THE GLUTAMINE TRANSAMINASE-ω-AMIDASE PATHWAY

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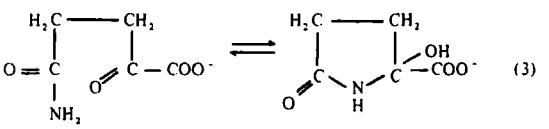
INTRODUCTION

In the course of studies on the enzymatic deamidation of glutamine, Greenstein and co-workers[1-11] discovered that the formation of ammonia from glutamine by rat liver extracts was greatly enhanced by the addition of pyruvate or certain other α-keto acids. The activity responsible for the α-keto acid-dependent reaction was termed "glutaminase II" and distinguished from another activity, "glutaminase I," a deamidation reaction that is activated by phosphate and to a lesser extent by arsenate and several other anions. Most of the "glutaminase I" activity could be removed from aqueous liver extracts by centrifugation, whereas most of the "glutaminase II" activity was associated with the soluble fraction. "Glutaminase II" was found to be more stable to heat and to low values of pH than "glutaminase I." "Glutaminase I," which was found to be associated with mitochondria,[11] hydrolyzes L-glutamine to L-glutamate and ammonia; it is now generally believed that this enzyme plays a major role in ammoniagenesis, especially in the kidney. It was initially thought that pyruvate acted catalytically in the "glutaminase II" reaction; thus it was proposed that the deamidation is a two-step process involving condensation of the amide moiety of glutamine with pyruvate to form a dehydropeptide, followed by enzymatic hydrolysis of the latter to glutamate, pyruvate, and ammonia.[1] However, in studies on the pyruvate-stimulated deamidation reaction, Meister and Tice[12] found that pyruvate (as determined with lactate dehydrogenase) disappears, while the total α-keto acid present (as determined by formation of α-keto acid 2,4-dinitrophenylhydrazone) remains unchanged; α-ketoglutarate was recovered at the end of the reaction as the corresponding 2,4-dinitrophenylhydrazone. They also established in studies with [^{15}N] amide-labeled glutamine that the ammonia formed is derived from the amide group of glutamine. In these and later studies,[12-21] it was found that a large number of α-keto acids can replace pyruvate and that the disappearance of pyruvate (or other α-keto acids) is accompanied by equimolar formation of the corresponding L-amino acids. No ammonia is formed in the absence of an α-keto acid, and with most of the α-keto acids studied, replacement of glutamine by glutamate led to markedly decreased transamination. When glutamine was replaced by γ-methylglutamine, transamination occurred but no ammonia was formed and α-keto-γ-methyl-

L-Glutamine + α-keto acid ⟷ α-ketoglutaramate + L-α-amino acid (1)

α-Ketoglutaramate + H_2O ⟶ α-ketoglutarate + NH_3 (2)

glutaramate was demonstrated as a product of the reaction. The findings were interpreted to indicate an initial transamination reaction between glutamine and α-keto acid to yield the corresponding amino acid and α-ketoglutaramate (Reaction 1), followed by an ω-deamidation reaction in which α-ketoglutaramate is converted to α-ketoglutarate (Reaction 2). In support of this interpretation, it was found that α-ketoglutaramate (prepared by enzymatic oxidation of L-glutamine[14]) is hydrolyzed by preparations that also catalyze the transamination-deamidation reaction of glutamine; on the other hand, α-keto-γ-methylglutaramate is not deamidated. The α-keto acid-ω-amidase was partially purified from rat liver and separated from the transaminase.[14,20,21] It was found to hydrolyze several other amides including α-ketosuccinamate, succinamate, and glutaramate, but it was inactive toward glutamine and asparagine. Rat liver preparations also contain a separate transaminase that catalyzes: (a) transamination between asparagine and a wide variety of α-keto acids to yield the corresponding L-α-amino acids and α-ketosuccinamate, and (b) transamination between α-ketosuccinamate and various L-amino acids to form asparagine.[19] In studies with enzyme preparations from *Neurospora crassa* which are deficient in ω-amidase activity, transamination between α-ketosuccinamate and glutamine was shown to yield asparagine and α-ketoglutaramate.[22] In the initial work on α-ketoglutaramate, it was found that this α-keto acid can exist in two interconvertible forms, one of which exhibits properties expected of the open-chain α-keto acid analogue of glutamine; the other form is relatively unreactive toward 2,4-dinitrophenylhydrazine and it is not decarboxylated by hydrogen peroxide.[14] The unreactive form of α-ketoglutaramate, which is not a substrate of ω-amidase, was shown to be 2-hydroxy-5-oxo-proline.[23] The equilibrium between the open-chain and cyclic forms lies markedly in favor of the latter under physiological conditions. Thus, more than 99% of the compound exists in the cyclic form:



(3)

Nonenzymatic conversion of the cyclic form to the open-chain form is promoted by increase of pH.[14] This is reflected in the pH-activity curve of ω-amidase which shows a sharp maximum at about pH 9.[14] In contrast, studies on the pH dependence of α-ketosuccinamate hydrolysis showed a broad plateau between pH 6.5 and 9.5. α-Ketosuccinamic acid can be obtained in a relatively stable open-chain reactive form and also in an unreactive form, which is a dimer[23] whose structure was shown to be 4,6-dihydroxy-2-oxo-piperidine-5-carboxamide-4,6-dicarboxylic acid.[24]

Although studies with relatively unpurified enzyme preparations indicated that the α-keto acid-dependent deamidation of glutamine is a two-step process, further progress in understanding this pathway of glutamine metabolism has required purification and study of the individual catalytic components. Preparations were obtained that had ω-amidase activity but lacked transaminase,[14,20,21] and a partially purified preparation of rat liver glutamine transaminase was isolated that did not exhibit ω-amidase activity.[25,26] Although the latter preparation was free of glutamate-aspartate, asparagine-α-keto acid, and glutamate-phenylalanine transaminases, it exhibited glutamate-alanine transaminase activity, and the possibility was considered that this activity and glutamine transaminase activity were properties of the same enzyme.[26] Another preparation of glutamine transaminase was later obtained from rat liver which did not exhibit glutamate-alanine transaminase activity.[27-29] In 1971, the first highly purified and apparently homogenous preparation of α-keto acid-ω-amidase was described by Hersh,[30] and at about the same time an apparently homogeneous preparation of soluble rat liver glutamine transaminase was obtained by Cooper and Meister.[31,32]

Early studies by Price and Greenstein[4] showed that the "glutaminase II" activity of liver was substantially higher than that of kidney. However, Kupchik and Knox[33,34] obtained a fourfold purified glutamine transaminase from rat kidney and noted that this activity was about six times higher than that found in rat liver. These apparently conflicting findings were resolved by further work.[35] Thus, Kupchik and Knox[33,34] used an assay in which transamination between

glutamine and phenylpyruvate was determined, whereas the glutamine-pyruvate reaction had been used in the earlier work. Reinvestigation of the α-keto acid specificities of rat liver and kidney homogenates showed that kidney homogenates are much less active than liver homogenates in catalyzing transamination between glutamine and pyruvate, while kidney homogenates are much more active than liver homogenates in catalyzing transamination between glutamine and phenylpyruvate. These findings culminated in the isolation of a new glutamine transaminase from rat kidney, and in the demonstration that both glutamine transaminases are present in liver and kidney.[35]

Our present knowledge of the enzymes involved in the glutamine transaminase-ω-amidase pathway may be summarized as follows. The predominant glutamine transaminase of liver (glutamine transaminase L) is most active toward methionine, glyoxylate, pyruvate, and several other substances. The predominant glutamine transaminase of kidney (glutamine transaminase K) is most active toward methionine, phenylalanine, tyrosine, and the α-keto acid analogues of these amino acids; this enzyme exhibits relatively less activity toward glyoxylate, pyruvate, and several other substrates. Transaminases K and L are present in liver and kidney, and both enzymes are present in these tissues as mitochondrial and soluble isozymes. There are thus at least four separate glutamine-α-keto acid transaminases in liver as well as in kidney. α-Keto acid-ω-amidase is present in substantial amounts in rat liver and kidney. It had long been known that ω-amidase is present in the soluble portion of rat liver homogenates, but it was shown recently[35] that there is also a mitochondrial ω-amidase isozyme. The data now available indicate that the glutamine transaminase-ω-amidase pathway is well represented in both the soluble and mitochondrial fractions of rat liver and kidney. There is evidence that α-keto acid-ω-amidase activity also occurs in a variety of other rat tissues.[14] Sugiura[36] found that glutamine transaminase activity is present in rabbit brain extracts. Recent studies indicate that homogenates of rat cardiac muscle and brain can catalyze transamination reactions between glutamine and a variety of α-keto acids.[35] Glutamine transaminase activity has been found in certain higher plants,[37] *Neurospora crassa*,[22] bacteria,[38] and insects.[39]

It is evident that much additional work needs to be done on the glutamine transaminases of various mammalian tissues and of other organisms. The wide distribution of ω-amidase in mammalian tissues suggests that glutamine transaminases and possibly also asparagine transaminases may be widely distributed. The detection of glutamine transaminase in tissue preparations may require the use of particular substrates and conditions. Study of the glutamine transaminase-ω-amidase system of brain may be of importance in relation to findings that α-ketoglutaramate accumulates in the cerebrospinal fluid of patients with hepatic coma.[40] This review considers some of the physical and catalytic properties of the enzymes of this pathway, especially those which have been obtained in highly purified form from liver and kidney. Consideration is given to the physiological function of this pathway in mammals in relation to amino acid metabolism.

