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Synthesis of rare sugars with L-fuculose-1-phosphate aldolase (FucA) from *Thermus thermophilus* HB8

Zijie Li [a,b,†], Li Cai [b,†], Qingsheng Qi [a], Thomas J. Styslinger [b], Guohui Zhao [b], Peng George Wang [b,*]

[a] National Glycoengineering Research Center and the State Key Laboratory of Microbial Technology, Shandong University, Jinan, Shandong 250100, China
[b] Department of Chemistry and Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210, USA

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ABSTRACT

We report herein a one-pot four-enzyme approach for the synthesis of the rare sugars D-psicose, D-sorbose, L-tagatose, and L-fructose with aldolase FucA from a thermophilic source (*Thermus thermophilus* HB8). Importantly, the cheap starting material DL-GP (DL-glycerol 3-phosphate), was used to significantly reduce the synthetic cost.

The aldol reaction has long been recognized as one of the most powerful methods for new carbon–carbon bond formation.[1,2] The high stereoselectivity of aldolases in C–C bond construction confers upon them tremendous applications as synthetic biocatalysts.[3–5] Among the aldolases, dihydroxyacetone phosphate (DHAP)-dependent aldolases are particularly attractive as a set of four possible diastereomers of vicinal diols can be synthesized conferring upon them the potential to be used in the synthesis of rare sugars and other hydroxylated natural products.[1,6,7] Unfortunately, the strict requirement for the donor substrate DHAP, a rather expensive and unstable compound, limits aldolase use in large-scale preparation.[6,8,9] Therefore, the capability to generate DHAP from inexpensive sources could ultimately broaden the scope of aldolase reactions making it an attractive challenge.[10,11]

Rare sugars are monosaccharides, and their derivatives, that are particularly uncommon in nature.[12] Importantly, rare sugars possess many potential applications in the food, pharmaceutical and nutrition industries.[13] In addition, rare sugars can be used as starting materials for the synthesis of intriguing natural products with important biological activities.[13,14] Unfortunately, most rare sugars are quite expensive, and their synthetic routes are both limited and costly due to the expense of costly starting materials. As a specific example, D-psicose, a rare sugar and C-3 epimer of D-fructose, has the unique property of being an ideal sucrose substitute. Compared with sucrose, it has 70% the sweetness but provides no energy due to its suppressive effect toward hepatic lipogenic enzymes.[15,16] Furthermore, it has been observed that foods supplemented with D-psicose exhibit higher antioxidant activity.[15] Furthermore, D-psicose can be used as a precursor in the synthesis of xylosylpsicoses, which are promising candidates for prebiotics, cosmetics and therapeutic uses.[17] However, only two enzymes D-tagatose 3-epimerase from *Pseudomonas cichorii*[18,19] and D-psicose 3-epimerase from *Agrobacterium tumefaciens*[20] have been reported for D-psicose production. In addition, due to the fact that the interconversion between D-fructose and D-psicose is an equilibrium process, the large scale and high yield production of D-psicose remains quite challenging.

A potential solution to the above mentioned problems with rare sugar production lies in the use of aldolase reactions (i.e., DHAP aldolase reactions), which are capable of generating vicinal diol diastereomers and are thus an ideal tool for the construction of rare sugars.[21] In fact, the preparation of rare sugars, as well as their derivatives, represents the most significant applications of DHAP-dependent aldolases.[8,14,22,23] In our previous work, we synthesized two rare sugars (D-sorbose and D-psicose) simultaneously with Rhamnulose-1-phosphate aldolase (RhaD) from *Escherichia coli* in good overall yields (Scheme 1). However, RhaD showed no stereo-preference for either product (syn/anti ~1:1) when accepting D-glyceraldehyde. Here we describe another enzyme, L-fuculose-1-phosphate aldolase, from *Thermus thermophilus* HB8 ($FucA_{T.HB8}$) capable of stereoselectively synthesizing D-psicose from L-glycerol 3-phosphate (L-GP) and D-glyceraldehyde. Alternatively, the rare sugars L-tagatose and L-fructose were synthesized efficiently with

* Corresponding author. Tel.: +1 614 292 9884; fax: +1 614 688 3106.
E-mail addresses: pgwang@chemistry.ohio-state.edu, wang.892@osu.edu (P.G. Wang).
† Authors who contributed equally to this work.

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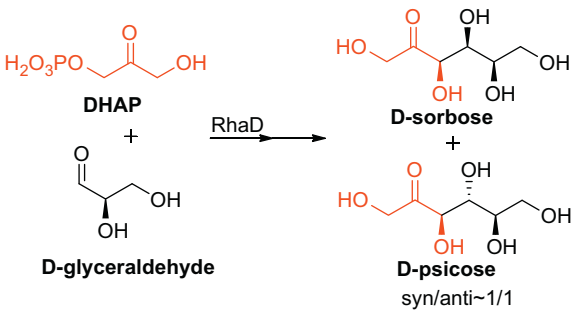


Scheme 1. Enzymatic synthesis of D-sorbose and D-psicose with RhaD.

