The Reaction of Glutathione with Amino Acids and Related Compounds as Catalyzed by γ-Glutamyl Transpeptidase*

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In a previous publication (1) methods were described which permit the quantitative estimation of the action of γ-glutamyl transpeptidase upon glutathione. In the present report these methods are employed to study the influence of amino acids, peptides, and other compounds upon the reaction. In addition, we present the results of attempts to account for all the main products formed in the reaction in both the presence and absence of the amino acid, arginine. Data on the distribution of γ-glutamyl transpeptidase activity in a variety of organs and animal species are also given.

EXPERIMENTAL

The reaction of glutathione with transpeptidase was followed by either the CO manometric method or the alloxan method (1). In the manometric method the rate was calculated from the period of the reaction when the CO absorption was linear with time. This was usually for a 30-minute period. Unless otherwise noted, the reaction was carried out at 37.8° and in a solution buffered near pH 8.7, the optimum for the reaction. The total volume of the reaction mixture was 3.0 ml. and contained 20 μmoles of glutathione and 200 μmoles of buffer. In the manometric method 7.5 μmoles of $FeSO_4$ were also added in the form of the monohydrate salt. The concentration of amino acids and other compounds under test was as indicated in the results.

γ-Glutamylarginine was determined by the method of Kinoshita and Ball (2). Glutamic acid was measured by a modification of the method of Schales and Schales (3). Carbon dioxide was released from glutamic acid by squash decarboxylase and collected in NaOH in the center well of an oversize Warburg flask. The NaOH was then analyzed for its CO_2 content in the Van Slyke manometric apparatus (4). The squash decarboxylase released no CO_2 from glutathione, pyrrolidone carboxylic acid, or arginine, and these compounds did not interfere with the quantitative recovery of CO_2 from glutamic acid.

pH values were measured with a glass electrode at room temperature. Kidney homogenates were prepared by blending fresh whole kidney with buffer solution or water in a Potter-Elvehjem type (5) homogenizer. When purified enzyme was used, it was prepared from beef kidney cortex by the method of Kinoshita and Ball (2) with slight modifications.

We are indebted to Dr. F. M. Strong for the sample of β-aminopropionitrile used. Some of the samples of D- and L-serine used were the gift of Dr. D. H. Elwyn. Glutathione and all other amino acids and peptides used were commercial samples.

RESULTS

In most of the results to be presented we have compared simultaneously the rate of the reaction in the presence of the amino acid or other compound under test with the rate in its absence. We have termed these two reactions "transpeptidation" and "hydrolysis," respectively. The ratio of the rate of the "transpeptidation" reaction to the "hydrolytic" reaction multiplied by 100 has been designated as the TP:H value. Thus a value for TP:H of 200 indicates that the rate of glutathione disappearance or cysteinylglycine appearance in the presence of a compound is twice that observed in its absence. This procedure is employed in order that a direct comparison may be made of the results obtained in a large number of experiments in which the absolute rates observed are not directly comparable because of variations in the activity of the particular enzyme preparation employed. In the "hydrolytic" reaction in our experiments the absolute amount of glutathione utilized varied between 0.10 and 0.20 μmoles per minute.

Factors Influencing Reactivity of Amino Acids—The addition of L-amino acids to a mixture of glutathione and transpeptidase increases the rate of cysteinylglycine appearance (1). In contrast, the addition of D-amino acids has little effect on the reaction rate. Table I presents the results obtained with alanine, methionine, and serine. The significance of the slight effects observed with D-methionine or with high concentrations of D-alanine is questionable, since it is possible that the commercial preparations employed contained some of the L-isomer. The presence of a racemase in the kidney enzyme preparations must also be considered. The slight inhibition observed with 300 μmoles of D-alanine parallels the behavior of L-alanine at this concentration (*cf.* Fig. 1). Table I shows that DL-alanine at a final concentration of 6.6×10^{-3} M has the same effect as 3.3×10^{-3} M L-alanine. Thus D-amino acids do not participate in, nor do they interfere with, transpeptidation reactions.

In general the rate of the reaction increases with increasing amino acid concentration up to a certain level. However, as may be seen from the results presented in Fig. 1, the relation of

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TABLE I

Comparison of behavior of D- and L-amino acids

All results were obtained by the manometric procedure with 1.25 mg. of a purified beef kidney enzyme preparation. Borate buffer was employed except when serine was the amino acid; then tris(hydroxymethyl)aminomethane was employed as the buffering agent. The final pH of this reaction mixture was approximately 8.6 in all cases. All other conditions were as described under "Experimental."

