Arch. Microbiol. 125, 97–104 (1980)

Archives of
Microbiology

Regulation of Chitin Synthesis During Germ-Tube Formation in *Candida albicans*

Yoke Y. Chiew, Maxwell G. Shepherd, and Patrick A. Sullivan[1]

[1] Department of Biochemistry, University of Otago, Dunedin, New Zealand

Abstract. The synthesis of chitin during germ-tube formation in *Candida albicans* may be regulated by the first and last steps in the chitin pathway: namely L-glutamine-D-fructose-6-phosphate aminotransferase and chitin synthase. Induction of germ-tube formation with either glucose and glutamine or serum was accompanied by a 4-fold increase in the specific activity of the aminotransferase. Chitin synthase in *C. albicans* is synthesized as a proenzyme. *N*-acetyl glucosamine increased the enzymic activity of the activated enzyme 3-fold and the enzyme exhibited positive co-operativity with the substrate, UDP-N-acetylglucosamine. Although chitin synthase was inhibited by polyoxin D (K_i = 1.2 μM) this antibiotic did not affect germination. During germ-tube formation the total chitin synthase activity increased 1.4-fold and the expressed activity (in vivo activated proenzyme) increased 5-fold. These results could account for the reported 5-fold increase in chitin content observed during the yeast to mycelial transformation.

Key words: L-glutamine-D-fructose-6-phosphate aminotransferase – Chitin synthase – Chitin – *Candida albicans* – Germ-tube formation – Dimorphism – Polyoxin D.

Induction of germ-tube formation by *Candida albicans* is the initial stage in the yeast to mycelial transition (Cassone et al., 1973; Scherwitz et al., 1978; Shepherd et al., 1980). According to Stewart and Rogers (1978) germ-tube formation may be regarded as a cell wall morphogenesis and hence the control of the developmental process should be reflected in the regulation of the enzymes required for the synthesis of cell wall polymers. Chitin is a minor constituent of yeast cell walls but the chitin content is 3 times higher in mycelial cells compared with blastospores (Chattaway et al., 1968), the incorporation of *N*-acetyl glucosamine (GlcNac) into the chitin fraction was ten times higher for hyphal cells than for blastospores (Brown and Calderone, 1978) and the chitin content increased 5-fold during germ-tube formation (Chiew, unpublished results). The pathway for chitin synthesis is summarised in Fig. 1. Although the regulation of this pathway has not been studied in detail, likely steps for overall regulation are the first and last reactions in this pathway. The enzyme L-glutamine: D-fructose-6-phosphate aminotransferase (E.C. 2.6.1.16) catalyses the first step in the pathway. Ghosh and Roseman (1962) were unable to detect any reaction in the reverse direction with a trapping system of phosphoglucoisomerase and glucose-6-phosphate dehydrogenase. Hence this reaction may be regarded as a committed step in the pathway. Chitin synthase (E.C. 2.4.1.16) catalyses the last reaction in the pathway and previous studies on this enzyme in other yeasts and fungi have indicated that enzymic activity is modulated by a variety of controls including the rate of synthesis, proenzyme activation and allosteric control (Cabib and Farkas, 1971; McMurrough and Bartnicki-Garcia, 1971; Schekman and Brawley, 1979). In this paper we describe properties of L-glutamine-D-fructose-6-phosphate aminotransferase and chitin synthase in blastospores and germ-tubes of *Candida albicans*.

Non-Standard Abbreviations: GlcNac = N-acetyl glucosamine; UDP-GlcNac = UDP-N-acetyl glucosamine; PMSF = phenylmethylsulphonylfluoride

Materials and Methods

Growth of C. albicans and Preparation of Germ-Tubes

Yeast cells and germ-tubes of *Candida albicans* (ATCC 10261) were prepared as described previously (Shepherd et al., 1980). The extent of germ-tube formation was assessed by microscopy.

0302-8933/80/0125/0097/$01.60

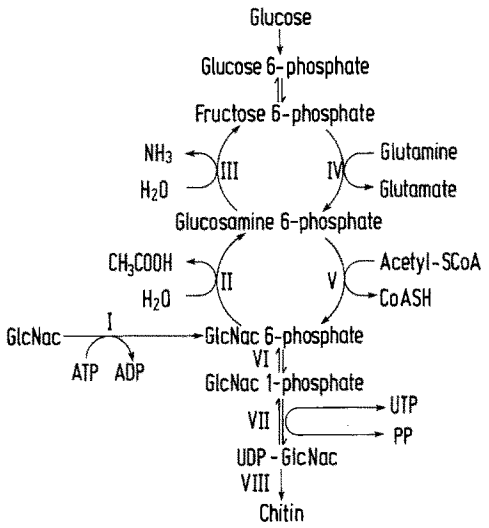

Fig. 1. Pathway for chitin synthesis. The enzymes involved in the metabolism of N-acetylglucosamine (GlcNac) and the synthesis of chitin are: (I) N-acetyl-D-glucosamine kinase; (II) N-acetyl-glucosamine-6-phosphate deacetylase; (III) glucosamine-6-phosphate deaminase; (IV) L-glutamine D-fructose-6-phosphate aminotransferase; (V) glucosamine phosphate acetyltransferase; (VI) glucosamine phosphate isomerase; (VII) UDP acetylglucosamine pyrophosphorylase; (VIII) chitin synthase

L-Glutamine: D-Fructose-6-Phosphate Aminotransferase

Cells were washed and resuspended in 0.5 M-potassium phosphate buffer, pH 6.45, containing 1 mM-EDTA and 1-mM-dithioerythritol, then disrupted in a French Press at an internal pressure of 20,000 lb/in^2. Cell-free extracts were obtained by centrifuging the homogenate at 27,000 g for 20 min.

