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Antibiotic tetaine – a selective inhibitor of chitin and mannoprotein biosynthesis in *Candida albicans*

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Abstract. The antibiotic tetaine inhibits in *Candida albicans* the biosynthesis of two important cell wall constituents, chitin and mannoprotein. This effect is a consequence of inactivation of the enzyme glucosamine-6-phosphate synthetase. Due to the lack of glucosamine-6-phosphate the effective secretion of mannoprotein enzymes, acid phosphatase and invertase, by *Candida albicans* spheroplasts is inhibited. In the presence of tetaine, probably a modified mannoprotein, lacking a branched polymannan, is synthesized. The antibiotic action decreases the viability of *Candida albicans* cells, especially that of mycelial forms of this fungus.

Key words: *Candida albicans* – Tetaine – Inhibition – Chitin – Mannoprotein – Dimorphism – Secretion of mannoproteins – Yeast spheroplast regeneration

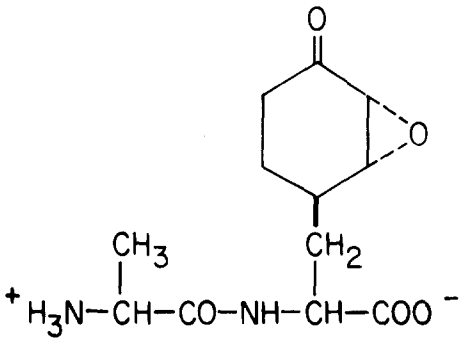

Fig. 1. Structure of the antibiotc tetaine

Candida albicans is a microorganism that causes severe systemic mycoses in humans. It can grow in the yeast-like (Y) or the mycelial (M) morphological form, depending on environmental factors (nutrients and temperature; for a review see Odds 1979). Its virulence is probably related to the mycelial form of this fungus (Borgers et al. 1983; Cassone 1984). Some reports suggest that inhibition of the transition of yeast-like to mycelial growth (Y → M transformation) may be of importance in the action of anticandidal agents of potential chemotherapeutic value (Cassone 1984). Anticandidal agents used in the therapy of systemic candidioses (amphotericin B, 5-fluorocytosine, ketoconazole) are strong inhibitors of Y → M transformation. This process is also affected by some agents interfering in protein and nucleic acid biosynthesis (Shepherd et al. 1980a).

Among known inhibitors of Y → M transformation there is a lack of compounds interfering in cell wall polysaccharide biosynthesis. Such inhibitors might be of interest since essential changes in amino sugar metabolism (Gopal et al. 1982; Sullivan and Shepherd 1982; Shepherd et al. 1980b) and chitin biosynthesis (Chiew et al. 1980) occur during germ-tube formation.

The antibiotic tetaine (Borowski 1952; Fig. 1), synonymous with bacilysin (Kamiński and Sokołowska 1973) and bacillin (Atsumi et al. 1975), has been previously shown to be a powerful inhibitor of growth of Y and M forms of *C.albicans* (minimal inhibitory concentrations were 3 μg/ml and 0.6 μg/ml, respectively) and of Y → M transformation, causing agglutination, deformation of cells and inhibition of septum formation (Milewski et al. 1983). Tetaine is transported into Y cells by a specific, dipeptide permease and into M cells by the oligopeptide transport system (Milewski et al. 1983). Inside the cell the antibiotic is cleaved by peptidases (Chmara et al. 1980). A C-terminal epoxy amino acid, identical with the antibiotic anticapsin (Neuss et al. 1970), is a powerful inhibitor of the enzyme, glucosamine-6-phosphate(GlcN-6-P) synthetase, [2-amino-2-deoxy-D-glucose-6-phoshate ketol isomerase, amino-transferring, EC 5.3.1.19 (Kenig et al. 1976; Chmara et al. 1980; Chmara et al. 1982)]. Recently it has been shown, that anticapsin acts as a glutamine analog, causing in vitro the irreversible inactivation of GlcN-6-P synthetase (Milewski et al. 1984).

In this paper we present results of some experiments, which may explain the morphological changes induced by tetaine.

Materials and methods

Candida albicans strain AMB 25, from culture collection of Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, was stored on Sabouraud agar slants at 4° C and transfered monthly, as described previously (Milewski et al. 1983).

