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Mechanism of the aromatic aminotransferase encoded by the *Aro8* gene from *Saccharomyces cerevisiea*

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ABSTRACT

The amino acid L-lysine is synthesized in *Saccharomyces cerevisiae* via the α-aminoadipate pathway. An as yet unidentified PLP-containing aminotransferase is thought to catalyze the formation of α-aminoadipate from α-ketoadipate in the L-lysine biosynthetic pathway that could be the yeast *Aro8* gene product. A screen of several different amino acids and keto-acids showed that the enzyme uses L-tyrosine, L-phenylalanine, α-ketoadipate, and L-α-aminoadipate as substrates. The UV–visible spectrum of the aminotransferase exhibits maxima at 280 and 343 nm at pH 7.5. As the pH is decreased the peak at 343 nm (the unprotonated internal aldimine) disappears and two new peaks at 328 and 400 nm are observed representing the enolimine and ketoenamine tautomers of the protonated aldimine, respectively. Addition, at pH 7.1, of α-ketoadipate to free enzyme leads to disappearance of the absorbance at 343 nm and appearance of peaks at 328 and 424 nm. The $V/\mathbf{E_t}$ and $V/K_{\alpha\text{-ketoadipate}}\mathbf{E_t}$ pH profiles are pH independent from pH 6.5 to 9.6, while the $V/K_{\text{L-tyrosine}}$ pH-rate profile decreases below a single pK_a of 7.0 ± 0.1. Data suggest the active enzyme form is with the internal aldimine unprotonated. We conclude the enzyme should be categorized as a α-aminoadipate aminotransferase.

Introduction

The α-aminoadipate pathway for the biosynthesis of lysine in *Saccharomyces cerivisiea* comprises seven enzyme-catalyzed reactions, Scheme 1. The pathway begins with Claisen condensation of α-ketoglutarate (α-Kg[1]) and acetyl-CoA to give homocitrate (homocitrate synthase, step 1 in Scheme 1), which is followed by its conversion to homoisocitrate (homoaconitase, step 2) and oxidative decarboxylation to give α-ketoadipate (α-KA)[1] (homoisocitrate dehydrogenase, step 3). Once α-KA is converted to α-aminoadipate via an aminotransferase reaction (step 4), the δ-carboxylate of aminoadipate is reduced to the aldehyde (aminoadipate reductase, step 5), followed by condensation with L-glutamate to give L-saccharopine, (saccharopine reductase, step 6), which is finally converted to L-lysine and α-ketoglutarate (saccharopine dehydrogenase, step 7). With the exception of the aminotransferase, LYS genes code for the enzymes of the α-aminoadipate pathway. The aminotransferase is presumably an existing enzyme that is "borrowed" for the aminoadipate pathway. Because the pathway is not present in humans, the enzymes in the pathway are potential candidates for drug development. Interruption of the lysine biosynthetic pathway is known to be a lethal event in fungi [1].

The identity of the aminotransferase that converts α-ketoadipate to L-α-aminoadipate is unknown. There are a number of known aminotransferases present in yeast for which the metabolic role has not been elucidated or the enzyme characterized. Our goal is to identify and characterize the aminotransferase that is likely involved in the lysine biosynthetic pathway. We are also interested in investigating the metabolic role of other aminotransferases from yeast that currently have not been characterized. A BLAST search of the yeast *Aro8* protein vs. the human genome revealed the greatest similarity with a α-aminoadipate aminotransferase. This aminotransferase is also known as L-kynurenine/α-aminoadipate aminotransferase. Based upon the BLAST sequence alignment the yeast enzyme is 27% identical and 47% similar to the human α-aminoadipate aminotransferase. The enzyme encoded by the *Aro8* gene also has some similarity to human aromatic aminotransferases. The sequence similarity with the human α-aminoadipate aminotransferase suggested the aminotransferase encoded by the *Aro8* gene may be a likely candidate for the enzyme that catalyzes the same reaction in the lysine biosynthetic pathway in yeast.

The *Aro8* gene encodes a protein with a subunit molecular mass of 56,168, which apparently exists as a homodimer [2]. On the basis of primary sequence, the *Aro8* gene product is in subgroup I

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[1] *Abbreviations used:* AATase, aspartate aminotransferase; Caps, 3-(cyclohexylamino)-propane-sulfonic acid; Ches, 2-(*N*-cyclohexylamino)ethane-sulfonic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane-sulfonic acid; α-KA, α-ketoadipate; α-kg, α-ketoglutarate; Mes, 2-(*N*-morpholine)ethane-sulfonic acid; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate.

