Enzymes acting on the first carbon of α-glucose are specific for a particular anomer. Two well known cases are the glucose oxidase from Penicillium notatum and glucose dehydrogenase from liver, both of them specific for β-glucose (1, 2). It is likely that enzymes acting at the same level of α-glucose 6-phosphate will also have an anomic specificity, but there was no definite information on this point, with the exception of phosphoglucomutases, which act on either α-α-glucose 1-phosphate (3) or its β anomer (4). The rate of spontaneous anomerization (mutarotation) of glucose and many other free sugars is rather slow at physiological pH (5) and there is an enzyme, mutarotase, that catalyzes the interconversion α-α-glucose = β-β-glucose (6-8). However, there was no information on the rate of spontaneous anomerization of glucose 6-phosphate or on the possibility of its enzymatic catalysis.

Hexokinase was known to act on both α- and β-glucose giving rise, presumably, to α- and β-glucose 6-phosphate, respectively (9). At the level of glucose 6-phosphate there is a metabolic crossroad in which at least three enzymes are involved, glucose phosphate isomerase, glucose 6-phosphate dehydrogenase, and phosphoglucomutase, whose anomic specificities for glucose 6-phosphate were unknown. The glucose phosphate isomerase reaction seems to involve the formation of an enediol as an intermediate, which presumably would require the prior opening of cyclic hexose 6-phosphate, spontaneously or catalyzed by glucose phosphate isomerase (10). On the basis of some observations of competitive inhibition of the enzyme by open and cyclic structurally related compounds, it has also been suggested that glucose phosphate isomerase would probably act on the open forms of its substrates (11, 12). These suggestions raised the problem of the conversion mechanism of glucose 6-phosphate, which is a product of hexokinase and phosphoglucomutase reactions, into the acyclic form. Taking into account the high glycolytic activity of yeast and its low intracellular pH (13), it is indicated otherwise. α and β forms refer to the pyranose ring.

Glucose 6-phosphate, it would probably be specific for one of its anomers.

The results in this paper show that yeast glucose 6-phosphate dehydrogenase is specific for β-glucose 6-phosphate and that the reaction product of muscle phosphoglucomutase with α-glucose 1-phosphate is α-glucose 6-phosphate. They also indicate that yeast glucose phosphate isomerase has an anomerase-like activity, specific for α-glucose 6-phosphate, the intermediate product of which is presumably acyclic glucose 6-phosphate. We have also found that the spontaneous anomerization of α-glucose 6-phosphate within the physiological pH range is some two orders of magnitude faster than that of α-glucose.

**Experimental Procedure**

**Enzymes**—Glucose phosphate isomerase, glucose-6-P dehydrogenase, and hexokinase from yeast, and aldolase and phosphoglucomutase from muscle were obtained from Boehringer und Soehne; α-α-glycerophosphate dehydrogenase was obtained from Mann Research Laboratories, Inc. Glucose oxidase and peroxidase were a gift from Worthington Biochemical Corporation. Brain hexokinase was prepared according to Crane and Sols (14). Phosphofructokinase was obtained from muscle according to Ling, Byrne, and Lardy (15). When more than 0.2 unit of enzyme was used, the ammonium sulfate suspensions were centrifuged and the sediment was dissolved in 1 mM EDTA, pH 7, except that of phosphoglucomutase which was dissolved in 0.1 M acetate, pH 6.

