Enzymes of *N*-Acetylglucosamine Metabolism during Germ-tube Formation in *Candida albicans*

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(Received 4 December 1981; revised 1 March 1982)

The enzymes of *N*-acetyl-D-glucosamine (GlcNAc) metabolism, GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase were found to be inducible in *Candida albicans*. The pattern of induction for these enzymes was the same under conditions of germ-tube formation (37 °C) and where yeast cells metabolized GlcNAc with no change in morphology (28 °C); this indicates that these enzymes are not control points in the dimorphic development of *C. albicans*. During induction there was a 40- and 25-fold increase in specific activity for the deacetylase and the deaminase, respectively, and the maximum specific activity corresponded to the time when all the GlcNAc had been metabolized. The presence of lomofungin (an inhibitor of transcription) or trichodermin (an inhibitor of translation) in cell suspensions of *C. albicans* containing GlcNAc prevented the increase in specific activity of these enzymes. 2-Deoxyglucose inhibited germ-tube formation, partially inhibited the induction of the deacetylase (43%) and the deaminase (60%), but did not affect the growth of *C. albicans* on either Glc or GlcNAc. GlcN-6-phosphate was a competitive inhibitor of the deacetylase with a K_i of 1.45 mM while the other product of the reaction, acetate, did not inhibit the enzyme. The K_m value for GlcN-6-phosphate on GlcN-6-phosphate deaminase was 0.24 mM. Incubation of starved yeast cells with GlcNAc produced a four-fold increase in the specific activity of UDP-GlcNAc-pyrophosphorylase at either 28 °C or 37 °C.

INTRODUCTION

A number of environmental factors have been described which effect the reversible transition from a yeast to mycelial habit of growth in *Candida albicans*, and these have been reviewed by Odds (1979). Cassone *et al.* (1973) and Scherwitz *et al.* (1978) demonstrated that germ-tubes of *C. albicans* are the precursors of the septate mycelial form of the organism. *N*-Acetylglucosamine (GlcNAc) can induce germ-tube formation in yeast cells of *C. albicans* (Simonetti *et al.*, 1974; Shepherd *et al.*, 1980a), and more than 90% of the blastospores form germ-tubes after 3 h incubation if they have been conditioned by starvation (Shepherd *et al.*, 1980a).

The metabolism of GlcNAc by *C. albicans* involves partitioning of GlcNAc-6-phosphate between the catabolic pathway to fructose-6-phosphate and the anabolic pathway to chitin and glycoproteins (Fig. 1). The induction of the enzymes for the conversion of GlcNAc to fructose-6-phosphate in *C. albicans* has been reported by Singh & Datta (1979a), but these studies were carried out with yeast cells incubated at 30 °C. It is well documented that germ-tube formation occurs only at 37 °C (for review, see Odds, 1979, and Mattia & Cassone, 1979). In a previous study (Shepherd *et al.*, 1980b), it was shown that the induction pattern of GlcNAc kinase (Fig. 1) was the same for yeast cells incubated at 28 °C and in cells incubated at 37 °C which formed germ-tubes. However, the induction patterns for GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase, have not been studied under conditions which elicit germ-tube formation.

Germ-tube formation may be regarded as a cell-wall morphogenesis and, hence, the control of

Fig. 1. Pathway for chitin synthesis in *C. albicans*. The enzymes involved in metabolism of *N*-acetylglucosamine (GlcNAc) and synthesis of chitin are: I, *N*-acetylglucosamine kinase; II, *N*-acetylglucosamine-6-phosphate deacetylase; III, glucosamine-6-phosphate deaminase; IV, glutamine-fructose-6-phosphate aminotransferase; V, glucosamine-phosphate acetyltransferase; VI, phosphoacetyl glucosamine mutase; VII, UDP-*N*-acetylglucosamine pyrophosphorylase; VIII, chitin synthase.

the developmental process should be reflected in the regulation of the enzymes for cell wall polymer formation. Chitin is a minor constituent of the *C. albicans* cell wall, but the chitin content is three times higher in mycelial cells than in yeast cells (Chattaway *et al.*, 1968). The incorporation of GlcNAc into chitin was ten times higher in cells which had formed germ-tubes compared with yeast cells (Braun & Calderone, 1978; Chiew *et al.*, 1980*a*). In addition there was a fivefold activation of chitin synthase during germ-tube formation (Chiew *et al.*, 1980*b*).

