THE MECHANISM OF THE PHOSPHOGLUCOMUTASE REACTION

By EARL W. SUTHERLAND, MILDRED COHN, THEODORE POSTERNAK, and CARL F. CORI

(From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis)

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Although many samples of glucose-1-phosphate had been prepared in this laboratory in the last 12 years, it was not until recently that an unusual observation was made with one of them. This sample was found by Dr. Najjar to be inactive as substrate for phosphoglucomutase, while it retained its full activity as substrate for phosphorylase. In pursuit of this observation other preparations of glucose-1-phosphate, both natural and synthetic, were tested with phosphoglucomutase. It was found that these preparations varied in the rate of conversion to glucose-6-phosphate from maximal activity to a barely perceptible rate of enzymatic conversion. Addition of an active to an inactive sample of glucose-1-phosphate resulted in a rapid disappearance of both, suggesting that the active sample contained a hitherto unrecognized activator for the phosphoglucomutase system.

It would undoubtedly have been a difficult problem to establish the nature of this activator, but this was brilliantly solved by Leloir and co-workers (1, 2) when they suggested that the activator might be glucose-1,6-diphosphate.

By way of confirmation, and because of the interest of this laboratory in both the enzyme and the 1-phospho sugars, it was thought appropriate to attempt a synthesis of this compound. Methods for the preparation of α-glucose-1,6-diphosphate are described in a preceding paper (3). On addition of catalytic amounts of this compound to inactive samples of glucose-1-phosphate, the rate of conversion reached that observed with active preparations of glucose-1-phosphate alone.

The mode of participation of glucose-1,6-diphosphate in the transformation of glucose-1-phosphate to glucose-6-phosphate was studied by means of isotopic tracers. P$^{32}$ was used to label the phosphate group and C$^{14}$ to label the glucose moiety of glucose-1-phosphate. On incubation with crystalline phosphoglucomutase and non-isotopic glucose-1,6-diphosphate, equilibration of the phosphorus and carbon between the two hexose monophosphates and the diphosphate was observed.
Activation of Phosphoglucomutase Reaction

Effect of Different Samples of Glucose-1-phosphate—The test system for phosphoglucomutase activity has been described in detail previously (4). Metal-binding agents and Mg ions are necessary for full enzyme activity, but, even when they are present in optimal amounts, one still observes that various samples of glucose-1-phosphate, either synthetic or natural, differ greatly in their rate of conversion to glucose-6-phosphate. This applies to relatively crude enzyme preparations from yeast (4) as well as to crystalline phosphoglucomutase from rabbit muscle (5). Figs. 1 and 2 show typical experiments with these two enzyme preparations. In Fig. 1 colorimeter readings (Klett-Summerson) rather than per cent conversion of glucose-1 to glucose-6-phosphate are plotted against time in order to permit direct comparison of rates of conversion in the presence of varying amounts of substrate. It may be seen that the addition of active to inactive glucose-1-phosphate allows the latter to be attacked by
the enzyme. Furthermore, the addition of the active to the inactive sample, either at the start of the reaction or after 33 minutes of incubation, increased the rate of conversion of the mixture to that of the active sample alone. The same result is obtained when a catalytic amount of glucose-1,6-diphosphate is added to inactive glucose-1-phosphate, as shown in the next section.

![Graph](http://www.jbc.org/)

**Fig. 2.** Rate of conversion of inactive glucose-1-phosphate to glucose-6-phosphate with and without the addition of glucose-1,6-diphosphate in a phosphoglucomutase (muscle) reaction mixture. The concentration of glucose-1,6-diphosphate was $1.7 \times 10^{-4} \text{M}$ in Curve 1 and $8 \times 10^{-4} \text{M}$ in Curve 2. Curve 3 shows the rate of conversion of the active sample of glucose-1-phosphate used in Fig. 1.

Activation by $\alpha$-Glucose-1,6-diphosphate—In Fig. 2 the rates of conversion of maximally active glucose-1-phosphate (Curve 3) and of an inactive sample with added glucose-1,6-diphosphate (Curves 1 and 2) are all shown to be nearly the same. Considering that a concentration of about $2 \times 10^{-4} \text{ M}$ gave maximal activation, the enzyme must have a high affinity for glucose-1,6-diphosphate. This is borne out in the experiment in Fig. 3, which shows that half maximal activity was observed with a con-
centration of α-glucose-1,6-diphosphate of the order of $5 \times 10^{-7}$ mole per liter.

