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Properties of D-Arabinose Isomerase Purified from Two Strains of *Escherichia coli*

JAMES R. BOULTER AND WILLIAM O. GIELOW

Department of Biological Sciences, Section of Biochemistry and Molecular Biology, University of California, Santa Barbara, California 93106

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D-Arabinose isomerase (EC 5.3.1.3) has been isolated from L-fucose-induced cultures of *Escherichia coli* K-12 and D-arabinose-induced cultures of *E. coli* B/r. Both enzymes were homogeneous in an ultracentrifuge and migrated as single bands upon disc electrophoresis in acrylamide gels. The $s_{20,w}$ was 14.5 $\times$ 10^{-13} sec for the *E. coli* K-12 enzyme and 14.3 $\times$ 10^{-13} sec for the *E. coli* B/r enzyme. The molecular weight, determined by high-speed sedimentation equilibrium, was 3.55 $\pm$ 0.06 $\times$ 10^5 for the *E. coli* K-12 enzyme and 3.42 $\pm$ 0.04 $\times$ 10^5 for the enzyme isolated from *E. coli* B/r. Both enzyme preparations were active with L-fucose or D-arabinose as substrates and showed no activity on any of the other aldopentoses or aldohexoses tested. With the *E. coli* K-12 enzyme, the K_m was 2.8 $\times$ 10^{-1} M for D-arabinose and 4.5 $\times$ 10^{-2} M for L-fucose; with the *E. coli* B/r enzyme, the K_m was 1.7 $\times$ 10^{-1} M for D-arabinose and 4.2 $\times$ 10^{-2} M for L-fucose. Both enzymes were inhibited by several of the polyalcohols tested, ribitol, L-arabitol, and dulcitol being the strongest. Both enzymes exhibited a broad plateau of optimal catalytic activity in the alkaline range. Both enzymes were stimulated by the presence of Mn^{2+} or Co^{2+} ions, but were strongly inhibited by the presence of Cd^{2+} ions. Both enzymes were precipitated by antisera prepared against either enzyme preparation. The amino acid composition for both proteins has been determined; a striking similarity has been detected. Both enzymes could be dissociated, by protonation at *p*H 2 or by dialysis against buffer containing 8 M urea, into subunits that were homogeneous in an ultracentrifuge and migrated as single bands on disc electrophoresis in acrylamide gels containing urea. The molecular weight of the subunit, determined by high-speed sedimentation equilibrium, was 9.09 $\pm$ 0.2 $\times$ 10^4 for the enzyme from *E. coli* K-12 and 8.46 $\pm$ 0.1 $\times$ 10^4 for the enzyme from *E. coli* B/r. On the basis of biophysical studies, both isomerases appear to be oligomeric proteins consisting of four identical subunits.

In 1955, Green and Cohen (4) observed that crude extracts of an *Escherichia coli* B strain adapted to either L-fucose or D-arabinose contained an enzyme activity capable of converting L-fucose to L-fuculose and D-arabinose to D-ribulose. It was postulated that a single enzyme might be responsible for the isomerization of both aldoses to their respective ketoses. Similar findings have been reported for *Aerobacter aerogenes* adapted to L-fucose, D-arabinose, or L-xylose (1). With *A. aerogenes*, activity for the isomerization of L-fucose, as well as D-arabinose and L-xylose, could be observed in extracts of cells grown with any one of the three sugars as a sole source of carbon and energy.

Although it is able to utilize L-fucose, wild-type *E. coli* K-12 is unable to utilize D-arabinose as a sole source of carbon and energy. Recent work by LeBlanc and Mortlock (6) has shown that growth on L-fucose of an *E. coli* K-12 strain, selected for its ability to grow on D-arabinose, results in the synthesis of the enzymes necessary for growth on D-arabinose. They suggest that the L-fucose isomerase is responsible for the isomerization of D-arabinose to D-ribulose in this organism.

E. coli B/r is able to utilize the pentose D-arabinose as a sole source of carbon and energy. This strain will grow on D-arabinose with a doubling time of 120 min, but cannot metabolize L-fucose. In *E. coli* B/r, both D-

arabinose and L-fucose, however, will induce the synthesis of the D-arabinose isomerase and D-ribulokinase (J. Boulter, *unpublished data*).

Biochemical and biophysical data are accumulating which suggest that the L-fucose and D-arabinose isomerase activities found in different strains of *E. coli* and related organisms are due to the same enzyme. In this paper, we present a detailed comparison of the properties of an L-fucose-induced isomerase activity from *E. coli* K-12 and a D-arabinose-induced isomerase activity from *E. coli* B/r. This comparison suggests strongly that the two enzyme preparations are virtually identical and bear a strong resemblance to a D-arabinose-induced isomerase activity purified from *A. aerogenes*.

MATERIALS AND METHODS

D-Arabinose isomerase was isolated from a mutant strain of *E. coli* B/r (strain da11) which produces lower levels of the D-ribulokinase and increased levels, approximately threefold, of the D-arabinose isomerase as compared with the wild-type strain. This mutant carries a mutation in the D-ribulokinase structural gene and possesses wild-type D-arabinose isomerase. (Details of the construction of this strain and other findings on the metabolism of D-arabinose in *E. coli* B/r will be the subject of a future paper.) Batch cultures were grown in a Fermacell fermentor, model F-130 (New Brunswick Scientific Co., Inc.). Approximately 500 g (wet weight) of cells was used for each purification. The growth medium contained 1% casein hydrolysate, a mineral base of 1% K_2HPO_4-KH_2PO_4 (*p*H 7), 0.01% $MgSO_4 \cdot 7H_2O$, and 0.1% $(NH_4)_2SO_4$, and 0.15% D-arabinose. The culture used to inoculate the fermentor was grown to log phase in the same medium without D-arabinose.