L-Glutamine: D-fructose-6-phosphate aminotransferase was assayed according to Chattaway et al., (1973) except that potassium phosphate buffer (0.03 M), pH 6.45, and dithioerythritol (1 mM) were used in place of sodium phosphate buffer (0.2 M) and dithiothreitol (1 mM). Assay mixtures were incubated for 30 min at 37° C. Glucosamine-6-phosphate was estimated according to the method described by Ghosh and Roseman (1962). Enzyme activity was expressed as μmoles of glucosamine-6-phosphate produced per h per ml of enzyme extract, or as specific activity (μmol h^{-1} mg protein^{-1}).

Chitin Synthase

For enzyme assays, yeast cells and germinating cells were washed by centrifuging, once in distilled water, twice with 0.05 M-potassium phosphate buffer, pH 6.45, containing 0.01 M-$MgCl_2$, 1 mM EDTA and 0.01 M-mercaptoethanol and then resuspended in the same buffer. Cell suspensions were treated in a Braun homogenizer (4000 rpm for 30 s), and the homogenate was centrifuged at 1000 g for 5 min. The supernatant was further centrifuged at 54,000 g for 45 min and the pellet, a mixed membrane fraction, was washed twice with the potassium phosphate solution described above. Chitin synthase activity in this fraction was unstable at 4° C or to freezing and thawing. The enzyme activity was stable, however, for over 2 weeks when the membrane fraction was stored at −15° C as a suspension in the potassium phosphate buffer solution containing 50% (v/v) glycerol.

Chitin synthase is synthesized as a proenzyme (Cabib and Farkas, 1971). The assay system used depended on the incorporation of labelled GlcNac from UDP-GlcNac into an insoluble residue. A similar assay has been described by Ruiz-Herrera and Bartnicki-Garcia (1976). In this work assays were designed to measure both the expressed and the total chitin synthase activity in the mixed membrane fraction. Expressed chitin synthase (i.e. enzyme activated in vivo) activity was assayed in a system containing 0.05 M-potassium phosphate buffer, pH 6.5, 39 mM-GlcNac (N-acetyl glucosamine), 0.27 mM-UDP-[U-^{14C}]GlcNac (UDP-N-acetyl glucosamine) (0.3×10^5 dpm), 10 mM-$MgCl_2$ and enzyme preparation, 20 μl. The toal volume was 0.15 ml. Activity was proportional to protein concentration in the range of 0—0.8 mg protein per assay. After incubation for 1 h at 25° C, glacial acetic acid (20 μl) was added and the incubation mixture was filtered through a Whatman GF/C glass-fibre filter. The filter was washed with 40 ml of a solution containing 1 M-acetic acid and 95% ethanol (80:20, v/v) and dried at 70° C. The radioactivity was determined by scintillation counting with POPOP-PPO-toluene scintillant in a Packard Tricarb Spectrometer. Total chitin synthase activity was assayed by preincubating the membrane preparation (20 μl) with 20 μl of protease solution from *Aspergillus oryzae*, 0.15 units/mg solid), containing 20 mg/ml, at 25° C for 30 min. PMSF (phenylmethylsulphonylfluoride) (10 μl of a solution containing 10 mg/ml methanol) was then added to the incubation. In preliminary experiments it was established that PMSF at this concentration did not affect the activity of activated chitin synthase but any subsequent protease activation of chitin synthase proenzmye was completely inhibited. Control incubations with methanol alone were carried out at the same time. Chitin synthase activity was expressed as nmoles of [U-^{14}C]GlcNac incorporated from UDP-[U-^{14}C]GlcNac into chitin per min per ml of enzyme preparation, or as specific activity (nmol min^{-1} mg protein^{-1}). The heat stability of chitin synthase was determined by preincubating 0.4 ml of mixed membrane fraction with 0.4 ml of protease (20 mg/ml) at 25° C for 30 min. PMSF, 0.2 ml (10 mg/ml methanol) was then added and 50 μl portions were transferred to test tubes containing 60 μl of the chitin synthase reaction mixture from which UDP-[U-^{14}C]GlcNac and GlcNac were omitted. After incubating at 45° C for the different time-intervals, each tube was rapidly chilled. UDP-[U-^{14}C]GlcNac and GlcNac were then added (final volume 150 μl) and the chitin synthase activity determined as described above.

Protein was measured by a modified Lowry method described by Eggstein and Kreutz (1967).

Radioactive Materials and Chemicals

N-acetyl-D-[1-^{14}C]glucosamine (58 Ci/mol), was purchased from the Radiochemical Centre, Amersham, England. Substrates and enzymes were obtained from the Sigma Chemical Co., St. Louis, U.S.A. All other chemicals were of analytical grade.

