Phosphoribokinase from *Pseudomonas saccharophila*

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**SUMMARY**

1. An enzyme catalyzing the formation of ribose 1,5-di-P from ribose 5-phosphate and ATP has been purified 40-fold from sonic extracts of *Pseudomonas saccharophila*. This preparation is free of both phosphoribomutase and phosphoglucomutase activity. It is specific for ribose 5-phosphate as substrate; ribose 1-phosphate, glucose 6-phosphate, and glucose 1-phosphate fail to react.

2. The apparent $K_m$ for both ribose 5-phosphate and ATP is about 1 mM. Inhibition is observed at higher concentrations of either substrate. The pH optimum is 9.1.

3. The ribose diphosphate formed was identified by the following characteristics: ability to stimulate the phosphoglucomutase reaction; chromatography on Dowex 1 (Cl−); mobility identical with that of authentic ribose diphosphate on paper electrophoresis; and acid lability, yielding a material which corresponds electrophoretically with ribose 5-phosphate and which no longer stimulates the mutase reaction.

4. It is suggested that the function of the ribose diphosphate formed is to serve as a cofactor in the conversion of ribose 5-phosphate to ribose 1-phosphate by the enzyme phosphoribomutase. The availability of ribose diphosphate could serve as a means for controlling this reaction.

Various sugar diphosphates are considered to participate in mutase reactions. The role of glucose-1,6-di-P in the phosphoglucomutase reaction is well established (1), and that of several other diphosphates, including ribose 1,5 di P, is presumed to be analogous. According to the hypothesis that the function of the enzyme is to catalyze the conversion of ribose 5-phosphate to ribose 1-phosphate by the enzyme phosphoribomutase, the availability of ribose diphosphate could serve as a means for controlling this reaction.
system contained in a final volume of 0.60 ml: 50 nm Tris-HCl buffer, pH 7.4, 6.6 nm MgCl₂, 4.1 nm EDTA, 6.6 nm glucose-1-P, 0.08 mg of gelatin, and 0.02 ml of boiled incubation product (or control). The reaction was started by the addition of 0.1 i.u. of phosphoglucomutase (as assayed with saturating cofactor) and was incubated for 15 min at 37°.

Standards containing 0, 4.2 × 10⁻³, and 8.3 × 10⁻³ μmole of glucose-1,6-di-P were assayed with each set of experimental samples. The reaction was terminated after 15 min by boiling, and 1 ml of Somogyi's reagent (5) was added to each incubation mixture. Mixture were placed in a boiling water bath for 15 min, then in an ice bath for 5 min before 1.5 ml of arsenumolybdate reagent (6) was added, followed by the addition of 3.0 ml of water. Samples were read in a Klett colorimeter with a No. 54 filter. One unit of enzyme activity is defined as the production of 1 μmole of ribose-1,5-di-P per min at pH 9.1.

Other Enzyme Assays—Phosphoglucomutase and phosphoribomutase activities in the crude bacterial extracts were assayed by the formation of reducing sugar from the corresponding aldose 1-phosphate under conditions of saturating cofactor. The assay mixture contained in a final volume of 0.30 ml: glucose-1-P or ribose-1-P, 4.2 mm; Tris-HCl buffer, pH 7.4, 50 mm; MgCl₂, 7 mm; EDTA, 4.2 mm; glucose-1,6-di-P, 0.20 μg; and enzyme solution as indicated. After incubation for 1 hour at 37°, the amount of reducing sugar in excess of that in a zero time control was determined as before. A standard curve, using glucose-6-P as a reference reducing sugar, was determined with each experiment. Orotidine 5-phosphate pyrophosphorylase activity was assayed by the spectrophotometric method of Lieberman, Kornberg, and Simms (7), in which the phosphoribomutase activities in the crude bacterial extracts were assayed by the formation of reducing sugar from the corresponding ribose-5-P as assayed by stimulation of the phosphoglucomutase reaction (Table I). Ribose-5-P was less effectively utilized; glucose-6-P and glucose-1-P were poor diphosphate precursors. Assays of the sonic extract for phosphoribomutase and phosphoglucomutase demonstrated the presence of both enzymes. Hence, it is possible that the diphosphate production from ribose-1-P involved its prior conversion to ribose-5-P. This is supported by experiments on the specificity of more purified fractions (below). All extracts of *P. saccharophila* examined showed a marked superiority (as high as 200-fold) with ribose-5-P over glucose-6-P as substrate for diphosphate production.