Amino acid	Concentration		TP:H
	μmoles/3 ml.	*M*	
D-alanine	0	0	100
	10	0.0033	99
	20	0.0066	106
	50	0.016	111
	100	0.033	122
	300	0.1	90
DL-Alanine	20	0.0066	206
L-Alanine	10	0.0033	195
D-Methionine	20	0.0066	115
L-Methionine	20	0.0066	440
D-Serine	20	0.0066	100
L-Serine	10	0.0033	130

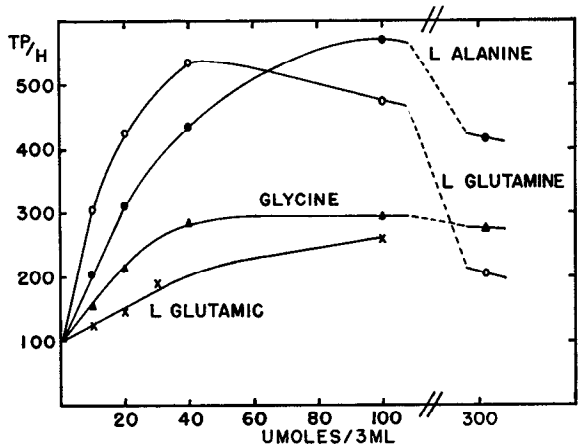

FIG. 1. The effect of the amino acid concentration on the rate of the reaction. All results were obtained by the manometric procedure in the presence of 20 μmoles of glutathione and the indicated concentration of amino acid, with a purified beef kidney preparation as the enzyme. Each vessel contained 1.25 mg. of enzyme and 2 ml. of 0.1 M borate, pH 9.0, in a total volume of 3.0 ml. Glutamic acid was converted to its monosodium salt before use. The final pH of the mixture was found to be 8.6 to 8.7. All other conditions were as given in "Experimental."

rate to concentration depends upon the particular amino acid employed. In the case of L-alanine and L-glutamine, the reaction rate increases and then falls as their concentration increases. In the case of glycine the rate rapidly reaches a plateau value and remains there as the concentration increases. The optimal concentration for each of these compounds is decidedly different. It is thus obvious that in any comparison of the effects of different amino acids upon the reaction rate, the relative values obtained will vary depending upon the concentration level of the amino acid which happens to be selected for the study. One procedure would be to run concentration curves for all compounds and to compare rates at the optimal concentration for each compound. However, in the survey of a large number of compounds this procedure is not practical and we have therefore chosen the value of 20 μmoles per 3 ml. as a suitable one to employ. This concentration value was selected in part because it is on the rising part of the curve for the four compounds presented in Fig. 1; it is the same concentration at which glutathione is present; and it permits the use of compounds of limited availability.

TABLE II

Relative reactivity of amino acids and peptides

The manometric procedure was used for all the compounds listed except cysteine. In this case the alloxan method was employed. Each value represents the average of at least two experiments. Results were reproducible to ±5 to 10 per cent. In all cases 20 μmoles of the compound were present in 3 ml. of reaction mixture and 1.25 mg. of a purified beef kidney enzyme preparation were employed. The pH of the reaction mixture in each case was measured by the glass electrode on a sample of the reaction fluid which contained all the components except enzyme. Values between 8.5 and 8.6 were found in all cases except as follows: L-glutamic acid, pH 8.45; diglycylglycine, pH 8.45; and DL-leucylglycylglycine, pH 8.30. All other conditions were as given in the "Experimental" section.

Compound	Amino pK	TP:H	Compound	Amino pK	TP:H
Glycylglycine	8.15	530	L-Leucine	9.60	165
L-Methionine	9.21	440	L-Glutamic acid	9.67	150
L-Leucylglycine		420	DL-Ornithine	8.65	145
L-Glutamine	9.13	400	DL-Homoserine		140
DL-Alanylglycine	8.20	390	Glycyl-DL-valine	8.25	140
Isoglutamine	7.90	334	Asparagine	8.80	130
L-Alanine	9.69	295	DL-Leucylglycylglycine		116
DL-Citrulline		260	Diglycylglycine	7.90	115
Glycyl-L-leucine	8.30	220	DL-Threonine		115
DL-Norleucine	9.76	210	Glycyl DL-serine		110
L-Arginine	9.04	205	DL-Valine	9.62	110
L-Tryptophane	9.39	205	L-Aspartic acid	9.60	105
Glycine	9.60	195	L-Isoleucine	9.68	100
L-Lysine	8.95	190	β-Alanine		100
L-Cysteine	8.18	175	L-Serine	9.15	25
DL-Phenylalanine	9.13	170			

