The inducible d-ketohexose-1,6-diphosphate aldolase that functions in the metabolism of lactose and d-galactose in *Staphylococcus aureus* was purified to electrophoretic homogeneity from an extract of d-galactose-grown cells. At saturating substrate concentrations, d-tagatose 1,6-diphosphate was cleaved to dihydroxyacetone phosphate plus d-glyceraldehyde 3-phosphate at twice the rate of d-fructose 1,6-diphosphate; *K*ₚ values for d-tagatose 1,6-diphosphate and d-fructose 1,6-diphosphate were 1.5 mM and 2.5 mM, respectively. The enzyme catalyzed the aldol condensation of dihydroxyacetone phosphate and d-glyceraldehyde 3-phosphate to yield a mixture of the 1,6-diphosphate derivatives of d-tagatose, d-fructose, d-sorbose, and d-psicose, indicating that it also catalyzes the cleavage of all four d-2-ketohexose 1,6-diphosphates. The enzyme was not inhibited by EDTA and it had no divalent metal ion requirement, but it did exhibit substrate-dependent inactivation by NaBH₄, indicating that it is a Class I (Schiff’s base) aldolase. Density gradient centrifugation and gel electrophoresis in the presence of sodium dodecyl sulfate indicated that the enzyme exists as a monomer with a molecular weight of about 37,000 and a sedimentation coefficient of 3.4 S. Data on the stability, pH optimum, and inducibility of the enzyme are also presented.

The preceding two papers in this series described the isomerization of d-galactose 6-phosphate to d-tagatose 6-phosphate (1) and the ATP-dependent phosphorylation of d-tagatose 6-phosphate to d-tagatose 1,6-diphosphate (2) by enzymes from *Staphylococcus aureus*. The present paper establishes that the next step in the metabolism of lactose and d-galactose in this organism is the enzymatic cleavage of d-tagatose 1,6-diphosphate to dihydroxyacetone phosphate plus d-glyceraldehyde 3-phosphate (Fig. 1), and reports on the isolation and some properties of the aldolase that catalyzes this reaction.

### RESULTS

**Purification of d-Tagatose-1,6-diphosphate Aldolase**

The aldolase was 155-fold purified with an overall recovery of 36% (Table 1). It appeared to be homogeneous by several criteria. The fractions from the DEAE-cellulose column which were combined had identical specific activities and were free from the constitutive d-fructose 1,6-diphosphate aldolase (4, 9). Examination of the enzyme by polyacrylamide gel electrophoresis performed either in the absence or presence of sodium dodecyl sulfate revealed only one protein band (Fig. 2). This aldolase preparation was used in all subsequent experiments.

**Properties of d-Tagatose-1,6-diphosphate Aldolase**

**Stability**—The DEAE-cellulose fractions were stable to storage at −20°C for several months.

**Effect of pH and Buffer Composition**—The aldolase was most active at about pH 7.1 in phosphate and Hepes buffers, and at about pH 7.8 in Tris and glycylglycine buffers (Fig. 3).

**Substrate Specificity and Kinetic Constants**—Of nine sugar phosphates tested, only d-tagatose 1,6-diphosphate and d-fructose 1,6-diphosphate served as substrates. Compounds that were not cleaved (<1% of the rate with d-tagatose 1,6-diphosphate) at 10 mM concentrations were d-fructose 6-phosphate, d-fructose 1-phosphate, t-tagatose 1,6-diphosphate, t-sorbose 1-phosphate, d-galactose 6-phosphate, d-glucose 6-phosphate, and d-mannose 6-phosphate. d-Fructose 1-phosphate was not cleaved even at 100 mM concentrations. Evidence presented below on the reversibility of the reaction indicated that d-sorbose 1,6-diphosphate and d-psicose 1,6-diphosphate also serve as substrates.

