On the Enzymology of Amino Acid Transport

Transport in kidney and probably other tissues is mediated by a cycle of enzymic reactions involving glutathione.

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Understanding of the mechanisms by which molecules of various types are transported across cell membranes is of major importance in many areas of biology and medicine. This article deals with the transport of amino acids in certain mammalian tissues in relation to a new metabolic cycle of enzyme-catalyzed reactions. The data that support the existence of this cycle and its role in amino acid transport have come from research on enzymes and from biochemical studies of patients.

The transport of amino acids, like that of a number of other compounds (*1*), is generally thought to be a multistep process in which the amino acid is first bound to an active site on the cell membrane. Then a translocation occurs in which the amino acid (or an amino acid–carrier complex) moves to the inside of the cell; this process is followed by release of the amino acid from the carrier. There is much evidence that energy is needed for transport and, according to some hypotheses, it is required for the reactivation or resynthesis of the carrier, which becomes inactivated or destroyed during transport. It thus seems probable that the transport of amino acids involves a number of chemical reactions, and, since most of the reactions that take place in the cell are catalyzed by enzymes, it is reasonable to suppose that the transport of amino acids requires the action of enzymes. Although many studies on amino acid transport are consistent with an enzyme-catalyzed process, the particular enzymes involved have not yet been identified.

In the course of our work on amino acid metabolism and the associated enzymology, we became interested in several enzymes that act on glutamic acid and on derivatives of this amino acid, such as glutamine (*2*) and glutathione (*3*). We have pursued studies on γ-glutamyl transpeptidase (*4*) and on γ-glutamyl cyclotransferase (*5*), enzymes which have been known for a number of years, but which have not yet been assigned specific metabolic functions and are not usually mentioned in textbooks of biochemistry. We have also investigated γ-glutamylcysteine synthetase (*6*) and glutathione synthetase (*3*), the two enzymes needed for the synthesis of glutathione (*7*). Recent work in our laboratory led to the finding of a new enzyme, 5-oxoprolinase, that catalyzes the energy-requiring conversion of 5-oxoproline to glutamate (*8, 9*). The catalytic functions of these enzymes are integrated and form a cycle of chemical events that involve almost all of the protein amino acids; we have called this the γ-glutamyl cycle (Fig. 1) (*10*).

The studies reviewed here support the thesis that the γ-glutamyl cycle is responsible for amino acid transport in mammalian kidney and that the cycle also functions in the brain and possibly in other tissues as well. Our findings indicate that a major function of glutathione lies in its role as a donor of the γ-glutamyl group, which serves as a carrier in amino acid transport. 5-Oxoproline, first described in 1882 (*11*), and believed for many years to be formed mainly by nonenzymatic reactions from glutamate or its derivatives, now appears to be a quantitatively significant intermediate of major metabolic importance.

Our studies were initially concerned principally with the kidney, which is very active in amino acid transport and would therefore be expected to contain high concentrations of the enzymes that function in amino acid transport. Indeed, it may be calculated that the transport of amino acids from the glomerular filtrate across the renal tubule is of the order of 50 grams per day in man. Furthermore, the reabsorption of amino acids from the glomerular filtrate is extremely efficient so that normally very little amino acid is excreted in the urine.

Utilization and Synthesis of Glutathione

When glutathione is incubated with homogenates of kidney, 5-oxoproline and cysteinylglycine are formed; this degradation of glutathione is catalyzed by the combined actions of γ-glutamyl transpeptidase and γ-glutamyl cyclotransferase (*12*). γ-Glutamyl transpeptidase catalyzes a reaction between glutathione and any one of a number of amino acids to form the corresponding γ-glutamyl–amino acid and cysteinylglycine

$$\underset{\text{(glutathione)}}{\gamma\text{-Glutamylcysteinylglycine}} + \text{amino acid} \rightarrow \gamma\text{-glutamyl–amino acid} + \text{cysteinylglycine} \quad (1)$$

All of the common protein amino acids except proline are active substrates in this reaction (*4, 12*).

Cysteinylglycine is cleaved by a peptidase (*13*) to yield free glycine and cysteine

$$\text{Cysteinylglycine} + H_2O \rightarrow \text{cysteine} + \text{glycine} \quad (2)$$

γ-Glutamyl cyclotransferase catalyzes the conversion of γ-glutamyl–amino acid to free amino acid and 5-oxoproline

$$\gamma\text{-Glutamyl–amino acid} \rightarrow \text{5-oxoproline} + \text{amino acid} \quad (3)$$

This enzyme has been obtained from several sources in highly purified form (*5, 14*). It should be mentioned that γ-glutamyl transpeptidase can also catalyze transpeptidation reactions involving γ-glutamyl–amino acids other than glutathione; the activity of the enzyme toward γ-glutamyl-*p*-nitroanilide (*4*) forms the basis of the currently used clinical determination of serum γ-glutamyl transpeptidase. Both γ-glutamyl transpeptidase and γ-glutamyl cy-

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clotransferase are widely distributed in mammalian tissues, both are most active in kidney.

