantitative paper chromatography. Glutamic acid, cysteinylglycine and peptide formation were measured by quantitative paper chromatography. Data obtained by chromatography are the average of two runs with two different solvents, propanol + water and tertiary butanol + formic acid + water.

Reaction mixture	GSH cleavage		Reaction products		
	Alloxan method (μ mole)	Chromatography (μ mole)	Glutamic acid (μ mole)	Cysteinylglycine (μ mole)	γ -glutamyl-peptide (o.d. $\times$ 100)
1. GSH	2.4	4.0	2.7	3.3	4.8
2. GSH + L-methionine	7.6	7.3	0	7.0	22.0
3. GSH + glycylglycine	9.9	10.5	0	10.0	20.0

In the presence of acceptors (glycylglycine or L-methionine) both methods gave identical results. Neither glutamic acid nor γ -glutamylglutathione was detected; cysteinylglycine in equivalent amount to the glutathione cleaved and a new compound were isolated. Acid hydrolysis of the new compound, formed in the presence of L-methionine, yielded equivalent amounts of glutamic acid and methionine. Hydrolysis of the DNP-derivative of this compound yielded DNP-glutamic acid and methionine. The peptide formed in the presence of glycylglycine yielded, upon

hydrolysis, glutamic acid and glycine in a ratio of 1 : 1.6. This ratio was apparently due to the presence of a dipeptidase acting on glycylglycine; glycylglycine together with glycine served as acceptors for the γ -glutamyl moiety. Hydrolysis of the DNP-derivative of this compound gave DNP-glutamic acid and glycine. The only reaction that took place in the presence of either of the acceptors tested was

At pH 8.5 (at which the reactions were tested), neither cysteinylglycinase nor γ -glutamylactamase activities could be detected. The former enzyme was present in the extract but showed no activity at pH 8.5.

Kinetics of γ -glutamyl transfer

The kinetics of the γ -glutamyl transfer from glutathione to amino acids, peptides and water were studied in cell-free extracts of *Proteus morganii*.

Enzyme concentration. The enzymic breakdown of glutathione in the presence or absence of acceptor was found to be proportional to the enzyme concentration (Fig. 1). At high enzyme concentrations, glutathione became limiting and the effect of the acceptor in increasing γ -glutamyl transfer was decreased. Experiments were therefore done at enzyme concentrations that cleaved 25–30% of the substrate in the absence of acceptor.

Substrate concentration. The effect of substrate concentration on enzyme activity is depicted in Fig. 2. A constant ratio of glutathione to acceptor was kept, while varying the substrate concentration.

Effect of time. The effect of time on enzyme activity in the presence of glycylglycine is shown in Fig. 3. It was measured by three different methods: disappearance of glutathione (alloxan method), formation of cysteinylglycine and synthesis of γ -glutamyl-peptide (quantitative paper chromatography). Similar progress curves were obtained with each method.

Reduced and oxidized glutathione as γ -glutamyl donors. Since both donors yielded peptides of identical chromatographic properties, the efficiency of these donors was measured by comparing the amounts of peptide formed. Oxidized glutathione (in equivalent amounts) was only 50% as active as reduced glutathione (Table 3). The higher efficiency of GSH was also found by Fodor *et al.* (1953), with a purified kidney extract.

Effect of pH value. The optimal pH value for γ -glutamyl transfer was 8.3–8.5 (Fig. 4). The pH activity curves resembled those obtained by Ball *et al.* (1956) for a kidney preparation but with some differences on the acid side of the optimal pH which will be discussed.

Effect of acceptor concentration. Both of the acceptors used increased the γ -glutamyl transfer; glycylglycine was found to be more effective than L-methionine (Fig. 5). High acceptor concentrations decreased the amount of glutathione cleaved. The inhibitory effect of high acceptor concentrations has been described in other reports (Fodor *et al.* 1953; Hird & Springell, 1954*a*; Revel & Ball, 1959). With the bacterial extract, the inhibition caused by the acceptor was competitive and could be annulled by increasing the concentration of the substrate (Table 4).

Acceptor efficiency. Data on the comparative efficiency of different amino acids and peptides, as acceptors in the γ -glutamyl transfer reaction, are given in Table 5.

