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Fructose 6-phosphate aldolase and 1-deoxy-D-xylulose 5-phosphate synthase from *Escherichia coli* as tools in enzymatic synthesis of 1-deoxysugars

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Abstract

We cloned the genes for a novel fructose 6-phosphate aldolase (FSA) and for 1-deoxy-D-xylulose 5-phosphate synthase (DXS) from *Escherichia coli* and investigated in their potential for enzymatic synthesis. FSA is the first example of a novel type of class I aldolases as it catalyzes the reversible formation of fructose 6-phosphate from dihydroxyacetone and D-glyceraldehyde 3-phosphate. It utilizes several aldehydes as acceptor compounds, and interestingly, hydroxyacetone is an alternative donor which can be used to generate 1-deoxysugars. DXS catalyzes the decarboxylation of pyruvate and transfers the covalently bound thiamin diphosphate-intermediate C_2-moiety to D-glyceraldehyde 3-phosphate. The reaction product, 1-deoxy-D-xylulose 5-phosphate, is a precursor to isoprenoids and vitamins. DXS also uses other sugar phosphates as well as short aldehydes as acceptor substrates. Apart from pyruvate, the two α-ketoacids hydroxypyruvate and α-oxobutyrate could be used as donor substrates. FSA and DXS were successfully used to synthesize 1-deoxyketoses from C_4 (1-deoxy-erythrulose) to C_7 (1-deoxy-sedoheptulose) in phosphorylated and non-phosphorylated form.

Keywords: Novel class I aldolase; 1-Deoxy sugars; Dihydroxyacetone; Hydroxyacetone; Thiamine diphosphate

1. Introduction

Fructose 6-phosphate aldolase (FSA) is a novel type of class I aldolases (Schiff base intermediate) that catalyses the reversible formation of fructose 6-phosphate from dihydroxyacetone (DHA) and D-glyceraldehyde 3-phosphate [1]. The enzyme utilizes the inexpensive donor compound DHA instead of the phosphorylated form, DHAP. DHAP is used by other aldolases as the classical fructose 1,6-bisphosphate aldolase which is widely used as biocatalyst for C−C bonding [2,3]. FSA could therefore be an interesting novel tool in chemoenzymatic synthesis since the reaction products do not require a subsequent dephosphorylation. Here we show that also hydroxycacetone (acetol) is a donor compound for FSA allowing access to various 1-deoxysugars.

1-Deoxy-D-xylulose 5-phosphate (DXP) has recently attracted much interest as it is the biosynthetic precursor for isoprenoids in most bacteria, in plant chloroplasts [4], and in the malaria parasite, *Plasmodium falciparum*. DXP synthase (DXS) is a transketolase-related enzyme which can be used to provide isotopically labeled species of this compound [5]. Moreover, DXP analogues and derivatives can be useful tools to unravel this not yet completely

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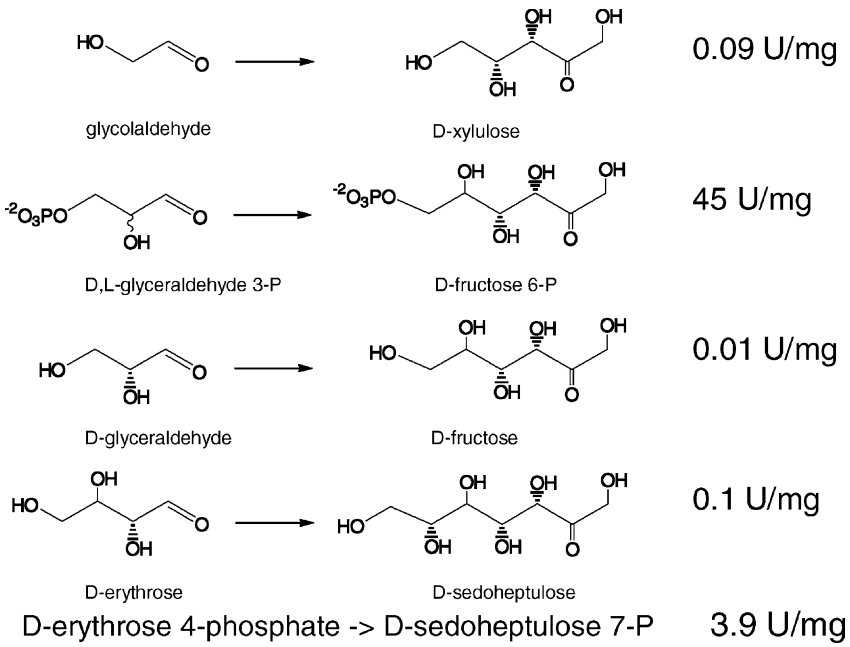


