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Enzymatic synthesis of 1-deoxysugar-phosphates using *E. coli* 1-deoxy-D-xylulose 5-phosphate synthase

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Abstract—The thiamine diphosphate-dependent enzyme 1-deoxy-D-xylulose 5-phosphate synthase from *E. coli* can use D-erythrose 4-phosphate and D-ribose 5-phosphate as alternative substrates. These reactions were used for the production of 1-deoxy-D-fructose 6-phosphate and 1-deoxy-D-sedoheptulose 7-phosphate and have potential application for the biosynthesis of other 1-deoxysugar phosphates. © 2002 Elsevier Science Ltd. All rights reserved.

In many bacteria, several green algae and plastids, isoprenoids are synthesized by a mevalonate-independent pathway known as the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway.[1] The first reaction of the MEP pathway (Fig. 1) involves the synthesis of 1-deoxy-D-xylulose 5-phosphate (DXP) **5** by a condensation of (hydroxyethyl)thiamine derived from the decarboxylation of pyruvate **1** with the C1 aldehyde group of D-glyceraldehyde 3-phosphate **2**.[2] This thiamine diphosphate-dependent reaction is catalyzed by the enzyme 1-deoxy-D-xylulose 5-phosphate synthase (DXS), a novel type of transketolase-like enzyme recently identified.[3]

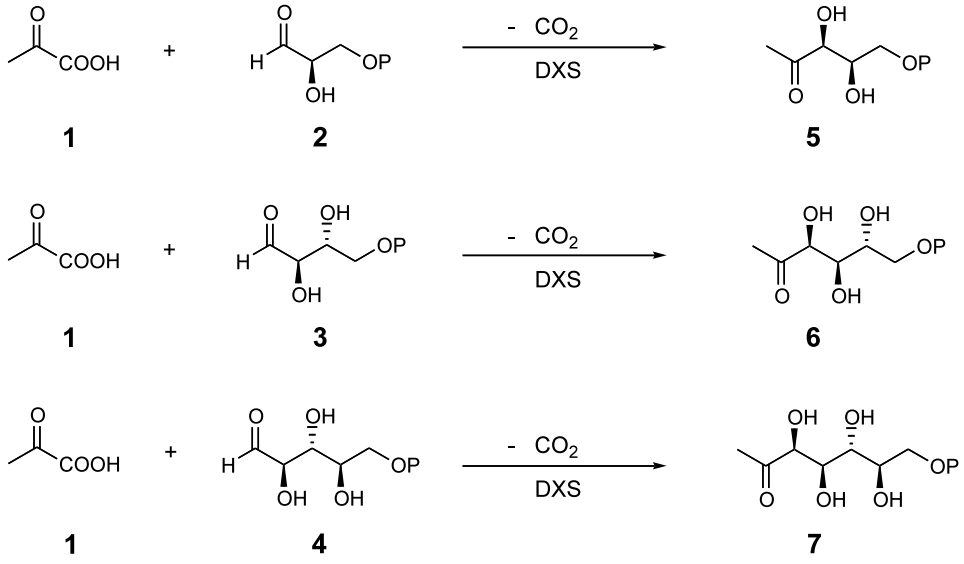

Figure 1. Enzymatic synthesis of 1-deoxy-D-xylulose 5-phosphate **5**, 1-deoxy-D-fructose 6-phosphate **6** and 1-deoxy-D-sedoheptulose 7-phosphate **7**.

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In addition to its role as the first intermediate in the synthesis of IPP, DXP is also a precursor for the synthesis of pyridoxol (vitamin B_6) and thiamine (vitamin B_1) in bacteria and plants.[4] The location of DXS at a branch point of three essential biosynthetic pathways in bacteria and higher plants, together with the absence of this enzyme in animals, makes DXS an attractive target for the design of drugs with potential applications as antibiotics and herbicides.[5] The recent report of the occurrence and essentiality of the MEP pathway in the parasite *Plasmodium falciparum* extends the interest of DXS inhibitors to the development of antimalarial drugs.[6]

Thiamine diphosphate-dependent enzymes are increasingly being used as catalysts in chemoenzymatic synthesis.[7] This is particularly important in carbohydrate chemistry where the inherent multifunctionality of sugars is an enormous task and a number of protective groups has to be used in order to prevent unwanted reactions of the hydroxyl, keto, or phosphate groups. A variety of enzymes, mostly lyases and aldolases, have been used so far to synthesize complex sugars, sugar analogues and other biologically important natural products.[8] One of the enzymes which has attracted most interest is transketolase. The enzyme from various micro-organisms or from plants has been used for various carbon–carbon bonding reactions since the enzyme can be produced in a good quality and at a reasonable price form highly productive recombinant micro-organisms.[9]

