A Kinetic Study of the Effect of \( \alpha \)-D-Galactose, \( \alpha \)-D-Mannose, \( \alpha \)-D-Glucosamine, \( N \)-Acetyl-\( \alpha \)-D-glucosamine, and \( \alpha \)-D-Ribose Diphosphate on the Activity of Phosphoglucomutase*  

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SUMMARY  

The specificity of phosphoglucomutase for various \( \alpha \)-D-sugar diphosphates was studied by examining the relative ability of these compounds to activate the dephosphorylated form of the enzyme prepared from rabbit muscle. The rate of the reaction measured spectrophotometrically at 340 m\( \mu \) with a coupled assay system containing TPN\(^+\) and excess D-glucose 6-phosphate dehydrogenase was linear with time only when the incubation was carried out in the presence of \( \alpha \)-D-glucose 1,6-diphosphate. When other sugar diphosphates were used, the rate of disappearance of \( \alpha \)-D-glucose 1-phosphate increased with time until a maximum linear rate was attained. The final rate, in each case, was dependent on the concentration of \( \alpha \)-D-sugar diphosphate added to the incubation mixture, but it was independent of the order of addition of reactants and the concentration of components of the coupled assay system. The apparent \( K_m \) values were as follows: \( \alpha \)-D-glucose 1,6-diphosphate, 0.1 \( \mu \)M; \( \alpha \)-D-mannose 1,6-diphosphate, 3.7 \( \mu \)M; \( \alpha \)-D-ribose 1,5-diphosphate, 3.2 \( \mu \)M; \( \alpha \)-D-glucosamine 1,6-diphosphate, 11 \( \mu \)M; \( N \)-acetyl-\( \alpha \)-D-glucosamine 1,6-diphosphate, 1.6 \( \mu \)M; \( \alpha \)-D-galactose 1,6-diphosphate, 13 \( \mu \)M. The most effective activator of phosphoglucomutase was \( \alpha \)-D-glucose 1,6-diphosphate. The \( K_m \) values of \( \alpha \)-D-galactose 1,6-diphosphate and \( \alpha \)-D-glucosamine 1,6-diphosphate, the poorest activators, were about 120-fold greater than that of \( \alpha \)-D-glucose 1,6-diphosphate. The maximum velocity varied only 2- to 3-fold when different sugar diphosphates were used to activate the reaction. The following maximum velocities, expressed as micromoles min\(^{-1}\) mg\(^{-1}\), were obtained: \( \alpha \)-D-glucose 1,6-diphosphate, 500; \( \alpha \)-D-mannose 1,6-diphosphate, 360; \( \alpha \)-D-ribose 1,5-diphosphate, 210; \( \alpha \)-D-glucosamine 1,6-diphosphate, 370; \( N \)-acetyl-\( \alpha \)-D-glucosamine 1,6-diphosphate, 220; \( \alpha \)-D-galactose 1,6-diphosphate, 240. Addition of the appropriate \( \alpha \)-D-sugar 1-phosphate or \( \alpha \)-D-sugar 6-phosphate decreased the velocity of the reaction being maintained by either \( \alpha \)-D-sugar 1,6-diphosphate or \( \alpha \)-D-glucose 1,6-diphosphate. The findings reported are consistent with and support a mechanism in which each of the sugar diphosphates combines with dephosphoenzyme to form a sugar diphosphate-enzyme complex which then further reacts to yield a phosphenzyme intermediate and free sugar monophosphate.  

The activation of phosphoglucomutase by \( \alpha \)-D-glucose 1,6-diphosphate was first demonstrated by Leloir et al. (2). The role of the sugar diphosphate in the reversible interconversion of \( \alpha \)-D-glucose 1-phosphate and D-glucose 6-phosphate and the intermittent formation of a phosphorylated enzyme were shown by Najjar and Pullman (3). They proposed a mechanism in which the phosphorylated enzyme reacted with a sugar monophosphate to yield dephosphoenzyme and \( \alpha \)-D-glucose 1,6-diphosphate. The inactive dephosphoenzyme was regenerated by the transfer of a phosphate group from either position of \( \alpha \)-D-glucose 1,6-diphosphate with the concomitant formation of \( D \) glucose 6-phosphate or \( \alpha \)-D-glucose 1-phosphate (3, 4). It is not yet certain whether the form of phosphoenzyme which reacts with \( \alpha \)-D-glucose 1-phosphate is the same as that which reacts with D-glucose 6-phosphate, and it is possible that two distinct forms of phosphenzyme exist.  

Phosphorylated and dephosphorylated forms of the enzyme have been isolated by Yankeelov, Horton, and Kosshland (5). These workers labeled phosphoglucomutase with a mixture of \( \alpha \)-D-glucose-\( 1\)-\(^32\)P and D-glucose-\( 6\)-\(^32\)P, and they were able to separate two radioactive peaks containing enzyme activity by chromatography on modified cellulose columns. The active fractions contained 1 mole of phosphate per mole of enzyme. Recently, Harshman, Bocchini, and Najjar (6) isolated a phosphopeptide from phosphoglucomutase which contains 2 serine residues, both of which can be labeled with \( \alpha \)-D-glucose-\( 1\)-\(^32\)P and D-glucose-\( 6\)-\(^32\)P. Joshi et al. (7) and Joshi and Handler (8) have also isolated two forms of phosphoglucomutase from rabbit, shark, flounder, and human muscle. The different forms in some species appear to be isozymes, since they are similar in molecular weight and catalytic properties, but they differ in charge and amino acid sequence. The amino acid sequence of the peptide containing the active serine was found to be identical in the case of human, rabbit, and flounder phosphoglucomutase (8, 9).  

Ray and Roscelli concluded from their kinetic studies on phosphoglucomutase that the most probable mechanism was one in  

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which there is only one form of phosphoenzyme that can react with either of the d-glucose monophosphate esters (4, 10). The results of their studies with ^32P-labeled substrates indicated that free \( \alpha-D \)-glucose 1,6-diphosphate was not an obligatory intermediate in the interconversion of sugar monophosphates. They estimated that the rate of turnover of free \( \alpha-D \)-glucose 1,6-diphosphate was only one-twentieth of the rate of interconversion of the \( \alpha \)-glucose monophosphates. More recent studies by Lowry and Passonneau indicate that this ratio may be as high as one-sixtieth (11). Phosphoglucomutase will catalyze the conversion of sugar 1-phosphates to sugar diphosphates in the presence of \( \alpha-D \)-glucose 1,6-diphosphate. Thus, \( \alpha-D \)-mannose 1-phosphate was converted to \( \alpha-D \)-mannose 1,6-diphosphate (12), \( \alpha-D \)-ribose 1-phosphate and \( \alpha-D \)-galactose 1-phosphate were converted to the corresponding sugar diphosphates (13), and \( D \)-fructose 1-phosphate was converted to \( D \)-fructose 1,6-diphosphate by this enzyme (11). Various sugar diphosphates can also be used to activate phosphoglucomutase; however, the specificity of the enzyme for different sugar diphosphates has not yet been examined.