Scheme 1. Reaction rates of FSA with DHA and various aldehydes as acceptors. Specific activities of FSA towards those substrates are given in U (μmol min mg^{-1} of protein). Note that unphosphorylated glyceraldehyde is not a significant acceptor compound.

known biosynthetic pathway or act as inhibitors of subsequent enzymatic reaction steps and are therefore potential antibacterial, herbicidal, and anti-malarial agents. Here, we present data which highlight the usefulness of both FSA and DXS showing both the donor and acceptor specificities of these enzymes.

2. Experimental

If not indicated otherwise, sugars, sugar phosphates, and other fine chemicals of highest available purity were purchased from Sigma (Deisenhofen, Germany) as well as the auxiliary enzyme sorbitol dehydrogenase. Aldehydes and D-erythrose were from Fluka (Neu-Ulm, Germany). Auxiliary enzymes 6-phosphoglucose isomerase, glucose 6-phosphate dehydrogenase, and calf intestinal alkaline phosphatase were from Roche Diagnostics Mannheim, Germany. SDS was from Serva (Heidelberg, Germany). 1-Deoxy-D-xylulose 5-phosphate was prepared enzymatically as described previously using purified DXS [5]. 1-Deoxy-D-xylulose was obtained by dephosphorylation of 1-deoxy-D-xylulose 5-phosphate with alkaline phosphatase. Homo-1-deoxy-D-xylulose (1,2-dideoxy-D-*threo*-3-hexulose) was a kind gift from W. Boland (Jena, Germany).

Fructose 6-phosphate aldolase and 1-deoxy-D-xylulose 5-phosphate synthase of *E. coli* were purified from recombinant *E. coli* strains and spectrophotometrical enzyme assays for FSA and DXS activity were performed as described elsewhere [1,6]. Enzyme-dependent conversion of non-physiological substrates and product formation by FSA and DXS was also monitored by analysis of the reaction mixtures on an HP87X-H column (Bio-Rad, Munich, Germany) using a LaChrom D-7000 HPLC-system (Merck, Darmstadt) with a UV detector and a refraction index monitor in series. H_2SO_4 (6 mM) was used as isocratic eluent. The formation of 1-deoxy-D-xylulose, homo-1-deoxy-D-xylulose, xylulose, sedoheptulose, DHA, HA, and glycolaldehyde by FSA or DXS was quantified by calibration with these substances. We wish to note that—due to the lack of proper standards and the use of HPLC—we currently cannot exclude the presence of minor amounts of

