2-KETO-3-DEOXY-L-ARABONATE ALDOLASE AND ITS ROLE IN A NEW PATHWAY OF L-ARABINOSE DEGRADATION

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Summary: A new aldolase which catalyzes the cleavage of 2-keto-3-deoxy-L-arabonate to pyruvate and glycolaldehyde was demonstrated in extracts of a pseudomonad (strain MSU-1). Extracts also catalyzed an oxidation of L-arabinose and a dehydration of L-arabonate. However, the dehydration and oxidation of 2-keto-3-deoxy-L-arabonate to α-ketoglutarate, which has been reported to occur in other pseudomonads, could not be demonstrated in extracts of pseudomonad MSU-1. Thus, the pathway of L-arabinose degradation in pseudomonad MSU-1 is believed to be: L-arabinose → L-arabonate → 2-keto-3-deoxy-L-arabonate → pyruvate + glycolaldehyde.

Three pathways are known for the degradation of L-arabinose. In Aerobacter aerogenes (1), Escherichia coli (2), Lactobacillus plantarum (3), and Bacillus subtilis (4), L-arabinose is isomerized to L-ribulose, which is sequentially phosphorylated at C-5 and epimerized at C-4 to yield D-xylulose 5-phosphate. In Penicillium chrysogenum (5), L-arabinose is metabolized through an alternating sequence of reductions and oxidations followed by phosphorylation as follows: L-arabinose → L-arabitol → L-xylulose → xylitol → D-xylulose → D-xylulose 5-phosphate. In Pseudomonas saccharophila (6-8) and Pseudomonas fragi (9, 10), L-arabinose is metabolized via a series of oxidation and dehydration reactions as follows: L-arabinose → L-arabonate → 2-keto-3-deoxy-L-arabonate → α-ketoglutarate semialdehyde → α-ketoglutarate. This conversion of 2-keto-3-deoxy-L-arabonate to α-ketoglutarate is the only previously known route for its metabolism. In this paper we present evidence for an aldolase which catalyzes the reversible cleavage of 2-keto-3-deoxy-L-arabonate. Evidence is also presented which implicates the participation of this aldolase in a

new pathway of L-arabinose metabolism, namely: L-arabinose → L-arabonate → 2-keto-3-deoxy-L-arabonate → pyruvate + glycolaldehyde.

MATERIALS AND METHODS

The pseudomonad (strain MSU-1) used in this investigation was isolated in our laboratory. It can grow well on D-glucose, D-fucose, or L-arabinose as a sole carbon source, but cannot grow on D-arabinose. For enzyme studies it was grown overnight on a rotatory shaker at 32°C in a mineral salts-sugar medium (11), the sugar being 0.5% L-arabinose unless indicated otherwise. Crude extracts were prepared by sonicating washed cells in 0.143 mM 2-thioethanol and 0.10 M Bicine buffer (12), pH 7.4, and centrifuging at 40,000 x g for 10 minutes. For some experiments, 2-keto-3-deoxy-L-arabonate aldolase was purified 17-fold by ammonium sulfate precipitation followed by chromatography on Sephadex G-200.

L-Arabonate was prepared by the method of Moore and Link (13). 2-Keto-3-deoxy-L-arabonate was prepared and isolated as described by Weimberg (7) and was determined with the 2-thiobarbituric acid-periodate procedure of Weissbach and Hurwitz (14). Pyruvate was determined with lactic acid dehydrogenase and NADH. Glycolaldehyde was determined with 3-methyl-2-benzothiazolone hydrazone (15).

