D-Phosphoarabinoisomerase and D-Ribulokinase in Escherichia coli*

(Received for publication, April 13, 1966)

Ramon Lim† and Seymour S. Cohen

From the Department of Biochemistry and the Department of Therapeutic Research, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

SUMMARY

An enzyme, D-phosphoarabinoisomerase, which stoichiometrically interconverts D-arabinose 5-phosphate and D-ribulose 5-phosphate, was isolated from Escherichia coli. At equilibrium, 75% of D-arabinose-5-P and 25% of D-ribulose-5-P are found. This unstable enzyme does not show any requirement for added cofactor, and is inhibited by Mn++, Co++, Zn++, Cd++, p-chloromercuribenzoate, and phosphate. The optimum pH is 8.0. The Km is 1.36 x 10⁻³ M for D-arabinose-5-P and 5.40 x 10⁻⁴ M for D-ribulose-5-P. The enzyme lacks activity toward D-arabinose, D-ribose, and D-ribose-5-P. This enzyme not only accounts for the metabolic origin of the precursor for Z-keto-3-deoxy-octonate (KDO), a constituent in the cell wall lipopolysaccharide of E. coli and some other gram-negative bacteria, but also fits into the schema suggested for the utilization of D-arabinose-1-P generated in the metabolism of D-arabinosyl nucleosides. The enzyme is absent from yeast and mouse fibroblasts.

D-Arabinose-5-P was synthesized by phosphorylating D-glucosamine followed by ninhydrin degradation. The product was contaminated with D-ribulose-5-P and was purified by precipitation of the latter with borate.

D-Ribulose-5-P was obtained by phosphorylating D-ribulose with a D-ribulokinase isolated from E. coli. The product showed a distinct carbonyl absorption band in its infrared spectrum. Upon periodate oxidation, phosphoglycolaldehyde was detected as a major degradation product, as was expected for D-ribulose-5-P. However, this purified preparation of D-ribulokinase also phosphorylated L-fuculose at position 1.

We are reporting studies on the isolation and characterization of D-phosphoarabinoisomerase and D-ribulokinase derived from Escherichia coli. Investigations on the biochemistry of D-arabinosyl nucleosides as well as the distribution, biosynthetic origin, and metabolic fate of D-arabinose have recently been reviewed (1). A metabolic sequence from D-arabinose-5-phosphate to the insertion of KDO into the cell wall of certain gram-negative bacteria has been clarified by several groups of workers (2-5). The interrelationships of these compounds, which are discussed in this paper, are presented in the following schema.

\[
\begin{align*}
\text{D-Arabinose} & \xrightarrow{A} \text{D-arabinose-5-P} \xrightarrow{B} \text{D-arabinose-1-P} \xrightarrow{C} \text{ATP} \\
\text{D-Ribulose} & \xrightarrow{A} \text{D-arabinose-5-P} \xrightarrow{B} \text{D-arabinose-1-P} \xrightarrow{C} \text{ATP} \\
\text{Phosphoenolpyruvate} \xrightarrow{A} \text{D-arabinose-5-P} \xrightarrow{B} \text{D-arabinose-1-P} \xrightarrow{C} \text{ATP} \\
\end{align*}
\]

Reactions A, B, and C have been suggested as reactions leading to the formation of the D-arabinosyl nucleosides found in the sponge Cryptothyra crypta (6). Step C has been demonstrated in E. coli by Tono and Cohen (9). The same authors (9) also showed the conversion of the D-arabinose moiety of arabinosyl uracil to the ribose moiety of uridylate in intact E. coli. This finding provides indirect evidence for the entire pathway. In the present work, we have shown the existence of a D-phosphoarabinoisomerase in E. coli, thus establishing the validity of Step A in this organism.

Although the same isomerase has previously been studied in Propionibacterium pentosaceum (10), the physiological significance of this enzyme in the two species might be different. Thus D-arabinokinase (11) is present in P. pentosaceum but has not been detected in E. coli, whereas KDO is known in E. coli but has not been reported in P. pentosaceum.

* This investigation was supported by Grant E-3963 from the National Institute of Allergy and Infectious Diseases of the United States Public Health Service. The data in this paper are taken from a dissertation presented by Ramon Lim to the faculty of the Graduate School of Arts and Sciences of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

† United States Public Health Service Trainee. Present address, Section of Biochemistry, Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan.
In the process of synthesizing D-ribulose-5-P as a substrate for D-phosphoarabinosilomerase, we have isolated the induced enzyme, D-ribulokinase, from E. coli. Studies on this enzyme have also revealed interesting divergencies between the metabolism of D-ribulose and the structurally related L-fuculose.

**MATERIALS AND METHODS**

**Materials**—The authors gratefully acknowledge gifts of the following compounds: samples of D-arabinose-5-P (barium salt) from Dr. W. A. Volk and Dr. E. C. Heath; a solution of KDO from Dr. E. C. Heath; crystalline N-acetyl-neuraminic acid from Dr. M. C. Glick; a solution of D-xylulose from Dr. I. A. Rose; a solution of L-ribulose from Dr. N. Lee; phosphoglycolic acid (barium salt) from Dr. I. Piser. Glucose-hydrochloride was purchased from Calbiochem; the sample was free of materials reactive in the orcinol or the cysteine-carmozole reactions. D-Arabinose was a product of Pfanzehl. L-Fucose was purchased from Sigma. D-Ribulose and L-fuculose were synthesized enzymatically from D-arabinose and L-fucose, respectively, by the method of Cohen (12). They were stored as the nitrophenylhydrazones and were converted to the free sugars shortly before use (12). A mixed bed resin was purchased from Fisher as Rexyn IRG-501 (H-OH). The pH of the water effluent from this resin was 5.5. The RF values of both D-arabinose and D-ribulose standards were not altered after passing through the resin. DEAE-cellulose was obtained from Brown.

**Colorimetry**—Phosphate was determined by the method of King (13). The ninhydrin reaction (14) was employed for the detection of amino sugar. Protein was determined by the method of Lowry et al. (15) and, for convenience in following enzyme purification, by a spectrophotometric method (16).

Bial's orcinol reaction, with reagent concentration recommended by Miller, Goldar, and Miller (17) and a heating period of 40 min, was used for the determination of pentoses. The relative molar extinction coefficients (at 660 μm) of various sugars in the Bial reaction are: D-arabinose and its phosphate, 100%; D-ribose and its phosphate, 100%; D-ribulose and its phosphate, 50%; D-xylulose, 40%; KDO, 2%.

Warren's modification (18) of the thiobarbituric acid test was used for the determination of 2-keto-3-deoxyoctonate. In estimation of the KDO content in cellular fractions, the heating period was extended to 20 min to ensure complete reaction. The reaction mixture, after heating and cooling, was extracted with 8 ml of isomyl alcohol to remove most of the 2-deoxyribose color (19), and 3 ml of the aqueuos phase were then extracted with 4 ml of cyclohexanone as usual. With standards as control, isomyl alcohol extracted 78% of the deoxyribose color, whereas 91.5% of the sialic acid and KDO chromophore remained in the aqueous phase. A correction was therefore made for the KDO color removed by isomyl alcohol. In addition, Warren's formula (18) was used to correct for the residual deoxyribose color after extraction. Neither D-arabinose, D-ribose, or D-ribulose, nor their phosphates, give any color with the thiobarbituric acid test.

