Stereospecificity of the sugar-phosphate isomerase reactions; a uniformity

It has been shown for all the phosphoaldose isomerases studied thus far that the interconversion of aldose and ketose in deuterated or tritiated water results in the incorporation of one atom of isotope in the C-2 and C-1 positions respectively[1-3]. Furthermore, it has been shown that the position labeled on C-1 of fructose 6-phosphate formed from glucose 6-phosphate with phosphoglucose isomerase is not the same as that labeled in the reaction of mannose 6-phosphate with phosphomannose isomerase[1].

The absolute configuration of the monotritiated dihydroxyacetone phosphate formed with triose phosphate isomerase in tritiated water has been established by conversion of that ketose to glycolic acid through HIO_4 oxidation and further oxidation to glyoxylic acid by the stereospecific reaction with glycolic acid oxidase[4]. In the present report, a similar approach has been used to determine the stereospecificity of labeling of fructose 6-phosphate and ribulose 5-phosphate formed by reaction with phosphoglucose isomerase and phosphopentose isomerase respectively. It is the purpose of this work to determine whether there is any uniformity underlying the stereospecificity of the different isomerase reactions.

D-ribose 5-phosphate was treated with spinach phosphopentose isomerase[5] in tritiated water until equilibrium had been established. The mixture was treated with HIO_4 and the glycolate isolated by ion-exchange chromatography and converted to glyoxylate enzymically[4]. D-[1-^{3}H]glucose was phosphorylated with yeast hexokinase and the isolated glucose 6-phosphate was treated with phosphoglucose isomerase until equilibrium amounts of fructose 6-phosphate had been formed. This mixture was then treated as above for the eventual isolation of glyoxylate derived from the first two carbons of the fructose 6-phosphate.

The results of this pair of experiments (Table I) show that in the phosphoglucose isomerase reaction the proton from water is not found in the final glyoxalate (that is, tritium is retained), and that in the phosphopentose isomerase reaction the tritium

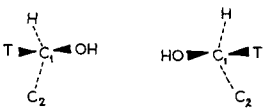
TABLE I

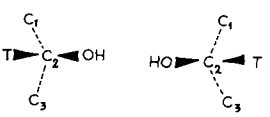
ANALYSIS OF GLYCOLIC AND GLYOXYLIC ACIDS DERIVED FROM EQUILIBRIUM MIXTURES OF PHOSPHOGLUCOSE AND PHOSPHORIBOSE ISOMERASE REACTION

In the first experiment D-ribose 5-phosphate (30 μmoles) was incubated 15 min in 0.3 ml ^3H_2O (156,000 counts/min/μatom hydrogen) with phosphopentose isomerase (an amount of enzyme to convert 10 μmoles substrate/min), 0.06 *M* triethanolamine-acetate buffer, pH 7.5, and 3.5 m*M* cysteine. In the second experiment D-[1-^{3}H]glucose 6-phosphate (6.3 μmoles and containing 140,000 counts/min/μmole) was incubated with phosphoglucose isomerase (an amount of enzyme to convert 3 μmoles of substrate/min) and 0.05 *M* triethanolamine-acetate, pH 7.5 in a final volume of 1.5 ml for 15 min at 37°. Both incubations were treated with HIO_4 and the remaining procedure for isolation and counting is as previously described[5].

Expt.	*Specific activity of glycolic acid (counts/min/μmole)*	*Specific activity of glyoxylic acid (counts/min/μmole)*	*% of tritium specifically located*
1. phosphoribose isomerase	178,000	7,525	96
2. phosphoglucose isomerase	90,000	107,000	108

derived from the water is lost in the oxidase step. From these data, the previous report on phosphotriose isomerase, and the observation that phosphomannose isomerase and phosphoglucose isomerase act on different C-1 positions of fructose 6-phosphate it may be concluded that dihydroxyacetone phosphate, D-ribulose 5-phosphate, and D-fructose 6-phosphate derived respectively from reaction of triosephosphate isomerase, phosphopentose isomerase and phosphoglucose isomerase in tritiated water have the configuration around C-1 shown in Fig. 1a, whereas fructose-6-phosphate derived from the phosphomannose isomerase reaction in tritiated water is as shown in Fig. 1b. On the other hand, it is clear from the structure of the aldosephosphates corresponding to the above sequence that the first three, D-glyceraldehyde 3-phosphate, D-ribose 5-phosphate, and D-glucose 6-phosphate formed in tritiated water have the configuration at C-2 shown in Fig. 2a and that D-mannose 6-phosphate is as shown in Fig. 2b. Thus a correlation seems to exist among the four enzymes for which data is available between the configuration of the aldose and that of the derived, tritiated ketose.





It has been proposed[1] that the mechanism of the isomerase reactions might involve the intermediate formation of a 1,2 enediol bound to the enzyme. If this is assumed to be correct then the present results may be generalized in the following terms: (a) If a cis-enediol is formed, proton attack on C-1 or C-2 must occur from the same side of the plane of the enediol structure. (b) If a trans-enediol is formed, proton attack on C-1 or C-2 must occur from opposite sides of the plane of the enediol structure. (c) A cis-enediol intermediate cannot be attacked by protons from opposite sides nor can a trans-intermediate be attacked at each carbon from the same side.

In the paper of TOPPER[1] it was suggested that the difference in stereospecificity of phosphoglucose and phosphomannose isomerases required that the enediols formed be cis in one case and trans in the other. However, it is possible that only (a) or (b) holds in both cases if one provides that opposite faces of the plane of the same enediol are bound in the complex with each enzyme.

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