RESULTS AND DISCUSSION

Table 1 shows specific activities of L-arabinose dehydrogenase, L-arabonate dehydratase, and 2-keto-3-deoxy-L-arabonate aldolase in crude extracts. The substantially higher specific activities in extracts of L-arabinose-grown cells indicate that the enzymes are inducible. In other experiments, half-maximal rates were obtained with 0.14 mM L-arabinose in the dehydrogenase reaction, 4.3 mM L-arabonate in the dehydratase reaction, and 1.8 mM 2-keto-3-deoxy-L-arabonate in the aldolase reaction. L-Arabinose isomerase (1) and L-arabinose reductase (5) activities could not be detected (< 1 nanomole of product formed per minute per milligram of protein). These findings are consistent with investigations of other pseudomonads (6-10)

TABLE 1. Enzyme Activities in Crude Extracts

Growth Substrate	Specific Activity*		
	L-Arabinose dehydrogenase	L-Arabonate dehydratase	2-Keto-3-deoxy-L-arabonate aldolase
L-Arabinose	0.112	0.071	0.071
D-Glucose	0.002	0.009	0.021
Nutrient broth (Difco)	0.001	0.006	0.018

*Micromoles of product formed per minute per milligram of protein.

L-Arabinose dehydrogenase was assayed spectrophotometrically at 340 nm and 25°C. The reaction mixture (0.15 ml) contained: 2.5 μmoles of L-arabinose, 0.3 μmole of $NADP^+$, 15 μmoles of Tris-HCl buffer, pH 8.1, and enzyme.

L-Arabonate dehydratase was assayed at 30°C by the semicarbazide method (16). The reaction mixture (0.15 ml) contained: 5 μmoles of L-arabonate, 5 μmoles of $MgCl_2$, 0.15 μmole of 2-thioethanol, 20 μmoles of Bicine buffer (12), pH 7.4, and enzyme.

2-Keto-3-deoxy-L-arabonate aldolase was assayed spectrophotometrically at 340 nm and 25°C. The reaction mixture (0.15 ml) contained: 5 μmoles of 2-keto-3-deoxy-L-arabonate, 1 μmole of $MgCl_2$, 0.15 μmole of 2-thioethanol, 0.07 μmole of NADH, 13 μgrams of lactic acid dehydrogenase, 15 μmoles of HEPES buffer (12), pH 7.8, and enzyme.

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except for the aldolase, which has not been previously reported.

The stoichiometry and equilibrium values for the cleavage of 2-keto-3-deoxy-L-arabonate by the aldolase, approached from both directions, are shown in Table 2. From these data, the equilibrium constant for the reaction,

$$K_{eq} = \frac{[\text{Pyruvate}][\text{Glycolaldehyde}]}{[\text{2-Keto-3-deoxy-L-arabonate}]}$$

was computed to be 0.35 (measured in the cleavage reaction) or 0.39 (measured in the condensation reaction).

The data in Table 2 provide evidence that the products of 2-keto-3-deoxy-L-arabonate cleavage are pyruvate and glycolaldehyde. Both pyruvate (measured with lactic acid dehydrogenase) and glycolaldehyde (measured by its reaction with 3-methyl-2-benzothiazolone hydrazone and $FeCl_3$ to form a tetra-

TABLE 2. Stoichiometry, Reversibility, and Equilibrium of the Aldolase-Catalyzed Reaction

Initial concentrations*			Equilibrium concentrations*		
2-Keto-3-deoxy-L-arabonate	Pyruvate	Glycolaldehyde	2-Keto-3-deoxy-L-arabonate	Pyruvate	Glycolaldehyde
4.93	0	0	3.81	1.23	1.07
0	10.0	10.0	8.45	1.85	1.77

*Concentrations are expressed as millimolarities.

The reaction mixture (0.40 ml) for the cleavage reaction contained: 1.97 μmoles of 2-keto-3-deoxy-L-arabonate, 2 μmoles of $MnCl_2$, 0.3 μmole of 2-thioethanol, 6 μmoles of HEPES buffer (12), pH 7.4, and 0.3 mg of 17-fold purified 2-keto-3-deoxy-L-arabonate aldolase. The reaction mixture was incubated at 28°C until equilibrium was established.