SOLUBLE RAT LIVER GLUTAMINE TRANSAMINASE L

Although certain characteristics of glutamine transaminase were uncovered by earlier work carried out on relatively unpurified preparations, its isolation in an apparently homogeneous state made it possible to define more exactly many of its physical and catalytic properties. Until such purification was achieved, the possibility existed that this activity was in fact a function of another transaminase. The earlier studies had not settled the question of whether this transaminase contained a vitamin B_6 cofactor. Studies on the purified enzyme have established the nature of its interesting substrate specificity and have made it possible to approach the question of its metabolic significance.

Isolation and General Properties

Glutamine transaminase L was purified from the soluble fraction of rat liver homogenates by a procedure involving selective heat denaturation of impurities, ammonium sulfate fractionation, and chromatography on DE-52 and hydroxylapatite.[31] The isolated enzyme, purified about 900-fold, was found to be homogeneous on polyacrylamide gel electrophoresis and ultracentrifugation ($S_{20,w}$ = 6.25S). Gel filtration studies indicated that the enzyme has a molecular weight of about 110,000, and polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate indicated a

single component of molecular weight about 54,000, suggesting that the enzyme is composed of two apparently identical subunits. It is of significance, especially in view of the suggestion that transamination of glutamine might be catalyzed by other transaminases,[26] that the purified rat liver glutamine transaminase was found to be completely devoid of glutamate-aspartate, glutamate-alanine, and α-keto acid-ω-amidase activities.

Earlier studies on the glutamine transaminase activity of liver homogenates from vitamin B_6-deficient rats were consistent with the view that glutamine transaminase is a vitamin B_6 enzyme;[41-43] however, the observations were of an indirect nature and some were inconclusive. Early indications that the cofactor is tightly bound to the enzyme have been supported by studies on the highly purified rat liver enzyme. The purified enzyme exhibits a spectrum characteristic of pyridoxal 5′-phosphate enzymes; thus, it has absorbance maxima at 278 and 415 nm (in 5 m*M* potassium phosphate buffer; pH 7.2).[31] Thus far, attempts to obtain a stable apoenzyme have been unsuccessful. Dialysis of the enzyme against solutions containing 20 m*M* L-glutamine, L-alanine, or L-cysteine, or storage of the enzyme in solutions containing 4 m*M* urea and 0.1 m*M* L-cysteine did not lead to loss of activity. Application of the phenylhydrazine procedure of Wada and Snell[44] for the determination of pyridoxal 5′-phosphate did not give a positive result. However, when the enzyme was treated with 2,4-dinitrophenylhydrazine, a hydrazone was obtained which had properties consistent with those expected of the pyridoxal 5′-phosphate hydrazone.[31] The enzyme was found to be inactivated after treatment with sodium borohydride, hydroxylamine, or isonicotinic acid hydrazide. Convincing evidence for enzyme-bound pyridoxal 5′-phosphate was obtained by application of the procedure of Dempsey and Christensen.[45] Thus, when the enzyme was reduced with sodium borohydride followed by hydrolysis with 6 *N* hydrochloric acid, ε-pyridoxyllysine could be identified in the hydrolysate. The collected findings indicate that the cofactor is more tightly bound than it is in many other vitamin B_6 enzymes. The possibility must be considered that the cofactor is covalently bound.

Catalytic Properties

Studies on the substrate specificity of highly purified soluble glutamine transaminase L are summarized in Table 1. In many respects the findings agree with earlier data obtained with less purified preparations of the enzyme, but several new observations were made. These data and those obtained with glutamine transaminase K (see below) make it possible to carry out the separate determination of glutamine transaminases L and K in crude tissue homogenates. The determination of glutamine transaminases in unpurified tissue preparations must be approached with caution

TABLE 1

Specificity of Purified Glutamine Transaminases L and K with Respect to α-Keto Acids[a]

α-Keto acid	Relative activities: Liver[b]	Kidney[b]
α-Keto-γ-ethiolbutyrate (5 m*M*)	250	
α-Keto-γ-methiolbutyrate (5 m*M*)	240	75
β-Mercaptopyruvate	195	
α-Ketoglutaramate (80 m*M*)	134	40
Glyoxylate	[100]	20
S-Methyl-β-mercaptopyruvate	80	
α-Ketoglutarate-γ-ethyl ester	77	23
α-Ketosuccinamate	62	
β-Hydroxypyruvate	50	28
Pyruvate	28	20
α-Ketobutyrate	24	21
α-Keto-*n*-caproate	24	42
α-Keto-γ-hydroxybutyrate	24	
Phenylpyruvate	17; <1[c]	[100][c]
α-Keto-δ-carbamidovalerate	10	18
α-Keto-δ-guanidinovalerate	6	
β-Sulfopyruvate	6	
Oxaloacetate	3	4
α-Ketoglutarate	3	3
α-Ketoisocaproate	3	18
α-Ketoisovalerate	2	22
Mesoxalate	Trace	
p-Hydroxyphenylpyruvate[c]	<1	65
Trimethylpyruvate	<0.1	<0.1
(D and L)-α-Keto-β-methylvalerate	<0.1	<0.1
β-Cyclohexylpyruvate[d]		47
α-Keto-*n*-valerate		24

[a] Adapted in part from Cooper and Meister (see References 31 and 35).

[b] The assay system (except as noted) contained 20 m*M* α-keto acid, 20 m*M* L-[^{14}C] glutamine, 50 m*M* Tris-HCl buffer (pH 8.4), and purified enzyme in a final volume of 0.1 ml. The rates of transamination were determined at 37°C by measuring the formation of [^{14}C] α-ketoglutaramate.[31]

[c] Assayed by the method of Kupchik and Knox;[33] the α-keto acid concentration was 0.4 m*M*.[35]

[d] Cooper, A. J. L., unpublished observation.

because of the likelihood that other transaminase and glutaminase activities are also present. It is evident that the overlap in specificity between the K and L glutamine transaminases tends to complicate their separate determination in crude tissue preparations. The presence of ω-amidase activity must also be considered. For example, in recent studies on transamination between glutamine and α-ketoisocaproate in crude tissue homogenates, the reaction was assayed by measuring α-ketoglutarate formation.[46-48] The validity of this assay method requires that a sufficient amount of ω-amidase activity be present. The choice of α-keto acid is also relevant. Thus, α-ketoisocaproate is a poor substrate for glutamine transaminase L, but a somewhat better substrate for glutamine transaminase K. However, since the activity of the K enzyme in rat liver is about 20% of that of the L enzyme, transamination between glutamine and α-ketoisocaproate will be catalyzed by both enzymes at comparable rates; thus with this α-keto acid substrate, both enzymatic activities will contribute substantially to the observed overall rate of transamination. Although this assay procedure does not distinguish between the two glutamine transaminase activities, a distinction can be made as discussed below.

Studies on the specificity of glutamine transaminase L show that the α-keto analogues of ethionine, methionine, cysteine, glycine, *S*-methylcysteine, asparagine, and serine are among the most active. High activity was observed with β-mercaptopyruvate, but this reaction was linear for only about 5 min under the conditions employed.[31] Substantial substrate inhibition was observed with the α-keto analogues of methionine and ethionine at concentrations greater than 5 m*M*. Evidence has also been obtained that the enzyme is irreversibly inhibited by β-mercaptopyruvate and α-keto-γ-methiolbutyrate; the mechanism of inhibition requires further study. Studies in which the amino acid specificity of the enzyme was examined with glyoxylate, pyruvate, and α-ketoglutaramate are summarized in Table 2. Glutamine was by far the most active substrate, but significant activity was observed also with γ-glutamylmethylamide, glutamic acid-γ-methyl ester, methionine, methionine sulfoxide, and ethionine. The reversibility of the glutamine transamination reaction was also shown in studies in which transamination between L-[^{14}C] glutamine and α-ketoglutaramate was measured. The equilibrium constants for the glutamine-glyoxylate, glutamine-pyruvate, and glutamine-α-keto-γ-methiolbutyrate reactions were determined in Tris buffer at pH 8.4; the respective values were 1.8, 1.0, and 0.2.[31] In these determinations the observed equilibrium constants were corrected for the concentration of the open-chain form of α-ketoglutaramate (0.3% of the total α-ketoglutaramate[30]). In agreement with observations on other transamination reactions, the equilibrium constants are not far from unity.

Data on the specificity and apparent affinity of the enzyme for substrates are summarized in Table 3. It seems notable that although the α-keto acid analogues of cysteine, glycine, asparagine, serine, alanine, α-aminobutyrate, and norleucine are moderate-to-good substrates, the corresponding amino acids are much less active, i.e., they are no more than about 2% as active as glutamine when examined at a concentration of 0.02 *M*. The K_m values for alanine and glycine are very much higher than those for the other substrates studied. It therefore appears that α-amino and α-keto acid substrates that have side chains of the type $-CH_2CH_2X$, where X = $CONH_2$, $CONHCH_3$, COOR, $S(O)CH_3$, SCH_3, and SC_2H_5, effectively interact with the enzyme. However, a number of compounds that lack such a side chain are also active, but only in the α-keto acid form. The data suggest that the binding of some substrates to the enzymes (e.g., glutamine, methionine) involves the carboxyl group as well as the moiety attached to C-4 of these molecules. Clearly this moiety is not required for the binding of certain α-keto acid substrates (e.g., β-mercaptopyruvate, glyoxylate). That certain α-keto acids bind effectively as compared to the analogous α-amino acids may possibly be ascribed to a more favorable geometry about the α-carbon atom, perhaps planar rather than tetrahedral.

