The Journal of Biological Chemistry
Vol. 250, No. 18, Issue of September 25, pp. 7277-7279. 1975
Printed in U.S.A.

Mechanistic Implications of the pH Independence of Inhibition of Phosphoglucose Isomerase by Neutral Sugar Phosphates*

(Received for publication, December 30, 1974)

JOHN M. CHIRGWIN,† THOMAS F. PARSONS,$ AND ERNST A. NOLTMANN¶
From the Department of Biochemistry, University of California, Riverside, California 92502

In contrast to the strongly pH-dependent inhibition of phosphoglucose isomerase by substrate analogues with a free carboxyl group, inhibition of this enzyme by neutral sugar phosphates is essentially invariant between pH 7 and 9. Competitive inhibition constants for glucitol 6-phosphate (40 μM), arabinose 5-phosphate (50 μM), and erythritol 4-phosphate (100 μM) were found to be of the same order of magnitude as that reported previously for substrate binding constants (50 to 240 μM). The unique exception is erythrose 4-phosphate whose $K_i$ (0.7 μM, independent of pH) reflects a tightness of binding similar to that found at pH values near or below neutrality for the transition state analogue 5-phosphorarabinonate. The pH independence of inhibition by erythrose 4-phosphate and other neutral sugar phosphates may reflect a mode and locus of binding to phosphoglucose isomerase different from that of the aldolate inhibitors.

Phosphoglucose isomerase is believed to catalyze the interconversion of glucose 6-phosphate and fructose 6-phosphate via five enzyme-bound metastable intermediates (1, 2): glucopyranose 6-phosphate ≡ aldehydo-glucose 6-phosphate ≡ cis-1,2-enediolate anion ≡ keto-fructose 6-phosphate ≡ fructofuranose 6-phosphate.

The acyclic (aldehydo- and keto-) and enediolate intermediates are predicted by transition state theory (3–5) to bind to the enzyme more tightly than the cyclic hemiacetals of the substrates, which are the predominant forms in solution (2). If analogous of these intermediates bind more tightly than substrates to the enzyme, this corroborates the occurrence of intermediates in the enzymatic reaction. Evidence of this kind has been obtained for analogues of the enediolate anions of triose phosphate isomerase (3) and phosphoglucose isomerase (6, 7). For both enzymes such analogues have been found to bind with a strong and characteristic pH dependence which appears to be caused by direct electrostatic interaction between enzyme and inhibitor (7).

It was therefore of interest to determine whether substrate analogues other than those mimicking the enediolate (all of which have a free carboxyl group) would show a pH dependence of inhibition. Compounds investigated with respect to their inhibition of phosphoglucose isomerase as a function of pH were: erythrose 4-phosphate, two other acyclic inhibitors, glucitol 6-phosphate and erythritol 4-phosphate, and a presumably cyclic one, arabinose 5-phosphate. Of these, erythrose 4-phosphate was of special interest since it had previously been studied by Grazi et al. (8) who determined its $K_i$ at pH 7.6 to be between 0.7 and 1.0 μM.

EXPERIMENTAL PROCEDURES

Rabbit muscle phosphoglucose isomerase prepared according to Blackburn et al. (9) was assayed spectrophotometrically at 30° in the direction from fructose 6-phosphate to glucose 6-phosphate by coupling to yeast glucose-6-phosphate dehydrogenase and NADP (10). Erythrose 4-phosphate was prepared from the dimethyl acetal (Calbiochem), and erythritol 4-phosphate was a gift from Dr. C. E. Ballou; other inhibitors were purchased from Sigma. The concentrations of stock solutions of these compounds were determined as total minus inorganic phosphate. The half-life of erythrose 4-phosphate as the free phosphate ester in aqueous solution was determined to be 130 days at pH 7.5 and 4°. Details of the procedures for $K_i$ determinations are provided in the preceding paper (7).

RESULTS AND DISCUSSION

Figs. 1 and 2 represent Dixon plots (11) of the $K_i$ values for erythrose 4-phosphate, erythritol 4-phosphate, arabinose 5-phosphate, and glucitol 6-phosphate as a function of pH. The data indicate that the competitive inhibition constants for all four compounds are independent of pH and have values of 0.7, 100, 50, and 40 μM, respectively. When $K_i$ values for erythrose 4-phosphate were determined in Tris buffer, which is a primary amine, they were found to be about 20 times larger than those obtained in buffers which do not have primary amino groups (see Fig. 1), suggesting that the amine and the aldehyde might form a Schiff base or carbinolamine.

