RESEARCH ARTICLE

Homoisocitrate dehydrogenase from *Candida albicans*: properties, inhibition, and targeting by an antifungal pro-drug

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Abstract

The *LYS12* gene from *Candida albicans*, coding for homoisocitrate dehydrogenase was cloned and expressed as a His-tagged protein in *Escherichia coli*. The purified gene product catalyzes the Mg^{2+}- and K^{+}-dependent oxidative decarboxylation of homoisocitrate to α-ketoadipate. The recombinant enzyme demonstrates strict specificity for homoisocitrate. SDS-PAGE of CaHIcDH revealed its molecular mass of 42.6 ± 1 kDa, whereas in size-exclusion chromatography, the enzyme eluted in a single peak corresponding to a molecular mass of 158 ± 3 kDa. Native electrophoresis showed that CaHIcDH may exist as a monomer and as a tetramer and the latter form is favored by homoisocitrate binding. CaHIcDH is an hysteretic enzyme. The K_M values of the purified His-tagged enzyme for NAD^{+} and homoisocitrate were 1.09 mM and 73.7 μM, respectively, and k_{cat} was 0.38 s^{-1}. Kinetic parameters determined for the wild-type CaHIcDH were very similar. The enzyme activity was inhibited by (*2R,3S*)-3-(*p*-carboxybenzyl)malate (CBMA), with IC_{50} = 3.78 mM. CBMA demonstrated some moderate antifungal activity in minimal media that could be enhanced upon conversion of the enzyme inhibitor into its trimethyl ester derivative (TMCBMA). TMCBMA is the first reported antifungal for which an enzyme of the AAP was identified as a molecular target.

Introduction

L-Lysine is an essential amino acid for mammals including humans, whereas bacteria, plants, and fungi have developed pathways of lysine biosynthesis. There are two versions of this pathway: the diaminopimelic acid pathway, that is characteristic for bacteria, plants, and lower fungi, and the α-aminoadipate pathway (AAP) present in higher fungi, *Ascomycetes* and *Basidiomycetes*, including most of the human pathogenic yeast and filamentous fungi but also in some archaea and thermophilic bacteria. Enzymes that catalyze reactions of the first of the above-mentioned pathways are considered promising molecular targets for antibacterial chemotherapy (Hutton *et al.*, 2003), whereas the second pathway could be a source of new targets for antifungal chemotherapy (Xu *et al.*, 2006). Homocitrate synthase (HCS), homoaconitase (HA) and homoisocitrate dehydrogenase (HIcDH) catalyzing biosynthetic reactions present only in fungal cells and having no counterparts in mammalian cells, are the most obvious candidates for the molecular targets. It was shown that disruption of both genes encoding HCS in *C. albicans* leads to lysine auxotrophy (Kur *et al.*, 2010) and diminished virulence in some systems of fungal cells lacking HCS of HA encoding genes was demonstrated (Liebmann *et al.*, 2004; Schöbel *et al.*, 2010).

Homoisocitrate dehydrogenase (EC 1.1.1.87) catalyzes the fourth reaction of the AAP, i.e., the NAD^{+}-dependent conversion of homoisocitrate to α-ketoadipate (α-Ka), as shown in Fig. 1. HIcDH is a member of the family of pyridine nucleotide-dependent β-hydroxyacid oxidative decarboxylases (Karsten & Cook, 2000). The reaction is metal ion-dependent and the enzyme selectively binds the Mg(II):homoisocitrate complex (MgHIc) (Lin *et al.*, 2007).

The genetics and biochemistry of homoisocitrate dehydrogenase have been studied to some extent for the enzyme from prokaryotic sources: *Deinococcus radiodurans*