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Fructose 1,6-Bisphosphate Aldolase Activity Is Essential for Synthesis of Alginate from Glucose by *Pseudomonas aeruginosa*

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We have isolated a mutant of *Pseudomonas aeruginosa* deficient in fructose 1,6-bisphosphate aldolase activity. This mutant, similar to the mutants deficient in any of the Entner-Doudoroff pathway enzymes, does not allow appreciable alginate formation from glucose and gluconate, but allows alginate synthesis from mannitol and fructose. This suggests that glucose and gluconate must be converted to fructose 1,6-bisphosphate via the Entner-Doudoroff pathway enzymes and fructose 1,6-bisphosphate aldolase.

We have previously reported (1) that triose phosphates must be produced from glucose and gluconate via operation of the Entner-Doudoroff pathway to generate alginic acid, which is a polymer of selectively acetylated D-mannuronic and L-guluronic acids (5). We could not, however, conclude whether it is triose phosphates, fructose 1,6-bisphosphate (FDP), or fructose 6-phosphate (F6P) that is the true alginate precursor in the absence of a FDP aldolase-negative mutant. We also were unable to detect phosphomannose isomerase (PMI) activity in mucoid or nonmucoid *Pseudomonas aeruginosa* strains as assayed by conversion of mannose 6-phosphate to F6P (1). Since such activity is essential for a proposed pathway of alginate synthesis which involves F6P, mannose 6-phosphate, mannose 1-phosphate, guanosine diphosphoomannose, and guanosine diphosphomannuronic acid as intermediates (8, 9), the undetectable level of PMI activity in the cystic fibrosis isolate of *P. aeruginosa* was intriguing. In the accompanying paper (4), we have reported that a cloned gene specifying PMI activity from *Escherichia coli* will complement alginate-negative (Alg^-) mutants of a stable alginate-producing strain of *P. aeruginosa* (3). Since the 1.5-kilobase-pair *E. coli* gene specifies a single polypeptide in the *E. coli* maxicell system and complements known *E. coli* PMI mutants, it is clear that the Alg^- mutant of *P. aeruginosa*, which is complemented by this cloned gene, is deficient in PMI activity. Introduction of the cloned gene allows the Alg^- mutants to become Alg^+, suggesting the essential role of PMI in alginate synthesis. Such roles of FDP aldolase and PMI suggest the conversion of glucose or gluconate to triose phosphates via the Entner-Doudoroff pathway (6) and their subsequent conversion to FDP and perhaps to F6P, for ultimate conversion to alginate.

We have isolated a FDP aldolase-negative mutant (ALD1) of *P. aeruginosa* strain FRD1 by ethyl methanesulfonate mutagenesis, as described previously (1). This mutant grew on LB or glutamate minimal medium on supplementation with 5 mM fructose or mannitol, but grew extremely slowly on fructose or mannitol minimal medium and failed to grow on glucose, gluconate, glutamate, glycerol, succinate, or lactate. Cell extracts of the mutant ALD1 examined for FDP aldolase activity demonstrated that the mutant had no detectable aldolase activity (Table 1). However, the other glycolytic enzyme activities were present in amounts comparable to those in the wild-type strain FRD1 (Table 1). Spontaneous reversion of ALD1 on gluconate plates led to the appearance of revertants that had regained FDP aldolase activity and produced the wild-type level of alginate from glucose. This suggested that the mutation had affected a single locus determining FDP aldolase activity. The defect in FDP aldolase enzyme activity is associated with the accumulation inside the cells of high levels of triose phosphates from gluconate (Fig. 1). Under identical conditions, small amounts of triose phosphates and also FDP accumulate in the wild-type cells from gluconate.