γ-Glutamyl transpeptidase is a membrane-bound enzyme. Histochemical studies indicate that γ-glutamyl transpeptidase is localized in the brush border of the proximal convoluted tubule of the kidney (*15*), the region believed to be the major site of amino acid reabsorption. In kidney homogenates the enzyme is associated with the insoluble fraction, and rather drastic procedures, such as extraction with deoxycholate, are needed to solubilize the enzyme (*4, 16*). Studies on the isolated brush border fraction of rat kidney indicate that γ-glutamyl transpeptidase represents about 1.5 percent of the membrane protein (*17*). The most purified preparations of this enzyme (about 1000-fold, from kidney) contain about 35 percent carbohydrate (*4, 16*). In contrast, γ-glutamyl cyclotransferase is a soluble enzyme and contains no bound carbohydrate.

That the kidney contains very substantial amounts of these enzymes, but nevertheless maintains a considerable concentration of gluthathione (4 to 5 millimolar) (*18*), led us to conclude that the kidney must also have active systems for the synthesis of glutathione, that is, γ-glutamylcysteine synthetase and glutathione synthetase. A procedure was designed for the determination of γ-glutamylcysteine synthetase which can be used for the assay of this enzyme in the presence of γ-glutamyl cyclotransferase and γ-glutamyl transpeptidase; it was then possible to obtain a highly purified preparation of γ-glutamylcysteine synthetase from rat kidney (*6*). Application of this assay procedure also led to the finding that γ-glutamylcysteine synthetase is widely distributed in mammalian tissues. Of the tissues examined, kidney exhibits the highest activity, and it was deduced that this enzyme represents 2 to 3 percent of the soluble protein of rat kidney. The available data indicate that mammalian tissues are capable of synthesizing glutathione; there seems to be no evidence that very much glutathione is transported by way of the blood plasma. Calculations based on the activities of γ-glutamylcysteine synthetase and glutathione synthetase in rat kidney homogenates indicate that the available synthesis activity is sufficient to catalyze the formation of several grams of glutathione per gram of kidney per day, which is of the same order as the estimated maximum rate of degradation of glutathione by γ-glutamyl transpeptidase. It appears then that there is a balance between the available enzymatic activity for glutathione synthesis and for glutathione breakdown.

It seems probable that the major metabolic fate of γ-glutamyl–amino acids is conversion to the corresponding free amino acids and 5-oxoproline, a reaction catalyzed by γ-glutamyl cyclotransferase. The γ-glutamyl derivatives of glutamine, methionine, glycine, alanine, and cysteine are good substrates of this enzyme. While certain γ-glutamyl–amino acids (for example, γ-glutamylvaline) are poor substrates, these may be converted by the action of γ-glutamyl transpeptidase to the corresponding γ-glutamyl-γ-glutamyl–amino acids; such di-γ-glutamyl compounds are excellent substrates of the cyclotransferase (*5*). Thus, coupled reactions of the following type can account for the conversion of a γ-glutamyl–amino acid to 5-oxoproline and free amino acid (where γ-GTP is γ-glutamyl transpeptidase; and γ-GCT is γ-glutamyl cyclotransferase)

$$\gamma\text{-Glutamylvaline} + \gamma\text{-glutamylvaline} \xrightarrow{\gamma\text{-GTP}} \gamma\text{-glutamyl-}\gamma\text{-glutamylvaline} + \text{valine} \quad (4)$$

$$\gamma\text{-Glutamyl-}\gamma\text{-glutamylvaline} \xrightarrow{\gamma\text{-GCT}} \text{5-oxoproline} + \gamma\text{-glutamylvaline} \quad (5)$$

The sum of Eq. 4 and Eq. 5 is

$$\gamma\text{-Glutamylvaline} \longrightarrow \text{5-oxoproline} + \text{valine} \quad (6)$$

Since γ-glutamyl transpeptidase can catalyze reactions between γ-glutamyl–amino acids and free amino acids, a γ-glutamyl–amino acid such as γ-glutamylvaline might also be cleaved by a pathway of the following type

$$\gamma\text{-Glutamylvaline} + \text{glutamine} \xrightarrow{\gamma\text{-GTP}} \gamma\text{-glutamylglutamine} + \text{valine} \quad (7)$$

$$\gamma\text{-Glutamylglutamine} \xrightarrow{\gamma\text{-GCT}} \text{5-oxoproline} + \text{glutamine} \quad (8)$$

The sum of Eq. 7 and Eq. 8 is

$$\gamma\text{-Glutamylvaline} \longrightarrow \text{5-oxoproline} + \text{valine} \quad (9)$$

It is possible that some γ-glutamyl–amino acid is hydrolyzed to glutamate and amino acid but, at least under the conditions thus far used, such reactions seem to take place at very low rates.