Since an active site lysyl residue of phosphoglucose isomerase can form a Schiff base with pyridoxal 5'-phosphate (12), the tight binding of erythrose 4-phosphate could be the manifestation of a similar type of covalent adduct formation.
Buffers without primary amino groups, Na⁺ or Cl⁻ form; performed at 30°C in 0.1 M piperazine-N,N′-bis(2-ethanesulfonic acid), N-2-hydroxyethylpiperazine-N'-2:ethane sulfonic acid, and N,N′-bis-(2-hydroxyethyl)glycine buffers, Na⁺ or Cl⁻ form; performed in 0.1 M Tris chloride buffer.

Further assays (unfilled symbols), however, showed that \( K_a \) is 20-fold higher in Tris, which carries a primary amino group.

However, activity was not lost when the enzyme was treated with sodium borohydride in the presence of erythrose 4-phosphate, even when non-denaturing amounts of urea or guanidine hydrochloride were added, nor was tritium from \([3H]NaBH_4\) incorporated into the protein in the presence of the inhibitor.

Our previous proposal (1) that the straight chain forms of the substrates are intermediates in the reaction mechanism of phosphoglucose isomerase is supported by the fact that the best inhibitors of the enzyme are the straight chain compounds 5-phosphoarabinonate, erythrose 4-phosphate and 6-phosphogluconate (6). As shown in Table I, the results of our inhibition studies as a function of pH with rabbit muscle phosphoglucose isomerase are in good agreement with those obtained at pH 8.0 with the yeast enzyme by Salas et al. (13). The present data thus affirm the conclusion of Salas et al. that strong inhibition of phosphoglucose isomerase requires an acyclic sugar phosphate possessing a free carbonyl group. Thus, 4-phosphoerythronate, in which the aldehyde at C-1 of erythrose 4-phosphate is replaced by a carboxylate, is a poor inhibitor of phosphoglucose isomerase, with a \( K_a \) of 140 μM at pH 8 (7). Erythritol 4-phosphate is a similarly poor inhibitor, with a \( K_a \) of 100 μM (Fig. 2), and glucitol 6-phosphate (acyclic) and arabinose 5-phosphate (cyclic) have \( K_a \) values much larger than that for erythrose 4-phosphate.

Whereas the specific molecular mechanism of the very tight binding of erythrose 4-phosphate to phosphoglucose isomerase is unknown at the moment, it is evident from the data in Fig. 1 that it does not involve an ionizing residue at the active site, as has been found with the aldolase inhibitors (6, 7). In fact, x-ray crystallographic data support the existence of a separate binding site for erythrose 4-phosphate. The zero slopes in the Dixon plots of Fig. 1 indicate that there is no dependence of the inhibition on pH. The relatively high \( K_a \) values for the tested sugar phosphates other than erythrose 4-phosphate (see Table I) indicate that they would only be able to compete effectively for binding with the Michaelis complexes occurring in the course of catalytic conversion from substrate to product.

It is interesting to note that Avigad has reported (14) that millimolar levels of the two substrates of phosphoglucose isomerase inhibit glycogen phosphorylase. Therefore, if the inhibitory effects of erythrose 4-phosphate and 6-phosphogluconate bear any physiological significance, it could lie in their potential to control the relative rates of glycogenolysis, glycolysis, and the pentose phosphate pathway by affecting the levels of glucose 6-phosphate and fructose 6-phosphate.

A final point to be made from our data is a note of caution regarding the determination of binding constants for free carbonyl sugars. As can be seen from Fig. 1, erythrose 4-phosphate interacts with certain buffers, apparently by Schiff base or carbinolamine formation with primary amines, and by so doing gives misleading binding constants.

Acknowledgments—We wish to thank Dr. C. E. Ballou, University of California, Berkeley, for a generous gift of erythritol 4-phosphate, Dr. K. D. Schnackertz, University of

\[ \text{Glucose 6-phosphate and fructose 6-phosphate.} \]

**Table I**

<table>
<thead>
<tr>
<th>Compound</th>
<th><strong>( K_a )</strong> μM</th>
<th><strong>( K_b )</strong> μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrose 4-phosphate</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>Erythritol 4-phosphate</td>
<td>100</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Glucitol 6-phosphate</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Arabinose 5-phosphate</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>d-Erythro-pentulose 5-phosphate</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

*Results with the rabbit muscle enzyme at 30°C. Average values over the physiological pH range.

*Results with the yeast enzyme in 0.04 M Tris at pH 8.0 and 20°C, taken from Salas et al. (13).
Würzburg, for performing some of the experiments on Schiff base formation, and Ms. Adele Register for technical assistance.

REFERENCES
Mechanistic implications of the pH independence of inhibition of phosphoglucose isomerase by neutral sugar phosphates.
J M Chirgwin, T F Parsons and E A Noltmann


Access the most updated version of this article at http://www.jbc.org/content/250/18/7277

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/18/7277.full.html#ref-list-1