ON THE MECHANISM OF ACTION OF PHOSPHOGLUCOSE ISOMERASE AND PHOSPHOMANNOSE ISOMERASE

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The interconversion of ketoses and aldoses or of their phosphate esters involves the migration of a carbon-bound hydrogen to an adjacent carbon atom. In reactions of this type, two mechanistic possibilities present themselves: (1) The hydrogen may shift as a hydride ion as follows:

\[
\begin{align*}
\text{H-C=O} & \rightarrow \text{H-C-OH} \\
\text{H-C-OH} & \rightarrow \text{H-C-O} + \text{H}
\end{align*}
\]

This transformation is intramolecular and would involve no exchange between the migratory hydrogen and hydrogen in the aqueous solvent. (2) The hydrogen may migrate as a proton (Fig. 1), in which case exchange with the solvent would occur. In order to distinguish between these alternatives the conversion of fructose-6-phosphate (F-6-P) into glucose-6-phosphate (G-6-P), catalyzed by phosphoglucose isomerase, was conducted in the presence of deuterium oxide.

With respect to possible products derivable from a given aldose, while it is conceivable that both a ketose and a second aldose might be formed, it becomes more and more apparent (1-5) that in isomerization reactions of this type there is not a direct conversion of one aldose into the corresponding diastereoisomeric aldehyde; instead, the corresponding ketose is first formed which can then, in some cases, give rise to the second aldehyde. Some of the mechanistic reasons for these relationships have been investigated.

EXPERIMENTAL

Conversion of Fructose-6-phosphate to Glucose-6-phosphate in Deuterium Oxide—A solution of 200 mg. of barium fructose-6-phosphate in 2.0 ml. of 99.5 per cent D₂O was decolorized with Norit A. After a seed of crystalline barium glucose-6-phosphate was introduced, the solution was permitted to stand at room temperature for 10 hours in order that G-6-P
present in the commercial material might separate from the mixture as the barium salt; this was removed by filtration. Then 0.08 ml. of a solution of rabbit muscle phosphoglucose isomerase (6) was added to the clear supernatant fluid; final concentration of D$_2$O was 95.7 per cent; final pH, 7.0. Barium glucose-6-phosphate began to crystallize from the solution after about 1 hour; total incubation time at 26° was 15 hours. The product was collected by filtration and recrystallized three times as the barium salt from water by alternate acidification and neutralization. Yield, 53 mg. The purity of the crystalline barium salt heptahydrate was about 99 per cent, based upon assay with glucose-6-phosphate dehydrogenase.

Barium glucose-6-phosphate so obtained was found to contain 0.91 atom excess deuterium per mole. The osazone derived from this material was devoid of isotope.

*Synthesis of Glucose-6-phosphate-1-D*—3.5 gm. of barium gluconate-6-phosphate (7) were treated with Amberlite IR-120 (H) in 20 ml. of 99.5 per cent deuterium oxide in order to remove barium ions and exchange oxygen-bound hydrogen for deuterium. The supernatant solution was lyophilized to dryness and the acidic residue was dissolved in 23 ml. of 99.5 per cent D$_2$O. This solution was then heated at 65° for 2 hours in order to lactonize the acid. Reduction to the aldehyde was effected by treating the solution containing gluconolactone-6-phosphate with 37 gm. of 2.5 per cent sodium amalgam; the amalgam was added slowly while the temperature was maintained at 0°, and the pH was kept at 4.0 by the dropwise addition of sulfuric acid in D$_2$O. Barium chloride in D$_2$O was added to the aqueous layer which had been separated from mercury, and barium sulfate was removed by centrifugation. The pH of the supernatant solution was brought to 7.0 by the addition of NaOD which had been prepared from D$_2$O and sodium amalgam. The white, amorphous precipitate which formed at this point was removed by filtration, and the filtrate was seeded with crystalline barium glucose-6-phosphate and permitted to remain at room temperature. Crystals began to appear after 15 hours; after 7 days the crystalline product was collected by filtration, recrystallized four times from H$_2$O as the barium salt, converted to the brucine salt, and finally reconverted to the barium salt. Yield, 320 mg. The purity of the crystalline barium salt heptahydrate, based upon assay with glucose-6-phosphate dehydrogenase, was 96 per cent. The product contained 0.92 atom excess of deuterium per mole.