**Phosphoric Esters**—Fructose-6-P was synthesized with crystalline hexokinase and isolated by ion exchange chromatography on Dowex 2-X8 in formate form; fructose-6-P was eluted with 0.5 M sodium formate, giving a quantitative separation from the nucleotides; glucose-6-P contamination in this preparation was 0.5%. Mannose-6-P was also prepared with crystalline hexokinase and isolated by the standard fractional precipitation with Ba(OH)₂ after removal of the nucleotides by adsorption with Norit (17). Glucosone-6-P, allose-6-P, and 3-deoxyglucose-6-P were similarly synthesized but not isolated. Sedoheptulose-7-P was prepared with brain hexokinase but it was not isolated; phosphorylation of sedoheptulose by this enzyme, not previously reported, indicates a phosphorylation coefficient with respect to glucose (9) of about 8 X 10⁻². Erythrose-4-P was prepared from its dimethyl acetal, cyclohexylammonium salt (18), kindly supplied by Dr. C. E. Ballou. Sorbitol-6-P was prepared by reduction of glucose-6-P with KBH₄ (19), and...
isolated by precipitation with Ba++-ethanol; glucose-6-P impurity in this preparation was less than 0.1%. Erythritol-4-P was prepared similarly from erythrose-4-P and used without isolation; residual erythrose-4-P was about 4%. L-Sorbose-6-P was obtained by mild acid hydrolysis from L-sorbose-1,6-P kindly supplied by Dr. H. A. Lardy. Other phosphoric esters were obtained as follows: potassium α-D-glucose 1-phosphate from Dr. C. Villar-Palasi; barium mannitol 6-phosphate from Dr. M. Liss; barium L-xylulose 5-phosphate from Dr. W. A. Wood; barium galactose 6-phosphate and 1,5-anhydroglucomel-6-P (cyclohexylammonium salt) from Dr. R. K. Crane; barium ribulose 5-phosphate from Dr. A. Bonsignore; barium ribose 5-phosphate from Schwarz BioResearch, Inc.; sodium salts of glucose-6-P and 6-P-gluconate (Sigma grade) and sodium 2-deoxyglucose 6-phosphate, from Sigma Chemical Company. 3-Deoxyglucose was a gift from Dr. T. Z. Csaky; α- and β-glucose were obtained from Pfanstiehl Laboratories.

Assay of Enzymatic Activities—Glucose-6-P dehydrogenase activity was measured spectrophotometrically following the increase in optical density at 340 mp accompanying the reduction of NADP. Glucose phosphate isomerase activity was usually studied following the formation of glucose-6-P with glucose-6-P dehydrogenase. In addition to other methods were used in certain cases: (a) a spectrophotometric method following the formation of fructose-6-P with ATPMg, NADH, and an excess of phosphofructokinase, aldolase and L-α-glycerophosphate dehydrogenase; (b) a colorimetric method measuring the appearance of ketose (20) with a standard of enzymatically synthesized fructose-6-P. Phosphoglucomutase activity was determined with glucose-6-P dehydrogenase or by measuring acid labile phosphate (21). Hexokinase activity was determined with glucose-6-P dehydrogenase or by measuring residual glucose with a glucose oxidase reagent (22).

Activity is expressed in international units (amount of enzyme which under optimal conditions can transform 1 μ mole of substrate per minute) at room temperature.

RESULTS

Glucose phosphate isomerase from several sources is competitively inhibited by 6-P-gluconate (23, 24) and sorbitol-6-P (25). Erythrose-4-P strongly inhibits the muscle enzyme with a Ki value somewhat larger than 100 μ M. At a fixed concentration of glucose-6-P (0.25 mM), erythrose-4-P had an inhibitory effect on the heart enzyme. At pH 6 we have obtained the same Ki value as Glaser and Brown (31) at pH 7, which agrees with that reported by Glaser and Brown (31); at pH 6 we have obtained the same K values for glucose-6-P dehydrogenase. They have also reported a noncompetitive inhibition of the activity of glucose-6-P dehydrogenase on glucose-6-P by high glucose concentrations. Thus, assuming a similar inhibition when glucose was used as substrate, large corrections would have to be applied to the apparent Kmax and Vmax for free glucose. Nevertheless, we have found

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative rates at 1 mm</th>
<th>Compound</th>
<th>Relative rates at 1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-P</td>
<td>100</td>
<td>3-Deoxyglucose-6-P</td>
<td>0.8</td>
</tr>
<tr>
<td>2-Deoxyglucose-6-P</td>
<td>1</td>
<td>Allose-6-P</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glucosone-6-P</td>
<td>0.25</td>
<td>Galactose-6-P</td>
<td>1</td>
</tr>
<tr>
<td>Mannose-6-P</td>
<td>&lt;0.1</td>
<td>Glucose</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

* This value was calculated from the data in Fig. 1.