The cysteine-carmozole reaction of Dische and Borenfreund (20) was used for the assay of ketopenetoses. For free D-ribulose the mixture was allowed to react at room temperature for 15 min and was read in a Klett photometric colorimeter with a No. 540 filter. The absorption spectrum in the visible range of the reaction product and the rate of color development had been described by Cohen (12). While D-ribulose attains its maximum color intensity before 15 min and decreases slightly thereafter, D-xylulose gives 50% of its color at 15 min and reaches its maximal height (equal to that of D-ribose) only after 5 hours (21).

For D-ribulose-5-P the reaction mixture was immersed in a 50° water bath for 30 min, at which time the molar extinction coefficient of the reaction product was 50% of that obtained with D-ribulose subjected to the same treatment (Fig. 1). L-Fuculose produces a maximum color intensity after 2 hours at room temperature. D-Arabinose, D-arabinose-5-P, D-ribose, D-ribose-5-P, and KDO do not participate in the cysteine-carmozole reaction.

**Assay for D-Ribulokinase**—The assay system (1.0 ml) contained 3.0 mM D-ribulose, 50 mM glycylglycine buffer (pH 8.0), 6 mM ATP, 20 mM magnesium diacetate, 10 mM NaF (omitted for reactions with the purified enzyme), and the enzyme preparation. After incubation at 37°, 0.20-ml aliquots were mixed with 0.4 ml of 5% ZnSO₄·7H₂O, followed by 0.4 ml of 0.3 N NaOH (22, 23). The precipitate, containing the phosphorylated sugar, was removed by centrifugation, and the supernatant fluid was assayed for free D-ribulose by the cysteine-carmozole method. A less accurate but more convenient method consists of adding 0.8 ml of 0.1 N HCl to 0.2-ml aliquots of the incubation mixture and subjecting the solution to the cysteine-carmozole reaction at room temperature for 15 min. The decrease in color is due to the fact that D-ribulose-5-P gives only 11.5% of the free D-ribose color. One unit of D-ribulokinase was defined as that amount of enzyme which phosphorylates 0.1 μmole of D-ribulose per min.

**Assay for D-Phosphoarabinosilomerase**—The assay system (1.0 ml) contained 3 mM D-arabinose-5-P, 50 mM glycylglycine or Tris-HCl buffer (pH 8.0), 10 mM NaF (omitted for reactions with the purified enzyme), and the enzyme preparation. After incubation at 37°, 0.8 ml of 0.1 N HCl was added to 0.2-ml aliquots of the incubation mixture and the entire solution was subjected to the cysteine-carmozole reaction at 50° for 30 min. Unless stated otherwise, all values of arabinose-5-P and ribulose-5-P pertaining to this enzyme refer to the amount present in the 1-ml original incubation mixture. One unit of D-phosphoarabinosilomerase was defined as that amount of enzyme which converts 0.01 μmole of D-arabinose-5-P to D-ribulose-5-P per min.
Culture Methods and Preparation of Extracts—Two strains of bacteria were generally used, *E. coli* B and *E. coli* B415. The mutant strain B415 had been selected from strain B by passage on d-arabinose (12). Unlike the parent strain B, B415 is capable of relatively rapid growth on d-arabinose, exhibiting a chauvin growth in mineral medium containing glucose plus d-arabinose. Two enzymes, d-arabinonoisomerase and d-ribulokinase, are inducible by d-arabinose on B415. The bacteria were grown in a mineral medium (24) supplemented with the desired sugars. For *E. coli* strain B, 3 mg of glucose were added per ml, and the cells were grown for 15 hours and harvested in the stationary phase. For *E. coli* strain B415, 0.5 mg of glucose and 1.5 mg of d-arabinose were added per ml, and the growth was followed up to the middle of the second logarithmic phase.

*E. coli* 15 TAU, a thymineless, arginineless, and uracil-less mutant, was grown in the mineral medium containing the following additions per ml: 0.016 μmole of thymine, 0.1 μmole of arginine, 0.1 μmole of uracil, and 1 mg of glucose (or 0.9 mg of glucuronate plus 0.1 mg of glucose).3

The yeast culture used was the *Candida utilis* (*Torulopsis utilis*), strain V-900, obtained from the American Type Culture Collection. The organisms were grown in the medium described by Lewis (25) at 28°C with constant shaking for 18 hours.

The *E. coli* and the yeast cells were harvested by centrifugation and with washed with 0.85% NaCl solution. The cells were suspended in 10 ml of 0.06 M Tris-HCl, pH 7.5, per g of pellet, weight wet, and were disrupted through a French pressure cell at 6,000 p.s.i. Extracts were obtained by removing the cellular debris by centrifugation at 14,000 × g for 30 min.

Mouse fibroblasts (L cells) were grown in suspension up to 300,000 cells per ml in a medium modified from the minimum essential medium of Eagle (26). The cells were centrifuged at 4°C and washed with cold buffer containing 0.1 M KCl and 0.02 M Tris-HCl, pH 7.4. The pellet was suspended in 0.05 M Tris-HCl, pH 7.5, and treated with a 5.9-kc Raytheon sonic oscillator, model R-223, for 10 min at 4°C. Cellular debris was removed by centrifugation at 1,200 × g for 15 min.

Enzyme Reagents—Prostatic acid phosphatase was generously supplied by Dr. G. Schmidt (27) and has been stored at −20°C for several years. One millilitre of this preparation contained less than 0.06 μmole of pentose (as arabinose) and less than 0.05 μmole of ketopentose or ketohexose. Each millilitre dephosphorylated 400 μmoles of sugar phosphate per hour at 37°C in 0.1 M sodium acetate buffer, pH 5.

D-Arabinonoisomerase was isolated by a slight modification of the method of Cohen (12). The nucleic acids were precipitated from the extract with 0.3 volume of 5% streptomycin sulfate. The 50 to 75% (NH₄)₂SO₄ fraction was dialyzed overnight against 100 volumes of water at 4°C before use. The enzyme thus prepared did not interfere with the orcinol or the cysteine-carbazole color. With pure D-arabinose or D-ribulose as substrate, 10 to 2% of D-ribulose remained at equilibrium when incubated in 0.1 or 0.05 M glycylglycine buffer, pH 8.0, at 37°C. This enzyme was assayed in 0.1 M sodium borate, pH 5.0, with 3.0 mM D-arabinose or L-fucose, at 37°C; a 0.2-ml aliquot of the incubation mixture was added to 0.5 ml of 0.1 N HCl and the mixture was subjected to the cysteine-carbazole test at room temperature.

EXPERIMENTAL DATA AND RESULTS

**Synthesis and Purification of D-Arabinose-5-P**

D-Glucosamine (24.6 mmoles) was phosphorylated with hexokinase and ATP according to the method of Jourdain and Rosenman (28). The purified D-glucosamine-6-P (97% yield) had a nitrogen to phosphorus ratio of 0.95. This product was degraded with ninhydrin by the method of Volk (29), and 13.8 mmoles of D-arabinose-5-P were isolated after adsorption and elution from Dowex 1-formate and precipitation of the barium salt with 80% ethanol. The ratio of D-arabinose to phosphorus was 0.95. The inorganic phosphate was less than 1% of the total.

