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Redesign of MST enzymes to target lyase activity instead promotes mutase and dehydratase activities ☆

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ABSTRACT

The isochorismate and salicylate synthases are members of the MST family of enzymes. The isochorismate synthases establish an equilibrium for the conversion chorismate to isochorismate and the reverse reaction. The salicylate synthases convert chorismate to salicylate with an isochorismate intermediate; therefore, the salicylate synthases perform isochorismate synthase and isochorismate-pyruvate lyase activities sequentially. While the active site residues are highly conserved, there are two sites that show trends for lyase-activity and lyase-deficiency. Using steady state kinetics and HPLC progress curves, we tested the "interchange" hypothesis that interconversion of the amino acids at these sites would promote lyase activity in the isochorismate synthases and remove lyase activity from the salicylate synthases. An alternative, "permute" hypothesis, that chorismate-utilizing enzymes are designed to permute the substrate into a variety of products and tampering with the active site may lead to identification of adventitious activities, is tested by more sensitive NMR time course experiments. The latter hypothesis held true. The variant enzymes predominantly catalyzed chorismate mutase–prephenate dehydratase activities, sequentially generating prephenate and phenylpyruvate, augmenting previously debated (mutase) or undocumented (dehydratase) adventitious activities.

Introduction

The redesign of enzyme active sites has been successful to interconvert substrate specificities in structurally homologous and functionally related enzymes. In some examples, the interconversion is as simple as a single amino acid substitution, such as the generation of a malate dehydrogenase from lactate dehydrogenase [1]. In other cases the interconversion of substrate specificity requires more complex changes including modification to the active site and surface loop changes, such as the generation of a trypsin serine protease with the substrate specificity of chymotrypsin [2–4]. Enzymes dependent on the cofactor pyridoxal 5′-phosphate, including ornithine decarboxylase [5] and amino acid aminotransferases [6–8], have been redesigned to have new activities when changes in the active site lead to a promiscuous addition of the proton to different locations on the substrate-cofactor covalent intermediate. Enzymatic activities have been interconverted in the *N*-acetylneuraminate lyase subfamily of (β,α)-barrel enzymes with a single point mutation [9]. More recently, design of enzymes with augmented adventitious or promiscuous activities by directed evolution, looking for an increase of a desired activity after cycles of random mutagenesis, has been successful [10,11].

The MST family of enzymes are structurally homologous, Mg^{2+}-dependent, chorismate-utilizing enzymes involved in *m*enaquinone, *s*iderophore and *t*ryptophan biosynthesis [12–14]. This work will focus on two enzymes that isomerize the chorismate ring to form isochorismate for siderophore biosynthesis (Fig. 1A): PchA[1], the isochorismate synthase from *Pseudomonas aeruginosa*, and Irp9, the salicylate synthase from *Yersinia enterocolitica*. These enzymes are required to make the salicylate cap of the structurally similar siderophores pyochelin and yersiniabactin (Fig. 2). Both enzymes generate isochorismate from chorismate, but Irp9 is then capable of generating salicylate from isochorismate. An interesting question may be posed: what are the features of Irp9 that makes this enzyme bifunctional, whereas PchA is not? This question can be extrapolated more broadly to include other enzymes of this same family in similar biosynthetic pathways, including the salicylate synthase for mycobactin biosynthesis (MbtI) and the

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[1] *Abbreviations used:* EntC, isochorismate synthase from *E. coli*; Irp9, salicylate synthase from *Yersinia enterocolitica;* MbtI, salicylate synthase from *Mycobacterium tuberculosis*; MenF, isochorismate synthase from *E. coli*; MST, protein family containing proteins of *m*enaquinone, siderophore or *t*ryptophan biosynthesis; PchA, isochorismate synthase from *Pseudomonas aeruginosa*; PchB, isochorismate-pyruvate lyase from *Pseudomonas aeruginosa*.