**FIG. 1.** Effect of glucose concentration on the activity of glucose-6-P dehydrogenase. The following reagents were added at about 20°C in a final volume of 2 ml: Tris, pH 8, 50 mM; NADP, 0.25 mM; glucose-6-P dehydrogenase, 0.8 unit; and glucose as indicated. Activity is expressed as micromoles of NADPH formed per minute per mg of protein.
that sorbitol at high concentration, like glucose, inhibited the oxidation of glucose-6-P by glucose-6-P dehydrogenase but it did not inhibit the activity when glucose was used as substrate. This contrast could be related to the marked difference in enzyme concentration involved in the comparison of the physiological substrate and a very poor one. Thus, 1 mM sorbitol did not inhibit the oxidation of 2-deoxyglucose-6-P with an enzyme concentration similar to that used with glucose. Accordingly, it seems that the inhibition observed at high sugar concentration is dependent on the use of diluted enzyme and therefore there is no reason to correct the kinetic constants obtained for glucose. Changes in the state of aggregation affected by the enzyme concentration have been reported for erythrocyte glucose-6-P dehydrogenase (33).

The activity of glucose-6-P dehydrogenase on free glucose offered a way of ascertaining its anomeric specificity. As shown in Fig. 2, yeast glucose-6-P dehydrogenase, when acting on free glucose, is specific for the \( \beta \) anomer. The experiment was carried out at pH 6 to slow down the spontaneous anomeration of glucose. A similar result was obtained independently by Colowick and Goldberg (32). By comparing the rate of glucose-6-P dehydrogenase with \( \beta \)-glucose (first order kinetics) with the rates with \( \alpha \)-glucose at different time intervals (Fig. 2), it was possible to calculate the amount of \( \beta \)-glucose formed from \( \alpha \)-glucose in each interval and hence the rate of anomeration of \( \alpha \)-glucose. A value of approximately 0.0005 pmole of \( \beta \)-glucose per second per pmole of \( \alpha \)-glucose at pH 6 and about 20' was obtained, which approximately corresponds to the spontaneous mutarotation rate known for \( \alpha \)-glucose from polarimetric (5) and infrared spectra (34) studies.

**Substrate Specificity of Yeast Glucose Phosphate Isomerase**—In Table II are shown the \( K_r \) values of several compounds for yeast glucose phosphate isomerase obtained with fructose-6-P as substrate, or with glucose-6-P when the compounds to be assayed as inhibitors of glucose phosphate isomerase were substrates of glucose-6-P dehydrogenase. Fig. 3 shows the inhibition of glucose phosphate isomerase by sorbitol-6-P with either glucose-6-P or fructose-6-P as substrates. No significant differences in the \( K_r \) values were obtained with one or another substrate. The apparent \( K_m \) value of glucose-6-P for yeast glucose phosphate isomerase is 0.3 mM and that of fructose-6-P is 0.15 mM.

![Fig. 2. Glucose-6-P dehydrogenase activity on \( \alpha \) and \( \beta \)-glucose. The following reagents were added at about 20' in a final volume of 2 ml: histidine, pH 6, 10 mM; NADP, 0.25 mM; glucose-6-P dehydrogenase, 20 units. The reaction was started by the addition of 100 pmoles of \( \alpha \)-glucose (O) or \( \beta \)-glucose (●) freshly dissolved in 5 mM phosphate buffer, pH 6.](http://www.jbc.org/)

![Fig. 3. Inhibition of glucose phosphate isomerase activity by sorbitol-6-P with fructose-6-P or glucose-6-P as substrates. The following reagents were added at about 20' in a final volume of 2 ml: A, Tris, pH 8, 40 mM; NADP, 0.25 mM; glucose-6-P dehydrogenase, 0.2 unit; sorbitol-6-P, 0.2 mM where indicated; fructose-6-P at the concentrations indicated; and glucose phosphate isomerase. Glucose-6-P contaminating the fructose-6-P preparation was rapidly oxidized by glucose-6-P dehydrogenase and the reaction was linear after 1 or 2 minutes; B, Tris, pH 8, 40 mM; ATPMg, 0.5 mM; NADH, 0.2 mM; phosphofructokinase, aldolase, and \( \alpha \)-glycerophosphate dehydrogenase, 0.2 unit; sorbitol-6-P, 0.1 mM where indicated; glucose-6-P at the concentrations indicated; and glucose phosphate isomerase. Activity is expressed as millimicromoles of NADPH or NAD formed, respectively, per minute per 0.01 ml of glucose phosphate isomerase.](http://www.jbc.org/)

### Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>( K_r^* ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrose-4-P</td>
<td>0.002</td>
</tr>
<tr>
<td>Erythritol-4-P</td>
<td>&gt;0.04</td>
</tr>
<tr>
<td>6-P-Glucosone</td>
<td>0.015</td>
</tr>
<tr>
<td>Sorbitol-6-P</td>
<td>0.025</td>
</tr>
<tr>
<td>1,5-Anhydroglucitol-6-P</td>
<td>2.5</td>
</tr>
<tr>
<td>Mannitol-6-P</td>
<td>0.13</td>
</tr>
<tr>
<td>Mannose-6-P</td>
<td>0.45</td>
</tr>
<tr>
<td>Ribulose-5-P</td>
<td>0.05</td>
</tr>
<tr>
<td>Ribose-5-P</td>
<td>0.5</td>
</tr>
<tr>
<td>2-Deoxyglucose-6-P</td>
<td>0.7</td>
</tr>
<tr>
<td>Galactose-6-P</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* The \( K_r \) values were calculated in the same way as described in Fig. 3.

† The \( K_r \) of 6-P-glucosone is markedly pH dependent. At pH 6 the \( K_r \) value is 0.0015 mM.
Nonlimiting Anomerization in Glucose-6-P Isomerization—The rate of glucose-6-P isomerization in glucose-fermenting yeast (about 0.5 μmole per second per g of fresh yeast at 30° (36)) is much greater than that usually obtained in experiments in vitro. If glucose phosphate isomerase worked only on the open form of glucose-6-P and the spontaneous anomerization of glucose-6-P were of the same order of magnitude as that of glucose, the anomerization of glucose-6-P could become limiting after the isomerization of the open form initially present in an equilibrated solution, when working at high enzyme concentrations and over very short times. However, even in only 10 seconds at 2° (conditions in which spontaneous anomerization of glucose would be very small), with increasing amounts of glucose phosphate isomerase, there was no indication of limiting anomerization (Fig. 4). This observation indicated that either the rate of spontaneous anomerization of glucose-6-P was much greater than that of glucose or that the glucose phosphate isomerase preparation had an anomerase activity, intrinsic or contaminating.

Mutarotation of Glucose-6-P—If the major factor in the opening of glucose-6-P prior to isomerization were an anomerase activity in the glucose phosphate isomerase preparation, there was the possibility of detecting mutarotation of glucose-6-P formed in situ with other enzymes. However, as shown in Fig. 5, there was no appreciable lag between synthesis and mutarotation by either the α-glucose-hexokinase or the α-glucose-1-P-phosphoglucomutase systems. The fact that these two enzyme preparations (as well as that of glucose phosphate isomerase in the above experiment) were crystalline, ruled out as a reasonable possibility the involvement, in the rate of mutarotation observed, of a contaminating anomerase. The obvious conclusion is that the spontaneous anomerization of glucose-6-phosphate is faster than that of free glucose. How much it actually was, could not be ascertained from the polarimetric measurements because of the time involved in these experiments.

Rate of Spontaneous Anomerization of α-Glucose-6-P—An actual measurement of the rate of anomerization of α-glucose-6-P synthesized with hexokinase was achieved by coupling this reaction with an enzyme specific for β-glucose-6-P. Results with free glucose had indicated that yeast glucose-6-P dehydrogenase is specific for the β anomer (see above). For a quantitative approach on the anomerization rate of glucose-6-P, the qualitative evidence that hexokinase acts readily on both α- and β-glucose (9) was not enough. From the experiment in Table III, at saturating glucose concentration (22), it can be calculated that the Vmax of yeast hexokinase with α-glucose is approximately 1.2 times and the Km 0.5 times as great as the values obtained with β-glucose. A detectable lag on the coupled system α-glucose-hexokinase-glucose-6-P dehydrogenase with respect to the similar one with β-glucose could afford a basis for the measurement of the anomerization rate of α-glucose-6-P. From the polarimetric results it was obvious that very short periods of time and a low temperature had to be employed. Fig. 6 shows a direct evidence of the specificity of glucose-6-P dehydrogenase for β-glucose-6-P. From the initial kinetics, shown in the figure, the rate of conversion of α-glucose-6-P in β-glucose-6-P can be approximately evaluated according to the expression:

$$v = \frac{1}{i} \frac{B}{A - B}$$

open forms of glucose-6-P and fructose-6-P may bind glucose phosphate isomerase more efficiently than the cyclic forms. It is a notable fact that erythrose-4-P has such a great affinity for the enzyme. It is possible that the open forms of glucose-6-P and fructose-6-P may bind glucose isomerase as indicated. At 10 seconds, the reaction was stopped with sulfuric acid. Fructose-6-P formed was determined as ketose.

