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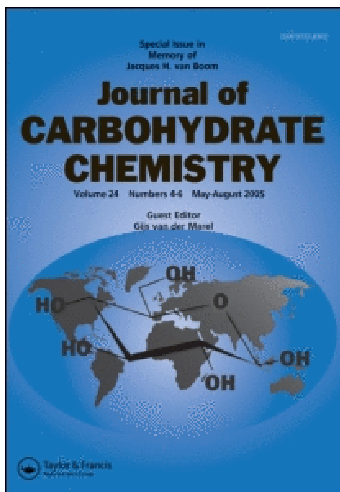

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THE CONVERSION OF D-XYLOSE TO XYLITOL AND D-XYLONIC ACID BY A BOVINE LENS PREPARATION[1]

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ABSTRACT

Incubation of D-xylose with an aqueous solution of bovine lens protein (100 mg/mL)at 37 °C and pH 7.0 gives both a reduced product (xylitol) and an oxidation product (D-xylonic acid), which were both unequivocally identified by GLC (TMS ether derivative) and by GLC/MS. Studies of the reaction at a variety of conditions suggest that both the reduction and oxidation reactions are protein (possibly enzyme) catalyzed and appear to be unique to lens protein (albumin and ovalbumin do not catalyze the reaction).

INTRODUCTION

It is a well known fact that lens protein preparations contain appreciable quantities of the enzyme aldose reductase,[2,3] which functions to convert aldoses into the corresponding alditol, and aldose sugars (including D-xylose) are frequently used to induce cataract formation in experimental animals.[4-6] It is assumed that the alditol, produced from the aldose by aldose reductase, accumulates in the lens, causing the accumulation of water and, as a result, damage to the lens tissue, thus inducing an experimental cataract. In the course of some experiments designed to examine the fate of aldose sugars in the presence of lens protein, we have discovered that D-xylose, when incubated with sterile, soluble lens protein, is both oxidized and reduced. Data with respect to these conversions are reported herein.

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RESULTS AND DISCUSSION

In some initial experiments, D-xylose-1-^{13}C (1.25 mg) was incubated in deuterium oxide (1.0 mL) which contained approximately 100 mg of sterile filtered, homogenized, water soluble bovine lens protein. The incubation solution was placed in a 5 mm NMR spin tube and the progress of the reaction qualitatively followed by observing the appearance of signals corresponding to C-1 of the sugar by ^{13}C NMR spectroscopy. Over a period of several days, the C-1 xylose signals (δ 99.1 and 94.7) diminished in intensity and two new signals appeared at δ 65.2 and at δ 180.9. These were tentatively[7] assigned to C-1 of xylitol and D-xylonic acid, respectively. Spectra for this experiment are shown in Figure 1. A parallel experiment at conditions identical to those described above (data not shown), using D-xylose-2-^{13}C provided support for these assignments, since signals corresponding to C-2 of xylitol (δ 73.2) and C-2 of D-xylonic acid (δ 74.2) were observed.[7] In order to verify this observation, an experiment was undertaken, using conditions as described above but on a larger scale, and 1 mL aliquots were removed from time to time and analyzed for D-xylose, xylitol and D-xylonic acid by GLC as the TMS ether derivatives (Figure 2). The GLC peaks for the compounds produced in the incubation had retention times identical to those for the TMS derivatives of authentic D-xylonic acid and xylitol. That the compounds produced in the reaction were produced from the D-xylose-1-^{13}C was confirmed by comparing the mass spectra of the observed peaks with the spectra of the known compounds. For the TMS derivative of authentic D-xylonic acid, peaks were observed at m/z 364 (M^+), 349 (M-15) and 259 (M-105), while the compound produced in the D-xylose-1-^{13}C experiment gave peaks at m/z 365, 350 and 260. Likewise, for the trimethylsilyl derivative of authentic xylitol, peaks at m/z 422 (M-90), and 307 (M-205) were observed, while for the ^{13}C experiment, peaks were observed at m/z 423, 307 and 308. While the enzymatic reduction of an aldose to an alditol by lens homogenates is to be expected (aldose reductase), an oxidative reaction is very surprising. There appear to be no examples of "oxidase" type enzymes in the lens or analogous products being produced, although it has been reported[8] that aldehyde oxidations can occur as a result of dismutation of NAD, initially produced as a result of liver alcohol dehydrogenase activity. It has also recently been reported that lens crystallins are members of the aldehyde dehydrogenase gene family,[9] thus suggesting that this conversion is protein catalyzed.