From Lineweaver-Burk plots (Fig. 4), the *K*ₚ values for d-tagatose 1,6-diphosphate and d-fructose 1,6-diphosphate were determined to be 1.5 mM and 2.5 mM, respectively. At saturating levels of substrate, d-fructose 1,6-diphosphate was cleaved at about 47% of the rate of d-tagatose 1,6-diphosphate.

Several lines of evidence indicate that the cleavage of d-tagatose 1,6-diphosphate and d-fructose 1,6-diphosphate is catalyzed by the same enzyme. First, the enzyme was electrophoretically homogeneous. Second, the two activities decayed coincidentally when heated at 50°C (Fig. 5). Third, the activities are competitive rather than additive (Table II).

**Effects of N-Ethylmaleimide and β-Mercaptoethanol**—When β-mercaptoethanol was removed by passing the aldolase over a column of Sephade G-25, the addition of the thiol-blocking reagent N-ethylmaleimide, at a concentration of 0.1 mM, caused substantial loss (>90%) of activity in 30 min. This inactivation could be partially reversed by the subsequent addition of 0.25% (v/v) β-mercaptoethanol. β-Mercaptoethanol also stabilized the enzyme during purification. When β-mer-
captoethanol was removed from the enzyme by dialysis, the recovery of activity was considerably less than the quantitative recovery obtained when dialysis was done with β-mercaptoethanol in the dialysis buffer. Time-dependent reactivation could be achieved by adding back β-mercaptoethanol (Table III).

**Effect of Metal Ions**—In assay mixtures from which monovalent cations other than Tris and cyclobexy lammonium were excluded, there was no effect on the activity of added NaCl, KCl, NH₄Cl, LiCl, RhCl, or CsCl at 6.67 mM concentrations.

EDTA (10 mM) did not affect the aldolase. Also, 1 mM MgCl₂, CoCl₂, CaCl₂, or CdSO₄ had no effect, whereas 1 mM FeSO₄, MnCl₂, ZnCl₂, BaCl₂, CuSO₄, and NiCl₂ caused 20 to 50% inhibition.

**Effect of NaBH₄**—NaBH₄ caused severe inhibition of the aldolase in the presence of substrate. At 10 mM NaBH₄, 90% of the activity was lost in less than 5 min; at 100 mM NaBH₄, all of the activity was lost in the same time period. In the absence of substrate, 100% and 67% of the activity were recovered in the presence of 10 mM and 100 mM NaBH₄, respectively. Since there is no divalent metal ion requirement but there was inhibition by NaBH₄, D-tagatose-1,6-diphosphate aldolase is a Class I (Schiff's base) aldolase (10).

![Fig. 1. Reaction for the cleavage of D-tagatose-1,6-diphosphate by D-tagatose-1,6-diphosphate aldolase.](image)

**Table I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (units/mg protein)</th>
<th>Specific activity (units/mg protein)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract (pH 5.2)</td>
<td>215</td>
<td>1092</td>
<td>138</td>
<td>0.126</td>
<td>100</td>
</tr>
<tr>
<td>Phosphocellulose I</td>
<td>150</td>
<td>27.2</td>
<td>118</td>
<td>4.34</td>
<td>85</td>
</tr>
<tr>
<td>Phosphocellulose II</td>
<td>72</td>
<td>4.52</td>
<td>57.9</td>
<td>12.8</td>
<td>42</td>
</tr>
<tr>
<td>DRAE-cellulose</td>
<td>8.4</td>
<td>2.52</td>
<td>49.1</td>
<td>19.5</td>
<td>36</td>
</tr>
</tbody>
</table>

**Fig. 2. Polyacrylamide gel electrophoresis of purified D-tagatose-1,6-diphosphate aldolase.** The amount of protein was 100 µg in each case, and the direction of migration was down. Details of the procedure are given in Ref. 2. A, native gel; B, sodium dodecyl sulfate gel.