**TABLE I**

**Diphosphate production by crude sonic extracts of *P. saccharophila***

The conditions for the reactions (at pH 8.0) and for assay of the product are given under “Experimental Procedure.” Each incubation mixture contained 0.025 ml of crude sonic extract with a protein concentration of 26 μg per ml.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Aldose diphosphate formed</th>
<th>μmoles × 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribose-5-P</td>
<td></td>
<td>10.2</td>
</tr>
<tr>
<td>Ribose-1-P</td>
<td></td>
<td>0.840</td>
</tr>
<tr>
<td>Glucose 6-P</td>
<td></td>
<td>0.252</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td></td>
<td>0.060</td>
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</table>

**TABLE II**

**Purification of phosphoribokinase**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Protein</th>
<th>Specific activity × 10⁻³</th>
<th>Total units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial extract</td>
<td>26.3</td>
<td>0.250</td>
<td>3.89</td>
</tr>
<tr>
<td>Streptomycin, supernatant solution</td>
<td>21.8</td>
<td>0.375</td>
<td>5.19</td>
</tr>
<tr>
<td>pH 5 precipitate</td>
<td>18.3</td>
<td>1.18</td>
<td>2.53</td>
</tr>
<tr>
<td>Ammonium sulfate, 0-40%</td>
<td>9.2</td>
<td>2.22</td>
<td>2.43</td>
</tr>
<tr>
<td>Ammonium sulfate, 20-30%</td>
<td>1.7</td>
<td>9.80</td>
<td>1.90</td>
</tr>
</tbody>
</table>

**Protein**—The protein concentration in crude extracts was determined by the biuret method of Gornall, Bardawill, and Davidson (10); that in more purified fractions, by the spectrophotometric method of Warburg and Christian (10).

**Materials**—Glucose-6-P, glucose-1-P, ribose-5-P, ribose-1-P (enzymatically prepared), ATP, creatine-P, 3-phosphoglycerate acid, and phosphoglucomutase were products of Boehringer Mannheim. Glucose-1-P was freed from contaminating diphosphate as previously described (4). Authentic glucose-1,6-di-P used as a standard was obtained from Sigma, as were PP-ribose-P and ribulose 1,5-diphosphate. 1,3-Di-phosphoglyceric acid was prepared as previously described. The sample of synthetic ribose-1,5-di-P was a gift from Dr. H. G. Khorana to Dr. S. C. Hartman. Ammonium sulfate was enzyme grade reagent purchased from Mann.

**31C-Ribose-5-P** was prepared from uniformly labeled 3C-AMP (obtained from New England Nuclear) and 10 mg of carrier AMP by the procedure of Khym, Doherty, and Cohn (11), involving hydrolysis by Dowex 50 (H⁺). The barium salt of ribose-5-P obtained from this procedure was dissolved by the addition of HCl and passed through a short Dowex 50 (Na⁺) column. The eluate from the column was neutralized with NaOH and was shown to be free of starting material by the lack of absorbance at 260 μg and the absence of charcoaal adsorbable radioactivity. Upon electrophoresis at pH 3.6, the material migrated as a single spot which coincided with a standard sample of ribose-5-P.

**RESULTS**

**Diphosphate Production in Crude Sonic Extracts of *P. saccharophila***

A crude sonic extract incubated with ATP produced significant amounts of diphosphate from ribose-5-P as assayed by stimulation of the phosphoglucomutase reaction (Table I). Ribose-5-P was less effectively utilized; glucose-6-P and glucose-1-P were poor diphosphate precursors. Assays of the sonic extract for phosphoribomutase and phosphoglucomutase demonstrated the presence of both enzymes. Hence, it is possible that the diphosphate production from ribose-1-P involved its prior conversion to ribose-5-P. This is supported by experiments on the specificity of more purified fractions (below). All extracts of *P. saccharophila* examined showed a marked superiority (as high as 200-fold) with ribose-5-P over glucose-6-P as substrate for diphosphate production.

**Purification of Phosphoribokinase**

All steps (Table II) were performed at 0-4°. Centrifugation, unless otherwise specified, was at 35,000 × g for 15 min.