Only L-amino acids were tested, since D-amino acids showed no activity. The effect of the acceptors on the γ -glutamyl transfer was assayed at two different concentrations at which neither glycylglycine nor L-methionine was inhibitory. All amino acids and peptides tested served as acceptors for the γ -glutamyl moiety; the exceptions were proline and hydroxyproline, which lack a free NH_2 group.

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Effect of enzyme concentration on γ -glutamyl transfer. ○ GSH, 20 μmole ; ▲, GSH 20 μmole + L-methionine 80 μmole ; ●, GSH 20 μmole + glycylglycine 80 μmole . The different reaction mixtures, total volume 1.0 ml., contained, in addition to the compounds listed, tris buffer (pH 8.5) 400 μmole and *Proteus morganii* extract as indicated in the figure. Incubation under nitrogen for 45 min. at 37°. GSH cleavage was determined by the alloxan method.

Fig. 2. Effect of substrate concentration on γ -glutamyl transfer. The reaction mixtures, total volume 1.0 ml., containing GSH in various concentrations (○—○) or various concentrations of GSH + glycylglycine (●—●) were incubated for 45 min. at 37° under nitrogen. The reaction mixtures also contained tris buffer (pH 8.5), 400 μmole , and *Proteus morganii* extract 1.6 mg. protein. GSH cleavage was determined by the alloxan method.

Fig. 3. Effect of time on γ -glutamyl transfer. The experiment was performed in Thunberg tubes under nitrogen. The reaction mixtures, total volume 1.0 ml., contained GSH, 20 μmole ; glycylglycine, 80 μmole ; tris buffer (pH 8.5), 400 μmole ; and *Proteus morganii* extract, 1.6 mg. protein. Incubation at 37°. At intervals tubes were removed and the amount of products determined. GSH cleavage (○—○) (alloxan method); cysteinylglycine formation (●—●) and γ -glutamyl peptide formation (▲—▲) (quantitative paper chromatography). Data obtained by paper chromatography are the average of two runnings using two different solvents (propanol + water and tertiary butanol + formic acid + water). Owing to the lack of γ -glutamyl peptide markers, quantitative data on peptide formation are represented as the optical density of the ninhydrin complex of the peptides at 505 $\text{m}\mu$.

The effect of acceptor concentration on the transfer reaction is also seen in Table 5. The inhibition by the acceptor has been shown to be competitive (see Table 4). It is worthy of note, therefore that the strongest inhibition of the transfer reaction was caused by glutamine and cysteine, which resemble closely or constitute parts of the glutathione molecule.

DISCUSSION

The search for γ -glutamyl transfer activity revealed a wide distribution of the enzyme in bacteria. In mammals this activity is restricted to kidney and pancreas (Woodward, Munro & Schroeder, 1935; Binkley & Nakamura, 1948; Revel & Ball

1959). In most of the bacterial strains tested here, addition of an acceptor increased the γ -glutamyl transfer. However, in a few organisms addition of acceptor was without effect as was also found with fish kidney extract (Ball, Cooper & Clarke, 1953). Since our experiments were performed with washed organisms, the lack of acceptor effect might be attributed to permeability barriers.

The cleavage of glutathione in the absence of acceptor (at alkaline pH values) yielded less glutamic acid than could be accounted for by the amounts of glutathione split or cysteinylglycine formed. Similar results have been described with

Fig. 4

Fig. 4. Effect of pH on γ -glutamyl transfer. (A) GSH 20 μ mole; (B) GSH 20 μ mole + L-methionine 80 μ mole; (C) GSH 20 μ mole + glycylglycine 80 μ mole. The different reaction mixtures, total volume 1.0 ml., contained, in addition to the compounds listed, *Proteus morganii* extract—1.6 mg. protein—and phosphate buffer 750 μ mole (O); tris buffer 400 μ mole ($\square$); or borate buffer 300 μ mole ($\triangle$). Incubation under nitrogen at 37° for 45 min. GSH cleavage was determined by the alloxan method. All pH values were determined at the end of incubation period with a glass electrode.