The cells were harvested in late log phase in a refrigerated Sharples centrifuge, type AS-14 (Pennsalt Chemicals Corp.). The cell pellet was resuspended in approximately 600 ml of 10 mM glycylglycine, *p*H 7.6, quick-frozen in a dry ice-acetone bath, and stored at −20 C until use.

The frozen cells were treated in 100-ml quantities for 8 min at level 8 of a Sonifier cell disrupter, model W185D (Heat Systems Ultrasonics, Inc.). The sonically treated suspension was centrifuged at 56,000 × *g* for 1 hr in a model L ultracentrifuge and the supernatant fluid was recovered. Unless otherwise indicated, this and all subsequent steps were carried out at 0 to 4 C.

A 1.0 M solution of $MnCl_2$ was added slowly, with stirring, to the cell-free extract to give a final concentration of 50 mM $MnCl_2$. At 15 min after the last addition of $MnCl_2$, the viscous solution was centrifuged at 66,000 × *g* for 1 hr and the supernatant fluid was recovered.

The supernatant fluid from the $MnCl_2$ precipitation was immediately adjusted to *p*H 7.6 by the addition of 3% NH_4OH. Solid $(NH_4)_2SO_4$ was added to 48% saturation over a period of 30 min, and the preparation was allowed to equilibrate with stirring for an additional 30 min. After centrifugation for 15 min at 35,000 × *g*, the supernatant fluid was recovered and brought to 60% saturation in the manner described above. The resulting precipitate was recovered by centrifugation at 35,000 × *g* for 30 min, suspended in 10 mM potassium phosphate buffer, *p*H 7.6, and dialyzed against at least 40 times the suspension volume of the same buffer. The dialysis buffer was changed after 8 hr, and dialysis was continued for an additional 8 hr.

The diethylaminoethyl (DEAE)-cellulose was prepared as reported by Patrick and Lee (14). The enzyme from the $(NH_4)_2SO_4$ step was loaded on a 1,200-ml DEAE-cellulose column and washed on with 600 ml of 10 mM potassium phosphate buffer, *p*H 7.3. Elution was carried out with a linear gradient of 0 to 0.35 M NaCl in a total volume of 6 liters of phosphate buffer, *p*H 7.3. Fractions of 15 ml were collected at a flow rate of 120 ml per hr. The enzyme was eluted at approximately 0.30 M NaCl. Those fractions of high specific activity were pooled, dialyzed extensively against 1 mM phosphate buffer, *p*H 8, and lyophilized to dryness.

The lyophilized protein from the *p*H 7.3 DEAE-cellulose column was dissolved in 6 ml of phosphate buffer and layered on a Sephadex G-200 column. Fractions of 5 ml were collected at a flow rate of 10 ml per hr. Fractions were pooled on the basis of high specific activity. The pooled material from the Sephadex G-200 column was applied to the same column to effect further purification. Fractions from the second G-200 column were pooled on the basis of homogeneity in the ultracentrifuge and disc electrophoresis studies.

The first stages of the purification of the D-arabinose isomerase from *E. coli* K-12 were identical to those described above for the D-arabinose isomerase from *E. coli* B/r. The batch culture growth medium was 1% Casamino Acids-mineral base supplemented with 0.06% L-fucose and 4 μg of thiamine per ml. The 50 to 65% $(NH_4)_2SO_4$ pellet contained the isomerase activity.

The enzyme preparation from the $(NH_4)_2SO_4$ step was loaded onto a DEAE-cellulose column (3.7 by 100 cm), washed on with one-half column volume of 10 mM potassium phosphate buffer, and eluted with a 6- or 8-liter linear NaCl gradient, 0 to 0.40 M, *p*H 7.6. The enzyme was eluted at approximately 0.35 M NaCl. Fractions of 15 ml were collected at a flow rate of 120 ml per hr. Those fractions of high specific activity were pooled and dialyzed as described earlier. The pooled material was lyophilized to dryness and stored under vacuum at −20 C.

The lyophilized enzyme from the *p*H 7.6 DEAE-cellulose column was dissolved in glass-distilled water (1% column volume or less) and layered above the bed of a Sephadex G-200 column that had been equilibrated with 10 mM potassium phosphate buffer, *p*H 7.6, containing 1 mM ethylenediaminetetraacetate (EDTA) and 0.1 mM dithiothreitol. Fractions of high specific activity were pooled, dialyzed as above,

and lyophilized to dryness. The pooled material from the first G-200 column was rerun on the same column to effect further purification. Fractions from the second G-200 column were pooled on the basis of homogeneity in the ultracentrifuge and disc electrophoresis studies.

Enzyme assay. The D-arabinose isomerase was routinely assayed in a reaction mixture containing 0.50 ml of 0.25 M glycylglycine (adjusted to *p*H 7.6 with 1 N NaOH), 0.05 ml of 0.5 mM $MnCl_2$, 0.30 ml of 0.5 M D-arabinose or L-fucose, and water plus extract to total 1.0 ml. The reaction mixture was warmed to 37 C, and the reaction was initiated by adding 5 to 15 units of isomerase activity. Samples of 0.1 ml were taken at 3-min intervals for 9 min and pipetted into 0.9 ml of 0.1 N HCl. The ribulose or fuculose in these samples was determined by the cysteine-carbazole method of Dische and Borenfreund (3). D-Ribulose and L-fuculose do not have the same molar absorptivity in the cysteine-carbazole reaction. The value for L-fuculose obtained by Green and Cohen (4) of 300 Klett units per 0.1 μmole was used, and a value of 225 Klett units per 0.1 μmole of D-ribulose was determined in this laboratory. All assays were read with a Klett-Summerson colorimeter equipped with a 540-nm filter. One unit of enzyme activity is that amount of enzyme that will convert 1 μmole of D-arabinose to D-ribulose or L-fucose to L-fuculose per min. All assays were calculated on the basis of three or four points over the 9-min assay, and, unless otherwise indicated, represent the average of duplicate assays. Single-point assays were often employed in which only one sample was assayed for ribulose or fuculose production after 6 min of incubation. The reaction mixture was the same as that for multipoint assays, except that only 0.1 ml of the reaction mixture was used with 1 to 10 μliters of enzyme being added. The 0.1 N HCl (0.9 ml) was pipetted directly into the reaction tube to stop the reaction. The ribulose or fuculose was determined as above.

