AN NMR STUDY OF THE PHOSPHOGLUCOSE AND PHOSPHORIBOSE ISOMERASE REACTIONS.*

Milton S. Feather and Michael J. Lybyer
Department of Agricultural Chemistry,
University of Missouri, Columbia, Missouri 65201

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SUMMARY. Examination of the NMR proton signals arising from C-1 of D-glucose-6-phosphate, as it is formed from D-fructose-6-phosphate in deuterium oxide via the phosphoglucose isomerase reaction verify that the transformation occurs by a combination of C-2 proton exchange with solvent, and intramolecular hydride transfer from C-1 of the ketose to C-2 of the aldose and, in addition, indicates the stereospecific formation of β-D-glucose-6-phosphate from D-fructose-6-phosphate during the interconversion. The NMR method was also applied to the phosphoribose isomerase system, but, because of the nature of the NMR spectrum of D-ribofuranose-5-phosphate, only proton exchange at C-2 could be absolutely verified.

INTRODUCTION. Aldose-ketose interconversions catalyzed by the sugar-phosphate isomerases constitute reactions which are widespread in nature. In studies thus far (1), it has been found that carbon bound hydrogen exchange occurs at C-1 of the ketose and at C-2 of the aldose during the interconversions, and, Rose and O'Connell (2) have recently reported that, in the case of the phosphoglucose isomerase reaction, intramolecular hydride transfer occurs from C-1 of the ketose to C-2 of the aldose in a substantial portion of the reaction pathway.

We wish to report the use of nuclear magnetic resonance spectrometry in studies on these interconversions. The methodology described herein, aside from providing data on the phosphoglucose and phosphoribose isomerase reactions, is of general

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utility in studying a variety of analogous transformations. The instrumentation was used primarily to observe signals arising from the C-1 protons of the aldose phosphates when the interconversions were run in deuterium oxide solution.

The C-1 proton of α-D-glucose-6-phosphate appeared as a doublet centered at τ 4.74, J=3.0cps, and that of the β anomer at τ 5.34, J=7.5cps, both signals being split by the carbon-bound proton at C-2. These assignations are similar to those reported for α and β-D-glucose (3) and indicate that D-glucose-6-phosphate exists in solution in a pyranose chair conformation similar to D-glucose itself (4). As with both D-glucose and α-D-glucose pentaacetate (5), the incorporation of deuterium at C-2 of glucose-6-phosphate would be expected to give rise to singlets arising from the C-1 protons due to the absence of significant coupling of the C-1 proton with the C-2 deuteron. This was experimentally observed when anomerized D-glucose-6-phosphate (180 mg), deuterium oxide (1.0 ml) and phosphoglucose isomerase (2.5 mg) were placed in a spin tube and incubated in the NMR spectrometer at 25°. The spectra was repeatedly scanned during a 1 hour reaction period. In addition to the above doublets, singlets appeared at τ 4.74 and τ 5.34 and increased in intensity at the expense of the doublets. At the end of the reaction period, these singlets were the only signals observable in this region.

To examine qualitatively the importance of hydride transfer in the reaction, an experiment similar to the first was performed using D-fructose-6-phosphate as the substrate. The simultaneous appearance of both the normally observed doublet centered at τ 5.34 and a singlet at τ 5.34 uniquely proves that a combination of both hydride transfer and solvent exchange are features of this mechanism and are consistent with previous conclusions (2) regarding

this reaction. The finding that C-1 proton signals were first observed after a 1 minute reaction time at τ 5.34 while signals at τ 4.74 did not appear until after 15 minutes of reaction time is taken as evidence that β-D-glucose-6-phosphate is the initially formed species in the reaction. Further evidence that an interconversion reaction had occurred was evident from the spectrum in the region τ 5.5-7.0 which became more complex during the reaction and, at the end, closely resembled that of a mixture of authentic D-glucose and D-fructose-6-phosphates. This reaction has been discussed in terms of a cis-acyclic 1,2-enediol (2,6) as the substrate which is, presumably, released into solution as an acyclic aldose (or ketose). Such structures would be expected to simultaneously produce both α and β anomers. The stereospecific production of only one anomer does not support this hypothesis and suggests the possibility of alternate mechanistic sequences.

An NMR spectrum of D-ribofuranose-6-phosphate revealed the presence of a doublet centered at τ 4.72, J=1.0cps. Because of the chemical shift value, and the magnitude of the coupling constant, it seems probable that this signal is due to the anomeric hydrogen of the sugar in a β configuration (7). Incubation of D-ribose-5-phosphate (180 mg) with phosphoribose isomerase (10 units, 0.1 mg) in 1.0 ml of deuterium oxide at 25° for 25 min. revealed that the doublet at τ 4.72 had collapsed to a singlet, thus demonstrating the complete incorporation of deuterium at C-2. The low coupling constant observed under these conditions precluded a determination of the relevancy of hydride transfer versus solvent exchange during this interconversion.

EXPERIMENTAL. NMR spectra were run at 60 MH_z in a Varian A-60 spectrometer and chemical shift values are reported relative

to sodium 3-(trimethylsilyl)-propane sulfonate. All carbohydrate derivatives and enzymes were obtained from Sigma Chemical Co., St. Louis, Missouri.

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