Since there is no evidence that 5-oxoproline accumulates in the kidney or that it is excreted to a significant extent in the urine, we looked for a reaction in which 5-oxoproline is metabolized. It is interesting that 5-oxoproline was thought to be metabolized in studies carried out many years ago, but we could find little definitive information in the literature about the metabolism of 5-oxoproline (*11*). Studies were therefore carried out in which 5-oxo-L-proline uniformly labeled with ^{14}C was administered to mice (*8–10*); in such experiments there was rapid appearance of a substantial amount of the administered label as [^{14}C]carbon dioxide in the expired breath, indicating clearly that 5-oxoproline is effectively metabolized. Independent studies of a similar nature were performed on rats by Ramakrishna *et al.* (*19*); these workers obtained very similar results. Further work on slices of mouse tissues in our laboratory showed that tissue slices (for example, of kidney, spleen, liver) were capable of oxidizing 5-oxoproline rapidly, and that of the tissues examined kidney was most active (*8, 9*). Subsequent work led to the finding and partial purification of an enzyme from kidney that catalyzes the conversion of 5-oxoproline to glutamate coupled with the cleavage of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (P_i) (*8, 9*)

$$\text{5-oxo-L-proline} + \text{ATP} + 2H_2O \longrightarrow \text{L-glutamate} + \text{ADP} + P_i \quad (10)$$

Unpurified preparations of this enzyme (5-oxoprolinase) catalyze the conversion of 5-oxoproline to glutamine, but this result was found to be associated with the presence of glutamine synthetase and ammonia in the enzyme preparation. When the reaction was carried out in the presence of methionine sulfoximine, an irreversible inhibitor of glutamine synthetase (*20*), only glutamate was formed. Furthermore, with more purified preparations (which do not exhibit glutamine synthetase activity), glutamate is the only amino acid product whether or not the reaction is carried out in the presence of methionine sulfoximine. The reaction catalyzed by 5-oxoprolinase is unusual and quite interesting in that it involves an energy-dependent cleavage of a peptide bond; the mechanism of the reaction is being studied.

The γ-Glutamyl Cycle

The reaction catalyzed by 5-oxoprolinase provides a link between that catalyzed by γ-glutamyl cyclotransferase and the first step in the biosynthesis of

glutathione, which is catalyzed by γ-glutamylcysteine synthetase. The six enzymes discussed above thus catalyze a cyclic series of reactions (Fig. 1). The operation of the cycle requires both amino acids and ATP; without either of these or without glutathione the cycle does not turn. Since γ-glutamyl transpeptidase can act on all but one (proline) of the amino acid building blocks of protein, the cycle is a general amino acid system. Each turn of the cycle requires a molecule of amino acid which is not altered chemically by the action of the cycle, and for each molecule of amino acid that is converted to γ-glutamyl–amino acid by the action of γ-glutamyl transpeptidase there will be one molecule of 5-oxoproline formed. Each turn of the cycle utilizes three molecules of ATP and produces three molecules each of ADP and inorganic phosphate. It is difficult to perceive any metabolic advantage that might be derived from forming a covalent linkage between glutamate and an amino acid and then cleaving this linkage. It does not seem likely that the γ-glutamyl cycle exists solely for the purpose of continually degrading and resynthesizing glutathione. It should be noted that the enzymes of the γ-glutamyl cycle in rat kidney exhibit rather high activity (from 50 to 460 nanomoles per minute per milligram of tissue protein) (*10*), and that the capacity of the intact organism to utilize 5-oxo-L-proline is also very great (see below).

Our hypothesis that the γ-glutamyl cycle is involved in amino acid transport was stimulated by the recognition that the cycle exhibits features that fulfill the requirements for active amino acid transport. Thus, the system contains a membrane-bound enzyme, γ-glutamyl transpeptidase, which can interact with the amino acid to be transported and also with glutathione, which serves as precursor of the carrier, the γ-glutamyl moiety. γ-Glutamyl cyclotransferase catalyzes the release of amino acid from the carrier. The cycle contains three energy-requiring recovery steps. Thus, the transport of 0.5 mole of amino acid per day by the kidneys of an average man would require 1.5 moles of ATP. This compares with a requirement of 2 moles of ATP high energy phosphate for the synthesis of 0.5 mole of urea, the average amount formed by man per day. The relatively high energy requirement of amino acid transport by the γ-glutamyl cycle may be related to the need for high efficiency of amino acid transport in the kidney. It is of crucial importance to maintain a substantial blood concentration of amino acids to support protein synthesis in a variety of tissues and cells including those of the central nervous system. Urea formation, synthesis of the peptide bonds of proteins, and synthesis of glycogen are other examples of biological processes that require more ATP than one might think would be minimally necessary. It is of interest in this connection that, while the synthesis of glutamine from glutamate requires the cleavage of one high energy phosphate bond of ATP, the synthesis of the analogous amino acid amide asparagine from aspartate requires cleavage of three or two high energy phosphate bonds of ATP, depending on the source of the enzyme.