In Table II is given the relative reactivity of 31 amino acids and peptides when they are present at the chosen concentration level of 20 μmoles per 3 ml. of reaction medium. The compounds are arranged in the order of decreasing activity. Also given in this table is the pK value at 25° of the amino group of the compound where such values were available in the literature. These pK values are given since evidence has been presented previously that it is the uncharged —NH_2 form of an amino acid or peptide which takes part in transpeptidation reactions (1). Some correlation between the reactivity and the pK value of the compound under test might therefore be expected, especially when the pH of the reaction is near 8.7 and the pK values of the compounds range from 8.1 to 9.8. However, no simple relationship of this sort is evident from the data presented in Table II. To be sure, the dipeptides, glycylglycine, L-leucylglycine, DL-alanylglycine, and glycyl-L-leucine, which do possess amino pK values that should make them most reactive, are near the top of the list. Yet the tripeptide, diglycylglycine, with an even lower amino pK value than the dipeptides, is a

poor reactor as is also the tripeptide, DL-leucylglycylglycine. It has been previously suggested by Fodor *et al.* (6) that there is a maximal length beyond which peptides become poor reactors.

That factors other than the pK value of the amino group may influence the reactivity of a compound is shown by the behavior of DL-norleucine, L-leucine, and L-isoleucine. These three isomeric forms have nearly identical amino pK values, but their reactivity decreases markedly as branching of the side chain occurs and particularly when branching occurs on the β-carbon atom as in isoleucine. Thus the bulkiness of the side chain in the immediate vicinity of the reactive amino group would appear to be important. Hird and Springell (7) have made similar observations.

There is some indication that a charge on the side chain of an amino acid may diminish its reactivity, although the conclusions to be drawn are equivocal. Thus, DL-ornithine, L-lysine, and L-arginine, all of which possess a positively charged side chain at the pH of the experiment, are less reactive than the structurally similar amino acid citrulline, which possesses an uncharged side chain. L-Glutamine is much more reactive than L-glutamic acid with its negatively charged side chain. Part of this increased reactivity of glutamine may be ascribed to the lower pK value of its amino group, but not all. This is best seen from the data presented in Fig. 1. The TP:H value is approximately 250 for glutamic acid when it is present at a final concentration of 100 μmoles per 3 ml. At the pH of the experiment it may be calculated from the pK value that about 10 μmoles of glutamic acid would be present with an uncharged amino group. A greater TP:H value (300) is found for glutamine when its total concentration is 10 μmoles per 3 ml. The amount of glutamine with an uncharged amino group would be less than half this concentration. Also of interest in this connection is the behavior of isoglutamine. This derivative of glutamic acid possesses a negatively charged side chain, yet it is almost as reactive as glutamine at the pH of these experiments. Here the markedly lower pK value of the amino group of isoglutamine is undoubtedly an important factor, and the fact that isoglutamine is present predominantly with an uncharged amino group outweighs the effect of the negatively charged side chain. A similar, although less striking, difference is to be noted for aspartic acid and asparagine.

β-Alanine was found to be unreactive at concentrations ranging from 20 to 500 μmoles per 3 ml. Thus an amino group in the beta position to the carboxyl appears to be unreactive. However, if the —COOH group of β-alanine is replaced by a —C≡N group as in β-aminopropionitrile, then a weakly reactive compound is obtained. This compound has little effect on the reaction at low concentrations, but gives a TP:H value of 157 when present at a concentration of 360 μmoles per 3 ml.

It should be pointed out that in those cases in which amino acids were employed as the DL-mixture the relative reactivity as shown in Table II is probably too low. This is because we have employed these compounds at a final concentration of 20 μmoles per 3 ml., which means that the active L-form is present at only one-half this concentration. Perhaps it would have been better to have used these compounds at a concentration level of 40 μmoles per 3 ml. in order that all the results might be directly comparable.

