A GLUTAMIC-α-KETOADIPIC TRANSAMINASE IN SACCHAROMYCES[1]

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A considerable body of evidence now supports a biosynthetic pathway to lysine via α-aminoadipic acid (α-AAA) in the fungi (Strassman and Weinhouse, 1953; Broquist and Trupin, 1966). The conversion of α-ketoadipic acid (α-KA) to α-AAA by a pyridoxal-phosphate (PALP)-dependent glutamic-α-ketoadipic transaminase (GKAT) was demonstrated in Torulopsis utilis by Broquist and Stiffey (1959), and in Neurospora crassa by DeBoever (1963). No PALP dependence was demonstrated for the GKAT of Saccharomyces cerevisiae. The current study demonstrates the existence of a PALP-dependent GKAT in wild-type and lysine auxotrophic mutant strains of Saccharomyces and indicates that GKAT is probably a different enzyme from glutamic-oxalacetic transaminase (GOT).

Yeast cultures were grown to stationary phase in conventional 1% glucose complex liquid media. Cells were harvested, washed, and broken by sonication in the Branson Sonifier for 4 minutes at scale setting 7 at a cell density of 1×10^{10} cells per ml. The broken cell preparations were centrifuged and the supernatant (18,800 x g), which contained most of the GKAT activity,

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was assayed as a crude enzyme preparation by following the conversion of DL-α-AAA-6-C^{14} to labelled α-KA. Incubation mixtures containing the labelled substrate, enzyme, and appropriate cofactors were separated by paper strip chromatography and scanned in a radiochromatogram strip scanner (Nuclear

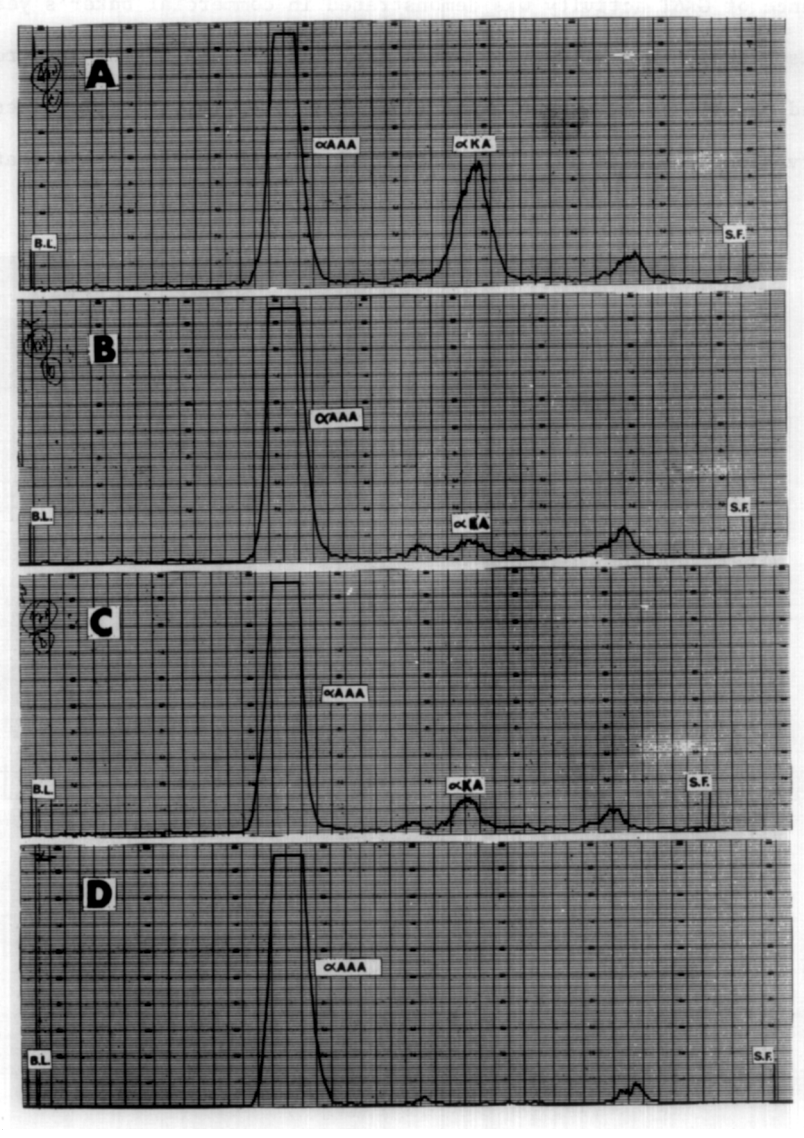

Figure 1. Radiochromatograms of four hour incubation mixtures of DL-α-AAA-6-C^{14} with the enzyme preparation of a ly_8 yeast mutant: (A) complete system containing PALP and α-KG, (B) minus α-KG, (C) minus PALP, and (D) minus PALP and α-KG. B.L. = base line. S.F. = solvent front. Solvent system - n-butanol/acetic acid/water (4:1:1, v/v/v). Migrational direction - descending.