Media YM1 and MA (Milewski et al. 1983) were used for growth of Y and M forms of *C. albicans*. PM medium (Kuo and Lampen 1974) was employed for cultivation of spheroplasts. Conditions for growth of Y cells, induction of germ-tube formation and cultivation of M forms were the same as described previously (Milewski et al. 1983).

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Abbreviations. GlcNAc, N-acetyl-D-glucosamine; GlcN-6-P, D-glucosamine-6-phosphate; ManNAc, N-acetyl-D-mannosamine; α-MM, α-methylmannoside; EGTA, 1,2 di/2-aminoethoxy/ethane-N,N,N′,N′-tetra-acetic acid

Inactivation of GlcN-6-P synthetase in vivo

Ten Erlenmeyer flaks, containing 500 ml of YM1 medium, were inoculated with 10^6 cells of *C. albicans* per ml. Suspensions were preincubated for 240 min at 30° C with vigorous shaking. Afterwards, tetaine at required concentrations was added to five flasks. Immediately and after 15, 30, 60 and 120 min, contents of two flasks (one with tetaine and one without antibiotic) were chilled to 8° C and centrifuged ($5{,}000 \times g$, 5 min). Pellets were washed twice with cold distilled water. Pyrophosphate extracts exhibiting GlcN-6-P synthetase activity were obtained as described previously (Milewski et al. 1985). Enzyme activity was determined using a modified Elson-Morgan procedure (Kenig et al. 1976). Protein concentration was estimated according to Bradford (1976).

Determination of cell viability

For the determination of cell viability a modified method based on the thin layer agar procedure for spheroplast regeneration (Ota 1972) was employed. Intact cells were used instead of spheroplasts. For the induction of yeast mode of growth, cells derived from overnight cultures were taken; for the cultivation of the mycelial form, cells were washed from a slant (for details see Milewski et al. 1983). Cells were immobilized in a thin layer of agar and treated in sequence by antibiotic solution in growth medium, distilled water and finally growth medium. Media and temperatures of growth for Y and M forms were the same as described previously (Milewski et al. 1983). By this procedure clumping of cells was avoided. Tetaine caused a strong agglutination of cells (Milewski et al. 1983) and for that reason, classical slide culture technique (Rose 1975) could not be employed.

Preparation of C. albicans spheroplasts

100 ml of PM medium was inoculated with 10^8 *C. albicans* cells from overnight culture. The suspension was incubated for 5 h at 30° C with vigorous shaking. Then, cells were harvested ($5{,}000 \times g$, 5 min), washed twice with distilled water and once with 12% mannitol solution. 5×10^8 cells were suspended in 1.5 ml of 0.1 M succinate buffer, pH 5.8. The suspension was mixed with the same volume of a solution containing 24% mannitol. 50 mM dithiothreitol, 30 mM $MgSO_4 \times 7H_2O$, and incubated for 30 min at 30° C. β-Glucuronidase from *Helix pomatia* was added; 10,000 Fishman units (Fishman et al. 1948) per 200 mg of wet weight of cells. Subsequently the suspension was incubated for 60 min at 30° C. After this time, 95–98% of cells were osmotically fragile. The spheroplast suspension was centrifuged ($800 \times g$, 5 min, 4° C), washed twice with 12% mannitol and suspended in 12% mannitol to 5×10^8 spheroplasts per ml.

Spheroplast cultivation.
Determination of secreted glycoprotein enzymes

PM medium was used for cultivation of spheroplasts. The medium contained as a carbon source, 2% w/v glucose or maltose for phosphatase or invertase experiments, respectively. 2×10^7 spheroplasts per ml were incubated at 30° C with gentle shaking. Tetaine was added after 3 h. Immediately and at 30 min, intervals, 1 ml samples were withdrawn and centrifuged ($800 \times g$, 5 min, 4° C). The activity of acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) and invertase (D-fructofuranoside fructohydrolase, EC 3.2.1.26) was determined in supernatants. Pellets were treated with 1 ml of distilled water for 3 min at 4° C. The activity of alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) was estimated in the suspension of burst spheroplasts.

The activity of alkaline and acid phosphatase was determined using p-nitrophenylphosphate as a substrate (Lampen et al. 1973). Sucrose was the substrate for invertase, glucose was estimated by the glucose oxidase method (Kuo and Lampen 1974).

