THE INHIBITION OF PHOSPHOGLUCOSE ISOMERASE BY D-ERYTHROSE 4-PHOSPHATE

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The following compounds are known to be inhibitors of phosphoglucose isomerase: 6-phosphogluconate,[1] sorbitol 6-phosphate,[2] D-glucosamine 6-phosphate[3] and 2-deoxyglucose 6-phosphate.[4] As these substances have the same chemical structure in carbon atoms 3-6 it is believed that this part of the molecule is responsible for the inhibitory effect. Erythrose 4-phosphate shares the same configuration in all carbon atoms, particularly if the hydrated form of the aldehydic group is considered. This compound has now been found to act as a competitive inhibitor of phosphoglucose isomerase and to possess an unusually high affinity for this enzyme.

MATERIALS

Phosphoglucose isomerase from rabbit muscle was a commercial preparation from the Sigma Chemical Company. Crystalline glucose 6-phosphate dehydrogenase was purchased from Boehringer and Sons. D-Erythrose 4-phosphate as the dimethyl acetal cyclohexylamine salt was kindly supplied by Dr. C.E. Ballou of the University of California. Other substrates were commercial preparations.

EXPERIMENTAL AND RESULTS

The assay methods were as follows:

(1) With fructose 6-phosphate as substrate, glucose 6-phosphate formation was followed spectrophotometrically at 340 mμ by TPN reduction The assay system (1 ml.) contained 0.2 micromoles of TPN;0.021 mg.

of phosphoglucose isomerase; 0.01 ml. of glucose 6-phosphate dehydrogenase; glycylglycine buffer, 0.05M, pH 7.6; and fructose 6-phosphate in the concentrations indicated. The temperature was 20°. Phosphoglucose isomerase was added to start the reaction and readings were taken at 30 second intervals.

(2) With glucose 6-phosphate as substrate, fructose 6-phosphate formation was evaluated following the colorimetric technique of Roe and Papadopoulos (5). The assay system (1 ml.) contained 0.03 mg. of phosphoglucose isomerase; glycylglycine buffer, 0.05 M, pH 7.6; and glucose 6-phosphate in the concentrations indicated. Isomerase was added to start the reaction. In both test systems other compounds were added as indicated.

The inhibition of phosphoglucose isomerase by erythrose 4-phosphate is competitive with both of the substrates for this enzyme (Figs. 1-2). With fructose 6-phosphate as substrate several experiments were carried out (Fig. 1), from which K_m for fructose 6-phosphate was calculated to be 8×10^{-5}M. K_i for erythrose 4-phosphate was found to lie between 0.7 and 1×10^{-6}M, indicating that the affinity of the enzyme for this compound is approximately 100 times that for fructose 6-phosphate.

The experiments with glucose 6-phosphate as substrate were carried out at 2° in order to allow enough time for the kinetic experiments, and therefore the affinity constants cannot be compared directly. However from the data in Figure 2 it is evident that the inhibition by erythrose 4-phosphate is also competitive with this substrate.

In order to compare the affinity constants for glucose 6-phosphate and fructose 6-phosphate the results were interpreted according to the integrated rate equation as suggested by Alberty.[6] The results obtained with two different concentrations of substrate (Fig. 3) indicate that the values of K_m for glucose 6-phosphate and fructose 6-phosphate are identical at 20°, and equal to 7.6×10^{-5}M.

In control experiments it was demonstrated that erythrose 4-phosphate was without effect on either triose phosphate isomerase or glucose 6-phosphate dehydrogenase. Phosphoglucose isomerase is not inhibited by the concentrations of cyclohexylamine which were present in