![Table IV](image)

**Table IV**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Diagnostic enzyme(s)</th>
<th>Compound(s) determined</th>
<th>Amount of product (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>α-Glycerol phosphate dehydrogenase</td>
<td>Dihydroxyacetone-P</td>
<td>7.4</td>
</tr>
<tr>
<td>B</td>
<td>α-Glycerol phosphate dehydrogenase + triose-P isomerase</td>
<td>D-Glyceraldehyde-3-P</td>
<td>14.8</td>
</tr>
<tr>
<td>C</td>
<td>D-Glyceraldehyde-3-P dehydrogenase</td>
<td>dihydroxyacetone-P</td>
<td>7.4</td>
</tr>
<tr>
<td>D</td>
<td>D-Glyceraldehyde-3-P dehydrogenase + triose-P isomerase</td>
<td>dihydroxyacetone-P</td>
<td>14.8</td>
</tr>
</tbody>
</table>

**Native Enzyme Molecular Weight and Sedimentation Coefficient**—Gel filtration of the aldolase on a standardized Sephadex G-100 column indicated an apparent molecular weight of 50,000 (Fig. 6). In a sucrose density gradient (Fig. 7), the aldolase sedimented with a calculated sedimentation coefficient of 3.37 S. Assuming a molecular weight of 39,800 and a sedimentation coefficient of 3.48 S for the porodix standard (6), the apparent molecular weight of D-tagatose-1,6-diphosphate aldolase was calculated (2) to be 37,900.

**Subunit Molecular Weight**—The aldolase exhibited a subunit molecular weight of 37,000 when measured by electrophoresis on dodecyl sulfate polyacrylamide disc gels against standards (Fig. 8).

**Product Identification and Reaction Stoichiometry**

As determined by enzymatic analyses, cleavage of 1 mol of D-tagatose-1,6-diphosphate by the aldolase yielded 1 mol each of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate (Table IV).

**Reversibility of the Aldolase-catalyzed Reaction**

A reaction mixture (5.0 ml) containing 200 µmol of Hepes buffer (pH 7.0), 100 µmol of DL-glyceraldehyde 3-phosphate, 50 units of triose phosphate isomerase, and 0.6 unit of D-tagatose-1,6-diphosphate aldolase was incubated at 25°C. When the concentration of ketohexose became constant (about 3 h), the solution was adjusted to pH 8.5 with NaOH, applied to a Dowex 1-X8 bicarbonate column (1.2 × 10 cm) and eluted with a stepwise gradient as described previously (1). Fractions containing ketohexose diphosphate, which eluted with 0.30 M KHCO₃, were combined and treated as described previously (1). The yield of ketohexose diphosphate was 7.2 µmol.

The aldolase was found to catalyze the condensation of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate to yield a mixture of all four D-2-ketohexose 1,8-diphosphates, as determined by the following data. All of the ketohexose product eluted from the Dowex column in the position of a diphosphate, and it had a phosphate/ketohexose ratio of 2.0. In separate reactions, the product reacted quantitatively through the sequences: (i) ketohexose diphosphate → dihy-
D-TAGATOSE-1,6-P\textsubscript{2} ALDOLASE FROM \textit{S. aureus}

