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A nuclear magnetic resonance study of the glucose oxidase reaction*

In addition to converting β-D-glucopyranose to δ-D-gluconolactone with the resultant production of H_2O_2 (refs. 1, 2), glucose oxidase is also known to react with 2-deoxy-D-glucose[3] and D-arabinohexosulose[2,4] (D-glucosone) with the former substrate reported to react at from 12 to 24% the rate of D-glucose and the latter about 30% that rate. Anomeric specificity has been observed in the reaction with respect to D-glucose as the substrate[2], but the complex polarimetric procedures and the physical state of D-glucosone[5] have precluded a more complete stereochemical study of the system. Herein, the use of nuclear magnetic resonance (NMR) spectroscopy, an instrumental method useful for determining anomeric specificity in enzyme reactions[6,7], is described for the glucose oxidase reaction.

The anomeric proton signals arising from the three substrates were well defined and separated from the rest of the spectra indicating that if one anomeric form of the sugar reacted in preference to another, it would be detectable by signal diminution measurements. For a determination of chemical shift values for anomeric proton signals, spectra were run at 60 MHz in deuterium oxide solution relative to sodium trimethyl propane sulfonate (τ 10.0). Consistent with previous observations and assignations, doublets at τ 4.78 and at τ 5.43, corresponding to the anomeric proton signals of α- and β-D-glucopyranose[8] and pairs of doublets in the regions τ 4.57–4.65 and τ 4.95–5.15, corresponding, respectively, to α- and β-2-deoxy-D-glucose[9], were observed. In the case of D-glucosone, three major singlet signals were observed at τ 4.96, 5.06 and 5.30. The signals are assigned to the anomeric protons of three different forms of glucosone in solution on the basis of their chemical shift values and the fact that they appear as the expected singlets. By analogy with other carbohydrate assignations[8], the most upfield signal at τ 5.30 is assigned to the β-D-pyranose structure.

The experimental procedure consisted in equilibrating the substrate in deuterium oxide buffered at pH 5.5 with phosphate salts, adding a precalculated amount of glucose oxidase (Sigma, Type II) and making an initial scan. Following this, the solution was incubated at 37° with aeration for 5 min and a spectrum again obtained. Finally, the solution was heated to 100° for 5 min to inactivate the enzyme and allow anomeric equilibrium to be reestablished, and scanned again. Although the enzyme caused substantial line broadening of the carbohydrate spectrum, the results (Fig. 1) clearly indicate that in all three cases, it is the β-pyranose form which is preferentially oxidized during the reaction. That the signal diminution is observed as a result of enzyme catalysis is evident from the fact that β signals reappear at the expense of α signals on heat inactivation of the enzyme.

In addition to the above experiments, which indicate that the reaction of both D-glucosone and 2-deoxy-D-glucose is stereochemically equivalent to D-glucose itself, chromatographic experiments showed that the products are also analogous, *i.e.* D-glucosone gives rise to 2-keto-D-gluconic acid and 2-deoxy-D-glucose to 2-deoxy-D-gluconic acid. The latter experiments involved comparing the solutions of the aldoses, which had been incubated with enzyme with standard 2-ketogluconic acid (obtained

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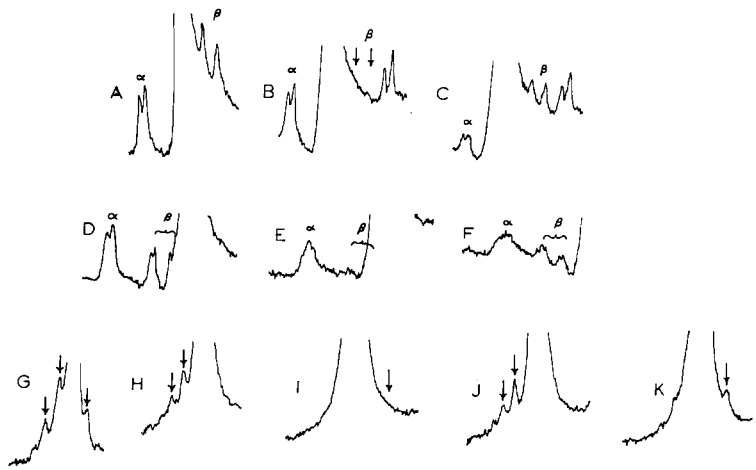

Fig. 1. 60-MHz NMR scans in the region τ 4.0–6.0 for the glucose oxidase reaction. The intense off scale signal in each item represents HO^2H. Items A–C show D-glucose (100 mg), oxidase (50 mg) in 1 ml of ^2H_2O at (A) zero time, (B) after 5 min of aeration and (C) after heating to 100° for 5 min. Items D–F show 2-deoxy-D-glucose (150 mg), oxidase (125 mg) in 1 ml of ^2H_2O at (D) zero time, (E) after 5 min and (F) after heating at 100° for 5 min. Items G–K show D-glucosone (200 mg), oxidase (125 mg) in 1 ml of ^2H_2O at (G) zero time, (H, I) after 5 min aeration at 37° (H) to show the downfield singlets and at 20° (I) to shift the HO^2H signal downfield to show the expected position of the upfield singlet, and (J, K) at the same temperatures after heating at 100° for 5 min. In all cases, the positions or expected positions of anomeric proton signals are indicated.

from Northern Regional Research Labs., Peoria, Ill.), and 2-deoxy-D-gluconic acid, prepared from the aldose by the procedures of LEVINE AND MIKESKA[10], on papergrams using ethyl acetate–acetic acid–formic acid–water (18:3:1:4, by vol.) and periodate–silver nitrate[11] as spray reagent and on thin-layer silica gel plates using butanone–acetic acid–water (3:1:1, by vol.) as irrigant and sulfuric acid as the spray reagent.

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