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Tetraphenylphosphonium is an indicator of negative membrane potential in *Candida albicans*

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The characteristics of the uptake of lipophilic cations tetraphenylphosphonium (TPP^+) into *Candida albicans* have been investigated to establish whether TPP^+ can be used as a membrane potential probe for this yeast. A membrane potential ($\Delta\psi$, negative inside) across the plasma membrane of *C. albicans* was indicated by the intracellular accumulation of TPP^+. The steady-state distribution of TPP^+ was reached within 60 min and varied according to the expected changes of $\Delta\psi$. Agents known to depolarize membrane potential caused a rapid and complete efflux of accumulated TPP^+. The initial influx of TPP^+ was linear over a wide range of TPP^+ concentrations (2.5-600 μM), indicating a non mediated uptake. Thus, TPP^+ is a suitable $\Delta\psi$ probe for this yeast.

In some yeasts e.g. *Endomyces magnusii* and *Pichia humboldtii* it has been possible to directly measure the membrane potential with microelectrodes [1–3]. However, because of the smaller size of most of the yeasts, indirect methods of measuring potential have been more frequently used [4–8]. Recently, Bakker et al. [2] measured the membrane potential of *E. magnusii* cells by both the direct (with microelectrode) and the indirect methods (uptake of lipophilic cation) and found comparable values of $\Delta\psi$. Among several indirect methods, the uptake of lipophilic cations is most frequently used for monitoring the membrane potential in yeast cells as well as in other systems [9,10]. It is observed that the use of the tetraphenylphosphonium ion (TPP^+) as an indicator of $\Delta\psi$ offers an additional advantage over other cations e.g. the dibenzyldimethylammonium ion (DDA^+) or the triphenylmethylphosphonium ion ($TPMP^+$), because the later cations may be translocated across the plasma membrane via an inducible thiamine transport system [8]. However, the use of TPP^+ as plasma membrane $\Delta\psi$ probe has also been severely questioned both in yeast [11] and in alga [12]. Because of this controversy it becomes important that the suitability of TPP^+ to indicate $\Delta\psi$ is tested among different yeasts. The results presented in this paper demonstrate that TPP^+ accumulation can be used as probe in the yeast *Candida albicans*.

Candida albicans (NCL 3100 = ATCC 46977) was obtained from the National Chemical Laboratory, Poona, India. The cells were maintained and grown as described earlier except that YPD medium was used [13,14]. A 10% (w/v) cell suspension was prepared in distilled water and was kept in ice for the experiments. The uptake of TPP^+ was measured by the method of Hauer and Höfer [4]. $\Delta\psi$ values were calculated by inserting the steady-state intra- and extracellular concentrations of TPP^+ into the Nernst equation. The

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intracellular concentration for *C. albicans* was calculated assuming a value of 2.0 μl intracellular water/mg dry wt.

In *C. albicans* cells, the steady-state equilibrium of TPP^+ was reached in about 60 min (Fig. 1), which was somewhat longer than in *R. glutinis* (20 min), however, it was considerably shorter as compared to several hours in some strains of *S. cerevisiae* [6,11]. The uptake of TPP^+ and its equilibration ratio in *C. albicans* did not change significantly even in cells aerated for 30 h in spite of about 25% reduction of cell respiration (data not shown). From the accumulation of TPP^+, a membrane potential negative inside can be inferred. It was of interest to test whether conditions known to depolarize biological membranes also decrease the accumulation of TPP^+ in *C. albicans* cells.

