Structural and kinetic characterization of *Agrobacterium tumefaciens* D-Psicose-3-Epimerase

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I. Introduction

D-Psicose-3-Epimerase (DPEase) is an enzyme that was demonstrated to be useful in the bioproduction of D-psicose from its carbon-3 (C3) epimer D-fructose[1]. D-Psicose is a uncommon hexose that is poorly absorbed in the digestive track, produces almost no energy and is not toxic. In diabetic patients, the supplemental D-psicose might be helpful in preventing post prandial hyperglycemia.

In this report, we will discuss the structural characterization, the catalytic mechanism and the biochemical properties of *Agrobacterium tumefaciens* D-Psicose-3-Epimerase.

II. Structural analysis

DPEase (EC 5.3.1) catalyzes the reversible conversion of D-fructose and D-psicose by changing the configuration the C-3 stereogenic center[2] (figure 1). Futhermore, its substrates, D-fructose and D-psicose, are free sugars; they are not phosphorylated as in some epimerization reactions. The enzymatic activity of DPEase requires the presence of Mn^{2+} or Co^{2+}, although it has a low basal activity without ions[3]. These characteristics distinguish DPEase from the majority of epimerases.

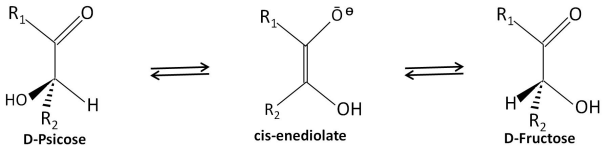

Figure 1. Reversible epimerization between D-Psicose and D-Fructose, where cis-enediolate is a intermediate state.

A. Overall structure

Native DPEase shows a tetrameric arrangement of 132 kDa (figure 2). Each of its subunits has of 289 amino acid residues with a molecular weight of 33 kDa. The topology of each subunit, in common with many other epimerases, is a TIM-barrel fold with a cluster of eight β-strands surrounded by twelve α-helices (figure 2).

B. Active site

The active site is located in the central cleft of the subunit, where there is a metal-binding site. A Mn^{2+} ion is bound with octahedral coordination by four residues (E150, D183, H209 and E244) and two water molecules[2] (figure 3).

There are no major conformational change between the apo- and holo-enzymes (RMSD of 0,6 Å for the α carbon backbone after superimposing the backbone of the tetrameres)[2] (figure 3). Thus, the binding of the substrate did not produce any large structural changes. However, some small alterations are noticeable, for example, the residues Y6, F246, W14 and W112 move towards the bound D-Fructose.

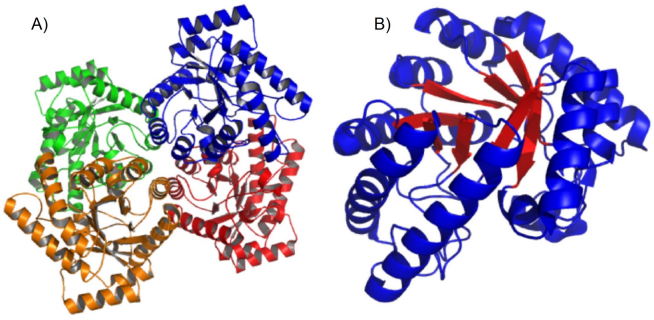

Figure 2. Cartoon representation of *A. tumefaciens* DPEase, each subunit in a different color. B) One subunit in blue, with the β-strands shown in red. (PDB ID: 2HK0)

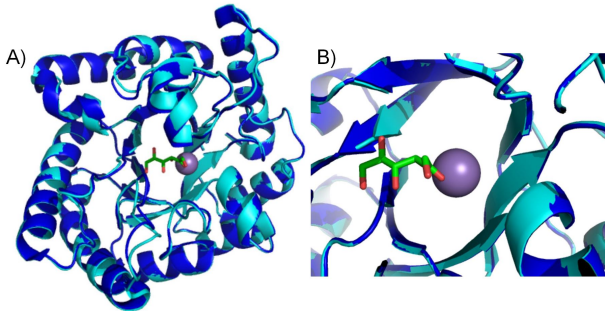

Figure 3. Cartoon representation of the superposition of the subunit (A) or active site (B) of DPEase with (blue) and without (cyan) D-Fructose. D-Fructose is shown in green and Mn^{2+} ion is in purple. (PDB ID: 2HK0 and 2HK1)

The binding of D-fructose shifts the loops between β4 and α4 and between α1' and α1. This permits to enclose the substrate in the active site[2]. With the concerted action of the D-fructose binding and the loop shift, all the water molecules leave the active site in the "closed" conformation, even those implicated in Mn^{2+} ion coordination, which are replaced by O2 and O3 from D-Fructose.