*Incubation of Glucose-6-phosphate-1-D with Phosphohexose Isomerases*—Rabbit muscle phosphoglucose isomerase free from phosphomannose isomerase was prepared by the method of Slein (6). The muscle phosphomannose isomerase (8) used in these experiments was a generous gift from Dr. Milton Slein of Camp Detrick, Maryland.
Glucose-6-phosphate-1-D, as the potassium salt, was incubated with phosphoglucone isomerase and also with a combination of this enzyme and phosphomannose isomerase. The water in the medium was then analyzed for deuterium by the optical spectroscopic method of Broida et al. (9); we are indebted to Dr. H. P. Broida and M. Chapman of the National Bureau of Standards for these determinations. The results are presented in Table I.

**Table I**

*Incubation of Glucose-6-phosphate-1-D with Phosphoheose Isomerases*

100 μmoles of glucose-6-phosphate-1-D (0.92 atom excess deuterium per mole) in total volume of 0.5 ml. Incubation, 1 hour at 26°, pH 6.8.

<table>
<thead>
<tr>
<th>System</th>
<th>Atom per cent excess deuterium in water medium</th>
<th>Atom deuterium per mole substrate released into water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoglucone isomerase*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>“ “ (boiled)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>“ “ + phosphomannose isomerase†</td>
<td>0.105</td>
<td>0.63</td>
</tr>
<tr>
<td>Phosphoglucone isomerase + phosphomannose isomerase (boiled)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* At the end of the incubation period this system contained 34 μmoles of F-6-P as determined by the method of Roe (10).
† At the end of the incubation, this system contained 36 μmoles of F-6-P. 5 times as much phosphoglucone isomerase as phosphomannose isomerase activity was present.

**DISCUSSION**

When fructose-6-phosphate was treated in deuterium oxide with muscle phosphoglucone isomerase, the reaction product, glucose-6-phosphate, was synthesized through the following scheme:

\[
\begin{align*}
H-\overset{\text{C}}{\text{O}}\text{H} & \quad \text{CH}_3\text{OPO}_3\text{H}_2 \\
H-\overset{\text{C}}{\text{O}}\text{H} & \quad \text{G-6-P} \\
\text{HO-}\overset{\text{C}}{\text{H}} & \quad \text{CH}_3\text{OPO}_3\text{H}_2 \\
\text{H-}\overset{\text{C}}{\text{O}}\text{H} & \quad \text{Phenylglucosazone-6-phosphate} \\
\text{H}-\overset{\text{C}}{\text{O}}\text{H} & \quad \text{CH}_3\text{OPO}_3\text{H}_2 \\
\text{H}-\overset{\text{C}}{\text{O}}\text{H} & \quad \text{CH}_3\text{OPO}_3\text{H}_2 \\
\text{H}-\overset{\text{C}}{\text{O}}\text{H} & \quad \text{CH}_3\text{OPO}_3\text{H}_2 \\
\end{align*}
\]

**Scheme 1**
found to have incorporated approximately 1 atom of carbon-bound deuterium per mole. The osazone prepared from the product was completely devoid of heavy hydrogen. Since the only carbon-bound hydrogen which the osazone lacks, as compared with the parent compound, is that originally present on C-2 (Scheme 1), the deuterium present in the hexose phosphate must have resided on C-2. These results suggest that the enzymatic isomerization proceeds via the following sequence (Fig. 1).

The hemiacetal linkage of the cyclic sugar is presumably first ruptured to give the open chain form; this is followed by labilization of an \( \alpha \)-hydrogen on C-1 as a proton and consequent enediol formation. It is Reaction \( e \) of the sequence which leads to deuterium incorporation on C-2 when the reaction is carried out in deuterium oxide. A hydride-ion mechanism, whether it be intramolecular or intermolecular, would lead to no deuterium uptake on carbon. In these respects the enzymatic transformation is similar to the alkali-catalyzed interconversion of glucose and fructose as demonstrated by Topper and Stetten (11) and Sowden and Schaffer (12).

It is not known whether the isomerase has mutarotase-like activity or whether the conversion of the cyclic to the open chain forms of the hexose phosphates is non-enzymatic.

Recently, Slein (13) has shown that a second phosphohexose isomerase, present in rabbit muscle and referred to as phosphomannose isomerase, interconverts F-6-P and mannose-6-phosphate (M-6-P). It thus appears that, whereas the ketohexose phosphate can give rise to one or the other aldose phosphate, according to which of the two isomerases acts upon it, the conversion of one aldose to the other does not occur directly but proceeds via the intermediary formation of fructose-6-phosphate. Some
stereochemical information relating to this point is recorded in Table I. Thus, whereas the dynamic equilibrium between G-6-P and F-6-P catalyzed by phosphogluco-isomerase does not lead to a loss of deuterium initially present on C-1 of synthetic glucose-6-phosphate-1-D, the simultaneous presence of phosphomannose isomerase does result in a release of deuterium from the substrate into the medium. Thus, the isomerases are able to distinguish between the 2 hydrogen atoms on C-1 of F-6-P; furthermore,

![Diagram of stereochemical relationships](http://example.com/diagram.png)

**Fig. 2. Stereospecificity of the phosphohexose isomerases.** PGI, phosphogluco-isomerase; PMI, phosphomannose isomerase.