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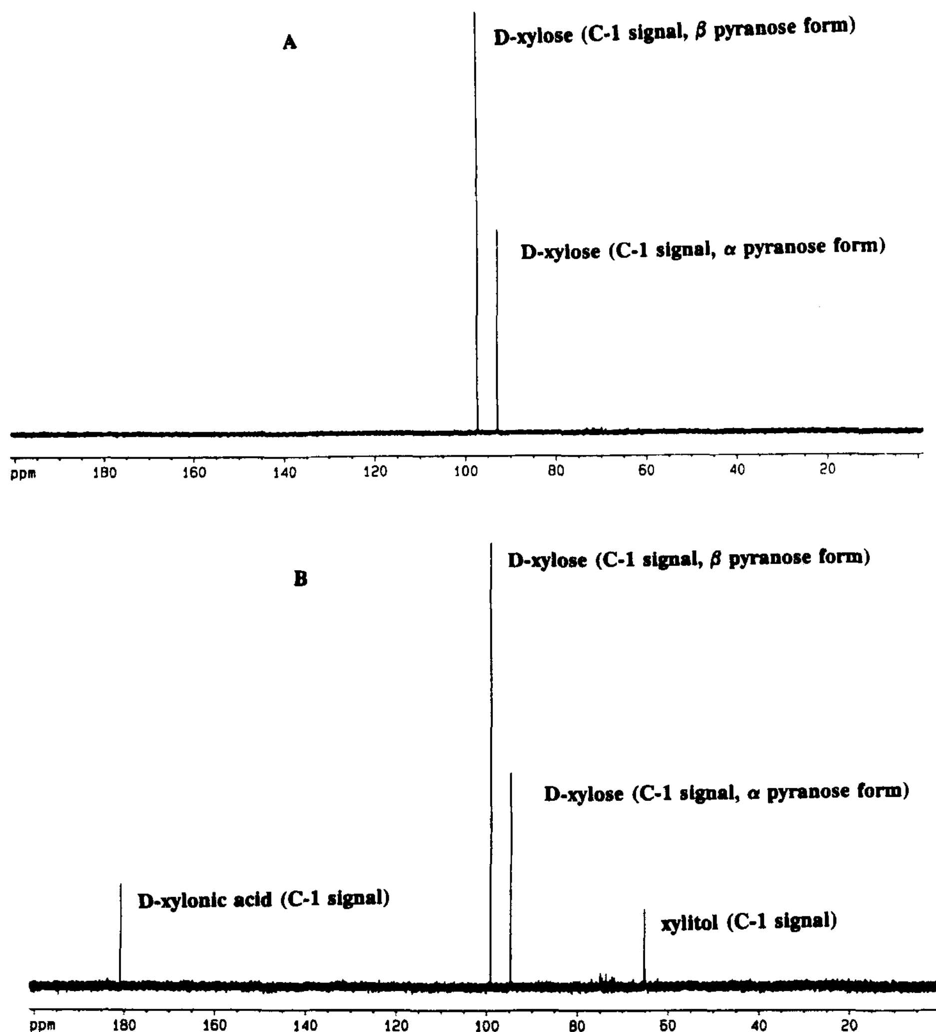

FIG. 1. ^{13}C NMR spectra (125 MHz) for, (A) a solution of D-xylose-1-^{13}C (1.25 mg per mL) at equilibrium, showing signals for the C-1 carbons (α and β) of the pyranose ring forms and (B) an incubation solution containing 1.25 mg of D-xylose-1-^{13}C, 100 mg of soluble lens protein in D_2O after 3 days of incubation. Signals corresponding to C-1 of xylitol, D-xylose and D-xylonic acid are indicated.

