Synthesis of 2-deoxyglucitol from 2-deoxy-D-glucose by the fungus *Puccinia graminis*

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After incubation of glucose-grown mycelium of *Puccinia graminis* with 2-deoxy-D-[U-^{14}C]glucose, all cellular ^{14}C was present in compounds soluble in 80% (v/v) ethanol. Metabolites identified included 2-deoxyglucitol, and free and phosphorylated forms of 2-deoxyglucose and 2-deoxygluconate. This is the first report of 2-deoxyglucitol as a metabolite of 2-deoxyglucose in any organism, and in *P. graminis*, this confirms previous proposals that free D-glucose is directly reduced to D-glucitol in vivo.

Puccinia *Rust* *2-Deoxyglucose* *2-Deoxyglucitol* *2-Deoxygluconate* *Glucitol*

1. INTRODUCTION

2-Deoxy-D-glucose (2-dG) is widely used as a glucose analogue for the study of glucose transport and assimilation by cells of many organisms (e.g., [1–5]). We report here the formation of 2-deoxyglucitol (2-dGol) as a novel metabolite of 2-dG by the wheat stem rust fungus, *Puccinia graminis* f.sp. *tritici*.

We have proposed that during the assimilation of D-[U-^{14}C]glucose by mycelium of *P. graminis*, glucose is metabolised to D-glucitol by two routes: (i) the direct reduction of free glucose; and (ii) less directly by isomerisation of glucose to free fructose followed by reduction [6]. In the present work to confirm route (i) independent of (ii), we offered 2-dG to the fungus as a glucose analogue. Because 2-dG cannot be isomerised to fructose (a 2-keto sugar), the direct synthesis of 2-deoxy-D-glucitol (2-dGol) from 2-dG would be consistent with route (i) being operative. The identification of 2-dGol as reported herein, is discussed in relation to the potential use of 2-dG to probe pathways in other organisms in which glucitol is an intermediate of glucose assimilation.

Abbreviations: 2-dG, 2-deoxy-D-glucose; 2-dGol, 2-deoxy-D-glucitol; 2-dGate, 2-deoxy-D-gluconate (lactone or open chain forms unspecified)

2. MATERIALS AND METHODS

2.1. *Chemicals*

Authentic 2-dG, 2-deoxy-D-glucose-6-phosphate and 2-deoxy-D-galactose were obtained from Sigma Chemical Co. (St Louis MO). The calcium salt of 2-deoxy-D-gluconate (2-dGate) was synthesised by bromine oxidation of 2-dG [7]; 2-dGol was prepared by reduction of 2-dG with sodium borohydride [8].

2.2. *Growth and incubation of the fungus*

Strain VIB of *Puccinia graminis* f.sp. *tritici* was maintained on media containing 200 mM D-glucose [6], and harvested by filtration 14 d after transfer. Mycelium from 300 ml pooled stock cultures was washed [6], and resuspended in three 50 ml portions (each 0.54 mg dry wt mycelium/ml) of glucose-free medium containing 2-deoxy-D-[U-^{14}C]glucose (Radiochemical Centre, Amersham) at: (1) 50 μM (38600 dpm/nmol); (2) 500 μM (3600 dpm/nmol); and (3) 5 mM (670 dpm/nmol). Cultures were incubated at 26°C with continuous, gentle agitation.

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2.3. *Analysis of samples*

Samples (2–10 ml) taken at intervals between 15–600 min after addition of 2-d[^{14}C]G were filtered, washed with cold H_2O, extracted with boiling 80% (v/v) ethanol, and separated into insoluble residues, lipids, and water-soluble compounds as in [6]. The water-soluble compounds (which contained all cellular ^{14}C) were analysed by descending chromatography (18 h) on unwashed Whatman no.1 paper as described in the text, using the following solvents: (1) ethyl acetate:acetic acid:water, 14:3:3, by vol. [9]; (2) ethyl acetate:pyridine:H_2O, 14:5:1 [9]; (3) ethyl methyl ketone:acetic acid:boric acid-saturated H_2O, 9:1:1 [10,11]. Standard compounds and radioactive components on chromatograms were detected as before [6]. All standard compounds gave single spots on chromatograms except 2-dGate, which gave 3 spots in solvent 3 (fig.2A), presumably due to a mixture of γ and δ lactones and the open chain form of the molecule. Putative phosphate esters were identified by elution from chromatograms, treatment with 5 units of potato acid phosphatase (Boehringer, Mannheim) in 200 μl 50 mM sodium citrate (pH 4.8) at 37°C for 3 h, followed by rechromatography to detect free deoxy sugars.