Results

Figure 2 shows the time course for the increase in the total activity and the specific activity of glutamine-fructose-6-phosphate aminotransferase during germ-tube formation. The induction of this enzyme correlated with the appearance of germ tubes when glucose and glutamine were used as germination substrates (Shepherd et al., 1980). Further, a significant proportion of the glucose and glutamine (70% and 50%, respectively) were removed from the medium after 2 h of incubation (Chiew, unpublished results). In typical experiments over 80% of the conditioned blastospores formed germ-tubes when incubated with glucose and

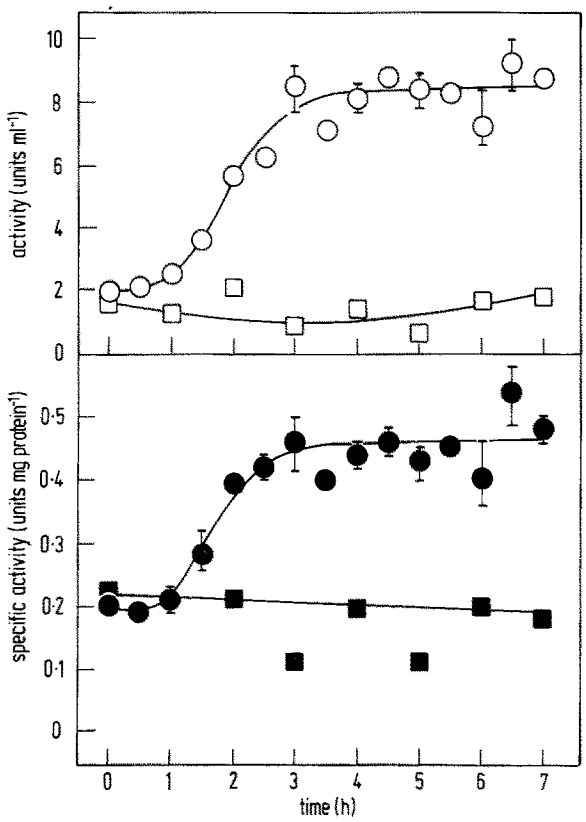

Fig. 2. L-glutamine: D-fructose-6-phosphate aminotransferase in germinating and non-germinating cells. Cells were incubated in germination medium with glucose plus glutamine and aminotransferase activity (total activity, ○ and specific activity, ● were measured in the 27,000 g cell-freee supernatants at the times indicated. The yield of germinated cells after 3 h of incubation was 60%. Conditioned blastospores were also incubated with glucose plus glutamine and cycloheximide (5 mg/ml) and cell-free supernatants were assayed for specific activity (■) and total activity (□). The yield of germinated cells with cycloheximide added was 0%

glutamine for 3 h (Shephered et al., 1980). Aged batches of conditioned cells gave decreased yields of germ tubes and it was found that there was a direct correlation between the increase in specific activity of the aminotransferase and the yield of germinated cells (Table 1). It is noteworthy that a concentration of cycloheximide sufficient to inhibit germ-tube formation and protein synthesis (Shepherd et al., 1980) also inhibits the induction of the aminotransferase.

After centrifuging at 70,000 g for 30 min, 96% of the total aminotransferase was found in the supernatant. The enzyme was unstable at 4° C ($t^1/_2$ = 2 days) but activity could be retained if the enzyme was stored at −15° C in the presence of 70 mM fructose-6-phosphate, 1 mM EDTA and 1 mM dithioerythritol.

The induction of germination with either serum (Shepherd et al., 1980) or glucose + glutamine resulted in a 3–4-fold increase in the specific activity of the

Table 1. Correlation between germ-tube formation and the specific activity of L-glutamine: D-fructose-6-phosphate aminotransferase

Germination (%)	Specific activity (μmol h^{-1} mg $protein^{-1}$)
0	0.23
50	0.37
70	0.55
80	0.65
90	0.80

Several batches of conditioned blastospores were incubated in germination medium with glucose plus glutamine. The yields of germinated cells were determined after 4 h of incubation and aminotransferase activity was assayed in the cell-free extract from each batch of cells

aminotransferase but germination with GlcNac as substrate produced only a 1.5-fold increase in enzyme activity.

The properties of the aminotransferase isolated from germinated cells and blastospores were similar except with respect to the apparent K_m values of L-glutamine. The apparent K_m (glutamine) for the yeast form (1.7 mM) is considerably higher than that of the enzyme from germinated cells (0.74 mmol). An active L-glutaminase in the cell-free extract could affect the value of the K_m for glutamate of the glutamine-fructose-6-phosphate aminotransferase. No glutaminase activity was detected in cell-free extracts of either yeast or germinating cells and the reaction rates were linear and proportional to enzyme concentration. The apparent K_m for fructose-6-phosphate (1.5 mM) and the optimum pH of 6.5 were the same for extracts from blastospores and germinated cells. The aminotransferase from blastospores and germinated cells also differed with respect to the effect of high concentrations of glutamine on the enzyme activity. Plots according to the method of Dixon (1953) showed that the enzyme from yeast cells was unaffected by saturating concentrations of glutamine whereas a marked inhibition was observed using the enzyme from germinated cells (Fig. 3). The K_s value was 18 mM.

The chitin synthase activity in *C. albicans* was associated with the membrane fractions of the cell: 32% of the total activity was with the 1000 g pellet, which contained unbroken cells and cell wall debris, and 68% was with the 54,000 g pellet. The 54,000 g pellet is a mixed membrane fraction; it was used in all subsequent experiments.

Chitin synthase is synthesized as a proenzyme (Cabib and Farkas, 1971); consequently, we have assayed for the expressed activity, i.e. the activity found immediately after cell disruption, and the total activity, i.e. protease-activated activity. Table 2 shows the activity of chitin synthase; expressed activity and total

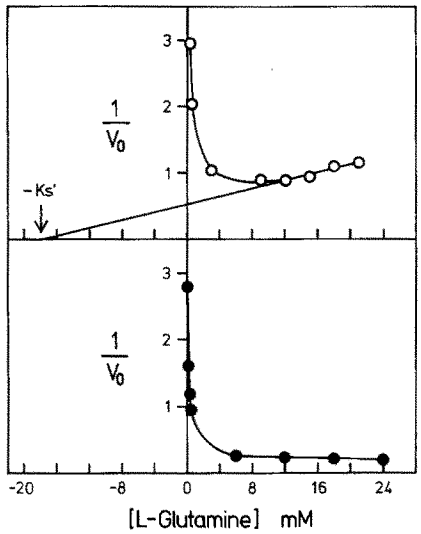

Fig. 3. The effect of excess L-glutamine on L-glutamine: D-fructose-6-phosphate aminotransferase activity. Enzyme was assayed as described in Methods. L-glutamine concentrations were varied in the assays while D-fructose-6-phosphate concentration was 18 mM in all the assays. Enzyme preparations were from yeast cells (●) and cells germinated for 4 h in glucose + glutamine medium (○)

