α-Ketoglutaramic acid as a product of enzymic transamination of glutamine in *Neurospora**

Evidence for the participation of α-ketoglutaramic acid (KGAM) as an intermediate in the transamination-deamidation reaction of glutamine in liver and kidney has been reported previously[1,2,3] Experiments with substituted glutamine derivatives[2] and with a purified ω-amidase obtained from rat liver[4] were consistent with the intermediate formation and hydrolysis of KGAM. The formation of KGAM as a product of the enzymic transamination of glutamine could not be directly demonstrated with the mammalian systems because of the presence of highly active ω-amidase which rapidly catalyzed its conversion to α-ketoglutaric acid and ammonia[4]. We now wish to report studies on a glutamine–asparagine transaminase system of *Neurospora*, in which it has been possible to demonstrate the formation of KGAM as a product of glutamine transamination.

These studies were carried out with extracts of an asparagine-requiring mutant** of *N. crassa* (S-1007a)[5] and its parent wild type strain (Y8743-21 (13-7) a). The mutant strain exhibited a growth response to α-ketosuccinamic acid as well as to L-asparagine, suggesting that it converted α-ketosuccinamic acid to asparagine. It was subsequently found that extracts of both the wild and mutant *Neurospora* strains catalyzed the formation of asparagine from α-ketosuccinamic acid and L-glutamine (Table I). Of many amino acids tested, glutamine was the only active amino donor in this system. The reaction was followed by observing the decrease in the characteristic absorption band exhibited by α-ketosuccinamic acid at 290 mμ in 0.1 *M* NaOH[1]; the formation of asparagine was demonstrated by paper chromatography. In contrast to the glutamine transaminase preparations which have been obtained from liver and kidney, the *Neurospora* preparations did not catalyze appreciable deamidation of added KGAM; when KGAM, α-ketosuccinamate, or L-glutamine was incubated with these enzyme preparations, less than 5% of the added amide was deamidated. Although added L-asparagine was hydrolyzed by the enzyme preparations (5 to 40%, depending on the method of preparation of the extract), we observed that the total evolution of ammonia during the course of the transamination reaction was not sufficiently great to account for complete deamidation of the KGAM presumably formed. These observations suggested that it might be possible to demonstrate the formation of KGAM in this system.

TABLE I

TRANSAMINATION BETWEEN GLUTAMINE AND α-KETOSUCCINAMATE CATALYZED BY *Neurospora* PREPARATIONS

The complete system consisted of 4 μmoles sodium α-ketosuccinamate, 4 μmoles L-glutamine, 0.02 μmole pyridoxal phosphate, 20 μmoles *tris*(hydroxymethyl)aminomethane–pyrophosphate buffer (pH 9.0) and 0.2 ml of enzyme preparation in a final volume of 0.3 ml; incubated for 60 min at 37°. *Neurospora* (wild strain) was grown at 30° on Fries medium[6] containing 0.00167 *M* L-asparagine; mycelia were harvested after 3 days, cooled to 0°, minced, and homogenized in 0.05 *M* sodium pyrophosphate buffer (pH 8.5); the homogenate was centrifuged at 1,000 × *g* for 20 min and the supernatant solution was dialyzed against 0.0005 *M* pyrophosphate buffer (pH 8.5) for 6 h at 5°. α-Ketosuccinamate was determined from the absorption at 290 mμ in 0.1 *N* NaOH, read against blanks in which α-ketosuccinamate was omitted.

+ = formation demonstrated by paper chromatography (asparagine) or as described in the text (KGAM); o = no formation.

	α-Ketosuccinamate disappearance (μmoles)	*KGAM*	*Asparagine*
Complete system	2.47	+	+
L-Glutamine omitted§	0.20	o	o
α-Ketosuccinamate omitted		o	o
Enzyme omitted	o	o	o

§ No activity was observed when L-glutamine was replaced by D-glutamine, NH_4Cl, glycine, or the L-isomers of valine, leucine, isoleucine, aspartic acid, glutamic acid, threonine, serine, lysine histidine, tryptophan, methionine, phenylalanine, tyrosine, alanine, and homoglutamine.