A somewhat higher value reported previously (6) was due to the fact that the synthetic preparation of the diphosphate then used was a mixture of the α and β forms. It has since been found that β-glucose-1,6-diphosphate does not have an activating effect on the enzyme.

**Assay for Glucose-1,6-diphosphate**—By means of the enzyme velocity-coenzyme concentration curve, expressed by $K = \left(\frac{(V - v)}{v}\right) \times c$, where

\[
\begin{align*}
X & \text{IMALLY ACTIVE G-1-P} \\
I & \text{NACTIVE G-I-P} \\
\text{MAXIMALLY ACTIVE G-1-P} \\
\text{INACTIVE G-1-P (5.6 \times 10^{-7} M)} \\
\end{align*}
\]

\[
\begin{align*}
0 & \text{MINUTES} \\
6 & \text{12} \\
17 & \text{MINUTES}
\end{align*}
\]

**Fig. 3.** Effect of a submaximal amount of glucose-1,6-diphosphate on the rate of conversion of inactive glucose-1-phosphate to glucose-6-phosphate in a phosphoglucomutase (muscle) reaction mixture.

$K$ corresponds to that concentration of coenzyme which gives half maximal velocity (in our case $5 \times 10^{-7}$ M), one can determine the amount of glucose-1,6 diphosphate in an unknown sample. Such determinations were of particular interest in the case of various preparations of glucose-1-phosphate. It was found that synthetic glucose-1-phosphate, depending on the method of preparation and purification, contained from practically 0 to 0.03 per cent of glucose-1,6-diphosphate. In the case of enzymatically prepared glucose-1-phosphate some samples contained as much as 0.1 per cent glucose-1,6-diphosphate or 3 times the amount necessary for maximal

1 Prepared by the method of Cori, Colowick, and Cori (7) as well as by methods referred to in a preceding paper (3).
activation of the enzyme (in the usual reaction mixture containing \(6 \times 10^{-3} \text{m} \) glucose-1-phosphate). The factors which lead to the formation of glucose-1,6-diphosphate in a system consisting of polysaccharide, inorganic phosphate, and phosphorylase are being investigated.

**Exchange Experiments**

*Preparation of Glucose-1-phosphate (P\(^{32}\))—* This was prepared by exchange of crystalline non-radioactive glucose-1-phosphate with radioactive inorganic phosphate (5 \(\gamma \) of P) in the presence of sucrose phosphorylase (8). Sucrose phosphorylase was obtained from dried *Pseudomonas saccharophila*\(^2\) by the method of Doudoroff (9). At the end of the reaction, the radioactive inorganic phosphate was diluted with non-radioactive inorganic phosphate and removed by precipitation with magnesia mixture; this procedure was repeated twice, the first time 8 mg. of inorganic P were added, the second time, 6 mg. The glucose-1-phosphate (P\(^{32}\)) was crystallized and recrystallized twice as the potassium salt. To the crystalline product were added 500 mg. of non-radioactive potassium salt of glucose-1-phosphate and the mixture was washed out with non-radioactive inorganic phosphate (16 mg. of P). The inorganic phosphate was removed with magnesia mixture and the potassium salt of glucose-1-phosphate was recrystallized twice.

*Preparation of Glucose-1-phosphate (C\(^{14}\))—* Glucose-1-phosphate containing radioactive carbon was prepared from radioactive starch\(^3\) (2 mg.) incubated with inorganic phosphate and crystalline muscle phosphorylase. At the end of 1 hour, salivary amylase was added and the reaction mixture was reincubated for a half hour and then heated for 10 minutes to destroy enzyme activity. The glucose-1-phosphate (C\(^{14}\)) formed was isolated in crystalline form after addition of 150 mg. of non-radioactive dipotassium glucose-1-phosphate as carrier. 10 mg. of non-radioactive starch were then added and the amylase digestion was repeated. The glucose-1-phosphate was again crystallized and recrystallized as the dipotassium salt from alcohol. Any precipitate appearing at pH 4 in alcohol was removed before crystallization of the glucose-1-phosphate.

*Separation of Phosphate Esters* The method of separation is based on the fact that the barium salt of the diphosphate ester precipitates almost quantitatively from hot, slightly alkaline solution, while glucose-1-phosphate and glucose-6-phosphate remain in solution.