The present communication reports studies relating to the mechanism of interaction of phosphoglucomutase with the \( \alpha \) anomers of \( D \)-glucose 1,6, \( D \)-galactose 1,6, \( D \)-glucosamine 1,6, \( N \)-acetetyl-\( \alpha-D \)-glucosamine 1,6, \( \alpha \)-mannose 1,6-, and \( D \)-ribose 1,5-diphosphate.

**EXPERIMENTAL PROCEDURE**

**Assay of Phosphotransferase Activity**—The spectrophotometric assay for phosphoglucomutase activity was based on the rate of conversion of \( \alpha-D \)-glucose-1-P to \( D \)-glucose-6-P in the presence of excess \( \beta-D \)-glucose-6-P dehydrogenase and TPN\(^+\) (14). The standard reaction mixture was incubated at 25\(^\circ\)C and contained in a final volume of 1 ml: 25 mM imidazole buffer (pH 7.0), 0.5 mM TPN\(^+\), 5 mM MgCl\(_2\), 1 mM EDTA, 0.005% bovine serum albumin, 6.4 \( \mu \)g of \( \beta-D \)-glucose-6-P dehydrogenase, 8.9 \( \times \)10\(^{-4}\) \( \mu \)M phosphoglucomutase, 250 \( \mu \)M \( \alpha-D \)-glucose-1-P, and varying amounts of \( \alpha-D \)-sugar diphosphate. Phosphoglucomutase was first incubated for 10 min at 3\(^\circ\)C in a solution containing 0.05 mM imidazole buffer (pH 7.0), 10 mM MgCl\(_2\), 2 mM EDTA, and 0.01% bovine serum albumin just before it was added to the reaction mixture (15). The reaction was initiated by the addition of \( \alpha-D \)-glucose-1-P or \( \alpha-D \)-sugar diphosphate, and the increase in absorbance at 340 nm was measured with a Gilford recording spectrophotometer. Glucose 6 P dehydrogenase was obtained from Calbiochem, and phosphoglucomutase was purchased from Sigma and it was further purified (5). One unit of activity is defined as the amount of enzyme required to convert 1 \( \mu \) mole of \( \alpha-D \)-glucose-1-P to \( D \)-glucose-6-P per min at 25\(^\circ\)C under the standard assay conditions. Specific activity is expressed as units per mg of protein. The concentration of protein was determined colorimetrically (16) and spectrophotometrically at 278 nm with an absorbance index of 7.7 for a 1% solution (17).

**Methods and Materials**—\( \alpha-D \)-Sugar diphosphates were prepared from acetylated sugar monophosphates by condensation with crystalline phosphoric acid as described by Hanna and Mendicino in the preceding communication (18). The analysis of these compounds indicated a ratio of acid-labile phosphate to acid-labile reducing sugar to total phosphate of 1:1:2, within the experimental error of the methods of assay used. The \( \alpha-D \)-sugar diphosphates and the products formed on acid hydrolysis of these compounds were chromatographically pure in each case. However, a very small amount of contamination by \( \alpha-D \)-glucose-1,6-di-P, not detectable by the colorimetric and chromatographic procedures used, could seriously interfere with the assay of the other \( \alpha-D \)-sugar diphosphates. Therefore, each of the samples was hydrolyzed and assayed spectrophotometrically with \( D \)-glucose-6-P dehydrogenase and TPN\(^+\) in order to determine whether a trace amount of \( \alpha-D \)-glucose 1,6-diphosphate was present.

\( D \)-Glucose-6-P, \( \alpha-D \)-glucose-1-P, \( \alpha-D \)-ribose-1-P, \( \beta-D \)-ribose-1-P, and \( \alpha-D \)-ribose-5-P were purchased from Sigma and Calbiochem. \( \alpha-D \)-Mannose-1-P, \( \alpha-D \)-galactose-1-P, and \( N \)-acetetyl-\( \alpha-D \)-glucosamine-1-P were synthesized from the corresponding pentacetates by a modification of the procedure of MacDonald (19). \( D \)-Mannose-6-P and \( D \)-glucosamine-6-P were prepared by phosphorylation of \( \alpha-D \)-mannose and \( D \)-glucosamine with ATP and crystalline yeast hexokinase (20). The \( D \)-glucosamine-6-P was \( N \)-acetetylated with acetic anhydride (21) and purified by ion exchange chromatography. All of the sugar monophosphate esters were completely freed from small amounts of \( D \)-glucose-6-P, \( \alpha-D \)-glucose-1,6-di-P, and any other diphosphate compounds by the following procedure. Approximately 500 \( \mu \)mole of the sugar monophosphate were incubated with \( D \)-glucose-6-P dehydrogenase and TPN\(^+\) to oxidize any \( D \)-glucose-6-P to 6-P-gluconic acid. The TPN\(^+\), TPNH, and 6-P-gluconic acid present in this reaction mixture were retained on the anion exchange column with other diphosphate esters. The incubation mixture was passed through a Dowex 1-Cl column (2 cm \( \times \)3 cm) and the column was washed with 200 ml of water. The sugar monophosphate ester was then eluted with 0.01 N HCl, and the eluant was neutralized with LiO\(_2\)H and concentrated under reduced pressure. The \( \alpha-D \)-ribose-1-P and \( \beta-D \)-ribose-1-P, which were somewhat labile in dilute acid, were eluted from the column with 0.05 M LiCl. The compounds were applied to washed Whatman No. 3MM paper and LiCl was removed by paper chromatography with a solvent containing ethanol and acetone (2:8). After development for 20 hours with this solvent, the band containing LiCl was well separated from the sugar phosphate ester. The lithium salt of the monophosphate ester was eluted from the paper with distilled water and the solution was passed through a Dowex 50-H\(^+\) column (2 cm \( \times \)2 cm). The phosphate ester, in each case, was converted to the imidazole salt by neutralizing the free acid with imidazole to pH 7.0.