Restoration of osmoresistance in spheroplasts

Thin layer agar method (Ota 1972) was used to study tetaine effect on the regeneration of cell walls in spheroplasts, obtained as described in Material and methods. The antibiotic was added at the beginning of the regeneration period. Colonies formed in Petri plates, which contained spheroplasts exposed to tetaine action, were compared to those in control plates, where regeneration proceeded in the absence of antibiotic.

Incorporation of ^{14}C glucose by C. albicans Y and M forms

Incorporation of labelled glucose was performed on YM1 medium for Y forms and on MA medium for M forms of *C. albicans*. Suspensions of exponentially growing Y or M cells were prepared as previously described (Milewski et al. 1983). Tetaine, amino sugars and 260 kBq/ml of [^{14}C]glucose were added simultaneously. At hourly intervals 10 ml samples were withdrawn, carrier cells were added and the whole suspension was centrifuged ($5{,}000 \times g$, 5 min, 4° C). Pellets were taken for the isolation of cell wall polysaccharides. In parallel, 1 ml samples of cell suspension were taken for determination of dry weight of cells.

Fractionation of cell wall polysaccharides

Pellets, obtained as described above, were washed twice with distilled water and then treated with 20 ml portions of 10% trichloroacetic acid at 0° C, for 20 min. The suspensions were centrifuged ($5{,}000 \times g$, 5 min, 4° C) and washed twice with cold distilled water. Pellets were suspended in 5 ml portions of 6% KOH. The general scheme of further fractionation is shown on Fig. 2. Chitinase preparate exhibited a specific activity equal to 0.3 glucose equivalent per milligram.

Isolation of labelled mannoproteins and their fractionation on Concanavalin A-Sepharose

Tetaine and 260 kBq/ml of [^{14}C]glucose were added to the suspension of *C. albicans* cells, growing exponentially on YM1 medium. After 3 h, 50 ml portions of tetaine treated and of control suspensions were withdrawn and centrifuged ($5{,}000 \times g$, 5 min, 4° C). Pellets were washed twice with cold distilled water. Mannoproteins were extracted by autoclaving of cell paste in 0.02 M citrate buffer, pH 7.0 at 121° C for 90 min. After cooling the solid material was removed by centrifugation and the supernatant was saved. Extraction and centrifugation were repeated with a pellet resuspended in a new portion of citrate buffer. Supernatants

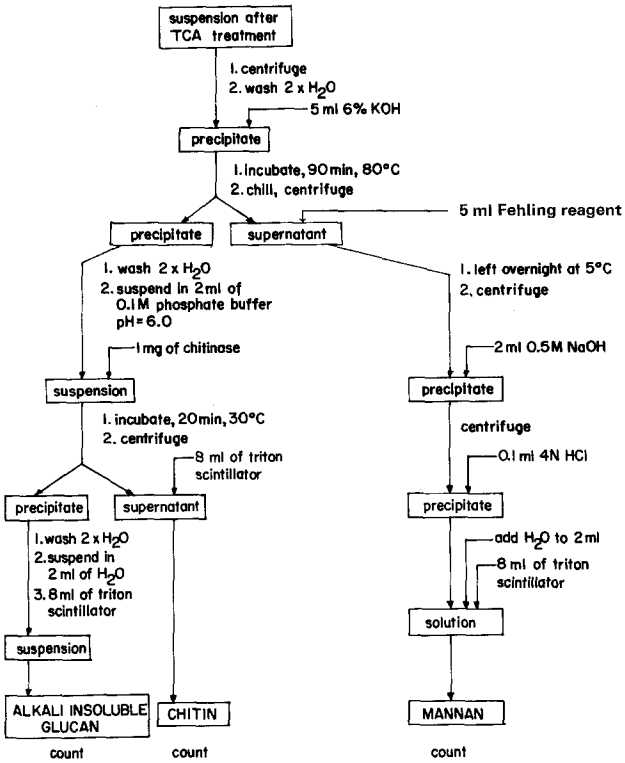

Fig. 2. Fractionation of cell wall polysaccharides

obtained after two extractions were combined. A volume of Fehling solution (Kocourek and Ballou 1969) equal to that of extract was added with stirring. The further procedure included repeated dissolving of precipitated mannan in 3 M HCl and reprecipitation with methanol: acetic acid (8:1). This step was repeated until the green colour of the supernatant disappeared. The mannan was collected on Whatman GF/A filters, washed several times with methanol, finally once with ethyl ether and allowed to dry at room temperature.

