2-Keto-3-deoxy-6-phosphogalactonate Aldolase as a Catalyst for Stereocontrolled Carbon−Carbon Bond Formation

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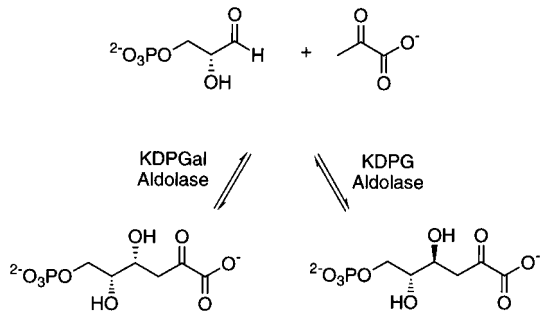
Received October 14, 1997

Aldolases are now firmly established as powerful tools for stereocontrolled carbon−carbon bond formation.[1−6] Aldolases are classified by nucleophile type into four groups: the dihydroxyacetone phosphate (DHAP) aldolases, the glycine aldolases, the acetaldehyde aldolases, and the pyruvate/phosphoenolpyruvate aldolases. The first three classes of enzymes have been extensively utilized for the synthesis of a variety of carbohydrate-like products.[7−15] The remaining and largest group of aldolases utilize pyruvate or phosphoenolpyruvate as the nucleophile. Two members of this group, *N*-acetylneuraminic acid aldolase (NeuAc aldolase, EC 4.1.3.3)[16−24] and 2-keto-3-deoxyoctulosonate aldolase (KDO aldolase, EC 4.1.2.23),[25] have been used to prepare analogues of their natural substrates. Both enzymes operate under thermodynamic control and frequently yield product mixtures.[20,22,24,25] In our laboratories, we have utilized 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPG aldolase, EC 4.1.2.14) to prepare a range of structurally varied products.[26−30] Unlike other pyruvate aldolases, KDPG aldolase operates exclusively under kinetic control providing stereochemically pure products.[28] *Here we report the isolation, properties, and synthetic utility of the complementary pyruvate aldolase, 2-keto-3-deoxy-6-phosphogalactonate aldolase (KDPGal aldolase, EC 4.1.2.21, Scheme 1).*

Scheme 1. *In Vivo* Reactions of KDPG Aldolase and KDPGal Aldolase



KDPGal aldolase has been detected in several sources including *Azotobacter vinelandii*,[32] *Pseudomonas saccharophila*,[33] *Pseudomonas fluorescens*,[34] *Rhizobium meliloti*,[35] *Mycobacterium* strains,[36] *Caulobacter crescentus*,[37] and *Escherichia coli*[38] wild-type and mutant strains. KDPG aldolase is a constitutive enzyme, while KDPGal aldolase is inducible and expressed only during growth on galactose or galactonate. In 1982, Meloche and co-workers reported an isolation of KDPGal aldolase from *P. saccharophila*.[33] Separation of KDPGal aldolase from KDPG aldolase was laborious and yields too little pure enzyme for synthetic utility.

To circumvent this problem, we investigated the isolation of KDPGal aldolase from bacterial strains lacking a KDPG aldolase. Specifically, we examined KDPGal aldolase from *Pseudomonas cepacia* eda$^-$ strain 249−27, a mutant reported by Lessie and co-workers.[39,40] When grown on glycerol augmented with 1% galactose, this strain yields 31.8 U L^{-1} of cell growth, a 10-fold improvement over previously reported sources.[33,41] The specific activity of crude protein was 0.39 U mg^{-1}. The enzyme was purified by ammonium sulfate fractionation followed by differential dye−ligand chromatography on a Procion Navy H-ER dye supported on Sepharose CL-4B.[27] Elution with pyruvate and (±)-α-glycerophosphate gave enzyme with a specific activity of 4.2 U mg^{-1}, suitable for synthetic application. No residual KDPG aldolase was detectable by enzymatic assay. The enzyme is stable during isolation, lyophilization, and for periods of at least months when stored at −78 °C. The pH−activity relationship of KDPGal aldolase was also investigated. The enzyme showed a classical bell-shaped pH−activity curve with a pH maximum near 7.5, characteristic of a requirement for two ionizable residues that likely act

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(41) One unit is defined as the amount of enzyme required to convert 1 μmol of substrate to product per minute at pH 7.5 and 25 °C.

S0022-3263(97)01881-1 CCC: $15.00
Published on Web 02/04/1998

Table 1. Substrate Specificity of KDPGal Aldolase from *P. cepacia* eda⁻ Strain 249−27

electrophile	nucleophile	V_{rel}[a] (%)
D-glyceraldehyde 3-phosphate	pyruvate	100
D-glyceraldehyde		++++
L-glyceraldehyde		---
glyoxylate		++++
D-erythrose		+++
chloroacetaldehyde		++
L-threose		+++
D-ribose		+++
D-ribose 5-phosphate		++++
2-pyridinecarboxaldehyde		+++
benzaldehyde		---
valeraldehyde		+
D-glyceraldehyde 3-phosphate	3-fluoropyruvate	---
D-glyceraldehyde 3-phosphate	2-oxobutyrate	---

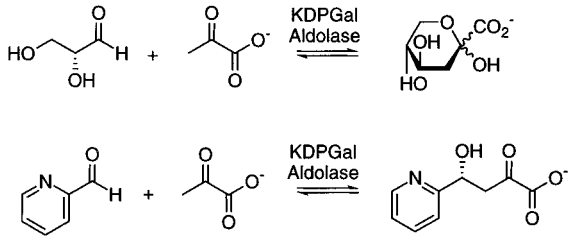
Relative rates to D-glyceraldehyde 3-phosphate, the in vivo substrate. Key: ++++, >1%; +++, 0.50−0.75%; ++, 0.25−0.50%; +, <0.25%; ---, 0%.

as general base and general acid catalysts during aldol addition. The enzyme shows a broad pH−activity profile and activities of >50% of maximum activity are available over the pH range 6.5−9.5.

