Mechanism of antifungal action of kanosamine

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The antibiotic kanosamine inhibited growth of Saccharomyces cerevisiae and a range of human pathogenic fungi, including Candida albicans. Kanosamine was transported into C. albicans cells by the glucose transport system and subsequently phosphorylated. The product of its intracellular metabolism, kanosamine-6-phosphate, was an inhibitor of the enzyme glucosamine-6-phosphate synthase. Inhibition was competitive in respect to one of the substrates, D-fructose-6-phosphate, with \( K_i \approx 9 \times 10^{-5} \) M, and was non-competitive in respect to the second substrate, L-glutamine. On the other hand, kanosamine-6-phosphate had no effect on the enzyme catalysing the next metabolic step, namely glucosamine-6-phosphate N-acetylase. The action of kanosamine on C. albicans cells resulted in profound morphological changes, inhibition of septum formation and cell agglutination. Experiments with S. cerevisiae mutants showed that the presence of the Cdr1p drug efflux pump did not affect the antifungal activity of kanosamine.

**Keywords** Candida albicans, chitin, enzyme inhibition, kanosamine

Introduction

Disseminated fungal infections remain one of the major problems in modern chemotherapy. Only a very limited number of antifungal agents are used in clinical practice, including the highly toxic amphotericin B as a ‘gold standard’ and less toxic but also less effective synthetic compounds, flucanazole and itraconazole [1]. The emerging challenge of multi-drug resistance (MDR) makes the situation even worse. Thus searching for alternatives for existing drugs is an urgent need. In this respect, it is worth re-evaluating some antibiotics which have not found clinical application but which have some properties making them interesting starting points for new drug development.

Kanosamine, 3-amino-3-deoxy-D-glucose, was first isolated as one of the products of acid hydrolysis of kanamycin [2], but later the same compound was identified as an antibiotic produced by Bacillus aminoglucosidicus [3]. The antibiotic was reported to exhibit some antibacterial in vitro activity [3] but very high minimal inhibitory concentrations (MICs) and narrow antibacterial spectrum precluded its further development. More recently, a strong inhibitory effect of kanosamine on growth of some plant-pathogenic fungi and oomycetes was demonstrated [4]. Studies on the mechanism of antibacterial action of kanosamine revealed that the antibiotic inhibited cell wall biosynthesis [5]. It was suggested that a product of possible intracellular metabolism of kanosamine might be an inhibitor of one of the enzymes catalysing the initial reactions of the hexosamine biosynthetic pathway [6]. However, the actual target has not been identified unequivocally.

In this paper we present studies on the antifungal action of kanosamine, aimed at identification of the actual kanosamine metabolite and its intracellular target.

Materials and methods

**Microorganisms and growth conditions**

B. aminoglucosidicus A-4722 was a generous gift from Dr Tomio Takeuchi, Institute of Microbial Chemistry, Tokyo, Japan. Composition of the growth medium and growth conditions allowing kanosamine production were as described previously [3]. Clinical strains of different Candida species were from the collection of the Department of Clinical Microbiology, Medical Academy
Two methods of preparation of kanosamine were used. A small amount of the antibiotic for analytical purposes was isolated from fermentation broth of *Kanamycin*, yeast hexokinase and RPMI 1640 medium were from Sigma-Aldrich (St. Louis, Missouri, USA). Dowex 50 WX 8 and 1X8 resins were from Serva Feinbiochemica, Heidelberg, Germany. Other reagents were of the highest grade commercially available.

**Chemicals**

Kanamycin, yeast hexokinase and RPMI 1640 medium were from Sigma-Aldrich (St. Louis, Missouri, USA). Dowex 50 WX 8 and 1X8 resins were from Serva Feinbiochemica, Heidelberg, Germany. Other reagents were of the highest grade commercially available.