Dry mannan fractions were dissolved in small volumes of 0.1 M phosphate buffer, pH = 7.0, containing 1 M NaCl, 1 mM each of $MnCl_2$, $MgCl_2$ and $CaCl_2$. These solutions were applied at the tops of columns containing Concanavalin A-Sepharose, bed volume 5 cm^3. The bed was washed with 0.1 M phosphate buffer and then mannoproteins were eluted with solutions containing 0.02 M and 0.1 M α-methylmannoside. The flow speed was 5 ml/h. 2 ml fractions were collected and 0.5 ml samples were withdrawn from each fraction, combined with 5 ml of Triton scintillator and the radioactivity was counted.

Chemicals

Tetaine was purified from culture filtrate of *Bacillus pumilus* "Theta" as described previously (Borowski 1952). Concanavalin A-Sepharose was from Pharmacia, Uppsala, Sweden. Peroxidase, glucose oxidase and β-glucuronidase were from Sigma, St. Louis, MI, USA. Chitinase was from Calbiochem, San Diego, CA, USA. D-[U-^{14}C]glucose, 80 GBq/mmol was from UVVVR, Prague, Czechoslovakia. Other chemicals were of the finest grade comercially available.

Triton scintillator contained: 7.5 g 2,5 diphenyloxazole (PPO); 642.5 ml Triton X-100; 265 ml ethanol; 92.5 ml ethylene glycol; 1,500 ml xylene.

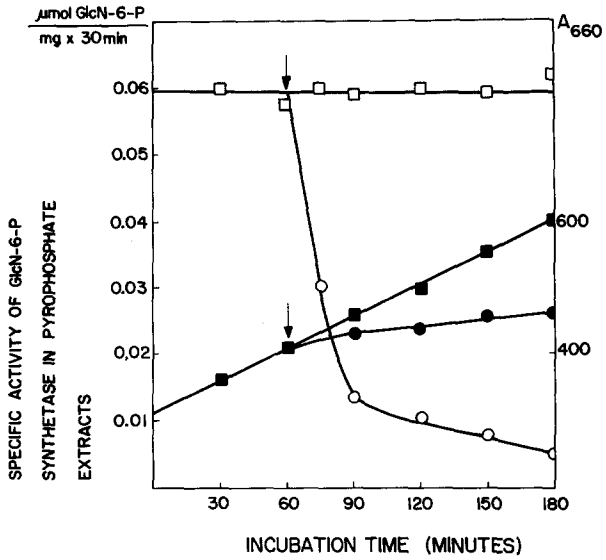

Fig. 3. Inactivation "in vivo" of GlcN-6-P synthetase by tetaine compared with the inhibition of growth. □, ■ control; ○, ● plus 10 μM tetaine. *Opened symbols* GlcN-6-P synthetase activity; *filled symbols* growth. Tetaine was added at the time indicated by arrows

Results

Inactivation of GlcN-6-P synthetase in vivo

The action of 10 μM tetaine on exponentially growing *C. albicans* cells caused inhibition of growth. Activity of the enzyme GlcN-6-P synthetase was determined in pyrophosphate extracts obtained from broken yeast cells. The procedure enabled a separation of GlcN-6-P synthetase from the unbound inhibitor in a cell free extract. Pyrophosphate extracts isolated from tetaine treated cells showed decreased GlcN-6-P synthetase activity (Fig. 3). After 30 min only about 20% of activity remained. The enzyme from pyrophosphate extracts isolated from control cells showed constant activity during the experiment.

Inhibition of biosynthesis of cell wall polysaccharides

The influence of tetaine on *C. albicans* cell wall constituents was determined by studying incorporation of ^{14}C from [^{14}C]glucose into mannan, glucan and chitin of Y and M forms (Fig. 4). The three cell wall polysaccharides were fractionated as described in Materials and methods.