Table 2. Protease activation of chitin synthase

	Additions	Activity	
		−ATP	+ATP
Expressed activity:	nil	0.30	0.25
Total activity:	*A. oryzae* protease (0.03 units)	4.68	3.99
	trypsin (250 units)	1.78	2.81
	pepsin (60 units)	0.00	0.11

Chitin synthase was assayed as described in Methods. The mixed membrane fraction (7 mg protein/ml) was obtained from cells which had been incubated in glucose + glutamine medium for 1 h. Protease preparations and ATP (60 nmol) were added to enzyme assays (total volume 150 µl) as indicated

activity in the presence of three different proteases. The most effective protease for activation was the *A. oryzae* preparation. The results found with pepsin and trypsin could indicate ineffective activation and/or rapid degradation of chitin synthase (active or proenzyme form) by these proteases. McMurrough and Bartnicki-Garcia (1971) found that the activity of chitin synthase in *Mucor rouxii* was enhanced by ATP. Table 2 shows the effect of ATP on chitin synthase activity. Both the expressed activity and the total chitin synthase activity in the presence of *A. oryzae* protease were inhibited by 0.4 mM-ATP (17% inhibition). In contrast, total chitin synthase activity in the presence of trypsin and pepsin was increased.

Figure 4 shows the time course for *A. oryzae* protease activation of chitin synthase. The activation was rapid and almost linear during the first 10 min of incubation and the maximum extent of activation was observed after 30 min of incubation. Enzyme activity rapidly declined with further incubation.

When chitin synthesis was allowed to proceed together with proenzyme activation (i.e. when protease was added together with the reaction mixture), there was an initial lag for enzyme activation (0 – 20 min) and then a linear phase of incorporation for 30 – 90 min (Fig. 4). Chitin synthase was activated by GlcNac; at 20 mM GlcNac, enzyme activity was increased 2.5-fold and maximum activation was observed with concentrations greater than 35 mM. The concentration of GlcNac used in routine assays was 39 mM. An analysis of GlcNac activation was carried out using mixed membrane preparations from both yeast cells and germinated cells, over the range of 1.3 mM to 78 mM GlcNac (Fig. 5). Both enzyme preparations yielded the same data and there was a sharp break in the plots at approx. 5 mM GlcNac. The extrapolates of the linear plots at low concentrations yielded an apparent K_a of 1.2 mM for GlcNac: the high concentrations of GlcNac yielded an apparent K_a of 6 mM.

Figure 6 shows the sigmoidal character of a plot of reaction velocity against substrate concentrations with a fixed concentration of GlcNac (39 mM). The S_{50} for UDP-GlcNac (i.e. concentration of UDP-GlcNac for 50% of the apparent V_{max}) was 0.2 mM. These data were replotted in a Hill plot according to the method of Atkinson et al. (1965). The slope of such a plot (n) is an index of the degree of co-operativity in this system. A value of $n = 1.7$ was obtained from these plots with enzymes from two separate batches of cells and at two activator concentrations, 26 mM and 39 mM-GlcNac (Fig. 7).

Polyoxin D was a potent inhibitor of chitin synthase. A Dixon plot (Dixon, 1953) of inhibitor concentrations against the reciprocal of reaction velocity at two substrate concentrations and with two enzyme preparations yielded an apparent K_I of 1.2 µM, (Fig. 8) and the point of intersection indicated that Polyoxin D was a competitive inhibitor for chitin synthase. Diflubenzuron, a benzoylphenyl-urea derivative, is an insecticide which inhibits chitin synthesis (Marx, 1977). This compound was tested with chitin synthase preparations used in this work. There was no effect on the enzyme activity at a concentration of 67 µg/ml.

The expressed and the total chitin synthase activities were measured during germ-tube development. Figure 9 shows that there was a 1.4-fold increase in total chitin synthase during the first 2 h of germ-tube formation. This shows that either there was synthesis of new enzyme during germination or a decrease in the