C. Stabilisation of D-Fructose

In the DPEase-fructose complex, the O2 and O3 of the sugar are in an eclipsed conformation that mimics the intermediate cis-enediolate of the epimerization reaction.

Three residues, E156, H186 and R215 are involved in a putative hydrogen bond with O1 of the D-fructose. The Mn^{2+} ion is within 2.4 Å of the O2 and O3[2]. O2 might be involved in hydrogen bonds with H186 or E244. O3 is also possibly stabilized by interactions with E150 or E244. For O4 there is a possible interaction with E150. O5 had no possible interaction within 4.0 Å. To finish, O6 seems to interact with A107 or I66 with hydrogen bonds to the backbone (figure 4).

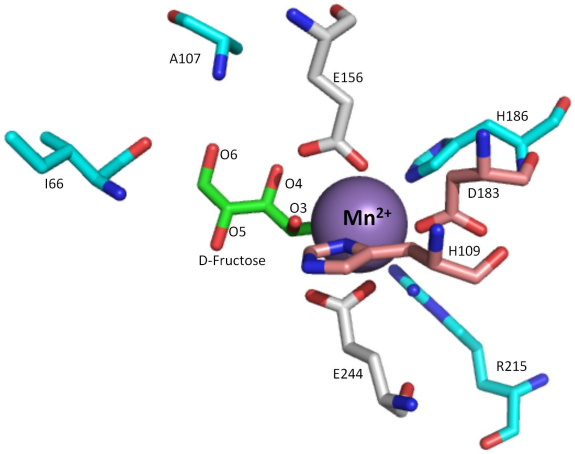

Figure 4. Active site of DPEase. D-Fructose is shown in green, the Mn^{2+} ion is in purple and all cyan residues are implicated in the D-Fructose stabilization. Pink residues are involved in Mn^{2+} ion coordination. Finally, white residues are involved in both. (PDB ID: 2HK1)

III. Biochemical Analysis

A. Catalytic mechanisms

The putative catalytic mechanism of isomerisation involve two glutamate acid residues coordinated with the Mn^{2+} ion : E150 and E244. One of the two residues removes a proton from C3 to generate a cis-enediolate intermediate, and the second one helps to protonate C3 from the other side, in order to form the epimer [3],[2].

In the case of the epimerizsation from D-fructose to D-Psicose, E244 removes the proton and E150 gives it back (figure 5).

B. Enzyme activity

A. tumefaciens DPEase has its enzymatic activity greatly enhanced in the presence of Mn^{2+} or Co^{2+} [3]. The bioconversion level of D-psicose is 32 % with a reaction buffer containing 15 U of enzyme and 700 g/L of D-Fructose at 50°C pH 8.0 for 100 minutes and the final specific activity found for DPEase is 8,89 U/mg[1].