The incorporation of radioactivity into cell wall polysaccharides was linear in uninhibited controls for 180 min. The only exception was incorporation into chitin of M forms.

The incorporation of ^{14}C into chitin and mannan of both morphological forms was strongly inhibited by tetaine, but incorporation into alkali insoluble glucan was practically not affected. During 60 min of incorporation, 2 μM and 20 μM tetaine did not exert a measurable effect on biosynthesis of cell wall polysaccharides in Y forms, while radioactive labelling of chitin and mannan of M forms was inhibited at 51 and 47%, respectively.

The incorporation of ^{14}C label was also measured in the presence of tetaine and some N-acetylated amino sugars (Table 1). The addition of 5 mM N-acetylglucosamine to tetaine (20 μM) treated cultures of Y and M forms reversed the inhibition of incorporation of labelled precursor. The

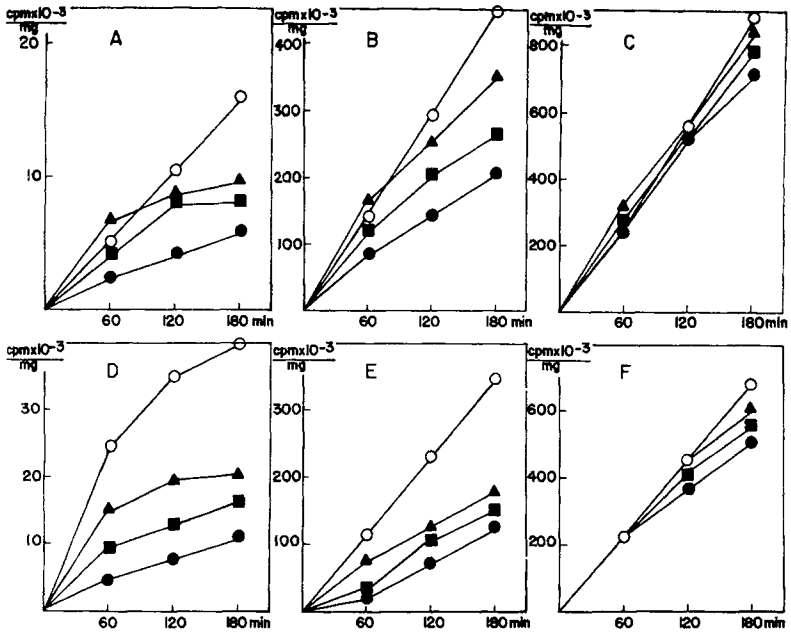

Fig. 4
Time course of incorporation of ^{14}C from [^{14}C]glucose into chitin (**A,D**), mannan (**B,E**) and glucan (**C,F**) of Y and M forms of *C. albicans*. **A–C** Y forms; **D–F** M forms. Tetaine concentrations: ○, none; ▲, 2 μM; ■, 20 μM; ●, 200 μM. Specific radioactivity is expressed in counts per min (cpm) per mg of dry weight of yeast or mycelial cells

Table 1. Incorporation of ^{14}C from [^{14}C]glucose to cell wall polysaccharides in the presence of tetaine and amino sugars. Incorporation was carried out for 180 min; % inh – percent of inhibition in regard to control

Tetaine concentration [μM]	Addition and concentration mM	Y forms – YM 1 medium			M forms – MA medium		
		Mannan % inh	Glucan % inh	Chitin % inh	Mannan % inh	Glucan % inh	Chitin % inh
0.0[a]	0.0	0(451540)	0(880210)	0(16100)	0(344760)	0(676720)	0(41040)
20.0	0.0	42	11	52	55	18	56
20.0	GlcNAc, 5	5	−3	14	8	−1	0
20.0	ManNAc, 5	27	3	33	17	N.T.	1

N.T. = not tested
[a] In brackets: levels of ^{14}C incorporation expressed in counts per min (cpm) per mg of dry weight of cells

effect was practically complete. N-acetylmannosamine was much more effective in reversing the tetaine effect with the mycelial than with the yeast-like form; it is particulary evident for the incorporation into chitin.