It will be noted that of all the compounds listed in Table II only L-serine caused the TP:H value to fall below 100. This means that L-serine not only failed to undergo transpeptidation, but that it inhibited the cleavage of glutathione by γ-glutamyl transpeptidase. Now all the results presented in Table II were obtained in borate buffer, and it will be seen from the data presented in Table III that the inhibitory action of L-serine manifests itself only when borate is the buffering agent. When either Veronal or tris(hydroxymethyl)aminomethane buffer is employed, the TP:H value is increased to values of 118 and 130, respectively, in the presence of L-serine. It should be noted also that the results presented in Table III were obtained by the alloxan method; the result for L-serine given in Table II, however, was obtained by the CO manometric method. Thus the inhibitory action of serine in the presence of borate is observable regardless of the method employed to follow the reaction. A more detailed account of this inhibition by serine in borate buffer will be published in a separate communication.

TABLE III

Reaction of L-serine in presence of different buffers

The data were obtained by the alloxan method. In each case the final reaction volume was 3 ml. and contained 2 ml. of 0.1 M buffer. Glutathione was added to give a concentration of 20 μmoles and L-serine, when present, a concentration of 10 μmoles per 3 ml. of reaction mixture. A water homogenate of rat kidney was used as the enzyme. Each vessel received 0.1 ml. of this homogenate which was equivalent to 7 mg. of wet kidney tissue.

Buffer	Glutathione utilized		TP:H	Final pH
	Serine absent	Serine present		
	μmoles/30 min.			
Tris,* HCl	5.3	6.9	130	8.3
Na Veronal	5.45	6.4	118	8.4
Na borate	5.65	2.9	51	8.4

* Tris, tris(hydroxymethyl)aminomethane.

Attempts to Determine Quantitatively Main Products of Reaction—It has been shown previously (1) that under the experimental conditions employed here there is a stoichiometric relation between cysteinylglycine appearance and glutathione disappearance. We have now attempted to account quantitatively for the fate of the γ-glutamyl moiety of glutathione in both the "hydrolytic" and "transpeptidation" reaction. Arginine was selected as the amino acid for use in the transpeptidation reaction since the γ-glutamylarginine formed could be determined quantitatively. The experiments were performed at two pH values, 6.2 and 8.6. At pH 6.2 glutathione disappearance was measured; at pH 8.6 both glutathione disappearance and cysteinylglycine formation were determined. Glutamic acid formation was measured in all experiments. The results obtained are presented in Fig. 2. When the "hydrolytic" reaction is carried out in a phosphate buffer at pH 6.2, practically all the glutathione which disappears is recovered as glutamic acid. The addition of arginine at this pH value causes a slight increase in glutathione disappearance and there is a concomitant appearance of γ-glutamylarginine to account for this increased glutathione utilization. However, only 5 to 8 per cent of the glutathione originally present in the reaction mixture is utilized at this pH during the 30-minute incubation period. At pH 8.6 a marked increase in the reaction rate occurs and 20 to 33 per cent of the glutathione is utilized during the experiment. Here the actual amount of glutamic acid formed is even less

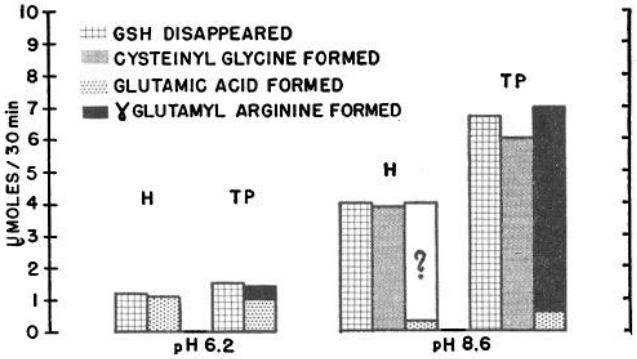

FIG. 2. The quantitative assay of the products formed from glutathione in the presence and absence of arginine. Each 3 ml. of reaction mixture contained 20 μmoles of glutathione, 1.25 mg. of a purified enzyme preparation from beef kidney, and 0.1 M buffer. A phosphate mixture was used as a buffer in the experiment at pH 6.2; borate was employed in the experiment at pH 8.6. When arginine was present its concentration was 20 μmoles per 3 ml. of the reaction mixture. Incubation of the reaction mixture was carried out for 30 minutes under N_2 in large Thunberg tubes shaken mechanically at 38°. At the end of the incubation period appropriate aliquots were withdrawn. The aliquot used to measure GSH disappearance was added to metaphosphoric acid to stop the reaction and precipitate the protein. In the case of the aliquot for glutamylarginine determination, trichloroacetic acid was used as the protein precipitant. In the case of the aliquot used for glutamic acid determination, the reaction was stopped by immersion of the aliquot in boiling water for 1 minute. Control experiments show that this treatment inactivated the enzyme and did not affect the recovery of glutamic acid. Cysteinylglycine formation was measured in Warburg vessels by the manometric procedure on a separate portion of the reaction mixture.