In the course of studies on various glutamine analogues, γ-glutamyl hydrazide was examined as a substrate of glutamine transaminase, and it was found that under the conditions employed the hydrazide reacted rapidly and spontaneously with α-keto acids such as glyoxylate and pyruvate to yield the corresponding γ-glutamyl hydrazones, i.e., 2-(γ-glutamyl)-1-α-keto acid hydrazones. It was subsequently discovered that L-γ-glutamyl hydrazones of α-keto acids are themselves good substrates of the enzyme.[49] Thus, when glutamine transaminase L was incubated with an L-γ-

TABLE 2

Amino Acid Specificity of Soluble Glutamine Transaminase L[a,b]

Amino acid	Relative rates		
	Glyoxylate	Pyruvate	α-Ketoglutaramate
L-Glutamine	[100]	28	134
L-γ-Glutamylmethylamide	63	17	
L-Glutamic acid-γ-methyl ester	57		20
L-Glutamic acid-γ-benzyl ester			6
L-Methionine	20	3	18
L-Ethionine	30		11
L-Methionine-SR-sulfoxide	36	5	17
L-Methionine sulfone	10	2	5
L-Methionine-SR-sulfoximine	12	3	
L-Methionine-SR-sulfoximine phosphate	6		
L-Homoserine	10		14
DL-Homocysteine (40 m*M*)	8		
L-Phenylalanine	8	1	4
L-2-Amino-4-oxo-5-chloropentanoic acid	4		
L-Asparagine	2		5
L-Cysteine	2		6
L-Alanine	2		2
L-Serine	2		4
Glycine	0.05	0.05	1
S-Methyl-L-cysteine	10		

[a]The reaction mixtures contained α-keto acid (pyruvate, 0.02 *M*; glyoxylate, 0.02 *M*; and α-ketoglutaramate, 0.09 *M*), amino acid (0.02 *M*, except as indicated), 0.05 *M* Tris-HCl buffer (pH 8.4), and enzyme. The initial rates of transamination are expressed relative to that for the glutamine-glyoxylate reaction, i.e., 300 units/mg of enzyme.

[b]The following amino acids were less than 1% as active as glutamine when tested (20 m*M*) with glyoxylate: L-glutamate, L-aspartate, L-ornithine, L-tyrosine (2 m*M*), L-cystine (2 m*M*), L-leucine, L-threonine, L-valine, L-isoleucine, L-arginine, L-cysteate, DL-2-amino-4-phosphonobutyrate, L-tryptophan, L-histidine, L-lysine, β-Alanine, L-2,4-diaminobutyrate, aminomalonate, L-α-aminobutyrate, and L-norvaline.

Adapted from Cooper, A. J. L. and Meister, A., *Biochemistry*, 11, 661, 1972.

glutamyl α-keto acid hydrazone (I, Figure 1), the substrate was converted to the corresponding L-α-amino acid and 3-hydroxy-tetrahydro-6-pyridazinone-3-carboxylic acid (II, Figure 1); the latter compound was found to undergo non-enzymatic dehydration in acid to yield 1,4,5,6-tetrahydro-6-pyridazinone-3-carboxylic acid (III, Figure 1). In the reaction in which glycine is formed from the γ-glutamyl hydrazone of glyoxylate, it was demonstrated that free glyoxylate is not formed during the reaction, nor does added free glyoxylate equilibrate with γ-glutamyl hydrazone-bound glyoxylate. The rates of reaction observed with a series of γ-glutamyl α-keto acid hydrazones paralleled those of the corresponding glutamine α-keto acid transamination reactions. In most instances the rates found with the γ-glutamyl α-keto acid hydrazones were greater than those of the corresponding glutamine α-keto acid reactions. For example, the rate observed with the γ-glutamyl hydrazone of α-ketoisovalerate was about ten times that observed for the glutamine α-keto-isovalerate reaction. The available data indicate that the transamination reaction with γ-glutamyl hydrazones of α-keto acids involves a transfer of the α-amino group of the glutamyl moiety to the α-keto acid moiety of the molecule without intermediate formation of a free α-keto acid. It would appear that the glutamyl moiety of the γ-glutamyl α-keto acid hydrazone, which may be regarded as a monosubstituted amide, binds to the portion of the active site that normally binds glutamine, and that the α-keto acid moiety subsequently moves into the α-keto acid binding site.

TABLE 3

Summary of Data on the Specificity of Soluble Glutamine Transaminase L

Parent amino acid substrate	Structure of amino acid substrate or α-keto analogue $R=^{-}OOC-\overset{O}{\overset{\|}{C}}-$ or $R=^{-}OOC-\overset{+NH_3}{\overset{\|}{CH}}-$	Relative rates: Amino acid + glyoxylate[a]	Relative rates: Glutamine + α-keto acid[b]	Approximate K_m value (m*M*): Amino acid	Approximate K_m value (m*M*): Keto acid
L-Glutamine	$R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-NH_2$	[100]	54	2	0.2
L-γ-Glutamylmethylamide	$R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-NHCH_3$	63			
L-Glutamic acid-γ-methyl ester (ethyl)	$R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$ (or OC_2H_5)	57	(31)	4	
L-Methionine-SR-sulfoxide	$R-CH_2-CH_2-\overset{O}{\overset{\|}{S}}-CH_3$	36		2	
L-Ethionine	$R-CH_2-CH_2-S-CH_2CH_3$	30	[100]		
L-Methionine	$R-CH_2-CH_2-S-CH_3$	20	96	2	3
L-Methionine-SR-sulfoximine	$R-CH_2-CH_2-\overset{O}{\overset{\|}{\underset{\|}{S}}}-CH_3$ NH	12			
L-Methionine-sulfone	$R-CH_2-CH_2-\overset{O}{\overset{\|}{\underset{\|}{S}}}-CH_3$ O	10			
L-Homoserine	$R-CH_2-CH_2-OH$	10	10		
S-Methyl-L-cysteine	$R-CH_2-S-CH_3$	10	32		

TABLE 3 (continued)

Summary of Data on the Specificity of Soluble Glutamine Transaminase L

Parent amino acid substrate	Structure of amino acid substrate or α-keto analogue O +NH$_3$ R=$^-$OOC–C– or R=$^-$OOC–CH–	Relative rates: Amino acid + glyoxylate[a]	Relative rates: Glutamine + α-keto acid[b]	Approximate K_m value (m*M*): Amino acid	Approximate K_m value (m*M*): Keto acid
DL-Homocysteine	R–CH_2–CH_2–SH	8			
L-Phenylalanine	R–CH_2–C_6H_5	8	17		
L-Cysteine	R–CH_2–SH	2	78		5
Glycine	R–H	0.05	40	>1000	0.7
L-Asparagine	R–CH_2–$CONH_2$	2	25		
L-Serine	R–CH_2–OH	2	20		8
L-Alanine	R–CH_3	2	11	>1000	11
L-α-Aminobutyrate	R–CH_2–CH_3	<1	10		
L-Norleucine	R–CH_2–CH_2–CH_2–CH_3	<1	10		

[a]From Table 1.
[b]Calculated from data given in Table 2.

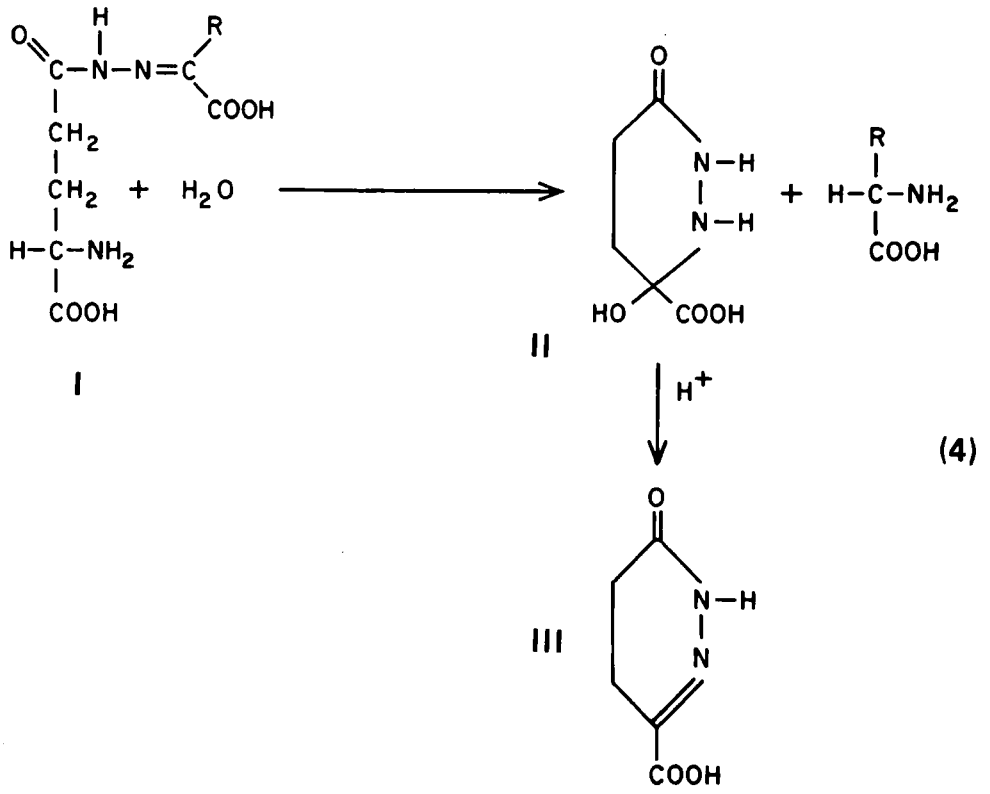


FIGURE 1. Transamination of L-γ-glutamyl hydrazones of α-keto acids catalyzed by glutamine transaminase L. (From Cooper, A. J. L. and Meister, A., *J. Biol. Chem.*, 248, 8489, 1973. With permission.)