**Pretreatment with Streptomycin Sulfate**—To 50 ml of crude sonic extract (20 mg of protein per ml), 5.0 ml of a freshly prepared solution (10%) of streptomycin sulfate were added. After stirring for 15 min, the mixture was centrifuged and the precipitate was discarded. This step consistently resulted in a significant increase in total activity, which might indicate the removal of an inhibitor.

**Pretreatment with pH 5**—The supernatant solution from the previous step was adjusted to pH 5.0 by the careful addition of 0.2 ml acetic acid, with constant stirring. After 15 min, the supernatant solution was discarded. The precipitate was suspended in 10 ml of phosphate buffer,
0.033 M, pH 6.7, and stirred for several hours. The material could be stored overnight at this stage, if necessary. A small amount of undissolved precipitate was removed by centrifugation. The activity lost in this step (approximately 30 to 40%) could be accounted for in the pH 5 supernatant. However, conducting the precipitation at lower pH to improve the yield resulted in the precipitation of other proteins.

**First Ammonium Sulfate Fractionation**—The dissolved pH 5 precipitate was brought to 40% saturation by the addition of solid ammonium sulfate, and was stirred for 15 min. After centrifugation, the precipitate, which contained all the enzyme activity, was dissolved in 10 ml of the same phosphate buffer.

**Second Ammonium Sulfate Fractionation**—Solid ammonium sulfate was added to 20% saturation, and the solution was stirred for 15 min. The slight precipitate after centrifugation was discarded. The solution was then brought to 30% saturation with solid ammonium sulfate, stirred again for 15 min, and centrifuged. The precipitate, dissolved in 10 ml of phosphate buffer, had a specific activity about 40-fold higher than that of the crude extract. Attempts at purification by column chromatography (carboxymethylcellulose, diethylaminoethylcellulose, and Sephadex G 100) were not successful. Assays of the partially purified enzyme demonstrated no detectable phosphoribokinase activity into a product more strongly anionic than the monophosphate precursor.

**Identification of Reaction Product**

With the use of $^{14}$C-ribose-5-P, the incorporation of radioactivity into a product more strongly anionic than the monophosphate precursor was demonstrated. The procedure was a modification of one used previously to separate $^{14}$C-glucose-1-P from $^{14}$C-glucose-1,6-di-P (12). The elution pattern from a Dowex 1 (Cl⁻) column (Fig. 1) demonstrates the formation of an anionic compound in the experimental mixture which does not appear in the mixture boiled at zero time. About 20% of the radioactivity originally added was eluted in the "diphosphate" region, which corresponds closely to the amount of ribose diphosphate formed, as was deduced by the usual assay for cofactor activity.

High voltage electrophoresis of the reaction mixture resulted in three distinct zones of radioactivity (Fig. 2). The peak at the origin is unidentified, but might consist of radioactive material bound to denatured protein. The other two peaks correspond well with standards of ribose-5-P and ribose-1,5-di-P, respectively. In contrast, the control mixture completely lacked the peak corresponding to the diphosphate. When the area of the electrophoretogram corresponding to the diphosphate peak was eluted with water, cofactor activity was present in the eluate of the incubation mixture but not of the zero time control. A portion of the diluted incubation mixture was hydrolyzed in 1 N HCl at 100°C for 7 min, evaporated to dryness in a vacuum desiccator, and taken up in 0.075 ml of distilled water. This hydrolyzed material no longer exhibited cofactor activity in the phosphoglucomutase reaction. Electrophoresis of the hydrolyzed material showed complete disappearance of the peak corresponding to ribose-1,5-di-P. This indicated that the

![Fig. 1. Elution of reaction product from Dowex 1 (Cl⁻). The reaction mixtures (experimental and zero time) were standard (pH 9.1), except that they were in a volume of 1 ml and contained 0.5 μ mole (215,000 cpm) of $^{14}$C-ribose-5-P, and 170 μg of 40-fold purified enzyme. After 2 hours at 37°C the mixtures were boiled for 2 min and diluted to 5.0 ml with water. A small sample of each was removed for assay of cofactor activity by the usual method. One milliliter from each mixture was passed through a column (1 X 4 cm) of Dowex 1 (Cl⁻). The $^{14}$C-ribose-5-P precursor was eluted with 50 ml of 0.65 N HCl. Elution was continued with 1 N HCl, and 3.0-ml fractions were collected. Samples (2.0 ml) of each fraction were transferred to glass scintillation vials and evaporated to dryness. Scintillation fluid (10 ml) (12) was added to each vial, and the radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer. – - – – , experimental mixture; ● ● ● ●, control boiled at zero time.