However, the D-arabinose-5-P thus synthesized gave a strong cysteine-carbazole color (15 min at room temperature) after treatment with acid phosphatase, and the ratio of color intensity before and after dephosphorylation was identical with that of D-ribulose-5-P. The result was the same whether the enzyme was Polidase-Schwarz or the prostatic phosphatase. When the dephosphorylated compound was incubated with D-arabinonoisomerase, the material reactive in the cysteine-carbazole test entered into equilibrium with D-arabinose. It was therefore concluded that the contaminant in the D-arabinose-5-P was D-ribulose 5-P. It amounted to 15.1% of the total pentose phosphate (molar basis). Batches of D-arabinose-5-P from other laboratories, which were also prepared by the inohydrin degradation of D-glucosamine-6-P, contained up to 20.4% D-ribulose-5-P when analyzed as described above.

Although D-ribulose-5-P is easily separable from D-ribose-5-P by Dowex 1-formate column (30), several attempts with this method resulted in very little separation between D-arabinose-5-P and the contaminating D-ribulose-5-P. With a borate column (31), a very large amount of borate buffer was needed to elute the sugar phosphates, resulting in excessive dilution of the eluted materials.

We had observed that when the barium salt of a sugar phosphate is mixed with a concentrated borate solution, the resulting precipitate of barium borate contains some of the sugar phosphate. D-Ribulose-5-P, containing cis-hydroxyls, is precipitated to a greater extent than is D-arabinose-5-P, which contains only trans-hydroxyl groups. This selective precipitation was used to separate D-ribulose-5-P from D-arabinose-5-P, and the optimal concentration and volume of the borate solution for a given amount of sugar phosphate were determined empirically.

The barium salt (1 mmole of D-arabinose-5-P) was suspended in 12.5 ml of water. To this were added 12.5 ml of 0.1 M sodium tetraborate, pH 9. The suspension was stirred for 5 min at room temperature. After it had been chilled in ice for 10 min, the white suspension was centrifuged at 2000 rpm at 4°C for 15 min, and the pellet was discarded. The supernatant, containing 580 μmoles of D-arabinose-5-P with 2.2% D-ribulose-5-P, was passed through a Dowex 50-H⁺ column (25-ml bed volume) and washed through with 25 ml of water. Pentose phosphate recovery was complete. The pH was adjusted to about 2.5 with 0.5 N NaOH. Boric acid
Phosphoarabinoisomerase in E. coli.

Isolation of D-Ribulokinase—An enzyme which phosphorylates D-ribulose at some undetermined position had previously been detected in this laboratory (12) in E. coli B44 grown on D-arabinose. In the present work this enzyme has been purified in order to synthesize a possible substrate for the study of D-phosphoribosinoisomerase. In the isolation of D-ribulokinase and other enzymes, the procedures were carried out at 4° with glass-distilled water.

A cell-free extract was prepared from 18 g of E. coli B44 cells, wet weight, which were grown on D-arabinose. After dialysis for 2 hours against 50 volumes of 0.002 M Tris-HCl, pH 7.5, 0.3 volume of 5% streptomycin sulfate in water was added slowly with rapid stirring. The mixture was stirred for an additional 5 min, and the white precipitate was removed by centrifugation. One volume of saturated (NH₄)₂SO₄, pH 7.5, was added slowly with stirring. After standing for 6 hours, the mixture was centrifuged at 14,000 × g for 30 min and the pellet was taken up in 21 ml of 0.002 M Tris-HCl, pH 7.5. This fraction (50% saturated ammonium sulfate precipitate) was free from D-arabinosioisomerase but was contaminated with D-phosphoribosinoisomerase and D-phosphoribosilomerase. The solution was made 0.01 M with respect to magnesium acetate and 5 × 10⁻⁴ M with respect to D-ribulose. The residual (NH₄)₂SO₄ was removed by passage through a Sephadex G-25 column, 4.5 × 16 cm, in 0.002 M Tris-HCl buffer, pH 7.5. The enzyme solution was then applied at 1 ml per min to a DEAE-cellulose column, 2.9 × 21.5 cm which had been equilibrated with 0.01 M Tris-HCl, pH 7.5. A batchwise elution was effected with the following solutions in succession: 150 ml of 0.01 M Tris-HCl, pH 7.5 (2 ml per min); 100 ml of the same buffer with 0.03 M NaCl (2 ml per min); 300 ml of the buffer with 0.1 M NaCl (2 ml per min); 500 ml of the buffer with 0.5 M NaCl (4 ml per min). The D-ribulokinase was eluted with the 0.5 M NaCl solution. The peak fraction contained only a trace of D-phosphoribosinoisomerase but was rich in D-phosphoribosilomerase. The enzyme was concentrated by (NH₄)₂SO₄ precipitation at 50% saturation and was resuspended in 6 ml of 0.005 M sodium glycylglycine buffer, pH 7.5. This solution was made 0.01 M with respect to magnesium acetate and 5 × 10⁻⁴ M with respect to D-ribulose, and was applied to a Sephadex G-100 column, 4.5 × 67 cm. The column was treated with 0.005 M sodium glycylglycine containing 0.001 M magnesium acetate, pH 7.5. The elution was effected with the same buffer at a rate of 0.5 ml per min. Fractions of 5 ml were collected and D-ribulokinase was detected in Fractions 65 to 85. This was preceded by the main protein peak and was followed by the D-phosphoribosinoisomerase peak. D-Phosphoribosinoisomerase, D-phosphoribosilomerase, and phosphatase were not detected in the fractions containing D-ribulokinase. Fractions 70 through 75, containing 346 units of enzyme, were pooled and made 0.01 M sodium glycylglycine containing 0.001 M magnesium acetate, pH 7.5. The elution was effected with the same buffer at a rate of 0.5 ml per min. Fractions of 5 ml were collected and D-ribulokinase was detected in Fractions 65 to 85. This was preceded by the main protein peak and was followed by the D-phosphoribosinoisomerase peak. D-Phosphoribosinoisomerase, D-phosphoribosilomerase, and phosphatase were not detected in the fractions containing D-ribulokinase. Fractions 70 through 75, containing 346 units of enzyme, were pooled and made 0.01 M with respect to magnesium acetate and 0.001 M with respect to D-ribulose. The procedure is summarized in Table 1 and Fig. 2.

Assayed by the Zn⁺⁺-Ba⁺⁺ method described for D-ribulokinase, the purified enzyme exhibited the following relative specificity: L-fuculose, 100%; D-ribulose, 42%; D-xylulose, 38%; L-ribulose (a 4-year-old solution stored at −20°), 0%; D-arabinose, 0%; D-ribose, 0%. One-half of the activity with respect to D-ribulose was lost after storage at −20° for 3 weeks.