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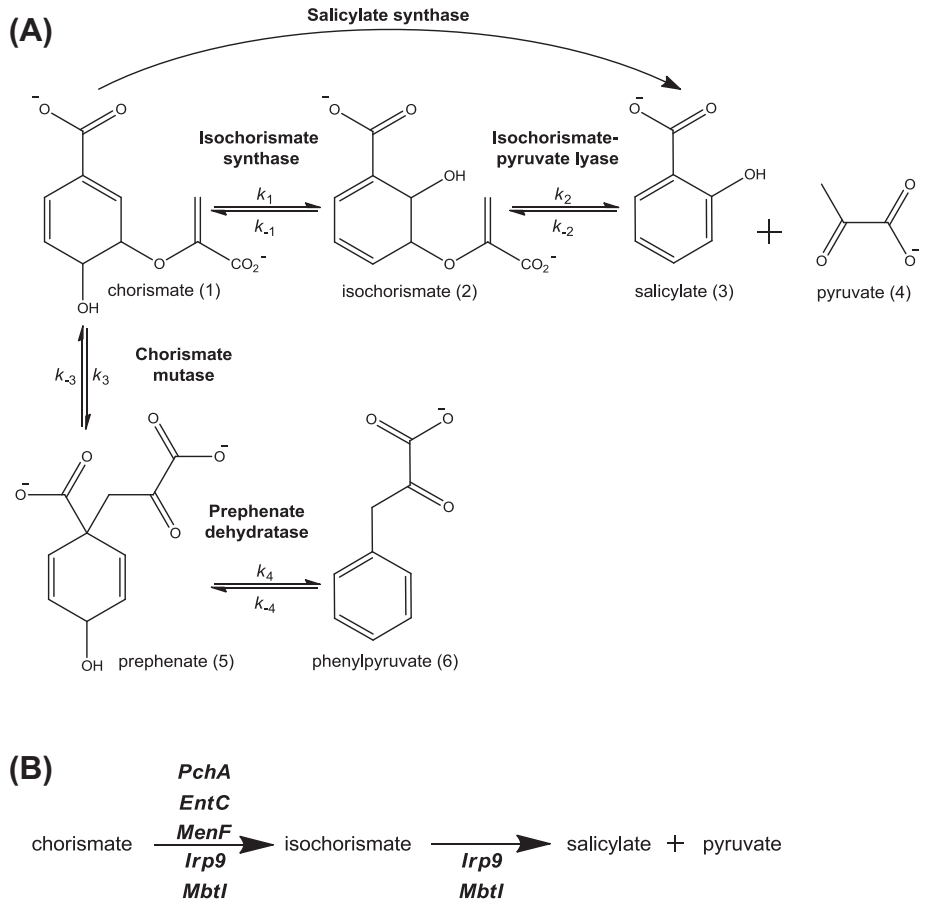

Fig. 1. Enzymatic activities of PchA and Irp9 and their variants. (A) Schematic of the isochorismate synthase, isochorismate-pyruvate lyase, salicylate synthase, chorismate mutase and prephenate dehydratase activities. (B) The physiological functions of the isochorismate synthases and salicylate synthases of the MST family discussed herein.

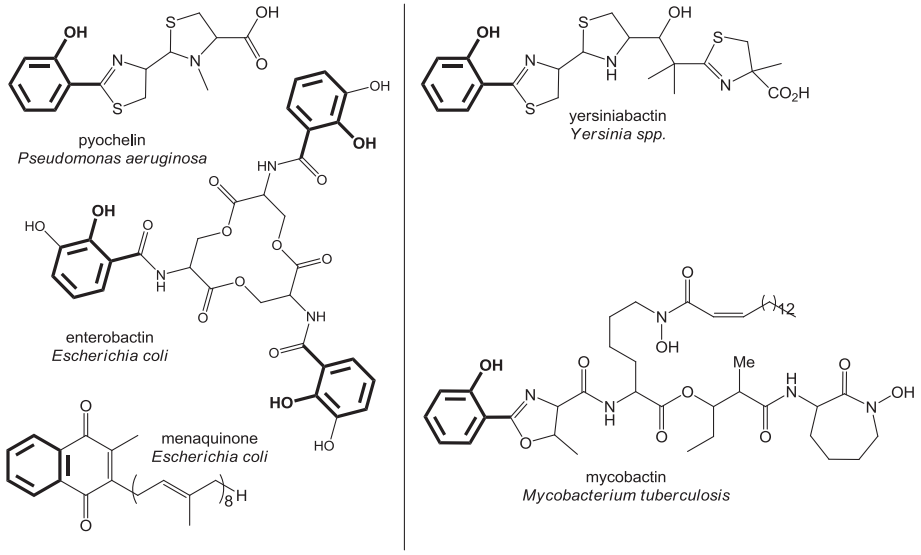

Fig. 2. Biosynthetic products for the isochorismate and salicylate synthases. The compounds in the left column are all generated in a pathway that includes an isochorismate synthase (PchA for the siderophore pyochelin, EntC for the siderophore enterobactin, and MenF for the electron carrier menaquinone). The compounds in the right column are both generated in siderophore biosynthetic pathways that include a salicylate synthase (Irp9 for yersiniabactin, and MbtI for mycobactin). The portion derived from chorismate is shown in bold.

isochorismate synthases from *Escherichia coli* (EntC of enterobactin biosynthesis and MenF of menaquinone biosynthesis).

The formation of isochorismate, common to all five enzymes, has been hypothesized to be catalyzed by general acid–general base chemistry, in which a lysine (K221 in PchA) is the general base for the activation of water to attack the C2 position and a glutamic acid (E269 in PchA) is the general acid for elimination of the C4-OH. This proposed mechanism is supported by mutational analyses