In this paper, we describe studies on the regulation of UDP-GlcNAc pyrophosphorylase, which is required for GlcNAc metabolism, and the catabolic enzymes GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase during germ-tube formation.

METHODS

Organism. *Candida albicans* (ATCC 10261) was used throughout this work. The organism was propagated and maintained on malt-extract agar slopes.

Chemicals. The following chemicals were obtained from Sigma: *N*-acetyl-D-glucosamine, UDP-*N*-acetylglucosamine, 2-deoxyglucose, α -methylglucopyranoside, 3-*O*-methylglucose, glucosamine-6-phosphate and daunomycin.

The radiochemicals, L-[U- 14 C]leucine (59 Ci mol $^{-1}$), [8- 3 H]adenine (25 Ci mmol $^{-1}$), [5,6- 3 H]UTP (41 Ci mmol $^{-1}$) and [1- 14 C]acetic anhydride (117.4 Ci mol $^{-1}$) were purchased from Amersham (1 Ci = 3.7×10^{10} becquerels). Trichodermin was a gift from Dr W. O. Godtfresden, Leo Pharmaceuticals. Lomofungin was obtained from the Upjohn Co., Kalamazoo, Mi., U.S.A. All other chemicals were of analytical grade. Commercial GlcN-6-phosphate (Sigma) was found to be contaminated (8%) with glucose-6-phosphate. The contaminating glucose-6-phosphate was removed by chromatography on Dowex 50-H $^+$ resin (20–40 mesh). After applying the sample the column (25 $\times$ 110 mm) was washed with four volumes of distilled water which eluted the glucose-6-phosphate. The glucosamine-6-phosphate was then removed from the column by washing with 1 M-formic acid. These fractions were pooled and then lyophilized. The preparation of *N*-[1- 14 C]acetylglucosamine-6-phosphate from [1- 14 C]acetic anhydride and glucosamine-6-phosphate was as described previously (Shepherd *et al.*, 1980*b*) except a Dowex-1 (mesh 200–400) acetate column (2 $\times$ 16 cm) was used for the removal of glucosamine-6-phosphate (Gopal *et al.*, 1981).

Preparation of yeast cells. Yeast cells of *C. albicans* were prepared in shake culture using glucose as a carbon

source as described previously (Shepherd & Sullivan, 1976). Cells were harvested after 18 h and then starved for germ-tube formation by aerating for 24 h at room temperature as described by Shepherd *et al.* (1980a).

Germ-tube formation. Starved yeast cells were resuspended in 0.01 M-imidazole/HCl buffer (pH 7.2) containing 0.2 mM-MnCl₂ and 2.5 mM-GlcNAc to give a cell density of 0.8×10^8 cells ml⁻¹. A 200 ml suspension was dispensed into a 500 ml Erlenmeyer flask and incubated in a gyratory shaker (200 rev. min⁻¹) at 37 °C. With the starved yeast cells, more than 90% of the blastospores formed germ-tubes after 3 h. At 28 °C no germ-tubes are formed.

Enzyme induction. The induction of the enzymes GlcNAc-6-phosphate deacetylase, GlcN-6-phosphate deaminase and UDP-GlcNAc pyrophosphorylase by GlcNAc was tested on exponential phase yeast cells and starved yeast cells of *C. albicans*. Under the conditions described for germ-tube formation the exponential phase yeast cells remain as blastospores. The extent of induction for each enzyme was determined at 30 min intervals on 200 ml samples.

Effect of inhibitors on enzyme induction. Cells, resuspended in 0.01 M-imidazole/HCl buffer as described above, were preincubated with an inhibitor for 30 min at 37 °C before the addition of GlcNAc. Incubation with shaking was then continued at 37 °C.

Preparation of cell-free extracts. At the end of the incubation period, cells were harvested by centrifugation and washed once with distilled water. Cells were then resuspended in 0.1 M-Tris/HCl buffer, pH 7.2 together with 0.5 mm glass beads (1:2:2, w/v/w). A cell-free extract was obtained by shaking the mixture at 4000 rev. min⁻¹ (2 × 20 s) in a Braun homogenizer which was cooled by compressed carbon dioxide. The glass beads and cell wall debris were removed by centrifugation at 1000 g and the supernatant obtained was centrifuged again at 27000 g for 45 min. The pellet was discarded and the supernatant was stored at either 4 °C or -20 °C.