$FucA_{T.HB8}$ using L-glyceraldehyde as the acceptor instead of the D-isomer.

FucA is a class II DHAP-dependent aldolase that catalyzes the reversible cleavage of L-fuculose 1-phosphate to DHAP and L-lactaldehyde, a central step for L-fucose metabolism in bacteria.[24] Furthermore, FucA from *E. coli* ($FucA_{E.coli}$) is a homotetramer of 215 amino acid residues containing one Zn^{2+} ion per subunit.[25] Importantly, FucA has demonstrated its value in aldol reactions to selectively afford vicinal diols with the anti configuration, which is advantageous for D-psicose construction.[26]

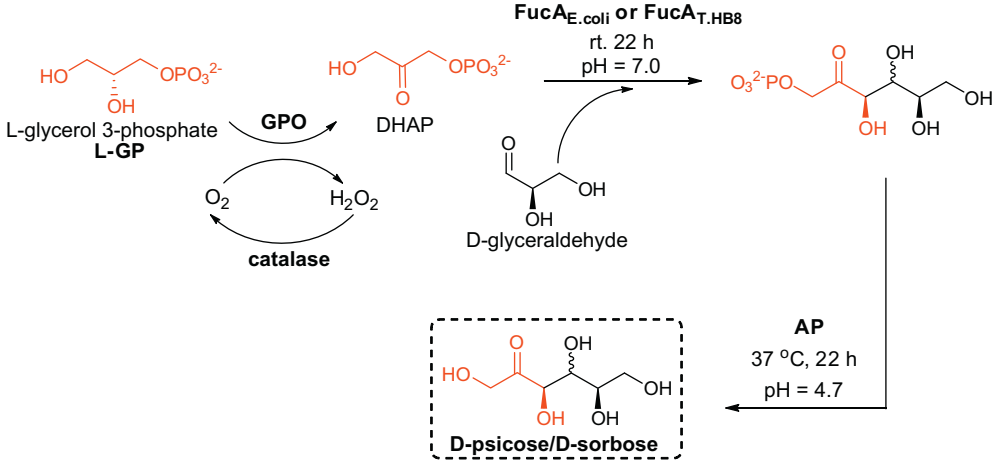
Consequently, we first employed $FucA_{E.coli}$ in the synthesis of D-psicose via a one-pot four enzyme reaction in which DHAP was generated from the oxidation of L-glycerol 3-phosphate (L-GP) by glycerol phosphate oxidase (GPO) (Scheme 2).[27] To degrade the corresponding by-product, hydrogen peroxide, and regenerate oxygen, catalase was added to the reaction mixture. Subsequently, the DHAP generated in situ was coupled with D-glyceraldehyde by FucA to give D-psicose 1-phosphate. Lastly, the phosphate group was removed under acidic conditions by acid phosphatase (AP) to furnish D-psicose. Unfortunately, after silica gel and gel filtration purification, only a 12% yield of D-psicose could be obtained (Table 1). Compared to the high yield of D-psicose 1-phosphate when DHAP was used directly for aldol addition[26], this low yield can most likely be attributed to the possibility that $FucA_{E.coli}$ may not be compatible with the one-pot system.

To improve the overall yield for this synthesis, we turned our attention towards enzymes from thermophilic bacteria, which show a great potential for biotechnological applications.[28] Upon investigation, we noticed that the crystal structure of FucA from *T. thermophilus* HB8 ($FucA_{T.HB8}$) was recently reported but the enzyme had yet to be used for synthetic purposes.[29] Consequently, we expressed and purified $FucA_{T.HB8}$ in *E.coli* (see Supplementary data) and then employed it in the one-pot reaction under the same conditions (Scheme 2). To our delight, the yield was greatly improved (Table 1 and 67% vs 12%) with the diastereomer D-sorbose being detected as a minor product when D-glyceraldehyde was used as the acceptor (Fig. 1 maroon line). The product ratio of D-psicose/D-sorbose was ~5:1 as determined by ion exchange HPLC (Fig. 1 maroon line).