Fig. 5

Fig. 5. Effect of acceptor concentration on γ -glutamyl transfer. GSH 20 μ mole + varying amounts of L-methionine (O—O) or glycylglycine (●—●) were incubated under nitrogen for 45 min. at 37°. The different mixtures, total volume 1.0 ml., contained, in addition to the compounds listed, tris buffer (pH 8.5) 400 μ mole and *Proteus morganii* extract, 1.6 mg. protein. GSH cleavage was determined by the alloxan method.

Table 3. Comparative efficiency of GSH and GSSG as γ -glutamyl donors

The reaction mixtures, total volume 1.0 ml., contained, in addition to the substrates listed, *Proteus morganii* extract—1.6 mg. protein—and tris buffer (pH 8.5) 400 μ mole. Incubation under nitrogen for 45 min. at 37°. Peptide formation was measured by paper chromatography, using propanol water as solvent. The data were expressed as the optical density $\times 100$ of the eluted ninhydrin-peptide complex.

Reaction mixtures	Peptide formation O.D. $\times 100$ (at 505 m μ)
20 μ mole GSH + 80 μ mole L-methionine	11.0
10 μ mole GSSG + 80 μ mole L-methionine	5.0
20 μ mole GSH + 80 μ mole glycylglycine	10.0
10 μ mole GSSG + 80 μ mole glycylglycine	5.5

Table 4. *Effect of ratio of acceptor: GSH on γ -glutamyl transfer*

The different reaction mixtures, total volume 1.0 ml., contained in addition to the listed compounds tris buffer (pH 8.5), 400 μ mole, and *Proteus morganii* extract, 1.6 mg. protein. Incubation for 45 min. at 37° under nitrogen. GSH cleavage was determined by the alloxan method. In the last column GSH cleavage is expressed as a percentage of the amount of GSH cleaved in the absence of added acceptor.

Reaction mixture			Acceptor: GSH	GSH cleaved	
GSH (μ mole)	Glycylglycine (μ mole)	L-methionine (μ mole)		μ mole	%
20	—	—	—	5.3	100
20	240	—	12	5.2	98
20	—	240	12	5.1	96
20	120	—	6	15.4	290
20	—	120	6	10.8	205
40	—	—	—	5.4	100
40	240	—	6	16.5	306
40	—	240	6	11.5	214

Table 5. *Efficiency of various amino acids and peptides in promoting γ -glutamyl transfer*

The different reaction mixtures, total volume 1.0 ml., contained GSH, 20 μ mole; acceptor as indicated in the table; tris buffer (pH 8.5), 400 μ mole; and *Proteus morganii* extract, 1.6 mg. protein. Incubation 45 min. at 37° under nitrogen. The data, which were obtained by the alloxan method, are expressed as the percentage of γ -glutamyl transfer as effected by the concentration of the corresponding acceptor.

Acceptor added	γ -glutamyl transfer			Amino pK*
	40 μ mole	80 μ mole	160 μ mole	
None	100	100	—	—
L-Alanine	116	106	—	9.87
L-Arginine	103	127	—	8.99
L-Asparagine	125	151	—	8.87
L-Cysteine	150	65	—	8.36
L-Glutamic acid	163	124	—	9.96
L-Glutamine	165	95	—	9.13
Glycine	116	151	—	9.78
L-Histidine	194	186	—	9.17
L-Hydroxyproline	101	101	—	9.62
L-Leucine	116	111	—	9.74
L-Lysine	141	117	—	9.18
L-Methionine	182	196	—	9.21
L-Phenylalanine	230	240	—	9.13
L-Proline	90	85	—	10.64
L-Serine	169	120	—	9.21
L-Threonine	128	142	—	9.10
L-Tyrosine	128	—	—	9.11
L-Valine	169	125	—	9.72
DL-Alanyl-DL-alanine	—	200	204	8.42
DL-Alanylglycine	—	207	204	8.18
Glycylglycine	213	222	—	8.25
Glycyl-L-methionine	213	200	—	—
Glycylglycylglycine	157	164	—	7.91

* Amino pK values from *Biochemist's Handbook* (C. Long, editor), 1961, pages 43–52, E. & F. N. & Son Ltd. London.