Several pathways for the action of rat liver glutamine transaminase on the γ-glutamyl hydrazone of α-keto acids are conceivable (Figure 2). Thus, it may be postulated that the substrate undergoes Schiff base formation with enzyme-pyridoxal 5′-phosphate to yield an aldimine (I) and then the corresponding ketimine (II). Attack of the hydrazone nitrogen atom of II on the α-carbon atom (Reaction 2) would yield III, which by attack of water displacing pyridoxamine and attack of pyridoxamine on the α-carbon atom of the keto acid moiety would give IV. Prototropic shift (Reaction 4) would give the pyridazinone and VIII, which would be expected to undergo specific stereochemical rearrangement to form the optically active aldimine (not shown). On hydrolysis, this would yield an L-amino acid and enzyme-pyridoxal 5′-phosphate. The ketimine (II) might hydrolyze (Reaction 8) to yield V; concomitant attack of the hydrazone nitrogen on the α-carbon atom of the glutarate chain and of the pyridoxamine nitrogen atom on the α-carbon atom of the α-keto acid moiety (Reaction 9) would yield IV. V might be converted to VI by attack of the pyridoxamine nitrogen atom on the α-carbon atom of the α-keto acid (Reaction 5), and VI would then yield VIII and the pyridazinone via intermediate formation of α-ketoglutaric acid γ-hydrazide (VII). Still other pathways are conceivable as discussed elsewhere.[49] The accumulated data suggest that the γ-glutamyl α-keto acid hydrazones bind initially at the glutamine site of the enzyme, followed by transformations associated with movement of the attached α-keto acid moiety into the active site, and that the tendency of the intermediates to cyclize provides the driving force for the reaction.

This novel intramolecular transamination reaction catalyzed by soluble glutamine transaminase L has not as yet been observed with other transaminases. Thus, glutamate-alanine transaminase, glutamate-aspartate transaminase, and glutamine-α-keto acid transaminase K (see below) were found to be inactive toward L-γ-glutamyl hydrazones of α-keto acids. This offers a useful means for distinguishing between the activities of glutamine transaminase L and glutamine transaminase K. The γ-glutamyl hydrazones of α-keto

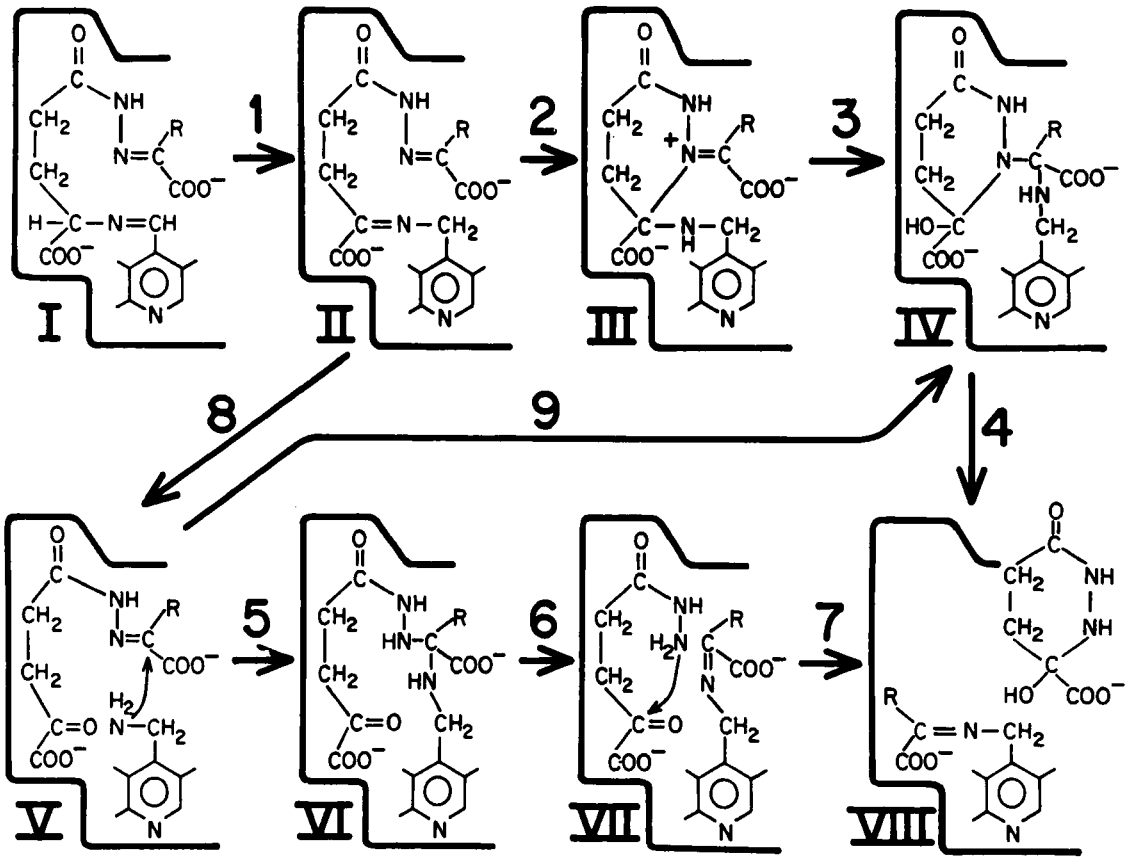
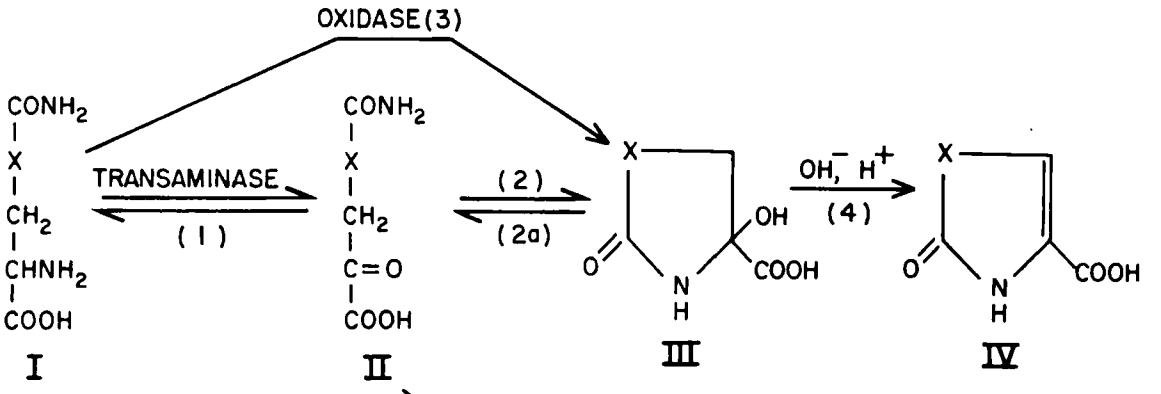


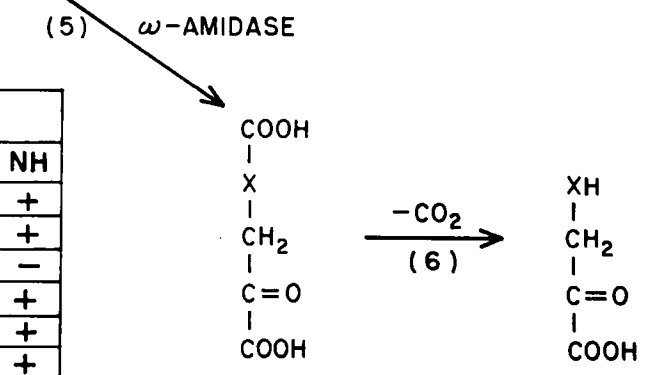
FIGURE 2. Possible pathways for the action of glutamine transaminase L on the γ-glutamyl hydrazones of α-keto acids. See text. (From Cooper, A. J. L. and Meister, A., *J. Biol. Chem.*, 248, 8489, 1973. With permission.)

acids are an interesting new type of α-keto acid derivative, which may prove useful in various studies on α-keto acids. Thus, they react with ninhydrin and exhibit high characteristic absorbance in the ultraviolet.[49]

Other interesting analogues of glutamine which have been examined as substrates of soluble glutamine transaminase L include those in which the 4-methylene moiety of glutamine is replaced by S, O, or NH.[50] Thus, it was observed that L-albizziin (L-α-amino-β-ureidopropionic acid, I, Figure 3, where X = NH), *S*-carbamyl-L-cysteine, and *O*-carbamyl-L-serine can effectively replace L-glutamine in the transamination reaction. In each of these reactions the α-keto acid product formed was found to cyclize to a lactam (III, Figure 3) analogous in structure to the cyclic form of α-ketoglutaramic acid. The data obtained indicate that the initial products of transamination of albizziin, *S*-carbamylcysteine, and *O*-carbamylserine are the corresponding α-keto acids which were found to be converted by ω-amidase, followed by spontaneous decarboxylation, to β-aminopyruvate, β-mercaptopyruvate, and β-hydroxypyruvate, respectively. These enzymatic and nonenzymatic reactions are summarized in Figure 3.