dihydroxyacetone phosphate + D-glyceraldehyde 3-phosphate \rightarrow 2 dihydroxyacetonate phosphate \rightarrow 2 α-glycerol phosphate through the participation of D-tagatose-1,6-diphosphate aldolase, triose phosphate isomerase, and α-glycerol phosphate dehydrogenase; and (ii) ketohexose diphosphate \rightarrow dihydroxyacetone phosphate + D-glyceraldehyde 3-phosphate \rightarrow 2 D-glyceraldehyde 3-phosphate \rightarrow 2 D-phosphoglycerate through the participation of D-tagatose-1,6-diphosphate aldolase, triose phosphate isomerase, and D-glyceraldehyde-3-phosphate dehydrogenase (conditions as in Table IV). This indicates that only the \( \Delta \) isomer of the commercial DL-glyceraldehyde 3-phosphate was utilized in the condensation reaction. The ketohexose phosphate was dephosphorylated with acid phosphatase (1) and the resulting free ketohexose chromatographed on paper. Four ketohexose spots were found, each of which migrated with a known ketohexose: tagatose, fructose (\( R_{\text{tagatose}} = 1.14 \)), sorbose (\( R_{\text{tagatose}} = 0.96 \)), and psicose (\( R_{\text{tagatose}} = 2.22 \)). The ketohexose spots were located using an orcinol spray for ketohexoses (8) or silver nitrate procedure for total carbohydrate (7). The presence of sorbose in the dephosphorylated product solution was verified with the cysteine-H\textsubscript{2}SO\textsubscript{4} reaction (11), which gives a distinctive absorption spectrum for sorbose. These data indicate that dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate are condensed to yield a mixture of the four D-ketohexose 1,6-diphosphates, and that L-glyceraldehyde 3-phosphate does not serve as a substrate.

\textbf{Induction of the Aldolase}

D-Tagatose-1,6-diphosphate aldolase is induced only by growth of the organism in the presence of d-galactose (specific activity, 0.065) and lactose (specific activity, 0.028). No activity (<0.0002 pmol of D-tagatose 1,6-diphosphate cleaved/min/mg protein) could be detected in extracts of cells grown in the medium of McClatchy and Rosenblum (12) when D-galactose was replaced with D-glucose, D-fructose, D-mannose, sucrose, maltose, or peptone.

\textbf{DISCUSSION}

The data in this paper establish that D-tagatose 1,6-diphosphate is cleaved by an enzyme from \textit{S. aureus} to yield equimolar amounts of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. Although the inducible aldolase that catalyzes this reaction can also cleave D-fructose 1,6-diphosphate, it is distinct from the constitutive D-fructose-1,6-diphosphate aldolase that also occurs in this organism. Because the inducible enzyme has a higher maximal velocity and a lower \( K_{m} \) with D-tagatose 1,6-diphosphate than with D-fructose 1,6-diphosphate, and because it is induced specifically in response to the need of cells to cleave D-tagatose 1,6-diphosphate, we have termed it D-tagatose-1,6-diphosphate aldolase rather than D-fructose-1,6-diphosphate aldolase. However, as will be discussed below, the specificity of this enzyme is even broader, encompassing more than these two ketohexose diphosphates.

Rabbit muscle D-fructose-1,6-diphosphate aldolase has also been reported to cleave D-tagatose 1,6-diphosphate (3). However, we have been unable to detect this activity (2, 4, 9), and our finding has been confirmed by Thomas (13).

It is of interest that whereas D-tagatose 1,6-diphosphate has the \textit{erythro} configuration at the site of cleavage (carbon atoms 3 and 4), D-fructose 1,6-diphosphate, which is also cleaved by D-tagatose-1,6-diphosphate aldolase, has the \textit{threo} configuration at C-3 and C-4. An aldolase that apparently requires the \textit{erythro} configuration at C-3 and C-4 is one from \textit{Escherichia coli} which cleaves L-fucose 1-phosphate (14) and D-ribulose 1-phosphate (15).