Inorganic phosphate and acid-labile phosphate were determined by the method of Fiske and SubbaRow (22), and total phosphate by the procedure of King (23). Reducing sugars were analyzed by the method of Somogyi and Nelson (24, 25), and for small quantities the Park and Johnson (26) assay was used. Glucosamine and \( N \)-acetetyl-\( D \)-glucoseamine and their phosphate esters were assayed by a modification of the procedure of Morgan and Elson (21, 27).

**RESULTS**

**Influence of \( \alpha-D \)-Glucose-1,6-di-P on Activity of Phosphoglucomutase**—Some phosphotransferase activity was observed in the absence of added \( \alpha-D \)-glucose-1,6-di-P, even when highly purified \( \alpha-D \)-glucose-1-P was added. The \( \alpha-D \)-glucose-1-P used in these studies was completely freed of sugar diphosphate by chromatography on Dowex 1-Cl columns as described in the previous section. The residual activity of the enzyme in the absence of...
α-D-glucose-1,6-di-P was less than 5% of the activity which could be obtained under similar conditions in the presence of the sugar diphosphate. The velocity was 0.24 nmole per min with 8.9 \times 10^{-4} \mu M enzyme and 250 \mu M α-D-glucose-1-P in the absence of α-D-glucose-1,6-di-P, and it was 5.34 nmole per min in the presence of 0.046 μM α-D-glucose-1,6-di-P. The velocity in the absence of α-D-glucose-1,6-di-P was strictly dependent on the concentration of enzyme. Under the standard assay conditions an activity of 0.24 μmole per min was obtained with 8.9 \times 10^{-4} \mu M enzyme and the velocity increased 10-fold (2.48 nmole per min) when 8.9 \times 10^{-3} \mu M enzyme was added. In order to eliminate the possibility that this residual activity was due to contamination of the enzyme preparation with α-D-glucose-1,6-diphosphate, the solution was slowly passed through a Dowex 1-imidazole column to exchange any anions present. After chromatography no residual activity was observed. The possible presence of tightly bound α-D-glucose-1,6-di-P in the enzyme preparation was examined by denaturing 8.9 \times 10^{-4} \mu M of enzyme with trichloroacetic acid at 3°. The resulting suspension was centrifuged and the supernatant was extracted with ether to remove trichloroacetic acid. The solution was then neutralized and concentrated under vacuum.

The reaction did not increase when this sample was added to the standard assay mixture in the absence of α-D-glucose-1,6-di-P. Since an extract prepared from 10 times the amount of enzyme normally used in the assay failed to activate the reaction, it is likely that the enzyme preparation is free of α-D-glucose-1,6-di-P. The residual activity could be due to the presence of some active phosphoenzyme in the preparation. However, when the enzyme was treated with p-glucose-6-P or α-D-glucose-1-P according to the procedure of Najjar and Pullman (3) to remove the phosphorylated form of phosphoglucomutase, the same level of residual activity was found. The α-D-glucose-1-P and p-glucose-6-P used to inactivate the enzyme preparation were completely free of α-D-sugar diphosphate esters, and, therefore, the persistence of a small amount of residual activity could not be attributed to a contaminant in these preparations which reactivated some of the enzyme. The final preparation was considered suitable for the present study since at low enzyme concentrations only a very small activity was observed in the absence of added α-D-sugar diphosphates.

The effect of the concentration of α-D-glucose-1-P on the activity of the enzyme was determined over a range of 0.25 mM to 15 mM α-D-glucose-1-P and 1.2 mM to 36 mM α-D-glucose-1,6-di-P (Fig. 1). Substrate inhibition was observed, especially at high concentrations of α-D-glucose-1-P. The apparent K_m for α-D-glucose-1,6-di-P in the presence of 15, 10, and 5 mM α-D-glucose-1-P was 25, 13.7, and 9.1 μM, respectively. There was little or no inhibition at low concentrations of α-D-glucose-1-P. Similar plots of 1/V against 1/α-D-glucose-1,6-di-P were obtained under these conditions with 0.25 mM and 0.5 mM α-D-glucose-1-P, as shown in Fig. 1. The apparent K_m for α-D-glucose-1,6-di-P at 0.25 mM α-D-glucose-1-P was calculated to be 0.1 μM. The activity and the dependence on α-D-glucose-1,6-di-P were strictly proportional to the concentration of enzyme. It should be noted that the inhibition of the enzyme by high concentrations of magnesium ion, caused by the formation of inactive magnesium complexes of α-D-glucose-1-P and the sugar diphosphates, was also avoided in the present studies by activating the enzyme prior to the assay and by using low concentrations of magnesium ion relative to the amount of phosphate esters present (28, 30). The rate of the phosphoglucomutase reaction in this assay system may also be limited by the fact that D-glucose-1-P dehydrogenase is specific for D-glucose-6-P, whereas α-D-glucose-6-P is formed in the phosphoglucomutase reaction (29). It has been noted that the addition of P-glucose isomerase, a mutarotase for the α and β forms of p-glucose-6-P, accelerates the rate of TPNH formation in the coupled assay by at least 4-fold (11, 20), presumably by increasing the spontaneous rate of anomerization of α-D-glucose-6-P to β-D-glucose 6-P. However, the addition of P-glucose isomerase to the assay system used in these studies did not accelerate the rate of reduction of TPNH. The components of the assay system used in the present studies were adjusted so that the rate-limiting factor was the concentration of the sugar diphosphate added.

**Activity of Phosphoglucomutase in Presence of α-D-Mannose-1,6-di-P**—The K_m of phosphoglucomutase for α-D-mannose-1,6-di-P has been reported to be about the same as that for α-D-glucose-1,6-di-P (31). The effect of α-D-mannose-1,6-di-P concentration on the enzyme activity was determined over a range of 0.46 μM to 6.9 μM (Fig. 2). An interesting characteristic of the system, when phosphoglucomutase was activated with any sugar diphosphate other than α-D-glucose-1,6-diphosphate, was the increase in velocity for approximately 10 min before a steady state was reached. As seen in Fig. 2, the rate of increase as well as the final velocity attained was dependent on the concentration of α-D-mannose-1,6-di-P. The velocity observed after the 1st min also increased with increasing concentrations of α-D-mannose-1,6-di-P. The same curves were obtained when the reaction was initiated with either α-D-mannose-1,6-di-P or α-D-glucose-1,6-di-P. Preliminary incubation of the reaction mixture for periods of up to 20 min before the addition of α-D-glucose-1-P or α-D-mannose-1,6-di-P had no effect on the velocity observed in the 1st min. Moreover, this treatment did not affect the rate of change of the velocity or the final velocity attained at each concentration of α-D-mannose-1,6-di-P.