Protein assay. Protein was determined by the method of Lowry et al. (8) or, with column eluants, by the application of Layne's (5) formula relating optical density readings at 260 and 280 nm to protein concentration.

Disc electrophoresis. Electrophoresis was performed at 4 C by the method of Ornstein (12) and Davis (2) with a gel bed (0.5 by 5.1 cm) of 7.5% acrylamide, tris(hydroxymethyl)aminomethane-glycine electrode buffer (*p*H 8.3), and a constant current of 3 ma per gel. When urea gels were used, the electrode buffers contained 0.1% 2-mercaptoethanol. Sodium dodecyl sulfate (SDS) gel electrophoresis was performed by the method of Weber and Osborn (18). After the tracking dye had moved to within 1 cm of the bottom of the gel, the gels were removed, stained for 2 to 8 hr in aniline blue-black (or for 2 hr in amido-Schwartz for urea gels and 1.5 hr in Coomassie blue for SDS gels), and destained by electrophoresis in 7.5% acetic acid at room temperature.

Amino acid analysis. The acid hydrolysis of the native enzyme preparations was performed by the method of Moore and Stein (9). Samples were hydrolyzed in sealed, evacuated ampoules for 16, 24, 48, 72, and 96 hr at 110 C. All analyses were done on a Beckman model 120 C amino acid analyzer, and all results are the average of duplicate assays. Norleucine, 0.50 μmole per hydrolysate, was added prior to hydrolysis to measure any losses due to handling and preparation of the samples.

Biophysical studies. All ultracentrifugal studies were conducted with a Spinco model E analytical ultracentrifuge equipped with a phase plate, RTIC, and open-end camera. Kodak Spectroscopic Plates, type II-G, were used for photographs with interference optics and Kodak Super XX panchromatic film for the photographs taken with schlieren optics. All photographs were read on a Nikon two-dimensional microcomparator, model 6 C. Sedimentation coefficients for both enzyme preparations were determined by sedimentation velocity experiments on enzyme solutions dialyzed against 0.1% KCl in phosphate buffer, with the use of an An-E rotor at a speed of 50,740 rev/min. Photographs were taken at 4- or 8-min intervals, and at least five such photographs were read to provide the data for the plot of the log of the distance from the axis of rotation versus time. Sedimentation coefficients calculated from the photographs (s_{obs}) were corrected to sedimentation coefficients in a solution with the density and viscosity of water at 20 C by the method of Schachman (16).

The molecular weight of the native enzymes and the dissociation products was determined by the sedimentation equilibrium method of Yphantis (19). Samples of the isomerases were dialyzed against 0.1% KCl in potassium phosphate buffer before use. The native enzymes were sedimented at either 9,945 or 10,137 rev/min at 20 C in the An-J rotor. The dissociation products were sedimented at 27,690 rev/min in an An-D rotor. The time to reach equilibrium was calculated from the equation of Weaver (17), and interference photographs were taken several hours after the calculated t_{eq} to verify the attainment of equilibrium. When the dialysis step also formed part of the dissociation procedure, a sedimentation velocity experiment was run prior to the sedimentation equilibrium run to determine the degree of homogeneity and the extent of dissociation of the sample.

Immunochemistry. Agar diffusion studies were done by the method of Ouchterlony (13) with 0.6% Ionagar No. 2. Antisera were prepared against each enzyme by injecting two rabbits with 1.5 mg of purified isomerase from *E. coli* K-12 or *E. coli* B/r. Three weeks later, a second injection of 2.5 mg of each enzyme preparation was made. Five days later the rabbits were bled, and sera were collected.

Reagents. All reagents were of the highest grade available. L-Fucose, D-arabinose, L-arabinose, and SDS were obtained from Sigma Chemical Co.; dulcitol and *i*-erythritol, from Phanstiehl Chemical Co.; D-xylose, D-fucose, L-rhamnose, L-glucose, D-glucose, and *i*-inositol from Nutritional Biochemicals Corp.; Casamino Acids were from Difco; urea, from Baker and Adamson; and acrylamide, from Eastman Kodak Co. The urea was recrystallized from methanol

TABLE 1. *Purification summary*[a]

Step and fraction	I-B/r			I-K-12		
	Vol (ml)	Protein (g)	Specific activity	Vol (ml)	Protein (g)	Specific activity
Crude extract	470	49.4	1.19	760	31.7	0.67
$MnCl_2$ supernatant	362	19.6	1.59	755		
$(NH_4)_2SO_4$ fraction	95	4.28	5.82	196	8.8	1.75
DEAE-cellulose pooled fractions	450	0.49	16.40		0.61	11.83
Sephadex G-200 pooled fractions	66	0.083	43.33		0.146	23.83
Sephadex G-200 pool (rerun)	19	0.018	63.70	43	0.041	63.33

[a] I-B/r = D-arabinose isomerase from *E. coli* B/r; I-K-12 = D-arabinose isomerase from *E. coli* K-12. Specific activity is expressed as units per milligram of protein.

and the acrylamide from acetone before use.

RESULTS

Purification of the enzymes. Several purifications of the isomerases have been performed. A description of the standard procedure is presented in Materials and Methods. An outline of the purification procedure is presented in Table 1.