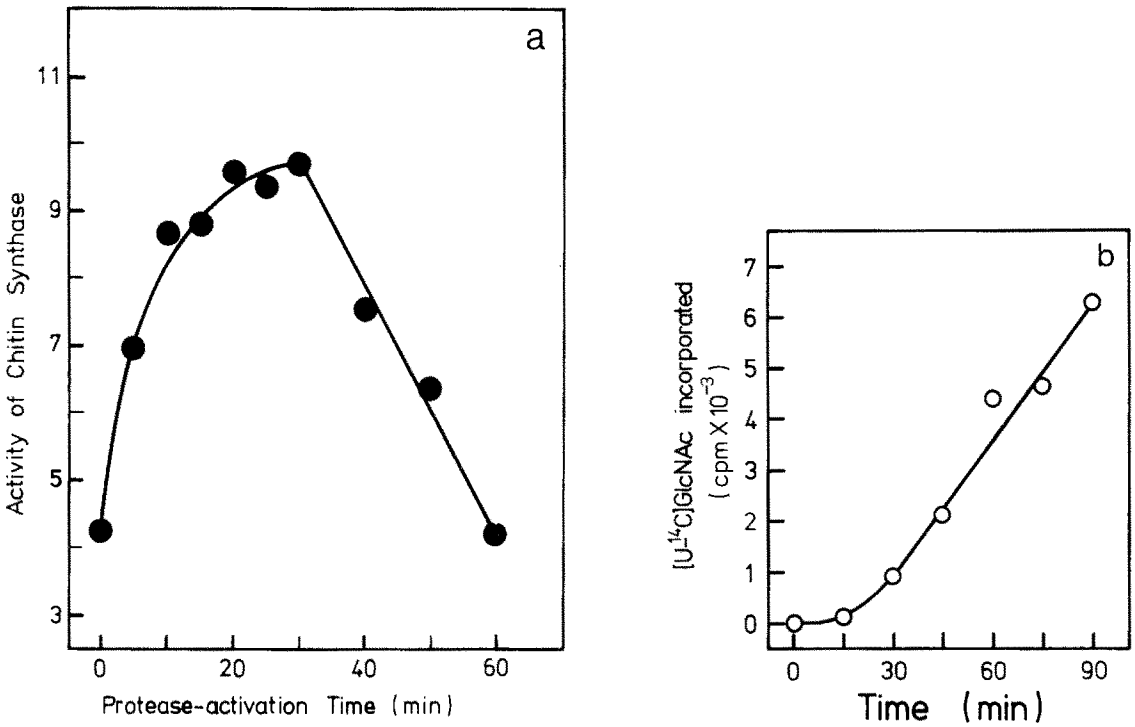

Fig. 4. a Time course for the activation of chitin synthase by *A. oryzae* protease. A series of incubations, each containing 20 µl of mixed membrane fraction (25 mg protein/ml) from yeast cells, were incubated with an equal volume (20 µl) of protease (20 mg/ml). Protease activity was stopped by the addition of PMSF at the times indicated and chitin synthase activity was assayed as described in Methods. Enzyme activity is expressed as nmol [U-^{14}C]GlcNac incorporated $min^{-1}ml^{-1}$. **b** Incorporation of [U-^{14}C]GlcNac by chitin synthase versus reaction time. Assays containing protease (20 µl of a 20 mg/ml solution) were incubated as described in Methods. At the time intervals shown the reaction was stopped by the addition of 20 µl glacial acetic acid

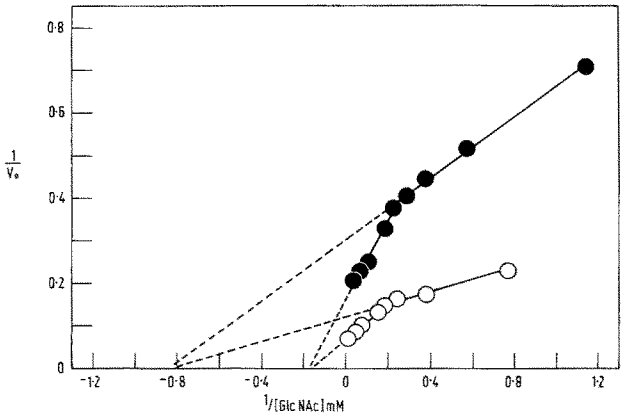

Fig. 5. Double reciprocal plots of chitin synthase activity versus GlcNac (activator) concentrations. Total chitin synthase activity was measured in each assay as described in Methods; only GlcNac concentrations were varied. Enzyme extracts were (●) mixed membrane fraction (21 mg protein/ml) from yeast cells and (○) mixed membrane fraction (22 mg protein/ml) from conditioned cells germinated for 2 h in Glucose + Glutamine medium

rate of degradation of this protein. More significant, however, is the increase in 'in vivo' active enzyme. The heat stability of the total chitin synthase activity was examined during germ-tube formation (Fig. 9). The half-life of this enzyme at 45° C increased from 2 min for yeast cells to 6 min for germ-tube preparations obtained 1, 2 and 3 h after germination was initiated.

Discussion

Ghosh and Roseman (1962) reported that the reaction catalysed by glutamine-fructose-6-phosphate aminotransferase was not detectably reversible even with a trapping system of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase, added to the system. Thus induction of the aminotransferase which accompanies both germ-tube formation and chitin synthesis when either serum or glucose and glutamine are used as substrates may be considered as a committed step in the overall pathway. In contrast germ-tube formation and chitin synthesis were not associated with induction of the aminotransferase when GlcNac was a substrate. These results are in accord with the metabolic scheme given for chitin synthesis from glucose and GlcNac (Fig. 1).

The specific activity of the aminotransferase in the 27,000 g supernatant correlated with the percentage of cells which formed germ-tubes and the change in

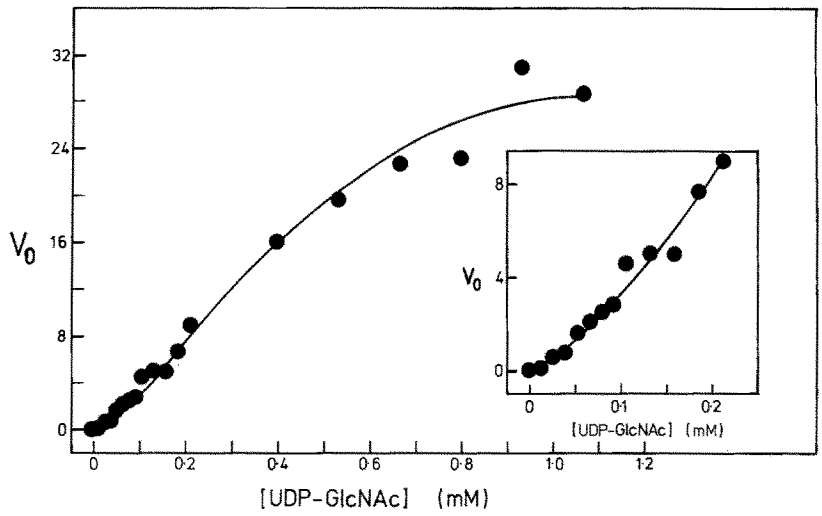

Fig. 6. Plot of chitin synthase reaction velocity versus UDP-GlcNac concentrations. Inserted is an enlarged plot of results obtained at low UDP-GlcNac concentrations. Mixed membrane preparation (22 mg protein/ml) from 2 h germinated cells was assayed for total chitin synthase activity as described in Methods. GlcNac (activator) concentration was 39 mM. UDP-GlcNac concentration was varied as shown

