

Molecular Cloning of Aldolases for Synthetic Applications^a

CLAUS H. VON DER OSTEN,^b
CATHERINE GIOANNETTI,^b CARLOS BARBAS,^c
RICHARD L. PEDERSON,^c YI-FONG WANG,^c
CHI-HUEY WONG,^c AKIO OZAKI,^{b,d} ERIC TOONE,^d
GEORGE M. WHITESIDES,^d
AND ANTHONY J. SINSKEY^b

^b*Department of Biology
and
Biotechnology Process Engineering Center
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139*

^c*Department of Chemistry
Research Institute of Scripps Clinic
La Jolla, California 92037*

^d*Department of Chemistry
Harvard University
Cambridge, Massachusetts 02138*

INTRODUCTION

Aldolases catalyze the formation of carbon-carbon bonds in monosaccharides, as exemplified by fructose-1,6-biphosphate (FBP), L-fucose-1-phosphate, and L-rhamnose-1-phosphate aldolases, which catalyze the formation of six-carbon sugars with complementary stereochemistries (FIGURE 1). FBP aldolase from rabbit muscle has been applied in the synthesis of several chiral products.¹ This enzyme accepts a variety of aldehydes in place of its natural substrate glyceraldehyde-3-phosphate while the stereospecificity of the aldol condensation is conserved, thus increasing the significance of this enzyme as a catalyst in organic synthesis.

ALDOLASES AS CATALYSTS IN ORGANIC SYNTHESIS

Rabbit muscle FBP aldolase and most other eukaryotic FBP aldolases are classified as class I aldolases that depend on the formation of an intermediate Schiff base.² Most prokaryotic FBP aldolases are class II aldolases, which do not form a Schiff base

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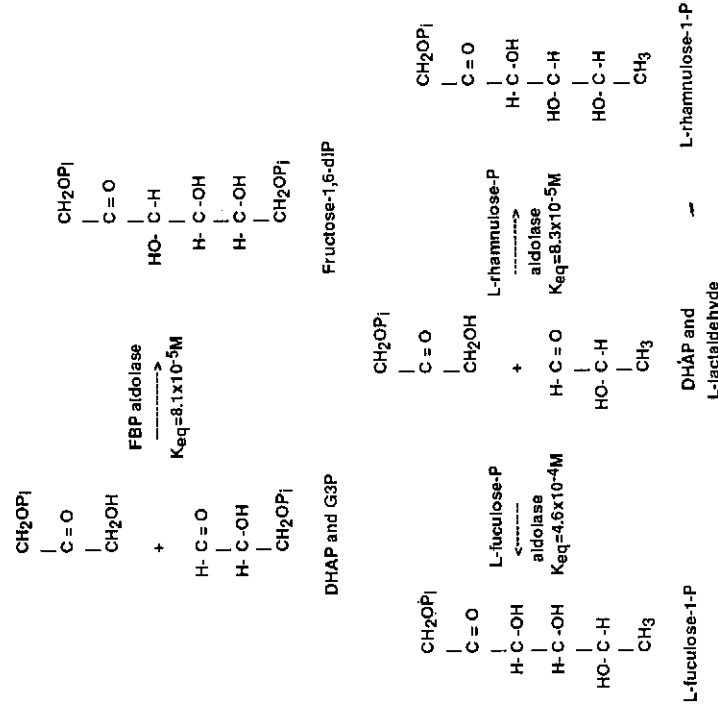


FIGURE 1. Stereospecific aldol condensations catalyzed by FBP aldolase, L-fucose-1-phosphate aldolase, and L-rhamnose-1-phosphate aldolase.

intermediate. Instead, a divalent metal ion, often Zn^{2+} , stabilizes an intermediate enolate.

To obtain an efficient source of a class II FBP aldolase, recombinant DNA technology was applied in the cloning and overproduction of a class II FBP aldolase from *Escherichia coli*.³ The *fdx* gene was isolated by complementation of an *E. coli* mutant, cloned into the multicopy vector pUC8, and expressed in *E. coli* JM83. A crude extract of the recombinant strain was used to catalyze the initial aldol condensation in the synthesis of several polyhydroxylated alkaloids such as 1-deoxynojirimycin and 1-deoxymannojirimycin. Both alkaloids are glucosidase inhibitors and can be used as precursors of anticancer and antiviral agents.⁴ The initial aldol condensation catalyzed by *E. coli* FBP aldolase in the synthesis of 1-deoxynojirimycin is shown in FIGURE 2. In the presence of 0.3 mM ZnCl_2 , the stability of the *E. coli* FBP aldolase under air and at room temperature was superior to that of rabbit muscle aldolase. The half-life of the cloned aldolase was 60 days, whereas that of rabbit muscle aldolase under the same conditions, except for the absence of ZnCl_2 , was 2 days. *E. coli* class II FBP aldolase appears to have identical substrate specificity to that of rabbit muscle class I aldolase in spite of the difference in enzyme mechanisms.³

Fucose-1-phosphate and rhamnose-1-phosphate aldolases catalyze carbon-

carbon bond formations with stereospecificities different from that of FBP aldolase. To extend the range of reactions that can be catalyzed by aldolases in organic synthesis, the *E. coli* gene encoding fuculose-1-phosphate aldolase was cloned⁵ and work is in progress to overexpress the *E. coli* rhamnulose-1-phosphate aldolase gene. The *E. coli* *fucA* gene encoding L-fuculose-1-phosphate aldolase was inserted into the prokaryotic expression vector pKK223-3⁶ and was expressed by the strong *tac* promoter in *E. coli* JM105. Preliminary substrate specificity data indicate that this aldolase will accept a range of aldehydes in place of its natural substrate L-lactaldehyde, thus increasing the value of this catalyst in the synthesis of monosaccharides and structurally related compounds.