Measurement of protein and RNA synthesis. Protein and RNA synthesis were measured by following the incorporation of L-[¹⁴C]leucine and [8-³H]adenine into trichloroacetic acid precipitates as described by Shepherd *et al.* (1980a).

Protein estimation. Protein was estimated by the modified Lowry method (Eggstein & Kreutz, 1967) with crystalline bovine serum albumin as a standard.

Enzyme assays. (i) *GlcNAc-6-phosphate deacetylase* (EC 3.5.1.25) GlcNAc-6-phosphate-deacetylase activity was determined by measuring the liberation of [¹⁴C]acetate from [¹⁴C-acetyl]-N-acetylglucosamine-6-phosphate (Gopal *et al.*, 1981). The assay system (final volume 500 µl) contained: 50 µmol Tris/HCl, pH 8.5; 4.5 µmol [¹⁴C]GlcNAc-6-phosphate (0.028 µCi) and cell-free extract. The mixture was incubated at 37 °C for 10 min and the reaction was terminated by heating in a boiling water bath for 1 min. After the addition of 50 µl 2 M-HCl the acetic acid was extracted with diethylether (4 × 1 ml). This extraction procedure removed 88–90% of the acetate and to obtain total enzyme units a 10% correction must be used. The ether extract was counted in toluene scintillation fluid. The [¹⁴C]GlcNAc-6-phosphate was not extracted into ether and a boiled enzyme extract was used as a control. A unit of deacetylase activity was defined as that amount of enzyme that produces 1 µmol of acetate min⁻¹. The specific activity is given as units (mg protein)⁻¹.

(ii) *GlcN-6-phosphate deaminase* GlcN-6-phosphate deaminase was measured in a coupled enzyme assay as described by White & Pasternak (1975). The reaction mixture contained in 0.8 ml: 30 µmol Tris/HCl pH 7.5; 2 µmol NADP; 1.5 units of glucose-6-phosphate dehydrogenase (EC 1.1.1.49); 2 units phosphoglucosomerase (EC 5.3.1.9); 2.0 µmol GlcN-6-phosphate and cell-free extract. The rate of NADPH formation at 30 °C was followed at 340 nm. A unit of deaminase activity was defined as that amount of enzyme that produced 1 µmol NADPH min⁻¹. The specific activity is given as units (mg protein)⁻¹.

(iii) *UDP-GlcNAc-pyrophosphorylase* (EC 2.7.7.23) UDP-GlcNAc-pyrophosphorylase activity was determined by measuring the formation of UDP-GlcNAc from UTP and GlcNAc-1-phosphate. The assay system (final volume 30 µl) contained 1.2 µmol Tris/HCl, pH 7.5; 0.2 µmol GlcNAc-1-phosphate; 0.160 µmol [5,6-³H]UTP (0.05 µCi); 0.3 µmol MgCl₂ and cell-free extract. A cocktail (20 µl) containing the Tris/HCl buffer, GlcNAc-1-phosphate, [³H]UTP and MgCl₂ was placed on a 20 mm square of parafilm and 10 µl of cell-free extract added. The incubation was carried out in a capillary tube (Gallenkamp Griffin MFB-210-538L) which had been drawn to a fine jet. Mixing was achieved by drawing the assay mixture into the capillary and transferring it back on to the parafilm. This procedure was repeated four times. The assay mixture was finally drawn up the capillary tube away from the tip. The tube tip was sealed in a micro-flame and the tube was then incubated at 37 °C for 10 min. The reaction was terminated by placing the capillary tube into a boiling water bath for 1 min. After cooling the tip was broken and the contents were spotted on to a 2.5 × 10 cm strip of PEI-cellulose paper (Macherey-Nagel). The chromatograms were developed at room temperature in 0.25 M-LiCl for 40 min (Randerath & Randerath, 1967) and spots were visualized under UV light. The following standards were also chromatographed: UTP, UDP, UMP, UDP-GlcNAc and UDP-Glc. The band containing the UDP-GlcNAc was cut out and placed in a scintillation vial containing PPO/toluene. Radioactivity was determined in a Packard Tri-Carb Model 3003 liquid scintillation spectrometer. A unit of UDP-GlcNAc pyrophosphorylase activity was defined as that amount of enzyme that produces 1 nmol of UDP-GlcNAc min⁻¹.