<table>
<thead>
<tr>
<th>Glucose phosphate isomerase, units</th>
<th>Fructose-6-P formed, jumoles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

Fig. 4. Isomerization of glucose-6-P in a short time with increasing glucose phosphate isomerase concentrations. The following reagents were added at 2° in a final volume of 0.1 ml: histidine, pH 6, 50 mM; glucose-6-P, 10 mM; and glucose phosphate isomerase as indicated. At 10 seconds, the reaction was stopped with sulfuric acid. Fructose-6-P formed was determined as ketose.
FIG. 5. Mutarotation of α-glucose-6-P at room temperature (about 20°). A, the following reagents were added in a final volume of 0.88 ml: α-glucose-1-P, pH 6, 100 mM; cysteine, pH 6, 10 mM; MgCl₂, 5 mM; the reaction was started with 30 units of phosphoglucomutase and an aliquot was taken out to follow the change in its optical rotation; at the times indicated, aliquots of 0.01 ml were added to 1 ml of 1 M HCl to determine residual seed labile phosphate. B, 18 mg of α-glucose were mixed with 120 μmols of ATPMg, pH 6 (0.8 ml) and the reaction was started with 40 units of yeast hexokinase (0.2 ml). An aliquot was rapidly taken out to measure the change in its optical rotation; at the times indicated, aliquots of 0.005 ml were added to 2 ml of a glucose oxidase reagent (containing 50 mM N-acetylglucosamine to inhibit hexokinase (22)) to estimate the amount of residual glucose.

### Table III
Anomeric specificity of yeast hexokinase

The following reagents were added at 2° in a final volume of 0.1 ml: ATPMg, pH 5.7, 10 mM; hexokinase, 0.1 unit; N-acetylglucosamine, 10 and 25 mM where indicated. The reaction was started with 0.1 μmole of α-glucose or β-glucose freshly dissolved in 5 mM phosphate buffer, pH 6. Free glucose remaining after 10 minutes was estimated with glucose oxidase. From the initial kinetics of color development of the glucose oxidase reagent it could be ascertained that no significant anomerization of free glucose occurred in the conditions of the hexokinase experiment.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glucose phosphorylated</th>
<th>α-Glucose (%)</th>
<th>β-Glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>48.5</td>
<td>41.0</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td></td>
<td>30.5</td>
<td>20.5</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>19.0</td>
<td>10.8</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this equation \( v \) = rate of anomerization of α-glucose-6-P to β-glucose-6-P; \( B \) is the amount of β-glucose-6-P oxidized by glucose-6-P dehydrogenase in the interval \( t \), starting with α-glucose; \( A \) is the amount of α-glucose-6-P formed by hexokinase (1.2 times greater than the β-glucose-6-P oxidized when starting with β-glucose) in the same interval. From the results in Fig. 6 the rate of spontaneous anomerization of α-glucose-6-P at pH 6.7 and approximately 5° is about 0.01 μmole of β-glucose-6-P formed per second per μmole of α-glucose-6-P. In a similar experiment at 20°, a rate of anomerization 4 times greater was obtained. This temperature effect is of the same order of magnitude as that reported for the mutarotation of free glucose (5).

From these results it can be concluded that spontaneous anomerization of α-glucose-6-P at pH 6.7 (about 0.04 μmole of β-glucose-6-P formed per second per μmole of α-glucose-6-P at 20°) is approximately 100 times greater than that of α-glucose. Variation in pH between 6 and 7 (the physiological range in yeast (13)), did not appreciably affect the rate of spontaneous anomerization. Even a further increase in pH to 8, at which the rate of spontaneous anomerization of free glucose markedly increases (5), did not significantly affect that of glucose-6-P.

