ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 5 (1998) 113–118

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Fructose-1,6-bisphosphate aldolase and transketolase: Complementary tools for the de novo syntheses of monosaccharides and analogues

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Received 1 October 1997; accepted 24 November 1997

Abstract

This paper reports a new synthesis of bromoacetol phosphate and dihydroxyacetone phosphate for use in fructose-1,6-bisphosphate aldolase (FB-aldolase) catalyzed syntheses. Then the activities of FB-aldolase and transketolase towards polyhydroxybutanal analogues of erythrose and erythrose-4-phosphate were studied. These activities were high enough to allow the syntheses of rare heptulose-1-phosphates of the D and L series.

Keywords: Carbon–carbon bond; Bromoacetol phosphate; Unnatural sugars

1. Introduction

The story of monosaccharide analogue syntheses by C–C bond forming reaction started in 1983 with the publication in the Journal of Organic Chemistry of two papers [1,2], both from G.M. Whitesides and C.H. Wong, related to the utilisation in synthesis of fructose-1,6-bisphosphate aldolase (FB-aldolase). Of course, various articles appeared previously [3,4] on the specificity of this enzyme but the approach of Whitesides, by its realism and its anticipation of the synthetic potential of aldolases, has really opened the way. In the following years, Whitesides and Wong developed this chemistry separately, with the success that everyone knows, followed by some other authors, most of them (but not all) having caught the 'enzymatic synthesis virus' in the Mallinckrot building of Harvard University where Whiteside's laboratory is located.

The first results concerned FB-aldolase. For our part, when we started working in this area, we looked for the utilisation of another enzyme playing a critical role in the biosynthetic pathway of sugars, transketolase, and we were the first to demonstrate its ability to catalyse the synthesis of unnatural sugars [5]. In this communication, we will present our results, together with some of the literature on the complementary utilisation of these two enzymes.

Fructose-1,6-bisphosphate aldolase catalyses in vivo the reversible retroaldolisation of fructose-1,6-bisphosphate into dihydroxyacetone phosphate (DHAP) and glyceraldehyde phosphate (GAP). At relatively high concentrations,

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1381-1177/98/$19.00
PII: S1381-1177(98)00014-9

the condensation is favoured. The enzyme is rather specific for DHAP, but numerous aldehydes can replace GAP leading to the synthesis of fructose analogues having the 3*S*,4*R* configuration [6]. Other DHAP specific aldolases are known to be specific for the three other possible configurations in 3 and 4 [7], but in this study we will focus on fructose aldolase.

Transketolase is a transferase which catalyses in vivo the reversible transfer of an α hydroxyacetyl group from a ketose phosphate to an aldose phosphate. Transketolase also accepts as a donor substrate hydroxypyruvate, and various aldehydes, preferably hydroxylated in the α position, can be acceptor substrates. The reaction becomes irreversible, and of obvious synthetic interest [8–10]. FB-aldolase allows the lengthening of the aldehyde backbone by three carbon units with the concomitant creation of two new asymmetric carbon atoms, whereas transketolase adds two carbon atoms and only one asymmetric center. However, due to the enantioselectivity of transketolase towards α-hydroxyaldehydes, both enzymes provide ketoses of D-threo configuration on carbon 3 and 4 (Fig. 1). Moreover, transketolase is also able to act on aldehydes lacking an α-hydroxyl group, then leading to 4-deoxyketoses which cannot be obtained by FB-aldolase-catalysed syntheses. Starting from the same substrate, transketolase and FB-aldolase will lead to homologous compounds, and a particular targeted compound with n carbon atoms can be reached either using FB-aldolase from a C($n-3$) aldehyde or using transketolase from a C($n-2$) aldehyde (Fig. 1). Various authors took advantage of this complementarity [11], as we did, to prepare precursors in the biosynthesis and/or synthesis of natural polyhydroxylated piperidines and pyrrolidines [12,13].