L-glycerol 3-phosphate is a reasonably stable starting material and commercially available, however it is still quite expensive, and thus not ideal for large-scale synthesis. In contrast, racemic glycerol 3-phosphate (DL-GP) is much cheaper and it has been reported that GPO can exclusively oxidize the L-isomer.[10,27] Therefore, it was envisioned that a racemic mixture could deliver a 50% yield of DHAP and the remaining D-glycerol 3-phosphate could be isolated during purification. Most significantly, the synthetic cost could be greatly decreased by utilizing racemic DL-glycerol 3-phosphate (DL-GP). Thus, under the same reaction conditions, staring from racemic DL-GP, a total yield of 58% (Table 1) of D-psicose/D-sorbose was obtained and the ratio was determined by HPLC (Fig. 1 green line, D-psicose/D-sorbose 8.4/1). The only difference for reactions starting with DL-GP is that DL-GP was used in excess and the reaction yield was calculated using the acceptor as the limiting reagent (see Supplementary data for reaction stoichiometry). After silica gel and gel filtration chromatography, the mixture containing D-psicose/D-sorbose could be easily separated by a cation exchange resin column (Ca^{2+} form) under elevated temperature (70 °C) (see Supplementary data).

Alternatively, when L-glyceraldehyde was used as the acceptor, instead of the D-isomer, two additional rare sugars, L-fructose and L-tagatose, could accordingly be synthesized as allowed by the stereoselectivity of FucA (Scheme 3). L-fructose is a well known non-nutritive sweetener[30] and an inhibitor of several glycosidases[31] with its enzymatic synthesis by the aldolase RhaD being greatly exploited by Wong and co-workers.[14,23] However, L-tagatose, which is a functional sweetener[32] and a promising starting material for the synthesis of high value-added complex compound,[33] has not yet been broadly utilized due to its high cost of production. L-Tagatose can be produced via oxidation of galactitol by *Klebsiella pneumoniae* 40b[34] or generated via epimerization of L-sorbose by D-tagatose 3-epimerase from *Pseudomonas sp.* ST-24[35], however, both methods give low yields.

As shown in Scheme 3, we were able to successfully carry out the synthesis of L-fructose and L-tagatose in a one-pot fashion using DL-GP as starting material and L-glyceraldehyde as the acceptor. It is interesting to note that the acid phosphatase (AP) we previously



Scheme 2. Synthesis of D-psicose with FucA and acid phosphatase (see Table 1 for yields and product ratio).

Table 1
Summary of FucA catalyzed reactions using different starting materials and acceptors

Enzyme	Starting saterial	Acceptor	Product(s)		Yield (%)	Ratio
$FucA_{E.coli}$	L-GP	D-Glyceraldehyde	D-Psicose		12	1:0
$FucA_{T.HB8}$	L-GP	D-Glyceraldehyde	D-Psicose	D-Sorbose	67	5.3:1
$FucA_{T.HB8}$	DL-GP	D-Glyceraldehyde	D-Psicose	D-Sorbose	58	8.4:1
$FucA_{T.HB8}$	DL-GP	L-Glyceraldehyde	L-Fructose	L-Tagatose	47	1.2:1

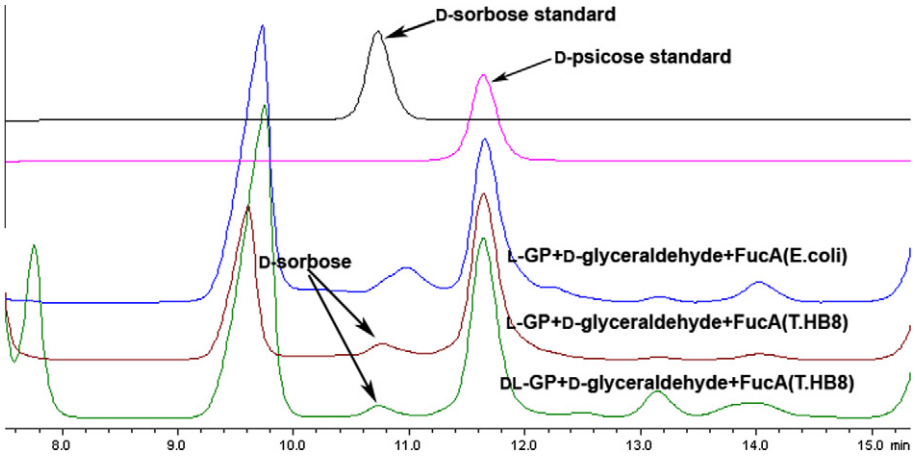
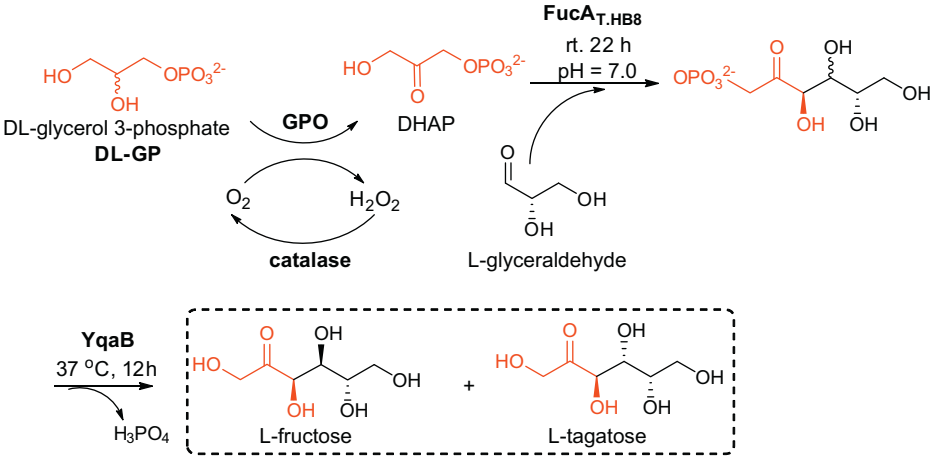