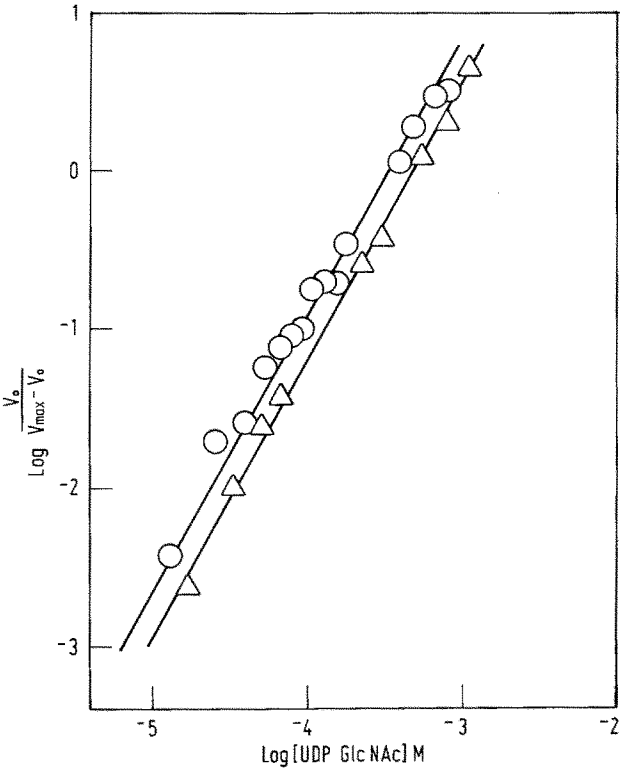

Fig. 7. Hill plots of chitin synthase activity. Plots of the reaction velocity versus UDP-GlcNac concentrations were constructed as described in Fig. 6. Enzyme preparations were the mixed membrane fraction (△, 21 mg/ml) from blastospores assayed with a fixed GlcNac concentration of 26 mM and (○) the membrane preparation (22 mg/ml) from 2 h germinated cells with a fixed GlcNac concentration of 39 mM

specific activity could be used as a marker for estimating germination.

Unlike the enzyme isolated from *Blastocladiella emersonii* (Norrman et al., 1975), or *Neurospora crassa* (Endo et al., 1970) the enzyme from *C. albicans* was not inhibited by UDP-GlcNac, the substrate for chitin synthase. The kinetics of the enzyme from blastospores and 4 h germ-tubes, were different in the apparent K_m values for L-glutamine; 1.7 mM and 0.7 mM respectively. This lower K_m value could contribute to the increased flux in the chitin pathway during germination. The inhibition of L-glutamine: D-fructose-6-phosphate aminotransferase from germ-tubes by high concentrations of L-glutamine, K_S, 18 mM, may not be physiologically significant. However, Gancedo and Gancedo (1973) have reported an L-glutamine concentration of 15–35 mM in *S. cerevisiae* cells growing in ammonium salts medium. This is approximately the K_S, value (18 mM) of L-glutamine for the aminotransferase from germ-tubes. It is not possible from these kinetic data obtained with crude extracts to conclude whether the enzyme from blastospores and germinated cells are different gene products.

Chitin synthase from *C. albicans* was found to be located entirely in the membrane fractions of the cell, in common with other fungal systems, e.g. *S. cerevisiae* (Duran et al., 1975) and *Mortierella vinacea* (Peberdy and Moore, 1975). The enzyme was present in the membrane fraction as a proenzyme but the *C. albicans* protease required for chitin synthase activation has not been identified. Treatment of the membrane fraction with *A. oryzae* protease resulted in rapid activation and subsequent inactivation, presumably due to proteolytic degradation. When chitin synthase was activated by protease in the presence of substrate and GlcNac the enzyme was not inactivated (Fig. 4b). This protection of chitin synthase by substrate and/or effectors could affect the rate of in vivo synthesis of chitin and the turnover of chitin synthase.

Chitin synthase was activated by GlcNac and the biphasic double reciprocal plots of 1/GlcNac versus $1/V_o$ indicated two different binding sites for GlcNac on

