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Note

Wet oxidation of radiolabeled D-glucose

CHRISTOPHER J. BIERMANN, GARY D. MCGINNIS, LEONARD L. INGRAM, JR.,
Mississippi Forest Products Utilization Laboratory, Drawer FP, Mississippi State, Mississippi 39762 (U.S.A.)

AND THOMAS F KELLOGG
Dept. of Biochemistry, Mississippi State University, Mississippi State, Mississippi 39762 (U.S.A.)

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Wet oxidation is a process of treating materials with water and air or oxygen at temperatures >100°. Recently, this process has been studied as a method for converting plant materials into organic chemicals[1–3]. At relatively low temperatures (120–170°) and oxygen pressure (827–1655 kPa), acid-catalyzed hydrolysis of the lignin and hemicellulose causes these fractions to be solubilized. At higher temperatures (170–220°) and pressures (1655–3310 kPa), and with the addition of metal catalysts, the major products are carboxylic acids (mainly formic and acetic acids) formed by oxidation and fragmentation of the polysaccharides[3,4].

Wet oxidation of model carbohydrates[5] has shown that C-1 of aldoses plays an important role in initiating the wet oxidation reaction. A 10% solution of D-glucose was shown to give 1.55 mol of formic acid and 0.18 mol of acetic acid per mol with 1655 kPa oxygen, 0.67% ferric sulfate, and a reaction time of 30 min. Under the same conditions without ferric sulfate, ~90% of the glucose remained after an experiment at 170°, but <10% remained after one at 180°.

The purpose of this study was to use D-[1-^{14}C]- and D-[6-^{14}C]-glucose to determine which carbon atoms of glucose appear in the products: formic acid, acetic acid, glycolic acid, and carbon dioxide.

EXPERIMENTAL

Materials. — D-[1-^{14}C]Glucose and D-[6-^{14}C]glucose were purchased from New England Nuclear. Approximately 3.5 μCi of labeled glucose was used for each experiment. The identity of glucose was confirmed by trapping and counting the glucose peak after separation and purification by high-performance liquid chromatography (l.c.).

An aliquot of D-[1-^{14}C]glucose was reduced to D-[^{14}C]glucitol by adding 0.1 g of sodium borohydride in 4 mL of water to 1 mL of solution containing the isotope.

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After one h, dilute acetic acid was slowly added until the evolution of gas ceased.

Oxidations. — The experiments were conducted in a 600-mL, stainless-steel reactor (Parr No. 316, Model 4521) with 100 mL of 10% glucose solution, to which the isotope and catalyst were added as required. The reactor was then sealed and charged with oxygen to 1725 kPa, unless otherwise specified. The reactor was heated with stirring to the required temperature (~15 min) and kept to ±3° for 30 min. Cooling was effected rapidly by immersing the reactor in cool water.

After the reactor had cooled, the gaseous contents were diverted to a bubble trap containing 100 mL of 0.200M sodium hydroxide. (A cold trap at −40° before the carbon dioxide trap proved unnecessary, since negligible radioactivity was trapped.) In most cases, more carbon dioxide was formed in the reactor than could be trapped, and so the total amount of carbon dioxide could not be determined, although the specific activity could be determined. Activity was determined by counting 0.5 mL of solution with 10 mL of Bioscint (ICN Pharmaceuticals) and an additional 0.5 mL of water. Two aliquots of the sodium hydroxide solution were titrated with aqueous HCl as required to the hydrogencarbonate endpoint with phenolphthalein as indicator. 2M NaOH was used in the bubble trap in the [6-^{14}C]glucose experiments.

Analyses. — A portion of each liquid sample was analyzed with a C-18 Sep-Pak [Waters Associates No. 51910, a cartridge (1.2 × 1.0 cm) of octadecyl-derivatized silica gel]. This column retains the less-polar compounds (e.g., furfural), but carboxylic acids and monosaccharides are eluted with water. Recovery studies indicated that >95% of acids could be recovered by this technique. The concentration of the acids was determined by injecting 50 μL of the solution into an l.c. apparatus equipped with a Bio-Rad HPX-87H ion-exchange column, using 0.60 mL/min of 7mM sulfuric acid as the eluant, and a u.v. detector at 210 nm. The peak components were trapped directly in vials containing 10 mL of Bioscint. Counting was performed with a Packard model 3255 liquid scintillation counter using the external standard ratio method to determine counting efficiency. Samples were counted for 20,000 counts or 20 min, whichever was the shorter.

