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Sequence-based screening and characterization of cytosolic mandelate oxidase using oxygen as electron acceptor

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ABSTRACT

Sequence-based screening was carried out to find a type of cytosolic mandelate oxidase that converted L-mandelate to phenylglyoxylate using oxygen as the final electron acceptor. The sequence features of the cytosolic mandelate oxidase were summarized, and were used in the screening process. Mandelate oxidases from *Streptomyces coelicolor* (Hmo_{SC}) and *Amycolatopsis orientalis* (Hmo_{AO}) were screened and then they were heterologously expressed and characterized. At pH 7.3 40 °C, the Hmo_{AO} showed k_{cat} and K_m values of 140 min^{-1} and 10.2 mM, the Hmo_{SC} showed k_{cat} and K_m values of 105.1 min^{-1} and 2.06 mM. The Hmo_{SC} was thermal stable and retained its 90% activity at 60 °C for up to 5 h, while Hmo_{AO} lost most of its activity at this temperature. The Hmo_{SC} could effectively catalyze the conversion of L-mandelate to phenylglyoxylate at higher temperature using oxygen as the final electron acceptor.

1. Introduction

Mandelate oxidase (MO) catalyzing the conversion of mandelate to phenylglyoxylate belongs to the family of homologous FMN-dependent alpha-hydroxyacid oxidizing enzymes. The extensively studied members in this family are glycolate oxidase (GOX), mandelate dehydrogenase (MDH) and flavocytochrome b_2. These enzymes have a common reductive half-reaction during which the electron is transiently transferred from the electron donor to FMN resulting in the reduction of FMN and the oxidization of substrates.

The family of homologous FMN-dependent alpha-hydroxyacid oxidizing enzymes can be divided into three groups based on their final electron acceptors in the reoxidization of FMN during the second oxidative half-reaction. The first group consists of oxidase such as the GOX from spinach [1]; it uses oxygen as the final electron acceptor and produces hydrogen peroxide which is subsequently decomposed to water. The second group includes flavocytochrome b_2 from *Saccharomyces cerevisiae*. It locates in the intermembrane space of the mitochondria and mainly catalyzes the oxidation of lactate to pyruvate. The electron is transferred from reduced FMN to intramolecular heme in the b_2 domain, and finally passed to dissociative cytochrome *c* [2]. The third group is membrane-bound bacterial dehydrogenases, typically MDH from *Pseudomonas* sp. [3]; they bind to the membrane and most likely use an external coenzyme NAD^+ as the final electron acceptor [4]. Although MDH does catalyze the conversion of mandalate to phenylglyoxylate its requirement of ubiquinone regeneration is not met *in vitro*. The GOX is soluble in the cytosol and uses O_2 to reoxidize FMN which are convenient *in vitro*; however it shows little affinity to mandelate [5].

Elegant research has been carried out to engineer soluble chimeric enzymes that convert mandelate to phenylglyoxylate. A chimeric enzyme MDH–GOX1 was constructed by replacing a 53 amino acid segment (residues 177–229, responsible for membrane-binding in MDH, Fig. 1) with a 34 amino acid segment from GOX [6]. MDH–GOX1 was soluble in the cytosol but it retained only 1% activity on mandelate substrate. Another MDH–GOX2 was constructed by replacing a shorter amino acid segment (residues 177–215, Fig. 1) with a 20 amino acid segment from the GOX [7]. The cytosolic MDH–GOX2 was similar to the MDH in its substrate specificity, catalytic activity, and kinetic mechanism, however it lacked the reactivity toward oxygen. Therefore mutation at G81 was carried out to improve the reactivity toward oxygen; the chimeric protein MDH–GOX2 G81A displayed modestly higher affinity to O_2 due to a more optimal orientation of the bound oxygen molecule [3], while it displayed ~100-fold lower reactivity comparing with the MDH–GOX2 [3,8]. Besides the chimeric MDH–GOX, the engineering of GOX also provide useful information for a proper MO. In the GOX, the residues R257, Y24/129, H254 and W108 were involved in the catalytic reaction; H254 was