The double reciprocal plot of the data, based on the maximal
FIG. 2. Influence of α-D-mannose-1,6-di-P concentration on the rate of the phosphoglucomutase reaction. The incubation mixture contained in 1 ml: 25 mM imidazole-HCl (pH 7.0), 5 mM MgCl₂, 1 mM EDTA, 0.005% bovine serum albumin, 0.5 mM TPN⁺, 6.4 µg of glucose-6-P dehydrogenase, 8.9 X 10⁻⁴ PM phosphoglucomutase, and varying amounts of the imidazole salt of α-D-mannose-1,6-di-P. The reaction was initiated by the addition of 0.25 µmole of the imidazole salt of α-D-glucose-1-P. The rate of the reaction was measured for 15 min; however, the rate was nearly constant after 10 min.

velocities attained at corresponding concentrations of α-D-mannose-1,6-di-P, is shown in Fig. 3. The apparent Kₘ and maximum velocity for α-D-mannose-1,6-di-P, calculated from the data by the method of least squares, was 3.7 X 10⁻⁴ m and 360 µmoles per min per mg, respectively (Table I). Thus, under identical conditions the Kₘ of muscle phosphoglucomutase for α-D-mannose-1,6-di-P was at least 40-fold higher than that of α-D-glucose 1,6-diphosphate. In order to be certain that the activity observed with α-D-mannose-1,6-di-P was not due to contamination of this preparation with α-D-glucose-1,6-di-P, 3 pmol of α-D-mannose-1,6-di-P were hydrolyzed with 0.1 N HCl at 100°C for 10 min and analyzed as described previously. No D-glucose-6-P was detected when the sample was assayed in the presence of TPN⁺ and glucose-6-P dehydrogenase. A small amount of D-glucose-6-P added to the cuvette after the sample was assayed resulted in the reduction of an equivalent amount of TPN⁺. Thus, a negative test in this assay could not be attributed to an inhibition of D-glucose-6-P dehydrogenase by products formed upon acid hydrolysis of α-D-mannose-1,6-di-P. As little as 0.02 µmole of D-glucose-6-P added to the mixture after the sample was assayed resulted in the reduction of an equivalent amount of TPN⁺.

Table I

| α-D-Sugar diphosphate          | Kₘ (µM) | Vₘax (µmoles/min/mg) |
|--------------------------------|--------|----------------------|
| Glucose-1,6-di-P               | 0.1    | 500                  |
| N-Acetyl-D-glucosamine-1,6-di-P| 1.6    | 220                  |
| Ribose-1,5-di-P                | 3.2    | 210                  |
| Mannose-1,6-di-P               | 3.7    | 300                  |
| Glucosamine-1,6-di-P           | 11     | 370                  |
| Galactose-1,6-di-P             | 13     | 240                  |

As little as 0.02 µmole of D-glucose-6-P added to the mixture after the sample was assayed resulted in the reduction of an equivalent amount of TPN⁺.

If α-D-mannose-1,6-di-P reacted with phosphoglucomutase to form a phosphorylated enzyme, then α-D-mannose-1-P and α-D-mannose-6-P should also be formed. Furthermore, the addition of these compounds should influence the rate of the reaction by suppressing the formation of phosphoenzyme. The influence of α-D-mannose-1-P and d-mannose-6-P on the rate of the phosphoglucomutase reaction in the presence of α-D-glucose-1,6-di-P and α-D-mannose-1,6-di-P was examined and the results are summarized in Fig. 4. It may be noted that a maximal velocity was obtained immediately with α-D-glucose-1,6-di-P, whereas a slow increase in velocity is observed for 10 min with α-D-mannose-1,6-di-P. As shown in Table II, the addition of α-D-mannose-1-P to the reaction mixture containing α-D-glucose-1,6-di-P decreased the velocity by 1.9 nmoles per min in less than 3 min. When D-mannose-6-P was added to the reaction mixture, the velocity decreased by 1.4 nmoles per min. A new lower steady state was attained in each case. The addition of α-D-mannose-1-P to a reaction mixture containing 0.46 µM α-D-mannose-1,6-di-P decreased the velocity by 1.9 nmoles per min, whereas adding d-mannose-6-P decreased the rate of the reaction by 1.5 nmoles per min. The addition of d-mannose-6-P decreased the velocity by approximately 50% (1.4:2.7 and 1.5:2.7) when a velocity of 2.7 nmoles per min was being maintained by either
ol-n-mannose-1-P or 1.46 pmoles of the imidazole salt of n-mannose-1-P would result in the formation of phosphoenzyme with a-n-sugar 1,6-diphosphate before the rate-limiting intermediate as cu-n-glucose-1,6-diphosphate. On the basis of evidence presently available, the reaction of de- 

appears that about 12 times (0.46 : 0.039) as much ar-n-mannose-1-P as or-n-glucose-1-P was added (with a 20% difference in the final rate of the reaction), these results would suggest that the same intermediates are formed from both sugar phosphates, and that the sugar monophosphates influence the steady state concentrations of these intermediates to the same extent. It appears that about 12 times (0.46:0.039) as much ar-n-mannose-1,6-di-P as or-n-glucose-1,6-di-P is required to maintain the same concentration of the rate-limiting intermediate as or-n-glucose-1,6-diphosphate. On the basis of evidence presently available, the reaction of dephosphoenzyme with ar-n-sugar 1,6-diphosphate before the addition of ar-n-glucose-1-P would result in the formation of sugar-1-P, sugar-6-P, and various derivatives of the enzyme represented as E-sugar 1,6-diphosphate and EP (Reactions 1 to 3).

\[
2E + 2 \text{sugar-1,6-di-P} \rightleftharpoons 2E \cdot \text{sugar-1,6-di-P} \quad (1)
\]

\[
E \cdot \text{sugar-1,6-di-P} \rightleftharpoons EP + \text{sugar-1-P} \quad (2)
\]

\[
E \cdot \text{sugar-1,6-di-P} \rightleftharpoons EP + \text{sugar-6-P} \quad (3)
\]

\[
2E + 2 \text{sugar-1,6-di-P} = 2[EP] + \text{sugar-1-P} + \text{sugar-6-P} \quad \text{(Sum 1-3)}
\]