![Diagram of configurational relationships](http://example.com/diagram2.png)

**Fig. 3. Configurational relationships about the first 3 carbon atoms of F-6-P.** Heavy bars indicate possible sites of attachment to enzyme.

it is clear that the hydrogen which is attacked by phosphogluco-isomerase is not the one which is acted upon by phosphomannose isomerase, and vice versa (Fig. 2).

Several long established stereochemical facts about these sugars should now be pointed out. In F-6-P, by virtue of the double bond on C-2, it is possible for C-1, C-2, their respective oxygen atoms, one of the H atoms on C-1, and C-3 to assume an essentially planar configuration (Fig. 3). In the aldehydes where the double bond is on C-1, C-3 cannot be coplanar with the 5 atoms mentioned above (Fig. 4). As demonstrated in an earlier section of this paper, an enediol is presumably formed as the intermediate in these isomerizations; in this form C-1, C-2, and the 4 atoms bound to
them are necessarily coplanar. It is necessary, therefore, to visualize two geometrical isomers, a cis and a trans enediol, the one giving rise to G-6-P and the other to M-6-P. Two facts are now known about the relationship between F-6-P and enediol formation; i.e., the ketose can give rise to one or the other geometrical isomer, depending upon which enzyme acts upon it, and one or the other of the 2 hydrogen atoms on C-1 is activated in the process. Stereospecificity for hydrogen such as has been described indicates that the enzymes bind the substrates at a minimum of three points. Let us suppose that three binding sites for F-6-P about C-1 and C-2 are as depicted in Fig. 3. If A is the binding pattern for the one isomerase, then B is the pattern for the other; it is reasonable to assume that in the enzyme-substrate complex the 6 atoms of the substrate mentioned above are coplanar, since the enediol subsequently formed is necessarily coplanar about these points and is presumably enzyme-bound. Accordingly, a cis-enediol is the intermediate in one isomerization while a trans-enediol is involved in the other. This, in turn, is directly related to stereospecificity for hydrogen (Fig. 2).

The binding pattern for the aldehydes would presumably be as depicted in Fig. 4, where C-3 is visualized as projecting out from the plane of the paper in each case. It appears, then, that the inability of C-3 of the aldehydes to achieve coplanarity with the groups about C-1 and C-2 is largely responsible for the fact that the aldoses can give rise to only one or the other of the enediols. Further, it may be deduced that C-3 is probably not a binding site since in the conversion of aldehyde into enediol a considerable movement of C-3 into the plane of the carbon-carbon double bond must occur.

Similar considerations probably apply to a variety of aldose isomerase systems reported by a number of investigators. Thus enzymes have been described which interconvert mannose and fructose (1), lyxose and xylulose

![Fig. 4. Configurational relationships about the first 3 carbon atoms of G-6-P and M-6-P. Heavy bars indicate possible sites of attachment to enzyme.](image-url)
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(1), rhamnose and rhamnulose (1), ribose-5-P and ribulose-5-P (2), arabinose and ribulose (3), xylose and xylulose (4), and fucose and fuculose (5). In none of these transformations is one aldose converted into the corresponding epimeric aldehyde; instead, the corresponding ketose is formed.

SUMMARY

It has been demonstrated, with the aid of deuterium, that the interconversion of glucose-6-phosphate and fructose-6-phosphate, catalyzed by phosphoglucone isomerase, probably proceeds via an enediol intermediate. A detailed mechanism to account for this over-all isomerization has been proposed.

It has also been shown that, of the 2 hydrogen atoms on C-1 of fructose-6-phosphate, one is activated by phosphoglucone isomerase and the other by phosphomannose isomerase. Because of such stereospecificity and other stereochemical considerations, it is concluded that fructose-6-phosphate can form either a cis- or trans-enediol, depending upon which of the two isomerases acts upon it, whereas glucose-6-phosphate and mannose-6-phosphate can form only one or the other of the two geometrical isomers. A rationalization of these apparent differences has been offered.

BIBLIOGRAPHY

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