Fig. 2

Fig. 3

Fig. 2. Induction profile of GlcNAc-6-phosphate deacetylase during germ-tube formation in *C. albicans*. Germ-tubes were formed from starved yeast cells and the deacetylase activity was measured (●) as described in Methods. The deacetylase activity was also monitored after the addition of trichodermin (arrows) at 60 min (▲) and 150 min (■). The values shown are the mean of at least duplicate determinations. Experiments were reproducible with variation of <10%.

Fig. 3. Induction profile of GlcN 6-phosphate deaminase during germ-tube formation in *C. albicans*. Starved yeast cells were germinated and the deaminase activity was measured (●) as described in Methods. The deaminase activity was also monitored after the addition of trichodermin (arrows) at 60 min (▲) and 150 min (■). The values shown are the mean of at least duplicate determinations. Experiments were reproducible with variation of <10%.

RESULTS

Induction of GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase

The time course for the induction of GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase during germ-tube formation at 37 °C is shown in Figs 2 and 3. A similar pattern of induction for both enzymes was observed in freshly harvested cells from the exponential growth phase and 24 h starved cells at 28 °C, under conditions in which germ-tubes are not formed. The maximum specific activity was reached in 3 h and during this period the increases in specific activity were 40- and 25-fold for GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase, respectively. The maximum specific activity corresponds to the time when all of the GlcNAc had been metabolized (Y. Y. Chiew, unpublished results), and a decline in specific activity occurs after 3 h (Figs 2 and 3). In order to establish that the increase in activity of these enzymes involved gene expression, the effect of a protein synthesis inhibitor was investigated. As shown in Figs 2 and 3, the addition of trichodermin at 1 h and 2.5 h prevented any further increase in specific activity of both GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase.

Properties of GlcNAc-6-phosphate deacetylase

When trichodermin and lomofungin were added before germ-tube formation was initiated, the change in morphology was completely inhibited and more than 90% of the deacetylase induction was inhibited (Table 1). Also trichodermin and lomofungin both inhibited growth at 28 °C. 2-Deoxyglucose inhibited germ-tube formation and caused partial (43%) inhibition of the GlcNAc-6-phosphate deacetylase induction (Table 1). However, 2-deoxyglucose did not inhibit the growth of *C. albicans* at either 28 °C or 37 °C when either Glc or GlcNAc were used as carbon sources. In *in vitro* studies 2-deoxyglucose was shown to have no effect on the activity of

Fig. 4. Inhibition of GlcNAc-6-phosphate deacetylase by GlcN-6-phosphate. The GlcNAc-6-phosphate deacetylase activity was measured in the presence (2 mM and 3 mM) and absence of GlcN-6-phosphate. The K_i was determined from the plot of the slope versus GlcN-6-phosphate concentration.

Table 1. Induction of GlcNAc-6-phosphate deacetylase in the presence of compounds that promote or inhibit germ-tube formation

The specific activity of GlcNAc-6-phosphate deacetylase was examined in germinated yeast cells. Germ-tube formation was initiated in the presence of the compounds listed (final concentration in parentheses) plus 2.5 mM-GlcNAc. Incubations were at 37 °C in imidazole buffer for 3 h and the percentage germination for each incubation was determined by light microscopy. The specific activity at zero time was 0.16×10^{-2} unit (mg protein) $^{-1}$ and values are the means of duplicate determinations.

Addition	Percentage germ-tube formation	$10^2 \times$ Specific activity [units (mg protein) $^{-1}$]
None	80–90	5.0
Trichodermin (20 μ M)	0	0.5
2-Deoxyglucose (2.5 mM)	0	2.9
3-O-Methylglucose (2.5 mM)	90–100	4.8
α -Methylglucopyranoside (2.5 mM)	90–100	4.4
Lomofungin (90 μ g ml $^{-1}$)	0	0.4

the enzyme. The glucose analogues, α -methylglucopyranoside and 3-O-methylglucose, however, had no effect on either the formation of germ-tubes or the induction of deacetylase.