The over-all equilibrium between the reactants would be dependent on the concentration of the components shown as Sum 1-3. The initial rate observed in the experiments described in Fig. 2 may depend on the amount of EP formed before ar-n-glucose-1-P is added. After the addition of ar-n-glucose-1-P the ar-n-glucose-6-P formed in Reactions 4 and 5 would be removed by the action of glucose-6-P dehydrogenase. The rate of conversion of ar-n-glucose-1-P to ar-n-glucose-6-P was adversely influenced by in-

| Sugar monophosphates | Sugar monophosphate added | Velocity |
|---------------------|---------------------------|----------|
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.5     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.2     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.2     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 0.3     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.1     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.8     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.2     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.1     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.8     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.2     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.1     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.7     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.8     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.8     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 2.4     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 1.9     |
| ar-glucone-6-P        | ar-glucone-1-P (E, P)     | 3.8     |
creasing concentrations of sugar-1-P and sugar-6-P. The rate of the reaction should increase with increasing concentrations of α-D-glucose-1-P and α-D-sugar-1,6-di-P. In other experiments, not shown, it was shown that increasing concentrations of α-D-glucose-1-P and α-D-glucose-1,6-di-P or α-D-mannose-1,6-di-P increased the velocity of the reaction in the presence of the sugar monophosphates.

The possibility of forming α-D-glucose-1,6-di-P by the reactions shown below in Equations 6 and 7 should also be considered. It is unlikely that there is very much of this conversion occurring under these conditions, since glucose-6-P dehydrogenase rapidly removes D-glucose-6-P formed in Reaction 5 and thereby competes very favorably with Reaction 7 for any glucose-1-P.E.P formed in Reaction 4. Ray and Roscelli (4) and Lowry and Passonneau (11) have estimated that the ratio of the rate of dissociation of α-D-glucose-1,6-di-P from the enzyme (Reaction 7) to the rate of formation of D-glucose-6-P (Reaction 5) is probably less than one-sixtieth.

Glucose-1-P.E.P $\rightarrow$ Glucose-1,6-di-P

E.Glucose-1,6-di-P $\rightarrow$ E + Glucose-1,6-di-P

Influence of α-D-Ribose-1,5-di-P on Activity of Phosphoglucomutase Phosphoglucomutase was activated by α-D ribose 1,5-di-P and curves similar to those shown in Fig. 2 were also obtained with this sugar diphosphate. Preliminary incubation of the enzyme with α-D-glucose-1-P or α-D-ribose-1,5-di-P did not alter the initial velocity observed or the final velocity attained in this system. The same curves were obtained when the reaction was initiated with either α-D-glucose-1-P or α-D-ribose-1,5-di-P. However, both the initial rate and final velocity increased with increasing concentrations of α-D-ribose-1,5-di-P. When the concentration of the sugar diphosphate was varied from 1.59 $\mu$M to 7.95 $\mu$M the maximum velocity increased from 5.1 nmoles per min to 9.2 nmoles per min. The apparent $K_m$ and maximum velocity, calculated from plots of 1/velocity against l/substrate, were 3.16 x 10$^{-4}$ M and 210 nmoles min$^{-1}$ mg$^{-1}$, respectively (Table I). The $K_m$ for α-D-ribose-1,5-di-P was approximately 35 times greater than the $K_m$ for α-D-glucose-1,6-di-P under these conditions.

In order to establish that the extent of activation of the dephosphoenzyme by α-D-ribose-1,5-di-P was related to the amount of d-ribose monophosphates formed in the reaction, experiments were carried out to examine the effects of α-D-ribose-1-P and d-ribose-5-P on the velocity of the reaction in the presence of sugar diphosphates. The results of experiments designed to compare the effects of d-ribose monophosphate esters on the activity in the presence of α-D-glucose-1,6-diphosphate are summarized in Table II. The addition of 1.1 μmoles of α-D-ribose-1-P decreased the observed velocity by 0.9 nmoles per min in less than 3 min, where the addition of 3.1 μmoles of d-ribose-5-P decreased the velocity by 1.4 nmoles per min. The d-ribose monophosphates also decreased the velocity of the reaction when the enzyme was activated with α-D-ribose-1,5-di-P. The addition of 1.1 μmoles of α-D-ribose-1-P decreased the velocity by 2.5 nmoles per min, and under the same conditions 3.1 μmoles of d-ribose-5-P decreased the velocity by 2.9 nmoles per min (Table II). In other experiments, in which the concentration of the d-ribose monophosphates were varied between 1 and 5 μmoles, it was found that d-ribose-1-P was consistently about 2 to 3 times as effective as d-ribose-5-P in decreasing the velocity of the phosphoglucomutase reaction. The addition of 1.1 μmoles of β-D-ribose-1-P, which is inactive as a substrate in the phosphoglucomutase reaction, was completely ineffective in altering the velocity of the reaction in the presence of either α-D-glucose-1,6-di-P or α-D-ribose-1,5-di-P. Since almost the same relative effects were observed with α-D-glucose-1,6-di-P and α-D-ribose-1,5-di-P, some common enzyme intermediates are probably formed from both of the sugar diphosphate esters. Moreover, the capability of the monophosphate sugars to decrease the level of these intermediates appears to be related to the nature of the sugar, and perhaps the position of the phosphate group. The two terminally phosphorylated derivatives of D-mannose were equally effective, whereas those of D-ribose were different.