Phosphorylation of D-Ribulose—ATP, 3 mmoles, was dissolved in 40 ml of water and the pH was adjusted to about 6.5. To this were added 1.2 mmoles of glycylglycine buffer (pH 8.0), 2.4

<table>
<thead>
<tr>
<th>Step</th>
<th>Total volume</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>135</td>
<td>2510</td>
<td>units</td>
<td>units/mg</td>
<td></td>
</tr>
<tr>
<td>Dialysis</td>
<td>135</td>
<td>2510</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>166</td>
<td>630</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>21</td>
<td>368</td>
<td>2350</td>
<td>6.4</td>
<td>1.00</td>
</tr>
<tr>
<td>Sephadex G-25</td>
<td>73</td>
<td>364</td>
<td>2620</td>
<td>7.2</td>
<td>1.12</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>40</td>
<td>200</td>
<td>2000</td>
<td>10.0</td>
<td>1.56</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>6</td>
<td>346</td>
<td></td>
<td>24.0</td>
<td>3.75</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>32</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Because of the presence of interfering enzymes, it was difficult to determine accurately the total amount of D-ribulokinase in the extract before the ammonium sulfate step.*
mmoles of magnesium acetate, and 3.0 mmoles of n-ribulose. The pH was adjusted to 7.5 with 5.5 N NaOH and the total volume was diluted to 95 ml. The purified d-ribulokinase (270 total units) in 25 ml was added, and the whole mixture was incubated in a 37° water bath with intermittent stirring; the pH was maintained at 7.5 by the addition of 0.5 N NaOH and 27 ml of 25% barium acetate were added. The solution was cooled to 4° and 8 ml of cold 50% trichloracetic acid were added. The pH dropped to 1.5. After removal of most of the protein precipitate by a brief centrifugation, the pH was adjusted to 3.5, and the mixture was centrifuged in the cold to remove all the precipitate. Finally, the cold solution was brought to pH 7.0 with 5 N NaOH, and 27 ml of 25% barium acetate were added. The precipitate, which consisted mostly of barium salts of ATP and ADP, was removed and 1 volume of cold 95% ethanol was added. After standing at 4° for 30 min, the white precipitate was centrifuged, washed with cold 80% and absolute ethanol, and dried in a vacuum over P2O5 in a current of warm air, the paper was sprayed with ammonium molybdate reagent (41) at 2 ml per 100 sq cm, dried at 85° for 2 hours (descending) in the tert-butyl alcohol-water-picric acid system (41). After drying in a current of warm air, the paper was irradiated with ultraviolet light (42). The color of picric acid disappeared upon spraying, although inorganic phosphate remained yellow. The results are shown in Table II. It should be noted that the LiCl eluent, consisting of the orcinol peak, were pooled. The pH was adjusted to 7 with NaOH, and 0.15 ml of 25% barium acetate and 4 volumes of cold absolute ethanol were added. After standing at 4° for 30 min, the white precipitate was centrifuged, washed with 95% and absolute ethanol, and dried over P2O5 in a vacuum at 4°. It is important to carry out the entire procedure rather rapidly, because both acidic and basic conditions are detrimental to d-ribulose-5-P.

The procedure described above yielded 40.4 μmoles of barium d-ribulose phosphate (determined after dephosphorylation). The d-ribulose to phosphorus ratio was 1.01. The inorganic phosphate was less than 1.0% of the total phosphate. At 100° in 1 N HCl, 23% and 75.5% of the organic phosphate were liberated after 7 min and 1 hour, respectively. The nucleotide content as determined by the ultraviolet absorption was less than 0.3% of the d-ribulose on a molar basis. In the cysteine-carbazole reaction at 50°, the color at 30 min was 50.7% that of free d-ribulose; the maximum absorbance was at 540 μA. The A540: A660 ratios in Bial's orcinol reaction were 0.40 and 0.76 before and after dephosphorylation, respectively (see Fig. 7). In a simultaneous determination, the corresponding value for the free d-ribulose standard freshly generated from the hydrazine was 0.79 (the ratio varies slightly with each determination with the batch of reagents used). At 600 μA in a Klett colorimeter, the Bial color intensity of the synthesized compound, either before or after dephosphorylation, was 40% that of free d-arabinose. The corresponding value for the free d-ribulose standard was 50%.

Various methods of characterization established the sugar moiety to be d-ribulose, free of d-ribose, d-arabinose, d-xylulose, or fructose. The data of such determinations are included in the section "Identification of Products" under "d-Phosphoarabinosimerase in E. coli."

**Position of Phosphate**—It was clear that the purified compound was a d-ribulose monophosphate. It was of particular importance to rule out d-ribulose-1-P because an enzyme from another strain of E. coli showing a similar substrate specificity had been found to phosphorylate L-fuculose at carbon 1 (38). d-Ribulose-5-P should be differentiable from d-ribulose-1-P by peridate oxidation. For d-ribulose-5-P, 2 moles of periodate should be consumed per mole of sugar (39), with formation of phosphoglycolaldehyde; for d-ribulose-1-P, 3 moles of periodate should be used, with formation of phosphoglycolic acid.

Periodate oxidation was followed by a spectrophotometric method (40). One mole of the purified d-ribulose monophosphate was found to consume 1.7 moles of periodate in the first 10 min, and a total of 2.0 moles were consumed in 2 hours; thereafter very little increase was noticed.

To identify the oxidation products, 1 μmole of the d-ribulose phosphate (sodium salt) and 2.5 μmoles of NaI04 in a total volume of 0.35 ml were allowed to react at room temperature in the dark. After oxidation the solution was treated with excess Dowex 50-H+ and it was filtered. The filtrate was applied to Whatman No. 1 paper (previously washed with 0.1 N HCl and water) and run for 90 min (descending) in the tert-butyl alcohol-water-picric acid system (41). After drying in a current of warm air, the paper was sprayed with ammonium molybdate reagent (41) at 2 ml per 100 sq cm, dried at 85° for 1.5 min, and irradiated with ultraviolet light (42). The color of picric acid disappeared upon spraying, although inorganic phosphate remained yellow. The results are shown in Table II. It should be
noted that the findings were essentially the same whether the oxidation was carried out for 5 hours or 25 hours.

In order to determine quantitatively the periodate oxidation products, 7 μmoles of the d-ribulose phosphate (sodium salt) were oxidized as described above, and the areas corresponding to phosphoglycolaldehyde and phosphoglycolic acid were cut out from the chromatogram and eluted for total phosphorus determination. The yellow color due to picric acid in the eluant completely disappeared after digestion with perchloric acid. The phosphate recovery was 96%, of which 90% was in phosphoglycolaldehyde; 10% was in the region of phosphoglycolic acid. Corrections were applied for this 10% contaminant in the results of the enzymatic studies.

Other evidence in support of the conclusion that the compound was d-ribulose-5-P was provided by infrared spectroscopy. Since d-ribulose-5-P exists in the open chain form while d-ribulose-1-P is in the furanose form, a carbonyl absorption band should be indicative of the former compound. Indeed, the infrared spectrum of the d-ribulose phosphate (Fig. 3) showed a band at 1725 cm⁻¹, which is in agreement with the stretching of an acyclic carbonyl group with adjacent electronegative groups. For comparison, a similar band was detectable in dihydroxyacetone as its phosphate, but not in d-fructose, d-fructose-6-P, d-arabinose, fuculose-1-P, or d-arabinose-5-P. Thus the compound synthesized enzymatically was established to be mainly d-ribulose-5-P. It was converted to the sodium salt for enzymatic studies.

Additional Studies on D-Ribulokinase

The following experiments were aimed at comparing the utilization of d-ribulose and L-fuculose together with their corresponding aldoses in E. coli B41 grown on either d-arabinose or L-fucose. E. coli B41 was grown in the mineral medium containing one of the following sugar combinations: (a) 0.5 mg of glucose and 1.5 mg of d-arabinose per ml, (b) 0.5 mg of glucose and 1.5 mg of L-fucose per ml, or (c) 2 mg of glucose per ml. The growth rate of the organism in fucose was found to be one-half of what had been reported previously (44). The three cultures were harvested in the exponential phase at a Klett reading of 230 to 250; crude extracts were prepared as usual, and some of these were partially purified up to the first (NH₄)₂SO₄ step.