![Fig. 2. Electrophoresis of reaction mixture. From each mixture from the experiment of Fig. 1, 3 ml were evaporated to dryness in a vacuum desiccator, and the dried material was taken up in 0.10 ml of water. For electrophoresis, 0.025 ml of each was applied in a single spot to the paper (Whatman No. 43), which had been equilibrated with buffer at pH 3.6 as previously described (3). Authentic samples of ribose-5-P (R-5-P) and ribose-1,5-di-P (R-1,5-DiP) were run concurrently. After 2 hours at 2500 volts, the paper was dried and cut lengthwise. The standards were stained for phosphate compounds by the method of Bandurski and Axelrod (13), while the strip containing the radioactive sample was counted on a Baird-Atomic chromatogram scanner. Full scale deflection within the spaces shown represents 1000 cpm.](http://www.jbc.org/content/250/11/3163 ControllerBase/pce/92962a38-65a0-45c3-abd5-5605b53907ba)
Requirements for phosphoribokinase

The conditions for the reaction and for assay of the product are the same as under "Experimental Procedure" with the following modifications. Each incubation mixture contained an ATP-generating system consisting of 6.25 μmoles of creatine phosphate and 0.05 mg of creatine kinase; the pH in this experiment was 8.0; and 0.10 μ mole of EDTA was added where indicated. Each incubation mixture contained 0.01 ml of 40-fold purified enzyme (1.7 mg of protein per ml).

<table>
<thead>
<tr>
<th>Description</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>100</td>
</tr>
<tr>
<td>-Mg++</td>
<td>88</td>
</tr>
<tr>
<td>-Mg++ + EDTA</td>
<td>0</td>
</tr>
<tr>
<td>-ATP, creatine-P, creatine kinase</td>
<td>4</td>
</tr>
<tr>
<td>-Creatine-P, creatine kinase</td>
<td>101</td>
</tr>
</tbody>
</table>

**Fig. 3.** Phosphoribokinase activity as a function of pH. The conditions for the incubation and assay are described under "Experimental Procedure." Each mixture contained 43 μg of 40-fold purified enzyme. ——, activities determined in Tris buffer, 100 mM; ---, activities determined in glycine buffer, 100 mM. The velocity represents millimicromoles per min per incubation mixture.

Requirements of Reaction

The kinase reaction showed an absolute dependence on added ATP (Table III). The omission of MgCl₂ from the incubation mixture resulted in a slight decrease in activity. EDTA caused a total inhibition. In a separate experiment (not shown), the EDTA inhibition was completely reversed by the addition of excess MgCl₂. A generating system for ATP was not beneficial with the purified preparation.

pH Studies

Maximal activity was observed at a pH of approximately 9.1, with either Tris-HCl or glycine buffer (Fig. 3). The enzyme was about twice as active in Tris-HCl as in glycine at a given pH.

Substrate Specificity

The partially purified enzyme, in contrast to the crude sonic extract, was specific for ribose-5-P as substrate. Ribose-1-P, glucose-6-P, and glucose-1-P were inactive. Similarly, deoxyribose 5-phosphate failed to generate a product that would stimulate the phosphoglucomutase reaction. Moreover, the presence of deoxyribose 5-phosphate in the incubation mixture did not inhibit diphosphate formation from ribose-5-P. Experiments with purified materials also demonstrated that neither PP-ribose-P nor ribulose 1,5-diphosphate could stimulate the phosphoglucomutase reaction.

Various nucleotides were substituted for ATP in the reaction as shown in Table IV. Of those tested, only adenosine tetraphosphate showed activity, but this may have been due to breakdown to ATP.

Kinetic Studies

Product was formed linearly with time for a period of at least 90 min (Fig. 4). Linearity with enzyme concentration was exhibited similarly. Concentrations of ribose-5-P of 2 mM or higher were inhibitory. In a double reciprocal plot, the apparent Kₘ for ribose-5-P at pH 9.1 was about 1 mM (Fig. 5A).