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In order to examine some of the parameters that might affect this conversion, yields of D-xylonic acid and xylitol were quantitatively measured for a series of incubations, each treated in a different manner. These data are shown in Table 1. The fact that neither ovalbumin or albumin (as evidenced by ^{13}C NMR spectral data), nor a heated lens preparation were effective in the reaction suggests that the

Table 1. Yields[a] of xylitol and D-xylonic acid in an incubation solution containing D-xylose (1.0 mg/mL) and soluble bovine lens protein (100 mg/mL).

Conditions	Xylitol	D-xylonic acid
Control (buffer only)	0.00%	0.00%
Lens Protein	17.6%	16.5%
Heated at 100 °C, 15 min	0.2%	0.1%
Oxygen free	20.6%	13.0%
ALDH[b] added	27.4%	63.3%
Disulfiram[c] added	1.3%	5.6%

a. Expressed as mole % yield based on starting D-xylose, after 2.5 days incubation. See Experimental for conditions.
b. One unit (in 0.05 mL) of aldehyde dehydrogenase (potassium activated from baker's yeast, Sigma Chemical) was added, along with 6.6 mg of NAD (10 mmol, Sigma Chemical).
c. Tetramethylthiuram disulfide, a reported inhibitor of aldehyde dehydrogenase.

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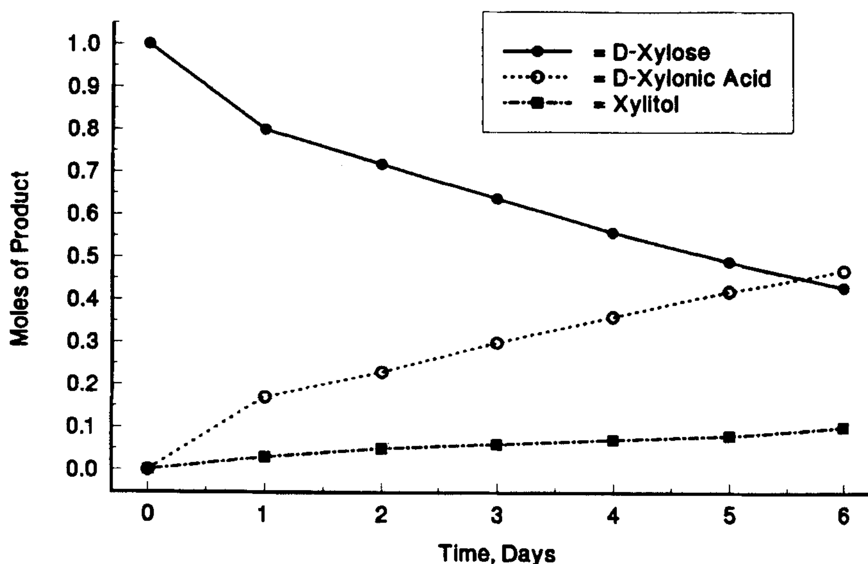

FIG. 2. Rates of disappearance of D-xylose and appearance of D-xylonic acid and xylitol at pH 7.0 and 37 °C. Data were collected as described in the Experimental Section.