STRUCTURAL COMPARISON OF CLASS I AND CLASS II FBP ALDOLASES

Corynebacterium glutamicum is an industrial food grade microorganism that is used in the production of primary metabolites such as amino acids and nucleotides. The genetic tools necessary for recombinant work in this organism have been established.⁷ The *C. glutamicum* *lda* gene was cloned and characterized to provide information on the structure of an FBP aldolase from a gram-positive bacterium and to contribute information on gene expression in *C. glutamicum*.⁸ Overexpression of the *C. glu-*

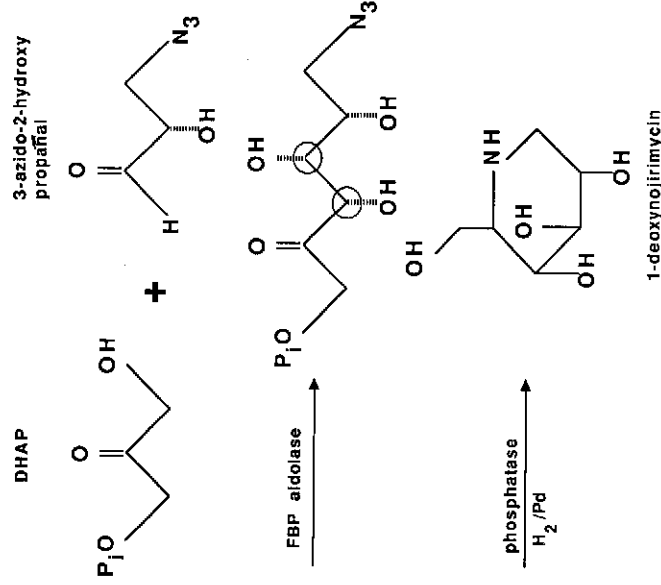


FIGURE 2. Synthesis of 1-deoxynojirimycin. Two chiral centers were generated in the initial aldol condensation by *E. coli* FBP aldolase.

tamicum fda gene by promoter replacement (*tac*) is in progress and the substrate specificity of this FBP aldolase will be compared to the *E. coli* and rabbit muscle FBP aldolases.

The amino acid sequence of the *C. glutamicum* FBP aldolase polypeptide as derived from the nucleotide sequence was aligned with amino acid sequences of two class II and four class I FBP aldolases. Regions of conserved primary structures across the classes were identified. The strongest level of homology was observed in a region containing the active-site lysyl residue of class I aldolases (FIGURE 3). A study involving site-directed mutagenesis of class II aldolases has been initiated to investigate the active site of class II aldolases and to examine the structural relationship between aldolases functioning by different mechanisms.

Class II	L	A	A	T	F	G	N	V	-	H	G	V	Y	K	P	G	N	V	K	L	R	P	E	V	L
<i>C. glut.</i>	I	A	A	A	F	G	N	C	-	H	G	L	Y	.	A	G	D	I	A	L	R	P	E	I	L
<i>S. cerev.</i>	I	A	A	S	F	G	N	V	-	H	G	V	Y	K	P	G	N	V	V	L	T	P	T	I	L
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Fruitfly	L	A	A	Y	K	A	L	S	D	H	H	V	Y	L	E	G	T	L	L	K	P	N	M	V	
Rab. musc	L	A	A	Y	K	A	L	S	D	H	I	Y	L	E	G	T	L	L	K	P	N	M	V		
Rat. liv	L	A	A	Y	K	A	L	N	D	H	H	V	Y	L	E	G	T	L	L	K	P	N	M	V	
<i>T. brucei</i>	W	S	E	V	V	S	A	L	H	R	H	G	V	V	E	G	L	L	L	K	P	N	M	V	
CLASS I																								*	

FIGURE 3. Primary structure homology between selected regions of class II (*C. glutamicum*, *S. cerevisiae*, and *E. coli*) and class I (fruit fly, rabbit muscle, rat liver, and *Trypanosoma brucei*) FBP aldolases. The active-site lysyl residue of class I aldolases is indicated by an asterisk. Gaps introduced to increase the homology within and across the classes are shown as dots and broken lines, respectively. Homologous amino acids are I = L = V, R = K, F = Y, E = D, T = S, G = A, and N = Q. Identical or homologous amino acids present in both class I and class II aldolase polypeptides are boxed.

CONCLUSIONS

Using molecular biology techniques, several bacterial aldolases have been cloned and overproduced. Initial experiments demonstrate the broad range of reactants that these biocatalysts will accept in place of their natural substrates and thus illustrate the potential use of aldolases in chiral synthesis. Cloning and expression of genes in prokaryotes provides a rich source of enzymes for the organic chemist.

Along with the overproduction of aldolases for organic synthesis, the cloning of the *C. glutamicum fda* gene will allow us to evaluate the classification of FBP aldolases into class I and class II aldolases.

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