ANOMERIC SPECIFICITY DURING SOME ISOMERASE REACTIONS*

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SUMMARY. An examination of the C-1 NMR proton signals arising from D-glucose and D-xylose as they are produced from the ketose via an isomerase-catalyzed reaction in deuterium oxide solution indicate that both conversions proceed via 1,2-proton transfer with no solvent exchange at C-2 of the aldose. In the latter case, the initial product appears to be α-D-xylopyranose. In a reinvestigation of the phosphoglucose isomerase reaction, a combination of 1,2 transfer and solvent exchange could be verified, but, contrary to an earlier report, the observed products are an equilibrium mixture of the α and β-D-glucose-6-phosphates.

INTRODUCTION. NMR spectroscopy has been used to study mechanistic aspects of a number of enzyme reactions (1-3), and a recent study in this laboratory (4) involving phosphoglucose isomerase and phosphoribose isomerase reported that, by observing NMR signals arising from the C-1 protons (the anomeric protons) of the aldose as it is produced from the ketose, it is possible, in theory, to determine the importance of 1,2 proton transfer (5) versus solvent proton exchange at C-2 of the aldose, and, to determine the anomeric form of aldose initially produced (anomeric specificity). This report describes such applications to some D-fructose-D-glucose and D-xylulose-D-xylose interconversions catalyzed by a crystalline manganese-requiring isomerase obtained from a Streptomyces species, and, to a reinvestigation of the phosphoglucose isomerase reaction with respect to anomeric

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specificity.

RESULTS AND DISCUSSION. The incubation of D-fructose at 60° for 12 hours and D-xylose at 37° for 3 hours with isomerase in buffered deuterium oxide produced equilibrium mixtures in which the anomeric proton signals of D-glucose ($H_1^{\alpha}=\tau 4.78$, J = 3.0 cps; $H_1^{\beta}=\tau 5.43$, J = 7.5 cps) and D-xylose ($H_1^{\alpha}=\tau 4.72$, J = 3.0 cps; $H_1^{\beta}=\tau 5.43$, J = 7.5 cps) all appeared as doublets produced by splitting by a proton at C-2, indicating a conversion involving exclusive 1,2 proton transfer when proceeding from ketose to aldose. This observation is in contrast to the phosphoglucose isomerase reaction (4) where considerable solvent exchange occurs at C-2 (as evidenced by initial, partial deuterium incorporation at C-2 and the resultant production of both doublet and singlet signals from H_1 protons), but is in agreement with recent conclusions reported by Mildvan and Rose (6) for a similar D-xylose isomerase.

Because the D-xylulose-D-xylose interconversion was substantially faster than the rate of spontaneous anomerization of D-xylose itself, it was possible to determine anomeric specificity in this system. Thus, D-xylulose and isomerase, in buffered deuterium oxide were placed in a spin tube, held at 37°, and NMR spectra scanned at intervals. The results (Fig. 1) indicate the initial, preferential appearance of H_1^{α} signals. The appearance of weak H_1^{β} signals after 20 minutes reaction is consistant with formation via spontaneous mutarotation of the α anomer under the conditions employed. Since signals produced by D-xylulose interfere with the detection of H_1^{β} signals only at very low levels, and, the measured ratio of H_1^{β}/H_1^{α} at equilibrium is approximately 2.0, the results clearly indicate that α-D-xylopyranose is the initial reaction product. The observed

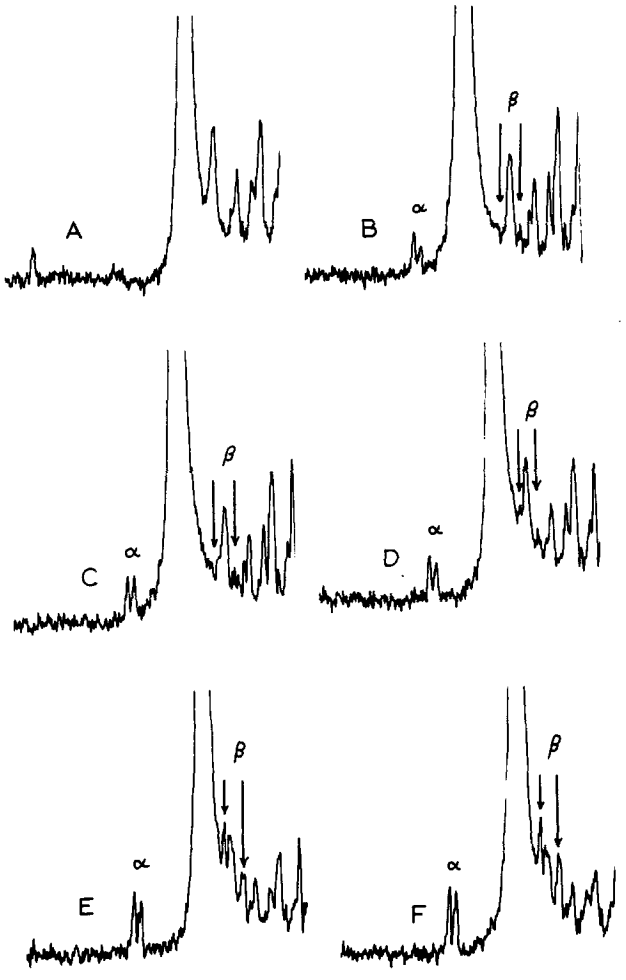

Figure 1. 60 MH_z NMR scans in the region τ 4.0 - 6.0 for a reaction mixture composed initially of 125 mg. of D-xylulose, 2.0 mg. of isomerase in 1.0 ml. of maleate-buffered D_2O at pH 6.8 at 25°. Positions and expected positions for H_1 signals are indicated. Reaction times correspond to (A) 1 min., (B) 10 min., (C) 20 min., (D) 30 min., (E) 60 min., and (F) 90 min.

stereochemical specificity is in agreement with recent suggestions (7) that the reaction intermediate in several manganese-dependent isomerase reactions is a cis-1,2 enediol. Such a structure would, in the case of D-xylose, give rise to an α-D configuration by addition of a proton to C-2.

The results of a previous NMR study of the phosphoglucose

isomerase reaction indicated the preferential formation of a β anomer (4). In view of the above findings, this reaction has been reinvestigated. The experiments were run with sufficient enzyme to produce measurable amounts of D-glucose-6-phosphate from D-fructose-6-phosphate within 90 seconds at 10°. Although the H_1^{β} signals are observed first, as reported previously, we now conclude that this is possible simply because the H_1^{β} signals are about twice as strong as those of H_1^{α}. The conclusions are based on the data collected, and, the fact that, even under these conditions, the rate of anomerization of D-glucose-6-phosphate at pH 7 (8) is sufficiently rapid relative to the isomerization rate that only an equilibrium mixture of the anomeric aldose phosphates can be found and this is, in fact, observed when the relative intensities of the two H_1 signals are taken into account.

EXPERIMENTAL. NMR spectra were obtained on a varian A-60 spectrometer relative to sodium 3-(trimethyl)-propane sulfonate (τ= 10.0). D-xylulose was prepared by previously described procedures (9), and was obtained as a colorless, chromatographically pure syrup. All other carbohydrates were obtained commercially. Phosphoglucose isomerase reactions were run using 150 mg. of D-fructose-6-phosphate (sodium salt), 60 mg. of crude phosphoglucose isomerase (obtained from Sigma Chemical Co., St. Louis, Mo.) in 1.0 ml. of deuterium oxide. Xylose isomerase reactions were run in maleate-buffered deuterium oxide at pH 6.8.

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