After incubation with phosphoglucomutase, the reaction mixture was heated at 100° for 10 minutes and the coagulated protein was removed by centrifugation. To precipitate the barium salt of glucose-1,6-diphosphate,
a concentrated barium acetate solution was added in slight excess to the supernatant solution and the solution was made alkaline to brom thymol blue with ammonium hydroxide. The precipitation was brought to maximum by heating at 100° for 2 minutes. The precipitate was separated by rapid centrifugation in a heated cup. In order to remove any contamination of the diphosphate ester by radioactive monophosphate esters, the product was washed three times with a boiling solution of an equilibrium mixture of glucose-6-phosphate and glucose-1-phosphate (95:5) containing 0.45 mg. of P per cc. and was subsequently washed three times with boiling water. The washed barium salt of the glucose-1,6-diphosphate was dissolved in ice-cold hydrochloric acid, just acid to Congo red paper; any trace of BaSO₄ which had formed due to the presence of ammonium sulfate in the enzyme preparations was removed by centrifugation. The glucose-1,6-diphosphate was reprecipitated by making the solution alkaline to brom thymol blue with ammonium hydroxide and heating. The precipitate was again centrifuged while hot. To replace Ba⁺⁺ by Na⁺, the precipitate was suspended in ice-cold water and ice-cold hydrochloric acid was added dropwise until the precipitate was completely dissolved. A slight excess of 5 per cent Na₂SO₄ solution was added, the BaSO₄ formed was centrifuged in the cold, and the supernatant solution was neutralized with NaOH.

The glucose monophosphate was obtained from the initial solution from which the diphosphate had been removed as the barium salt. Enough alcohol was added to yield a 20 per cent solution. The precipitate formed at this alcohol concentration was discarded, since it contained small amounts of glucose-1,6-diphosphate, and the alcohol concentration was increased to 80 per cent. The precipitate which formed was centrifuged, washed with alcohol, then with ether, and dried in vacuo. The precipitate was dissolved in a small amount of hot water and any undissolved material was discarded. The Ba⁺⁺ was removed from solution with Na₂SO₄ as already described for the diphosphate. Aliquots of the solutions of the sodium salts of the monophosphate and diphosphate esters, each of whose phosphorus content had been determined, were evaporated to dryness under an infra-red lamp for measurement of radioactivity.

*Measurement of Radioactivity*—The radioactivity of all samples was measured with a thin mica window counter. The samples which contained both P³² and C¹⁴ were measured with and without an aluminum filter 4 mils in thickness. This filter absorbed all the carbon radiation and 32 per cent of the phosphorus radiation under the conditions used. The radioactivity of the phosphorus in samples about 1 to 1.5 mg. per sq. cm. was determined directly from measurements with the filter by which only the phosphorus radiation was recorded. The radioactivity of carbon was measured in samples whose thickness (0.1 to 0.2 mg. per sq. cm.) was
so low that no absorption correction was necessary. The radioactivity due to carbon was calculated by subtracting from the total radioactivity, as measured without the filter, the radioactivity due to phosphorus; the latter was measured on the same sample with the filter and then corrected by the known filter factor.

Equilibration of Glucose-1-phosphate (P$^{32}$) with Glucose-1,6-diphosphate——In a preliminary experiment, the exchange of phosphate between glucose-1-phosphate labeled only with radioactive P and glucose-1,6-diphosphate was investigated. The monoester and diester, each containing 1.5 mg. of P, were incubated in a total volume of 6 cc. at 30° for 3 hours in a reaction mixture containing phosphoglucomutase, once recrystallized, 0.005 M MgCl$_2$, and saturated with 8-hydroxyquinoline. Equilibrium in the conversion of glucose-1-phosphate to glucose-6-phosphate had been attained in about 10 minutes.

The glucose-1,6-diphosphate and glucose monophosphates were separated in the manner already described. A comparison of their radioactivities indicated that the diphosphate had attained 65 per cent of the value calculated for complete equilibration of both phosphates in the diphosphate with the phosphate of the monophosphate. It was later shown (see the preceding paper (3)) that the diphosphate preparation used in this experiment was a mixture of the α and β forms. Since the β form is enzymatically inactive, the apparent incomplete equilibration becomes explicable.

The glucose-1,6-diphosphate isolated from the reaction mixture was hydrolyzed in 1 N HCl for 10 minutes at 100° in order to split off the P in position 1. The solution was then made alkaline with ammonium hydroxide, 5 times the calculated amount of magnesia mixture were added, and the solution was allowed to stand in the cold for 1 hour. The inorganic phosphate which had precipitated was filtered and washed with magnesia mixture. The precipitate was dissolved in HCl, made neutral to methyl orange, and its phosphorus content and radioactivity were measured. This fraction had 925 counts per minute per mg. of P.