Estimation of purity. The purity of the pooled purified enzymes was estimated by their homogeneity with respect to both size and charge. Samples of the second G-200 pooled column eluant for both isomerase preparations were examined in an ultracentrifuge. With both preparations, a single schlieren peak was observed, with no indications of heterogeneity (see Fig. 1). Identical samples were examined for purity by disc electrophoresis with 10 to 20 μg of enzyme per gel. A single protein band was observed in both cases (see Fig. 2b). Faint "contaminant" bands that appear in polyacrylamide disc gels of the native enzymes and the SDS-dissociated enzymes apparently result from the conditions to which the enzymes are subjected prior to or during electrophoresis.

Catalytic properties: optimal pH. The optimal pH was determined by testing a constant amount of enzyme in reaction mixtures buffered from *p*H 4.0 to 10.8. Both enzymes exhibited maximal activity between *p*H 8.0 and 10.0. With the isomerase from *E. coli* K-12, no decrease in activity was observed between *p*H 7.6 and 10.6. Both enzyme activities decreased sharply below *p*H 7, with no activity below *p*H 5.2.

Michaelis constant. The K_m values for both enzymes were determined by the method of Lineweaver and Burk with purified enzyme (7). A constant amount of enzyme was assayed in reaction mixtures containing different amounts of D-arabinose or L-fucose. With the isomerase from *E. coli* K-12, the K_m was 2.8×10^{-1} M for D-arabinose and 4.5×10^{-2} M for L-fucose. With the isomerase from *E. coli* B/r, the K_m was 1.7×10^{-1} M for D-arabinose and 4.2×10^{-2} M for L-fucose. Initial rate studies show that both enzymes are more active on L-fucose than on D-arabinose as a substrate. At v_{max}, however, the ratios of activity on L-fucose and D-arabinose for both enzymes is 1:1 (see Table 2).

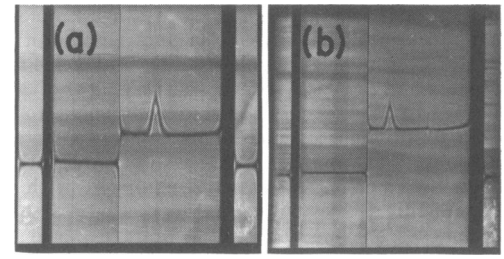

FIG. 1. *Sedimentation velocity profiles of purified D-arabinose isomerase from E. coli B/r (a) and from E. coli K12 (b). Photograph (a) was taken at a bar angle of 55 degrees, 20 min after reaching a speed of 50.740 rev/min. Photograph (b) was taken at a bar angle of 65 degrees, 8 min after reaching a speed of 50,740 rev/min. In both photos, the direction of sedimentation is from left to right.*

Inhibition. A variety of compounds was tested for the ability to inhibit the action of the isomerases. A constant amount of enzyme was assayed in the presence of 0.075 M D-arabinose and 0.1 M concentrations of the compounds to be tested for inhibition (Table 3). Ribitol, L-arabitol, and dulcitol were the three strongest inhibitors, all inhibiting the action of the isomerases by more than 70% under these conditions.

Substrate specificity. A number of sugars was tested for the ability to act as substrates for the enzymes. Of all the substrates tested, only L-fucose and D-arabinose would act as substrates for the enzymes. No activity (i.e.,

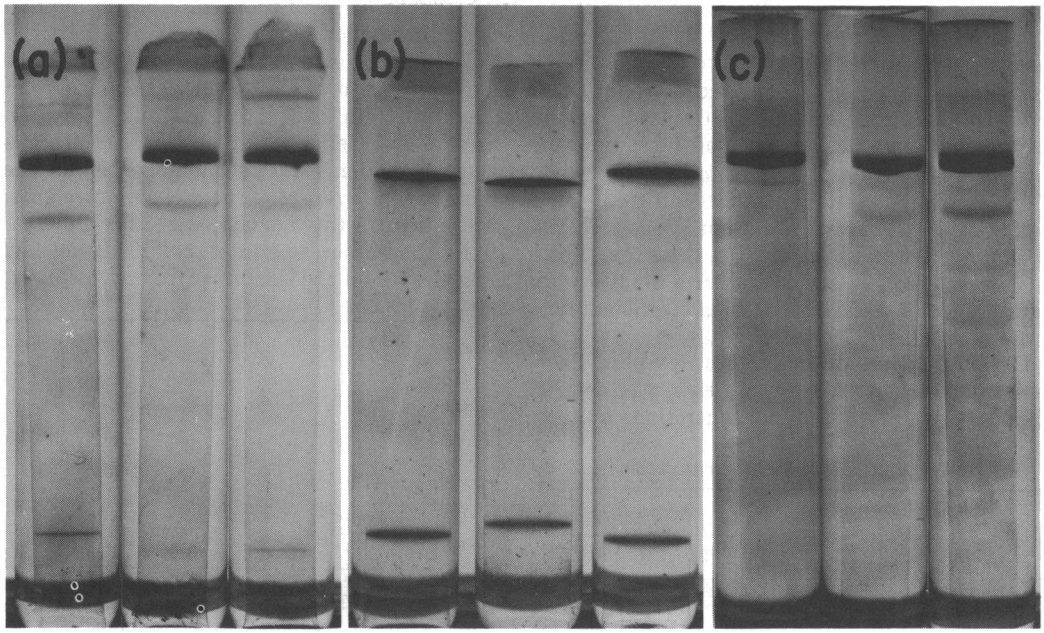

FIG. 2. *Polyacrylamide disc gel electrophoresis of (a) native D-arabinose isomerase from E. coli B/r (left), D-arabinose isomerase from E. coli K-12 (middle), and a mixture of both proteins (right); (b) urea-dissociated D-arabinose isomerase from E. coli B/r (left), isomerase from E. coli K-12 (middle), and a mixture of both proteins (right); (c) SDS gels of enzyme from E. coli B/r (left), from E. coli K-12 (middle), and a mixture of both proteins (right). The same enzyme preparation was used for the various gels. All gels were run as described in Materials and Methods with 10 to 20 μg of protein per gel. The dark band at the bottom of the plain and urea gels is the bromophenol blue tracking dye. The direction of migration is from the top of the gel to the bottom.*