The finding that albizziin can effectively replace glutamine in the glutamine α-keto acid transamination reaction catalyzed by glutamine transaminase L provides a highly sensitive assay for this activity. Thus, transamination between albizziin and glyoxylate yields glycine and 2-imidazolidone-5-hydroxy-5-carboxylic acid (III, Figure 3, where X = NH); the latter compound undergoes dehydration in acid or base (Reaction 4, Figure 3, X = NH) to form 2-imidazolinone-5-carboxylic acid, which exhibits intense ultraviolet absorbance. A number of kinetic studies on the transamination reaction between L-albizziin were carried out in a manner similar to those previously done on glutamate-aspartate transaminase.[49] The kinetic findings on glutamine transaminase L were found to be similar to those found earlier with





REACTION	X			
	CH_2	O	S	NH
1	+	+	+	+
2	+	+	+	+
2a	+	−	−	−
3	+	+	+	+
4	−	−	+	+
5	+	+	+	+
6	−	+	+	+

FIGURE 3. Enzymatic and nonenzymatic reactions of glutamine and glutamine analogues. (From Cooper, A. J. L. and Meister, A., *J. Biol. Chem.*, 248, 8499, 1973. With permission.)

glutamate-aspartate transaminase[51,52] and are consistent with a ping-pong mechanism in which the substrates bind to and react separately with the enzyme. However, such a mechanism does not necessarily mean that all of the substrates attach to the enzyme at the same binding site. While with glutamate-aspartate transaminase it is plausible to postulate that all four substrates bind at the same enzyme site since the substrates are of similar size and charge and can assume similar conformations, if glutamate and α-ketoglutarate bind to this enzyme in their respective extended conformations, it would appear that an additional binding site might be needed for the β-carboxyl groups of aspartate and oxaloacetate. The possibility must be considered that the substrate binding sites overlap and that only a portion of each substrate, e.g., the α-carboxyl group, binds to the same enzyme site. Such an interpretation may be applicable also to transaminases that catalyze reactions between amino and keto acids of markedly different structure, such as the glutamine transaminases which interact effectively with two types of substrates whose structures differ considerably. In other words, the carboxyl groups of substrates such as glyoxylate and glutamine might bind to the same enzyme site; thus, the binding of one substrate would prevent binding of the other. It is also possible to envision an active site in which the pyridoxal 5′-phosphate is bound at the bottom of a cleft in the enzyme and in which the substrates bind to separate binding sites on either side of the cleft. The binding of one substrate might provide steric hindrance sufficient to prevent binding of the other substrate. Indeed, other factors might influence the binding of substrates to transaminases, e.g., conformational changes in the enzyme and alterations in the spacial relationships between the cofactor and the enzyme.

SOLUBLE GLUTAMINE TRANSAMINASE K FROM RAT KIDNEY AND LIVER

Isolation and Properties

Evidence that kidney contains a glutamine transaminase different from the predominant

soluble glutamine transaminase of liver (glutamine transaminase L) was obtained in studies of the comparative substrate specificities of liver and kidney homogenates as discussed above. The predominant soluble glutamine transaminase of rat kidney (glutamine transaminase K) was purified by a procedure involving selective denaturation of impurities, ammonium sulfate fractionation, and chromatography on DE-52 and on hydroxylapatite.[35,53] Polyacrylamide gel electrophoresis of the purified enzyme revealed a major band containing about 85% of the total protein and all of the enzyme activity, as well as three enzymatically inactive smaller bands. Kidney glutamine transaminase K exhibited a mobility significantly different from that of glutamine transaminase L when subjected to polyacrylamide gel electrophoresis at pH 8.9. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate revealed a major band which exhibited the same mobility as that found under these conditions with purified rat liver glutamine transaminase L. It thus appears that the soluble glutamine transaminases K and L have similar molecular weights and subunit structures. Purified kidney glutamine transaminase K exhibited absorbance maxima at 278 and 415 nm. At no time during purification of the enzyme was activation observed on addition of pyridoxal 5′-phosphate. Dialysis of the purified kidney enzyme against 20 m*M* L-glutamine or L cysteine did not lead to loss of enzymatic activity. Evidence for the presence of pyridoxal 5′-phosphate was obtained by application of the method of Dempsey and Christensen;[45] thus, ε-pyridoxyllysine was found in the hydrolysate of the reduced enzyme.

Catalytic Properties

Studies on the specificity of glutamine transaminase K indicate a significantly different pattern than found for soluble glutamine transaminase L (Table 1). The most active α-amino acid substrates are glutamine, methionine, phenylalanine, and tyrosine, and the most active α-keto acid substrates are the α-keto acid analogues of these amino acids. It is notable that glutamine transaminase K exhibits only slight activity toward the γ-glutamyl hydrazones of α-keto acids. However, like liver glutamine transaminase L, the K enzyme catalyzes transamination between glutamine and a wide variety of α-keto acids. Other differences between the two enzymes include the findings that α-keto-γ-methiolbutyrate, α-ketoglutaramate, α-ketoglutarate-γ-ethyl ester, and β-hydroxypyruvate are more active with glutamine transaminase L than with the K enzyme. It is also of note that α-ketoisovalerate, only slightly active with glutamine transaminase L, is substantially active with the K enzyme. The apparent K_m value for phenylpyruvate is less than 0.1 m*M* and considerable substrate inhibition occurs with concentrations of phenylpyruvate greater than 4 m*M*. Relatively low apparent K_m values were also obtained with *p*-hydroxyphenylpyruvate, α-ketoglutaramate, and α-keto-γ-methiolbutyrate; these α-keto acids and phenylpyruvate showed the highest V_{max} values in transamination with L-glutamine (Table 4). Soluble glutamine transaminase K was also found in rat liver. In preparations carried through the last steps of the isolation procedure for soluble glutamine transaminase L from rat liver, partial separation of the glutamine-glyoxylate transaminase activity from the glutamine-phenylpyruvate activity was achieved on chromatography on DE-52, and a clear separation of the two activities was obtained by the subsequent chromatography on hydroxylapatite. Rat kidney was found to contain a small amount of glutamine transaminase L.[35]

MITOCHONDRIAL GLUTAMINE TRANSAMINASES

It was initially believed that virtually all of the glutamine transaminase activity of mammalian tissues is associated with the cytosol. However, Yoshida[27] reported partial purification of glutamine transaminase from rat liver mitochondria and his data, when interpreted in the light of later work,[31,35] suggest that this enzyme preparation contained predominantly glutamine transaminase L. Cooper and Meister[35] found considerable glutamine transaminase activity (L and K) in rat liver mitochondria and discovered that these activities can be readily solubilized by rapid freeze-thawing or by sonication. Solubilized mitochondrial glutamine transaminase L and soluble glutamine transaminase L exhibit different mobilities on polyacrylamide gel electrophoresis at pH 8.9. Rat kidney mitochondrial glutamine transaminase K exhibited the same mobility as that of rat liver mitochondrial glutamine transaminase K, but a different mobility than that of soluble glutamine transaminase K and both mitochondrial and sol-

TABLE 4

Apparent K_m and V_{max} Values for Soluble Kidney Glutamine Transaminase K

Substrate	K_m (m*M*)	V_{max}
Glyoxylate	4.8	0.35
Pyruvate	27	0.39
α-Ketobutyrate	18	0.26
α-Keto-*n*-valerate	7.8	0.32
α-Keto-*n*-caproate	8.7	0.64
α-Keto-γ-methiolbutyrate	0.92	1.7
α-Ketoglutaramate	0.10	1.2
Phenylpyruvate	<0.1	1.7
p-Hydroxyphenylpyruvate	<0.1	1.1
L-Glutamine	1.4	1.9
L-Methionine	4.2	3.4
L-Ethionine	4.4	2.7
L-Phenylalanine	0.58	0.97

Adapted from Cooper, A. J. L. and Meister, A., *J. Biol. Chem.*, 249, 2554, 1974.

uble glutamine transaminases L. In the course of these studies, it was found that soluble transaminase K isolated from kidney exhibited mobility which was identical to that of the soluble K enzyme from liver. Further work needs to be done on the purification and characterization of the mitochondrial liver and kidney glutamine transaminases, in order to further document the differences and similarities found between these and the corresponding soluble enzymes with respect to substrate specificity and electrophoretic behavior. It is notable, as discussed below, that other tissues (e.g., brain) also contain appreciable mitochondrial glutamine transaminase activity.