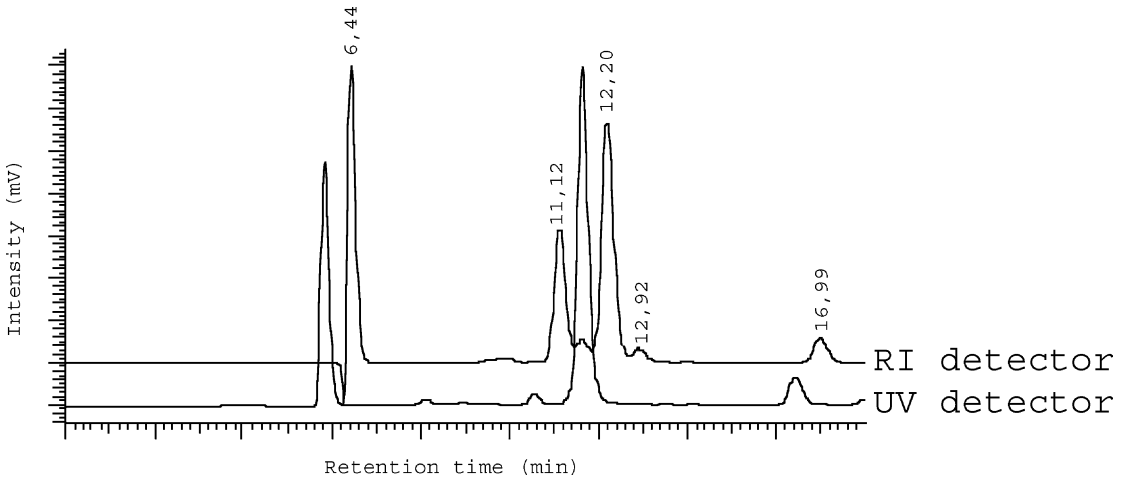
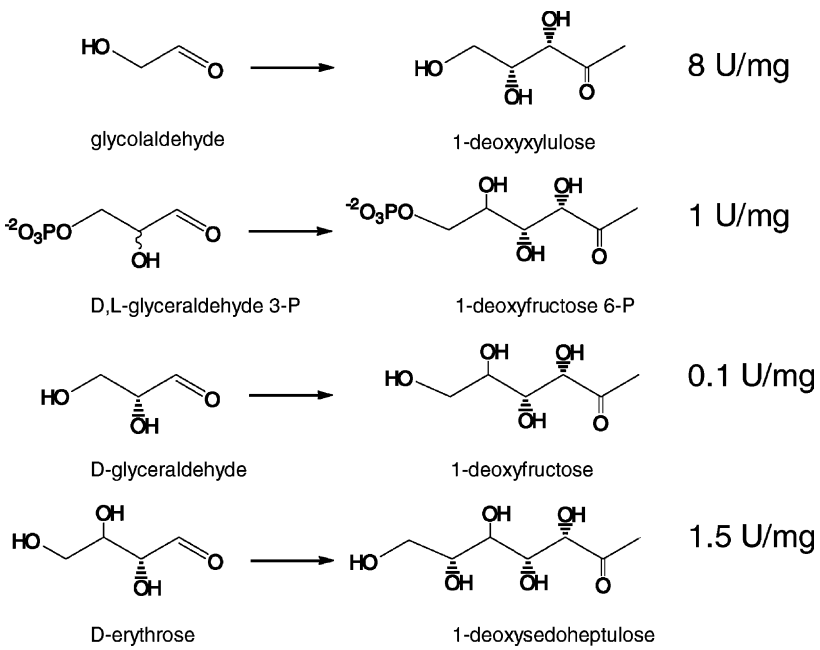


Fig. 1. FSA reaction with hydroxyacetone as donor and glycolaldehyde as acceptor. HPLC chromatogram with RI and UV detector of reaction mixture after 12 h, with residual hydroxyacetone (16.99 min) and glycolaldehyde (12.92 min). Product 1-deoxy-D-xylulose is at 12.20 min. Other peaks: at 6.44 min = void volume (protein), at 11.22 min = buffer substance (glycylglycine).

other diastereomers or the other enantiomer (in the case of 1-deoxy-L-erythrulose), respectively. This has to be taken into consideration when the enantiospecificity of the two enzymes is addressed.

3. Results and discussion

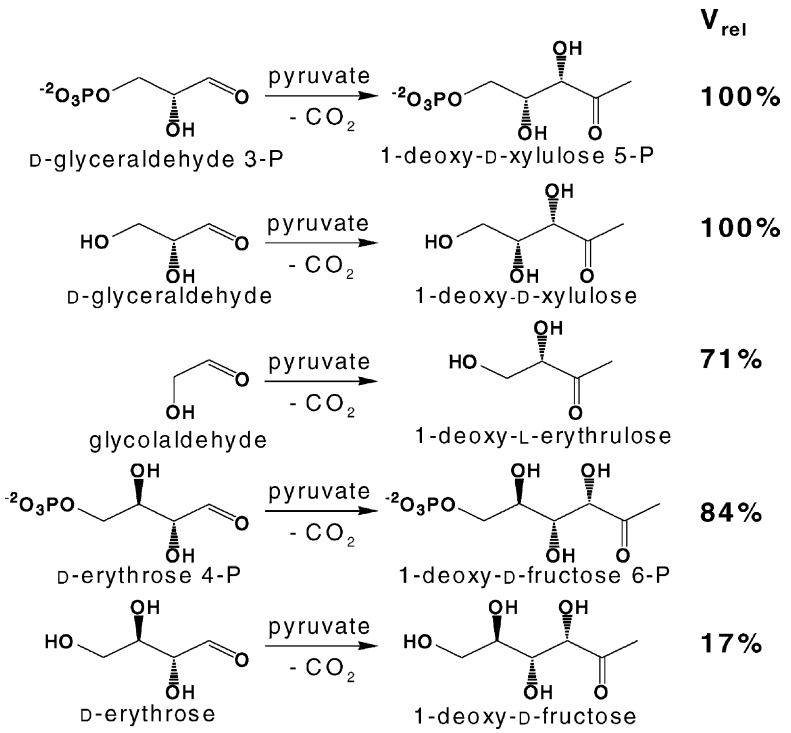
The usefulness of C−C bonding enzymes as aldolases in chemoenzymatic synthesis has been well