Assay of Induced Enzymes—The induced enzymes were assayed with different substrates and the results are shown in Table III. In agreement with the observation of Green and Cohen (44), the activity of d-arabinosimerase toward L-fucose is 2.5 times that toward d-arabinose, although d-arabinose is a better inducer. D-Ribulokinase is present in about the same amount (with d-ribulose as substrate) in L-fucose-grown and in d-arabinose-grown cells, but the activity toward L-fucose was found to be low; only one-third that on d-ribulose in d-arabinose-grown cells and one-fourth in L-fucose-grown cells.

Although it is difficult to extrapolate from the enzyme activity of the soluble extract to internal activity of the cells, the values in the table suggest that in the bacteria grown on d-arabinose, d-arabinosimerase is more limiting for growth than is d-ribulokinase. On the other hand, in the bacteria grown on L-fucose, the kinase seems to be more limiting than is the isomerase. In neither case has an excess of either enzyme in terms of the utilization of substrate during growth been detected in the extracts.

**Table II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf</th>
<th>Color</th>
<th>Time of color appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Ribulose phosphate (not oxidized)</td>
<td>0.50</td>
<td>Blue</td>
<td>After heating, before radiation</td>
</tr>
<tr>
<td>Phosphoglycolaldehyde</td>
<td>0.41</td>
<td>Blue</td>
<td>After radiation</td>
</tr>
<tr>
<td>D-Ribulose phosphate (oxidized)</td>
<td>0.44</td>
<td>Blue</td>
<td>After radiation</td>
</tr>
<tr>
<td>Phosphoglycolic acid</td>
<td>0.67</td>
<td>Blue</td>
<td>After radiation</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>0.61</td>
<td>Yellow</td>
<td>After spraying, before heating</td>
</tr>
</tbody>
</table>

*All solutions were adjusted to pH 2 with Dowex 50-H⁺ before application. Phosphoglycolaldehyde was generated from the diethylacetal dicyclohexylammonium salt according to Ballou (43).*  
*4 A very faint blue spot also appeared at Rf 0.61 (after ultraviolet irradiation).*

**代谢—**Each of the partially purified enzyme preparations was allowed to phosphorylate d-ribulose and L-fuculose, under the conditions described for the synthesis of d-ribulose-5-P. The reactions were terminated by deproteinization with 1 volume of absolute ethanol at room temperature. The sugar phosphates were isolated as dry barium salts as described above. The dried products were characterized by periodate oxidation as follows. One micromole of sugar phosphate (barium salt) suspended in 0.1 ml of water was mixed with 0.1 ml of 0.1 M NaOH (10 μmoles) and was allowed to stand in the dark at room temperature for 24 hours. Insoluble materials were removed by centrifugation. After treatment with dry Dowex 50-H⁺ to pH 2, the filtrates were analyzed by paper chromatography in tert-butyl alcohol-picric acid as described previously. While the oxidation products of d-ribulose phosphate consisted of about 90% phosphoglycolaldehyde and 10% phosphoglycolic acid, those of L-fuculose phosphate consisted mainly of phosphoglycolic acid and a faint trace (about 10%) of a slowly moving component extending from the region of Rf 0.20 to Rf 0.59. Although standard phosphohalacetaldheyde, the periodate oxidation product of L-fuculose-5-P, was not available for comparison, the faint trace of unidentified phosphate material appears to be derived from contaminating nucleotides rather than from L-fuculose-5-P. No carbonyl absorption band (less than 10% of that in d-ribulose-5-P) can be detected in the infrared spectrum of the L-fuculose phosphate (Fig. 3). In addition, in all the phosphorylated d-ribulose isolated at three different periods of incubation (at 35% phosphorylation, at 100% phosphorylation, and at twice the time needed for the complete phosphorylation), there was no significant change in the ratio of phosphoglycolic acid to phosphoglycolaldehyde (about 1:10 by visual comparison) upon periodate oxidation.
FIG. 3. Infrared spectra of L-arabinose-P, L-ribulose-5-P, L-fuculose-1-P, and standards. Each of the following samples was mixed with 300 mg of KBr, and the disk was prepared in the usual manner. 1, 1.08 mg of L-ribose; 2, 1.0 mg of L-ribose-5-P (sodium); 3, 1.12 mg of L-arabinose-5-P (barium); 4, 1.00 mg of L-ribulose-5-P (barium); 5, 1.0 mg of L-fructose-6-P (barium); 6, 1.1 mg of L-fructose; 7, 1.03 mg of deoxyribose; 8, 2.25 mg of barium deoxyribose-5-P converted to the sodium salt; 9, 1.22 mg of oxidation. These results were the same whether the enzyme preparation used was obtained from L-arabinose-grown or from L-fucose-grown cells. The evidence, therefore, indicates that both L-arabinose and L-fucose induce an enzyme system in E. coli B:\(^-\) which phosphorylates L-ribose at position 5 and L-fucose at position 1.

**d-Phosphoarabinoisomerase in E. coli**

**Growth Conditions and Enzymatic Activity**—The activity of this enzyme in E. coli was low (less than 10% of the activity of d-phosphoriboisomerase in the same bacteria), and was only about 9% that of the d-phosphoarabinoisomerase reported in P. pentosaceum. d-Phosphoarabinoisomerase was found in equal amounts in E. coli and in its d-arabinose-utilizing mutant, E. coli B:\(^-\). In the latter strain, the enzyme levels were similar when the bacteria were grown on glucose or on d-arabinose. In the case of E. coli B, there was a slight increase in total activity as the cells entered the stationary phase, although the specific activity did not change significantly in the different phases of growth. For convenience, therefore, E. coli B grown overnight for 15 hours was used for the preparation of extracts.

A comparison of the enzyme level has also been made between
gluconate-grown and glucose-grown cells, with E. coli 15 TAU, and no difference has been noted. In this strain the specific activity of the enzyme appears higher than that in E. coli B.

**Fate of D-Arabinose-5-P in Crude Extracts**—Extracts of E. coli B, prepared from cells grown on glucose, were incubated with 3.2 mm D-arabinose-5-P at 37° in the presence of one of the following buffers at pH 8.0: 0.05 M Tris-HCl; 0.05 M Tris-HCl with 0.01 M NaF; 0.1 M sodium borate; 0.1 M sodium borate with 0.01 M NaF. Aliquots were removed at intervals and were subjected directly to the cysteine-carbazole reaction. Also, supernatant fluids after precipitation by 5% trichloroacetic acid were assayed by the Bial reaction, thiobarbituric acid test, and inorganic phosphate method.

The results, expressed for 1 ml of incubation mixture, are plotted in Fig. 4. Material reactive in the cysteine-carbazole test is plotted as D-ribose-5-P, and orcinol-reactive material as D-arabinose-5-P, without correction for other compounds taking part in the color reactions. A decrease in orcinol-reactive material and a transient increase in cysteine-carbazole-reactive material were detected upon incubating D-arabinose-5-P with the crude extract. The cysteine-carbazole peak could not be explained by the possible dephosphorylation of the substrate with subsequent isomerization into free D-ribose, since in a control experiment the activity of D-arabinose isomerase in the same E. coli B extract acting on the same amount of free D-arabinose could account for no more than 2% of such color changes. It was therefore inferred that a direct conversion of D-arabinose-5-P to D-ribose-5-P must have occurred.

With crude extracts, material reactive with cysteine-carbazole could be trapped in borate buffer although borate reduced the initial rate of conversion (Fig. 4). The trapping was expected (31) because cis-hydroxy groups exist in D-ribose-5-P and not in D-arabinose-5-P. The inhibitory effect increased with borate concentration, but trapping was optimal only at 0.1 M and pH 8.0. Among the carbonyl reagents tested (hydrazine, hydroxylamine, and sodium bisulfite), at pH 8.0 and at various concentrations, the inhibition of rate was much more pronounced than the trapping effect, when compared with borate.