than at the lower pH value. In the presence of arginine the γ-glutamyl moiety of the glutathione which disappears can be accounted for nearly quantitatively as glutamic acid and γ-glutamylarginine. However, in the hydrolytic reaction at pH 8.6 only a small portion of the γ-glutamyl moiety of the glutathione which disappears can be accounted for as glutamic acid. We have not been able to account quantitatively for the rest of the γ-glutamyl moiety. The formation of pyrrolidone carboxylic acid has been reported to take place under conditions such as are employed here (6, 8). Others have denied that this compound is formed (9). We have attempted to determine the presence of pyrrolidone carboxylic acid both qualitatively by paper chromatography and quantitatively by the use of a Dowex 50 resin column in the H^+ cycle. Our results have been so inconsistent that we prefer to draw no conclusions at this time as to the formation of pyrrolidone carboxylic acid. Another compound whose formation could account for the γ-glutamyl moiety is γ-glutamyl glutathione. If the amino group of glutathione is as reactive as that of glutamine, then appreciable amounts of this compound could be formed. We have found no way as yet to determine quantitatively γ-glutamyl glutathione. If it is formed, we suspect that it may react in the alloxan method just as if it were glutathione. Hence its formation would not be detected by this procedure. Whatever the product, its formation seems to be markedly repressed when arginine is present.

Distribution of Transpeptidase Activity in Various Tissues—Homogenates of the following tissues of the rat were examined by the manometric procedure for transpeptidase activity: brain, heart, blood, salivary glands, liver, pancreas, stomach, intestines, spleen, lymph nodes, testis, and kidney. Only homogenates of the kidney and the pancreas were found to have activity. The addition of homogenates of liver, brain, or intestine to a kidney homogenate produced very little alteration in its activity. It thus appears unlikely that the inactivity of tissues such as liver, brain, or intestine is due to the presence of an inhibitor of γ-glutamyl transpeptidase. The tissues of other animals were examined for transpeptidase activity. These included the kidney, liver, brain, and pancreas of the leopard frog; the kidney and the pancreas of the rooster; and the kidney and the liver of the hamster. Again, as in the rat, activity was found only in homogenates of the kidney and pancreas of these animals. A black snake and a pigeon were examined and no enzyme activity was found in the liver, the pancreas, or the kidney of these two animals.

The relative enzyme activity of the kidneys from the various animals examined is shown in Table IV. The kidney of all the mammals displayed good enzyme activity. Among the lower animals only the kidney of the frog shows activity comparable to that found in the mammals. Ball *et al.* (10) examined the γ-glutamyl transpeptidase activity of various marine forms. In a variety of tissues examined in fishes they found enzyme activity to be present only in the kidney. The highest value reported by them for the kidney of these cold blooded animals was 1.75 μmoles of glutathione utilized per 30 minutes per 100 mg. at 30° for the hydrolytic reaction. Oddly enough, the addition of alanine to fresh kidney homogenates produced no change in the rate of the reaction. Through the cooperation of Dr. C. A. Villee and of Dr. R. Schwartz it was possible to

TABLE IV

Relative γ-glutamyl transpeptidase activity of kidney of various species

All results were obtained by the manometric procedure at pH 8.7 in borate buffer and 37.8°. In each case a 1:10 homogenate of the fresh tissue was prepared with either water or borate buffer and 0.1 ml. of this homogenate was used per vessel. When alanine was present, its concentration was 20 μmoles per 3 ml. Results are expressed in terms of 100 mg. of wet tissue. All other conditions were as described under "Experimental." We are indebted to Dr. Charles P. Lyman for furnishing us with the hamster kidneys.