Regeneration of cell wall and secretion of mannoprotein enzymes by C. albicans spheroplasts in the presence of tetaine

Spheroplasts prepared from exponentially growing *C. albicans* cells could regenerate their cell wall in a soft agar layer (see Materials and methods). The essence of the method is an immobilization of spheroplasts in a thin layer of agar. After 7 h of regeneration, a subsequent treatment with water resulted in lysis of spheroplasts which have not resynthesised the osmoresistant envelope. Osmotically stable cells formed microcolonies during the following incubation on normal growth medium.

The soft agar layer method was used for the investigation of the influence of tetaine on the resynthesis of cell wall (Table 2). Tetaine, when added during the regeneration period, inhibited resynthesis of the osmoresistant layer. But even at the very high concentration of 300 μM, tetaine, 31% of spheroplast population regenerated their cell wall. When 2 mM GlcNAc was present in the regeneration medium together with 300 μM tetaine, only 19% of spheroplasts did not regenerate the wall.

Table 2. Regeneration of the osmoresistant layer by *C. albicans* spheroplasts. Negative control – sample, where spheroplasts were lysed in water before regeneration. This enabled us to calculate the percentage of not osmotically fragile cells. Values are the means of two determinations

Sample	Microcolonies count	Percent of regeneration
Control	160	100
Tetaine 3 μM	125	78
Tetaine 30 μM	100	62
Tetaine 300 μM	50	31.5
Tetaine 300 μM + GlcNAc 2 mM	130	81
Negative control	10	6

Mannoproteins are effectively synthesised and secreted into medium, when yeast spheroplasts are cultivated on

liquid media containing an osmotic stabilizer (Necas 1971). Some of these proteins exhibit enzymatic activity. The influence of tetaine on the secretion of two mannoprotein enzymes was investigated (Fig. 5). Secretion of acid phosphatase and invertase was inhibited by tetaine. 10 μM antibiotic inhibited secretion of acid phosphatase to about 40% (Fig. 5A) and of invertase to about 70% (Fig. 5B). In contrast, biosynthesis of the cytoplasmic enzyme, alkaline phosphatase, was practically not affected (Fig. 5C).

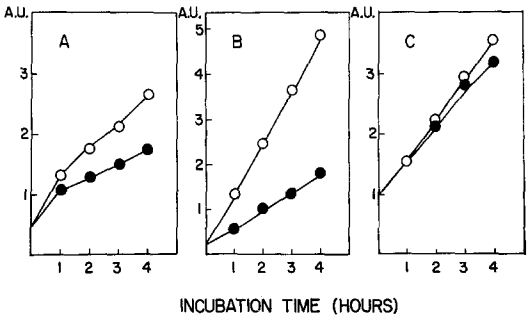

Fig. 5. Tetaine effect on the concurrent synthesis of acid phosphatase (**A**), invertase (**B**) and alkaline phosphatase (**C**) by *C. albicans* spheroplasts. Activities of enzymes are expressed in arbitrary units. ○, control, ●, 10 μM tetaine

Investigation of influence of tetaine on the structure of mannoproteins

C. albicans cells were treated with 20 μM tetaine and mannoproteins labelled with ^{14}C derived from [^{14}C]glucose were isolated according to the procedure described in Materials and methods. We obtained 11.25 mg of dry mannoproteins from tetaine treated and 25.20 mg from control cells; each from 1 g of wet weight of cells.

These mannoproteins were fractionated on Concanavalin A-Sepharose columns. The elution profiles are shown on Fig. 6A, B. Mannoproteins from control cells were eluted as a bulk peak with 0.1 M α-MM. The respective peak on diagram B is considerably smaller. Additionally, one can see some small peaks in 0.02 M α-MM region on diagram B, clearly separated from the bulk peak.

Viability of Y and M forms of C. albicans cells treated with tetaine

Viability of *C. albicans* cells exposed to tetaine was assessed as colony forming ability of cells immobilized in a thin agar layer (Table 3). Even at a very high concentration of tetaine, 300 μM, 7.5% of yeast cells remained viable. The same antibiotic concentration was completely fungicidal for M cells. At low, 3 μM, concentration, tetaine was fungistatic for Y cells and partially fungicidal for mycelial cells.