The concept of the γ-glutamyl cycle introduces several new ideas. Thus, if the enzymes that catalyze the uptake and release of amino acid to and from γ-glutamyl linkage are separated physically by a membrane, the cycle can serve as a system for transporting amino acids across the membrane. In addition,

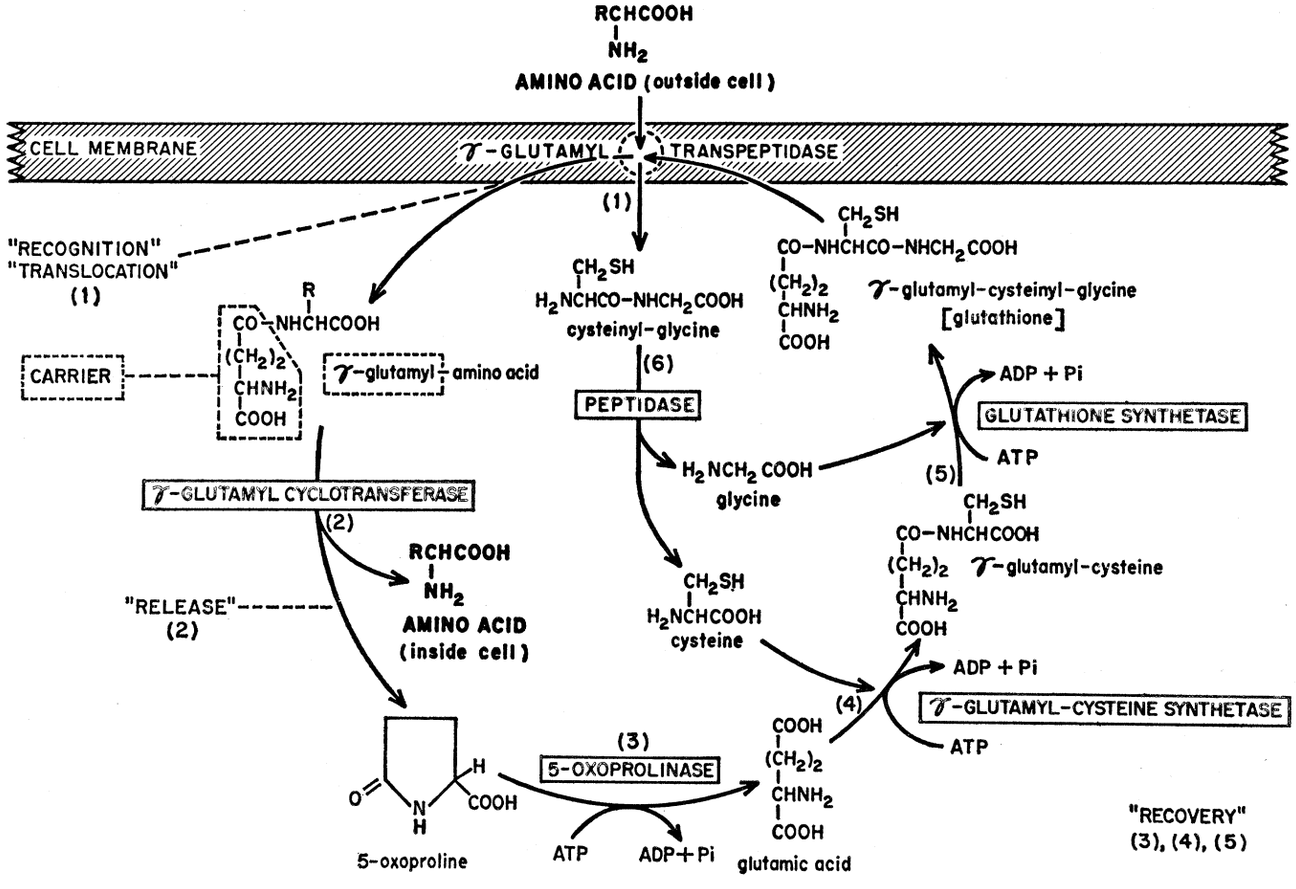


Fig. 1. The γ-glutamyl cycle.

the data indicate that 5-oxoproline is a metabolite of major quantitative importance. Finally, recognition of the cycle seems to have uncovered a new function, perhaps one of the most important functions, of glutathione.

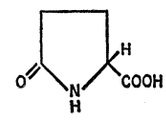
Although the intimate details of the translocation step require much additional study, it is possible to formulate mechanisms based essentially on enzymological considerations. Thus, according to the hypothetical scheme illustrated in Fig. 2, the amino acid to be transported binds noncovalently to a site on the membrane (A); a group X on the membrane-bound γ-glutamyl transpeptidase interacts with the γ-glutamyl moiety of glutathione to yield a γ-glutamyl enzyme (B), and cysteinylglycine is released. Attack of the amino acid nitrogen atom on the γ-carbon atom of this complex yields the γ-glutamyl–amino acid (Fig. 2C); in this step, the formation of the γ-glutamyl–amino acid linkage is associated with removal of the amino acid from its binding site on the membrane and movement of the amino acid moiety into the cell, perhaps through a space in the membrane or possibly facilitated by a conformational change in the membrane. The amino acid–carrier complex, now intracellular, serves as a substrate for γ-glutamyl cyclotransferase which releases the amino acid from the carrier. The 5-oxoproline formed in this reaction together with the cysteine and glycine formed by hydrolysis of cysteinylglycine are, in three energy-requiring recovery steps, reconverted to glutathione.

