Overproduction and Substrate Specificity of a Bacterial Fuculose-1-phosphate Aldolase: A New Enzymatic Catalyst for Stereocontrolled Aldol Condensation

Akio Ozaki, Eric J. Toone, Claus H. von der Osten, Anthony J. Sinskey,* and George M. Whitesides*†

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received October 27, 1989

The preparation of carbohydrates is a challenging area of organic synthesis.1 Enzymes are becoming valuable catalysts in this area.2-3 This report describes the cloning, overproduction, and substrate specificity of a bacterial fuculose-1-phosphate aldolase (Fuc-1-P aldolase, EC 4.1.2.17) that permits the aldol reaction between dihydroxyacetone phosphate (DHAP) and aldehydes to be carried out with a stereochemistry different from that of rabbit muscle fructose-1,6-diphosphate aldolase (RAMA, EC 4.1.2.13) (Scheme I). This new aldolase will be useful in the synthesis of monosaccharides and related structures.

RAMA yields products with the D-three configuration at C3 and C4 (3R,4R).3 RAMA is a valuable catalyst, but its application is limited to compounds containing this stereochemical unit. We required aldolases capable of generating compounds diastereomeric at C3 and C4 to the products generated by RAMA. Several bacterial aldolases have recently been cloned,4-6 Among these, Fuc-1-P aldolase from Escherichia coli generates products analogous to those produced by RAMA, but with 3R,4R stereochemistry.7

In order to generate Fuc-1-P aldolase in the large quantities required for practical synthesis, an overproducer of this enzyme was constructed in E. coli. Figure 1 shows the result of overproduction of fuculose-1-phosphate aldolase. The activity of the enzyme in crude cell extracts was 6.6 units/mg, and a total activity was obtained from the culture was 1.9 × 103 units/L.8

A partially purified preparation of the enzyme was used to determine the substrate specificity of Fuc-1-P aldolase (Table I). This aldolase accepts a sufficiently large range of substrates to suggest that its breadth of specificity may be similar to that of RAMA.9 To confirm the stereochemical configuration of the vicinal diol formed, D-ribulose was synthesized via a Fuc-1-P aldolase catalyzed condensation between DHAP and glycolaldehyde, on a 10-mmol scale. The product was identical with commercial D-ribulose, thus confirming the 3R,4R configuration of the adduct.

Table I. Substrate Specificity of Fuc-1-P Aldolase

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Rel Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lactaldehyde</td>
<td>105</td>
</tr>
<tr>
<td>2. D-glyceraldehyde</td>
<td>83</td>
</tr>
<tr>
<td>3. (EtO)₂CHCHO</td>
<td>~70*</td>
</tr>
<tr>
<td>4. OCH₂CH₂CH₂OH</td>
<td>65</td>
</tr>
<tr>
<td>5. (CH₃)₂CHCHO</td>
<td>44</td>
</tr>
<tr>
<td>6. Acetaldehyde</td>
<td>56</td>
</tr>
<tr>
<td>7. Glycolaldehyde</td>
<td>59</td>
</tr>
</tbody>
</table>

*The stereochemistry of the three known aldolases which utilize DHAP as the nucleophile are shown, as well as the stereochemistries that are available by utilizing the "inversion" strategy. "OP" refers to a protected aldehyde, usually a ketal; "OP" is phosphate. Three steps are involved in the route to the inverted aldehyde: aldolase-catalyzed condensation, stereospecific reduction, and deprotection of the remaining aldehyde.10

We have recently described a strategy for extending the range of applicability of aldolases from the preparation of ketose to aldose sugars, by using as substrates half-protected dialdehydes (e.g., 3 and 4 in Table I). This "inverted" strategy (Scheme I), when...
used with RAMA, may change the position of the \( \text{d-three-vic-diol} \) group, but does not change its absolute configuration, since the \( nS,(n+1)R \) stereochemistry is retained on inversion of the sense of the carbon backbone. This strategy used with Fuc-1-P aldolase changes the absolute stereochemistry of the vic-diol unit from \( 3R,4R \) to \( nS,(n+1)S \) by inverting the sense of the carbon backbone. Thus, the Fuc-1-P aldolase offers, in principle, a stereo-controlled synthetic route to two configurationally distinct \(-\text{CHOHCHOH}^-\) units.

This work makes Fuc-1-P aldolase readily available for use in organic synthesis and establishes that this enzyme can be expected to have usefully broad specificity in its aldehyde reactant. The stereochemistry of the vicinal diol unit generated by Fuc-1-P aldolase is complementary to that generated by RAMA; it has the further advantage that its use with aldehydes of the type exemplified by 3 and 4 provides access to a third stereochemical unit. The availability of Fuc-1-P aldolase will significantly extend the range of application of aldolases as a class of enzymes in carbohydrate synthesis.

Work directed toward cloning and overexpression of a third stereochemically distinct aldolase, L-rhamnulose-1-phosphate aldolase (EC 4.1.2.19) (Scheme 1), is now in progress.

**Acknowledgment.** This research was supported by the NIH, Grants GM-30367 and GM-39589, and the NSF under the Engineering Research Center Initiative to the Biotechnology Process Engineering Center (Cooperative Agreement CDR-88-03014). A.O. was supported by Kyowa Hakko Kogyo Co. Ltd., and C. H.v.d.O. acknowledges the Carlsberg Foundation for support. We are especially grateful to Professor E. C. C. Lin of the Harvard Medical School for the gift of plasmid pfuc41a, which contained the fuculose-1-phosphate aldolase gene, and to our colleagues Andrew Spaltenstein and Chris Borysenko for the gift of substrates 3 and 4.

This paper is dedicated to Günther Ohloff.

**Supplementary Material Available:** Experimental procedure for the preparation of \( \text{d-ribulose} \) (3 pages). Ordering information is given on any current masthead page.