The GlcNAc-6-phosphate deacetylase did not lose any activity when stored for 1 week at 4 °C in 100 mM-Tris/HCl pH 7.2. The optimum pH for activity was pH 8.5, but the pH optimum was broad and more than 90% of the activity can be measured at pH 7.0 or pH 9.5. The K_m values for GlcNAc-6-phosphate determined from double reciprocal plots and from the direct linear plot (Eisenthal & Cornish-Bowden, 1974) were 1.11 mM and 1.05 mM, respectively (Gopal *et al.*, 1981). GlcN-6-phosphate was a competitive inhibitor of the deacetylase with a K_i of 1.45 mM (Fig. 4). The other product of the reaction, acetate, did not inhibit the enzyme. The compounds UDP-GlcNAc (10 mM), UTP (7.5 mM) and fructose-6-phosphate (40 mM) caused 35%, 21% and 75% inhibition of the deacetylase at the final concentration indicated in parentheses.

Properties of GlcN-6-phosphate deaminase

Daunomycin and lomofungin both inhibited RNA synthesis in *C. albicans* as judged by [3 H]adenine incorporation into yeast cells undergoing germ-tube formation (Table 2). In addition, these compounds inhibited both germ-tube formation and the induction of GlcN-6-phosphate deaminase. In the presence of 2-deoxyglucose (2.5 mM) the time course for the induction of the deaminase was the same as the control cells, however only 40% as much enzyme

Table 2. *Effect of RNA synthesis inhibitors on GlcN-6-phosphate deaminase activity and germ-tube formation in C. albicans*

Germ-tubes of *C. albicans* were formed from yeast cells at 37 °C in GlcNAc in the presence of [8-³H]adenine. Daunomycin and lomofungin both at 90 µg ml⁻¹ were added at the times indicated. The incorporation of [8-³H]adenine into germ-tubes was determined as described by Shepherd *et al.* (1980a). The specific activity of the deaminase and the extent of germ-tube formation were determined as described in Methods.

Inhibitor	10 ² × Specific activity of deaminase [units (mg protein) ⁻¹]		Germ-tube formation after 3 h (%)	[8- ³ H]Adenine incorporated after 3 h [c.p.m. (mg dry wt) ⁻¹]
	After 1 h	After 3 h		
None	2.6	4.8	90	15640
Daunomycin added at zero time	<0.1	<0.1	nil	2210
Daunomycin added after 1 h	2.6	2.4	nil	ND
Lomofungin added at zero time	<0.1	<0.1	nil	5940
Lomofungin added after 1 h	2.3	2.5	nil	ND

ND, Not determined.

was induced. Although the 2-deoxyglucose blocked germ-tube formation, in *in vitro* assays it did not inhibit GlcN-6-phosphate deaminase. The optimum pH for the enzyme was 7.5 and the enzyme was stable at 4 °C. The K_m values for GlcN-6-phosphate on GlcN-6-phosphate deaminase determined by the direct linear plot method (Eisenthal & Cornish-Bowden, 1974) and from a double reciprocal plot were 0.24 mM and 0.23 mM, respectively.

Properties of UDP-GlcNAc-pyrophosphorylase

The assays for UDP-GlcNAc pyrophosphorylase activity in the direction of UTP formation (Yamamoto *et al.*, 1976) are not applicable with yeast cell extracts which contain pyrophosphatase activity. In this work we used a radiochemical microassay for UDP-GlcNAc formation (Gopal *et al.*, 1982). UDP-GlcNAc pyrophosphorylase was found in the supernatant after centrifuging the cell-free extract at 27 000 *g* for 90 min and was stable at 4 °C for at least 3 d if dithiothreitol (1 mM) was added. The K_m values for UTP and GlcNAc-1-phosphate were 0.8 mM and 2.1 mM, respectively, and the optimum pH in Tris/HCl buffer was 7.5 (Gopal *et al.*, 1982). The relative activity of UDP-GlcNAc pyrophosphorylase was studied under different conditions. In freshly harvested cells activity was found to be 2.2 nmol mg⁻¹ min⁻¹, whereas when cells were starved for 24 h the activity dropped to 1.3 nmol mg⁻¹ min⁻¹. On incubation of starved cells with GlcNAc (2.5 mM), the activity after 3 h increased to 5.5 nmol mg⁻¹ min⁻¹ at both 28 °C and 37 °C.