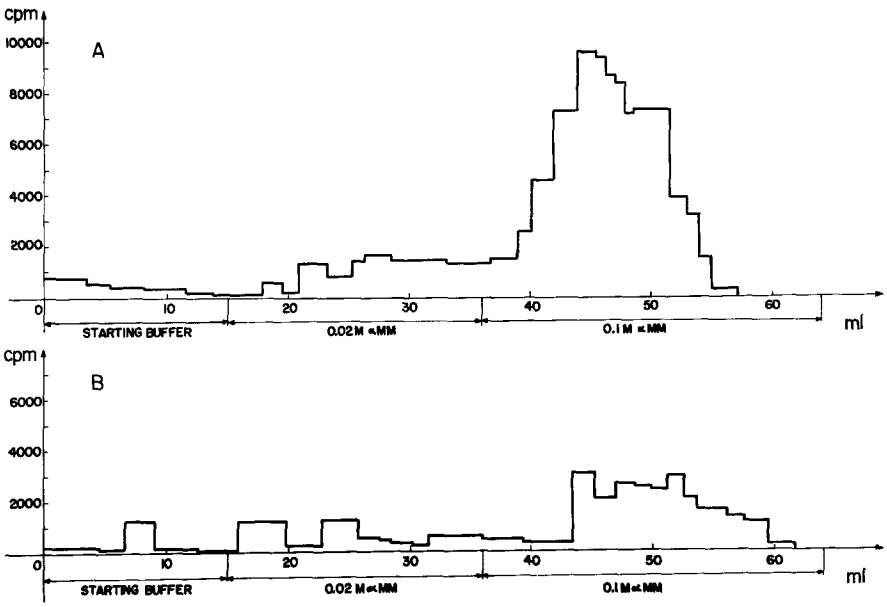

Fig. 6. A, B
Elution pattern of ^{14}C labelled mannoproteins from Concanavalin A-Sepharose columns. **A** mannoproteins from control cells; **B** mannoproteins from cells treated with 20 μM tetaine for 3 h

Table 3
Viability of Y and M forms of *C. albicans* exposed to tetaine for 12 h. Values are the means of three estimations

Tetaine concentration [μM]	Y forms – YM 1 medium					M forms – MA medium			
	0	3	15	60	300	0	3	60	300
Microcolonies count	200	210	150	100	15	140	93	25	0
Percent of viable cells	100	105	75	50	7.5	100	66	17	0

Discussion

Glucosamine-6-phosphate synthetase is a key enzyme for *C. albicans* in biosynthesis of cell wall constituents. N-acetylglucosamine is present in two substantial macromolecule types existing in the wall, namely mannoprotein and chitin.

In this paper we report, that the action of the dipeptide tetaine on *C. albicans* caused inactivation of the GlcN-6-P synthetase in intact cells. After 30 min of incubation of cell suspension with 10 μM tetaine, inactivation exceeded 75%.

Tetaine is not by itself an inhibitor of the target enzyme, because the antibiotic inside the cell is very quickly cleaved by peptidases (Chmara et al. 1982) and only anticapsin, the C-terminal amino acid derived from tetaine interacts with GlcN-6-P synthetase (Milewski et al. 1984). The rate of inactivation of the enzyme by anticapsin "in vitro" is markedly faster (Milewski et al. 1984), than inactivation "in vivo" by tetaine, reported in this paper. Transport of antibiotic into the cell, cleavage by peptidases and the necessity of competition with intracellular glutamine for the active centre of the enzyme may be factors contributing to the slower inactivation rate in vivo.

We have studied the consequences of a shortage in GlcN-6-P supply for the biosynthesis of cell wall polysaccharides. As was shown, the action of tetaine resulted in a strong inhibition of incorporation of ^{14}C derived from [^{14}C]glucose into chitin, somewhat lower into mannan and negligible into alkali insoluble glucan. Yeast glucan is a homopolymer of glucose and it does not contain glucosamine residues. Thus a small decrease of glucan biosynthesis rate, observed after prolonged [^{14}C]glucose incorporation, could be interpreted as a result of general inhibition of intracellular metabolism.