RESULTS AND DISCUSSION

Tables I and II show the results of wet oxidation of the labeled compounds. The percent yield is based on a theoretical yield of 6 mol of formic acid or carbon dioxide per mol of glucose and 1 mol of acetic acid or glycolic acid per mol of glucose. The percent label in all cases is the ratio of labeled molecules in the product compared to the ratio of the original labeled glucose molecules expressed as a percentage. For example, if all of the formic acid came from the 1-^{14}C position of glucose, the percent label would be expected to be 100%.

The information shows that more carbon dioxide is formed from C-1 than from C-6 and, by reducing the aldehyde group to the alcohol, the specific activity of the carbon dioxide from C-1 is decreased. In either case, the specific activity is

TABLE I

LOCATION OF LABEL IN PRODUCTS OF WET OXIDATION OF D-[1-^{14}C]GLUCOSE

Reactor conditions	*Carbon dioxide*		*Formic acid*		*Acetic acid*		*Glycolic acid*	
	Label, %[a]	*Yield,* %[b]	*Label,* %	*Yield,* %	*Label,* %	*Yield,* %	*Label,* %	*Yield,* %
160°	8.4	1.5	32.0	1.9	—	1.7	—	1.7
175°	10.9	—	28.9	13.2	—	1.4	52.3	5.0
165°[c]	9.0	8.5	21.9	19.1	—	0.5	26.5	15.8
165°[d]	9.7	—	20.3	31.8	6.3	5.0	26.5	14.1
165°[e]	10.7	8.8	26.5	12.9	—	3.6	43 0	6.7
170°[f]	5.2	2.1	20.5	1.2	—	—	63.2	1.5

[a]Percent of molecules with the label incorporated compared to labeled glucose molecules. [b]Based on 6 mol of CO_2 or formic acid per mol of glucose, and one mol of the other products per mol of glucose. [c]100 mg of ferric sulfate catalyst added. [d]100 mg of aluminum chloride catalyst added [e]lb.in^{-2} oxygen in the reactor (3450 kPa). [f]D[1-^{14}C]glucitol.

TABLE II

LOCATION OF LABEL IN PRODUCTS OF WET OXIDATION OF D-[6-^{14}C]GLUCOSE

Reactor conditions	*Carbon dioxide*		*Formic acid*		*Acetic acid*		*Glycolic acid*	
	Label, %[a]	*Yield,* %[b]	*Label,* %	*Yield,* %	*Label,* %	*Yield,* %	*Label,* %	*Yield,* %
170°	3.5	—	18.8	27.3	38.9	1.9	56.9	5.8
160°	1.8	2.4	—	1.9	—	—	—	—
160°[c]	2.9	4.5	20.1	7.2	—	0.2	80.0	5.6
165°[d]	5.6	29.4	26.0	27.6	38.2	4.4	50.1	14.1

[a]Percent of product with the label incorporated compared to original molecules of labeled glucose. [b]Based on 6 mol of CO_2 or formic acid per mol of glucose, and one mole of the other products per mol of glucose. [c]100 mg of ferric sulfate catalyst added. [d]100 mg of aluminum chloride catalyst added.

<16.7%, indicating that carbon dioxide is preferentially produced from the inner carbon atoms (C-2–C-6). The addition of aluminum chloride greatly increased the yield of carbon dioxide. It also led to an exothermic reaction that was difficult to control in the reactor. Both catalysts also increased the yield of other products.

Formic acid, on the other hand, was preferentially produced from the terminal carbon atoms, especially C-1. As the reaction conditions became more severe (higher temperature), the yield of labeled formic acid from [1-^{14}C]glucose decreased because of formation of formic acid from carbon groups other than the terminal aldehyde group.

Limited data are available for the specific activity of acetic acid, because the large amount of formic acid produced caused some tailing on the chromatogram. Therefore, if a small acetic acid peak were trapped, there would be substantial

error because of activity from formic acid. The limited data do indicate that acetic acid is preferentially produced from C-6 of glucose rather than from C-1.

Glycolic acid (succinic acid may also be present here in small amounts as it has a retention time close to that of glycolic acid) has a high specific activity resulting from preferential incorporation of both terminal carbon atoms of glucose, particularly C-6. When C-1 was reduced to yield glucitol, the specific activity also increased. When ferric sulfate was used as a catalyst, 80% of the glycolic acid incorporated C-6 of glucose, indicating that ferric sulfate may be catalyzing a very specific reaction. Wet oxidation of glycerol with and without addition of catalysts could lead to more information regarding specific reactions.

The data are limited; however, it is apparent that the terminal carbon atoms of glucose are being oxidized to formic acid, but not to carbon dioxide to nearly the same extent.

Previous work on extraction by dichloromethane of the liquid from the reactor shows that very little (<1%) of the C-1 from D-[1-^{14}C]glucose is converted into such products as methanol, ethanol, and other less-polar compounds.

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