DISCUSSION

Although many systems have been described for the production of germ-tubes in *C. albicans* (Odds, 1979), the observation that GlcNAc alone is sufficient to induce the formation of the germ-tube in this organism (Simonetti *et al.*, 1974; Shepherd *et al.*, 1980a) suggests that the metabolism of this hexosamine is important in determining the morphology of the organism. This study has shown that GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase were induced by GlcNAc and both the time course and the extent of induction were similar at 28 °C (yeast cells) and 37 °C (germ-tubes). Similar results were reported for GlcNAc kinase at 28 °C and 37 °C (Shepherd *et al.*, 1980b). Also it has been shown that UDP-GlcNAc pyrophosphorylase did not increase to the same extent (fourfold) as the catabolic enzymes (20- to 40-fold). The concentrations of all three enzymes were the same in induced cells grown at 28 °C and 37 °C. Although GlcNAc is a specific inducer for germ-tube formation at 37 °C, these

studies show that the induction of the catabolic enzymes and the amount of UDP-GlcNAc pyrophosphorylase activity do not play a critical role in the developmental process. Thus the regulatory role of GlcNAc remains unexplained at this time.

The large increase in specific activity (25- to 40-fold) was characteristic of an induction system. After the GlcNAc has been utilized there was a rapid decline in the specific activity which involves either the cessation of enzyme synthesis or enhanced enzyme degradation.

Singh & Datta (1979*b*) have reported on the induction of GlcN-6-phosphate deaminase in *C. albicans*, but their data are at variance with the results presented here. Firstly, they showed only a two- to threefold increase by comparison with a 25-fold increase in specific activity. Secondly, they reported a basal level of 10^{-2} units (mg protein) $^{-1}$ whereas in *C. albicans* ATCC 10261 the basal level of the deaminase from both exponentially growing yeast cells and starved yeast cells was 2×10^{-3} units (mg protein) $^{-1}$. The assay system for the deaminase uses a coupled enzyme system with glucose-6-phosphate isomerase and glucose-6-phosphate dehydrogenase where the rate of production of NADPH is measured. Contamination of the substrate GlcN-6-phosphate with glucose-6-phosphate leads to spurious results unless the contaminant is removed. In addition, the cell-free extract from growing yeast cells must be dialysed to remove endogenous glucose-6-phosphate; dialysis is not required with cell-free extract from starved cells.

The inhibition of the induction of these enzymes by trichodermin, lomofungin and daunomycin indicates that the increases in the activity of the deaminase and deacetylase are due to *de novo* protein synthesis requiring both transcription and translation. In a previous study (Shepherd *et al.*, 1980*b*), a similar result was observed for GlcNAc kinase and the observation that the DNA content remains constant over the first 4 h of germ-tube formation (Shepherd *et al.*, 1980*a*) indicates that DNA synthesis is not required. The similarity in the time course for induction for the three enzymes of GlcNAc catabolism, kinase (Shepherd *et al.*, 1980*b*), deacetylase and deaminase suggests that these enzymes could all be under the control of one operon.

This is the first report on the induction of GlcNAc-6-phosphate deacetylase in *C. albicans*. The introduction of the novel enzyme assay (Gopal *et al.*, 1981) allowed the deacetylase activity to be measured in the presence of the interfering activity due to phosphoacetylglucosamine mutase. One of the products, acetate, did not inhibit the deacetylase. However, the other product, GlcN-6-phosphate, was a competitive inhibitor with a K_i similar to that of the K_m for GlcNAc-6-phosphate. These data indicate a compulsory ordered mechanism for this enzyme reaction (Dixon & Webb, 1979) with GlcNAc-6-phosphate adding to the enzyme first. Acetate is the first product to be produced followed by the GlcN-6-phosphate as follows:

The glucose analogue 2-deoxyglucose did not inhibit growth of yeast cultures but inhibited germ-tube formation and partially inhibited (40%–60%) induction of both the deacetylase and the deaminase. In *in vitro* assays 2-deoxyglucose did not affect the activity of these enzymes. Lampen *et al.* (1973) have shown that accumulation of 2-deoxyglucose-6-phosphate affects the uptake of sugars and the synthesis of mannans and glucans, and inhibits some enzymes of yeast

protoplasts. It is not known how 2-deoxyglucose prevents germ-tube formation and inhibition of enzyme induction in *C. albicans*.

The fourfold increase in activity of UDP-GlcNAc-pyrophosphorylase during germination was not as dramatic as the 25- to 40-fold increase found for the GlcNAc catabolic enzymes. Nevertheless, this increase in activity could be sufficient to account for the observed 10-fold increase in GlcNAc incorporation into chitin during germ-tube formation (Braun & Calderone, 1978; Chiew *et al.*, 1980a).

This work was supported in part by a grant from the Medical Research Council of New Zealand. P. G. holds a New Zealand University Grants Committee Scholarship.

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