The demonstration that D-tagatose-1,6-diphosphate aldolase catalyzes the condensation of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate to a mixture of the four D-ketohexose 1,6-diphosphates, whose structures are compared in Fig. 9, indicates that the enzyme also catalyzes the cleavage of D-sorbose 1,6-diphosphate and D-psicose 1,6-diphosphate. However, the aldolase does not cleave ketohexose 1- or 6-monophosphates and does not utilize L-glyceraldehyde 3-phosphate in the condensation reaction. These observations indicate that D-tagatose-1,6-diphosphate aldolase is specific for carbon atoms 1, 2, 5, and 6 of the substrate molecule, but is nonspecific with respect to the configuration at carbon atoms 3 and 4, and thus may appropriately be termed a D-ketohexose-1,6-diphosphate aldolase. Nonspecificity at carbon 4 has been observed previously for 2-keto-4-dihydroxybutarate aldolase from rat liver (16), an unidentified soil bacterium (17), and \textit{E. coli} (18), for 2-keto-4-dihydroxyacetone-lactonemalate aldolase from \textit{Acinetobacter} (19), for 2-keto-4-dihydroxy-4-methylbutarate aldolase from \textit{Pseudomonas putida} (21), and for 2-keto-3-deoxyhexarate aldolase from \textit{E. coli} (22). However, we know of no previous report of an aldolase that is nonspecific with respect to the configuration of hydroxyl groups at C-3 or at both C-3 and C-4. Although most aldolases so far investigated mechanistically, including those that catalyze pyruvate-lyase reactions, proceed with retention of configuration at C-3 (23, 24), an apparent exception has recently been reported (25) in the case of deoxyribose-5-phosphate aldolase (an acetaldehyde lyase). Isotopic studies indicated that, during the cleavage of deoxyribose 5-phosphate, rotation of the methyl group of enzyme-bound acetaldehyde and enolization occurred prior to product release, resulting in an apparent lack of stereospecificity with respect to proton uptake at C-2 (equivalent to C-3 of a pyruvate-lyase substrate). Similarly, the apparent C-3 epimerization that we observed with D-tagatose-1,6-diphosphate aldolase suggests to us that, during cleavage or synthesis of the D-ketohexose 1,6-diphosphate, rotation of the hydroxymethyl group of enzyme-bound dihydroxyacetone phosphate occurs, and that enolization and C=C bond cleavage or synthesis proceed at faster rates than does release of the products from the enzyme.

Other distinguishing characteristics of this aldolase include its lack of a divalent metal ion requirement and its inhibition by NaBH\textsubscript{4}. These properties make D-tagatose-1,6-diphosphate aldolase a Class I aldolase even though it occurs in a representative of the bacteria, which typically have Class II aldolases (10). However, the occurrence of class I aldolases in bacteria is more widespread than once thought (26-32). The class I D-fructose-1,6-diphosphate aldolase from \textit{Micrococcus aerogenes} was considered to be unique among these enzymes since it is a monomer \( M_{r} = 33,000 \) (20). D-Tagatose 1,6-diphosphate aldolase from \textit{S. aureus} constitutes the second example of a monomeric Class I aldolase, and both of these enzymes are of bacterial origin.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig9.png}
\caption{Diagrammatic structures showing the epimeric relationships of the four D-ketohexose 1,6-diphosphates that serve as substrates for D-tagatose-1,6-diphosphate aldolase. The dashed lines indicate the sites of cleavage.}
\end{figure}
The use of substrate-facilitated elution has been reported for the purification of D-fructose-1,6-diphosphate aldolase (33) and of D-fructose 1,6-diphosphatases (33-35). A similar procedure was used in this study to purify D-tagatose-1,6-diphosphate aldolase. The enzyme eluted from phosphocellulose at about 0.20 M KCl. In the presence of substrate (1 mM D-fructose 1,6-diphosphate), it eluted from phosphocellulose at about 0.12 M KCl, providing an excellent separation from the remainder of the protein, which apparently did not interact with the substrate. Electrophoretically homogeneous enzyme was then obtained by KCl elution from DEAE-cellulose.

The apparent molecular weight of 50,000 observed by Sephadex G-100 chromatography is probably due to anomalous behavior of the aldolase on the gel since the subunit molecular weight and the native enzyme molecular weight determined in a sucrose density gradient were almost identical (about 37,000). An enzyme which behaves similarly is peroxidase, whose molecular weight is 39,800 by sedimentation and diffusion measurements (6), but which behaves like a 49,000 molecular weight protein on Sephadex G-100 (36). In this light, our data are consistent with D-tagatose-1,6-diphosphate aldolase being a monomer with a sedimentation coefficient of 3.4 S.

