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Review

Identification and characterization of new family members in the tautomerase superfamily: Analysis and implications

Jamison P. Huddleston, Elizabeth A. Burks, Christian P. Whitman *

Division of Medicinal Chemistry, College of Pharmacy, University of Texas, Austin, TX 78712, United States

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ABSTRACT

Tautomerase superfamily members are characterized by a β–α–β building block and a catalytic amino terminal proline. 4-Oxalocrotonate tautomerase (4-OT) and malonate semialdehyde decarboxylase (MSAD) are the title enzymes of two of the five known families in the superfamily. Two recent developments in these families indicate that there might be more metabolic diversity in the tautomerase superfamily than previously thought. 4-OT homologues have been identified in three biosynthetic pathways, whereas all previously characterized 4-OTs are found in catabolic pathways. In the MSAD family, homologues have been characterized that lack decarboxylase activity, but have a modest hydratase activity using 2-oxo-3-pentynoate. This observation stands in contrast to the first characterized MSAD, which is a proficient decarboxylase and a less efficient hydratase. The hydratase activity was thought to be a vestigial and promiscuous activity. However, this recent discovery suggests that the hydratase activity might reflect a new activity in the MSAD family for an unknown substrate. These discoveries open up new avenues of research in the tautomerase superfamily.

Introduction

4-Oxalocrotonate tautomerase (4-OT) converts 2-hydroxy-2, 4-hexadienedioate, or 2-hydroxymuconate (**1**, Scheme 1), to 2-keto-3-hexenedioate (**2**) [1,2]. The enzyme is part of the meta-fission pathway, which is a bacterial pathway for the degradation of aromatic hydrocarbons such as benzene, toluene, and alkyl-substituted derivatives [3]. Bacterial strains with this pathway can use aromatic hydrocarbons as sole sources of carbon and energy because these compounds are processed to products (i.e., pyruvate and acetyl CoA) that are channeled into the Krebs Cycle [4].

4-OT is also a founding member of the tautomerase superfamily (TSF) [5–7]. Members of this superfamily are characterized by a β–α–β building block and a catalytic amino terminal proline (Pro-1) [6,7]. TSF members are made up of short monomers (61–84 amino acids) or long monomers, which are about twice as long. The short monomers code for the signature β–α–β module (Fig. 1A) and the long monomers code for two β–α–β modules connected by a short linker (Fig. 1B). Pro-1 can function as a general base or acid depending on its pK_a value. There are five known families in the TSF, which are named for the first characterized member. The five families are represented by 4-OT [7], 5-(carboxymethyl)-2-hydroxymuconate isomerase (CHMI) [6,8], macrophage migration inhibitory factor (MIF) [9,10], *cis*-3-chloroacrylic acid dehalogenase (*cis*-CaaD)[1] [11], and malonate semialdehyde decarboxylase (MSAD) [12]. CHMI is part of a bacterial pathway for the degradation of aromatic amino acids (e.g., phenylalanine and tyrosine) [8], whereas *cis*-CaaD and MSAD are part of a bacterial pathway for the degradation of the soil fumigant known as 1,3-dichloropropene (see **5** in Scheme 4) [13–15]. MIF is a pro-inflammatory cytokine with a phenylpyruvate tautomerase (PPT) activity. The physiological relevance of the PPT activity is not known, but it is not related to MIF's properties as a cytokine. In all of the enzymes characterized thus far, Pro-1 functions as a general base if it has a low pK_a value (~6.4) and as a general acid if it has a higher pK_a value (~9.2).

This review will focus on two recent developments in the TSF, which came about with the characterization of new 4-OT and MSAD family members. The first development was a result of the characterization of TomN, which is a 4-OT homologue found in the tomaymycin biosynthetic pathway [16]. TomN is noteworthy because it is the first known 4-OT (and the first known TSF

* Corresponding author. Fax: +1 (512) 232 2606.
E-mail address: whitman@austin.utexas.edu (C.P. Whitman).

[1] *Abbreviations used:* *cis*-CaaD and CaaD, *cis*- and *trans*-3-chloroacrylic acid dehalogenase, respectively; CHMI, 5-(carboxymethyl)-2-hydroxymuconate isomerase; FG41 MSAD, malonate semialdehyde decarboxylase from *Coryneform* bacterium strain FG41; hh4-OT, heterohexamer 4-oxalocrotonate tautomerase; MIF, macrophage migration inhibitory factor; Pp MSAD, malonate semialdehyde decarboxylase from *Pseudomonas pavonaceae*; 4-OT, 4-oxalocrotonate tautomerase; PPT, phenylpyruvate tautomerase; TSF, tautomerase superfamily.

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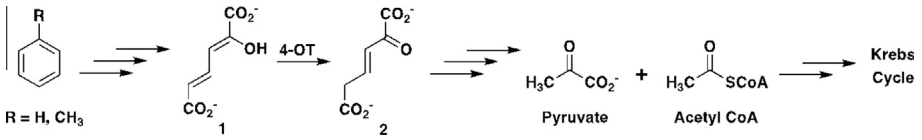


Scheme 1. The 4-OT-catalyzed reaction shown in the context of the meta-fission catabolic pathway.