The maximum velocities for the interconversion of α-D-mannose-1-P ($V_{max} = 10.4$ μmoles mg$^{-1}$ min$^{-1}$) and D-mannose-6-P ($V_{max} > 0.2$) or α-D-ribose-1-P ($V_{max} = 2.0$) and D-ribose-5-P ($V_{max} = 0.24$) are very low compared to that of D-glucose-1-P ($V_{max} = 328$) (11). The $K_m$ values for α-D-mannose-1-P (245 μM), D-mannose-6-P (500 μM), α-D-ribose-1-P (900 μM), and D-ribose-5-P (400 μM) are correspondingly high compared to that of D-glucose-1-P (8 μM) (11). It is, therefore, rather unlikely that any extensive equilibration of the two sugar monophosphate esters occurs under the conditions used in these experiments. However, the data obtained from preliminary incubation studies strongly indicate that complete and rapid equilibration of the sugar monophosphates with the corresponding α-D-sugar diphosphate does occur under these conditions. From this evidence and the fact that at equal concentrations α-D-ribose-1-P was more effective than D-ribose-5-P in removing phosphoenzyme, it might be tentatively suggested that after the new steady state is reached 2 to 3 times more P-enzyme-α-D-ribose-1-P is formed than P-enzyme-D-ribose-5-P as shown in the following general equations:

$EP + α-D-sugar-1-P \rightleftharpoons α-D-sugar-1-P.EP$ (2a)

$EP + D-sugar-5-P \rightleftharpoons D-sugar-5-P.EP$ (3a)

α-D-Sugar-1-P.EP $\rightleftharpoons E.sugar-1,5-di-P$ (2b)

D-Sugar-5-P.EP $\rightleftharpoons E.sugar-1,5-di-P$ (3b)

The compounds interconverted in these reactions represent the appropriate d-ribose phosphate intermediates of Equations 2 and 3.

Activation of Phosphoglucomutase with N-Acetyl-α-D-Glucosamine-1,6-di-P In Fig. 3 are shown the change in velocity with concentration and the Lineweaver-Burk plot of data obtained when phosphoglucomutase was activated with N-acetyl-α-D-glucosamine-1,6-di-P. The velocity increased for about 10 min at all concentrations of N-acetyl-α-D-glucosamine-1,6-di-P tested. The final maximum rate attained was dependent on the amount of sugar diphosphate added. Essentially identical curves were obtained when the enzyme was first incubated for various times with either sugar diphosphate or α-D-glucose-1-P, thus supporting the previous observation that rapid and complete equilibration of enzyme and sugar diphosphate occurs in this system. These results, as well as the data obtained with the other sugar diphosphates, are consistent with the mechanism shown in Equations 1 through 7. Calculations based on data obtained from the double reciprocal plots of 1/velocity with respect to 1/substrate concentration in Fig. 3 showed a $K_m$ and maximum velocity of 1.6 x 10$^{-4}$ M and 220 μmoles min$^{-1}$ mg$^{-1}$, respectively.
The reaction mixture contained in 1 ml: 25 mM imidazole-HCl (pH 7.0), 5 mM MgCl₂, 1 mM EDTA, 0.005% bovine serum albumin, 0.5 mM TPN⁺, 6.4 µg of glucose-6-P dehydrogenase, 8.9 × 10⁻⁴ µM phosphoglucomutase, and 1 µM imidazole salt of N-acetyl-α-D-glucosamine-1,6-di-P. The reaction was initiated with the addition of 0.25 µmole of α-D-glucose-1-P. At times the indicated 1.42 and 2.84 µmoles of N-acetyl-α-D-glucosamine-1-P or 1 µmole of N-acetylglucosamine-6-P was added.

As seen in Table II, when N-acetyl-α-D-glucosamine-1-P was added to a reaction mixture containing α-D-glucose-1,6-di-P the velocity decreased by 1.7 nmoles per min. The velocity decreased by 2.3 nmoles per min when N-acetyl-β-glucosamine-6-P was added. In this case, the decrease was greater when the 6-phosphate derivative was added. The ratio of the differences in the velocities was 1.8 (2.3:1.7 × 1.42) after correction for the concentrations of the sugar monophosphates used. When 1.42 µmoles of N-acetyl-α-D-glucosamine-1-P were added to a reaction mixture containing 1 µM N-acetyl-α-D-glucosamine-1,6-di-P, the velocity decreased by 1.1 nmoles per min, whereas the addition of 2.84 µmoles of the 1-phosphate sugar caused a decrease of 2.2 nmoles per min (Fig. 5). Similar results were obtained with all of the sugar monophosphates tested, indicating that the extent of decrease was directly proportional to the amount of sugar monophosphate added. The addition of 1 µmole of N-acetyl-β-glucosamine-6-P to the same reaction mixture decreased the velocity by 3.7 nmoles per min, as shown in Table II. It is noteworthy that, in contrast to the results obtained with the n-ribose monophosphate esters, N-acetyl-β-glucosamine-6-P was at least 3 times more active than N-acetyl-α-D-glucosamine-1-P in decreasing the velocity of the phosphoglucomutase reaction.

The data obtained in these experiments again suggest that unequal amounts of the intermediate forms of P-enzyme-N-acetyl-β-D-glucosamine-1-P and P-enzyme-N-acetyl-β-D-glucosamine-6-P may be formed, and that the free forms of these monophosphates are far from equilibrium even after the new steady state is reached.

Effect of α-D-glucosamine-1,6-di-P and α-D-galactose-1,6-di-P on Activity of Phosphoglucomutase—Phosphoglucomutase was activated by α-D-glucosamine-1,6-di-P. The rate of conversion of α-D-glucose-1-P to D-glucose-6-P was measured with levels of the sugar diphosphate ranging from 0.9 µM to 3.6 µM. The characteristic increase in velocity with time with was also obtained with this compound. The initial velocity and final rate of the reaction were unaffected by preliminary incubation and the order of addition of the reaction components, but increased with increasing concentrations of the sugar diphosphate. The Km and maximum velocity calculated from the data shown in Fig. 3 were 1.1 × 10⁻⁴ µM and 370 µmoles min⁻¹ mg⁻¹, respectively. The apparent Km for α-D-glucosamine-1,6-di-P was 7 times larger than that for N-acetyl-α-D-glucosamine-1,6-di-P.

The C-4 isomer, α-D-galactose-1,6-di-P, was a very poor activator of the phosphoglucomutase reaction. At low concentrations, the Km and maximum velocity calculated from the data shown in Fig. 6 were 1.3 × 10⁻³ µM and 240 µmoles min⁻¹ mg⁻¹, respectively. Not only was α-D-galactose-1,6-di-P a poor activator of phosphoglucomutase relative to α-D-glucose-1,6-di-P and the other sugar diphosphates, but at very high concentrations it was also a competitive inhibitor. The fact that the velocity increases with time to a maximum, as shown in Fig. 6, strongly indicates that some phosphoenzyme is being formed from this sugar diphosphate.