**Isolation of kanosamine**

Two methods of preparation of kanosamine were used. A small amount of the antibiotic for analytical purposes was isolated from fermentation broth of *B. aminoglucosidicus*, essentially as described previously [3]. Alternatively, a bulk amount of kanosamine was isolated from acid hydrolysate of kanamycin according to our modification of the previously published procedure [2]. Kanamycin, 300 mg, was hydrolysed with 6 M HCl at 60 °C for at least 2 h. The resulting hydrolysate was fractionated by thin layer chromatography (TLC) on silica gel plates, using solvent system 1 ([n-butanol–acetic acid–H₂O; 4:1:1]). Compounds containing amino groups were detected using the ninhydrin reagent. After evaporation at reduced pressure, the solid residue was re-dissolved in solvent system 2 ([n-butanol–acetic acid–H₂O; 4:2:1]) and the resulting solution was chromatographed on a cellulose column developed with the same solvent system. Fractions which showed a single ninhydrin-positive spot on TLC plates developed with solvent system 1 (Rf = 0.28) were collected and evaporated. The solid residue was dissolved in water and passed through the Dowex 50 WX 8 (H⁺) column. Pure kanosamine hydrochloride was eluted with 40 mM HCl. The solution was lyophilized to give a very hygroscopic white powder. The nuclear magnetic resonance (NMR) spectrum was consistent with the expected structure. The melting point and chromatographic properties were identical with those of the *B. aminoglucosidicus* fermentation product and with those published in the literature [2,3].

**Determination of antifungal activity of kanosamine**

MICs were determined by a serial dilution microtiter plates method in Yeast Nitrogen Base medium containing 1% glucose or glycerol as a carbon source. Wells containing serially diluted kanosamine and control wells were inoculated with 10⁶ cells ml⁻¹ of an overnight culture of fungal cells and incubated for 24 h at 30 °C. MIC was defined as the lowest antifungal agent concentration preventing visible growth. Alternatively, MICs were determined in RPMI 1640 medium buffered with 3-[N-morpholino]propanesulphonic acid (MOPS) to pH 7.0, under conditions recommended by NCCLS [8]. In all cases, reproducible sharp end points were obtained and trailing effects were not observed.

**Phosphorylation of kanosamine in vitro, isolation and identification of the phosphorylation product**

Kanosamine, 60 mg, was phosphorylated in 7.5 ml of 0.1 M Tris-HCl buffer, pH 8.0, containing 60 mM MgCl₂ 0.3 mM ethylenediaminetetraacetic acid (EDTA). 342 mg of adenosine 5′-triphosphate (ATP) and hexokinase (EC 2.7.1.1) from baker’s yeast, 30 units ml⁻¹. The resulting solution was incubated for several hours at 30 °C with stirring. The course of reaction was followed by TLC analysis (solvent systems 1 and 3 [iso-propanol–H₂O; 1:1]) at 90-min intervals. Components of the reaction mixture were detected with ninhydrin, Hanes reagent (specific for sugar phosphates), aniline phthalate (specific for reducing sugars) and alkaline solution of KMnO₄ (specific for polyols) [9]. When the reaction was completed after 12 h, the Hanes-, ninhydrin- and aniline phthalate-positive product was isolated by means of consecutive ion-exchange chromatography on Dowex 1X8 (OH⁻) and Dowex 50WX8 (H⁺) columns and lyophilized to give 40 mg of a white powder. The purified compound had the following properties: TLC data, Rf = 0.14 in solvent system 1, Rf = 0.55 in solvent system 3; NMR spectral data (D = deuterium; δ = chemical shift; m = multiplet; s = singlet; H = hydrogen; P = phosphorus), ¹H NMR (D₂O), δ: 3.50 (m, 1H), 3.59 (m, 2H), 3.82 (m, 1H), 3.97 (m, 3H); ³¹P NMR (D₂O), δ: 1.4 (s, 1P).

**Uptake studies**

*C. albicans* ATCC 10261 cells, in exponential growth in YNB medium at 30 °C, were harvested, washed with saline and resuspended in 50 mM potassium phosphate buffer, pH = 6.5 containing 1% glucose or 1% glycerol to a final cell density of 10⁶ cells ml⁻¹ (about 1.0 mg dry weight cells ml⁻¹). The suspension was pre-incubated at 30 °C for 10 min, kanosamine dissolved in a minimal amount of ethanol was added, and the reaction was terminated by addition of 30% trichloroacetic acid. The suspension was centrifuged and the supernatant was removed. The pellet was washed with 50% ethanol and analysed by ¹H NMR. The suspension was incubated at 30 °C for 1 h. Samples were withdrawn at intervals and treated as described above. The data are presented as mean ± SD.
aliquot of potassium phosphate buffer was added to the final concentration of 5 mg ml$^{-1}$ (23 mm) and incubation was continued. At that moment and after 5, 10, 15, 30, 60 and 90 min, samples of 100 µl were withdrawn and diluted 1:100. The samples were immediately filtered through GF/C glass fibre filters (Whatman International Ltd., Maidstone, UK) under suction and filtrates were collected. The kanosamine concentration in filtrates was measured by a colorimetric method. Briefly, 0.5 ml portions of filtrates were combined with 0.625 ml portions of the solution containing 0.8 mg ml$^{-1}$ of 2,4,6-trinitrobenzenesulphonic acid (TNBS) and 4% Na$_2$B$_4$O$_7$$\times$10 H$_2$O. The resulting mixtures were incubated at 37 °C for 30 min and absorption at $\lambda = 420$ nm was measured.