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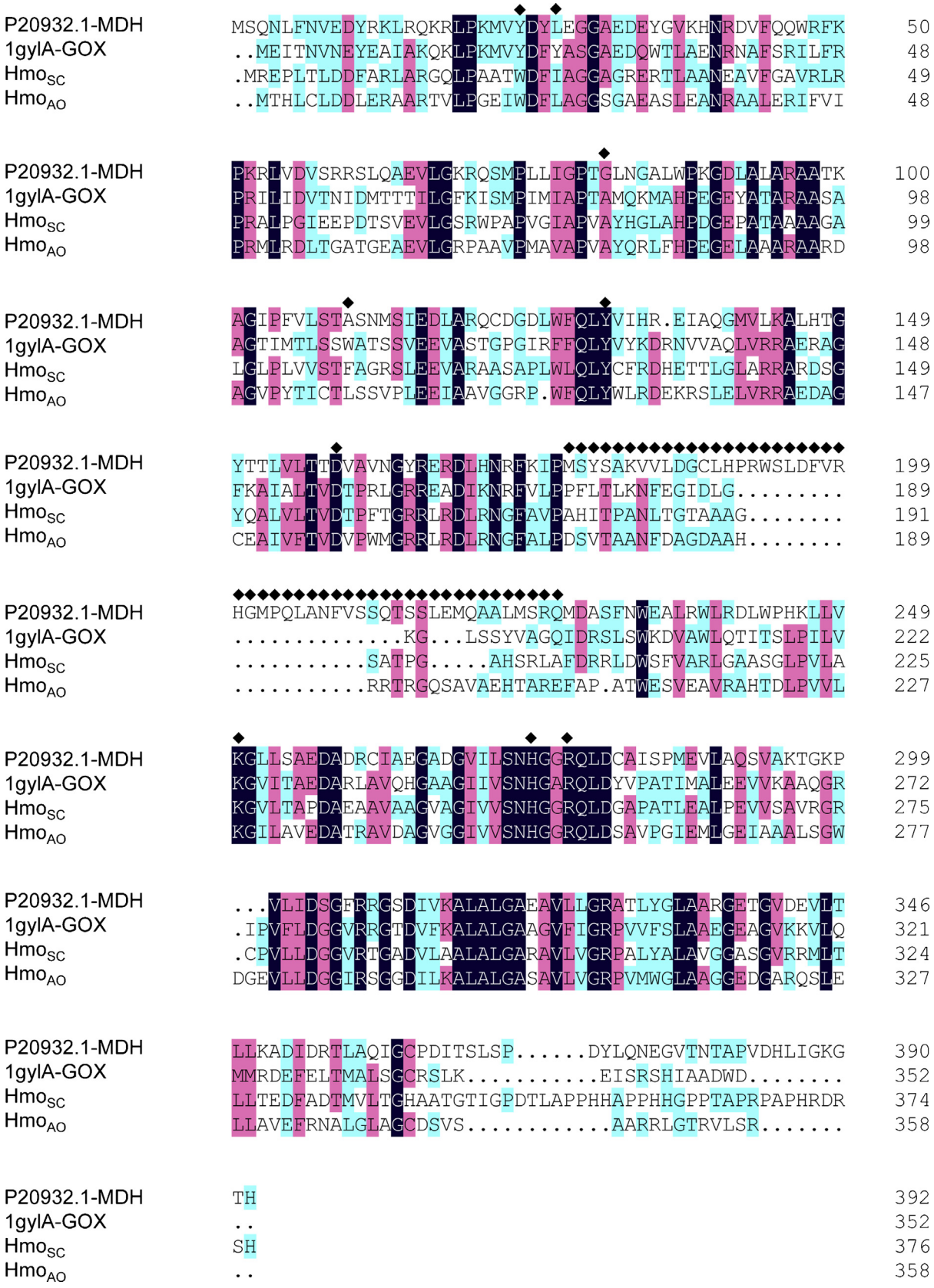

Fig. 1. Multiple sequence alignment of amino acid sequences of Hmo$_{SC}$, Hmo$_{AO}$, MDH and GOX. The black rhombus above the sequences indicated the key residues for sequence-based screening. The residues in dark blue showed positions at which all sequences were identical. The residues in lightcoral had high sequence identity. The residues in green had low sequence identity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)