TABLE 2. *Ratios of enzyme activity*[a]

Enzyme[b]	Substrate	Amt (μmoles/min)	F-A ratio[c]
I-B/r	D-Arabinose	0.246	1:0.99
	L-Fucose	0.248	
I-K-12	D-Arabinose	0.258	1:0.98
	L-Fucose	0.263	

[a] The reaction mixture was the same as that described in Materials and Methods with 150 mM D-arabinose or L-fucose as substrates. Single-point assays (in duplicate) were performed with 2 μliters of enzyme preparation (0.33 mg of protein/ml).

[b] I-B/r = D-arabinose isomerase from *E. coli* B/r; I-K-12 = D-arabinose isomerase from *E. coli* K-12.

[c] Ratio of L-fucose to D-arabinose.

TABLE 3. *Inhibition of enzyme activity*[a]

Addition	Activity (%)	
	I-B/r	I-K-12
None	100	100
i-Inositol	100	100
Glycerol	87	96
Sorbitol	77	67
i-Erythritol	56	41
L-Arabitol	30	24
Ribitol	20	15
Dulcitol	9	6

[a] The reaction mixture was the same as that described in Materials and Methods except that the D-arabinose concentration was 0.075 M and the inhibitor concentration was 0.10 M. Activity in the absence of any inhibitor was set to 100%.

there was no difference between the values obtained for the substrate alone and the substrate plus enzyme after 6 min of incubation) was observed with L-arabinose, L-rhamnose, D-xylose, D-ribose, D-glucose, L-glucose, D-mannose, L-mannose, D-galactose, or D-fucose.

Effect of divalent cations on enzyme activity. Both isomerases have been routinely assayed in the presence of Mn^{2+} ions (10). The results in Table 4 indicate that Co^{2+} ions as well as Mn^{2+} ions stimulated the activity of the enzymes. Other cations did not serve as activators, and Cd^{2+} ions inhibited the activity of both enzymes over 90% under these conditions.

Biophysical properties: determination of sedimentation coefficient. The effect of protein concentration on the sedimentation coefficient of the D-arabinose isomerase from *E.*

coli B/r was determined in 0.1% KCl in 0.01 M potassium phosphate buffer, *p*H 7.6. The sedimentation coefficient adjusted to the viscosity and density of water at 20 C and extrapolated to infinite dilution was 14.3×10^{-13} sec. There was no significant dependence of the sedimentation coefficient on protein concentration over the range tested. Sedimentation coefficients for the D-arabinose isomerase from *E. coli* K-12 were determined in an identical fashion. The $s_{20,w}$ was 14.5×10^{-13} sec with a significant dependence of the sedimentation coefficient on protein concentration (see Fig. 3).

Determination of molecular weight. The molecular weight of both proteins was determined at several protein concentrations by the high-speed sedimentation equilibrium method of Yphantis. The plots of the log of the fringe displacement versus the square of the distance from the axis of rotation for both proteins at several protein concentrations are shown in Fig. 4. The absence of any upward curvature is taken as evidence for the homogeneity of the samples. The average of the molecular weights, determined in this fashion, was $3.55 \pm 0.06 \times 10^5$ for the isomerase from *E. coli* K-12 and $3.42 \pm 0.04 \times 10^5$ for the isomerase from *E. coli* B/r.

TABLE 4. *Activation by divalent cations*[a]

Addition as the chloride[b]	Activity (%)	
	I-B/r	I-K-12
None	72	79
Mn^{2+}	100	100
Mg^{2+}	84	88
Ca^{2+}	78	81
Co^{2+}	150	174
Cd^{2+}	4	7

[a] The reaction mixture was the same as that described in Materials and Methods except that the cations listed above replaced the $MnCl_2$. The activity in the presence of Mn^{2+} was set to 100%.

[b] Added at 2.5 mM.

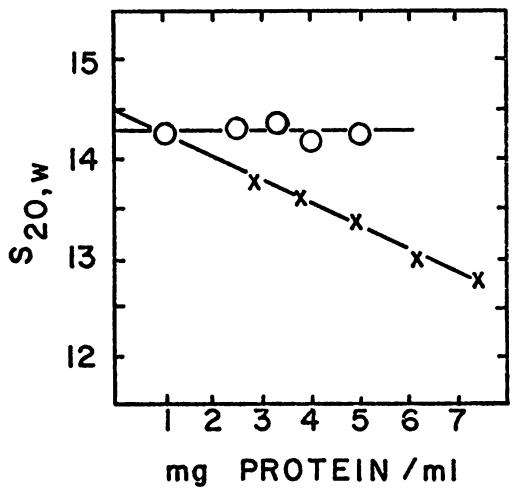

FIG. 3. *Effect of protein concentration on the sedimentation coefficient of the D-arabinose isomerase from E. coli B/r (O) and from E. coli K-12 (×). Sedimentation coefficients were determined as described in Materials and Methods. All runs were performed at pH 7.6.*