The extract was rich in KDO-8-P synthetase. Even without exogenous phosphoenolpyruvate, a significant amount of KDO-8-P was formed from D-arabinose-5-P (Fig. 4). The synthesis of KDO-8-P from D-arabinose-5-P in the crude extract could be enhanced by adding phosphoenolpyruvate. Synthesis of KDO was reduced by adding NaF, which was inhibitory to endolase. There was evidence of phosphatase activity, as the liberation of inorganic phosphate from phosphoenolpyruvate by KDO-8-P synthetase could not account for all the inorganic phosphate detected. The phosphatase was also inhibited by NaF (Fig. 4).

D-Phosphoarabinisomerase was unstable in the crude extract. One-half of the activity was lost by storage at 4° for 2 days, and two-thirds at -20° for 1 week. Heating at 60° for 1 min completely inactivated the enzyme. The enzyme was inhibited by phosphate at 0.025 M or higher. This inhibition was reversible by dialysis.

**D-Arabinose-5-P and KDO Content**—Phosphoarabinisomerase, KDO-8-P synthetase, and KDO content were studied in extracts of E. coli at different stages of growth. E. coli B, grown in the mineral medium containing 3 mg of glucose per ml, was harvested at different time intervals and the extracts were obtained after centrifuging the disrupted cells at 14,000 X g for 30 min. Enzyme assays were made on the supernatant fluids. KDO content was determined both on the supernatant fluid and on the pellet after hydrolysis at pH 2 for 1 hour.

A slight increase in activity for both enzymes was noted as the culture approached the stationary phase. The activity of D-phosphoarabinisomerase appeared to be slightly higher than that of the synthetase, under the assay conditions. In view of the high enzyme level of the D-phosphoarabinisomerase and the irreversible nature of the synthetase reaction, it seems unlikely that large amounts of D-arabinose-5-P would accumulate at normal conditions. The KDO content in the cell wall also increased slightly with aging. At any stage of the development of the culture, the KDO-8-P synthetase and the D-phosphoarabinosomerase activities in the bacteria under the experimental condition were enough to account for all the KDO present in the entire cell in a matter of 2 to 3 min.

**Table III**

Comparison of induced enzymes of E. coli B<sub>15</sub>

The values represent micromoles of substrate reacted per ml of extract per min. The extracts were prepared, with 10 ml of buffer per g of bacteria, wet weight, from E. coli B<sub>15</sub> grown on three different carbon sources. The protein content of the extracts was 14 mg per ml.

<table>
<thead>
<tr>
<th>Cells grown on</th>
<th>D-Arabinosiose/</th>
<th>D-Ribulokinase/</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Fucose</td>
<td>0.40</td>
<td>0.91</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>0.60</td>
<td>1.30</td>
</tr>
<tr>
<td>Glucose</td>
<td>&lt;0.01</td>
<td>&lt;0.06</td>
</tr>
</tbody>
</table>

*a* Assayed in 0.1 M borate, pH 8, with crude extracts.

*b* Assayed with 50% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitated fractions (free of D-arabinosiose), and the results calculated for the crude extract, since very little ribulokinase is present in the 50% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> supernatant (M. Green and S. S. Cohen, unpublished data). The glucose-grown extract, however, was assayed without preliminary treatment. The Zn<sup>++</sup>-Ba<sup>++</sup> precipitation method was used for the assay.

![Fig. 4. Fate of D-arabinose-5-P in the crude extract, showing the formation of different products in various buffer systems.](http://www.jbc.org/issue/issue10)
TABLE IV

Purification of D-phosphoarabinoinosomerase

<table>
<thead>
<tr>
<th>Step</th>
<th>Total volume</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Purifi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>135ml</td>
<td>2510mg</td>
<td>2969 units</td>
<td>1.18 units/mg</td>
<td>1.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>170ml</td>
<td>2710mg</td>
<td>2692 units</td>
<td>1.08 units/mg</td>
<td>1.0</td>
</tr>
<tr>
<td>Dialysis</td>
<td>168ml</td>
<td>1380mg</td>
<td>1521 units</td>
<td>1.14 units/mg</td>
<td>1.0</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>22ml</td>
<td>307mg</td>
<td>325 units</td>
<td>1.5 units/mg</td>
<td>1.5</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>80ml</td>
<td>107mg</td>
<td>112 units</td>
<td>0.9 units/mg</td>
<td>0.9</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>1.5ml</td>
<td>101mg</td>
<td>104 units</td>
<td>0.9 units/mg</td>
<td>0.9</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>7.5ml</td>
<td>12mg</td>
<td>14 units</td>
<td>1.2 units/mg</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Fig. 5. Purification of D-phosphoarabinoinosomerase on DEAE-cellulose (A) and Sephadex G-100 (B) columns. Fraction volume: A, 10 ml per tube; B, 2.5 ml per tube. The unit for D-phosphorabinosomerase is defined in the same way as for D-phosphoarabinosomerase.

Purification of Enzyme—The D-phosphoarabinoinosomerase of E. coli B is insufficiently stable to withstand numerous fractionation procedures. However, the purification of this enzyme could be carried out in E. coli B₄₁ grown on D-arabinose. The D-ribulose-5-P generated from D-arabinose in these cells seemed to stabilize the enzyme. Cell-free extract was prepared from 18 g of E. coli B₄₁, wet weight, grown on D-arabinose. To this was added 0.3 volume of 5% streptomycin sulfate in 0.05 M Tris-HCl, pH 7.5. After removal of the white precipitate by centrifugation, the enzyme solution was dialyzed for 2 hours against 50 volumes of 0.002 M Tris-HCl at pH 7.5, and the insoluble material thus formed was again removed. One volume of saturated (NH₄)₂SO₄, pH 7.5, was added slowly with rapid stirring. After standing for 1 hour, the mixture was centrifuged at 14,000 × g for 30 min. The pellet was taken up in 23 ml of 0.02 M Tris-HCl, pH 7.5, and dialyzed for 2 hours against 100 volumes of the same buffer. The enzyme solution was then diluted with the Tris buffer to a total of 50 ml and was applied to a DEAE-cellulose column, 2.9 × 21.5 cm, previously washed with 0.02 M Tris-HCl, pH 7.5. The column was eluted with 550 ml of the Tris buffer at 2 ml per min without removing much of the enzyme activity or protein. A linear gradient was applied at 1 ml per min with 400 ml of 0.02 M Tris-HCl, pH 7.5, in the mixing chamber and 400 ml of the same buffer with 0.5 M NaCl in the reservoir. Tubes 33 to 40 (80 ml) of the gradient eluent were pooled. Solid (NH₄)₂SO₄ was added to 50% saturation, and the precipitate, redissolved in 1.5 ml of 0.005 M sodium glycylglycine solution, pH 7.5, was applied to a Sephadex G-100 column, 2 × 21.5 cm, pretreated with the same buffer. Elution was carried out with the glycylglycine buffer at 0.33 ml per min. Fractions 9 to 11 (7.5 ml) were pooled. The purified enzyme was free of D-phosphorabinosomerase and phosphatase. The entire procedure is summarized in Table IV and Fig. 5.