A similar plot in which the ATP concentration was varied yielded comparable results (Fig. 5B). The apparent Kₘ for ATP was about 1 mM; concentrations higher than 2 mM were inhibitory. The same results were obtained whether the ATP

1 No evidence is available whether deoxyribose 1,5-diphosphate, if produced, would in fact stimulate the phosphoglucomutase reaction.
concentration was varied alone or both ATP and Mg^{++} were varied together to maintain a constant ratio.

**Effects of Phosphoglycerates**

The effect of phosphoglycerates was tested in view of the findings from this laboratory (12, 14) that the accumulation of glucose-1,6-di-P with phosphoglucomutase in the presence of glucose-1-P is accelerated by the addition of 1,3-diphosphoglycerate (or of a 1,3-diphosphoglycerate-generating system), which phosphorylates the enzyme. The presence of 3-phosphoglycerate did not affect the production of diphosphate from ribose-5-P by a crude sonic extract, while it caused a 3-fold increase in diphosphate production from glucose-6-P (Table V). Similarly, neither 3-phosphoglycerate nor 1,3-diphosphoglycerate affected ribose diphosphate production when the partially purified enzyme was used.

**Stability**

The partially purified enzyme was stored at -56° in 0.033 M sodium phosphate buffer, pH 7.0, and thawed immediately before each use. Activity declined to about 25% over a period of 6 months.

**Table V**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Aldose diphosphate formed (μmoles × 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude sonic extract</td>
<td>10.8</td>
</tr>
<tr>
<td>+ 3-Phosphoglycerate</td>
<td>10.8</td>
</tr>
<tr>
<td>Purified enzyme</td>
<td>6.19</td>
</tr>
<tr>
<td>+ 3-Phosphoglycerate</td>
<td>5.94</td>
</tr>
<tr>
<td>+ 1,3-Diphosphoglycerate</td>
<td>0.19</td>
</tr>
</tbody>
</table>

**Table VI**

**Orotidine 5-phosphate pyrophosphorylase activity of P. saccharophila**

The assay procedure is described under "Experimental Procedure." The concentration of PP-ribose-P or ribose diphosphate, or both, was 0.08 mg per ml. The reaction was initiated by the addition of 0.01 ml of crude sonic extract with a protein concentration of 28.0 μg per ml. Enzyme activity was calculated from the initial reaction rate, and the absorbance decrease was measured at 255 μm after conversion of orotate to UMP, with a molar absorption coefficient of 3050 used for the reaction (7). A unit of activity is defined as the amount of enzyme causing the removal of 1 μmole of orotate per hour (7).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP-ribose-P</td>
<td>0.95</td>
</tr>
<tr>
<td>Ribose-1,5-di-P (synthetic)</td>
<td>0</td>
</tr>
<tr>
<td>Ribose-1,5-di-P (from kinase reaction)</td>
<td>0</td>
</tr>
<tr>
<td>PP-ribose-P + ribose-1,5-di-P (synthetic)</td>
<td>1.04</td>
</tr>
</tbody>
</table>

**Riboce Diphosphate As Possible Phosphoribosyl Donor**

One possible function for the ribose diphosphate formed in the kinase reaction would involve transfer of the phosphoribosyl group to an acceptor molecule as originally proposed by Safran and Scarano (15) for the production of AMP from adenine. Two reactions of this type were investigated, in which ribose diphosphate might function like PP-ribose-P.

The first involved the production of orotidine monophosphate from orotic acid, as with the enzyme orotidine 5-phosphate pyrophosphorylase (16). The results (Table VI) indicated that crude sonic extracts of Pseudomonas contained considerable orotidine 5-phosphate pyrophosphorylase activity, but that ribose diphosphate (either synthetic or prepared with phosphoribokinase) could not substitute for the PP-ribose-P as reactant. Further, ribose 1,5 di P did not act as an inhibitor of the enzyme when both compounds were present simultaneously.

The second reaction was the formation of AMP from adenine, as with the enzyme adenosine 5-phosphate pyrophosphorylase.
The assay involved incubation with 14C-adenine and separation of the more anionic product on a Dowex 1 (Cl−) column. In this case, product formation was not observed with either PP-ribose-P or ribose-1,5-di-P, indicating that the crude sonicate of P. saccharophila lacked the enzyme adenosine 5-phosphate pyrophosphorylase.