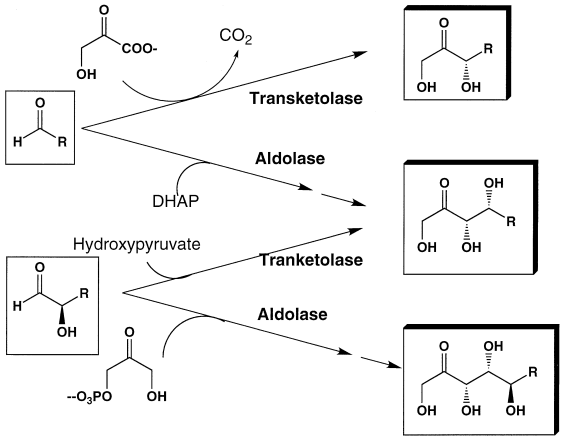


Fig. 1. Complementarity of FB-aldolase and transketolase.

2. FB-aldolase utilisation: bromoacetol phosphate and dihydroxyacetone phosphate syntheses

FB-aldolase can come from various sources. The commercial enzyme most often used is extracted from rabbit muscle and called RAMA. Alternatively, microbial FB-aldolase has been used [14], and we also showed that the enzyme from plants can be of interest [15].

Making use of an aldolase catalysed synthesis implies that the necessary substrate DHAP is available. Current preparations of DHAP involve both enzymatic and chemical methods. The easiest synthesis relies on the formation of DHAP in situ from fructose 1,6-bisphosphate (FBP) in the presence of triosephosphate isomerase (TPI) [16,17]. This method, although restricted to FDP-aldolase, is commonly used. However, GAP competes with the unnatural aldehyde and low yields result. Furthermore, the presence of FDP can complicate product isolation [6,18].

Two other enzymatic methods have been developed: phosphorylation of dihydroxyacetone (DHA) using glycerol-kinase and ATP [19–21], and oxidation of L-glycerol-1-phosphate by molecular oxygen in the presence of glycerophosphate-oxidase [22]. Both methods present some drawbacks and chemical preparation is thus preferred.

The most efficient syntheses of DHAP are based on the original method of Colbran et al. [23] and Effenberger and Straub [24] recently improved by Jung et al. [25]. However, the

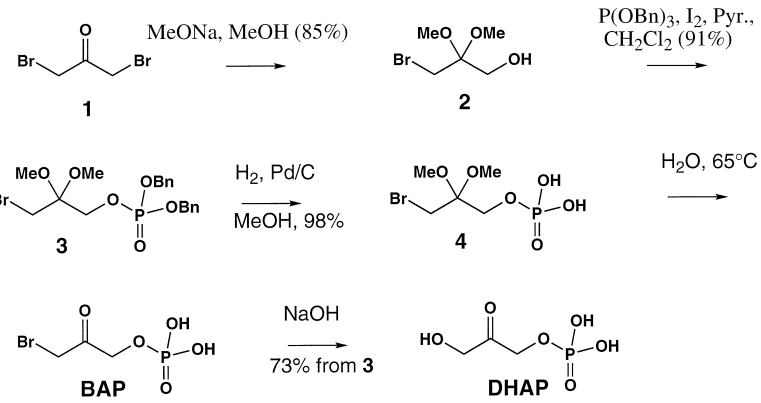


Fig. 2. Synthesis of bromoacetol phosphate (BAP) and dihydroxyacetone phosphate (DHAP).

limiting step of this synthesis remains to be the final hydrolysis of the precursor which gives DHAP in just 66% yield.

Looking for an alternative route to DHAP, our attention was attracted by the works of Hartman [26] who showed that DHAP can be formed by basic hydrolysis of bromoacetol phosphate (BAP). This close analogue of DHAP is also of special interest for aldolase catalysed syntheses because it behaves as a strong irreversible inhibitor of triose phosphate isomerase (TPI) [27,28], an enzyme often present and unwanted. BAP has already been incorporated in synthetic pathways by several groups to inhibit TPI [29,30]. Two syntheses of BAP have been described [27,28] but they are difficult to carry out on a large scale.