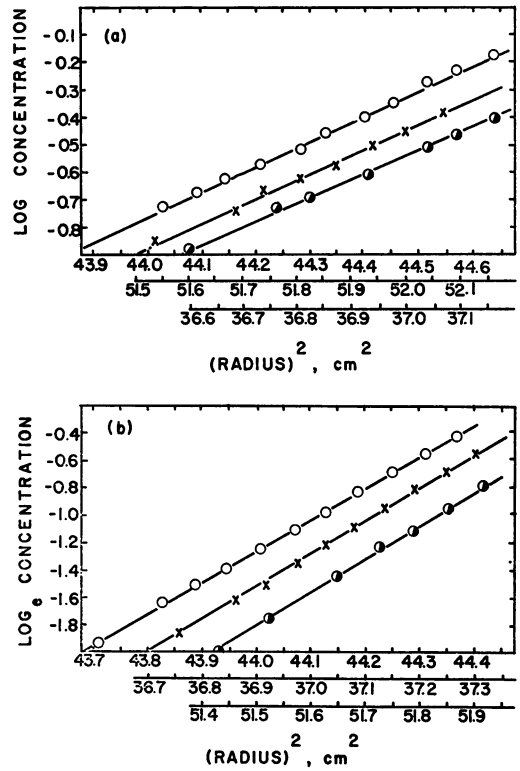

FIG. 4. *Determination of the molecular weight of the native enzymes by high-speed sedimentation equilibrium. The samples were prepared as described in Materials and Methods. The D-arabinose isomerase from E. coli B/r (a) was sedimented at 10,137 rev/min at 20 C in an An-J rotor. The data in this graph were taken from an interference photograph taken at 18.3 hr after reaching speed. Symbols: ×, 0.1 mg/ml, molecular weight 345,000; O, 0.2 mg/ml, molecular weight 345,000; ◑, 0.4 mg/ml, molecular weight 335,000. The isomerase from E. coli K-12 (b) was sedimented at 9,945 rev/min in an An-D rotor at 20 C. The data in this graph were obtained from an interference photograph taken 23.3 hr after reaching speed. Symbols: ◑, 0.25 mg/ml, molecular weight 386,000; O, 0.50 mg/ml, molecular weight 361,000; ×, 0.75 mg/ml, molecular weight 349,000. Both determinations were performed at pH 7.6 in. 0.01 M potassium phosphate buffer containing 0.1 M KCl.*

The molecular weight of the *p*H 2 dissociated enzymes was determined in a similar fashion (Fig. 5). The subunit molecular weight was 9.09 $\pm$ 0.2 $\times$ 10^4 for the enzyme from *E. coli* K-12 and 8.46 $\pm$ 0.1 $\times$ 10^4 for the enzyme from *E. coli* B/r. Thus, both enzymes appear to be oligomers of four identical subunits.

Electrophoretic studies. Disc electrophoresis in acrylamide gels was performed as described in Materials and Methods. Plain acrylamide gels, and gels containing SDS or urea, were run to compare the electrophoretic mobilities of the native enzymes and their dissociation products (see Fig. 2). Both of the native enzymes migrated as single bands on acrylamide gels (plain) and as single bands in gels containing SDS or urea. When the isomerases were run together, they migrated as a single band in plain, SDS, and urea gels. The native enzymes and their dissociation products appear to have the same electrophoretic mobility.

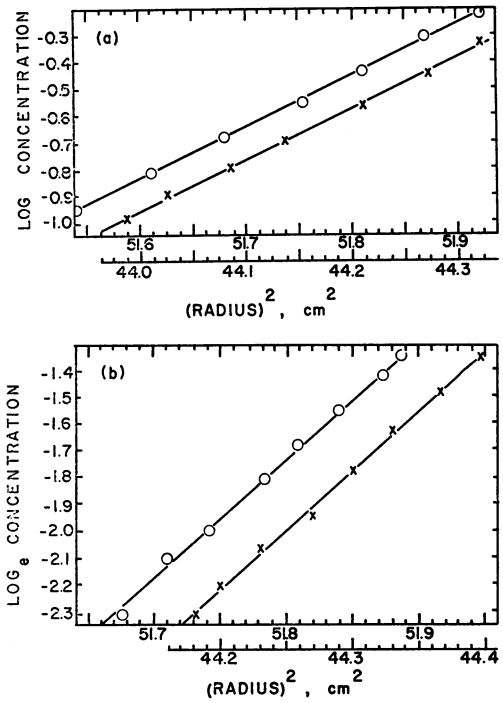

FIG. 5. *Determination of the molecular weight of the pH 2 dissociated enzymes by high-speed sedimentation equilibrium. The dissociated* D*-arabinose isomerase from E. coli B/r (a) was sedimented at 27,690 rev/min at 20 C in an An-D rotor. The data in this graph were taken from an interference photograph taken 5 hr after reaching speed. Symbols:* O, *0.2 mg/ml, molecular weight 85,700;* $\times$ *0.4 mg/ml, molecular weight 84,000. The dissociated* D*-arabinose isomerase from E. coli K-12 (b) was sedimented in an identical fashion. The data in this graph were obtained from an interference photograph taken 13 hr after reaching speed. Symbols:* O, *0.25 mg/ml, molecular weight 88,900;* $\times$, *0.50 mg/ml, molecular weight 92,900. Both samples were dialyzed against 0.05* M *potassium phosphate buffer, 0.1* M *KCl, plus 0.1* mM *dithiothreitol, pH 2.0, before use.*

Amino acid composition. A sample of the second Sephadex G-200 column pooled material of each isomerase was used for the acid hydrolysis: 1.0 mg per hydrolysis of the enzyme from *E. coli* K-12 and 0.97 mg per hydrolysis of the enzyme from *E. coli* B/r. The results of the time-dependent hydrolysis are presented in Tables 5 and 6. In Table 4, a comparison of the amino acid composition for three proteins (isomerase from *E. coli* B/r, isomerase from *E. coli* K-12, and L-arabinose isomerase from *E. coli* B/r) is presented. The micrograms found per hydrolysate have all been normalized with respect to the values obtained for the isomerase from *E. coli* B/r to facilitate comparison.