Animal	Glutathione utilized	
	Alanine absent	Alanine present
	μmoles/30 min./100 mg. tissue	
Leopard frog (*Rana pipiens*)	16.0	22.7
Waterdog (*Necturus maculosus*)	0	0
Black snake (Species unknown)	0	0
Rooster (*Gallus gallus*)	2.2	4.1
Squab (*Columba livia*)	0	0
Rat, Wistar (*Rattus norvegicus*)	24.0	44.0
Rat, Sprague-Dawley	20.7	65.0
Dog (*Canis familiaris*)	26.5	29.5
Rabbit (*Oryctolagus cuniculus*)	25.6	33.4
Hamster (*Cricetus cricetus*)	23.7	44.0
Hamster, hibernating	22.4	50.7
Beef (*Bos taurus*)	27.5	
Man (*Homo sapiens*), 5-month fetus	0	0
Man (*Homo sapiens*), hydrocephalus	7.1	17.4
Man (*Homo sapiens*), Fanconi syndrome	3.0	5.0

examine some kidneys of human origin. Two 5-month-old fetuses obtained shortly after abortion showed no transpeptidase activity. However, a kidney obtained at a surgical intervention for hydrocephalus in a child (2 months old) was active, but a biopsy sample in a case of the rare Fanconi syndrome (23-month-old child) showed a lower activity.

No transpeptidase activity was found in commercial preparations of trypsin, chymotrypsin, carboxypeptidase, pepsin, cysteine-activated papain, protease, or pancreatin. A highly purified preparation of renin, kindly sent to us by Dr. E. Haas, was also inactive as was a sample of pankrin, the new pancreatic enzyme, kindly furnished by Drs. Grant and Robbins (11).

DISCUSSION

Hanes *et al.* (12) showed by paper chromatography that γ-glutamyl peptides were formed by a variety of amino acids when these amino acids were allowed to react in the presence of a kidney extract with glutathione or other γ-glutamyl peptides. The data presented here permit a quantitative comparison of the ability of a variety of amino acids and related compounds to undergo such a transpeptidation reaction with glutathione. Hird and Springell (7) have previously made a similar study of the comparative reactivity of various amino acids with glutathione. Their results agree in some cases with those presented here. In other cases they disagree. This is perhaps not too surprising since their experimental conditions, namely pH 7.4, temperature 30°, amino acid concentration 132 μmoles per 5 ml., and kidney homogenate corresponding to 300 mg. of wet tissue per 5 ml., were markedly different from ours. Moreover, their method for following the reaction depended upon the measurement of cysteine formed in a secondary reaction from cysteinylglycine. Fodor *et al.* (6) have also presented values for the comparative ability of various amino acids and peptides to undergo transpeptidation reactions. Their experimental conditions correspond more closely to ours than those of Hird and Springell (7). They used a modification of the Sullivan and Hess reaction to determine cysteinylglycine formation. On the whole their results and ours are in general agreement, although the precise relative activities of different amino acids vary significantly. Fodor *et al.* (6) carried out their reactions in the presence of 0.02 M KCN to prevent oxidation of sulfhydryl groups. These workers have indicated that cyanide may not be without effect upon the enzymatic reaction. We feel that these differences in results serve to emphasize the great variety of factors that come into play when such a comparative study is made with this system. Certainly of great importance is the pH of the reaction mixture in relation to the pK of the amino group of the compound undergoing transpeptidation (1). Perhaps of equal importance is the concentration of the reactants of the system as shown by the data presented here in Fig. 1 and in Figs. 3 and 4 of the paper by Hird and Springell (7).