Scheme 2. Reactions of FSA with hydroxyacetone and various aldoses. The activities of FSA with hydroxyacetone towards those substrates are given in U (μmol min mg^{-1} of protein).

documented in past years [2,3,7,8]. A certain drawback for the synthetic use of, e.g. as rabbit muscle aldolase (RAMA) or its bacterial counterparts, however, is the necessity to use the expensive and unstable donor compound, DHAP [2,3]. Not only is DHAP expensive but it also yields a phosphorylated sugar product which may have to be dephosphorylated to obtain the final desired product.

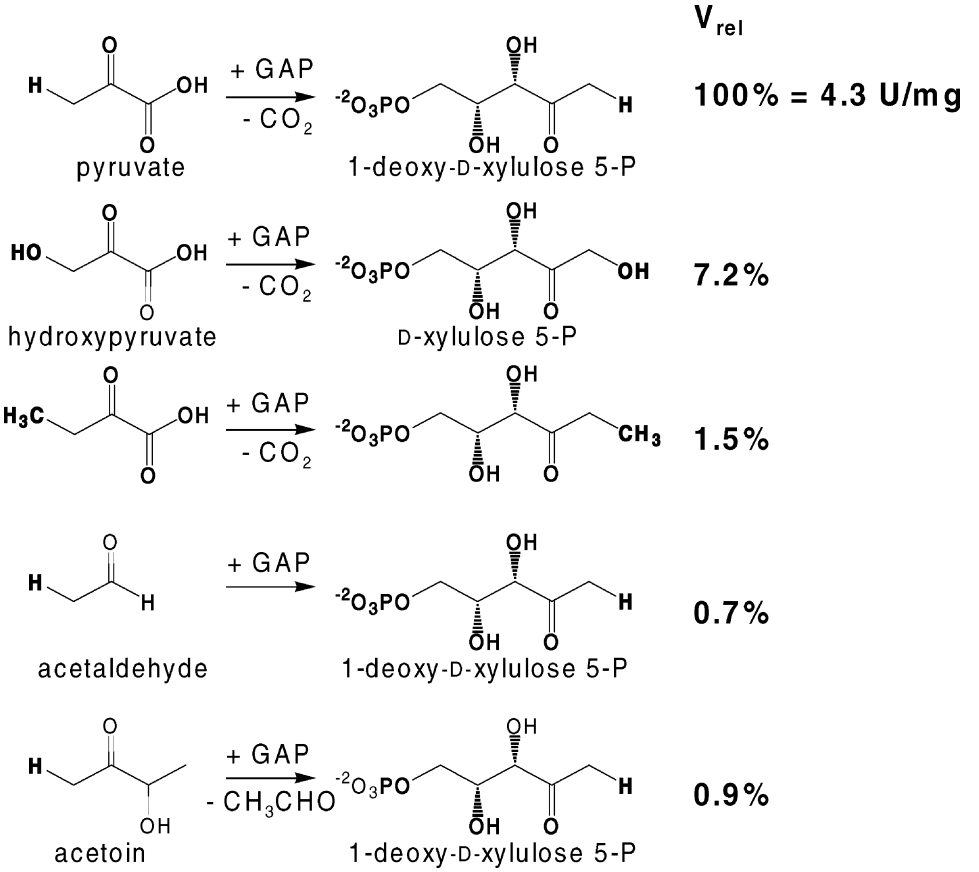
Recently, we reported on the discovery of a novel class I aldolase (Schiff base forming, no metal ion requirement), recombinant fructose 6-phosphate aldolase (FSA) from *Escherichia coli* [1] which utilizes dihydroxyacetone (DHA) instead of DHAP as C3-donor compound for the synthesis of fructose 6-phosphate. Favorable features of this enzyme for a prospective synthetic use are its broad pH range (from 5.5 to 11.0) and a remarkably high temperature stability ($t_{1/2}$ of 16 h at 75 °C) although the enzyme stems from a mesophile microorganism. The enzyme is enantiospecific (forming 3*S*, 4*R* products) and accepts several hydroxyaldehydes and their respective phosphorylated forms as substrates [1]. In order to assess the future use for chemoenzymatic synthesis, we established kinetic data for various aldehydes (see Scheme 1) and found a novel aldolase donor compound, hydroxyacetone (acetol) which can be used for synthesis of rare 1-deoxysugars and derivatives (Fig. 1). Most prominently, the enzyme displays a relatively high reaction rate with hydroxyacetone and glycolaldehyde (8 U/mg of protein, Scheme 2) yielding 1-deoxyxylulose. Presently we are at a loss to explain why glycolaldehyde as acceptor was much preferred when hydroxyacetone was used as donor instead of DHA (compare Schemes 1 and 2). 1-Deoxy-D-xylulose was formed at an even higher rate than with DXS, the cognate catalyst of this reaction [4,6]. Thus, various 1-deoxysugars from C5 to C7 are now available with FSA and the inexpensive hydroxyacetone as donor. As the equilibrium of the reaction strongly favors product formation [1] this is a novel approach to the class of rare 1-deoxysugars.