While the considerations outlined above seem to offer support for the hypothesis that the γ-glutamyl cycle is responsible for amino acid transport in the kidney, it is clear that studies on an in vivo system in which one of the enzymes in this cycle is specifically blocked would be of considerable importance. This type of blocked system has recently come to light through the finding by Eldjarn and his colleagues of a mentally retarded patient who excretes 25 to 50 grams of 5-oxoproline per day (*21*). The patient's blood contains 5 mg of 5-oxoproline per 100 ml; the concentration of 5-oxoproline in the cerebrospinal fluid is 30 mg per 100 ml. Normally little or no 5-oxoproline is found in these body fluids or in urine. The patient's excretion of urea is unusually low, and the urine also contains relatively large amounts of ammonia even when the urinary *p*H is brought to about 7 by administration of sodium bicarbonate orally. Although the patient was at first tentatively considered to have a defect in the pathway leading to urea formation (*21*), subsequent studies (*22*) indicated that the block is in the γ-glutamyl cycle. Thus, when the patient was given ^{14}C-labeled 5-oxoproline, only 1.7 percent of the injected radioactivity was expired as CO_2 in 3 hours, and about 65 percent was excreted, mainly as 5-oxoproline, in the urine in 24 hours. In a comparable study on a healthy control, the formation of labeled respiratory carbon dioxide accounted for 17 percent of the injected dose in 2.5 hours, and only 6 percent of the label, mainly as glutamate, appeared in the urine. On the other hand, both the patient and the healthy control metabolized ^{14}C-labeled glutamate, and to about the same extent. The findings indicate that most of the urinary 5-oxoproline arises from the kidneys (*22*) and that the patient has a block in the utilization of 5-oxoproline, presumably a marked reduction in 5-oxoprolinase activity. It is of interest that after injection of ^{14}C-labeled glutamate, the patient excreted a ^{14}C-labeled compound, which became labeled earlier than urinary glutamate or 5-oxoproline, and which gave glutamate on hydrolysis; this compound may be a γ-glutamyl–amino acid. It is relevant to note that γ-glutamyl–amino acids have been found in normal human urine (*23*).

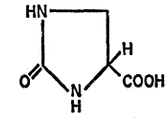
The rate of excretion of 5-oxoproline by the patient was not altered by administration of glutamate, glutamine, ammonium or sodium bicarbonate, or by the feeding of a low protein or high protein diet (*22*). However, when the serum concentration of amino acids was increased about threefold by intravenous infusion of a mixture of amino acids, the urinary excretion of 5-oxoproline increased about twofold. Under these conditions, there was massive aminoaciduria. It should be noted that the patient does not usually have aminoaciduria; this is discussed further below. Although it might well be expected that such an increased serum concentration of amino acids would be accompanied by aminoaciduria, the large increase in 5-oxoproline excretion seems to reflect a considerable (about 100 percent) increase in the turnover of the γ-glutamyl cycle.

We have recently learned of another patient with very similar chemical findings (*24*). There may well be instances of this error of metabolism in other individuals. 5-Oxoproline does not give a color when treated with ninhydrin, and 5-oxoproline would therefore not be detected by screening procedures based on this color reaction.

It should be emphasized that, until direct enzyme studies are carried out, we cannot conclude unequivocally that 5-oxoprolinase is absent or reduced in these patients. Indeed, it is possible that the enzyme activity is present, but that it is inhibited in some manner. It is conceivable, but less likely, that the block in the cycle occurs at the glutathione synthetase step. The findings would then be explained if (i) γ-glutamylcysteine served in place of glutathione in transpeptidation reactions with amino acids, (ii) much more than normal amounts of γ-glutamylcysteine were formed (and converted by γ-glutamyl cyclotransferase to 5-oxoproline), and (iii) if the amount of 5-oxoproline formed exceeded the capacity of 5-oxoprolinase to utilize it. However, the studies on the patient with ^{14}C-labeled 5-oxoproline point to a deficiency in utilization of this compound (*22*). Furthermore, the available information indicates that the normal capacity to metabolize 5-oxoproline is quite large. In addition, it would seem that the flow of γ-glutamylcysteine into glutathione must in some way be protected from the action of γ-glutamyl cyclotransferase—perhaps by compartmentalization within the cell or by a close linkage between the two synthetases.