To test whether FDP aldolase activity was essential for the synthesis of alginic acid from hexoses and gluconeogenic precursors, buffered cell suspensions of the mutant ALD1 were incubated with various substrates, and the alginic acid in the culture filtrate was estimated (Fig. 2). Whereas the wild-type strain synthesized alginic acid from all the substrates studied, the aldolase-negative mutant failed to produce the polysaccharide from gluconate and all other gluconeogenic compounds, but continued to synthesize it from fructose, mannitol, and, to some extent, glucose. The synthesis from fructose was the most extensive, mannitol being a close second, whereas from glucose the synthesis was very little, although measurable. The synthesis of alginate only from fructose, mannitol, and glucose and the failure of all the gluconeogenic precursors to support synthesis in the aldolase mutant indicate that FDP or F6P, rather than the triose phosphates, is the precursor of alginate.

Although F6P is on the catabolic pathway of fructose and mannitol utilization by *P. aeruginosa*, it may be derived directly from glucose via glucokinase and phosphoglucose isomerase reaction (6). However, Lynn and Sokatch (7) have recently reported a preferential incorporation in alginate of isotope from [6-^{14}C]glucose relative to that from [1-^{14}C]- or [2-^{14}C]glucose in *P. aeruginosa*, indicating a split of the glucose molecule before the alginate precursor is formed. We have examined the incorporation of isotopic carbon from glucose during alginate synthesis in the aldolase mutant. The results in Table 2 compare the relative incorporation into alginate of ^{14}C from glucose labeled specifically at positions 1 or 6 with resting cells of both the aldolase mutant ALD1 and the parental strain FRD1. In the wild type, [6-^{14}C]glucose contributed about fivefold more radioactivity to the alginate compared with [1-^{14}C]glucose. However, this asymmetry was extinguished in the mutant lacking FDP aldolase. This result suggested that in the aldolase

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TABLE 1. Enzyme activities in the wild type (FRD1) and a mutant defective in fructose 1,6-bisphosphate aldolase (ALD1)[a]

Enzyme	Enzyme activity (mU/mg of protein)	
	FRD1	ALD1
Phosphoglucose isomerase	28	22
G6P dehydrogenase	13	11
Entner-Doudoroff dehydrase	19	15
Entner-Doudoroff aldolase	50	46
Triose phosphate isomerase	408	375
Fructose 1,6-bisphosphate aldolase	27	<1.0
Fructose bisphosphatase	34	39
Glyceraldehyde 3-phosphate dehydrogenase (NADPH)	211	209
Glyceraldehyde 3-phosphate dehydrogenase (NADH)	18	14
3-Phosphoglycerate kinase	190	208
Phosphoglycerate mutase	12	14
Enolase	57	68
Pyruvate kinase	95	106

[a] The FRD1 and ALD1 cultures were grown overnight in Clarke minimal medium (2) with 50 mM glutamate plus 10 mM fructose. Enzymes were assayed as described previously (1), except pyruvate kinase was estimated in extracts with 5 mM Mg^{2+} and without EDTA.

mutant the precursor of small quantities of alginate was produced by a pathway that failed to distinguish the carbon 1 of glucose from carbon 6. Presumably the alginate precursor molecule was obtained directly from glucose without its conversion to triose phosphates by the Entner-Doudoroff pathway, although the nature of such direct conversion remains unknown.

In conclusion, the data presented in this paper demonstrate the essential role of FDP aldolase in the conversion of glucose or gluconate to alginate. The data of Darzins et al. (4) demonstrate the essential role of PMI in alginate synthesis from glucose or gluconate. Together, the data appear to suggest that glucose or gluconate must be converted to fructose 1,6-bisphosphate, and presumably to F6P, for its conversion to mannose 6-phosphate and ultimately to alginate.