oxidative conversion is unique to lens crystallin and that it is protein catalyzed. Performing the reaction in the absence of oxygen had little effect on yield, ruling out the participation of oxygen free radicals in the reaction. The oxidative portion of the reaction resembles a typical aldehyde dehydrogenase (ALDH) reaction and, as expected, the addition of commercially available ALDH greatly increases yields of D-xylonic acid. Similarly, the addition of disulfiram (tetraethylthiuram disulfide, an inhibitor of ALDH) causes significant diminution in the yield of D-xylonic acid. It thus appears that the oxidative portion of the reaction is protein catalyzed, possibly by ALDH or a protein that has ALDH activity. The yield of D-xylonic acid in the reactions is sufficiently large that this could have significant effects on the results of kinetic experiments that are many times done in conjunction with assaying enzyme levels in lens tissue. It is also noteworthy that lens preparations also catalyze similar, but more complex reactions (as evidenced by NMR spectra) when other aldoses, such as D-glyceraldehyde and L-threose are used as substrates. In these cases, the formation of (presumably) alditol is observed, along with the appearance of several signals in the 180 ppm region of the NMR spectrum that correspond to carboxylic acid carbon signals (2 for D-glyceraldehyde and 3 for L-threose), which have not yet been identified.

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EXPERIMENTAL

Materials and Methods. GLC-FID measurements were made using a Varian model 3400 chromatograph equipped with an on board computer. All Separations were performed using a Quadrex 007 OV-17 0.25 mm by 25 m capillary column. GLC-MS data were collected using either a Hewlett-Packard 5890 series chromatograph coupled to a Hewlett Packard 5970 mass selective detector or, using a Kratos MS-25 spectrometer interfaced with a DS-55 data system by direct probe insertion at 70 eV and an ion source temperature of 200 °C. ^{13}C NMR (125 MHz, dioxane, external reference, D_2O as solvent) spectra were obtained on a Brucker AMX 500 instrument. D-xylonic acid was prepared as described by Isbell.[10]

Preparation of Soluble Bovine Lens Protein. Approximately 15 young Pellfreeze Bovine lenses were selected by size and lack of discolorization (yellow). The lenses were decapsulated and gently stirred in 15 mL of a solution composed of 50% D_2O

and 50% H_2O with a stirring bar to remove the outer cortex. The outer cortex material was then disrupted in a Dounce homogenizer and then centrifuged at 30,000 (times) g for 15 min. The supernatant liquid was then diluted to 100 mg per mL, as determined using the BCA protein assay (Pierce Chemical, Rockford, IL). This solution was then sterile filtered (Gelman Science 37 GF Acrodisc, 0.2 μm) into a sterile screw capped test tube and used in the experiments described below.

Collection of NMR Spectral Data. For qualitative NMR experiments, 1 (or 2)-^{13}C D-xylose (1.25 mg) was dissolved in a solution of sterile, soluble bovine lens protein (1.0 mL total, containing 0.5 mL of D_2O), and the solution placed in a sterile 5 mm spin tube. For each sample, 600 scans were accumulated for each spectrum.

Assay for Xylitol and D-Xylonic Acid. Assays for D-xylose, D-xylonic acid and xylitol were performed as follows for a typical experiment. Solutions of bovine lens protein and D-xylose, at concentrations identical to the NMR experiments, but with perseitol added to a concentration of 1.0 mg per mL (internal standard) were placed in an incubator at 37 °C. For sampling, a 1.0 mL aliquot was removed at timed intervals and protein precipitated by adding 0.5 mL of trifluoroacetic acid. After centrifuging, the aqueous supernatant was transferred to a 3.0 mL reactivial. This solution was then concentrated to dryness under a stream of nitrogen and then by vacuum desiccation overnight. The dried samples were then converted to the TMS derivatives by treatment with TMSI (Pierce Chemical Co., Rockford, IL) at 100 °C for 2 h. A 1 μL sample was injected for GLC. The following parameters were used for all GLC runs: Initial temperature: 140 °C, 3 min hold, 5 °C per min ramp to 250 °C with a final hold time of 10 min. At these conditions the TMS derivatives had the following retention times: D-xylose (2 peaks) 7.35 and 8.50 min, D-xylonic acid, 8.23 min and xylitol, 6.83 min.

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