Chicago, Actigraph III). Figure 1 represents a series of incubation mixtures containing (A) complete system, (B) complete system minus α-KG, (C) complete system minus PALP, and (D) complete system minus α-KG and PALP. The stimulation of GKAT activity by both PALP and α-KG is apparent. The presence of GKAT activity was demonstrated in commercial baker's yeast (Fleischmann), in a wild-type strain (F_2), and in all lysine auxotrophs tested capable of growth on α-AAA (ly_6, ly_7, ly_8, ly_{11}, ly_{12}). These observations indicate that the yeast GKAT is PALP-dependent and that none

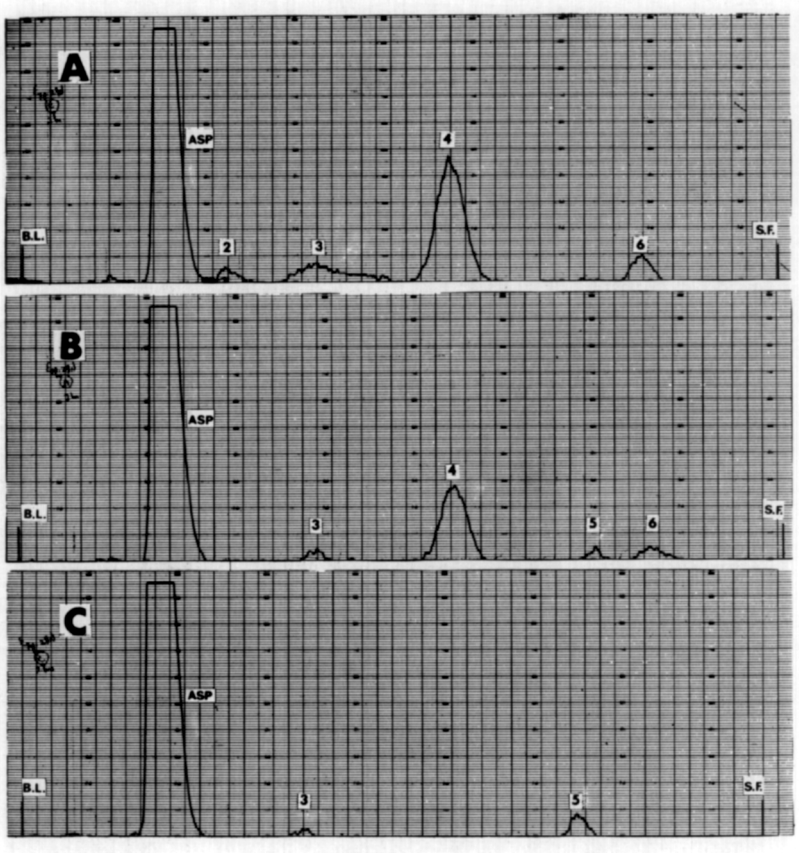

Figure 2. Radiochromatograms of two hour incubation mixtures of L-aspartate-4-C^{14} with the enzyme preparation of a baker's yeast (Fleischmann): (A) complete system containing PALP and α-KG, (B) minus α-KG, and (C) minus PALP and α-KG. B.L. = base line. S.F. = solvent front. Solvent system n-butanol/acetic acid/water (4:1:1, v/v/v). Migrational direction - descending. See text for peak identification.

of the lysine mutants isolated in our laboratory to date are blocked at the GKAT step.

The question of the possible identity of GKAT and GOT was tested with three mutant strains (ly_6, ly_8, and thr_5). If GKAT and GOT were the same enzyme, any mutant lacking this enzyme might be expected to exhibit a dual requirement for glutamate and lysine. The ly_6 and ly_8 mutants do in fact exhibit a dual requirement for glutamate and lysine. The thr_5 mutant, which also exhibits some glutamate and lysine stimulation of its growth on aspartate, is already known to lack GOT activity (deRobichon-Szulmajster et al., 1966), and it should also lack GKAT activity if they are in fact the same enzyme.

The assay for GOT activity was based upon the use of L-aspartate-4-C^{14} as substrate. Figure 2 represents the results of the assay for GOT activity on the 18,800 x g supernatant of a sonified baker's yeast preparation. The complete system (Figure 2A) containing labelled aspartate, enzyme, PALP, and α-KG produced one new major peak (#4) and two new minor peaks (#2 and #6). Minor peaks #3 and #5 are impurities present in the original labelled aspartate. None of these peaks corresponded to labelled oxalacetate (R_f 0.66 in this solvent system). The major peak (#4) was found to have R_f values comparable to α-KG in four solvent systems (Table I). The reduced level of peak #4 when the unlabelled amine acceptor (α-KG) was omitted, (Figure 2B) may be due to endogenous transaminase acceptors. Omission of both PALP and α-KG (Figure 2C) abolished all metabolism of labelled aspartate. The conversion of aspartate label beyond oxalacetate to α-KG probably arises via intervening steps of the TCA cycle. We have, therefore, followed the conversion of labelled aspartate to labelled α-KG in the complete system in assays of GOT activity in the ly_6, ly_8, and thr_5 mutants.

Employing the GKAT and GOT assays described above, we have demonstrated GKAT activity in the ly_6, ly_8, and thr_5 mutants comparable to that of the

TABLE I

COMPARISON OF R_f VALUES OF PEAK 4 WITH AUTHENTIC α-KG

	Solvents			
	1	2	3	4
α-KG	0.56	0.64	0.02	0.65
Peak 4	0.56	0.62	0.02	0.65

Solvents: 1) n-butanol/acetic acid/water (4:1:1, v/v/v)
2) t-butanol/formic acid/water (70:15:15, v/v/v)
3) n-propanol/ammonia (70:30, v/v)
4) t-butanol/methylethylketone/formic acid/water (40:30:15:15, v/v/v/v)

wild-type. We have also confirmed the absence of GOT activity by the more sensitive tracer assay in the thr_5 mutant already known to lack the enzyme by previous optical assay procedures (deRobichon-Szulmajster et al., 1966). The ly_8 mutant was, however, found to possess GOT activity. It is thus evident from the mutant data that GKAT and GOT are different enzymes under separate genetic control. The dual requirement for glutamate and lysine in the ly_6 and ly_8 mutants has been found to reside in the lack of the aconitase and homoaconitase enzymes (Scheifinger et al., 1966).

Additional work dealing with the physical separation and properties of the yeast transaminases will be published separately (Matsuda and Ogur, 1968).

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