Scheme 3. Alternative acceptor substrates of DXS with pyruvate as donor substrate. Relative activities of the enzyme are given as per cent of the physiological reaction with pyruvate and GAP which is 4.3 U/mg of protein (= 100%).

A prominent 1-deoxy sugar is 1-deoxy-xylulose 5-phosphate (DXP), the precursor for biosyntheses leading to isoprenoids in many bacteria, green algae, and in plant chloroplasts (for references see [4]) as well as in the malaria parasite, *Plasmodium falciparum* [9]. The cognate enzyme DXP synthase (DXS) has been cloned from various sources but no analysis of its use in organic syntheses has been reported so far. We studied several aldehydes as acceptors using pyruvate as donor. Besides D-glyceraldehyde and its phosphorylated form, also acetaldehyde, glycolaldehyde, erythrose and ribose (and the phosphates of the latter two) are accepted by DXS. The reaction is enantiospecific and practically irreversible as carbon dioxide is cleaved off. Thus, products as acetoin, 1-deoxy-L-erythrulose, 1-DX, 1-deoxy-fructose, and 1-deoxy-*altro*-heptulose are formed (Scheme 3).

Besides pyruvate, DXS also uses acetaldehyde and acetoin as C2-donors for the thiamine-dependent reaction. Interestingly, also hydroxypyruvate (about 7% relative rate) and α-oxobutyrate (1.5% relative rate) are used as donor compounds (Scheme 4). Hydroxypyruvate yields sugars as xylulose or fructose (thiamine-bound intermediate dihydroxyethyl), whereas α-oxobutyrate donates a C3 group (hydroxypropyl-) yielding 1,2-dideoxy-3-uloses as shown exemplarily for homo-deoxyxylulose (1,2-dideoxy-3-hexulose, Scheme 4).



Scheme 4. Alternative donor substrates of DXS with glyceraldehyde 3-phosphate (GAP) as acceptor substrate. Relative activities of the enzyme are given as per cent of the physiological reaction with pyruvate and GAP. Note that α-oxobutyrate as donor yields 1,2-dideoxy-3-uloses as products.

4. Conclusions

We provide kinetic data and substrate specificities of two novel C−C bonding enzymes from *E. coli* which could serve as useful biocatalysts in the organic synthesis of 1-deoxysugars. Fructose 6-phosphate aldolase FSA both reacts with dihydroxyacetone and hydroxyacetone yielding either typical aldolase products (e.g. fructose 6-phosphate) or novel 1-deoxysugars. DXS utilizes a variety of aldehydes as acceptors yielding the same stereochemistry as FSA when pyruvate (or acetaldehyde or acetoin) is used as donor. Using α-oxobutyrate as C3 donor, novel 1,2-dideoxy-3-uloses can be generated.

Acknowledgements

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