Tetaine inhibited the incorporation of labelled precursor into mannan and chitin of M forms stronger than that of Y forms. This difference is pronounced at low concentrations of antibiotic. When 5 mM GlcNAc was added together with 20 μM tetaine, inhibition of incorporation into mannan and chitin practically did not occur. Because N-acetylated amino sugars do not affect tetaine transport and intracellular hydrolysis (Milewski unpublished work), the decrease of label incorporation caused by tetaine is a consequence of inhibition of GlcN-6-P synthetase by anticapsin. When GlcNAc was replaced by ManNAc, the reversion of inhibition of ^{14}C incorporation was observed especially for M forms of *C. albicans*. A similar phenomenon was previously observed in growth inhibition experiments (Milewski et al. 1983). It may be explained on the basis of elevated activity of amino sugar metabolism in mycelial forms of *C. albicans* in comparison to yeast forms (Chiew et al. 1980; Gopal et al. 1982; Shepherd et al. 1980 b; Sullivan and Shepherd 1982).

The lack of inhibition of glucan biosynthesis by tetaine has been confirmed in investigations of cell wall regeneration in *C. albicans* spheroplasts. Yeast protoplasts and spheroplasts are able to regenerate their walls on solid media (Peberdy 1979), but the proportion of main constituents are different from that of the "normal" wall (Garcia-Mendoza and Novaes-Ledieu 1968; Popov et al. 1980). Regenerated walls contain much more chitin and glucan, whereas the content of mannan is reduced (Popov et al. 1980). Because, even at very high concentration of tetaine, 31% of spheroplast population were able to resynthesise an osmoresistant layer, one can suppose, that glucan synthesised by spheroplasts can partially protect them from lysis. In fact, branched β-(1→3)glucan is considered to be the constituent of yeast cell wall responsible for its rigidity and mechanical resistance (Farkas 1979; Cassone 1984).

The stronger fungicidal effect of tetane in regard to M forms may be explained on the basis of difference in chitin content between Y and M cells. In Y forms chitin constitutes only about 1% of the cell wall (Chattaway et al. 1968) and is localized mainly in septum and bud scar regions (Cabib and Bowers 1971; Cabib et al. 1982). In mycelial forms, the chitin content is about 5-fold higher (Chattaway et al. 1968), activity of GlcN-6-P synthetase is 4-fold increased (Chiew et al. 1980) and the role of chitin as a biopolymer enhancing the mechanical resistance of fungal cell wall was postulated (Gooday and Gow 1983).

Some of yeast mannoproteins, exhibiting enzymatic activity are localized in a periplasmic space (Farkas 1979). When yeast protoplasts are cultivated on liquid media containing osmotic stabilizers, these enzymes are secreted into the medium (Lampen 1968; Lampen et al. 1973). The antibiotic tunicamycin, an inhibitor of protein glycosylation, markedly decreases the rate of secretion (Kuo and Lampen 1974). In our experiments presented in this paper, we have shown that tetaine inhibits secretion of the mannoprotein enzymes, acid phosphatase and invertase, while it has no effect on the biosynthesis of intracellular alkaline phosphatase, enzyme of non-glycosylated (Bartels and Cristophersen 1970).

Our results confirm an earlier suggestion, that the glycosylation with branched mannan is indispensable for the export of protein through the cytoplasmic membrane (Pazur and Aronson 1972).

However, is still not known whether the biosynthesis of mannoproteins containing only short, linear mannan chains may take place when the glycosylation with branched mannan is stopped. We tried to answer this question using tetaine as an experimental tool. Mannoproteins isolated from tetaine treated cells appeared to contain some small fractions, eluted with low concentration of α-MM, which we suppose to be consisted of proteins linked with linear mannan polymers. This is in agreement with a previously described fact of the considerably lower affinity of the Concanavalin A to a linear mannanns (Okubo and Suzuki 1975; Goldstein and Hayes 1978). However this suggestion requires further confirmation.

Structural mannoproteins of yeast cells constitute an external wall layer (Zlotnik et al. 1984). *C. albicans* mutant cells with reduced mannan content exhibited a tendency to form agglomerates (Shimokawa and Nakayama 1984). We have shown previously, that *C. albicans* cells treated with tetaine agglutinate (Milewski et al. 1983). Our present results indicate that such an agglutination may be due to the lack of branched mannan.

The antibiotic tetaine is the first known inhibitor of chitin and mannan biosynthesis in pathogenic yeasts. One can assume, that this compound could be utilized as a tool in studies of the biosynthesis of fungal cell wall. Moreover it is noteworthy as a potential chemotherapeutic anticandidal agent.

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