Properties of Purified Enzyme—The purified enzyme has a pH optimum of 8.0. There is no cofactor requirement but there is a 20% increase in activity in the presence of 0.001 M EDTA. The relative effects of metals at 5 mM are: no addition, 100%; MgCl₂, 100%; MnCl₂, 62%; cobalt acetate, 81%; BaCl₂, 100%; ZnCl₂, 5%; CdCl₂, 3%. Sodium iodacetate at 5 mM does not inhibit the enzyme. Sodium p-chloromercuribenzoate at 0.25 mM reduces the relative activity to 16%. There is a 50% inhibition of initial rate when the reaction mixture is incubated in 0.05 M sodium phosphate buffer at pH 8 as compared with sodium glycylglycine or Tris-HCl buffer. Sodium borate (0.1 M) at pH 8 reduces the initial rate to 57% but does not affect the equilibrium.

The enzyme interconverts D-arabinose-5-P and D-ribulose-5-P without a lag period. The Km is 1.36 × 10⁻⁴ M for D-arabinose-5-P and 5.4 × 10⁻⁴ M for D-ribulose-5-P. At equilibrium, 29% of D-ribulose-5-P and 75% of D-arabinose-5-P are found (Fig. 6). In the incubation mixture, the change in D-ribulose-5-P concentration as detected by the cysteine-carbazole reaction is accompanied by a stoichiometric change in D-arabinose-5-P concentration as detected by the Bial reaction. The latter test is indicative of D-arabinose-5-P after correcting for the color contributed by D-ribulose-5-P (50%) that of D-arabinose-5-P. The enzyme has no activity toward D-arabinose, D-ribulose, D-ribose, and D-ribose-5-P.

The enzyme is relatively unstable in its purified state. When stored at −20°C, it lost 50% of its activity after a week, and 80% after a month.

Identification of Products—D-Arabinose-5-P and D-ribulose-5-P were separately incubated at 37°C with the purified D-phosphoarabinoinosomerase in 0.05 M sodium glycylglycine buffer, pH 8.0, until equilibrium was reached. The incubation mixtures were added to 50% saturation, and the precipitate, redissolved in 1.5 ml of 0.005 M sodium glycylglycine solution, pH 7.5, was applied to a Sephadex G-100 column, 2 × 21.5 cm, pretreated with the same buffer. Elution was carried out with the glycylglycine buffer at 0.33 ml per min. Fractions 9 to 11 (7.5 ml) were pooled. The purified enzyme was free of D-phosphorabinosomerase and phosphatase. The entire procedure is summarized in Table IV and Fig. 5.

Properties of Purified Enzyme—The purified enzyme has a pH optimum of 8.0. There is no cofactor requirement but there is a 20% increase in activity in the presence of 0.001 M EDTA. The relative effects of metals at 5 mM are: no addition, 100%; MgCl₂, 100%; MnCl₂, 62%; cobalt acetate, 81%; BaCl₂, 100%; ZnCl₂, 5%; CdCl₂, 3%. Sodium iodacetate at 5 mM does not inhibit the enzyme. Sodium p-chloromercuribenzoate at 0.25 mM reduces the relative activity to 16%. There is a 50% inhibition of initial rate when the reaction mixture is incubated in 0.05 M sodium phosphate buffer at pH 8 as compared with sodium glycylglycine or Tris-HCl buffer. Sodium borate (0.1 M) at pH 8 reduces the initial rate to 57% but does not affect the equilibrium.

The enzyme interconverts D-arabinose-5-P and D-ribulose-5-P without a lag period. The Km is 1.36 × 10⁻⁴ M for D-arabinose-5-P and 5.4 × 10⁻⁴ M for D-ribulose-5-P. At equilibrium, 29% of D-ribulose-5-P and 75% of D-arabinose-5-P are found (Fig. 6). In the incubation mixture, the change in D-ribulose-5-P concentration as detected by the cysteine-carbazole reaction is accompanied by a stoichiometric change in D-arabinose-5-P concentration as detected by the Bial reaction. The latter test is indicative of D-arabinose-5-P after correcting for the color contributed by D-ribulose-5-P (50%) that of D-arabinose-5-P. The enzyme has no activity toward D-arabinose, D-ribulose, D-ribose, and D-ribose-5-P.

The enzyme is relatively unstable in its purified state. When stored at −20°C, it lost 50% of its activity after a week, and 80% after a month.

Identification of Products—D-Arabinose-5-P and D-ribulose-5-P were separately incubated at 37°C with the purified D-phosphoarabinoinosomerase in 0.05 M sodium glycylglycine buffer, pH 8.0, until equilibrium was reached. The incubation mixtures...
were adjusted to pH 5 with sodium acetate buffer and were dephosphorylated with prostatic acid phosphatase. Two control solutions containing either substrate alone were also dephosphorylated.

Aliquots were removed and incubated with D-arabinoisomerase at pH 8 until equilibrium was reached. The amount of free D-ribulose at equilibrium, as determined by the 15-min cysteine-carbazole reaction at room temperature, was 10 ± 2% of the total pentose for the two reaction mixtures and the substrate controls, indicating the absence of sugars other than D-arabinose and D-ribulose in the dephosphorylated solutions. Furthermore, the kinetics of the color development up to 5 hours in the cysteine-controls, indicating the absence of sugars other than D-arabinose and D-ribulose in the dephosphorylated solutions. The amount of contaminating D-xylulose in each solution was shown to be less than 3.5% of the D-ribulose content.

Other aliquots, each corresponding to 0.20 μmole of total pentose, were subjected to the Bial reaction and the spectra are plotted in Fig. 7. Note that the spectra of the equilibrium mixtures lie somewhere between those of the two substrates.

Other aliquots were desalted with a mixed bed resin and chromatographed on 0.25-mm-thick cellulose MN 300 plates. One plate was developed with ethyl acetate-acetic acid-H₂O (3:1:3) (v/v) and the sugars were detected by the silver nitrate method (45). Under these conditions, dephosphorylated D-arabinose-5-P had an Rf of 0.15, which was identical with that of the D-arabinose standard; dephosphorylated D-ribulose-5-P had an Rf of 0.26, identical with that of the D-ribulose standard. When D-arabinose-5-P was incubated with D-phosphorabinoisomerase and then dephosphorylated, a second spot (lighter), corresponding to D-ribose, appeared; when D-ribulose-5-P was used instead of D-arabinose-5-P, a second spot, corresponding to D-arabinose, was demonstrable. Another thin layer plate was developed with 86% aqueous n-butyl alcohol and sprayed with m-phenylenediamine (46). The distribution of different spots is similar to what was just described except that the Rf values for D-arabinose and D-ribulose in this solvent were 0.14 and 0.22, respectively. In addition, with the m-phenylenediamine spray, characteristic colors appeared with each sugar under visible and ultraviolet lights. The Rf, coupled with the color of the spots, ruled out the presence of D-ribose, D-xylulose, and D-fructose in the dephosphorylated substrates and their isomerization products.

We conclude therefore that D-ribulose-5-P and D-arabinose-5-P are direct products of isomerization by D-phosphorabinoisomerase.

**Absence of D-Phosphorabinoisomerase in Yeast and Mouse Fibroblasts**—Freshly prepared extracts of yeast and mouse fibroblasts (L cells) were assayed for D-phosphorabinoisomerase. A fresh extract of E. coli B was also used for comparison. As another control, D-phosphoribosilomerase was tested in all of these extracts with the assay method described for D-phosphorabinoisomerase, with D-ribose-5-P instead of D-arabinose-5-P as substrate. Table V shows the results of the determinations. As can be seen, the activity of D-phosphorabinoisomerase in L cells and yeast was found to be less than 2% of that in E. coli. In all of the three extracts, D-phosphoribosilomerase level was high. In order to rule out any inhibitor in L cells and yeast preparations, both extracts were mixed with an equal volume of the E. coli extract and assayed for D-phosphorabinoisomerase. In both instances the results obtained were as if E. coli extract alone were present.

