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STEREOCHEMICAL EVIDENCE FOR A *CIS*-ENEDIOL INTERMEDIATE IN Mn-DEPENDENT ALDOSE ISOMERASES

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SUMMARY

Three Mn^{2+}-dependent bacterial aldose-ketose isomerases were examined to determine the stereochemistry of the C-1 proton of the ketose that is abstracted in the formation of the aldehyde. In the cases of D-xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5) and L-arabinose isomerase (L-arabinose ketol-isomerase, EC 5.3.1.4) it is the pro-R position of the D-xylulose and L-ribulose that is activated, whereas in the L-fucose isomerase reaction it is the pro-S position of L-fuculose that is labeled in tritiated water. Little proton exchange occurs in these reactions, especially the xylose isomerase, where retention of the substrate proton is complete.

These stereochemical results conform to those of four other sugar isomerases: triose-*P*, D-ribose-5-*P*, D-mannose-6-*P*, and D-glucose-6-*P* isomerase. Protonation at C-1 or C-2 from the same side of the plane of a *cis*-enediol gives the correct stereochemical result for all seven isomerases.

INTRODUCTION

It has been reported previously that the three enzymatic reactions: D-glucose-6-*P* $\rightleftarrows$ D-fructose-6-*P*, D-ribose-5-*P* $\rightleftarrows$ D-ribulose-5-*P*, and D-glyceraldehyde-3-*P* $\rightleftarrows$ dihydroxyacetone-*P*, when catalyzed in ^3H_2O, give rise to labeling at C-2 of the aldose and C-1 of the ketose which are isosteric[1]. On the other hand, the reaction D-mannose-6-*P* $\rightleftarrows$ D-fructose-6-*P*, has opposite stereochemistry at C-2 of the aldose and opposite labeling at C-1 of the ketose[2]. Since it had been observed that glucose-6-*P* isomerase catalyzed the vicinal transfer of ^3H (ref. 3), and since a similar conclusion pertains to mannose-*P* isomerase[4], a mechanism of proton donation from a single conjugate acid group to either C-1 or C-2 of an enediol was proposed[5]. With the restriction that proton additions occur from one side of the enediol plane, only a *cis*-enediol intermediate fitted the observed stereochemistries[5]. The present investigation concerns the stereochemical course of the following three Mn^{2+}-dependent isomerase reactions: D-xylose $\rightleftarrows$ D-xylulose, L-arabinose $\rightleftarrows$ L-ribulose, and L-fucose $\rightleftarrows$ L-fuculose. As was done previ-

ously, the stereochemistry of tritium labeling of the C-1 position of each ketose was determined by making use of the stereospecifiicity of glycolate oxidase acting on the glycolate formed by periodate oxidation of the ketose[1].

EXPERIMENTAL

Preparation of enzymes

The procedure of K. YAMANAKA was followed for the preparation of D-xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5) from *Lactobacillus brevis* (ATCC 14869) grown with D-xylose[6]. L-Arabinose isomerase (L-arabinose ketol-isomerase, EC 5.3.1.4) was obtained from the same preparation as a later peak from the DEAE-cellulose column. Both enzymes were homogeneous in the ultracentrifuge*.

L-Fucose isomerase was prepared from *Aerobacter aerogenes* beyond the state reported by MORTLOCK[7], to electrophoretic homogeneity**.

Preparation of tritium-labeled reactants

Incorporation of label from ^3H_2O was obtained upon incubation of L-arabinose or L-fucose with the appropriate homogeneous isomerase. Although these reactions had reached equilibrium, the extent of labeling of the ketose was much less than expected. These low values may be explained by a large isotope effect and by the transfer, in large part, of the C-2 proton to the keto-product. In the case of D-xylose isomerase the incorporation of tritium into an equilibrium mixture occurred at $< 0.4\%$ of the velocity of the forward reaction and this was probably due to non-enzymatic exchange since it was uninhibited by a concentration of xylitol that inhibits the

TABLE I

STEREOCHEMISTRIES OF ^{3}H-GLYCOLATES FROM PERIODATE OXIDATION OF THE ISOMERASE REACTION MIXTURES

Reaction mixture	*Glycolate from IO_4^- oxidation of product (counts/min per μmole)*	*Product of glycolate oxidase*	
		Water (counts/min per μmole of glycolate oxidized)	*Glyoxalate (counts/min per μmole)*
[1-^{3}H]-D-xylose (0.1 M, 4030 counts/min per μmole) + xylose isomerase (13 units/ml) + maleate$^-$ (50 mM, pH 6) + $MnCl_2$ (0.5 mM) (30 min at 35° → 19 mM D-xylulose)	4 050	38	5 200
L-Fucose (6 mM) + D-arabinose isomerase (10 units/ml), Tris (30 mM, pH 7) + $MnCl_2$ (6 mM) + ^3H_2O (61 500 counts/min per μatom H) (11 min at 37° → 0.6 mM L-fuculose)	14 900	264	14 970
L-Arabinose (20 mM) + L-arabinose isomerase (27 units/ml) + Tris (0.1 M, pH 7.4) + $MnCl_2$ (2 mM) + ^3H_2O (366 000 counts/min per μatom) (3 h at 25° → 3.5 mM L-ribulose)	40 000	39 000	1 040