Anomerase Activity of Glucose Phosphate Isomerase—If glucose phosphate isomerase worked only on open glucose-6-P, taking into account the rate of spontaneous anomerization indicated above (some 0.04 μmole of β-glucose-6-P formed per second per μmole of α-glucose-6-P at 20°) and the rate of yeast fermentation...
glucose (0) freshly dissolved in 5 mM phosphate buffer, pH 6.

enzyme. Anomerization and anomerization can occur in a single binding site of the enzyme. The simplest possibility was that the inhibition by erythrose-4-P (Fig. 7B) abolished the anomerase-like activity. These results suggested that isomerization of glucose phosphate isomerase equivalent to that in the extract could catalyze the anomerization of glucose-6-P, there was need of an additional catalyst. Indeed, it was found that addition of a fresh yeast extract in amount corresponding to 0.8 unit of hexokinase (a), accelerated the anomerization of o-glucose-6-P to the same extent as it was accelerated by the yeast extract. Where indicated, 8 units of glucose phosphate isomerase and 0.5 µmole of erythrose-4-P were added.

FIG. 7. Catalysis of the anomerization of a-glucose-6-P by glucose phosphate isomerase. A, the following reagents were added at about 3°C in a final volume of 2 ml: Tris-histidine, pH 6.5, 30 mM; ATPMg, 5 mM; MgCl₂, 5 mM; NADP, 1 mM; glucose-6-P dehydrogenase, 7.5 units; yeast hexokinase, 0.5 unit (○, △), or a yeast extract in amount corresponding to 0.8 unit of hexokinase (○). The reaction was started with 5 µmoles of a-glucose (○, △) or β-glucose (■) freshly dissolved in 5 mM phosphate buffer, pH 6. B, the same reagents as in A were added, except for the absence of yeast extract. Where indicated, 8 units of glucose phosphate isomerase was added. (about 0.2 to 0.3 µmole of glucose fermented per second per g of fresh yeast at 20°C), an intracellular concentration of glucose-6-P of about 5 µmoles per g of fresh yeast would be necessary in order that the spontaneous anomerization of glucose-6-P should not be limiting. To have an accurate knowledge of the glucose-6-P concentrations present in glucose-fermenting yeast was important. There have been reports of concentrations between 1 µmole per g of fresh yeast and 5 to 8 µmoles per g (38).

In this laboratory, in conditions that minimize changes during extraction, values of about 1 µmole per g have been obtained. It appeared that, in spite of the rapid rate of spontaneous anomerization of glucose-6-P, there was need of an additional catalyst. Indeed, it was found that addition of a fresh yeast extract could markedly accelerate the anomerization of glucose-6-P (Fig. 7A). Dialysis of the extract was without effect on this catalytic activity, while boiling the extract for 5 minutes abolished the anomerase-like activity. These results suggested an enzymatic activity. The simplest possibility was that the glucose phosphate isomerase of the extract could catalyze the anomerization. Fig. 7B shows that an amount of crystalline glucose phosphate isomerase equivalent to that in the extract used in Fig. 7A, accelerated the anomerization of glucose-6-P to the same extent as it was accelerated by the yeast extract. This glucose phosphate isomerase-catalyzed anomerization was inhibited by erythrose-4-P (Fig. 7B), a strong inhibitor of glucose phosphate isomerase. This observation suggests that isomerization and anomerization can occur in a single binding site of the enzyme.

The above experiments indicate that glucose phosphate isomerase can catalyze the anomerization of glucose-6-P. To ascertain if it could also act on β-glucose-6-P, glucose phosphate isomerase activity with α-glucose-6-P and β-glucose-6-P were compared, as shown in Table IV, in conditions that minimized spontaneous anomerization. The apparent glucose phosphate isomerase activity on β-glucose-6-P can be roughly accounted for by spontaneous anomerization.

**DISCUSSION**

The greater affinity of yeast glucose phosphate isomerase for open than for cyclic structurally related compounds, shown in
this paper, suggests that the enzyme has more affinity for the open than for the cyclic forms of glucose-6-P and fructose-6-P. Whether glucose phosphate isomerase actually acts on the open forms of its substrates could not be concluded from these results. Assuming that the amount of open form in glucose-6-P is similar to that found in glucose, Hilnes and Wolfe (39) have recently suggested that the actual substrates for glucose phosphate isomerase must be the cyclic forms. Taking into account the fact that there appears to be a relationship between mutarotation rate and proportion of open form (40) and the results in this paper, which indicate that the rate of spontaneous anomerization of α-glucose-6-P is some 100 times greater than that of α-glucose, the amount of open form in an equilibrated solution of glucose-6-P must be greater than that in glucose. Stepanov and Stepanenko (41) found that phosphorylated sugars react with HCN more rapidly than free sugars, which also seems to indicate a greater proportion of open form in phosphorylated as compared to free sugars.