The reaction mixture (1.0 ml) for the condensation reaction contained: 10.0 μmoles of sodium pyruvate, 10.0 μmoles of glycolaldehyde, 5 μmoles of $MnCl_2$, 1.2 μmoles of 2-thioethanol, 15 μmoles of HEPES buffer, pH 7.4, and 0.6 mg of 17-fold purified 2-keto-3-deoxy-L-arabonate aldolase. The reaction mixture was incubated at 28°C until equilibrium was established.

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azopentamethine cyanine dye) were formed in amounts equimolar to the amount of 2-keto-3-deoxy-L-arabonate that disappeared. Furthermore, pyruvate and glycolaldehyde served as substrates for the reverse reaction, yielding the same equilibrium values as in the forward reaction. The identity of the cleavage products as pyruvate and glycolaldehyde was confirmed by two-dimensional thin-layer chromatography (Table 3).

The only previously described pathway for the degradation of 2-keto-3-deoxy-L-arabonate involves dehydration to α-ketoglutarate semialdehyde followed by dehydrogenation to α-ketoglutarate (6-9). These two reactions have been measured in crude extracts of P. saccharophila and P. fragi (6-9) by following the 2-keto-3-deoxy-L-arabonate-dependent reduction of a pyridine nucleotide coenzyme. We made many attempts to detect activity in extracts of pseudomonad MSU-1 with this assay, using both NAD^+ and $NADP^+$, in the presence of various kinds and concentrations of buffers, metal ions, and thiols. In no case did we detect any activity (< 0.1 nanomole of

TABLE 3. Thin-Layer Chromatography of Derivatives of the 2-Keto-3-deoxy-L-arabonate Cleavage Products

2,4-Dinitrophenylhydrazone of:	Color after KOH spray	R_f Values	
		1st Dimension	2nd Dimension
Pyruvate standard	Purple-brown	0.38	0.40
Cleavage product A	Purple-brown	0.37	0.41
Glycolaldehyde standard	Yellow-brown	0.59	0.52
Cleavage product B	Yellow-brown	0.60	0.52

A reaction mixture (1.0 ml) containing 10 μmoles of 2-keto-3-deoxy-L-arabonate, 5 μmoles of $MgCl_2$, 7.35 μmoles of 2-thioethanol, 15 μmoles of HEPES buffer, pH 7.4, and 0.5 mg of 17-fold purified 2-keto-3-deoxy-L-arabonate aldolase was incubated at 27°C for 1 hour. The reaction was quenched by the addition of 1.5 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl. The hydrazones were allowed to form for 20 minutes, after which the mixture was extracted with three 10-ml portions of toluene. The toluene extracts were combined and concentrated under reduced pressure to 0.5 ml. Thin-layer plates (Brinkman, Silica Gel F_{254}) were spotted with 50 μliter aliquots and were developed two-dimensionally: (i) n-butanol-ethanol-0.5 N NH_4OH (70:10:20); (ii) benzene-tetrahydrofuran-glacial acetic acid (57:35:8). The compounds on the developed chromatogram were visualized with a 10% KOH spray. No 2,4-dinitrophenylhydrazones were detected on chromatograms of zero-time controls treated as above. Standard 2,4-dinitrophenylhydrazones of pyruvate and glycolaldehyde were prepared by the methods of Hawary et al. (17), and Powers et al. (18), respectively, and were dissolved in toluene before spotting.

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product formed per minute per milligram of protein). Neither could we detect NADH or NADPH oxidation by crude extracts in the presence of α-ketoglutarate. Also, we could not detect 2-keto-3-deoxy-L-arabonate dehydratase activity in fractionated extracts by measuring the disappearance of substrate with 2-thiobarbituric acid-periodate; when extracts of pseudomonad MSU-1 were chromatographed on Sephadex G-200, the only fractions that catalyzed a disappearance of 2-keto-3-deoxy-L-arabonate were those that cleaved it by aldolase action. Thus, we conclude that pseudomonad MSU-1 does not convert 2-keto-3-deoxy-L-arabonate to α-ketoglutarate, but possesses a new L-arabinose pathway involving cleavage of 2-keto-3-deoxy-L-arabonate to pyruvate and glycolaldehyde.

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