As seen in Table II, when N-acetyl-α-D-glucosamine-1-P was added to a reaction mixture containing α-D-glucose-1,6-di-P the velocity decreased by 1.1 nmoles per min. The velocity decreased by 2.3 nmoles per min when N-acetyl-β-glucosamine-6-P was added. In this case, the decrease was greater when the 6-phosphate derivative was added. The ratio of the differences in the velocities was 1.8 (2.3:1.7 × 1.42) after correction for the concentrations of the sugar monophosphates used. When 1.42 µmoles of N-acetyl-α-D-glucosamine-1-P were added to a reaction mixture containing 1 µM N-acetyl-α-D-glucosamine-1,6-di-P, the velocity decreased by 1.1 nmoles per min, whereas the addition of 2.84 µmoles of the 1-phosphate sugar caused a decrease of 2.2 nmoles per min (Fig. 5). Similar results were obtained with all of the sugar monophosphates tested, indicating that the extent of decrease was directly proportional to the amount of sugar monophosphate added. The addition of 1 µmole of N-acetyl-β-glucosamine-6-P to the same reaction mixture decreased the velocity by 3.7 nmoles per min, as shown in Table II. It is noteworthy that, in contrast to the results obtained with the α-D-glucosamine-1,6-di-P. The reaction was initiated with the addition of 0.25 µmole of α-D-glucose-1-P. At times the indicated 1.42 and 2.84 µmoles of N-acetyl-α-D-glucosamine-1-P or 1 µmole of N-acetylglucosamine-6-P was added.

As seen in Table II, when N-acetyl-α-D-glucosamine-1-P was added to a reaction mixture containing α-D-glucose-1,6-di-P the velocity decreased by 1.7 nmoles per min. The velocity decreased by 2.3 nmoles per min when N-acetyl-β-glucosamine-6-P was added. In this case, the decrease was greater when the 6-phosphate derivative was added. The ratio of the differences in the velocities was 1.8 (2.3:1.7 × 1.42) after correction for the concentrations of the sugar monophosphates used. When 1.42 µmoles of N-acetyl-α-D-glucosamine-1-P were added to a reaction mixture containing 1 µM N-acetyl-α-D-glucosamine-1,6-di-P, the velocity decreased by 1.1 nmoles per min, whereas the addition of 2.84 µmoles of the 1-phosphate sugar caused a decrease of 2.2 nmoles per min (Fig. 5). Similar results were obtained with all of the sugar monophosphates tested, indicating that the extent of decrease was directly proportional to the amount of sugar monophosphate added. The addition of 1 µmole of N-acetyl-β-glucosamine-6-P to the same reaction mixture decreased the velocity by 3.7 nmoles per min, as shown in Table II. It is noteworthy that, in contrast to the results obtained with the α-D-glucosamine-1,6-di-P. The reaction was initiated with the addition of 0.25 µmole of α-D-glucose-1-P. At times the indicated 1.42 and 2.84 µmoles of N-acetyl-α-D-glucosamine-1-P or 1 µmole of N-acetylglucosamine-6-P was added.

As seen in Table II, when N-acetyl-α-D-glucosamine-1-P was added to a reaction mixture containing α-D-glucose-1,6-di-P the velocity decreased by 1.7 nmoles per min. The velocity decreased by 2.3 nmoles per min when N-acetyl-β-glucosamine-6-P was added. In this case, the decrease was greater when the 6-phosphate derivative was added. The ratio of the differences in the velocities was 1.8 (2.3:1.7 × 1.42) after correction for the concentrations of the sugar monophosphates used. When 1.42 µmoles of N-acetyl-α-D-glucosamine-1-P were added to a reaction mixture containing 1 µM N-acetyl-α-D-glucosamine-1,6-di-P, the velocity decreased by 1.1 nmoles per min, whereas the addition of 2.84 µmoles of the 1-phosphate sugar caused a decrease of 2.2 nmoles per min (Fig. 5). Similar results were obtained with all of the sugar monophosphates tested, indicating that the extent of decrease was directly proportional to the amount of sugar monophosphate added. The addition of 1 µmole of N-acetyl-β-glucosamine-6-P to the same reaction mixture decreased the velocity by 3.7 nmoles per min, as shown in Table II. It is noteworthy that, in contrast to the results obtained with the α-D-glucosamine-1,6-di-P. The reaction was initiated with the addition of 0.25 µmole of α-D-glucose-1-P. At times the indicated 1.42 and 2.84 µmoles of N-acetyl-α-D-glucosamine-1-P or 1 µmole of N-acetylglucosamine-6-P was added.

As seen in Table II, when N-acetyl-α-D-glucosamine-1-P was added to a reaction mixture containing α-D-glucose-1,6-di-P the velocity decreased by 1.7 nmoles per min. The velocity decreased by 2.3 nmoles per min when N-acetyl-β-glucosamine-6-P was added. In this case, the decrease was greater when the 6-phosphate derivative was added. The ratio of the differences in the velocities was 1.8 (2.3:1.7 × 1.42) after correction for the concentrations of the sugar monophosphates used. When 1.42 µmoles of N-acetyl-α-D-glucosamine-1-P were added to a reaction mixture containing 1 µM N-acetyl-α-D-glucosamine-1,6-di-P, the velocity decreased by 1.1 nmoles per min, whereas the addition of 2.84 µmoles of the 1-phosphate sugar caused a decrease of 2.2 nmoles per min (Fig. 5). Similar results were obtained with all of the sugar monophosphates tested, indicating that the extent of decrease was directly proportional to the amount of sugar monophosphate added. The addition of 1 µmole of N-acetyl-β-glucosamine-6-P to the same reaction mixture decreased the velocity by 3.7 nmoles per min, as shown in Table II. It is noteworthy that, in contrast to the results obtained with the α-D-glucosamine-1,6-di-P. The reaction was initiated with the addition of 0.25 µmole of α-D-glucose-1-P. At times the indicated 1.42 and 2.84 µmoles of N-acetyl-α-D-glucosamine-1-P or 1 µmole of N-acetylglucosamine-6-P was added.
D-glucose-6-P, which was not present before the addition of the limiting intermediate must have been very rapid, under these conditions. The slow step in the overall conversion was the obligatory enzyme intermediate between α-D-glucose-1-P and d-sugar phosphate. The formation of enzyme-D-glucose-1-P to enzyme-α-D-glucose-1,6-diphosphate or some other enzyme complex with the sugar diphosphates. However, the mechanism does appear to involve the formation of both enzyme-D-sugar diphosphate and enzyme-phosphate intermediates. Furthermore, the steady state level of d-glucose-6-P remains high because of their slow rate of synthesis and breakdown. The rate of interconversion of sugar monophosphates appears to be much slower than the rate at which they react with phosphate-enzyme to form phosphate-enzyme-sugar monophosphate, enzyme-α-D-sugar diphosphate, or enzyme and free α-D-sugar diphosphate. The two enzyme intermediates, not common to both approaches which would not be expected to equilibrate rapidly under these conditions, are the products of the reaction of phosphate-enzyme with each of the sugar monophosphates.