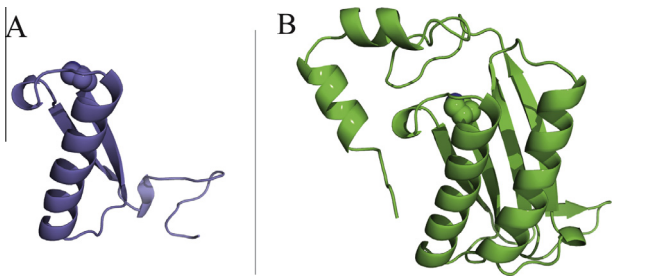
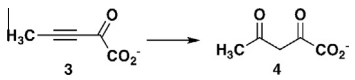


Fig. 1. Ribbon diagrams of the signature monomers in the tautomerase superfamily. (A) The 4-oxalocrotonate tautomerase monomer showing a single β–α–β unit (PDB code 4OTA). (B) The *cis*-3-chloroacrylic acid dehalogenase monomer showing the two β–α–β units covalently linked (PDB code 2FLZ). In both structures, Pro-1 is shown in space filling form.

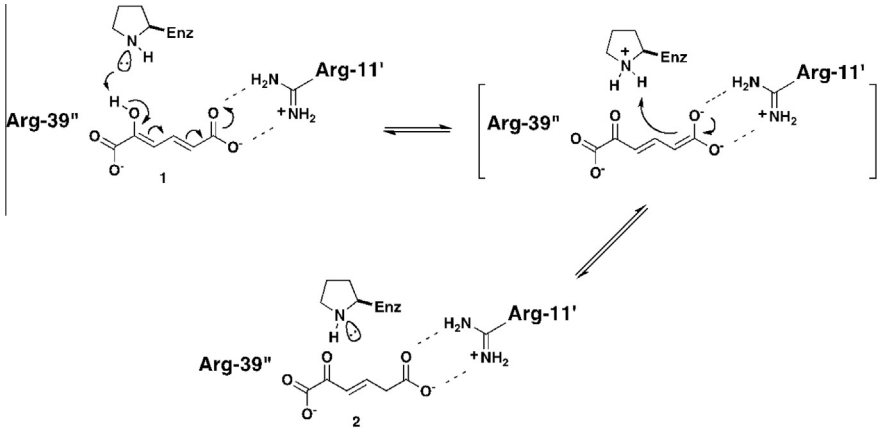


Scheme 2. The enzyme-catalyzed hydration of **3** to generate **4**.

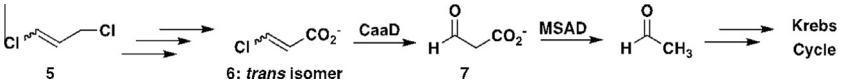
member) that participates in a biosynthetic pathway. All previously characterized 4-OT homologues (and TSF members) are found in catabolic pathways. Moreover, TomN appears to be part of a growing group of biosynthetic 4-OTs. The discovery of the biosynthetic 4-OTs suggests that there might be more metabolic diversity in the TSF than previously thought and opens up new avenues of research. The second development arose with the identification and characterization of five MSAD homologues. Although four of these homologues do not have assigned functions or genomic contexts that suggest functions, a homologue designated Bphy 4401 (or Bp4401) from *Burkholderia phymatum* strain STM815, has a modest hydratase activity, converting 2-oxo-3-pentynoate (**3**, Scheme 2) to acetopyruvate (**4**). In fact, the hydratase activity with **3** ($k_{cat}/K_m \sim 3 \times 10^3$ M^{-1} s^{-1}) is comparable to that observed for *trans*-3-chloroacrylic acid dehalogenase (k_{cat}/K_m - $\sim 6.4 \times 10^3$ M^{-1} s^{-1}) [17], which shows the highest hydratase activity measured for any TSF member thus far. Moreover, there is no detectable MSAD activity. The purpose of the hydratase activity in MSAD (and the MSAD homologues) is not yet clear, but it could remove covalent adducts between Pro-1 and reactive aldehydes (e.g., **7** and acetaldehyde). However, the observation that some homologues such as Bp4401 exhibit significant hydratase activity (on the order of that observed for *trans*-3-chloroacrylic acid dehalogenase) without decarboxylase activity is intriguing because it suggests that this activity might reflect a new function in the MSAD family once a biological substrate is identified.

The canonical 4-OT-catalyzed reaction

Mechanistic and structural studies have been carried out on 4-OT for more than 20 years. The enzyme is a homohexamer where each subunit is made up of 62 amino acids [18,19]. It does not require any coenzymes or metal ions. Pro-1, Arg-11, Arg-39, and Phe-50 (from 3 different monomers) are key players in the 4-OT-catalyzed conversion of **1** to **2** [18–27]. Pro-1 has a pK_a of ~6.4 (determined by an ^{15}N NMR titration study), and transfers a proton from the 2-hydroxy group of **1** to C-5 of **2** (Scheme 3, where the primes indicate the different subunits) [19–21]. The proton transfer is highly stereoselective, and generates (5*S*)-[5-D]**2**, when the reaction is carried out in D_2O [22]. The interaction between



Scheme 3. The catalytic mechanism for 4-OT with the roles of key residues indicated.



Scheme 4. The 1,3-dichloropropene catabolic pathway.