5-OXOPROLINE



2-IMIDAZOLIDONE-4-CARBOXYLIC ACID

An opportunity to develop an experimental animal system blocked at the 5-oxoprolinase step of the cycle presented itself with the finding that L-2-imidazolidone-4-carboxylic acid is a very effective competitive inhibitor of 5-oxoprolinase (*25*). Both L- and D-2-imidazolidone-4-carboxylic acid were prepared in our laboratory (*26*); only the L-isomer inhibits the enzyme. L-2-Imidazolidone-4-carboxylate was found to effectively inhibit the oxidation of 5-oxoproline by kidney slices. It was also found that mice treated with L-2-imidazolidone-4-carboxylic acid exhibited a marked reduction in ability to oxidize injected ^{14}C-labeled 5-oxoproline to respiratory carbon dioxide; under these conditions, labeled 5-oxoproline appeared in the urine. Thus, animals treated with 2-imidazolidone-4-carboxylate, like the human patients with 5-

oxoprolinuria, are blocked in the conversion of 5-oxoproline to glutamate. It was also found that administration of L-2-imidazolidone-4-carboxylate to mice leads to appreciable excretion of 5-oxo-L-proline. In the tissues (brain, kidney, liver, and skin) of such mice the concentrations of 5-oxo-L-proline are much higher than normal (*25*).

Consideration of the quantitative aspects of the 5-oxoprolinuria exhibited by the patient described above indicates that the excretion of 5-oxoproline is in the range of 0.2 to 0.4 mole per day, which is not far from the estimated amount of amino acid transported in the kidney per day. It thus appears that the γ-glutamyl cycle represents a quantitatively significant, perhaps the major, pathway for amino acid transport. It is particularly notable that increasing the blood amino acid concentration by amino acid infusion was accompanied by greatly increased formation of 5-oxoproline; this finding is clearly in accord with the cycle.

The unusually low excretion of urea and the rather high excretion of ammonia exhibited by the patient with 5-oxoprolinuria may be readily understood in terms of established biochemical considerations. Thus, it appears that a metabolic adaptation has occurred in which there is a sparing of urea formation by the need to produce extra glutamate for the kidney. It is well known that the kidney contains considerable glutaminase activity and that glutamine is a major amino acid constituent of the blood. Since the patient is blocked in the formation of glutamate from 5-oxoproline, he must then obtain glutamate for the γ-glutamate cycle by using other pathways, probably for the most part by hydrolysis of glutamine, which yields glutamate and ammonia; the glutamate is used in the cycle and the ammonia formed appears in the urine. The blood glutamine utilized by the kidney is replenished by glutamine synthesis in the liver; such synthesis requires ammonia for the amination of α-ketoglutarate to glutamate, and also for the amidation of glutamate to form glutamine. The increased requirement for ammonia for these two reactions, which are required for the formation of glutamine, would be expected to decrease the ammonia available for carbamylphosphate synthesis. In addition, the increased utilization of glutamate for glutamine formation would decrease the amount of glutamate available for transamination with oxaloacetate to form aspartate. The decreased formation of carbamylphosphate and aspartate would reduce the rate of urea formation. There might also be a decrease in available oxaloacetate because of the increased utilization of α-ketoglutarate for glutamate formation. This interpretation of the altered ammonia metabolism of the patient is consistent with the finding (*22*) that oral administration of glutamine led to an increase in urea formation (but had no effect on excretion of 5-oxoproline). The metabolic adaptation discussed above compensates for the glutamate deficiency in the kidney and permits the γ-glutamyl cycle to function in amino acid transport. Thus, patients blocked at the 5-oxoprolinase step of the cycle do not exhibit aminoaciduria.

One might expect aminoaciduria to occur if the synthesis of glutathione were blocked or markedly reduced. Quite recently, Richards and his colleagues described two patients with severe hemolytic anemia who have a marked deficiency of erythrocyte γ-glutamylcysteine synthetase (*27*). The enzyme deficiency is apparently not restricted to the red blood cell; thus, the glutathione content of the white blood cells and muscles, as well as that of the red blood cells, is markedly reduced. The patients also exhibit evidence of central nervous system disease (mental retardation, psychosis, spinocerebellar degeneration). It is of interest that these patients have a significant generalized aminoaciduria involving the neutral and dibasic amino acids (*28*). These findings are consistent with and support the view that the γ-glutamyl cycle functions in the renal transport of amino acids.

Discussion

The considerations reviewed above suggest that membrane-bound-γ-glutamyl transpeptidase can serve as a binding site for many amino acids; competition between amino acids for binding to the transpeptidase may ex-