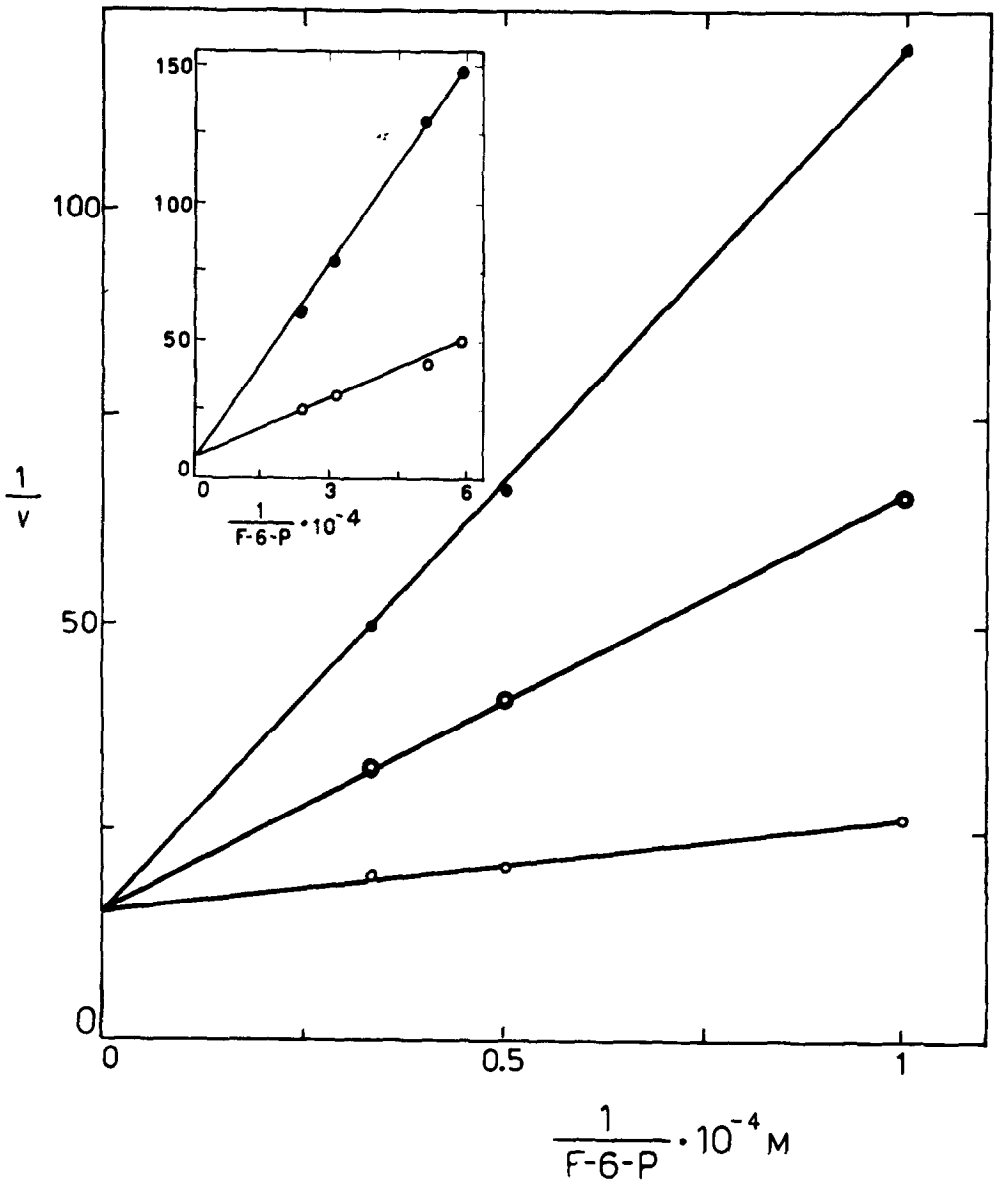

Fig. 1. Inhibition of phosphoglucose isomerase as a function of the concentration of fructose 6-phosphate. The concentrations of erythrose 4-phosphate were as follows: filled circles, 3.5×10^{-6}M; heavy unfilled circles, 7×10^{-6}M. In the insert the concentration of erythrose 4-phosphate was 1.7×10^{-6}M. In both parts of the figure the light open circles were without erythrose 4-phosphate.

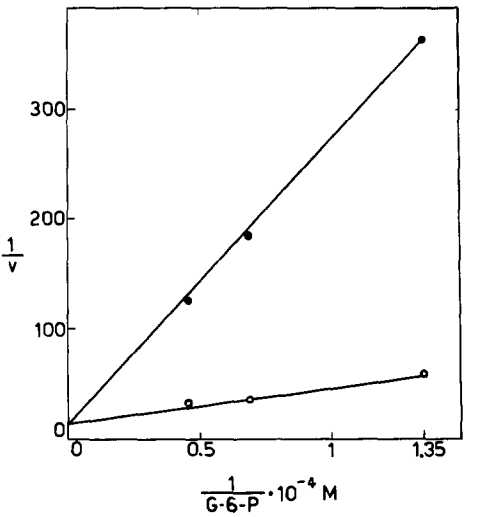

Fig. 2. Inhibition of phosphoglucose isomerase as a function of the concentration of glucose 6-phosphate. In the experiment indicated by the filled circles the concentration of erythrose 4-phosphate was 0.7×10^{-6}M. The temperature was 2°.

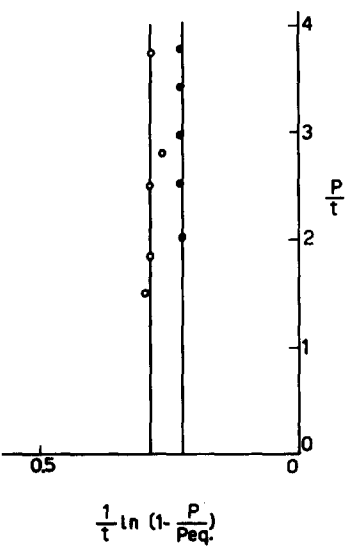

Fig. 3. Evaluation of K_m of glucose 6-phosphate isomerase for glucose 6-phosphate and fructose 6-phosphate according to the integrated rate equation. The experimental conditions were as in Method 2. The concentration of fructose 6-phosphate was 2.17×10^{-4}M in the experiment indicated by the open circles and 2.9×10^{-4}M in the experiment indicated by the filled circles.

these experiments.

The inhibition of phosphoglucose isomerase by erythrose 4-phosphate and remarkably high affinity of the enzyme for this substrate suggests that it may function in a regulatory manner in carbohydrate metabolism. Under conditions where the metabolism of fructose 6-phosphate by the transketolase-transaldolase sequence might lead to an accumulation of the tetrose ester it would prevent the further formation of the substrate through its effect on phosphoglucose isomerase. Inhibition by erythrose 4-phosphate may also explain the lack of proportionality experienced with the transaldolase assay by Horecker and Smyrniotis,[7] since at high enzyme levels removal of erythrose 4-phosphate by the action of muscle aldolase might be expected to be incomplete. Further support for this notion must await the demonstration of erythrose 4-phosphate accumulation in tissues or tissue extracts.

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