kidney extracts (Woodward & Reinhart, 1942; Fodor *et al.*, 1953; Revel & Ball, 1959). In some of these cases the lack of stoichiometry was accounted for by pyrrolidone carboxylic acid formation (Woodward & Reinhart, 1942; Fodor *et al.*, 1953), probably through γ -glutamyl lactamase activity (Connel & Hanes, 1956), but no pyrrolidone carboxylic acid formation was detected with the *Proteus morganii* extract. On the other hand, glutathione served both as acceptor and as donor of the γ -glutamyl residue. Transfer of the γ -glutamyl residue to glutathione has not been reported previously. The possibility of such a reaction was discussed by Revel & Ball (1959), who did not, however, obtain any evidence for such a reaction product. In the presence of acceptor the transfer reaction yielded equivalent amounts of γ -glutamyl-acceptor and cysteinylglycine. These results are in agreement with those obtained with kidney extracts (Revel & Ball, 1959).

The kinetic data obtained with the *Proteus morganii* extract are similar to those obtained with kidney extracts (Fodor *et al.*, 1953; Hird & Springell, 1954*a*; Ball *et al.*, 1956; Revel & Ball, 1959). However, some differences were encountered regarding the correlation between acceptor ability and pK value. It is assumed that the acceptor acts in its unprotonated form (Johnston, Mycek & Fruton, 1950; Fruton, Johnston & Fried, 1951; Revel & Ball, 1959; Avi Dor, 1960). If this is correct, acceptor activity should be inversely proportional to the pK value of the amino group. Comparison of acceptor activity and pK value (Table 5) shows that this was generally the case with the bacterial extract. However, there were a few exceptions to this rule; e.g. glycylglycylglycine, with a pK value lowest of all acceptors tested (7.91), showed poor acceptor ability. It seems, therefore, that factors other than the pK value may influence the suitability of the acceptor. Similar findings were reported also with kidney extracts (Fodor *et al.*, 1953). On the other hand, there were some differences between the *Proteus morganii* enzyme and the kidney extract in regard to the efficiency of certain acceptors (Fodor *et al.*, 1953; Ball *et al.*, 1956). Thus, glutamine (pK 9.18), which was one of the best acceptors for the kidney extract, showed moderate acceptor activity similar to that of glutamic acid (pK 9.96). Dipeptides, although exhibiting better acceptor capacity than amino acids in the bacterial system, were considerably less active there than in the kidney system. And, most important, lower pH values should have increased the acceptor effectivity of dipeptides as compared with amino acids. However, no such increase in the relative activities at acid pH values was encountered, when acceptor abilities of glycylglycine and of L-methionine were compared using the bacterial extract (Fig. 4).

The above-mentioned discrepancies can be explained by the presence of glutaminase and peptidase activities in the bacterial extract. The rapid deamination of glutamine resulted in the formation of a less active acceptor—glutamic acid. The partial degradation of peptides, by peptidases present in the crude extract, decreased the apparent acceptor ability of the dipeptide.

REFERENCES

- AVI DOR, Y. (1960). A study on the effect of particle-bound γ -glutamyl transpeptidase on the product of interaction of fluoropyruvate with glutathione. *Biochem. J.* **76**, 370.
BALL, E. G., COOPER, O. & CLARKE, E. C. (1953). On the hydrolysis and transpeptidation of glutathione in marine forms. *Biol. Bull. mar. biol. Lab., Woods Hole*, **105**, 370.