D-Galactose 6-phosphate is an intermediate in the metabolism of D-galactose and lactose in S. aureus (see Ref. 1 for a review). Fig. 10 summarizes the pathway of D-galactose 6-phosphate catabolism in S. aureus as we have elucidated it in this paper and in those preceding (1, 2, 4, 9). D-Galactose 6-phosphate is isomerized by D-galactose-6-phosphate isomerase to D-tagatose 6-phosphate (1), which is phosphorylated with ATP to D-tagatose 1,6-diphosphate by D-tagatose-6-phosphate kinase (2). The diphosphate is then cleaved by D-tagatose-1,6-diphosphate aldolase to yield dihydroxyacetone phosphate plus D-glyceraldehyde 3-phosphate, as established in this communication. In S. aureus this is the sole pathway of D-galactose metabolism (4). In group N streptococci, this pathway functions in addition to the Leloir pathway (37). The last two reactions of the pathway, ATP-dependent phosphorylation of D-tagatose 6-phosphate and aldol cleavage of D-tagatose 1,6-diphosphate, also function in the catabolism of galactitol (but not D-galactose or lactose) in Klebsiella pneumoniae (38).

REFERENCES

FIG. 10. Pathway of D-galactose 6-phosphate catabolism in S. aureus as elucidated in this paper and in those preceding (1, 2, 4, 9). The reactions leading to D-galactose 6-phosphate formation are shown for perspective.
**Supplementary Material**

**To**

Lactose and D-Tagatose Metabolism in *S. aureus* (2)

**in**

**3,6-Dehydro-D-Tagatose-1,6-P2 Aldolase from S. aureus**

Donald L. Small and Richard L. Anderson

**MATERIALS AND METHODS**

Materials and methods not detailed below are described in the preceding publications (1,2). Preparation of Cell Extracts. Cell extracts were prepared from a culture with 10 mM sodium acetate at pH 5.2. The buffer was 3% DMSO, 0.1% sodium acetate at pH 5.2. The pH of the assay was 5.2. The assay was adjusted to pH 5.2 with 0.05 M acetic acid prior to assay of cellular debris by centrifugation.

**D-Tagatose-1,6-P2 Aldolase Assay.** The standard assay (0.15 M) in a microtiter plate at 30°C in a 0.125 M glycine buffer (pH 5.2) contained 0.5 mg (50 μg) D-Tagatose-1,6-P2 aldolase from *S. aureus* per plate of D-Tagatose-1,6-P2 aldolase, D-Tagatose-1,6-P2, and rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (1). The reaction was monitored by recording the absorbance at 235 nm with a Gilford spectrophotometer equipped with a 1 cm cuvette. The reaction was started with tri-thiolan oxide in the buffer and was followed at 235 nm. The rate of the reaction was determined by the change in absorbance at 235 nm. The absorbance was determined at 10 min intervals.

**Assays to Determine the Effect of Monosaccharides on the Activity of D-Tagatose-1,6-P2 Aldolase.** Assays were performed as described above, except that the concentration of each monosaccharide was varied from 0.1 M to 0.3 M. The effect of each monosaccharide on the activity of D-Tagatose-1,6-P2 aldolase was determined at each concentration. The rate of the reaction was determined by the change in absorbance at 235 nm. The absorbance was determined at 10 min intervals.

**pH-Dependent Regulation of D-Tagatose-1,6-P2 Aldolase Activity.** The standard assay was used except that the pH was varied from 5.0 to 7.0. The optimal pH for the reaction was 6.0. The rate of the reaction was determined by the change in absorbance at 235 nm. The absorbance was determined at 10 min intervals.

**Table I.** The relative activity of D-Tagatose-1,6-P2 aldolase at different pH values. The data were determined by monitoring the reaction at 235 nm.

