

Homocitrate synthase as a novel target for antifungal agents.

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Invasive fungal infections have increased dramatically in recent years to become important causes of morbidity and mortality in hospitalized patients, yet only a few antifungal agents are used in clinical practice. Lysine biosynthesis is one example of a biochemical difference between higher fungi, which use the α -aminoadipic acid pathway (distinct from the diaminopimelic acid pathway used by bacteria and plants), and human host cells, which cannot synthesize lysine. The uniqueness of the enzymes catalyzing the first steps of the α -aminoadipate pathway makes them potentials target for antifungal chemotherapy.

In our laboratory we have studied the enzyme homocitrate synthase (EC 2.3.3.14) from human pathogenic yeast *Candida albicans* as a possible new target for antifungals. This enzyme catalyzes the first and committed step of the α -aminoadipate pathway, which involves the condensation of α -ketoglutarate with acetyl-CoA to yield homocitrate and CoA. In order to characterize the molecular properties of the target enzyme and to provide enzyme preparations for the purpose of inhibitor screening, the *C. albicans* *LYS21* and *LYS22* genes, encoding two isozymes of homocitrate synthase, were cloned. Expression plasmids containing recombinant genes encoding both enzyme versions were constructed and overexpressed in *E. coli*. The histidine-tagged enzymes were purified to near homogeneity and characterized in terms of physico-chemical and biological properties. Information gained from these studies were used for the design of putative enzyme inhibitors.

In order to validate the potential usefulness of homocitrate synthase as a target for antifungals, the *C. albicans* *LYS21* and *LYS22* knockout strains were constructed, using the FLP site-specific recombination system. The double-disruption $\Delta lys21 \Delta lys22$ mutant cells demonstrated lysine auxotrophy in minimal media and strongly diminished ability to grow in defined media used for tissue cultures, while the single disruptants, $\Delta lys21$ and $\Delta lys22$, showed growth phenotype intermediate between the double disruptant and the wild-type cells.