- BALL, E. G., REVEL, J. P. & COOPER, O. (1956). The quantitative measurement of γ -glutamyl transpeptidase activity. *J. biol. Chem.* **221**, 895.
- BINKLEY, F. (1961). Purification and properties of renal glutathionase. *J. biol. Chem.* **236**, 1075.
- BINKLEY, F. & NAKAMURA, K. (1948). Metabolism of glutathione. I. Hydrolysis by tissue of the rat. *J. biol. Chem.* **173**, 411.
- CONNEL, G. E. & HANES, C. S. (1956). Enzymic formation of pyrrolidone carboxylic acid from γ -glutamyl peptides. *Nature, Lond.* **177**, 377.
- ELLFOLK, N. & SYNGE, R. L. M. (1955). Detection of pyrrolidone carboxylic acid. *Biochem. J.* **59**, 523.
- FODOR, P. J., MILLER, A. & WAELSCH, H. (1953). Quantitative aspects of enzymatic cleavage of glutathione. *J. biol. Chem.* **202**, 551.
- FRUTON, J. S., JOHNSTON, R. B. & FRIED, M. (1951). Elongation of peptide chains in enzyme catalyzed transamidation reactions. *J. biol. Chem.* **190**, 39.
- HANES, C. S., HIRD, F. J. R. & ISHERWOOD, F. A. (1950). Synthesis of peptides in enzymic reactions involving glutathione. *Nature, Lond.* **166**, 288.
- HANES, C. S., HIRD, F. J. R. & ISHERWOOD, F. A. (1952). Enzymic transpeptidation reactions involving γ -glutamyl peptides and α -amino-acyl peptides. *Biochem. J.* **51**, 25.
- HANSON, H., BLECH, W., HERMANN, P. & KLEINE, R. (1959). Vorkommen und quantitative Erfassbarkeit proteolytischer Enzymaktivitäten in Zellbestandteilen von Leber und Niere der Ratte. *Hoppe-Seyler's Z. physiol. Chem.* **315**, 181.
- HASCHEN, R. J. (1956). Aktivitätsbestimmung von Peptidasen durch quantitative Papierchromatographie. *Clinica chim. Acta*, **1**, 242.
- HIRD, F. J. R. & SPRINGELL, P. H. (1954*a*). The enzymic reactions of amino acids with glutathione. *Biochem. J.* **56**, 417.
- HIRD, F. J. R. & SPRINGELL, P. H. (1954*b*). The enzymic hydrolysis of the γ -glutamyl bond in glutathione. *Biochim. biophys. Acta*, **15**, 31.
- JOHNSTON, R. B., MYCEK, M. J. & FRUTON, J. S. (1950). Catalysis of transpeptidation reactions by chymotrypsin. *J. biol. Chem.* **187**, 205.
- KAWERAU, E. & WIELAND, T. (1951). Conservation of amino acid chromatograms. *Nature, Lond.* **168**, 77.
- KINOSHITA, J. H. & BALL, E. G. (1953). A transpeptidation reaction between glutathione and arginine. *J. biol. Chem.* **200**, 609.
- MEHL, J. W. (1945) The biuret reaction of proteins in the presence of ethylene glycol. *J. biol. Chem.* **157**, 173.
- PATTERSON, J. W. & LAZAROW, A. (1955). Determination of glutathione. *Meth. biochem. Analysis*, **2**, 273.
- REITH, W. S. (1957). A device for concentrating and eluting the spots from chromatograms. *Nature, Lond.* **179**, 580.
- REVEL, J. P. & BALL, E. G. (1959). The reaction of glutathione with amino acids and related compounds as catalyzed by γ -glutamyl transpeptidase. *J. biol. Chem.* **234**, 577.
- ROSENTHAL, S. M. & TABOR, C. W. (1956). The pharmacology of spermine and spermidine distribution and excretion. *J. Pharmac. exp. Ther.* **116**, 131.
- RYDON, H. N. & SMITH, P. W. G. (1952). A new method for the detection of peptides and similar compounds on paper chromatograms. *Nature, Lond.* **169**, 922.
- SAMUELS TALALAY, P. (1954). Glutathione breakdown and transpeptidation reactions in *Proteus vulgaris*. *Nature, Lond.* **174**, 516.
- WOODWARD, G. E. & REINHART, F. E. (1942). The effect of pH on the formation of pyrrolidone carboxylic acid and glutamic acid during enzymatic hydrolysis of glutathione by rat kidney extract. *J. biol. Chem.* **145**, 471.
- WOODWARD, G. E., MUNRO, M. P. & SCHROEDER, E. F. (1935). Glyoxalase. IV. The antiglyoxalase action of kidney and pancreas preparations. *J. biol. Chem.* **109**, 11.