GLUTAMATE AUXOTROPHS IN SACCHAROMYCES[1] I. THE BIOCHEMICAL LESION IN THE glt_1 MUTANTS[2]

Maurice Ogur, Lowell Coker, and Sylvia Ogur

Department of Microbiology and Biological Research Laboratory
Southern Illinois University, Carbondale

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Glutamate auxotrophs of Saccharomyces are induced with relatively low frequency (compared to other amino acid auxotrophs) by ultraviolet irradiation or by nitrous acid treatment and have been isolated only in the last few years. Since glutamate appears to arise principally in yeasts by the amination of α-ketoglutarate, a lesion in glutamate biosynthesis may be sought either in the amination system or in the production of α-ketoglutarate. Glutamate auxotrophs in E. coli studied by Gilvarg and Davis (1956) proved to be lacking in condensing enzyme. The current report describes some of the principal characteristics associated with a glutamate requirement in the glt_1 yeast pedigree derived from a mutant isolated in Carbondale by ultraviolet irradiation.

In addition to a total requirement for glutamate in the presence of eighteen other common amino acids, the glt_1 strains were also characterized by either poor or negligible growth on media containing nonfermentable principal carbon and energy sources (acetate, lactate, ethanol,

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glycerol). In crosses to glutamate independent strains, the dual phenotype segregated as though controlled by a single gene. Selected revertants to glutamate independence, occurring spontaneously with a frequency of approximately 1 in 10^6 cells, or induced by u.v. irradiation, had recovered the ability to utilize nonfermentable substrates, confirming the single gene control of the dual phenotype.

The general inability to grow on nonfermentable substrates in Saccharomyces strains has previously been found exclusively associated with cytochrome deficient (petite) mutants (Ogur, Lindegren, and Lindegren, 1954) although the possible expression of other lesions by the same phenotype has been recognized.

To test whether glt_1 strains were also segregational petites, their cytochrome spectra and respiratory ability were examined. The spectral lines of cytochromes a, b, and c were detected in a number of glt_1 strains studied. Cultures of glt_1 strains grown in low sugar-lactate broth with agitation for several transfers exhibited strong respiratory capacity with glucose, ethanol, acetaldehyde, and lactate but failed to respire with acetate. Table I summarizes some of the above mentioned characteristics.

TABLE I

COMPARISON OF GLUTAMATE INDEPENDENT AND REQUIRING STRAINS WITH A REVERTANT TO GLUTAMATE INDEPENDENCE

Strain	Phenotype	Growth on Glucose -Glutamic	Growth on Lactate Complete	Q_{O_2}(N) Ethanol	Q_{O_2}(N) Acetate
37891	GLT	+	+	807	372
37892	glt_1	-	-	629	0
37892BM UV-1	GLT	+	+	1123	281

With the substrates ethanol and acetaldehyde, O_2 uptake occurred without CO_2 production. Assays of the stoichiometry of O_2 uptake with limiting

amounts of ethanol and acetaldehyde were consistent with oxidation proceeding only to the level of acetate with glt_1 strains, whereas GLT strains were able to carry oxidation beyond this level as indicated in Table II.

TABLE II

EXTENT OF OXIDATION OF VARIOUS SUBSTRATES BY GLUTAMATE REQUIRING AND GLUTAMATE INDEPENDENT STRAINS

	Oxygen uptake, moles per mole substrate			
	Theoretical		Observed	
Substrate	For Complete Oxidation	For oxidation to level of Acetate	37892 glt_1 AER	37891 GLT AER
Ethanol	3.0	1.0	1.0	2.0
Acetaldehyde	2.5	0.5	0.43	1.5
Glucose	6.0	2.0	1.47	3.3

These observations suggested that glt_1 strains were characterized by a functional electron transport system to O_2, but a nonfunctional TCA cycle, blocked before α-ketoglutarate. Such a block would enable one to account for single gene control of the glutamate requirement and the inability to utilize acetate. The most probable sites for such a block might be expected either at condensing enzyme, aconitase, or isocitric dehydrogenase. The absence of either of the two latter enzymes might be expected to lead to citrate accumulation. When media used for cultivating glt_1 strains was assayed, significant accumulation of citrate was observed, compared to GLT strains, as indicated in Table III.

TABLE III

CITRATE ACCUMULATION BY GLUTAMATE INDEPENDENT AND GLUTAMATE REQUIRING STRAINS

Strain	mg citrate per liter culture media
37891 GLT	7.7
37892 glt_1	210.

Enzyme assay of broken cell preparations of glt_1 strains revealed the presence of normal levels of NADP-isocitric dehydrogenase and glutamic-aspartic transaminase but failed to detect measurable amounts of aconitase as indicated in Table IV.

TABLE IV

ENZYMES IN THE GLUTAMATE PATHWAY

Strain	Aconitase[a] (Chemical)	Aconitase[a] (Optical)	Specific Activity NADP-isocitric Dehydrogenase[b]	Glutamic-Aspartic Transaminase[c]
37891 GLT	174.	125.	22.4	19.2
37892 glt_1	<0.5	<0.1	19.4	19.4

[a](Anfinson, 1955) -- Chemical assay unit = muM citrate/min/mg protein; optical assay unit = decrease of 0.001 in A_{240}/min/mg protein.

[b](Kornberg, 1955) -- Optical assay unit = increase of 0.001 in A_{340}/min/mg protein.

[c](Gunsalus and Stamer, 1955) -- Optical assay unit = increase of 0.001 in A_{280}/min/mg protein.

Aconitase assays were carried out on the 8000g supernatant of a glass homogenate. NADP-isocitric dehydrogenase and glutamic-aspartic transaminase assays were carried out on the pellet obtained by centrifuging the 8000g supernatant of a Vertis homogenate at 20000g. Protein was determined by the method of Lowry et. al., 1951. Citrate was determined by a modification of the method of Natelson, et. al., 1948.

In summary, the biochemical lesion in the glt_1 mutant strains involves the inability to synthesize aconitase and results in a non-functional TCA cycle. These are the first TCA cycle mutants described in yeast and represent a second major category of mutants with lesions in the aerobic pathway (the first being the cytochrome deficients). The glt_1 (aconitaseless) mutants are capable of respiring with various substrates which are oxidized to the level of acetate but are incapable of degrading two carbon substrates to CO_2 via the TCA cycle. The block at aconitase leads to citrate accumulation when glucose is metabolized. The inability to reach α-ketoglutarate via the TCA cycle is expressed

as a total growth requirement for glutamate in the presence of eighteen other common amino acids.

More detailed accounts of the genetic and biochemical data concerning a number of independently derived glutamicless mutants will be published separately.

It is anticipated that these mutants may be useful in biochemical studies of possible alternate pathways to the TCA cycle in acetate metabolism or glutamate biosynthesis in yeast.

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