The addition of an excess of barium acetate (1.5 times) and 3 volumes of ethyl alcohol to the filtrate of MgNH$_4$PO$_4$ precipitation yielded a precipitate of glucose-6-phosphate. This precipitate was centrifuged, washed with alcohol and ether, and dried in vacuo. The precipitate was dissolved in dilute HCl and made alkaline with ammonium hydroxide; a small precipitate formed and was discarded. After Ba$^{++}$ was removed in the usual way with Na$_2$SO$_4$, the phosphate content and radioactivity of the glucose-6-phosphate derived from the diphosphate were determined. The radioactivity of this fraction was 985 counts per minute per mg. of P, which agrees, within experimental error, with the value for the P of position 1.

Equilibration of Glucose-1-phosphate (P$^{32}$, C$^{14}$) with Glucose-1,6-diphos-
phate—Two experiments were carried out with glucose-1-phosphate labeled with P$^{32}$ and C$^{14}$. In the first experiment, the reaction mixture contained the following components in a total volume of 13.5 cc.: 8-hydroxyquinoline, 7 mg.; sodium salt of glucose-1,6-diphosphate, 4.25 mg. of P; potassium salt of glucose-1-phosphate (C$^{14}$), 0.88 mg. of P; potassium salt of glucose-1-phosphate (P$^{32}$), 4.30 mg. of P; MgCl$_2$, 0.1 mM; third phosphoglucomutase crystals, 2.2 mg. of protein. Before the addition of the enzyme, a sample was withdrawn for phosphate analysis, labile and total. The reaction mixture was incubated at 30° at pH 7.1. At the end of 8 minutes, a sample was withdrawn for analysis of labile phosphate; equilibrium had been reached in the conversion of glucose-1-phosphate to glucose-6-phosphate, since the amount of labile phosphate remaining corresponded to the amount of labile phosphate in the glucose-1,6-diphosphate initially added plus 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>P$^{32}$</th>
<th>C$^{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>40 min.</td>
</tr>
<tr>
<td>Glucose monophosphate</td>
<td>*</td>
<td>870</td>
</tr>
<tr>
<td>Glucose-1,6-diphosphate</td>
<td>0</td>
<td>430</td>
</tr>
</tbody>
</table>

* This sample was inadvertently lost.

per cent of the total hexose monophosphate P. Half of the remaining solution was withdrawn after 40 minutes for separation of the diphosphate and monophosphates and the determination of their P$^{32}$ and C$^{14}$ content. After 9 hours of incubation, the diphosphate and monophosphate fractions were separated from the remainder of the reaction mixture and their radioactivity measured. As is shown in Table I, both the P$^{32}$ and C$^{14}$ values for the diphosphate ester at 40 minutes and 9 hours were approximately 50 per cent of the values found for the monophosphate esters, instead of the equal values to be expected for complete equilibration. Again, this was due to the fact that the glucose-1,6-diphosphate used in this experiment was a mixture of the $\alpha$ and $\beta$ forms.

In the second experiment, pure $\alpha$-glucose-1,6-diphosphate was used. The reaction proceeded under the same conditions as in the first experiment on a somewhat smaller scale, the amount of diphosphate corresponding to 1.5 mg. of P, glucose-1-phosphate (C$^{14}$) to 0.42 mg. of P, and glucose-1-phosphate (P$^{32}$) to 2.2 mg. of P. An analysis for labile phosphate after
8 minutes of incubation showed that equilibrium had been established in so far as the interconversion of glucose-1-phosphate and glucose-6-phosphate was concerned. At the end of 2 hours, the diphosphate and monophosphates were separated and their radioactivities determined as given in Table II. In this case, complete equilibration had been achieved between the diphosphate and monophosphates.

**Table II**  
*Equilibration of Glucose Monophosphate (CÈ, PÈÈ) and 1,6-Diphosphate in Phosphoglucomutase Reaction*

Pure α-glucose-1,6-diphosphate was used in this experiment. The results are expressed in counts per minute per mg.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PÈÈ</th>
<th>CÈÈ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final, 2 hrs.</td>
</tr>
<tr>
<td>Glucose monophosphate</td>
<td>5,060</td>
<td>3310</td>
</tr>
<tr>
<td>Glucose-1,6-diphosphate</td>
<td>0</td>
<td>3230</td>
</tr>
</tbody>
</table>

**Discussion**

The activation of the phosphoglucomutase reaction by synthetic glucose-1,6-diphosphate confirms the work of Leloir and coworkers (1, 2). The apparent lack of necessity of a coenzyme in previous work in this laboratory, in particular in the work of Najjar (5) with crystalline phosphoglucomutase, was due to the presence of the coenzyme as an impurity in the glucose-1-phosphate used as substrate. Various samples of glucose-1-phosphate were found to contain from 0.03 to 0.1 per cent of glucose-1,6-diphosphate, amounts sufficient to give maximal activation of the enzyme but certainly too low to be detected by ordinary chemical analysis. The mechanism of the formation of glucose-1,6-diphosphate during the enzymatic preparation of glucose-1-phosphate is not clearly understood at the present time.