<table>
<thead>
<tr>
<th>pH</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>0.280</td>
</tr>
<tr>
<td>5.5</td>
<td>0.250</td>
</tr>
<tr>
<td>6.0</td>
<td>0.350</td>
</tr>
<tr>
<td>6.5</td>
<td>0.550</td>
</tr>
<tr>
<td>7.0</td>
<td>0.850</td>
</tr>
</tbody>
</table>

**Table II.** The relative activity of D-Tagatose-1,6-P2 aldolase at different monosaccharide concentrations. The data were determined by monitoring the reaction at 235 nm.

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.15</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.25</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.30</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.40</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.50</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.60</td>
</tr>
<tr>
<td>Galacturonate</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**Fig. 1.** The inhibition of the reaction by D-Tagatose-1,6-P2 aldolase activity at different pH values. The standard assay was used except that the pH was varied from 5.0 to 7.0. The optimal pH for the reaction was 6.0. The rate of the reaction was determined by the change in absorbance at 235 nm. The absorbance was determined at 10 min intervals.

**Fig. 2.** The inhibition of the reaction by D-Tagatose-1,6-P2 aldolase activity at different monosaccharide concentrations. The standard assay was used except that the monosaccharide concentration was varied from 0.1 M to 0.3 M. The activity was determined by monitoring the reaction at 235 nm. The absorbance was determined at 10 min intervals.

**Fig. 3.** The inhibition of the reaction by D-Tagatose-1,6-P2 aldolase activity at different substrate concentrations. The standard assay was used except that the substrate concentration was varied from 0.1 M to 0.3 M. The activity was determined by monitoring the reaction at 235 nm. The absorbance was determined at 10 min intervals.

**Fig. 4.** The inhibition of the reaction by D-Tagatose-1,6-P2 aldolase activity at different substrate concentrations. The standard assay was used except that the substrate concentration was varied from 0.1 M to 0.3 M. The activity was determined by monitoring the reaction at 235 nm. The absorbance was determined at 10 min intervals.
**d-Tagatose-1,6-P₂ Aldolase from S. aureus**

Fig. 1. Thermal inactivation of d-tagatose 1,6-diphosphate aldolase. The purified aldolase (1 ml) in 0.05 M phosphoric acid buffer (pH 6.2) was heated at 50°C in a 50°C water bath, and samples were withdrawn at timed intervals and assayed for aldolase activity using either d-tagatose 1,6-diphosphate (a) or D-fructose 1,6-diphosphate (b) as the substrate.

Fig. 3. Sedimentation coefficient determination of d-tagatose 1,6-diphosphate aldolase by sucrose density gradient centrifugation. For experimental details, see "Materials and Methods" in this paper and in a companion paper (2). D-fructose 1,6-diphosphate aldolase; a, hortensis peroxidase (S. aureus; Mr = 184,500).

Fig. 4. Molecular weight estimation of d-tagatose 1,6-diphosphate aldolase by chromatography on Sephadex G-150. The molecular weight standards used were: a, E. coli alkaline phosphatase (Mr = 40,000); b, D-fructose 1,6-diphosphate aldolase (Mr = 50,000); c, yeast hexokinase (Mr = 51,000); and d, ovalbumin (Mr = 43,000).

Fig. 5. Sedimentation coefficient determination of d-tagatose 1,6-diphosphate aldolase by sucrose density gradient ultracentrifugation. For experimental details, see "Materials and Methods" of a companion paper (2). The standards used were: a, horse serum albumin (Mr = 66,000); b, beef liver catalase (Mr = 65,000); c, ovalbumin (Mr = 43,000); d, yeast alcohol dehydrogenase (Mr = 37,000); and e, beef pancreas a-chymotrypsinogen A (Mr = 25,000). D-fructose 1,6-diphosphate aldolase migrated with the alcohol dehydrogenase subunit.