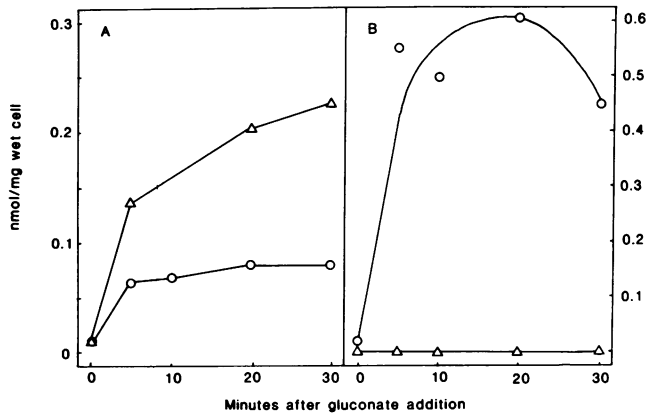

FIG. 1. Time course of accumulation of triose phosphates (○) and FDP (△) from gluconate in the wild-type strain FRD1 (A) and FDP aldolase-negative mutant ALD1 (B). Cells were grown overnight in Clarke medium (2) containing either 50 mM gluconate (FRD1) or 50 mM glutamate and 10 mM fructose (ALD1). The mutant cells were further induced with 50 mM gluconate for 6 h. Cells were harvested, washed, suspended in 50 mM potassium phosphate buffer (pH 7.2), and aerated with air. After 10 to 15 min, 20 mM gluconate was added at time 0. Aeration was continued; at the times indicated, samples were withdrawn into a final concentration of 0.5 M cold perchloric acid and centrifuged, and the supernatant was neutralized and assayed by the fluorometric method (1).

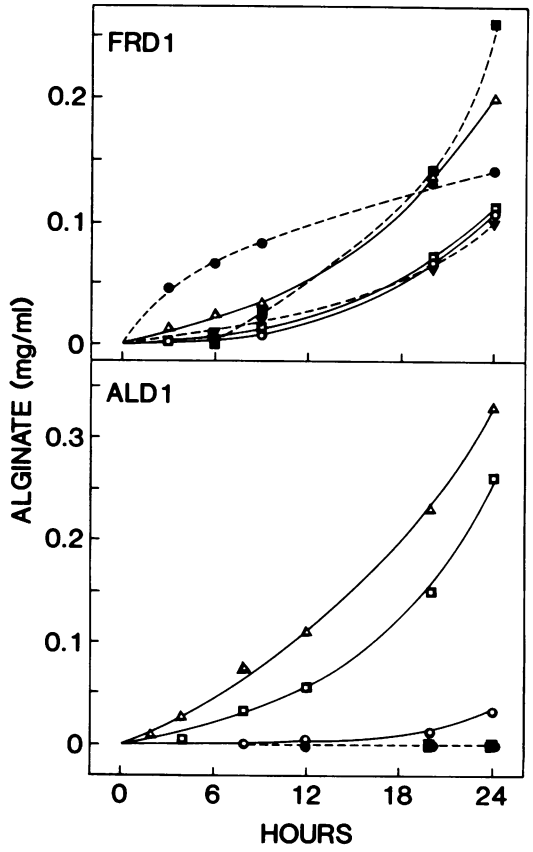

FIG. 2. Alginate synthesis in resting cell suspensions of aldolase mutant ALD1 and the wild-type parent FRD1. Overnight cultures of the strains grown on glutamate-fructose-modified synthetic medium (1) were harvested, washed, and suspended in buffered saline; 20 mM fructose (△), mannitol (□), glucose (○), gluconate (▼), glycerol (■), or glutamate (●) was added. Incubation was at 37°C. Cell-free supernatant fluids were assayed for alginic acid after dialysis (1). The initial cell protein concentration was 1.2 mg/ml.

TABLE 2. Incorporation in alginic acid of carbon from specifically labeled glucose in aldolase mutant ALD1 and the wild-type strain FRD1

Strain	^{14}C-glucose (nmol/μg of alginate)	
	[6-^{14}C]glucose	[1-^{14}C]glucose
FRD1	6.2	1.2
ALD1	5.5	6.1

[a] FRD1 and ALD1 cells were grown in L broth medium and supplemented with 5 mM each of fructose and glucose. The incubation medium contained 8 mM glucose. After 1 h, 2 μCi of [1-^{14}C]glucose or [6-^{14}C]glucose was added per ml, and incubation was continued for an additional 5 h. Alginic acid in the culture filtrate was dialyzed for 48 h, initially against distilled water and finally against 2 mM glucose. The amount of alginate produced during the incubation was measured in a parallel incubation mixture not containing any radioactive glucose.

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