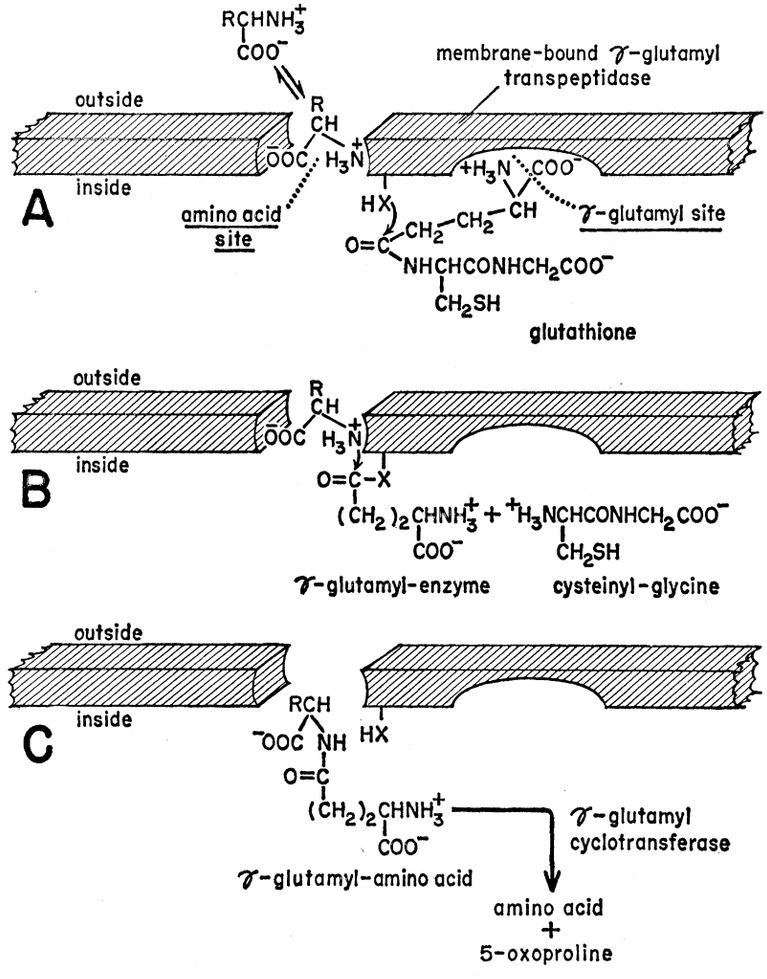


Fig. 2. Scheme for amino acid translocation.

plain a variety of in vivo studies in which competition between amino acids for transport was observed. For example, Pitts (*29*) reported evidence for a single transport system in the kidney tubule for glycine, alanine, glutamate, and arginine, and Kamin and Handler (*30*) found that the infusion of a large amount of almost any amino acid led to the urinary excretion of most of the others. The extensive literature now available on amino acid transport describes many in vitro and in vivo studies on a variety of amino acids and amino acid analogs in various cell and tissue systems. Many efforts have been made to identify various transport systems by measuring the transport of specific amino acids in the presence and absence of other amino acids, generally in an effort to determine the extent of competition. Interpretation of such data may be difficult because of overlapping specificities and because it is possible that an amino acid may interfere with the transport of another amino acid without being transported itself. Previous studies on amino acid transport in kidney, including work on inborn errors of amino acid transport in man, suggest that there may be separate systems for the neutral amino acids and for certain individual amino acids or groups of these, for example, for cystine (alone), for cystine, lysine, arginine, and ornithine, or for only the three basic amino acids (*31, 32*). The function of the γ-glutamyl cycle may involve isozymic forms of γ-glutamyl transpeptidase and γ-glutamyl cyclotransferase which exhibit different amino acid specificity; such an arrangement might offer an explanation for some of the observations that have been reported. On the other hand, it seems possible that a single system of enzymes exhibiting different affinities for different amino acids might also explain the findings. That certain amino acids may follow a pathway involving more than one transpeptidation step (as described above) may also influence "specificity" as observed in vivo. We cannot exclude the possibility that there are specific binding proteins for various amino acids and that the amino acids are transferred from these to the transpeptidase; if this is true, the γ-glutamyl cycle might then be primarily involved in translocation rather than in recognition. There is evidence that proline, hydroxyproline, and glycine share a single renal transport system, and that separate systems also exist for glycine and for the imino acids (*32, 33*). Since γ-glutamyl transpeptidase does not act on the imino acids, it seems probable that these are transported by a separate system. Studies on "unmetabolizable" amino acids, such as α-aminoisobutyric acid (which is not a substrate for the transpeptidase), suggest that these analogs are taken up by various cells; however, it is not clear how effectively they are transported by the renal tubular system as compared to the natural amino acids. A recent study suggests that α-aminoisobutyric acid may be transported by the same system that promotes the transport of proline (*34*).

The available data on amino acid transport indicate that different cells may have different transport systems and that a given cell may use more than one mechanism to transport amino acids. It should be emphasized that we are not proposing that the γ-glutamyl cycle mediates all amino acid transport in the kidney (or in all other tissues and cells); furthermore, at the present stage of our knowledge we cannot define the extent to which the cycle may participate in the various diffusion and exchange phenomena which have been reported in studies on systems containing intact cells. We certainly do not exclude the very likely possibility that other types of amino acid transport systems exist in the kidney and in other tissues and cells; definite conclusions in this regard must await development of information about the enzymology of such systems.