A comparison of the two isomerases studied yields a percent deviation of 3.7, suggesting, within experimental accuracy of the determinations, almost identical amino acid compositions for the two proteins. Another isomerase, the L-arabinose isomerase from *E. coli* B/r, was selected for comparison because it was functionally related to the two isomerases studied, and the L-arabinose isomerase molecular weight, 362,000, is similar to that of the two isomerases studied. A comparison of the isorase purified from *E. coli* B/r and the L-merase yields a percent deviation of 24.1, suggesting quite dissimilar amino acid compositions for the two proteins.

Antigenic studies. Agar diffusion studies and preparation of antisera were carried out as described in Materials and Methods. Antisera made against either isomerase would precipitate both enzyme preparations under the conditions described (see Fig. 6).

DISCUSSION

From the biochemical and biophysical data presented in this paper, it is clear that the D-arabinose isomerases purified from *E. coli* K-12 and *E. coli* B/r are extremely similar proteins. In the absence of data regarding their primary sequence, however, it is impossible to determine the exact extent of homology for the two proteins. The enzymes were isolated from two different strains of *E. coli*, and it is not unreasonable to assume that generic differences might account for single or even multiple amino acid differences in the native enzymes.

Although the conditions of the sedimentation velocity experiments were identical for both

TABLE 5. *Amino acid composition of* D-*arabinose isomerase from E. coli B/r*

Amino acid	16 hr	24 hr	48 hr	72 hr	96 hr	Corrected
Lysine	0.301[a]	0.308	0.310	0.310	0.306	0.307
Histidine	0.187	0.193	0.184	0.185	0.185	0.187
Ammonia	0.830	0.829	0.923	0.926	0.948	0.805
Arginine	0.381	0.373	0.374	0.366	0.385	0.376
Aspartic acid	0.731	0.704	0.753	0.762	0.760	0.742
Threonine	0.408	0.407	0.409	0.380	0.371	0.419
Serine	0.379	0.400	0.353	0.328	0.308	0.395
Glutamic acid	0.775	0.775	0.775	0.775	0.775	0.775
Proline	0.283	0.259	0.296	0.279	0.299	0.283
Glycine	0.657	0.662	0.661	0.666	0.659	0.661
Alanine	0.782	0.786	0.783	0.779	0.788	0.783
Half-cystine	0.034	0.037	0.077	0.058	0.047	0.051
Valine	0.429	0.425	0.466	0.472	0.475	0.475
Methionine	0.264	0.256	0.241	0.235	0.238	0.247
Isoleucine	0.341	0.360	0.405	0.413	0.417	0.416
Leucine	0.522	0.525	0.528	0.561	0.535	0.535
Tyrosine	0.199	0.200	0.204	0.212	0.210	0.205
Phenylalanine	0.204	0.204	0.211	0.220	0.220	0.212
Tryptophan	0.090	0.082	0.041	0.015	0.032	0.110

[a] All values in micromoles per hydrolysate, normalized with respect to glutamic acid. Corrected values were obtained by extrapolating serine, threonine, tyrosine, and ammonia to zero time of hydrolysis assuming first order kinetics. All other amino acids were averaged over all hydrolysis times.

TABLE 6. *Amino acid composition of* D-*arabinose isomerase from E. coli K-12*

Amino acid	16 hr	24 hr	48 hr	72 hr	96 hr	Corrected
Lysine	0.246[a]	0.246	0.252	0.254	0.258	0.251
Histidine	0.141	0.146	0.147	0.143	0.150	0.145
Ammonia	0.725	0.598	0.672	0.810	0.777	0.500
Arginine	0.308	0.324	0.311	0.315	0.326	0.317
Aspartic acid	0.568	0.573	0.568	0.586	0.587	0.576
Threonine	0.299	0.305	0.293	0.281	0.283	0.310
Serine	0.304	0.303	0.279	0.257	0.228	0.322
Glutamic acid	0.625	0.625	0.625	0.625	0.625	0.625
Proline	0.226	0.237	0.232	0.229	0.232	0.231
Glycine	0.533	0.531	0.532	0.548	0.548	0.539
Alanine	0.657	0.650	0.645	0.660	0.661	0.655
Half-cystine[b]	—	—	—	—	—	0.116
Valine	0.321	0.340	0.343	0.353	0.365	0.359
Methionine[b]	—	—	—	—	—	0.217
Isoleucine	0.282	0.308	0.330	0.329	0.335	0.331
Leucine	0.418	0.425	0.428	0.442	0.460	0.451
Tyrosine	0.164	0.166	0.164	0.166	—[c]	0.165
Phenylalanine	0.170	0.167	0.165	0.167	—[c]	0.167
Tryptophan	—	—	—	—	—	—

[a] All values, in micromoles per hydrolysate, normalized with respect to the average for glutamic acid, 0.625 μmoles per hydrolysate. Corrected values were obtained by extrapolating serine, ammonia, and threonine to zero time of hydrolysis assuming first order kinetics. Valine and leucine were obtained by averaging the 72- and 96-hr values; isoleucine, by averaging the 48-, 72-, and 96-hr values.

[b] Half-cystine and methionine were obtained by the determination of half-cystine as cysteic acid and of methionine as methionine sulfone by performic acid oxidation of purified isomerase.

[c] Not determined.

enzyme preparations, the D-arabinose isomerase from *E. coli* K-12 showed an appreciable concentration dependence of the sedimentation coefficient, whereas the D-arabinose isomerase from *E. coli* B/r showed no such effect. This, and other minor differences in the biophysical properties of the two enzyme preparations (molecular weight of the native enzyme and subunits, sedimentation coefficient), most probably are due to slight differences in the

amino acid composition affecting the secondary or tertiary structure of the isomerase molecules.