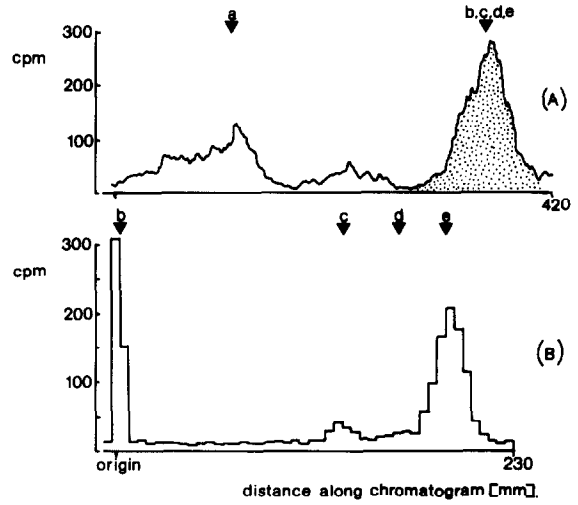

Fig.1. Chromatographic analysis of the water-soluble compounds extracted from *P. graminis* after 2.5 h incubation in 5 mM 2-deoxy-D-[U-^{14}C]glucose; positions of the following authentic standards are as indicated: (a) 2-deoxy-D-glucose-6-phosphate; (b) 2-deoxy-D-gluconate; (c) 2-deoxy-D-glucitol; (d) 2-deoxy-D-galactose; (e) 2-deoxy-D-glucose. (A) Strip-counter (Nuclear Chicago) scan after development in solvent (1). (B) Rechromatography of shaded area (fig.1A) in solvent (2); ^{14}C was determined in 5 mm strips of chromatogram (cf. [6]).

3. RESULTS

Water-soluble metabolites of 2-deoxy-D-[U-^{14}C]glucose were resolved into three major zones after chromatography in solvent (1), as shown in fig.1A. The most mobile peak (shaded in fig.1A) co-chromatographed with authentic 2-dG, 2-dGol, 2-dGate and 2-deoxy-D-galactose.

To resolve component(s) of the most mobile peak in fig.1A, radioactive compounds were eluted and rechromatographed in solvent (2) (fig.1B). Peaks of radioactivity corresponding to 2-dGate (origin), 2-dGol ($R_{2\text{-dG}}$ = 0.66) and 2-dG (most mobile) were revealed. These tentative identifications were confirmed by eluting each peak separately followed by rechromatography in solvent (3) (fig.2). The mobility of putative 2-dGol in solvent (3) was unaffected after treatment with sodium borohydride, suggesting that it was not a free aldose or ketose. Furthermore, putative 2-dGol was not removed from solution by shaking at 25°C for 20 min with a mixture of a sulphonic acid resin (Dowex 50, H^+ form) and a quaternary ammonium resin (Amberlite IRA-402, $^-$OH form), indicating that it contained no acidic or basic groups. Putative and authentic 2-d[^{14}C]Gol showed similar losses (15–30%) of ^{14}C from solution after treatment by these resins for 20 min at 100°C, indicating similar resistance to acid and base degradation. Solvent (2) (cf. fig.1B) therefore appears to be the most useful system for rapid identification of 2-dGol amongst non-phosphorylated derivatives of 2-dG. Intracellular concentrations of 2-dG, 2-dGol and 2-dGate reached a maximum after 5 h (table 1). At similar exogenous concentrations of 2-dG (5 mM) and glucose (1–20 mM, [6]), intracellular concentrations of 2-dG and glucose were in the same range (~1 mM), but intracellular 2-dGol (0.15 mM) was low compared to glucitol (5–8 mM).

