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Crystal Structure of *Escherichia coli* L-Arabinose Isomerase (ECAI), The Putative Target of Biological Tagatose Production

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Escherichia coli L-arabinose isomerase (ECAI; EC 5.3.1.4) catalyzes the isomerization of L-arabinose to L-ribulose *in vivo*. This enzyme is also of commercial interest as it catalyzes the conversion of D-galactose to D-tagatose *in vitro*. The crystal structure of ECAI was solved and refined at 2.6 Å resolution. The subunit structure of ECAI is organised into three domains: an N-terminal, a central and a C-terminal domain. It forms a crystallographic trimeric architecture in the asymmetric unit. Packing within the crystal suggests the idea that ECAI can form a hexameric assembly. Previous electron microscopic and biochemical studies supports that ECAI is hexameric in solution. A comparison with other known structures reveals that ECAI adopts a protein fold most similar to *E. coli* fucose isomerase (ECFI) despite very low sequence identity 9.7%. The structural similarity between ECAI and ECFI with regard to number of domains, overall fold, biological assembly, and active site architecture strongly suggests that the enzymes have functional similarities. Further, the crystal structure of ECAI forms a basis for indentifying molecular determinants responsible for isomerization of arabinose to ribulose *in vivo* and galactose to tagatose *in vitro*.

Keywords: L-arabinose isomerase; tagatose production

Introduction

The *Escherichia coli* araBAD operon consists of three metabolic genes, *araB*, *araA* and *araD*, encoding L-ribulokinase, L-arabinose isomerase, and L-ribulose-5-phosphate 4-epimerase, respectively; these enzymes convert L-arabinose to D-xylulose-5-phosphate, an intermediate in the pentose phosphate pathway (Scheme 1). This pathway allows microorganisms to utilize arabinose as a sole carbon source. The first step in the catabolism of L-arabinose by *E. coli* is catalyzed by the enzyme L-arabinose isomerase (ECAI, EC 5.3.1.4; swiss-prot P08202), which is the product of the structural gene *ara*A (M_r = 56,043 Da, 500 amino acid residues (aa), calculated pI of 6.3). Expression of structural gene *araA* is coordinated with *araB* (566aa) and *araD* (231aa) within the araBAD operon in *E. coli*.[1,2] The enzyme ECAI acts as a galactose isomerase *in vitro* catalyzing the conversion of galactose into tagatose in addition to its *in vivo* participation in the conversion of arabinose into ribulose (Scheme 2).[3–5] Therefore, it has been suggested that ECAI may have utility as a catalyst in commercial tagatose production†.[3,6]

Several enzyme sources of AI from mesophilic, thermophilic and hyperthermophilic organisms have been reported or patented as enzyme sources for the enzyme-mediated galactose-tagatose isomerization reaction.[3,4,7–11] This method of tagatose production is cost effective and easier than methods such as tagatose production by microorganisms through oxidation of galacitol[12,13] or when galactose derived from lactose hydrolysis is isomerised by a calcium catalyst‡.[2] In view of the

Abbreviations used: AI, L-arabinose isomerase; ECAI, *Escherichia coli* L-arabinose isomerase; ECFI, *E. coli* fucose isomerase; aa, amino acid residue(s); SAS, solvent accessible surface; SeMet, selenomethionine; PDB, Protein Data Bank.

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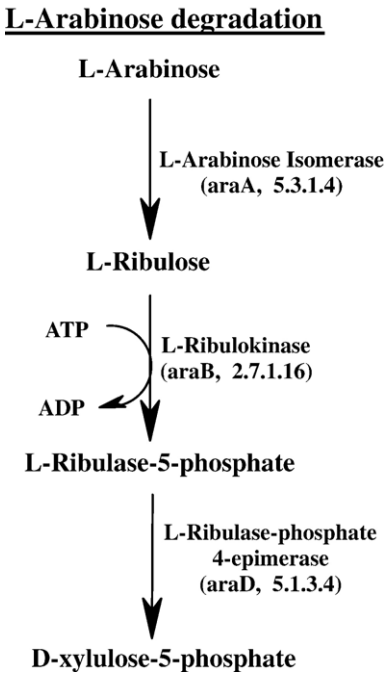
† World Patent pending (PCT/KR99/00661).