TISSUE DISTRIBUTION OF GLUTAMINE TRANSAMINASES

As stated above, phenylpyruvate is a poor substrate of glutamine transaminase L, and at the low concentration of phenylpyruvate employed in assays for glutamine-phenylpyruvate transaminase, virtually no activity is detected with purified glutamine transaminase L preparations. Therefore, the glutamine-phenylpyruvate reaction may be used for the assay of glutamine transaminase K in tissue preparations. Similarly, the γ-glutamyl hydrazone of glyoxylate is an excellent substrate for glutamine transaminase L, but it is not a significantly active substrate of glutamine transaminase K. Thus, the two glutamine transaminase activities can be distinguished in tissue homogenates by appropriate assays. Rat kidney contains mainly the soluble and mitochondrial K isozymes. Liver contains soluble and mitochondrial L isozymes and smaller but significant amounts of soluble and mitochondrial K isozymes. Some data on the tissue distribution of the glutamine transaminases in liver, kidney, brain, and cardiac muscle are summarized in Table 5. Thus far, glutamine transaminase activity has not been detected in homogenates of other tissues, but further studies along these lines would be desirable. The findings given in Table 5 are consistent with the observation of Sugiura[36] that rabbit brain preparations exhibit glutamine-pyruvate transaminase activity; glutamine transaminase activity has also been found in the brains of other species.[35,54-60] The available data indicate that most, if not all, of the glutamine transaminase activity of brain is mitochondrial; this conclusion is strongly supported by studies in which rat brain mitochondria were isolated by means of a discontinuous sucrose gradient and examined for glutamine transaminase activity with appropriate enzyme markers for the cytosol and mitochondria.[61] Van Leuven[59,60] purified the enzyme from rat brain and found that it has a molecular weight and subunit structure similar to the glutamine transaminases previously purified from rat liver[31] and kidney.[35] Purified brain mitochondrial glutamine transaminase exhibits high activity toward glutamine and methionine and the α-keto analogues of methionine and phenylalanine.[60,61]

α-KETO ACID-ω-AMIDASE

Introduction

α-Ketoglutaramic acid and α-ketosuccinamic acid were first prepared because of their possible significance as intermediates in the α-keto acid-stimulated deamidation reactions of glutamine and asparagine, respectively.[14] α-Ketoglutaramate was shown to exist in solution in equilibrium between the reactive open-chain form and a cyclic form (later[23] shown to be 2-hydroxy-5-oxoproline). α-Ketosuccinamic acid can be obtained in the open-chain form or in an unreactive dimeric form.[14,24] The open-chain forms of these α-keto acids are substrates of α-keto acid-ω-amidase, an enzyme present in considerable amounts in rat liver and kidney.[14,20,21] α-Keto acid-ω-amidase activity was also found in other

TABLE 5

Distribution of Glutamine Transaminase and ω-Amidase Activities in Various Rat Tissues[a]

Tissue	Soluble ω-amidase	Mitochondrial ω-amidase	Soluble glutamine transaminase L	Soluble glutamine transaminase K	Mitochondrial glutamine transaminase L	Mitochondrial glutamine transaminase K
Liver	179,[b] 1310,[c] 340[d]	135[d]	83[f]	25[h]	27[i]	15[k]
Kidney	136,[b] 726,[c] 390[d]	40[d]	~5[g]	135[h]		20[k]
Brain	24,[b] 19,[c] 24[e]					8,[j] 7[k]
Heart	82,[b] 74[d]	8[d]				

[a]Specific activity; μmol/hr/g at 37°C.
[b]α-Ketoglutaramate (15 m*M*); pH 9.0.[14]
[c]α-Ketoglutaramate (20 m*M*); pH 8.4.[58]
[d]α-Ketoglutaramate (5 m*M*); pH 8.4.[31]
[e]α-Ketoglutaramate (20 m*M*); pH 8.4.[61]
[f]Glutamine (20 m*M*), glyoxylate (20 m*M*); pH 8.4.[31]
[g]Assayed with the γ-glutamyl hydrazone of glyoxylate.[31]
[h]Glutamine (20 m*M*), phenylpyruvate (0.4 m*M*); pH 8.5.[35]
[i]Glutamine (20 m*M*), glyoxylate (20 m*M*); ph 8.4. This value is tentative since the activity may overlap with the mitochondrial glutamine transaminase K. It is the only one of the four known glutamine transaminases that remains to be purified.
[j]Glutamine (20 m*M*), glyoxylate (20 m*M*); pH 9.0.[61]
[k]Glutamine (10 m*M*), phenylpyruvate (10 m*M*); pH 9.0.[59]

mammalian tissues including spleen, pancreas, cardiac muscle, skeletal muscle, testes, brain, Novikoff hepatoma, mouse liver, yeast, *Escherichia coli, Streptococcus faecalis,* spinach leaves, and lettuce leaves. The ω-amidase was purified about 40-fold from the soluble fraction of rat liver, and the purified enzyme was shown to be free of glutaminase and asparaginase, as well as glutamine and asparagine transaminase activities.[14,21] In addition to α-ketoglutaramate and α-ketosuccinamate, the enzyme also hydrolyzes a number of other amides including succinamate, glutaramate, and succinyl hydroxamate.[20]

Highly Purified Soluble Rat Liver ω-Amidase

Hersh[30,62] purified ω-amidase about 140-fold from the soluble fraction of rat liver homogenates by a procedure involving ammonium sulfate fractionation, and chromatography on DEAE-cellulose, CM-Sephadex, Sephadex G-75, and hydroxylapatite. The purified enzyme was found to be homogeneous on polyacrylamide gel electrophoresis. The molecular weight, determined by sedimentation equilibrium, was found to be about 58,000; $S_{20,w}$ = 4.7S. The isolated enzyme was dissociated into subunits of molecular weight approximately 27,000 to 28,000 by treatment with 7 *M* guanidine hydrochloride or 7 *M* urea.

In confirmation of earlier studies,[14,20,21] the enzyme was found to catalyze hydrolysis of α-ketoglutaramate, α-ketosuccinamate, glutaramate, succinamate, and succinyl hydroxamate, as well as hydroxamate formation from hydroxylamine and glutaramate and succinamate.[30,62] The enzyme was also found to catalyze methylamide formation as measured by incorporation of [^{14}C] methylamine into several amide and ester substrates, such as the monomethyl and monoethyl esters of α-ketoglutarate, succinate, and glutarate.

Hersh[30] reported that ω-amidase does not hydrolyze *N*-methyl-α-ketoglutaramate and also that *N*-methyl succinamate and *N*-ethyl succinamate are not hydrolyzed by the enzyme. While these observations could be explained by assuming that the enzyme does not act on *N*-methyl substrates, earlier studies showed that L-γ-glutamylmethylamide transaminates readily with pyruvate in a system containing glutamine transaminase and ω-amidase to yield products which include methylamine.[18] In addition, rat liver ω-amidase was found to hydrolyze *N*-methyl-α-ketoglutaramate at a slow but definite rate;[14] recent studies with highly purified ω-amidase gave similar results (Cooper, A. J. L., unpublished data, 1976). It was shown that human kidney slices can convert L-γ-glutamylmethyl [^{14}C] amide to

[^{14}C] methylamine in the presence, but not in the absence, of added pyruvate;[63,64] similar results were obtained in studies with dog kidney.[65] These results can be readily explained in terms of intermediate formation of *N*-methyl-α-ketoglutaramate, followed by hydrolysis of the latter to α-ketoglutarate and methylamine. Studies on the preparation and properties of *N*-methyl-α-ketoglutaramate have shown that this compound exhibits a marked tendency to cyclize to *N*-methyl-2-hydroxy-5-oxoproline.[14] *N*-Methyl-α-ketoglutaramate does not readily form a 2,4-dinitrophenylhydrazone, nor is it decarboxylated when incubated with hydrogen peroxide. It seems probable that the open-chain form of this keto acid cyclizes rapidly during preparation; however, the open-chain form seems to be formed as a transient intermediate during preparation of this compound by enzymatic oxidation of L-γ-glutamylmethylamide, since the action of L-amino acid oxidase on L-γ-glutamylamide yields carbon dioxide in the absence of catalase.[14] It is of interest that an enzyme similar to mammalian α-keto acid-ω-amidase has been obtained from a pseudomonad which is reported to catalyze the slow hydrolysis of *N*-methyl-α-ketoglutaramate to α-ketoglutarate and methylamine;[66-68] it also catalyzes the reverse reaction, yielding almost stoichiometric amounts of cyclic product from α-ketoglutarate and methylamine. Presumably, cyclization of *N*-methyl-α-ketoglutaramate provides the driving force for this amide bond synthesis. Hersh[66] proposed that the hydrolysis of the cyclic form of *N*-methyl-α-ketoglutaramate proceeds by a nonenzymatic conversion of the cyclic form to the open-chain form which serves as the actual substrate of the reaction. It is of interest that the pseudomonas enzyme hydrolyzes α-ketoglutaramate but that it does not hydrolyze α-ketosuccinamate, glutaramate, or succinamate, all of which are substrates for rat liver ω-amidase.

