Deoxyribose-5-phosphate Aldolase as a Synthetic Catalyst

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Enzyme-catalyzed stereocentered aldol condensations are valuable in organic synthesis, particularly in the synthesis of carbohydrates and related substances. We report here an initial study on the synthetic utility of a bacterial 2-deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) overexpressed in Escherichia coli. The enzyme DERA catalyzes the reversible aldol reaction of acetaldehyde and 2-glyceraldehyde 3-phosphate to form 2-deoxyribose-5-phosphate (eq 1). This enzyme is unique among the aldolases in that it is the only aldolase that condenses two aldehydes. Other aldolases use ketones as aldol donors and aldehydes as acceptors.

(1)

The purified DERA showed optimal activity at pH 7.5 with the following kinetic constants: \( V_{\text{max}} = 210 \text{ units/mg} \) based on the cleavage of 2-deoxyribose-5-phosphate (Ka = 521.1 M\(^{-1}\)) and Ka for 2-deoxyribose-5-phosphate = 1.93 mM. At 25 °C in 0.1 M triethanolamine buffer (TEA), pH 7.5, the enzyme is fairly stable, with 70% of the original activity retained after 10 days.

Examination of the substrate specificity of DERA (Tables I and II) indicates that acetone, fluoroacetone, and propionaldehyde can replace acetaldehyde as the nucleophilic component in the aldol reaction. Substitution at C-2 of acetaldehyde with another than a single methyl group is not tolerated. It is of particular interest that the bond formation for fluoroacetone occurs regioselectively at the nonfluorinated carbon. With regard to the specificity of acceptors, many aldehydes as well as aldose sugars and their phosphates are accepted as weak substrates.

Although the enzyme possesses such a broad substrate specificity, the rates of condensation with unnatural substrate are relatively low and a relatively large amount of enzyme is required to achieve useful synthesis. The high stability and specific activity and the ready availability of the enzyme, however, appear to outweigh this shortcoming.

The following are representative syntheses with DERA.

(5) 4-Hydroxy-5-methylhexan-2-one

A 100-mL solution containing 0.2 M acetone, 0.1 M isobutyraldehyde, 0.1 M TEA, and 1 mM EDTA was added 1000 units of DERA in a dialysis bag. The reaction vessel was stopped, and the solution was stirred for 3 days at room temperature and then continuously extracted with ether for 16 h. The solvent was removed by slow fractional distillation to yield the crude product, which was chromatographed on silica gel (ether/hexane, 1:1), to yield 0.56 g, 44% yield of the title compound: [α]D -55.0° (c 1.4, CHCl₃).


Supplementary Material Available: Characterization data for 1 and 3-5 and tables of crystal, data collection, and refinement parameters, atomic coordinates and isotropic displacement parameters, bond distances and angles, and hydrogen atom coordinates for 5 (14 pages). Ordering information is given on any current masthead page.

(7) C₇H₁₆N₂Si: monoclinic, 

(1) Taken from the Ph.D. Thesis of C.F.B. at Texas A&M University, 1989. This work was supported by the NIH (GM 41354-01).

(2) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. Tetrahedron 1989, 45, 5165 and references cited.

(3) Wong, C.-H. Science 1984, 244, 1145 and references cited.

(4) To construct an overproducing E. coli species, plasmid p VH 17 which contains the deo C gene (Valentin-Hansen, P.; Alija, H.; Schumpe, D.; EMBO J. 1982, 1, 317) was introduced into E. coli EM2929. A 6-L growth of E. coli EM2929/pVH17 produced approximately 124,000 units of the aldolase. One unit = 1 μmol of 2-deoxyribose-5-phosphate cleaved per minute. About 3.1 × 10⁶ units can be prepared for synthesis.

(5) The equilibrium constant for the condensation is 4.2 × 10⁻⁴ M⁻¹ (Pricer, W. E.; Horecker, B. L. J. Biol. Chem. 1960, 235, 1295).

(6) The substrate specificity of E. coli DERA was not reported previously. The enzyme from Lactobacillus planatarum was reported to accept glycoaldehyde phosphate, D-ribose 5-phosphate, and D-erythrose 4-phosphate as acceptor substrate, and propionaldehyde, glucose and according to the activity assay (Rosen, O. M.; Hoffee, P.; Horecker, B. L. J. Biol. Chem. 1965, 240, 1517).
Table I. Substrate Specificity of DERA*  

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>Presumed Product</th>
<th>Rf</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehyde</td>
<td>Phosphate</td>
<td>5-phosphate</td>
<td>0.38(1)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.41(1)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.62(1)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.56(1)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.34-0.55(1)</td>
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<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.64(1)</td>
</tr>
<tr>
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<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.79(1)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.26(1)</td>
</tr>
<tr>
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<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.56(2)</td>
</tr>
<tr>
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<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.39(2)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.65(2)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>5-phosphate</td>
<td>5-phosphate</td>
<td>0.62(2)</td>
</tr>
</tbody>
</table>

*Reactions were conducted in a 1-mL solution containing 0.1 M triethanolamine, 0.1 mM EDTA, 0.1 M donor, 0.1 M acceptor, and 30 units of DERA. A control reaction was performed containing all components except the enzyme. After incubation overnight, TLC (silica gel) was used to identify the appearance of product by staining with p-anisaldehyde reagent. Solvent systems: solution containing 0.2 M fluoroacetone, 0.1 M isobutyraldehyde, 0.1 M TEA, and 0.1 mM EDTA; 0.1 M donor, 0.1 M acceptor, and 30 units of DERA. After incubation overnight, TLC (silica gel) was used to identify the appearance of product by staining with p-anisaldehyde reagent. Solvent systems: solution containing 0.2 M fluoroacetone, 0.1 M isobutyraldehyde, 0.1 M TEA, and 0.1 mM EDTA.

Infrared Multiple Photon Dissociation of Butyrophenone Cation. A Stepwise McLafferty Rearrangement

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Although the McLafferty rearrangement (eq 1) is one of the most extensively studied unimolecular reactions in mass spectrometry, its mechanism is still controversial. Theoretical studies have supported both stepwise and concerted pathways. Experimental studies on the benzyl ethyl cation rearrangement have also supported conflicting mechanisms. For a few systems, supplementary material is available.