* We are indebted to Miss Betty Travaglini for these determinations.

** According to an unpublished procedure of E. J. Oliver and R. P. Mortlock.

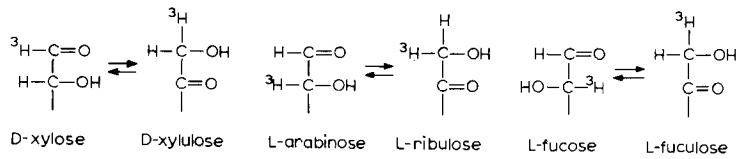
isomerization rate 70%. The failure of xylose to incorporate ^2H from an ^2H_2O medium was indicated by the fact that the C-1 proton of xylose remained a doublet signal in nuclear magnetic resonance measurement for many hours in the presence of a large amount of enzyme*.

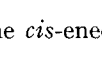
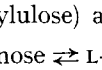
To introduce tritium into the C-1 of D-xylulose it was therefore necessary to begin with tritiated xylose. [1-^3H]-D-xylose was prepared from [1-^3H]glucose by the procedure of SOWDEN[8]** modified to make use of $NaBH_4$ in the reduction of the 5-aldo-monoacetonexylose at pH 6.0.

The three reaction mixtures described in Table I were taken to dryness to remove ^3H_2O, or in the case of the xylose isomerase incubation, to determine volatile radioactivity. The residue was dissolved in 0.05 M H_2SO_4 and 0.1 M H_5IO_6, 10 μmoles/μmole of pentose, was added. After 1 h at 25°, excess ethylene glycol was added and insoluble material removed by centrifugation. The supernatant was diluted to < 0.01 M anion and adjusted to pH 7 with NaOH. Glycolate, representing C-1 and C-2 of the ketose was separated from the formate derived from C-1 of the aldose by ion exchange on Dowex-1 (acetate) by elution with 4 M acetic acid[9]. The acetic acid was removed by evaporation *in vacuo* at 25°. The glycolate was converted to glyoxalate by reaction with glycolate oxidase of spinach[10] which is known to remove the pro-R position† only[10,12]. Radioactivity was determined in the water recovered by sublimation and in the glyoxalate isolated on Dowex-1 (acetate)[9]. Glycolate[13] and glyoxalate[14] were assayed chemically.

RESULTS

Table I indicates the following interconversions:



These pairs are obtained from the *cis*-enediol  by proton additions from above the plane (D-xylose ⇄ D-xylulose) and from  by tritium additions from above the plane (L-arabinose ⇄ L-ribulose) or from below the plane (L-fucose ⇄ L-fuculose).

In no case is it possible to obtain the proper labeling of the ketose from the stated aldose by additions to the same side of a *trans*-enediol. These results extend to the three Mn^{2+}-dependent enzymes the previously noted correlation with the Zn^{2+}-containing[15] mannose-*P* isomerase and three nonmetal-dependent isomerases.

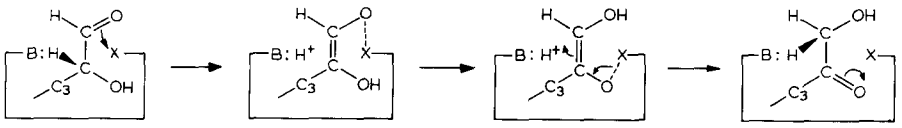
The probability that this conformity occurred by chance in nature is 1 in 64, $(1/2)^6$. On the grounds that only that which is functionally important is conserved in

* We are indebted to Dr. A. Mildvan for this observation.

** We are indebted to Dr. R. Schaffer, National Bureau of Standards for calling this method to our attention.

† According to HANSON's pro-R/pro-S system for designating identical ligands of a tetrahedral carbon[11].

evolution, it seems valid to conclude that the geometric form of the intermediate is critical to the aldose–ketose interconversion mechanism. It thus would follow that a single electrophilic center (acid group or metal ion) is used for the polarization of either carbonyl group as shown:



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