Mitochondrial α-Keto Acid-ω-Amidase

When homogenates of rat liver are centrifuged, virtually all of the ω-amidase activity is found in the soluble supernatant fraction. This finding, and the observation that the pellet contains only a small fraction of the activity found in the supernatant, led to the conclusion that ω-amidase is solely a cytosolic activity. This view was apparently supported by the finding that suspensions of intact mitochondria exhibited only weak ω-amidase activity. However, after the mitochondria were subjected to freeze-thawing or sonication, the total ω-amidase activity found was equivalent to about 40% of that present in the soluble fraction[35] (Table 5). The findings are in accord with earlier observations by Yoshida,[29] who reported that rat liver mitochondria exhibit ω-amidase activity. Studies in which mitochondrial and soluble rat liver fractions were subjected to polyacrylamide gel electrophoresis have shown that the ω-amidases of these sources have different mobilities.

PHYSIOLOGICAL ROLE OF THE GLUTAMINE TRANSAMINASE-ω-AMIDASE PATHWAY

The studies reviewed here have shown that the transamination of glutamine is catalyzed by several separate transaminases, which exhibit high affinity for glutamine and certain α-keto acids. The extensively studied glutamate-aspartate and glutamate-alanine transaminase and the transaminases that catalyze various α-ketoglutarate-amino acid transamination reactions (e.g., glutamate-phenylalanine, glutamate-branched chain amino acid) do not catalyze transamination of glutamine at significant rates. The work on purified glutamine transaminases has made it possible to examine their properties, including amino acid and α-keto acid specificity, more fully than was previously possible, and the data obtained support the early suggestion that transamination is one of the major metabolic functions of glutamine.[17,69] Although both the L and K enzymes exhibit relatively broad specificity with respect to α-keto acids, and a somewhat narrower specificity with respect to amino acids, glutamine is clearly the most active amino acid substrate. Methionine and its α-keto acid analogue are highly active substrates of both glutamine transaminase K and glutamine transaminase L, and phenylpyruvate and *p*-hydroxyphenylpyruvate are excellent substrates of glutamine transaminase K.

By obtaining these enzymes free of ω-amidase activity, it has been possible to study transamination reactions leading to the formation of glutamine and to obtain the equilibrium constants for several of the transamination reactions. The

reactions catalyzed by the glutamine transaminases are, like other transamination reactions, freely reversible and have equilibrium constants close to unity. Nevertheless, in contrast to reactions such as those catalyzed by glutamate-aspartate transaminase, it appears probable that under physiological conditions, the glutamine transaminase reaction proceeds in the direction of glutamine utilization rather than its synthesis. It is notable in this respect that the glutamine transaminases exhibit a much higher affinity for certain α-keto acids than for the corresponding amino acids. The steady state concentrations of α-ketoglutaramate in mammalian tissues are quite low,[70] and there is no evidence for the formation of α-ketoglutaramate except by pathways from glutamine. Glutamine might be oxidatively deaminated to α-ketoglutaramate in mammalian tissues, but the rate of this reaction, at least as catalyzed by glutamate dehydrogenase,[71] seems to be of such a low order of magnitude as to be insignificant physiologically. The rapid nonenzymatic cyclization of α-ketoglutaramate to 2-hydroxy-5-oxoproline effectively drives the glutamine transamination reaction in the direction of glutamine utilization in vitro. Under physiological conditions in the presence of α-keto acid-ω-amidase, the open-chain form of α-ketoglutaramate formed in the transamination of glutamine probably undergoes rapid enzyme-catalyzed hydrolysis. Transamination of glutamine is therefore essentially irreversible under physiological conditions, and it may therefore be concluded that its metabolic role must be associated with utilization of glutamine, formation of ammonia, and the utilization of certain α-keto acids for the synthesis of the corresponding amino acids.

The glutamine transaminases may play a role in the regulation of tissue glutamine levels. However, it is evident that any of the many pathways of glutamine utilization that exist within cells may also influence glutamine levels. One might think that the most likely control point for glutamine would be glutamine synthetase, and indeed it has been suggested that a regulatory mechanism exists in which products produced by glutamine transamination can inhibit the synthesis of glutamine; thus, rat liver glutamine synthetase is inhibited by L-alanine, glycine, and L-serine.[72-74]

It has also been suggested that the glutamine transaminase-ω-amidase pathway functions in ammoniagenesis. It has long been known that the amide nitrogen atom of glutamine is a major source of urinary ammonia.[75,76] A substantial fraction of urinary ammonia is also derived from the α-amino group of glutamine.[76] There is now a large body of evidence that renal phosphate-dependent glutaminase is a major catalyst for the production of urinary ammonia,[77] and it seems probable that the action of glutamate dehydrogenase on glutamate derived from glutamine also plays a significant role in ammonia formation. Nevertheless, there are data suggesting that the glutamine transaminase-ω-amidase pathway may function in renal ammonia formation,[77,78] and one cannot exclude the possibility that it may contribute to some extent to the total production of urinary ammonia. Thus, α-keto acids might serve as catalysts for the deamidation of glutamine, as shown in Reactions 5 to 8. Such a pathway, which would require an additional transamination reaction (Reaction 7), has been suggested by the finding of increased transaminase K activity in the kidneys of acidotic rats and the presence of a substantial concentration of phenylpyruvate (about 0.4 m*M*) in rat kidney.[79,80] Ammoniagenesis does not seem, however, to be the major function of the glutamine transaminase-ω-amidase system in liver, an organ which is geared to the utilization of ammonia rather than to its production. However, one must also consider the idea that this α-keto acid-stimulated system might function to produce ammonia at particular sites within the liver and under physiological conditions in which its utilization for α-amino group or amide group synthesis is metabolically desirable.

An attractive idea concerning the physiological role of the glutamine transaminase-ω-amidase pathway is that it functions in the conversion of

$$\alpha\text{-Keto acid} + \text{glutamine} \longrightarrow \alpha\text{-amino acid} + \alpha\text{-ketoglutaramate} \quad (5)$$

$$\alpha\text{-Ketoglutaramate} + H_2O \longrightarrow \alpha\text{-ketoglutarate} + NH_3 \quad (6)$$

$$\alpha\text{-Amino acid} + \alpha\text{-ketoglutarate} \longrightarrow \text{glutamate} + \alpha\text{-keto acid} \quad (7)$$

$$\text{SUM: Glutamine} + H_2O \longrightarrow \text{glutamate} + NH_3 \quad (8)$$

α-keto acids to amino acids. The degradative metabolism of many amino acids proceeds via the corresponding α-keto acids, but certain amino acids, e.g., phenylalanine and methionine, are metabolized principally by routes not involving the analogous α-keto acids. The amino acid levels of various mammalian tissues probably fluctuate, depending on nutritional and other factors, and it is therefore likely that certain amino acids are temporarily accumulated in amounts that exceed those necessary for the synthesis of proteins and other products derived from amino acids. Such accumulated amino acids may be converted to the corresponding α-keto acids by α-ketoglutarate-amino acid transamination reactions. It has long been known that liver preparations can catalyze transamination between α-ketoglutarate and a wide variety of amino acids,[81-83] and recent studies have shown that amino acids such as phenylalanine, tyrosine, and methionine are substrates for certain forms of glutamate-aspartate transaminase.[84,85] Rat liver mitochondrial "tyrosine transaminase activity" (identical with mitochondrial glutamate-aspartate transaminase) acts on phenylalanine, methionine, and other amino acids.[84] Transamination of tyrosine is also catalyzed by a soluble enzyme, and this reaction is thought to be on the normal degradative pathway of tyrosine. However, *p*-hydroxyphenylpyruvate produced in excess in the mitochondria may evidently escape the action of the soluble *p*-hydroxyphenylpyruvate hydroxylase;[86] mitochondrial glutamine transaminase K could function in the reamination of this α-keto acid. It is evident that the carbon chains of such amino acids as phenylalanine, tyrosine, and methionine might be lost by excretion or degradation and thus become unavailable for protein synthesis if accumulations of these amino acids led to formation of their α-keto acid analogues by transamination. The metabolic importance of retaining these essential carbon chains is in accord with our postulate that the glutamine transaminases normally function in this "salvage" process. Such transamination reactions are driven by the removal of the α-keto acid amide product, and the metabolic balance tends to be further stabilized by the production of ammonia which can be incorporated into the amide group of glutamine. One can thus envision a process in which the glutamine transaminases serve as part of a homeostatic mechanism for the preservation of amino acid balance. In such a mechanism, transamination reactions might be metabolically coupled with the endergonic synthesis of glutamine, as shown in Reactions 9 to 12. The overall reaction involves a large free energy change and phenylalanine formation. Such a system emphasizes the metabolic importance of glutamine transaminase and glutamine synthetase in amino acid formation. Although ammonia produced in the deamidation of α-ketoglutaramate (Reaction 10) might be utilized directly for glutamate synthesis by the action of glutamate dehydrogenase, the apparent K_m value for NH_4^+ for this enzyme is sufficiently high (>20 m*M*) as to suggest that its physiological role in mammalian tissues is the deamination of glutamate rather than its synthesis. Glutamate synthase, an enzyme that catalyzes the glutamine- and TPNH-dependent reductive amination of α-ketoglutarate to glutamate according to Reaction 13, has been found in certain bacteria,[87-90] higher plants,[91] and yeasts.[92] Although it has not yet been found in mammalian tissues, its presence might explain the reported utilization of glutamine amide nitrogen (in preference to NH_4^+) for α-amino group synthesis in kidney particulate preparations.[93]