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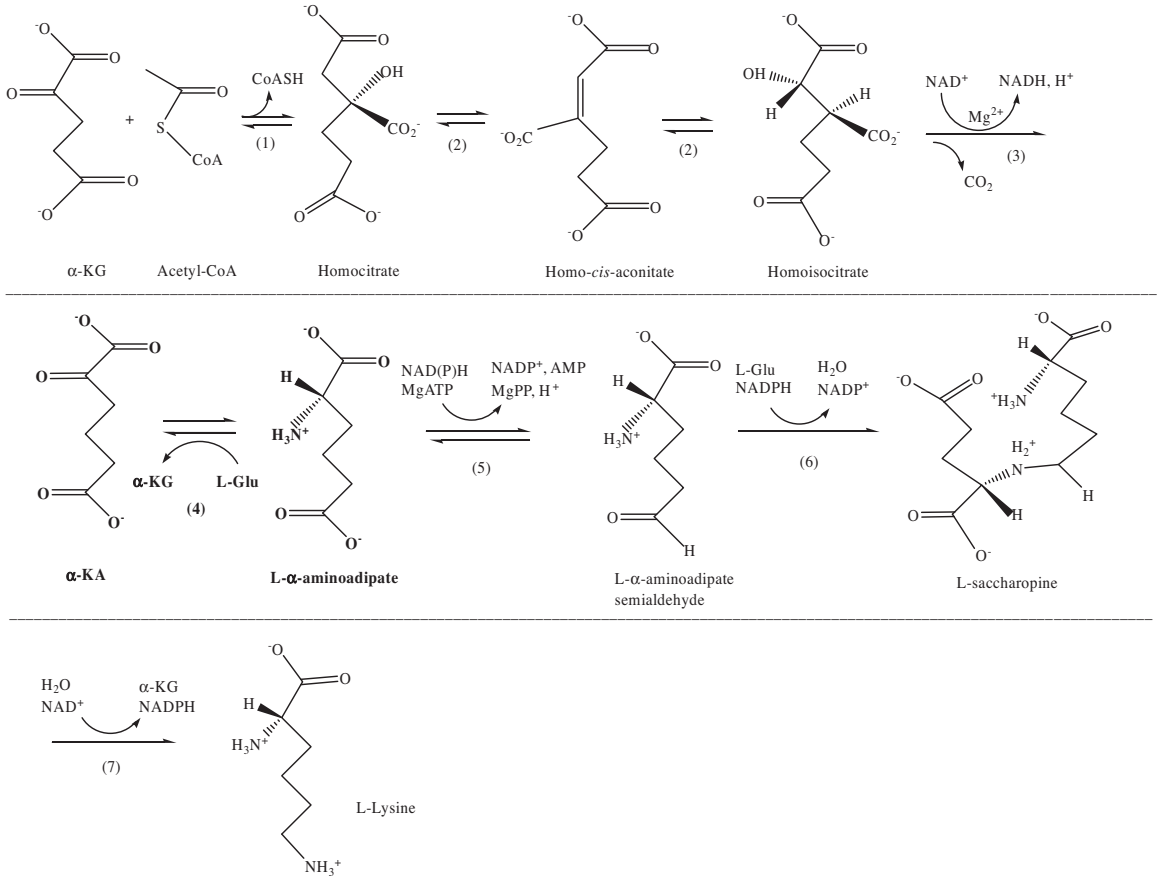
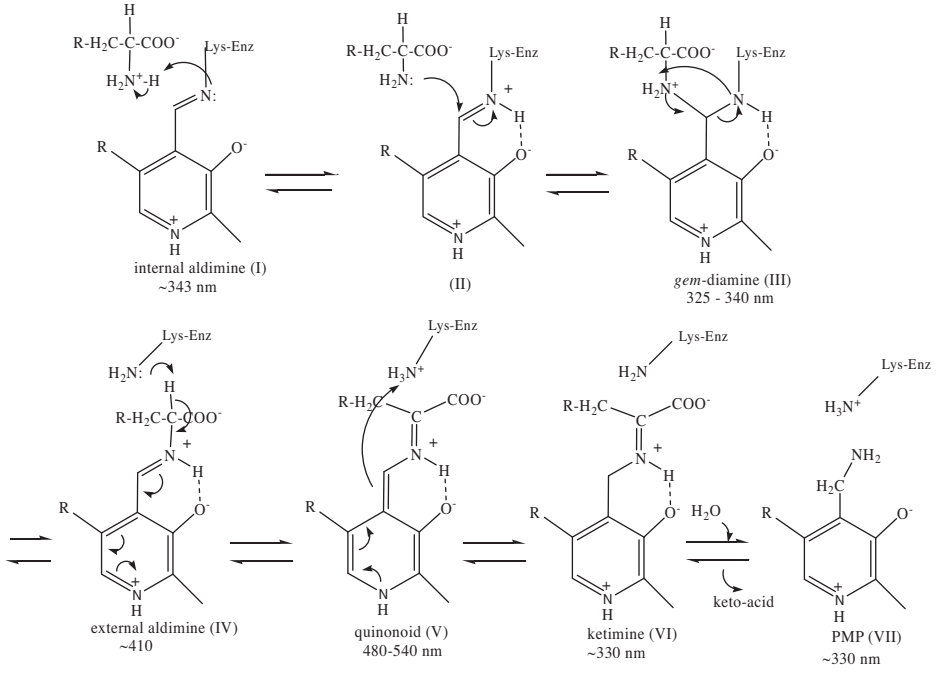


Scheme 1. α-Aminoadipate pathway for lysine synthesis in fungi. The enzymes are (1) homocitrate synthase, (2) homoaconitase, (3) homoisocitrate dehydrogenase, (4) α-Aminoadipate aminotransferase, (5) α-aminoadipate reductase, (6) L-Saccharopine reductase, and (7) L-Saccharopine dehydrogenase. The reaction of interest is indicated in bold.



Scheme 2. Proposed acid–base mechanism for the aromatic/aminoadipate aminotransferase.

of the PLP-dependent aminotransferases as classified by Mehta and Christen [3]. Aminotransferase subgroup I comprises aspartate, alanine, histidinol-phosphate, phenylalanine, and all described aromatic aminotransferases such as the tyrosine:2-oxoglutarate