The fact that glucose phosphate isomerase catalyzes the anomerization of α-glucose-6-P but not that of β-glucose-6-P, and that it has greater affinity for open than for cyclic related compounds, strongly suggests that the intermediate product in the anomerase activity of glucose phosphate isomerase is open glucose-6-P. Occasional dissociation of the enzyme-open glucose-6-P complex without isomerization would be the basis of the observed anomerase activity of glucose phosphate isomerase. The catalysis of the anomerization of α- and β-glucose by mutarotase from P. notatum also takes place by formation of open glucose as intermediate (40). The above results also suggest that a direct formation of the enzyme-open glucose-6-P complex can take place allowing direct isomerization.

Until now there is no information to ascertain if glucose phosphate isomerase can act on fructofuranose-6-P. It seems likely that spontaneous anomerization could be fast enough to permit the observed rates of isomerization of fructose-6-P.

The product of the phosphoglucomutase reaction has been shown to be α-glucose-6-P and, under physiological conditions, that of hexokinase is a mixture of α- and β-glucose-6-P. Taking into account the anomeric specificities of glucose-6-P dehydrogenase, phosphoglucomutase, and glucose phosphate isomerase for glucose-6-P indicated in this paper, it is important to consider the anomerization problem in the metabolism of glucose-6-P (Scheme 1). The anomerase activity of glucose phosphate isomerase, specific for α-glucose-6-P, is an important step when this anomer of glucose-6-P is formed by hexokinase or phosphoglucomutase. β-Glucose-6-P is also formed by hexokinase, but since neither glucose phosphate isomerase nor phosphoglucomutase can act on this anomer, the spontaneous anomerization of glucose-6-P must be an important reaction in glucose-6-P metabolism. The question was raised by Topper (10) some years ago whether the opening of cyclic hexose 6-phosphates for the activity of glucose and mannose phosphate isomerases is nonenzymatic or is catalyzed by a mutarotase-like activity of the isomerases. This problem is now solved for glucose phosphate isomerase in terms of both spontaneous opening of glucose-6-P and anomerase-like activity of glucose phosphate isomerase. There is the possibility that mannose phosphate isomerase and other aldose-ketose isomerases, the substrates of which can exist in cyclic forms, may also have anomerase activities. In any case, the activating effect of the phosphate group on the rate of spontaneous anomerization, shown here for glucose-6-P, is likely to have general significance in the metabolism of other phosphorylated sugars.

**SUMMARY**

The anomerase specificity of glucose 6-phosphate dehydrogenase, glucose phosphate isomerase, hexokinase, and phosphoglucomutase have been studied. Yeast glucose 6-phosphate dehydrogenase is specific for β-glucopyranose 6-phosphate. Yeast hexokinase acts only slightly more effectively on α- than on β-glucopyranose and produces the corresponding anomers of glucopyranose 6-phosphate. The primary product of the reaction of muscle phosphoglucomutase with α-glucopyranose 1-phosphate is α-glucopyranose 6-phosphate. Yeast glucose phosphate isomerase has a greater affinity for open than for cyclic structurally related compounds, and apparently can act directly on the open forms of its substrates. In addition, this enzyme has an anomerase-like activity specific for α-glucopyranose 6-phosphate, the intermediate product of which is probably the open chain of glucose 6-phosphate. The rate of spontaneous anomerization of α-glucopyranose 6-phosphate between pH 6 and 8 at 20°C is approximately 0.04 μmole of β-glucopyranose 6-phosphate formed per second per μmole of α-glucopyranose 6-phosphate; this value is about two orders of magnitude greater than that of α-glucopyranose at pH 6. Both spontaneous anomerization and that catalyzed by glucose phosphate isomerase are involved in the metabolism of glucose 6-phosphate. It is suggested that the marked increase of the spontaneous anomerization of glucose 6-phosphate by the presence of the phosphoryl group may have general significance for other phosphorylated sugars which can exist in interconvertible anomic forms.

**REFERENCES**

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Spontaneous and Enzymatically Catalyzed Anomerization of Glucose 6-Phosphate and Anomeric Specificity of Related Enzymes
Margarita Salas, Eladio Vinuela and Alberto Sols