The possibility that the glutamine transaminases are physically linked with other enzymes, such as ω-amidase, has been considered. Although glutamine transaminase, ω-amidase, and perhaps other enzymes may be closely associated within the cell, there is as yet no direct evidence for

Phenylpyruvate + glutamine ⟶ α-ketoglutaramate + phenylalanine (9)

α-Ketoglutaramate ⟶ α-ketoglutarate + NH_3 (10)

Glutamate + NH_3 + ATP ⟶ glutamine + ADP + Pi (11)

SUM: Phenylpyruvate + glutamate + ATP ⟶ phenylalanine + α-ketoglutarate + ADP + Pi (12)

Glutamine + α-ketoglutarate + TPNH + H^+ ⟶ 2 glutamate + TPN^+ (13)

physical linkage. Studies on the transamination-deamidation of glutamine in the presence of phenylpyruvate by a crude rat kidney preparation failed to indicate evidence of linkage; thus, the disappearance of phenylpyruvate occurred much more rapidly than the appearance of ammonia.[70] In studies on rat brain, it was found that most of the glutamine transaminase is mitochondrial, whereas most of the ω-amidase activity is present in the cytosol.[61]

It is also conceivable that the glutamine transaminases play a role in transport phenomena. Thus, it is possible that certain amino acids are transported into or out of cells or intracellular organelles as the corresponding α-keto acids. In such a transport process the α-keto acid might be formed on one side of the membrane (or within it) and reaminated on the other.

The ideas considered above are consistent with a large body of data derived from a variety of approaches which indicate that there is extensive deamination and reamination of most of the amino acids in the mammal. These studies began with the work of Schoenheimer and colleagues, who found that when $^{15}NH_4^+$ or ^{15}N-labeled amino acids were administered to rats, the isotope appeared in almost all of the amino acids.[94-96] Later it was shown that the α-keto analogues of L-arginine, L-histidine, L-isoleucine (and L-alloisoleucine), L-leucine, L-methionine, L-phenylalanine, L-tyrosine, L-tryptophan, and L-valine could replace the corresponding L-amino acids in supporting the growth of rats (see Reference 97 for a summary of the literature).

Other studies which indicate in vivo conversion of α-keto acids to amino acids include findings on patients with phenylketonuria, in which administration of glutamine led to substantially decreased urinary excretion of phenylpyruvate.[98] This result is consistent with the occurrence of transamination between glutamine and phenylpyruvate, perhaps catalyzed by glutamine transaminase K, and indicates that a metabolic abnormality associated with accumulation of an α-keto acid can be partially corrected by a mechanism that evidently involves transamination. Recently, several attempts have been made to treat patients with "nitrogen accumulation diseases" with α-keto acids. Thus, patients with chronic renal failure, who accumulate urea and other nitrogen-containing compounds, were treated with mixtures containing the α-keto analogues of essential amino acids.[99-103,105,106] Such treatment led to decreased urea formation and clinical improvement, presumably associated with utilization of nitrogen for amination of the administered α-keto acids and the consequent promotion of protein synthesis by the amino acids thus formed. In similar studies on grossly obese patients undergoing a starvation regime, administration of α-keto acids decreased the nitrogen loss and was thought to improve the efficiency of utilization of amino acids because the decrease in urea formation persisted for several days after α-keto acid administration was stopped.[104] α-Keto acids have also been used in the therapy of patients with portal-systemic encephalopathy,[107] congenital hyperammonemias,[108] and in similar conditions. Presumably such therapy reduces accumulation of urea by diverting nitrogen from the urea pathway to amino acid synthesis by pathways involving transamination. The utilization of ammonia for these pathways in the liver involves the actions of carbamyl phosphate synthetase and glutamine synthetase, respectively. Increased transamination associated with α-keto acid administration would appear to lead to increased utilization of nitrogen for glutamate and glutamine formation, and decreased utilization of nitrogen by the urea cycle. There is no evidence that the administration of α-keto acids leads to decreased hydrolysis of urea in the intestine (by bacterial urease), nor is there evidence for an increase of such urea hydrolysis in nitrogen accumulation diseases. However, it is possible that some of the administered α-keto acids are transaminated to the corresponding amino acids by organisms of the intestinal flora.[109,110]

In studies in which isolated rat liver preparations were perfused with solutions containing the α-keto acid analogues of valine, isoleucine, leucine, methionine, and phenylalanine, increases were found in the corresponding amino acids.[111] The amount of extra amino acid formed from each of the five α-keto acids was in the order: methionine > leucine = phenylalanine + tyrosine > valine > isoleucine. It is of interest that this is approximately the order that would have been predicted if the two glutamine transaminases of liver were responsible for the amination of these α-keto acids. However, it is probable that the utilization of α-keto acids in such a system is catalyzed by several transaminases. Studies of metabolites in freeze-clamped liver have shown that there is a decrease in glutamine concentration after per-

fusion with α-keto acids; the decrease in glutamine was sufficient to account for the nitrogen that appeared in the amino acids concomitantly synthesized. These findings are consistent with the in vivo function of glutamine transaminases.

Recent studies by Vergara et al. have shown that α-ketoglutaramate accumulates in the cerebrospinal fluid of patients with hepatic coma.[40] In this work, α-ketoglutaramate was determined by an enzymatic procedure involving the coupled activities of α-keto acid-ω-amidase and glutamate dehydrogenase. The high levels of α-ketoglutaramate in the cerebrospinal fluid of such patients were also demonstrated through use of an isotope dilution procedure.[70] Although brain contains substantial glutamine transaminase activity, it has relatively less ω-amidase activity as compared to the respective activities found in liver and kidney. On the assumption that the relationships between these enzyme activities are similar in human brain, the accumulation of α-ketoglutaramate in the cerebrospinal fluid in hepatic coma may be explained as follows. Patients with hepatic coma have increased cerebrospinal fluid glutamine concentrations,[112,113] which probably reflect elevated brain glutamine concentrations associated with increased utilization of ammonia by glutamine synthetase. There is also evidence for an increase in α-ketoglutarate and pyruvate in brains of ammonia-intoxicated rats.[114] An increase in the concentrations of glutamine and α-keto acids in the brain would be expected to lead to increased transamination of glutamine to α-ketoglutaramate, which would accumulate in the absence of appreciable ω-amidase. This interpretation is consistent with the observation that the concentrations of α-ketoglutaramate in the cerebrospinal fluid of patients with hepatic coma are almost linearly related to those of glutamine.[115] However, two patients were found to have elevated cerebrospinal fluid glutamine levels without increased α-ketoglutaramate levels, suggesting that other factors may play a role. These findings and the observation that α-ketoglutaramate is present in several rat tissues[70] should serve to stimulate further work on the metabolism of glutamine, in particular additional studies on the glutamine transminase-α-keto acid-ω-amidase pathway. The adult brain does not utilize ammonia via the urea cycle; thus, ammonia is probably utilized in the brain almost exclusively by the glutamine synthesis reaction. These considerations are in accord with the observation that patients with hepatic coma and experimental animals given high doses of ammonia show increased synthesis of glutamine. There is evidence that glutamine itself is not toxic to the central nervous system[116] and that brain ATP levels are not significantly decreased in experimental animals acutely intoxicated with ammonia.[117] Vergara et al.[40,112] have speculated that a metabolite of glutamine, perhaps α-ketoglutaramate, may be the toxic substance that produces symptoms and signs of ammonia-induced encephalopathy. Whether or not α-ketoglutaramate is toxic, its formation is clearly of potential significance in relation to the pathogenesis of hepatic encephalopathy.

In considering the role of glutamine and the glutamine transaminases in mammalian amino acid metabolism, it should be emphasized that the concentrations of glutamine in blood plasma and tissues are relatively high compared to those of the other amino acids, and that glutamine synthetase is present in a number of mammalian tissues. Furthermore, there is substantial evidence that glutamine is transported across cell membranes more effectively than glutamate. There is much support for the view that the reactions catalyzed by carbamyl phosphate synthetase and glutamine synthetase are the quantitatively major pathways of ammonia utilization in mammalian tissues. The pathway by which ammonia is incorporated into the α-amino groups of amino acids requires further investigation; although reductive amination of α-ketoglutarate by glutamate dehydrogenase may play a role in this process, the possibility that there is a glutamate synthase-type enzyme in mammalian tissues is very attractive and should be investigated. It is well known that the amide moiety of glutamine is utilized for the synthesis of many compounds of biological importance in mammals as well as in bacteria and plants. Although less emphasis has been placed by biochemists on the utilization of the α-amino group of glutamine, the accumulated data strongly suggest that such reactions are of considerable metabolic and physiological importance. Thus, in mammalian tissues there appears to be a homeostatic metabolic mechanism for preservation of amino acid balance, in which dietary nonessential amino acids, especially glutamine, function to maintain the tissue levels of amino acids and to prevent loss of essential carbon chains. The glutamine transaminase-ω-amidase system as well as other transaminases emerge as physiologically significant catalysts in this process.

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