The effects of the various sugar diphosphates and sugar monophosphates on the rate of conversion of α-D-glucose-1-P to d-glucose-6-P by phosphoglucomutase may be summarized in the following series of interactions:

\[
\begin{align*}
\text{α-D-sugar diphosphate + enzyme} & \quad \rightleftharpoons \quad \text{P-enzyme} \quad \text{D-sugar diphosphate} \\
\text{P-enzyme} \quad \text{D-sugar diphosphate} & \quad \rightleftharpoons \quad \text{P-enzyme} \quad \text{D-sugar 1,6-diphosphate} \\
\text{P-enzyme} \quad \text{D-sugar 1,6-diphosphate} & \quad \rightleftharpoons \quad \text{P-enzyme} \quad \text{n-glucose-6-P} \\
\text{P-enzyme} \quad \text{n-glucose-6-P} & \quad \rightleftharpoons \quad \text{α-D-Glucose-1-P} \\
\text{α-D-Glucose-1-P} & \quad \left\downarrow \quad \text{P-enzyme} \\
\text{P-enzyme} \quad \text{n-glucose-6-P} & \quad \left\downarrow \quad \text{α-D-Glucose-1,6-di-P + enzyme} \\
\end{align*}
\]

The observations made in the present study may be explained in terms of this scheme and the relative rates of the reactions involved as they influence the rate of formation of D-glucose-6-P. The formation of enzyme-sugar diphosphate or enzyme-phosphate complexes by equilibration of enzyme and sugar diphosphate is a relatively rapid reaction, and it is probable that the same enzyme-phosphate complex is formed from all of the sugar diphosphates. Thus, in no case did preliminary incubation of the enzyme and sugar diphosphate increase the initial rate of the reaction after the addition of α-D-glucose-1-P. However, both the initial and final velocities increased with increasing concentrations of α-D-sugar diphosphate. The large variation in the initial and final rates observed with the five activators tested indicates that the sugar component of the sugar diphosphate as well as the stereo orientation or the phosphate groups may be responsible for the differences observed in the Kₐ of these compounds.

The results further indicate that when the enzyme is activated by a sugar diphosphate, other than α-D-glucose-1,6-diphosphate, the increase in the velocity of the reaction may be limited by the slow rate of accumulation of a phosphate-enzyme-D-sugar phosphate, enzyme-α-D-glucose-1,6-diphosphate, or some other obligatory enzyme intermediate between α-D-glucose-1-P and n-glucose-6-P, which was not present before the addition of α-D-glucose-1-P. The final concentration and rate of accumulation of this intermediate were dependent on the amount of sugar diphosphate added. A maximum level was attained in about 10 min in most cases. In marked contrast, a maximal rate was obtained almost immediately when the enzyme was activated with α-D-glucose-1,6-diphosphate. The formation of the rate-limiting intermediate must have been very rapid, under these circumstances. The difference in the activation by other sugar diphosphates and α-D-glucose-1,6-diphosphate appears to involve the obligatory formation of an enzyme-phosphate intermediate, and, after the addition of α-D-glucose-1-P, a phosphate-enzyme-α-D-glucose-1-P complex with the sugar diphosphates. These intermediates may be subsequently converted to the same compound formed directly from α-D-glucose-1,6-diphosphate and free enzyme. Thus, it would seem that the conversion of phosphate-enzyme-α-D-glucose-1-P to enzyme-α-D-glucose-1,6-diphosphate or phosphate-enzyme-D-glucose-6-P is the relatively slow step in the over-all conversion.

The reaction of sugar monophosphates with enzyme-phosphate to form enzyme-α-D-sugar-1,6-diphosphate, a reversal of the activation process, is at least 3 times as fast as the formation of the rate-limiting intermediate after the addition of α-D-glucose-1-P (less than 3 min compared to 10 min). This result may be explained if the rate of conversion of α-D-glucose-1-P to d-glucose-6-P becomes dependent on the concentration of another intermediate, enzyme-phosphate, when relatively large amounts of sugar monophosphate are added. High concentrations of the sugar monophosphates, about 1 mm, were required to remove sufficient enzyme-phosphate to make this complex rate-limiting. Low concentrations, up to 300 μM, had little or no effect on the velocity, although they would be expected to remove some enzyme-phosphate. In this manner the decrease in the velocity of the reaction and the attainment of a new lower steady state occurs in less than 3 min, even though the concentration of the original rate-limiting enzyme complex (phosphate-enzyme-D-glucose monophosphates or enzyme-α-D-glucose diphosphate) remains high because of their slow rate of synthesis and breakdown. The rate of interconversion of sugar monophosphates appears to be much slower than the rate at which they react with phosphate-enzyme to form phosphate-enzyme-sugar monophosphate, enzyme-α-D-sugar diphosphate, or enzyme and free α-D-sugar diphosphate. The two enzyme intermediates, not common to both approaches which would not be expected to equilibrate rapidly under these conditions, are the products of the reaction of phosphate-enzyme with each of the sugar monophosphates. The results obtained in the present study show that D-mannose-6-P and α-D-mannose-1-P were equally effective in depressing the velocity of the reaction. D-Ribose-5-P was less active than α-D-ribose-1-P, and N-acetyl-D-glucosamine-6-P was more active than N-acetyl-α-D-glucosamine-1-P. The reaction of phosphate-enzyme and sugar monophosphate appears to be the step which is dependent on the structure of the sugar. The effective concentrations of the sugar monophosphates containing a free aldehyde group might be lower than the amount that was added, since only the α anomers would be expected to react with phosphoglucomutase. This factor would further alter the results obtained with each sugar monophosphate.

In agreement with the results of other studies (4, 10, 11), free α-D-sugar-1,6-diphosphates do not seem to be obligatory intermediates in the interconversion of the corresponding sugar monophosphates. However, the mechanism does appear to involve the formation of both enzyme-α-D-sugar diphosphate and enzyme-phosphate intermediates. Furthermore, the steady state level of these intermediates maintained during the reaction was dependent on the concentration of both α-D-sugar diphosphate and α-D-glucose-1-P.

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