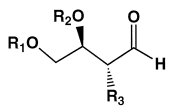
We found that bromoacetol phosphate (BAP) and dihydroxyacetone phosphate (DHAP) can be easily prepared in good yields from dibromoacetone phosphate according to Fig. 2. In a fast reaction, dibromoacetone is converted to ketal **2** by treatment with sodium methylate in methanol at $-10^{\circ}C$. This unusual ketalisation in basic conditions brings both protection of the carbonyl group and desymmetrization of the substrate in a single step. Phosphorylation of **2** was realized using dibenzylphosphoroiodidate (DBPI) generated from tribenzylphosphite and iodine [31] in 91% yield. Removal of the benzyl groups was easily achieved by hydrogenolysis and **4** was obtained in quantitative yield. Dimethyl acetal group was hydrolysed by heating in water at $65^{\circ}C$ leading to BAP in 90% yield. BAP solution can be stored frozen after adjusting the pH to 4.5. DHAP was finally generated by addition of 1 M sodium hydroxide (3 equivalents) to the aqueous solution of BAP (73% from **3**). A stock solution of DHAP adjusted at pH 3.5 was stored frozen for months without noticeable decomposition.

This short and facile procedure [32] represents a completely new route for the preparation of BAP and DHAP from acetone or 1,3-dibromoacetone and inexpensive reagents. BAP and DHAP obtained with 69 and 56.5% overall yield respectively were successfully applied to aldolase catalyzed syntheses in our laboratory.

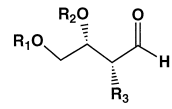
3. FB-aldolase and transketolase catalyzed syntheses from C_4 aldehydes

In this communication we will describe the reactivity of transketolase and FB-aldolase with substituted butanals as substrates, especially 2-deoxy-D- and L-erythrose derivatives (Fig. 3). By transketolase catalysis, these compounds would lead to 4-deoxy-D-fructose or 4-deoxy-L-sorbose, potential inhibitors of the glycolytic pathway. Moreover, by reaction with aldolase, they will give rare heptulose derivatives.

Some C_4-aldehydes were already studied as substrate of these enzymes. So, D-erythrose and



	R1	R2	R3
(R)-5a	H	H	H
(R)-5b	C(CH3)2		H
(R,S)-5c	PO3^{--}	H	H
6a	H	H	OCH3
6b	C(CH3)2		OCH3



	R1	R2	R3
(S)-5a	H	H	H
(S)-5b	C(CH3)2		H
(R,S)-5c	PO3^{--}	H	H
7a	H	H	OCH3
7b	C(CH3)2		OCH3

Fig. 3. Substrates tested with transketolase and FB-aldolase.

L-threose are substrates of transketolase [5] but not of aldolase [6] and 2,3-dihydroxybutyraldehydes are substrates for both enzymes [33–36].

3.1. Preparation of the substrates

We prepared compounds **5a**, **5b**, **6a**, **6b**, **7a** and **7b** from D-isoascorbic and L-ascorbic acids and racemic **5c** was synthesized by reaction of inorganic phosphate on the epoxide of 1,1-diethoxy-3-butene.

3.2. Transketolase activity

Compounds **5**–**7** were assayed as substrates for transketolase, using the assay developed in our laboratory [37]. The results are reported in Table 1. All the unphosphorylated substrates present reaction rates of 5 to 20% relatively to ribose-5-phosphate, but high K_M values indicating a weak affinity for the active site of the enzyme. The best substrate is **6a**, which presents the configuration of D-erythrose. This confirms that the presence of the α-hydroxyl group is very important for the activity, although not absolutely necessary. The steric hindrance of the dioxolane ring could explain the low reactivity of **5b**, **6b** and **7b**, but the deprotection of the diol in **5a**, **6a** and **7a** does not improve it significantly. This could result in the presence of hydrated or cyclic hemiacetal forms in solution. If the free aldehyde is the actual substrate, the presence of the other forms would reduce the reactivity. Careful examination of ^{13}C NMR spectra shows that the open hydrated form is present at 12% for **5** and undetectable in **6a** and **7a**. The free aldehyde is not present. Finally, the

Table 1
Kinetic data for the reactions of substituted butanals with transketolase and FB-aldolase

