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

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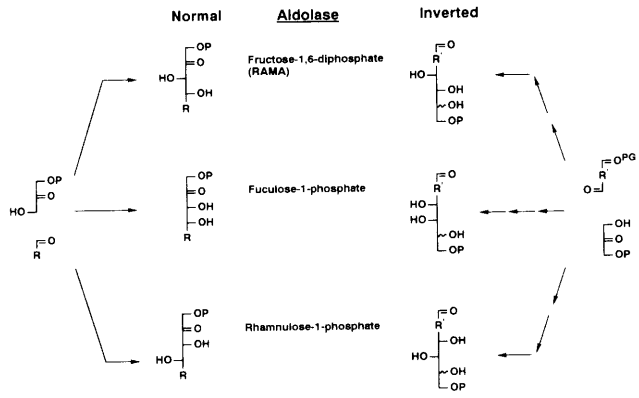
The preparation of carbohydrates is a challenging area of organic synthesis.[1] Enzymes are becoming valuable catalysts in this area.[2,3] This report describes the cloning, overproduction, and substrate specificity of a bacterial fuculose-1-phosphate aldolase (Fuc-1-P aldolase, EC 4.1.2.17) that permits 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-threo configuration at C3 and C4 (3*S*,4*R*).[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 3*R*,4*R* 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 obtained from the culture was 1.9×10^3 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.[3,9] 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 3*R*,4*R* configuration of the adduct.

Scheme I. Known Dihydroxyacetone Phosphate Aldolases[a]



[a] 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. "O^{PG}" 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]

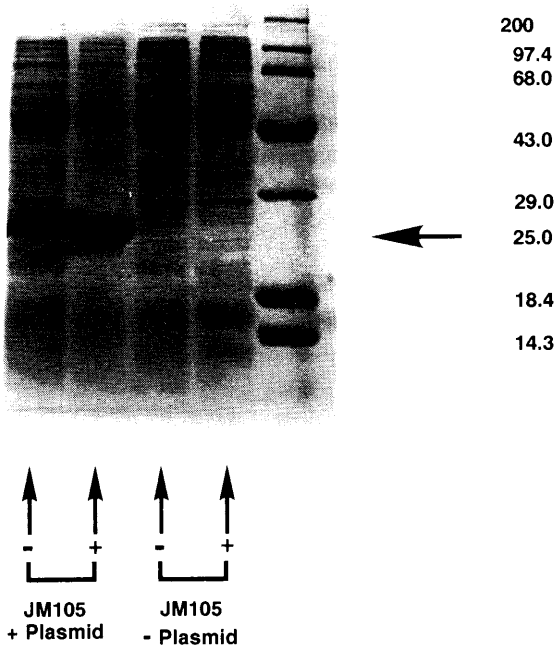


Figure 1. Overproduction of fuculose-1-phosphate aldolase. SDS-PAGE gel of crude cell extracts isolated from *E. coli* JM105 strains with and without plasmid pFUCA-5 are shown prior to (−) and following (+) the addition of β-isopropylthiogalactoside (IPTG). Note the significant overproduction of aldolase (the band at 25.0 kD) in the plasmid-containing strain even in the absence of IPTG. Molecular weight markers are shown on the right.

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

aldehyde	rel rate
1, lactaldehyde	100
2, D-glyceraldehyde	83
3, $(EtO)_2CHCHO$	~70[a]
4, $OCH_2CH_2CH_2OCHCH_2CHO$	65
5, $(CH_3)_2CHCHO$	44
6, acetaldehyde	56
7, glycolaldehyde	59

[a] Aldehyde **3** is sparingly soluble, and the rate is approximate.

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).[10] This "inverted" strategy (Scheme I), when

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(7) Fuculose-1-phosphate aldolase has previously been utilized synthetically, with whole cells being used (Drueckhammer, D. G.; Durrwachter, J. R.; Pederson, R. L.; Crans, D. C.; Daniels, L.; Wong, C.-H. *J. Org. Chem.* **1989**, *54*, 70).
(8) One unit of enzyme catalyzes the conversion of 1 μmol of fuculose-1-phosphate to DHAP and L-lactaldehyde per minute.
(9) Substrates were assayed by monitoring the disappearance of DHAP during a Fuc-1-P aldolase catalyzed condensation. A description of the assay can be found in ref 3.

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used with RAMA, may change the position of the D-*threo-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 stereocontrolled synthetic route to two configurationally distinct –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 I), is now in progress.

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This paper is dedicated to Günther Ohloff.

Supplementary Material Available: Experimental procedure for the preparation of D-ribulose (3 pages). Ordering information is given on any current masthead page.

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