The less mobile fractions after chromatography in solvent (1) (fig.1A) contained phosphorylated derivatives of 2-dG and an oligosaccharide (most probably dideoxytrehalose). Treatment of these

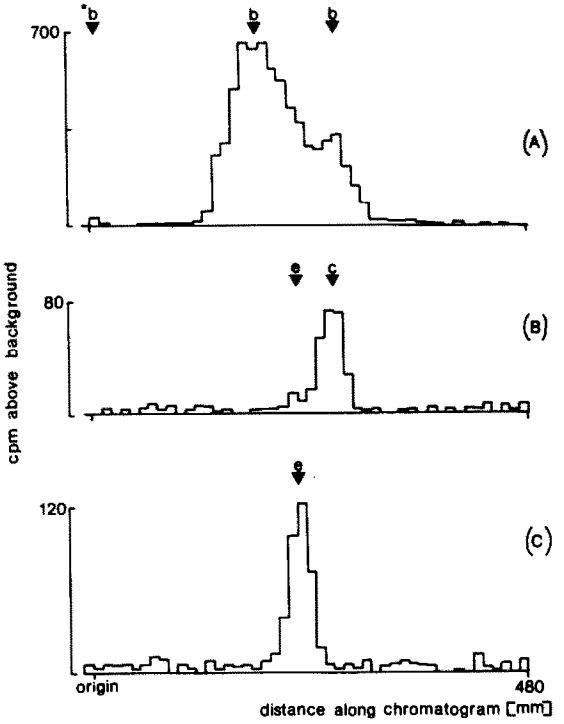

Fig.2. Rechromatography (solvent (3)) of fractions corresponding to the 3 major peaks of radioactivity shown in fig.1B. Notation for standard compounds is given in fig.1, and ^{14}C was determined as in fig.1B. (A) 2-Deoxy-gluconate (pooled eluates from position b in several chromatograms developed in solvent (2), e.g., fig.1B); authentic 2-dGate gave 3 spots corresponding to positions indicated by b in the figure (asterisk indicates trace on origin). (B) 2-Deoxy-glucitol (pooled eluates from position c, fig.1B). (C) 2-Deoxy-glucose (pooled eluates from position e, fig.1B).

Table 1

Intracellular concentrations[a] (mM) of some metabolites of 2-deoxyglucose in mycelium of *P. graminis* after 5 h incubation in 2-deoxy-D-[U-^{14}C]glucose

Compound	Exogenous 2-deoxy-D-[U-^{14}C]glucose		
	0.05 mM	0.50 mM	5.0 mM
2-Deoxyglucose	0.074	0.305	1.03
2-Deoxyglucitol	0.036	0.059	0.15
2-Deoxygluconate	0.650	0.917	1.32

[a] Cell volume estimated as 2.4 μl.(mg dry wt)$^{-1}$ as in [15]

components with acid phosphatase followed by rechromatography in solvents (1) and (2) resulted in the release and identification of free 2-dG and 2-dGate but not 2-dGol in all samples, thereby indicating the absence of 2-deoxyglucitol phosphate(s) amongst the metabolites of 2-dG.

4. DISCUSSION

Our results clearly indicate that 2-dGol, a novel metabolite of 2-dG, was formed after uptake of 2-dG by mycelium of *P. graminis*. Phosphorylated derivatives of 2-dGol could not be detected amongst the relatively large amounts of other phosphate esters of 2-dG and its metabolites which accumulated within mycelium. Our data are thus consistent with 2-dGol being synthesised by the direct reduction of 2-dG rather than via phosphorylated intermediates. The analogous reaction during glucose assimilation would be the direct reduction of free D-glucose to D-glucitol. The other route proposed for glucitol synthesis [6,12], involving the reduction of free fructose, presumably operates simultaneously with the reduction of free glucose.

D-glucitol has been proposed as an intermediate of glucose/fructose interconversion in a number of organisms (e.g., [13]). If the initial reaction of such pathways is the reduction of glucose to glucitol, administration of 2-dG under appropriate conditions should lead to the accumulation of 2-dGol. However, 2-dGol had not been reported as a metabolite of 2-dG, possibly because most procedures for analysis of metabolites of 2-dG were devised to distinguish 2-dG from its phosphorylated and acid derivatives. In locusts, the reduction of another glucose analogue (3-deoxy-3-fluoro-D-glucose) to its corresponding sugar alcohol [14] has suggested the occurrence of previously undetected role for glucitol (sorbitol) in carbon metabolism in this organism. Procedures described herein may prove useful in future studies using 2-dG to probe the role of D-glucitol in glucose assimilation in diverse organisms.

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