In this laboratory the only tissues of all those examined which have shown γ-glutamyl transpeptidase activity were kidney and pancreas. This is true for a number of species. As shown here, no activity could be detected by the manometric procedure in any of the following tissues from the rat: liver, spleen, brain, heart, testicle, intestine, blood, salivary gland, stomach, and lymph nodes. There seems little doubt now that γ-glutamyl transpeptidase is identical with the factor showing antiglyoxalase activity first described by Dakin and Dudley (13) in 1913. These workers reported that this antiglyoxalase activity was present in the pancreas of man, dog, cat, rabbit, fowl, and toad. They also showed that pancreatic juice from the dog was active and postulated that this might account for activity found by them in the intestine. Woodward *et al.* (14) in 1935 reported that antiglyoxalase activity was present in the kidney and pancreas of the rabbit, horse, and pig. These workers at the same time confirmed the report of Girsavicius (15) that antiglyoxalase activity was due to the destruction of glutathione, which acted as a coenzyme for the enzyme glyoxalase. Schroeder *et al.* (16) reported that extracts of rat kidney, but not liver and spleen, were able to hydrolyze glutathione as measured by the glyoxalase reaction. Binkley and Nakamura (17) studied the hydrolysis of glutathione by means of the Sullivan reaction for the detection of the cysteinylglycine or cysteine released. In the rat they examined kidney, liver, spleen, heart, skeletal muscle, testicle, and thymus and found activity only in the kidney. In the pig, activity was found in the kidney and pancreas, but not in the duodenum. Thus the results of all these workers are in agreement with our findings that of all tissues examined only the kidney and pancreas contain enzymes capable of hydrolyzing glutathione. On the other hand, Fodor *et al.* (6) have reported that they have prepared γ-glutamyl transpeptidase from sheep kidney cortex, calf liver, and sheep brain, but that kidney yielded the most potent extracts. We have not tested calf liver or sheep brain by our procedures. The only other tissue in which γ-glutamyl transpeptidase activity has been reported is the lactating mammary gland. This finding of Hanes, *et al.* (12) has recently been confirmed by Bailie and Morton (18).

From the data in the literature it would appear that γ-glutamyl transpeptidase and conjugase are separate enzymes. Conjugase is the name given to an enzyme which cleaves the γ-glutamyl linkage in glutamic acid conjugates of folic acid. According to Laskowski *et al.* (19), it is found in nearly all the organs of the rat, in contrast to γ-glutamyl transpeptidase. Kazenko and Laskowski (20) have shown that the conjugase of chicken pancreas does not cleave glutamic acid from glutathione, but does cleave 1 molecule of glutamic acid from *p*-aminobenzoyl-γ-glutamylglutamic acid or synthetic pteroyltriglutamate. These workers conclude that two terminal carboxylic groups of glutamic acid must be present in addition to a γ-glutamyl linkage for conjugase to be active.

The presence of γ-glutamyl transpeptidase activity in the pancreas raises the question as to whether this may be due to the action of some of the known proteolytic enzymes of this tissue. We have been unable, however, to detect any transpeptidase activity in commercial crystalline preparations of trypsin, chymotrypsin, or carboxypeptidase, or in a preparation of pankrin, the new pancreatic enzyme described by Grant and Robbins (11). Whether this means that a specific γ-glutamyl transpeptidase is present in pancreas in addition to these enzymes must remain at the moment an open question since we have only tested each of these enzymes separately. It is possible that a certain combination of these enzymes might be responsible for the activity observed. Grassmann *et al.* (21), for example, have reported that pancreatic enzymes are able to cleave glycine from glutathione. It is possible that the dipeptide, γ-glutamylcysteine, which is thus formed might be more susceptible to cleavage by one or more of the pancreatic enzymes than is glutathione.

SUMMARY

A study has been made of the reaction of glutathione with amino acids and related compounds as catalyzed by the enzyme, γ-glutamyl transpeptidase. D-amino acids do not undergo transpeptidation nor do they interfere with the reaction of the corresponding L form. In general the rate of the reaction of the L form increases with increasing amino acid concentration up to a certain level, although the relation of rate to concentration varies from one amino acid to another. The relative rates at which 31 different amino acids and peptides undergo transpeptidation have been quantitatively determined. No simple relationship between structure and reactivity is discernible. In general those compounds which possess an amino group with a low pK value are the most reactive, although there are striking exceptions to this correlation. Variations in the bulkiness or charge of the side chain of the compounds also seem to influence reactivity. Serine in the presence of borate inhibits the enzymatic reaction.

An attempt has been made to account quantitatively for the fate of the γ-glutamyl moiety of glutathione when acted upon by the enzyme in the presence and absence of arginine. In the presence of arginine at either pH 6.2 or 8.6 the glutathione that disappears is matched by the sum of the glutamic acid or γ-glutamylarginine formed. In the absence of arginine at pH 6.2 the glutathione that disappears is matched by the glutamic acid formed. However, at pH 8.6 in the absence of arginine the glutamic acid formed accounts for only a small portion of the glutathione that has disappeared. Attempts to identify quantitatively the other product or products of the reaction have not given consistent results.

Out of a dozen tissues examined in the rat only kidney and pancreas were found to possess γ-glutamyl transpeptidase activity. The kidney and pancreas from a variety of other species were also found to contain the enzyme. The relative enzyme activity of the kidneys from various animals is given.

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