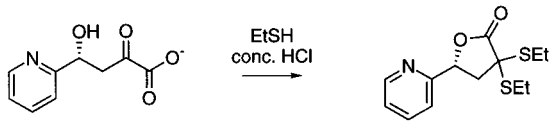
To establish the utility of KDPGal aldolase as a synthetic tool, we explored the substrate specificity of the enzyme with respect to unnatural substrates. Table 1 indicates clearly that the substrate specificity of this enzyme is useful for organic synthesis. As with KDPG aldolase, removal of the phosphate group from the *in vivo* electrophile greatly affects the rate of conversion: D-glyceraldehyde is converted to 2-keto-3-deoxygalactonate at about 1% of the rate that D-glyceraldehyde 3-phosphate is converted to 2-keto-3-deoxy-6-phosphogalactonate. Although the natural substrate possesses a negative charge at C3, a charged or polar moiety at C2 or C4 of the electrophile promotes substrate conversion. Two of the most reactive unnatural electrophiles, D-ribose 5-phosphate and glyoxylate, are accepted at >100% the rate of D-glyceraldehyde. Unlike the dihydroxyacetone phosphate aldolases and deoxyribose aldolase but similar to KDPG aldolase, aliphatic aldehydes are accepted very poorly as substrates by KDPGal aldolase. Again in keeping with KDPG aldolase, benzaldehyde is not accepted as a substrate while 2-pyridinecarboxaldehyde is an excellent substrate, suggesting the presence of a hydrogen-bonding residue near the active site. KDPGal aldolase appears to share with KDPG aldolase a specificity for the D-configuration at C2 of the electrophile; racemic aldehydes can be utilized and aldol addition will proceed with kinetic resolution to establish two stereocenters. KDPGal aldolase does not accept nucleophilic substrates other than pyruvate, in contrast to the KDPG aldolases.[28]

Preparative-scale reactions are essential to explicitly authenticate product identity and stereochemical fidelity during enzyme-catalyzed addition. We conducted preparative scale reactions with D-glyceraldehyde and 2-pyridinecarboxaldehyde as electrophilic substrates to produce 2-keto-3-deoxy-galactonate (KDGal) and (*R*)-4-hydroxy-2-keto-4-(2′-pyridyl)butyrate, respectively (Scheme 2). The stereochemical configuration of the adduct and the diastereoselectivity of the enzyme during addition was determined by comparison with the ^{1}H NMR of the literature compound prepared from D-galactono-1,4-lactone.[42] KDGal exists in solution as a mixture of α- and β-anomers of both the furanose and pyranose forms, in agreement with literature spectra.[42] The α-pyranose anomer is the predominant form, while the remaining anomers are present in approximately equimolar amounts. The enantioselectivity of KDPGal aldolase was determined by conversion of (*R*)-4-hydroxy-2-keto-4-(2′-pyridyl)butyrate to the corresponding dithiolactone (Scheme 3). Chiral GLC chromatography (Chrompack Chirasil-L-Val column) of the dithiolactone exhibited a single peak whereas racemic material, prepared by the method of Cornforth, exhibited baseline separation of the enantiomers giving an enantiomeric excess for the enzyme-catalyzed product of >99.7%.[43,44]

Scheme 2. Synthesis of 2-Keto-3-deoxygalactonate and (*R*)-4-Hydroxy-2-keto-4-(2′-pyridyl)butyrate



Scheme 3. Synthesis of 2,2-Bis(3thylthio)-4-(2′-pyridyl)-4-(*R*)-butyro-γ-lactone from (*R*)-4-Hydroxy-2-keto-4-(2′-pyridyl)butyrate



Our results demonstrate that KDPGal aldolase stereospecifically catalyzes the expected aldol addition with unnatural electrophilic substrates at rates practical for preparative scale organic synthesis. We are currently exploring additional sources of KDPGal aldolase and pursuing syntheses of natural products utilizing aldolases.

Acknowledgment. This research was supported by the National Institutes of Health (GM 48181). E.J.T. is a Camille Dreyfus Teacher−Scholar and a fellow of the Alfred P. Sloan Foundation. We also gratefully acknowledge P. V. Phibbs, Jr. (East Carolina University) for the gift of *P. cepacia* strain 249-27.

Supporting Information Available: Experimental details for the growth, isolation, and purification of KDPGal aldolase, unnatural substrate investigations, and the preparation of (*R*)-4-hydroxy-2-keto-4-(2′-pyridyl)butyrate, 2,2-bis(ethylthio)-4-(2′-pyridyl)-4-(*R*)-butyro-γ-lactone, and KDGal (4 pages).

JO9718814

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