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The formation of KGAM was demonstrated in the presence of α-ketosuccinamic acid by the following procedure, in which α-ketosuccinamic acid (as well as other α-keto acids) are selectively destroyed by H_2O_2; under these conditions KGAM (present in the ketolactam form)[7] is not attacked. A reaction mixture (4.4 ml) containing α-ketosuccinamate, glutamine, and enzyme (complete system, Table I) was treated with 13 ml absolute ethanol and the precipitated protein was removed by centrifugation. The supernatant solution was concentrated by evaporation to 7 ml and brought to about pH 12 by addition of NaOH; after standing at 26° for 10 min (to promote conversion of α-ketosuccinamate to the monomer[7]), the mixture was adjusted to pH 5.6 by addition of glacial acetic acid. H_2O_2 (0.5 ml of 30%) was added, and after incubation at 37° for 20 min, an excess of crystalline beef liver catalase was added to destroy the peroxide. The resulting mixture was treated with 2 ml 0.5% 2,4-dinitrophenylhydrazine in 2 *N* HCl, and then incubated for 6 h at 37°. The solution was extracted successively with ethyl acetate, sodium carbonate, and (after acidification) with ethyl acetate. The hydrazone solution was hydrogenated with platinum oxide catalyst as described;[8] hydrogenation of α-ketoglutaramic acid—2,4-dinitrophenylhydrazone under these conditions has previously been shown to yield glutamine and glutamic acid[8]. After hydrogenation, paper chromatography in pyridine–water–methanol (4:20:80), formic acid–*tert*-butanol–water (15:75:15), and phenol–water (80:20) revealed glutamine (R_F: 0.35, 0.29, and 0.43, respectively) and smaller quantities of glutamic acid (R_F: 0.55, 0.42, and 0.22, respectively). The results obtained with the phenol solvent are diagrammatically represented in Fig. 1 which also gives the composition of the reaction mixtures. The presence of glutamic acid may be ascribed to deamidation of the 2,4-dinitrophenylhydrazone of KGAM or of glutamine during hydrogenation. Hydrolysis of the products of hydrogenation in 1 *N* HCl at 100° followed by paper chromatography gave only glutamic acid. In similar experiments in which enzyme preparation, α-ketosuccinamic acid, and glutamine were separately omitted, no glutamine was formed. Added KGAM (Expts. 5 and 6, Fig. 1) was also recovered as glutamine and glutamic acid.

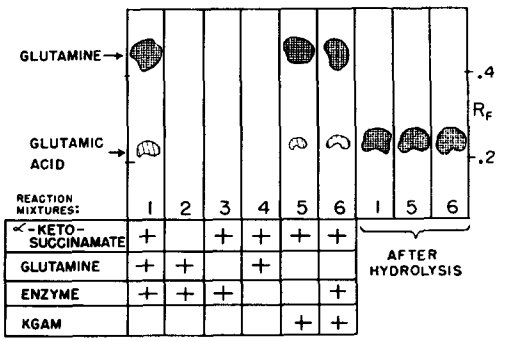

Fig. 1. Diagrammatic representation of chromatogram. (The experimental details are given in the text.)

The present observations provide direct evidence for the formation of KGAM as a product of enzymic transamination of glutamine in *Neurospora*, and are consistent with the conclusions drawn from studies on similar mammalian enzyme systems[1,2,3]. The formation of asparagine from α-ketosuccinamate by *Neurospora* extracts and the observation that α-ketosuccinamate can substitute for L-asparagine in supporting the growth of the *Neurospora* mutant suggest that the growth response of this mutant to D-asparagine[5] may be due to conversion of D-asparagine to α-ketosuccinamate followed by L-specific transamination to L-asparagine. Although conversion of α-ketosuccinamate to asparagine has now been observed in *Neurospora* as well as in mammalian systems, it is evident that further research is required to establish the pathway (or pathways) of asparagine formation and the nature of the block of asparagine biosynthesis in this *Neurospora* mutant.

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