There have been several attempts to elucidate the mechanism of action of phosphoglucomutase. Meyerhof et al. (10) investigated the occurrence of an exchange between the glucose monophosphates and radioactive inorganic phosphate and found no exchange. Schlamowitz and Greenberg (11) investigated the possibility of an exchange of glucose (CÈÈ) with glucose monophosphates in this reaction and again found no evidence of exchange. These authors postulated a mechanism which involved the intermediate formation of a compound with the phosphate attached simultaneously to
C-1 and C-6 of glucose. Recently Jagannathan and Luck (12) have reported that they observed an exchange between glucose-1-phosphate (P\textsuperscript{32P}) and a labile phosphate group in the enzyme. However, the crystalline phosphoglucomutase used in the present study contained no measurable phosphate.

The mechanism suggested by Leloir et al. (2), based on glucose-1,6-diphosphate as the coenzyme of the reaction, envisages the enzyme catalyzing the transfer of the phosphate from position 1 of the coenzyme to position 6 of glucose-1-phosphate. In this way, the diphosphate becomes glucose-6-phosphate and glucose-1,6-diphosphate is regenerated from glucose-1-phosphate. In the reverse reaction, the phosphate from position 6 of the diphosphate would be transferred to position 1 of glucose-6-phosphate, yielding glucose-1-phosphate and again regenerating glucose-1,6-diphosphate. This reaction may be represented in the following way.

\[
\begin{align*}
\text{C-6-phosphate} & \rightarrow \text{C-1-phosphate} \\
\text{C-1-phosphate} & + \text{C-6-phosphate} \\
\text{C-6-phosphate} & \rightarrow \text{C-1-phosphate} \\
\end{align*}
\]

The data presented in this paper on the exchange between the phosphate group of glucose monophosphates with the phosphate groups of glucose-1,6-diphosphate, and the exchange of the glucose moiety as well, are consistent with such a mechanism. The exchange of the phosphate alone could be explained by other mechanisms but the exchange involving the carbon clearly demonstrates that the monophosphate is transformed to the diphosphate and the diphosphate, in turn, is transformed to the monophosphate.

It should be pointed out that this mode of participation of a coenzyme in a reaction, namely its conversion to substrate and its simultaneous regeneration from substrate, is unique among enzyme mechanisms studied heretofore. How generally such a mechanism occurs remains a question for further investigation. It has already been shown for the phosphoglyceric acid mutase reaction (13) that the coenzyme 2,3-diphosphoglyceric acid plays an analogous rôle to glucose-1,6-diphosphate in the phosphoglucomutase reaction.

**SUMMARY**

1. Certain preparations of glucose-1-phosphate, both natural and synthetic, were found to be inactive, while others showed varying rates of
conversion to glucose-6-phosphate in the reaction catalyzed by phosphoglucomutase. Inactive preparations of glucose-1-phosphate could be made fully active by the addition of catalytic amounts of synthetic α-glucose-1,6-diphosphate, $5 \times 10^{-7}$ mole per liter being sufficient for half maximal rate. On this basis, samples of glucose-1-phosphate which were maximally active without the addition of glucose-1,6-diphosphate contained from 0.03 to 0.1 per cent of this cofactor; others which were not fully active contained less.

2. The mechanism of the reaction was investigated with glucose-1-phosphate labeled with C$^{14}$ and P$^{32}$ as substrate, crystalline phosphoglucomutase from muscle as enzyme, and non-labeled glucose-1,6-diphosphate (in this case added in large amount) as coenzyme. Complete equilibration of the phosphate and glucose moieties of the monophosphate esters and diphosphate ester occurred. This finding is consistent with a mechanism proposed by Leloir and coworkers for this reversible reaction, whereby the glucose-1,6-diphosphate transfers a phosphate group to the glucose-1-phosphate or glucose-6-phosphate. The diphosphate ester is thereby transformed to the product of the reaction and simultaneously the diphosphate is regenerated from the monophosphate. The net effect is a continuous interconversion of substrate and coenzyme, which explains the exchange.

BIBLIOGRAPHY

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