Figure 1. HPLC (hydrogen form, sulfonated divinyl benzene–styrene copolymer support and eluted with 5 mM H_2SO_4) profile of final reaction mixture in Scheme 2 compared with authentic samples.



Scheme 3. Synthesis of L-tagatose and L-fructose with $FucA_{T.HB8}$ using L-glyceraldehyde.

used could not dephosphorylate L-tagatose 1-phosphate completely to give the desired sugar products. However, upon searching relevant literature we found that YqaB phosphatase from *E.coli*, belonging to the haloacid dehalogenase (HAD) superfamily, shows a remarkably broad substrate range, among which the dephosphorylation activity toward D-fructose-1-phosphate is the highest.[36] We thus expressed and purified YqaB phosphatase from *E.coli* and the dephosphorylation reaction went quite smoothly under neutral conditions. L-Fructose and L-tagatose were afforded in moderate yield (see Table 1, total yield 47%) and the ratio was determined by HPLC (Fig. 2, L-fructose/L-tagatose 1.2/1). After silica gel and gel filtration chromatography, the mixture containing L-fructose/L-tagatose could be easily separated by cation exchange resin column (Ca^{2+} form) under elevated temperature (70 °C) (see Supplementary data). As shown in Table 1, one thing worth noting is that $FucA_{T.HB8}$ seems to lose its stereoselectivity when accepting L-glyceraldehyde–similar to our previous discovery that L-rhamnulose-1-phosphate aldoalse (RhaD) produces a single product (L-fructose) when using L-glyceraldehyde while losing its stereoselectivity when D-glyceraldehyde is the acceptor. Nonetheless, this property of aldolases could be well utilized for the synthesis of various types of rare sugars.

In summary, we developed a one-pot four-enzyme approach for the synthesis of the rare sugars D-psicose, D-sorbose, L-tagatose, and L-fructose with aldolase FucA. All synthesized rare sugars were characterized by ^1H NMR and compared with commercially authentic samples (see Supplementary data). To the best of our knowledge, this is the first use of FucA from a thermophilic source (*T. thermophilus* HB8), which proved to be more efficient than its *E.coli* counterpart. Importantly, the one-pot four-enzyme approach

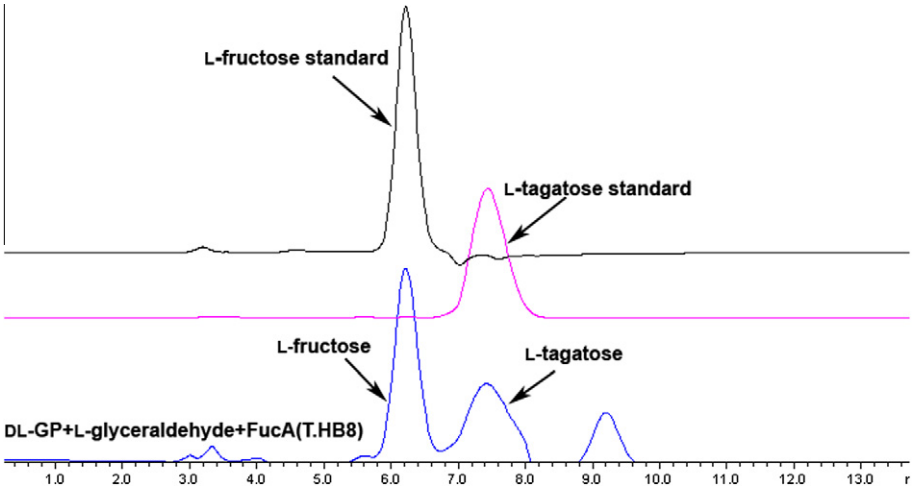


Figure 2. HPLC (calcium form, sulfonated divinyl benzene–styrene copolymer support and eluted with H_2O) profile of final reaction mixture in Scheme 3 compared with authentic samples.

does not require the use of expensive DHAP and most significantly, the inexpensive starting material DL-GP was used to greatly reduce the synthetic cost. We believe this approach could ultimately contribute to the synthesis of other rare sugars and their derivatives as well.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.072.

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