**Metabolism of kanosamine in cell free extract**

Kanosamine was added to the cell-free extract, prepared from *C. albicans* ATCC 10261 cells as described previously [10], to give a final concentration of 5 mg ml$^{-1}$. The mixture was incubated at 30 °C and 1 ml samples were collected hourly and de-proteinized by addition of 1 ml of ethanol. Precipitates were removed by filtration and the course of reaction was followed by TLC analysis of the filtrates (solvent system 1). Alternatively, *C. albicans* ATCC 10261 cells were grown in YNB liquid medium at 30 °C in the presence or absence of kanosamine, 1 mg ml$^{-1}$. Antibiotic was added when the density of the cell suspension measured at $\lambda = 660$ nm reached 0.6. At hourly time intervals samples of the cell suspension were collected, cells were harvested by centrifugation and broken by the small-scale glass beads procedure [11]. Crude extracts were analysed for the activity of glucosamine-6-phosphate (GlcN-6-P) synthase and GlcN-6-P N-acetylase and by TLC (solvent system 1, aniline phthalate spray reagent), as described above.

**Isolation of the kanosamine metabolite**

*C. albicans* cells from the overnight culture in YNB were harvested by centrifugation and suspended in 100 ml of the fresh YNB medium, to the cell density of 10$^8$ cells ml$^{-1}$. Kanosamine was added to the final concentration of 1 mg ml$^{-1}$, and the cell suspension was incubated for 6 h at 30 °C. Cells were then harvested, washed with cold distilled water, suspended in a minimal amount of water and broken with a French press. Cellular debris was removed by centrifugation (3000g, 15 min, 4 °C). The supernatant was de-proteinized by heating (5 min, 100 °C). Components of the resulting solution were separated by ion-exchange chromatography, essentially as described above for the isolation of kanosamine-6-phosphate prepared by enzymatic synthesis. Fractions containing a substance identical with kanosamine, as determined by TLC analysis, were combined and lyophilized. The white product was analysed by NMR.

**GlcN-6-P synthase purification and determination of enzymatic activity**

*C. albicans* GlcN-6-P synthase overproduced by *S. cerevisiae* YRS C-65 was purified to homogeneity as described previously [10]. Enzymatic activity was determined by the modified Elson–Morgan procedure [12]. Reactions were carried out in 25 mM phosphate buffer pH 6-9, containing 1 mM EDTA and 1 mM dithiothreitol. $\alpha$-Fructose-6-phosphate and 1-glutamine concentrations were either fixed (7.5 mM and 10 mM respectively, for general activity and for determination of concentrations inhibiting 50% of enzymatic activity [IC$_{50}$]) or variable (0.5–7.5 mM and 0-625–10 mM respectively), for inhibitory constants ($K_i$) determinations. $K_i$ values were then determined from the secondary plots of apparent Michaelis constants vs. inhibitor concentration, derived from Lineweaver–Burk plots.

**Determination of activity of GlcN-6-P N-acetylase**

Activity of this enzyme was determined in crude extracts prepared as described above from *C. albicans* ATCC 10261 cells, according to the previously described procedure [13].

**Other methods**

$^1$H and $^{32}$P NMR spectra were recorded on Gemini 200 and Unity 500+ spectrometers, respectively (Varian Scientific Instruments Inc., Palo Alto, Ca, USA). Morphological changes of *C. albicans* cells upon the action of kanosamine were documented by photomicrographs using the Olympus BX 60 F5 microscope (Olympus Optical Co., Shinjuku-ku, Tokyo, Japan). Protein was determined by the Bradford procedure [14].

**Results and discussion**

As the yield of kanosamine produced by the *B. aminoglucosidicus* strain was very low, we used the small amount of this compound as an analytical reference and decided to isolate kanosamine hydrochloride from the acid hydrolysate of kanamycin. For this purpose we elaborated a purification procedure which afforded 180 mg of pure kanosamine hydrochloride from 1500 mg of kanamycin. The NMR data for the isolated compound were consistent with the expected structure. TLC analysis and melting point were in
agreement with values reported previously for kanosamine hydrochloride ($R_t = 0.28$; solvent system 1, m.p. 120 °C (decomposition) [3].