**Growth Experiments**

The organism was grown in the presence of a number of substrates in an attempt to influence the level of enzyme activity. In two experiments, glucose or pyruvate (92.5%) were substituted for sucrose as the sole carbon source. Other experiments involved growing the organism on 0.25% sucrose to which was added 0.05% of the substrate to be tested. Compounds tested in this way were ribose, inosine, adenosine, adenine, uridine, and uracil. In all cases, the enzyme activity of the crude sonic extract was compared with that of a sonic extract tested in this way were ribose, inosine, adenosine, adenine, uridine, and uracil. In all cases, the enzyme activity of the crude sonic extract was compared with that of a sonic extract from a replicate culture grown with sucrose as the sole carbon source. These compounds did not influence the activity of phosphoribokinase or of phosphoribomutase, phosphoglucomutase, or phosphoglucookinase in crude extracts.

**DISCUSSION**

Although crude extracts of P. saccharophila can utilize either ribose-5-P or ribose-1-P as a diphosphate precursor, the reaction with the latter compound almost certainly proceeds through the phosphoribomutase reaction, since the partially purified kinase is specific for ribose 5-phosphate.

This production of ribose-1,5-di-P is independent of added 3-phosphoglycerate, although the production of glucose-1,6-di-P by the crude sonic extract is stimulated by 3-phosphoglycerate. Such a stimulation by phosphoglyceric acid has been reported previously by Levey and Alpers (4) in rat liver, and has been shown to be mediated by effects of 1,3-diphosphoglycerate on the phosphoglucomutase reaction (12, 14). The production of ribose-1,5-di-P in a partially purified preparation where mutase enzymes have been shown to be absent, and where phosphoglycerates are without effect, is clearly by an entirely different process.

The role of the phosphoribokinase described here is not clear in spite of the observation that ribose-5-P is a better precursor of diphosphate than glucose-6-P in crude extracts. The possibility that ribose-1,5-di-P might act as a phosphoribosyl donor has been discounted by experiments which demonstrated that it could not replace PP-ribose-P in the production of orotidine 5-phosphate from orotic acid in the presence of Pseudomonas sonicate extracts, nor did it react with adenine to form the corresponding mononucleotide.

There are other reactions involving PP-ribose-P which should also be investigated. It may be noted in this connection that for at least one well known PP-ribose-P mediated reaction, an alternative path has been recently detected. The production of 5-phosphoribosylamine, an early intermediate of the purine biosynthetic path, has now been described from ribose-5-P and ammonia, catalyzed by an hepatic enzyme (17), whereas it was previously considered that this intermediate could arise only from the interaction of PP-ribose-P and glutamine.

A likely role for the diphosphate produced would be to function in the conversion of ribose-5-P to ribose-1-P via the enzyme phosphoribomutase. Assays of crude sonic extract have demonstrated the presence of phosphoribomutase activity, and this appears to be distinct from phosphoglucomutase. The quantity of phosphoribomutase activity was found to vary greatly in various Pseudomonas extracts, due to factors that have not been elucidated. Such variation is not altogether unexpected; in the case of glucose-1,6-di-P, the correlation with the activity of phosphoglucomutase in tissues is extremely variable, i.e. the ratio of mutase activity to the concentration of glucose diphosphate shows large variation from tissue to tissue (18).

The existence of a phosphoribomutase which was distinct from phosphoglucomutase was first reported by Guarino and Sable (19) in extracts of yeast, uterine muscle, and human blood hemolysates. The enzyme from bovine uterus was purified 30-fold and was found to be stimulated by ribose-1,5-di-P (20). Vanderheiden (21) has isolated and identified ribose-1,5-di-P from human erythrocytes, where it is likely to play a similar role. Since it has been demonstrated that P. saccharophila can readily obtain ribose 5-phosphate via the pentose monophosphate shunt (22), the mutase reaction might rather serve for production of ribose-1-phosphate.

One possible fate for ribose-1-P involves its reaction with a nucleoside triphosphate to form an "activated" derivative of ribose. Initial experiments have been performed in which 14C-ribose-5-P, incubated with phosphoribomutase, crude P. saccharophila extract, and thymidine triphosphate formed a product which was charcoal-adsorbable and acid-labile. The exact nature of the product is currently under investigation.

**REFERENCES**

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1 L. R. DeChatelet and J. B. Alpers, unpublished experiments.
Phosphoribokinase from *Pseudomonas saccharophila*
Lawrence R. DeChatelet and Joseph B. Alpers


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