Substrates	Transketolase		FB-aldolase	
	V_{max} (%) relative to ribose-5-phosphate	K_M (mM)	V_{max} (%) or (∗) V at 50 mM relative to glyceraldehyde-3-phosphate	K_M (mM)
(*S*)-**5a**	4	34.5	0.25∗	
(*S*)-**5b**	5	31.3	1.18∗	
(*R*)-**5a**	8	22.4	0.25∗	
(*R*)-**5b**	6	28.7	1.18∗	
(*R,S*)-**5c**	61 (at 15 mM)	3.8	7.8	6.4
6a	27	12.2	0.62	10
6b	11	36.4	1.74	23
7a	6	31.7	0.42	58
7b	10	34.7	0.39	50

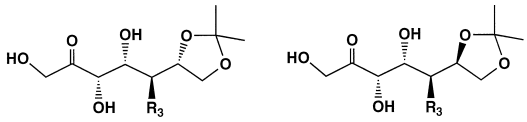


Fig. 4. Heptuloses synthesized by FB-aldolase catalysis: $R_3 = H$ or OCH_3.

K_M values for these compounds can be explained if the enzyme is unable to specifically stabilise the aldehyde form as probably happens for the natural substrates.

The phosphorylation of the 4-hydroxyl group makes **5c** a very good substrate for transketolase as expected, because the open form is the only one present and the phosphate group allows an anchorage in the active site of the enzyme as is the case for the natural substrate, D-erythrose-4-phosphate.

3.3. Aldolase activity

Compounds **5**–**7** were submitted to the action of FB-aldolase (Fig. 3). (*S*)-**5a** and (*R*)-**5a** are poor substrates although racemic mixture allowed the synthesis of the corresponding heptulose [38] (Fig. 4). Protection of the diol in (*S*) and (*R*)-**5b** significantly increases the reactivity. As for transketolase the phosphorylated compound **5c** is a good substrate. Among the 2-methoxy analogues **6** and **7**, compound **6a** and **6b** with the D-erythrose configuration presents unexpected good values.

3.4. Transketolase catalyzed syntheses

We did not succeed in carrying out a synthesis with unphosphorylated substrates. **5c**, which appears as a better substrate, could be submitted to the transketolase catalysed reaction. This synthesis is in progress in our laboratory.

3.5. Aldolase catalyzed syntheses, reaction of 3,4-dihydroxybutanal-4-phosphate

In a first run, the aldehyde was reacted with an equimolecular amount of dihydroxyacetone phosphate. After 12 h, the products were isolated as their barium salt in 65% yield. ^{13}C NMR of the product shows that the two diastereoisomers expected are present in a 1:2.5 ratio. This result suggests that although both enantiomers are substrates, the enzyme is able to discriminate between them. The synthesis was repeated with a half equimolecular amount of dihydroxyacetone phosphate. In this case, indeed, only one compound is obtained which, by analogy with the reactivity of the unphosphorylated 3,4-dihydroxybutyraldehyde, is supposed to be the heptulose of the L series (L-*galacto*-hept-2-ulose-1-phosphate), which is more stable in the cyclic form.

*3.6. Reaction of the protected polyhydroxybutyraldehyde (S)-**5b**, (R)-**5b**, **6b** and **7b***

The syntheses were performed on a 2 or 3 mmol scale, using an excess of aldehyde in order to completely consume the dihydroxyacetonephosphate. The product is isolated by precipitation of its barium salt. The expected heptuloses-1-phosphate are easily characterized by ^{13}C NMR spectroscopy as well as mass spectroscopy. Although (*S*) and (*R*)-**5b** present the same initial velocity at 50 mM in the aldolisation reaction, the *S* isomer (D series) gave the 6,7-*O*-isopropylidene-5-deoxy-D-*altro*-hept-2-ulose-1-phosphate in a 65% yield, while the *R* isomer (L series) led to the corresponding L-*galacto*-3-hept-2-ulose-1-phosphate in a modest 21% yield. The yields obtained with the 2-methoxy compounds **6b** and **7b** are also higher for the D-isomer (83%) than for the L- (10%), which in view of the kinetic constants appears to be a very poor substrate.

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