The previous literature data on biological properties of kanosamine confirmed the antibacterial activity of this compound [4]. We found that kanosamine inhibited growth of some fungi, but the minimal inhibitory concentration values determined by the microplate broth dilution method in minimal YNB medium containing 1% glucose and in buffered RPMI 1640 medium were very high (Tab. 1). Similar values were found when the experiment was performed in Sabouraud medium (data not shown). The presence of 10 mM N-acetyl-d-glucosamine (GlcNAc) completely abolished the growth inhibitory effect of kanosamine, while $N$-acetyl-$D$-mannosamine and $N$-acetyl-$D$-galactosamine had no effect.

In a separate experiment we compared the growth inhibitory activity of kanosamine against $S$. cerevisiae JG 436 and PS 124 cells. The JG 436 cells have a disrupted $PDR5$ gene, which normally encodes the major drug extruding protein of yeast. The PS 124 cells are derived from JG 436, but have been transformed with a plasmid containing the $CDR1$ gene encoding the $C$. albicans analogue of Pdr5p [7]. Exactly the same MIC values were noted for both JG 436 and PS 124 (Table 1), demonstrating that the presence of the Cdr1p drug efflux pump does not affect the antifungal activity of kanosamine in vitro.

The microscopic examination of cells treated with kanosamine revealed morphological changes characteristic for yeast cells affected by compounds inhibiting chitin and/or mannoprotein biosynthesis. These alterations included cell agglutination, visible destruction of cell integrity, cell swelling and inhibition of septum formation (Fig. 1). Similar morphological changes were previously described as a result of the action of oligopeptides containing an inhibitor of GlcN-6-P synthase [15]. On the other hand, nikkomycins and poloxyins, known inhibitors of chitin synthase, inhibit septum formation and cause cell swelling and lysis [16,17] while tunicamycin, a selective inhibitor of mannoprotein biosynthesis, causes mainly cell agglutination [18].

Uptake of low-molecular-weight compounds is usually measured by radiochemical methods. Since the radioactively labelled kanosamine was not available, we developed a chemical method allowing the colorimetric determination of kanosamine concentration. Kanosa-

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**Table 1** Antifungal activity of kanosamine determined in minimal media

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>RPMI 1640</th>
<th>YNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$. albicans ATCC 10261</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>$C$. albicans ATCC 26278</td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td>$+$ GlcNAc, 10 mM</td>
<td>NT</td>
<td>&gt;20</td>
</tr>
<tr>
<td>$+$ ManNAc, 10 mM</td>
<td>NT</td>
<td>5</td>
</tr>
<tr>
<td>$+$ GalNAc, 10 mM</td>
<td>NT</td>
<td>5</td>
</tr>
<tr>
<td>$C$. albicans MAG 122</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td>$C$. humicola MAG 325</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>$C$. glabrata MAG 421</td>
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<td>&gt;20</td>
</tr>
<tr>
<td>$C$. arborea MAG 067</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$C$. krusei MAG 011</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>$C$. parapsilosis MAG 502</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td>$C$. famata MAG 333</td>
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<td>5</td>
</tr>
<tr>
<td>Candida sp. MAG 006</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae ATCC 9763</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>$S$. cerevisiae JG 436</td>
<td>NT</td>
<td>5</td>
</tr>
<tr>
<td>$S$. cerevisiae PS 124</td>
<td>NT</td>
<td>5</td>
</tr>
</tbody>
</table>