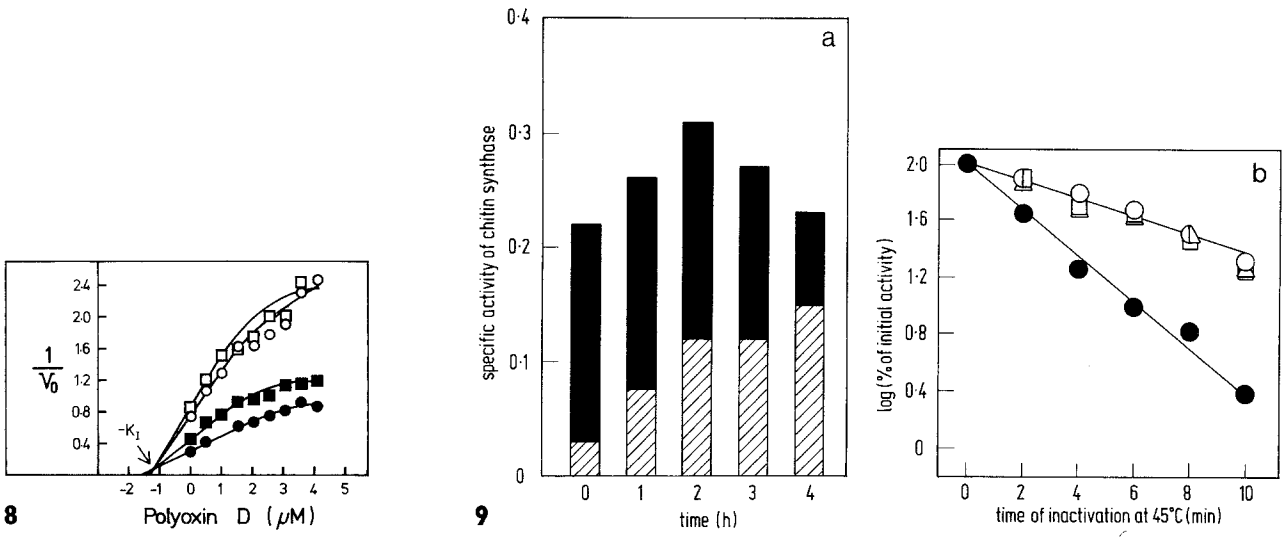

Fig. 8. Dixon plots showing polyoxin D inhibition of chitin synthase activity. In each reaction mixture total chitin synthase activity was assayed, as described in Methods, in the presence of varying polyoxin D concentrations. Mixed membrane fractions (9 mg protein/ml) from cells germinated for 2 h in glucose + glutamine medium were assayed at fixed UDP-GlcNac concentrations of 0.13 mM (○) and 0.27 mM (●). Mixed membrane fractions (13.6 mg protein/ml) from cells germinated for 2 h in medium containing 5 mM glucose plus 2.5 mM GlcNac were assayed at fixed UDP-GlcNac concentrations of 0.13 mM (□) and 0.27 mM (■)

Fig. 9. a The specific activity of chitin synthase during germ-tube formation. Each bar shows total specific activity (shaded plus hatched area) and the expressed specific activity (Hatched area only). Total chitin synthase activity and expressed chitin synthase activity were assayed as described in Methods. The specific activity is expressed as nmol U-^{14}C GlcNac incorporated min^{-1} mg $protein^{-1}$. Conditioned batch cells were germinated in glucose + glutamine medium. **b** Heat inactivation of chitin synthase preparations obtained at different times of germination. Heat stability assays were as described in Methods. Mixed membrane fractions were prepared from conditioned cells incubated in glucose + glutamine medium. (●) 0 h (yeast cells only), 23 mg protein/ml; (○) 1 h, 24 mg protein/ml; (□) 2 h, 21 mg protein/ml; (△) 3 h, 21 mg protein/ml

the enzyme. Activation of chitin synthase by GlcNac could have physiological significance: Gooday (1978) has suggested that chitinase activity, which softens the cell wall during cell wall synthesis, produces localized regions with elevated GlcNac concentrations which then activate chitin synthase.

The positive co-operativity of chitin synthase with the substrate, UDP-GlcNac (S_{50} value 0.2 mM, and a Hill coefficient for n of 1.7) was not unexpected. Chitin synthase from other fungal sources, e.g. *Coprinus cinereus* (de Rousset-Hall and Gooday, 1975); *Aspergillus nidulans* (Ryder and Peberdy, 1977) and *M. rouxii* (McMurrough and Bartnicki-Garcia, 1971) showed similar co-operativity with UDP-GlcNac.

Polyoxin D did not inhibit germ-tube formation and did not cause the germ-tubes to lyse. This can be contrasted with the lysis of the hyphal tips of *M. rouxii* (Bartnicki-Garcia and Lippman, 1972), and the bursting of *S. cerevisiae* at the birth site of the daughter cells (Bowers et al., 1974). The result with *C. albicans* cannot be accounted for by the presence of peptides which inhibit polyoxin D action (Mitani and Inoue, 1968). It is concluded, therefore, that polyoxin D is unable to permeate the cell envelope to the chitin synthase which is located at the cytoplasmic surface of the plasma membrane (Duran et al., 1979; Braun and Calderone, 1978). The general ineffectiveness of polyoxins towards pathogenic fungi has been commented on by Gooday (1977).

During germ-tube formation, the specific activity of total chitin synthase activity increased 40% after 2 h of germination and then returned to the original activity found in blastospores. There was also a 5-fold increase in the expressed activity (in vivo activated enzyme) during germ-tube formation. This would account for the increase in chitin content during the yeast to mycelial transformation (Chattaway et al., 1968; Braun and Calderone, 1978; Chiew, unpublished results). The difference in the heat stability of the chitin synthase from blastospores and germinating cells could reflect changes in the membrane composition, or different forms of chitin synthase. The understanding of the regulation of chitin synthase aptly exemplifies the central problem involved in dimorphism. The basic elements involved have been identified; a proenzyme distributed along the plasma membrane, activation by a protease and laying down of chitin fibrils at the membrane (Duran et al., 1979).

Acknowledgement. This work was supported by grants from the Medical Research Council of New Zealand and New Zealand Lottery Board of Control. These studies were completed during the tenure of a New Zealand Universities Grants Committee Post-graduate Scholarship to Chiew Yoke Yin.