**DISCUSSION**

2-Keto-3-deoxyoctonate is a constituent of the cell wall lipopolysaccharide of many gram-negative organisms, such as *Escherichia*, *Salmonella*, and *Shigella*. Its presence in gram-positive organisms has not yet been reported. Since KDO-8-P is a condensation product of D-arabinose-5-P and phosphoenolpyruvate, D-phosphorabinoisomerase is inferred to be present in all organisms in which KDO is found. Conversely, the presence of D-phosphorabinoisomerase may suggest the possible existence of KDO in the cell wall. In this connection it would be of interest to test for KDO or other D-arabinose derivatives in structures of *P. pentosaceum*, a gram-positive bacterium in which the isomerase has been detected (10). The attachment of KDO is probably an early event in cell wall synthesis since it is glycosidically bound to

![Fig. 7. Bial spectra of the dephosphorylated reaction mixtures of D-phosphorabinoisomerase. D-Ribulose-5-P and D-arabinose-5-P were isomerized with D-phosphorabinoisomerase and then dephosphorylated with prostatic acid phosphatase as described in the text. The dephosphorylated mixtures were subjected to the Bial reaction and the spectra are shown.](image)

**Table V**

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>D-Phosphorabinoisomerase</th>
<th>D-Phosphoribosilomerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>&lt;0.8</td>
<td>4,200</td>
</tr>
<tr>
<td>Mouse fibroblasts (L cells)</td>
<td>&lt;1.8</td>
<td>2,070</td>
</tr>
<tr>
<td>E. coli</td>
<td>100^a</td>
<td>19,000</td>
</tr>
</tbody>
</table>

^a This corresponds to 0.02 μmole of substrate reacted per mg of protein per min.
the lipid and serves as a link between the polysaccharide and the lipid moieties (47, 5). This suggests that all the enzymes leading to the synthesis of KDO, including d-phosphoribokinase, must have arisen early in the evolutionary scale. In addition, it can be suspected that d-phosphoribokinase is present in Mycobacterium tuberculosis, Mycobacterium leprae, some eubacteria, and some Nocardiaceae, where n-arabinose is a major structural element within the polysaccharide chains (48–50).

The absence of d-phosphoribokinase in yeast and in mouse fibroblasts (L cells) suggests that the distribution of the enzyme is limited to bacteria, a fact which may be of therapeutic significance. However, the sponge Cryptotheca crypta, which contains d-arabinonucleotides in large quantities, is an animal. It is possible, then, that d-phosphoribokinase is present in some animal cells, such as the sponge. On the other hand, it is conceivable that Cryptotheca synthesizes d-arabinonucleotides through a different mechanism, possibly involving accumulation of d-arabinose derived from a symbiont. It should be noted that some animal cells utilize d-arabinose readily (51–53), although the intermediates are not known.

L-Fucose can be considered as a 5-C-methyl derivative of d-arabinose. The same relationship in the ketose series holds true with d-ribulose and L-fucose. When E. coli strain B45 is grown on either d-arabinose or L-fucose, a series of induced enzymes capable of metabolizing the two sugars appears in the cell. The first enzyme, n-arabinokinase (12, 44), converts d-arabinose and L-fucose to the ketoses. The second enzyme is a kinase which phosphorylates the ketoses. The data in this paper suggest that the induced kinase phosphorylates both d-ribulose at position 5 and L-fucose at position 1. The formation of L-fuculose-1-P had been described by Heath and Ghalambor (58) in Escherichia freundii and E. coli O-111 B4, adapted to L-fucose. Although the L-fuculokinase of Heath and Ghalambor has a substrate specificity quite similar to the n-ribulokinase described in the present work, these investigators did not characterize the phosphorylated n-ribulose produced by their enzyme.

It may well be that only a single enzyme phosphorylates two structurally related sugars at two different positions. The hydroxyl group at C-5 of d-ribulose and that at C-1 of L-fucose are both primary alcohols, and the presence or absence of the methyl group may affect the active site of the enzyme sufficiently to direct phosphorylation to one or the other site. Alternatively the enzyme may consist of subunits, the type of aggregation of which is substrate-dependent, so that different substrates are phosphorylated at different positions.

However, two other possibilities may be considered. Conceivably a single enzyme which could be induced by either d-arabinose or L-fucose is capable of phosphorylating both d-ribulose and L-fucose only at position 1. This possibility requires the presence of a d-phosphoribulokinase which transfers the phosphate from C-1 of d-ribulose to C-5. This is supported by the fact that a small amount of ribulose-1-P always contaminates the synthesized ribulose-5-P. Also, ribulose-5-P-carboxylisomutase, which reacts with d-ribulose 1,5-diphosphate, is present in some gram-negative bacteria. The energy levels of d-ribulose-5-P and n-ribulose-1-P might not be very different. However, phosphoketomutases have not been reported previously. The failure to detect a large amount of d-ribulose-1-P compared with d-ribulose-5-P in the time course experiment implies that the mutase, if present, should be very active; or one single enzyme might serve the dual function of a kinase and a mutase, so that very little d-ribulose-1-P intermediate could be isolated.

In another, more prosaic explanation we may suppose that our preparations contain two separate enzymes, a d-ribulokinase that phosphorylates d-ribulose at position 5 only and an L-fuculokinase that phosphorylates L-fucose at position 1 only. These could be induced by either d-arabinose or L-fucose. This is suggested by the fact that the reactivity of the enzyme system toward the two substrates seems to differ as a function of the extent of purification. The small amount of ribulose-1-P detected might be due to some degree of cross reactivity of the L-fuculokinase on d-ribulose.

Ghalambor and Heath (54) reported the presence of L-fuculose-1-P aldolase as one of the enzymes induced by L-fucose on E. coli O-111 B4. This enzyme reversibly cleaves L-fuculose-1-P to dihydroxyacetone phosphate and L-lactaldehyde. Although the ultimate fate of L-lactaldehyde in this organism is not certain, dihydroxyacetone phosphate is a well known intermediate in carbohydrate metabolism. It is believed that the same enzyme is induced by L-fucose in E. coli B45. Green and Cohen (44) made the interesting observation that E. coli B45 adapted to L-fucose grows on d-arabinose as well as on L-fucose, whereas the same organism adapted to d-arabinose exhibits a lag period before it can grow on L-fucose to an extent equal to d-arabinose. They suggested that an enzyme present in L-fucose-adapted cells is essential for the metabolism of this sugar is absent from d-arabinose-adapted cells. In the light of present knowledge, this enzyme could be the L-fuculose-1-P aldolase. Although the aldolase also acts on d-ribulose-1-P, it is not essential for the utilization of d-arabinose, because in this instance the phosphorylated product is mainly d-ribulose-5-P.

REFERENCES
16. WALLBURG, O., AND CHRISTIAN, W., Biochem. Z., 310, 384 (1941).
d-Phosphoarabinose isomerase and d-Ribulokinase in *Escherichia coli*

Ramon Lim and Seymour S. Cohen


Access the most updated version of this article at http://www.jbc.org/content/241/19/4304

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/241/19/4304.full.html#ref-list-1