NT, not tested; MAG strains, clinical isolates from the collection of Medical Academy of Gdańsk.
mine reacted with TNBS to give a yellow product. As the absorbance of the resulting solution measured at $\lambda = 420$ nm showed a linear dependence on kanosamine concentration in the 5–100 $\mu$g ml$^{-1}$ range, this method could be validly used to determine the kanosamine concentration. Figure 2 shows that kanosamine was accumulated by C. albicans cells suspended in phosphate buffer containing 1% glucose. The initial uptake rate was $8.6 \pm 0.3$ nmol min$^{-1}$ mg$^{-1}$ dry weight. This rate was practically constant for at least 30 min. Assuming that the internal volume of $10^8$ C. albicans cells is roughly 6 $\mu$l [19], one can estimate that the intracellular concentration of kanosamine after 30 min treatment with the antibiotic was higher than 50 $\mu$M. The uptake was totally inhibited in the presence of 100 $\mu$M NaN$_3$, suggesting that there was active transport against the concentration gradient. A remarkably higher rate, $36 \pm 0.5$ nmol min$^{-1}$ mg$^{-1}$ dry weight, was noted when glucose in the incubation buffer was substituted by glycerol. Therefore, although we have not identified the actual permease transporting kanosamine, any energy-dependent glucose transporter is the likely candidate. Unfortunately, there is very little known about sugar transport systems in C. albicans. On the other hand, such systems have been extensively studied in S. cerevisiae. Several yeast sugar permeases have been identified and characterized, including those encoded by the $HEX1$–$HEX7$ genes [20]. They differ in a number of features but share one, i.e. they do not involve sugar phosphorylation in their mechanism of action [21]. It is possible that a similar situation exists in C. albicans.

Literature data suggests that kanosamine may be phosphorylated by yeast hexokinase [22]. We have developed conditions for enzymatic phosphorylation allowing an almost quantitative conversion of kanosamine into kanosamine-6-phosphate as well as a procedure for purification of the phosphorylated product (yield 90%). NMR data of the isolated compound were consistent with the structure of kanosamine-6-phosphate. The TLC analysis data were as follows: $R_f = 0.14$ (solvent system 1), $R_f = 0.55$ (solvent system 3). The phosphorylated compound at 20 mg ml$^{-1}$ was completely ineffective when tested for antifungal activity.

In previous papers on the mechanism of kanosamine’s antibacterial action, it was suggested that this amino sugar could be phosphorylated during transport or in the cytoplasm [6]; no evidence, however, was presented. In our studies we used TLC for semi-quantitative analysis of kanosamine metabolism in C. albicans cells treated with kanosamine, 5 mg ml$^{-1}$. Cells were harvested at time intervals and broken. Crude extracts were deproteinated by heating and remaining aliquots were examined by TLC using different colour-developing, specific reagents. At zero time and after 1 h, only the spot corresponding to kanosamine could be detected ($R_f = 0.28$, solvent system 1, nihydrin- and aniline phthalate-positive, Hanes-negative). However, the chromatograms of the samples collected at 2 h and thereafter showed another spot ($R_f = 0.14$, solvent system 1, nihydrin-, aniline phthalate- and Hanes-positive). The chromatographic behaviour of the newly seen metabolite was identical with that of kanosamine-6-phosphate synthesized enzymatically in vitro. Conclusive confirmation was provided by NMR analysis of the isolated metabolite. The NMR spectrum of this compound was almost identical with that of kanosamine-6-phosphate prepared by enzymatic synthesis from kanosamine.

The spot corresponding to kanosamine-6-phosphate was also detected by TLC analysis of the crude extract prepared from C. albicans cells, incubated in vitro for several hours at 30 $^\circ$C with kanosamine added at an initial concentration 5 mg ml$^{-1}$. Presence of kanosamine-6-phosphate was first detected after 5 h. Therefore it is certain that kanosamine is phosphorylated at C6-OH by the cytoplasmic kinase within C. albicans, most likely by the hexokinase. The rate of this process is rather low. This is not surprising in light of the report of Machado de Domenech and Sols [22], who noted that kanosamine was phosphorylated in vitro by yeast hexokinase at a rate only 0.3% of that observed for $D$-glucose.