References

Atkinson, D. E., Hathaway, J. A., Smith, E. C.: Kinetics of regulatory enzymes: Kinetic order of the yeast NAD isocitrate dehydrogenase reaction and a model for the reaction. J. Biol. Chem. **240**, 2682–2690 (1965)

Bartnicki-Garcia, S., Lippman, E.: Inhibition of *Mucor rouxii* by Polyoxin D: Effects on chitin synthetase and Morphological Development. J. Gen. Microbiol. **71**, 301–309 (1972)

Bowers, B., Levin, G., Cabib, E.: Effects of Polyoxin D on chitin synthesis and septum formation in *Saccharomyces cerevisiae*. J. Bacteriol. **119**, 564–575 (1974)

Braun, P. C., Calderone, R. A.: Chitin synthesis in *Candida albicans*: Comparison of yeast and hyphal forms. J. Bacteriol. **133**, 1472–1477 (1978)

Cabib, E., Farkas, V.: The control of morphogenesis: An enzymatic mechanism for the initiation of septum formation in yeast. Proc. Natl. Acad. Sci., USA, **68**, 2052–2056 (1971)

Cassone, A., Simonetti, N., Strippoli, V.: Ultrastructural changes in the wall during germ-tube formation from blastospores of *Candida albicans*. J. Gen. Microbiol. **77**, 417–426 (1973)

Chattaway, F. W., Holmes, M. R., Barlow, A. J. E.: Cell wall composition of the mycelial and blastospore forms of *Candida albicans*. J. Gen. Microbiol. **51**, 367–376 (1968)

Chattaway, F. W., Bishop, R., Holmes, M. R., Olds, F. C., Barlow, A. J. E.: Enzymic activities associated with carbohydrate synthesis in the yeast and mycelial forms of *Candida albicans*. J. Gen. Microbiol. **75**, 97–109 (1973)

de Rousett-Hall, A., Gooday, G. W.: A kinetic study of a solubilized chitin synthetase preparation from *Coprinus cinereus*. J. Gen. Microbiol. **89**, 146–154 (1975)

Dixon, M.: The determination of enzyme inhibitor constants. Biochem. J. **55**, 170–171 (1953)

Duran, A., Bowers, B., Cabib, E.: Chitin synthetase zymogen is attached to the plasma membrane. Proc. Natl. Acad. Sci. USA **72**, 3952–3955 (1975)

Duran, A., Cabib, E., Bowers, B.: Chitin synthetase distribution on the yeast plasma membrane. Science **203**, 363–365 (1979)

Eggstein, M., Kreutz, F. H.: Techniques in Protein Chemistry, (Bailey, J. L. ed.), p. 340. London; Elsevier 1967

Endo, A., Kakiki, K., Misato, T.: Feedback inhibition of L-glutamine D-fructose-6-phosphate aminotransferase by uridine diphosphate N-acetylglucosamine in *Neurospora crassa*. J. Bacteriol. **103**, 588–594 (1970)

Gancedo, J. M., Gancedo, C.: Concentrations of intermediary metabolites in yeast. Biochemie **55**, 205–211 (1973)

Ghosh, S., Roseman, S.: Methods Enzymol. **5**, 414–417 (1962)

Gooday, G. W.: Biosynthesis of the fungal wall-mechanisms and implications. J. Gen. Microbiol. **99**, 1–11 (1977)

Gooday, G. W.: In: Developmental Mycology Vol. 111 (J. E. Smith and D. R. Berry, eds.), pp. 51–77. London: Arnold 1978

Marx, J. L.: Chitin synthesis inhibitors: a new class of insecticides. Science **197**, 1170–1172 (1977)

Mitani, M., Inoue, Y.: Antagonists of antifungal substance polyoxin. J. Antibiot. **21**, 492–496 (1968)

McMurrough, I., Barnicki-Garcia, S.: Properties of a particulate chitin synthetase from *Mucor rouxii*. J. Biol. Chem. **246**, 4008–4016 (1971)

Norrman, J., Giddings, T. H., Catino, E. C.: Partial purification and properties of L-glutamine: D-fructose-6-phosphate aminotransferase from zoospores of *Blastocladiella emersonii*. Phytochemistry **14**, 1271–1274 (1975)

Peberdy, J. F., Moore, P. M.: Chitin synthase in *Mortierella vinacea*: properties, cellular location and synthesis in growing cultures. J. Gen. Microbiol. **90**, 228–236 (1975)

Ruiz-Herrera, J., Bartnicki-Garcia, S.: Proteolytic activation of chitin synthetase from *Mucor rouxii*. J. Gen. Microbiol. **97**, 241–249 (1976)

Ryder, N. S., Peberdy, J. F.: Chitin synthase in *Aspergillus nidulans*: properties and proteolytic activation. J. Gen. Microbiol. **99**, 69–76 (1977)

Scheckman, R., Brawley, V.: Localized deposition of chitin on the yeast cell surface in response to mating pheromone. Proc. Natl. Acad. Sci. USA **76**, 645–649 (1979)

Scherwitz, C., Martin, R., Veberberg, H.: Ultrastructural investigation of the formation of *Candida albicans* germ-tubes and septa. Sabouraudia **16**, 115–124 (1978)

Shepherd, M. G., Chiew, Y. Y., Ram, S. P., Sullivan, P. A.: Germ-tube induction in *Candida albicans*. Cand. J. Microbiol. (in press, 1980)

Stewart, P. R., Rogers, P. J.: In: Developmental mycology, Vol. 111 (J. E. Smith and D. R. Berry, eds.), pp. 164–196. London: Arnold 1978

Received September 28, 1979