DXS is a thiamine diphosphate-dependent enzyme which catalyses a transketolase-like reaction. Therefore, it is a potential enzyme to be used in chemoenzymatic synthesis. The enzyme from *E. coli* has been overexpressed in *E. coli* BL21 DE3 pLys cells and purified.[10] This enzyme has already been widely utilized for the synthesis of labeled DXP or free 1-deoxy-D-xylulose using pyruvate **1** and D-glyceraldehyde 3-phosphate **2** or free D-glyceraldehyde, respectively.[11] In our studies on the characterization of DXS from *E. coli*, we tested in addition a panel of different aldoses and aldehydes as substrates. These assays were carried out using radiolabeled pyruvate. The enzyme uses D-erythrose, D-ribose and their phosphorylated forms as substrates. Reaction products and substrates were separated by thin-layer chromatography and analyzed by autoradiography.[12] Under these experimental conditions, DXP **5** (the normal reaction product of DXS) and the alternative products obtained using D-erythrose 4-phosphate **4** and D-ribose 5-phosphate **5** as substrates (Fig. 1) showed very similar R_f values on TLC plates. However, the different nature of the products was first suggested by their differential staining with *p*-anisaldehyde/sulfuric acid. DXP **5** and the reaction products **6** and **7** stained purple, orange and brown, respectively. In order to characterize the products obtained from D-erythrose 4-phosphate and D-ribose 5-phosphate, the reaction was carried out at a semi-preparative scale,[13] and the products were purified, dephosphorylated and analyzed by NMR spectroscopy.[14]

As shown in Fig. 1, *E. coli* DXS catalyses the synthesis of 1-deoxy-D-fructose 6-phosphate **6** and 1-deoxy-D-sedoheptulose 7-phosphate **7** using pyruvate **1** and the alternative substrates D-erythrose 4-phosphate **3** and D-ribose 5-phosphate **4**, respectively, as aldehyde acceptors in the transketolase reaction. Under the experimental conditions used, most of the substrate was transformed into product since the reaction involves the release of CO_2, making the process irreversible. The non-phosphorylated aldoses D-erythrose and D-ribose were also transformed, although not completely, indicating that the reactions proceeded at much lower rates. Therefore, these aldoses are also substrates of DXS although poorer than their corresponding aldose-phosphates (data not shown). D-Glucose 6-phosphate, D-galactose 6-phosphate and free aldoses such as D-threose, D-xylose, D-arabinose or D-glucose were, however, not utilized as substrates.

Compared to the rather complicated chemical synthesis of these chiral compounds, the enzymatic procedure reported in this study is certainly advantageous, since it does not require group-protection. It is a single step process and is carried out under mild conditions with a high yield.

Enzymatic syntheses of a variety of 1-deoxyketoses via a similar acyloin enzymatic reaction have been previously reported from mostly free aldoses and either pyruvate using the pyruvate decarboxylase or acetoin or methylacetoin and using the acetoin dehydrogenase.[15a] Mostly non-phosphorylated products were described, as aldose phosphates were apparently not always converted: the reaction was successful with ribose 5-phosphate, but failed with D-glyceraldehyde 3-phosphate.[15b,c] The process described here can be used for the easy synthesis of 1-deoxysugar phosphates labeled with radioactive or stable isotopes for metabolic, inhibition and incorporation studies. As an example, it has been demonstrated that tumor cells use predominantly the pentose phosphate pathway for the highly increased synthesis de novo of nucleic acids during tumor cell proliferation.[16a] The deoxysugar phosphates reported here could be assayed as putative transketolase inhibitors such oxythiamine that shows inhibitory effect on cell proliferation in cultured Mia pancreatic adenocarcinoma cells and Ehrlich's ascitic tumor.[16b]

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12. Radiometric assay of DXS activity. The enzyme reaction mixture consisted of 40 mM Tris–HCl, 2.5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 1 mM thiamine diphosphate, 0.2 mM [2-^{14}C]pyruvate (15.9 mCi/mmol, DuPont/NEN), 10 mM sodium pyruvate, 10 mM D-erythrose 4-phosphate or D-ribose 5-phosphate and the DXS sample in a final volume of 50 μL. After 30 min incubation, reactions were stopped by heating at 80°C for 5 min, and 5 μL aliquots were loaded onto silica gel plates 60–200 UV254 (Merck). Labeled products formed during the reaction were separated from [2-^{14}C]pyruvate by TLC using *n*-propanol/ethyl acetate/H_2O (6:3:1) as eluent and detected by autoradiography.
13. Preparative synthesis of 1-deoxy-D-fructose 6-phosphate **6** and 1-deoxy-D-sedoheptulose 7-phosphate **7**. The enzyme reaction mixture consisted of 100 mM Tris–HCl, 2.5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 1 mM thiamine diphosphate, 10 mM sodium pyruvate, 10 mM D-erythrose 4-phosphate or D-ribose 5-phosphate and the DXS sample in a final volume of 10 mL. After incubation for 4 h at 37°C, reactions were stopped by heating at 100°C for 10 min and deproteinized by centrifugation at 13,000×*g*.
14. The NMR spectra were recorded on a Brucker AC 200 or ARX500 spectrometers at 200 and 500 MHz for ^{1}H NMR and 125.85 MHz for ^{13}C NMR. Experiments were carried out in $CDCl_3$ using as standard $CHCl_3$ ($\delta = 7.26$ ppm) for ^{1}H NMR and $CDCl_3$ ($\delta = 77.03$ ppm) for ^{13}C NMR. The TLC plates were developed with an ethanol solution of *p*-anisaldehyde (2.5%), sulfuric acid (3.5%) and acetic acid (1.6%) by heating up to 100°C. For the complete identification of the reaction products, the sugar phosphates were enzymatically dephosphorylated in the crude reaction mixture and converted into the corresponding peracetylated derivatives.