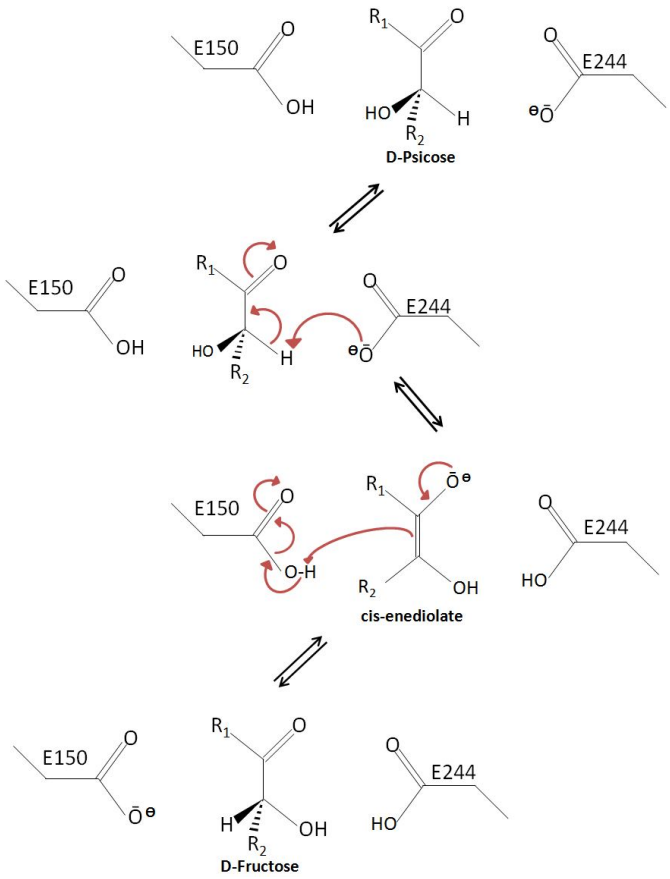

Figure 5. Proposed catalytic mechanisms for DPEase.

When the enzyme is at 50°C and with a pH of 8.0 and EPPS as buffer, it reaches its maximum activity and can produce D-psicose with a concentration of 230 g/L[1].

The equilibrium ratio at 30°C between D-psicose and D-fructose is 32:68. DPEase shows a Michaelis Menten constant (K_M) of 12 ± 0.11 mM for D-Psicose and 24 ± 0.15 mM for D-Fructose [1]. Which means that the enzyme has a better affinity for D-Psicose rather than D-Fructose.

Moreover, the turnover number (k_{cat}) are equivalent with 2068 ± 28 min^{-1} for D-Fructose and 2381 ± 22 min^{-1} for D-Psicose[1].

Therefore, the catalytic efficiency (k_{cat}/K_M) values are 85 ± 1.27 mM/min^{-1} for D-Fructose and 205 ± 2.67 mM/min^{-1} for D-Psicose The conversion rate is better with D-Psicose.

Finally, the thermostability of DPEase has been measured and the half-life of the enzyme is about 8.9 min at 55°C.

TABLE I
KINETIC PARAMETERS OF *C. cellulolyticum* AND *A. tumefaciens* DPEASE [4]

	C. cellulolyticum DPEase			*A. tumefaciens* DPEase		
substrate	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M (mM/min^{-1})	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M (mM/min^{-1})
D-psicose	3243.5±56.5	17.4±0.2	186.4±1.9	2381±22	12±0.11	205±2.67
D-fructose	3354.5±47.2	53.5±1.8	62.7±1.5	2068±28	24±0.15	85±1.27

IV. Discussion

A. tumefaciens DPEase seems to be a promizing bioconvertion tool of D-Fructose to D-Psicose. However, a new DPEase coming from *Clostridium cellulolyticum* has been found. They posses almost the same bioconversion rate of 32% but *C. cellulolyticum* DPEase has a remarkable thermostability and a half-life of 9.5h at 55°C[3].

Moreover, both enzymes showed equivalent kinetic parameter with *C. cellulolyticum* DPEase show with D-Psicose a K_M of 17.4±0.2 mM, a k_{cat} of 3243.5±56.5 mM/ min^{-1} and a k_{cat}/K_M of 186.4 ± 1.9mM/min^{-1} (table I)[4].

References

[1] Kim, H.-J., Hyun, E.-K., Kim, Y.-S., Lee, Y.-J. and Oh, D.-K. Characterization of an *Agrobacterium tumefaciens* D-psicose 3-epimerase that converts D-fructose to D-psicose. *Appl. Environ. Microbiol.* 72, 981985 (2006).

[2] Kim, K., Kim, H.-J., Oh, D.-K., Cha, S.-S. and Rhee, S. Crystal structure of D-psicose 3-epimerase from *Agrobacterium tumefaciens* and its complex with true substrate D-fructose: a pivotal role of metal in catalysis, an active site for the non-phosphorylated substrate, and its conformational changes. *J. Mol. Biol.* 361, 920931 (2006).

[3] Chan, H.-C. et al. Crystal structures of D-psicose 3-epimerase from *Clostridium cellulolyticum* H10 and its complex with ketohexose sugars. *Protein Cell 3*, 123131 (2012).

[4] Mu, W. et al. Cloning, Expression, and Characterization of a d-Psicose 3-Epimerase from *Clostridium cellulolyticum* H10. *J. Agric. Food Chem.* 59, 77857792 (2011).