The catalytic properties of the D-arabinose isomerases from *E. coli* K-12 and *E. coli* B/r are virtually identical. There were no significant differences observed in substrate specificity, inhibitor specificity, cation activation, or K_m values for the two enzyme preparations. The catalytic properties of the D-arabinose isomerase indicate that it could function in the metabolism of L-fucose and D-arabinose in *E. coli* K-12 and *E. coli* B/r. This evidence suggests that it is some other aspect of metabolism (induction specificity, kinase specificity, an enzyme deficiency, etc.) which prevents these strains from being able to utilize both L-fucose and D-arabinose as sources of carbon and energy.

As part of their continuing studies on the metabolism of D-arabinose and other pentoses, Oliver and Mortlock (11) recently reported the purification of a D-arabinose isomerase from *A. aerogenes*. In virtually every respect, a comparison of the properties of the D-arabinose isomerase purified from *E. coli* K-12, *E. coli* B/r, and *A. aerogenes* demonstrates that they are, in fact, the same enzyme. All three enzymes have the same molecular weight (350,000), and all are active on both L-fucose and D-arabinose as substrates and exhibit similar K_m values with these pentoses. The ratios of activities (at v_{max}) with L-fucose and D-arabinose as substrates are 1:1 for all three enzymes, and all are stimulated by the presence of Mn^{2+} or Co^{2+} ions in the reaction mixture and are inhibited by ribitol (10). Furthermore, Table 7 presents evidence that demonstrates the striking similarity in amino acid composition for the D-arabinose isomerase from *E. coli* K-12 and *E. coli* B/r (similar data for the D-arabinose isomerase from *A. aerogenes* were not available).

Work in this laboratory is now in progress to establish the genetic and biochemical relationships between the metabolism of L-fucose and D-arabinose in *E. coli* K-12 and *E. coli* B/r. It

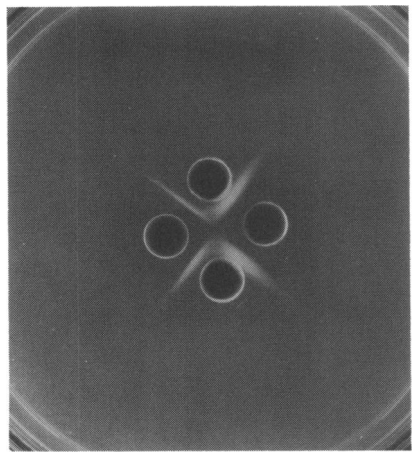

FIG. 6. *Agar diffusion studies were performed as described in Materials and Methods. In the bottom well is D-arabinose isomerase from E. coli B/r (125 μg) and in the top well is D-arabinose isomerase from E. coli K-12 (125 μg). The right well contains antiserum made against the isomerase from E. coli B/r; in the left well is antiserum made against the isomerase from E. coli K-12.*

TABLE 7. *Comparison of the amino acid composition for three proteins*[a]

Amino acid	I-B/r[b]	I-K-12[c]	LAI[c]	I-B/r / I-K-12	I-B/r / LAI
Aspartic acid	85.4	82.6	101.2	1.03	0.84
Threonine	42.4	39.1	50.4	1.08	0.84
Serine	34.4	35.0	22.8	0.98	1.51
Glutamic acid	100.0	100.8	113.5	0.99	0.88
Glycine	37.7	38.4	34.9	0.98	1.08
Alanine	55.7	58.1	46.4	0.96	1.20
Valine	47.1	44.5	52.4	1.06	0.90
Methionine[d]	—	—	—	—	—
Isoleucine	47.1	46.9	41.3	1.00	1.14
Leucine	60.6	63.8	85.0	0.95	0.71
Tyrosine	33.5	33.4	28.6	1.00	1.17
Phenylalanine	31.2	30.7	50.5	1.02	0.62
Half-cystine[d]	—	—	—	—	—
Lysine	39.3	40.3	46.1	0.98	0.85
Histidine	25.6	24.9	43.9	1.03	0.58
Arginine	58.7	61.9	57.5	0.95	1.02
Proline	27.5	28.2	30.5	0.98	0.90
Tryptophan[d]	—	—	—	—	—
Per cent average deviation[e]				3.7	24.1

[a] I-B/r = D-arabinose isomerase from *E. coli* B/r; I-K-12 = D-arabinose isomerase from *E. coli* K-12; LAI = L-arabinose isomerase.

[b] Micrograms found per hydrolysate.

[c] Micrograms found per hydrolysate for the LAI and I-K-12 samples have been normalized on a total weight basis to the values obtained for the D-arabinose isomerase from *E. coli* B/r.

[d] Methionine and half-cystine were calculated by different methods for each protein, and were not used in the comparison (methionine was calculated as the sulfone and cystine as cysteic acid for the I-K-12 and LAI samples). No basic hydrolysis was done on the I-K-12 or I-B/r samples, so the values for tryptophan have also been deleted.

[e] If the proteins in question were identical in terms of their amino acid composition, then the ratios I-B/r/I-K-12 and I-B/r/LAI should equal unity. The percent average deviation (from unity), then, is a measure of their dissimilarity.

appears that in bacterial systems it is not uncommon for the same, or similar, proteins to be produced by an organism, or organisms, grown on different carbon sources. In a variety of publications, the D-arabinose and L-fucose isomerase activities found in strains of *E. coli* and *A. aerogenes* have been called both an L-fucose isomerase and a D-arabinose isomerase. It has been suggested to us, and we agree, that the name D-arabinose isomerase and the International Union of Biochemistry number EC 5.3.1.3 be used for this enzyme activity to avoid further confusion of the literature.

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