    The lyophilized crude enzymatic system was dissolved in water (200 μL). To this solution (100 μL) were added Tris–HCl buffer (100 μL, 50 mM Tris, pH 9.8) and alkaline phosphatase (Sigma, P 3681, 2 μL). The reaction mixture was incubated in a shaker at 37°C for 3 h and then lyophilized. The residue (38 mg in the case of the incubation of D-erythrose 4-phosphate **4** and 29 mg in the case of the incubation of D-ribose 5-phosphate **5**) was directly acetylated using acetic anhydride/pyridine (1:1, 0.5 mL) overnight at room temperature in presence of dimethylaminopyridine (3 mg). The reaction mixture was repeatedly evaporated with toluene. The acetylated crude was purified by repeated TLC (hexane/EtOAc, 60/40) and yielded either 3,4,5,6-tetra-*O*-acetyl-1-deoxyfructose **6** (1 mg, $R_f = 0.27$) or 3,4,5,6,7-penta-*O*-acetyl-1-deoxysedoheptulose **7** (2.2 mg, $R_f = 0.35$).

    3,4,5,6 - Tetra - *O* - acetyl - 1 - deoxyfructose (acetylated 6)

    ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.06 (3H, s, CH_3COO-), 2.07 (3H, s, CH_3COO-), 2.08 (3H, s, CH_3COO-), 2.09 (3H, s, CH_3COO-), 2.18 (3H, s, CH_3COO-), 2.22 (3H, s, H-1), 4.18 (1H, dd, $J_{5,6} = 4.4$ Hz, $J_{6,6'} = 12.6$ Hz, H-6), 4.35 (1H, dd, $J_{5,6'} = 2.5$ Hz, $J_{6,6'} = 12.6$ Hz, H-6′), 5.25 (1H, ddd, $J_{5,6'} = 2.5$ Hz, $J_{5,6} = 4.4$, $J_{4,5} = 9.1$, H-5), 5.27 (1H, d, $J_{3,4} = 2.2$ Hz, H-3), 5.70 (1H, dd, $J_{3,4} = 2.2$ Hz, $J_{4,5} = 9.1$ Hz, H-4).

    ^{13}C NMR (102.85 MHz, $CDCl_3$): δ (ppm) = 20.45 (CH_3COO-), 20.48 (CH_3COO-), 20.72 (CH_3COO-), 20.79 (CH_3COO-), 26.80 (C-1), 61.63 (C-7), 67.86 (CHOAc), 68.27 (CHOAc), 75.78 (CHOAc), 169.46 (CH_3COO-), 169.56 (CH_3COO-), 170.17 (CH_3COO-), 170.51 (CH_3COO-), 201.50 (C-2).

    3,4,5,6,7-Penta-*O*-acetyl-1-deoxy-keto-D-*altro*-heptulose (3, 4,5,6,7 - penta - *O* - acetyl - 1 - deoxysedoheptulose, acetylated 7)[17]

    ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.05 (3H, s, CH_3COO-), 2.06 (3H, s, CH_3COO-), 2.07 (3H, s, CH_3COO-), 2.11 (3H, s, CH_3COO-), 2.17 (3H, s, CH_3COO-), 2.21 (3H, s, H-1), 4.18 (1H, dd, $J_{6,7} = 7.4$ Hz, $J_{7,7'} = 12$ Hz, H-7), 4.34 (1H, dd, $J_{6,7'} = 3.7$ Hz, $J_{7,7'} = 12$ Hz, H-7′), 5.21 (1H, d, $J_{3,4} = 2$ Hz, H-3), 5.26 (1H, dd, $J_{6,7'} = 3.7$ Hz, $J_{6,7} = 7.4$, H-6), 5.37 (1H, dd, $J_{5,6} = 3.1$ Hz,

$J_{4,5}$ = 8.6 Hz, H-5), 5.62 (1H, dd, $J_{3,4}$ = 2 Hz, $J_{4,5}$ = 8.6 Hz, H-4)

^{13}C NMR (102.85 MHz, $CDCl_3$): δ (ppm) = 20.43 (CH_3COO-), 20.53 (CH_3COO-), 20.59 (CH_3COO-), 20.68 (CH_3COO-), 20.79 (CH_3COO-), 26.81 (C-1), 61.51 (C-7), 68.34 (CHOAc), 68.79 (CHOAc), 69.85 (CHOAc), 75.63 (CHOAc), 169.16 (CH_3COO-), 169.61 (CH_3COO-), 169.91 (CH_3COO-), 170.16 (CH_3COO-), 170.56 (CH_3COO-), 201.49 (C-2).

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