‡ US Patent no 5,078,796.



Scheme 1. Part of the pentose phosphate pathway. The conversion of arabinose into D-xylulose-5-phosphate is shown.

commercial interest in AIs, biochemical studies to understand differences in catalytic activity and thermostability related to tagatose production have been reported.[14,15] However, no crystal structure of any AIs is available to date, thus it is not yet possible to account for these differences. Electron microscopy and biophysical studies suggest that the biological unit of ECAI (mesophilic) is a hexamer[16–18] whereas thermophilic AIs exist as tetramers in solution.[4] The catalytic activity of ECAI is metal ion dependent; Mn^{2+} binding produces an enzyme with greater intrinsic activity and heat stability.[19,20] The metal ion dependence of catalytic activity in mesophilic and thermophilic AIs have recently been investigated.[15] In addition, although the ECAI is specific for L-arabinose, it can also convert other aldoses, such as D-galactose, DL-fucose and D-xylose, into their ketose forms at low rates.[21,22]

AIs have very low sequence homology (<10%) with the known crystal structures of other isomerases, such as xylose isomerase (XI);[23] fucose isomerase (FI);[24] glucose-phosphate isomerase,[25] triose-phosphate isomerase;[26] and mannose-phosphate isomerase.[27] Two types of reaction mechanism are proposed for ketol isomerases in the literature: the enediol mechanism[24,26] and the hydride-shift mechanism.[28,29] The crystal structure of *E. coli* FI bound with the substrate analogue L-fucitol demonstrated that the reaction is of the enediol type in which the hydroxyl groups of the catalytic center are bound by Mn^{2+}.[24] A hydride shift mechanism is accepted for D-xylose isomerase based on the known crystal structures of XIs.[30,31] Moreover, the isomerases acting on sugars without phosphate groups use a metal ion in catalysis.[32] It has been noted that all of the simple sugar isomerases are metalloproteins, and it has been suggested that the metal ions play the same role in these enzymes that phosphate plays in the phosphosugar isomerases.[19]

The compound D-tagatose has attracted commercial attention for several reasons. First, tagatose is a low calorie, full-bulk natural sugar, and has been considered a generally recognised as safe (GRAS) substance under United States Food and Drug Administration (FDA) regulations§.[33] Second, tagatose is approved for use in foods and beverages in Korea, Australia, New Zealand and the USA. Tagatose has proven to be virtually indistinguishable in taste from sucrose. It is 92% as sweet as sucrose when tested in 10% aqueous solutions.[34] The caloric value of tagatose and sucrose are 1.5 kcal/g‖ and 40 kcal/g, respectively¶. Tagatose is a ketose (stereoisomer of D-fructose) which occurs naturally in various foods, such as sterilized and powdered cow's milk, hot cocoa, a variety of cheeses, yogurt and other dairy products.[34–37] Moreover, people have been chronically exposed to tagatose through use of two common drugs, Chronulac® and Cephulac®.[38] Since the tagatose was first described,[34] the attractiveness of this compound has grown dramatically attracting the commercial trademark of Naturlose™§. Naturlose™ is a unique product, ideally suited for use in oral care products (toothpaste and mouthwash) and cosmetics (flavoured lipstick), as a safe sweetener.[39,40] Tagatose has been found to have a number of reported health benefits including treatment for type 2 diabetes, as a anti-hyperglycemic agent, promotion of weight loss, glucose spike blunting; anti-anemic, and anti-hemopilia; and promoting fetal survival and development[a],[41–46] Due to increasing commercial interest in tagatose, studies on AIs to identify the molecular determinants of AI activity at the atomic level are warranted. We have undertaken the X-ray crystallographic studies of AI's. Here, we describe the first crystal structure of the mesophilic enzyme ECAI in its apo form.

Results and Discussion

Overall fold of arabinose isomerase

The overall subunit structure of the ECAI monomer includes 16 β-strands and 17 α-helices, which are numbered and labelled as represented in Figure

§ April 2001, Joint Sperix Incorporated/Arla Foods press release.

‖ July 1998: Report prepared for Arla Foods submitted to the US food and Drug Administration.

¶ http://www.naturlose.com

[a] US Patent no.5 447, 917.