It is evident that the γ-glutamyl cycle must be connected with other metabolic phenomena. For example, it would be expected that interference with the supply of ATP—for example, by inhibition of oxidative phosphorylation—would inhibit the activity of the cycle. Interrelation must also exist between other energy-dependent processes including the transport of other substances such as carbohydrates, sodium, and potassium; under certain conditions these could compete for energy whose availability may involve a common intermediate (*35*). It is possible that the function of the γ-glutamyl cycle is influenced by sodium ions and other cations and that it is in some way connected with the transport of cations. Additional studies are still needed to elucidate the nature of various reported coupling phenomena between the transport of amino acids and other compounds.

That the γ-glutamyl cycle functions in other mammalian tissues is suggested by the wide distribution of the enzymes of the cycle and the ubiquitous occurrence of glutathione. Histochemical studies show that the γ-glutamyl transpeptidase activity of the brain is localized in the ependymal cells and the epithelium that covers the leaves and villi of the choroid plexus as well as in the epithelium of brain capillaries (*36*). In addition to γ-glutamyl transpeptidase, brain contains substantial amounts of γ-glutamyl cyclotransferase and indeed human brain is an excellent source of the enzyme (*5*). Brain also contains 5-oxoprolinase (*9*) and γ-glutamylcysteine synthetase (*6*). It is notable that a number of γ-glutamyl–amino acids, that is, the γ-glutamyl derivatives of glutamine, glycine, alanine, valine, isoleucine, glutamate, and serine have been found in mammalian brain (*37*). It is also significant that the patient with 5-oxoprolinuria has a cerebrospinal fluid concentration of 5-oxoproline which is about six times greater than that of the concentration of this substance in his blood (*21*). This finding suggests that brain, like the kidney, synthesizes 5-oxoproline. The collected evidence therefore supports the view that brain has an active γ-glutamyl cycle.

Histochemical studies have shown that γ-glutamyl transpeptidase of the intestine is localized in the apical portion of the epithelial cells covering the jejunal villi (*38, 39*). γ-Glutamyl cyclotransferase has also been found in the intestine. Histochemical studies indicate that γ-glutamyl transpeptidase is localized in the submaxillary glands, prostate, bronchi, seminal vesicles, lactating breast, endometrium, epididymis, and fallopian tubes (*38*). γ-Glutamyl cyclotransferase has been found in kidney, spleen, liver, and intestine. That the γ-glutamyl cycle may be of general biological occurrence is suggested by the wide distribution of the enzymes of the cycle and of glutathione. γ-Glutamyl transpeptidase has been found in plants (*40*) and in insects (*41*). Plants are known to contain a variety of γ-glutamyl–amino acids (*42*). It is conceivable that glutathione functions in conjunction with enzymes different from those described here in other cells or in other types of transport phenomena. Even though highly purified preparations of γ-glutamyl transpeptidase have been obtained from kidney, these still exhibit considerable heterogeneity. In early stages of purification, other enzymes, especially α-peptidases, are present, and it is possible that γ-glutamyl transpeptidase is oriented in a complex mem-

brane structure that functions also in the transport or degradation of peptides. In this connection, it may be noted that glycylglycine is an excellent acceptor of the γ-glutamyl group of glutathione. The γ-glutamyl cycle might in some cells serve as a transport mechanism involved in the secretion of amino acids or of certain peptides.

Earlier studies have emphasized the function of glutathione as a cofactor for certain enzymes, and glutathione has generally been thought to play a role in the maintenance of protein sulfhydryl groups (*43*). Our work indicates that glutathione also functions to provide the reactive γ-glutamyl moiety which serves as a carrier in amino acid transport. The γ-glutamyl bond of glutathione is resistant to hydrolysis by the various tissue peptidases, and glutathione is not a substrate of γ-glutamyl cyclotransferase. Glutathione thus seems well adapted to functioning as the carrier precursor molecule. Glutathione and γ-glutamyl amino acids constitute a special category of peptides which are metabolized by enzymes that are separate and distinct from the enzymes that form and cleave α-peptide bonds. It should be mentioned also that the function of glutathione in the γ-glutamyl cycle does not seem to depend crucially on the integrity of its sulfhydryl group; thus, oxidized glutathione is a substrate of the transpeptidase in vitro as are glutathione analogs which lack a sulfhydryl group such as γ-glutamyl-α-aminobutyrylglycine and γ-glutamylalanylglycine.

There appears to be some analogy between the function of the γ-glutamyl group in amino acid transport and its role in ammonia transport. Thus, glutamine functions in the storage and transport of ammonia in a number of organisms. The amide group of glutamine is more reactive than is ammonia in several reactions of metabolic importance (*44*). The mechanisms of the reactions catalyzed by the glutamine amidotransferases (*44*) and by γ-glutamyl transpeptidase may involve formation of intermediate γ-glutamyl enzyme complexes.

Much remains to be done on the structure, function, and cellular disposition of γ-glutamyl transpeptidase. This glycoprotein, which seems to be an amino acid recognition site of the cell membrane, deserves further attention from enzymologists. The other enzymes of the cycle also require additional study. However, our present understanding of the γ-glutamyl cycle suggests that it will continue to serve as a useful working hypothesis in the design of new experiments on the enzymology of amino acid transport.

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