The structural analogy between kanosamine-6-phosphate and glucosamine-6-phosphate and reversal of antifungal action of kanosamine by GlcNAc suggested either $L$-glutamine: $D$-fructose-6-phosphate amidotrans-
ferase (GlcN-6-P synthase) or GlcN-6-P N-acetylase as possible targets for kanosamine or its metabolite in *C. albicans* cells. These enzymes catalyse consecutive steps in a cytoplasmic pathway leading to the eventual formation of uridine-5'-diphospho-N-acetylglucosamine (UDP-GlcNAc) which, in turn, provides GlcNAc for the biosynthesis of the fungal aminosugar-containing cell wall macromolecules, chitin and mannanproteins. Therefore, kanosamine and kanosamine-6-phosphate were tested as potential inhibitors of both enzymes. Kanosamine did not inhibit GlcN-6-P synthase activity in crude extract prepared from *C. albicans* cells or in the studies using the pure enzyme. On the other hand, kanosamine-6-phosphate inhibited the enzyme. Inhibition of 50% of the enzymatic activity was observed at 9.8 mM. Kinetic analysis of the inhibition, shown in Figure 3, revealed that it was competitive with regard to Fru-6-P ($K_i = 5.9$ mM) and non-competitive with regard to μ-Gln ($K_i = 21$ mM). It is thus clear that kanosamine-6-phosphate interacts with the enzyme at the Fru-6-P binding site. It should be therefore mentioned that the natural product of the reaction catalysed by GlcN-6-P synthase, i.e. d-glucosamine-6-phosphate, was previously demonstrated to be an inhibitor of the bacterial enzyme [23], with an inhibition pattern identical to that found by us for kanosamine-6-phosphate. On the other hand, neither kanosamine nor kanosamine-6-phosphate, 50 mM, inhibited GlcN-6-P N-acetylase activity. Inhibition of GlcN-6-P synthase was also demonstrated in *C. albicans* cells treated with kanosamine, 5 mg ml$^{-1}$. Figure 4 shows that the enzymatic activity determined in crude extracts of *C. albicans* progressively decreased with time, falling after 8 h to less than 50% of that noted for control cells. As mentioned above, the inhibitory effect of kanosamine on *C. albicans* was fully reversed by GlcNAc but not by other N-acetyl aminosugars. On the other hand, it is known that GlcNAc is transported into *C. albicans* by a specific inducible permease and subsequently phosphorylated [24]. GlcNAc-6-P may then enter the catabolic pathway or supplement a possible intracellular shortage of aminosugars, thus overcoming the kanosamine-induced glucosamine depletion. Therefore, there is little doubt that GlcN-6-P synthase is the only target for kanosamine-6-phosphate in *C. albicans* cells, and is crucial for their growth and division.

Giordani *et al.*, reported that 200 mM D-glucosamine caused a 25–35% inhibition of the growth of *S. cerevisiae* [25]. The authors explained that effect as a consequence of inhibition of the extracellular enzyme, N-acetyl-β-D-glucosaminidase. Inhibition of yeast growth on non-fermentable carbon sources by D-glucosamine was also demonstrated [26]. Despite the close structural analogy

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**Fig. 3** Kinetic analysis of inhibition of pure *C. albicans* GlcN-6-P synthase by kanosamine-6-phosphate. (a) Competitive inhibition of the enzyme with respect to Fru-6-P; (b) non-competitive inhibition with respect to L-Gln. Kanosamine-6-phosphate concentrations are shown in inset boxes.

**Fig. 4** Time course of inhibition of GlcN-6-P synthase activity in *C. albicans* cells treated with kanosamine, 5 mg ml$^{-1}$. Enzyme activity was determined in cell-free extracts prepared from cells harvested at time intervals. Bars represent standard deviations.
between D-glucosamine and kanosamine, the mechanism of antifungal action of the latter is very different. Our results clearly demonstrate that kanosamine is transported into fungal cells and phosphorylated in the cytoplasm, and that the kanosamine-6-phosphate thus formed interacts with GlcN-6-P synthase. Intracellular kanosamine phosphorylation is crucial for antifungal activity, as was demonstrated by the apparent inability of kanosamine to inhibit GlcN-6-P synthase and by the lack of antifungal activity of kanosamine-6-phosphate.

GlcN-6-P synthase has been already proposed as a potential target in antifungal chemotherapy [27]. Rationally designed oligopeptides, containing N\(^2\)-(4-methoxyfumaroyl)-L-2,3-diaminopropionic acid (FMDP), show promising antifungal in vitro and in vivo activity [28]. Unfortunately, their poor stability in serum [29] precluded possible further development as potential drugs. The anti-candidal activity of kanosamine is obviously very poor and this antibiotic cannot be considered as a drug candidate. However, we believe that the mechanism of its action could be exploited as a molecular basis for the rational design of a new class of GlcN-6-P synthase inhibitors as potential antifungals, active also against multidrug-resistant fungi. As a phosphorylation was obviously the step limiting the anti-candidal activity of kanosamine, it seems that further studies should be focused on modifications of aminosugar analogues enhancing the recognition of these compounds by phosphorylating enzyme(s).

Acknowledgements

This work was supported by the grant No 4 P05F 00216 from the Committee for Scientific Research (KBN). The generous gifts of Dr. R. Prasad and Dr. T. Takeuchi are gratefully acknowledged.

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