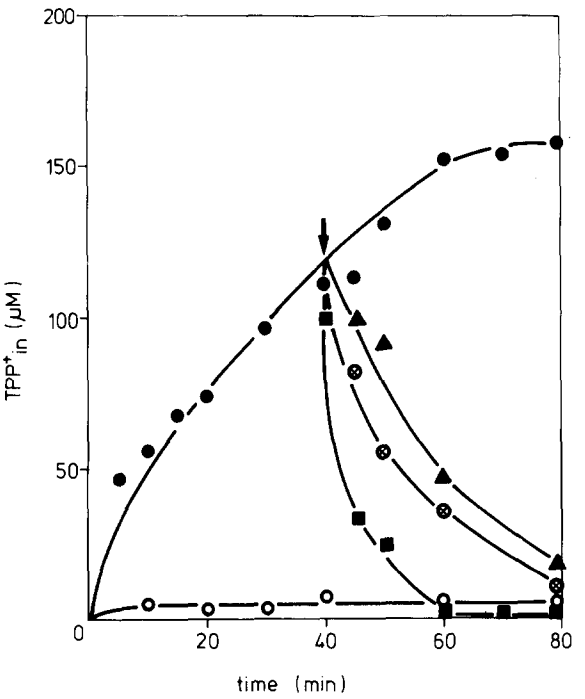

Fig. 1. Uptake to TPP^+ in *C. albicans* cells. The arrows (↓) on the graph indicate the time when cells were divided into flasks containing nystatin, 10 μM (⊗——⊗); CCCP, 40 μM (■——■); HCl to give pH 3.0 (▲——▲). (○——○) also indicate TPP^+ uptake when 10 μM nystatin was added from the beginning of the experiment. The assay conditions for TPP^+ uptake were similar to those described earlier [4]. The uptake mixture contained 100 mM Tris-citrate (pH 7.5) and cells (10–15 mg dry wt/ml). The uptake measured at 30°C, was started by the addition of ^3H-TPP^+ (5 μM). At indicated times, samples were withdrawn and quickly centrifuged (10 s). The radioactivity of the supernatants was measured in the Packard Scintillation counter.

It has been shown for *R. glutinis* [4], *S. cerevisiae* [6], and for the green alga *Chlorella vulgaris* as well [9] that $\Delta\psi$ becomes depolarized with increasing H^+ concentration in the medium, due to electrogenic back-diffusion of H^+ along its electrochemical gradient into the cells [15]. Fig. 2A demonstrates a similar effect of pH on TPP^+ accumulation in *C. albicans*. The highest $\Delta\psi$ value of above −120 mV was calculated from TPP^+ accumulation at pH 8.5 whereas it dropped to about a half at pH 4.5 (Fig. 2B). The membrane potential was also sensitive to other permeant cations, such as K^+, Na^+ or Ca^{2+}. When added at 100 mM concentrations all caused a significant efflux of TPP^+ from preloaded cells (data not shown). The impermeable choline cation as well as uncharged compounds like mannitol were ineffective in inducing TPP^+ efflux.

The polyene antibiotic nystatin is known to interact with membrane sterols to form nonselective pores [16]. The sensitivity of *C. albicans* towards nystatin has already been reported [17,18]. The authors demonstrated a rapid efflux of accumulated amino acids and K^+ following the addition of nystatin. As shown in Fig. 1, 10 μM nystatin also caused a rapid and complete efflux of accumulated TPP^+ from preloaded cells. If the antibiotic was added prior to the addition of labelled TPP^+, no detectable uptake of TPP^+ was

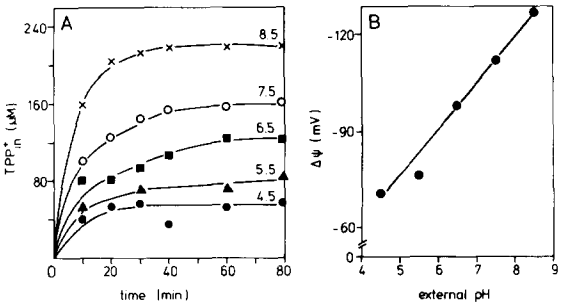

Fig. 2. Uptake of TPP^+ at different pH values. (A) The conditions described for Fig. 1 were also used for this experiment except that Tris-citrate buffer (100 mM) of different pH values was used. The values on top of each line indicate the pH value of the experiment. (B) The plot illustrates the relationship between $\Delta\psi$ and external pH.

found. Nystatin depolarizes plasmamembrane by increasing its permeability to ions. TPP^+ efflux was also observed when preloaded cells were depolarized by proton conductors (40 μM CCCP, Fig. 1) or by inhibitors of energy metabolism (data not shown). The rapid and complete outflow of accumulated TPP^+ from deenergized cells also indicated that there was no significant unspecific binding of TPP^+ to cellular or cell wall structures.

One important aspect of TPP^+ accumulation is the question whether its uptake is mediated by a carrier system. The plot of the initial influx of TPP^+ against TPP^+ concentrations (Fig. 3) was linear and non-saturable over a wide concentration range (2.5 to 600 μM). The lack of saturating tendency of TPP^+ uptake strongly indicates its nonmediated translocation into *C. albicans* cells. However, the calculation of $\Delta\psi$ from the steady-state equilibration of TPP^+ over the same concentration range revealed that the plasma membrane was gradually depolarized with increasing concentrations of TPP^+ (Fig. 3). At very high concentrations of TPP^+ (3–75 mM) the uptake kinetics deviated from linearity probably due to the participation of surface potential [19], and $\Delta\psi$ was further reduced to values about -40 mV. The

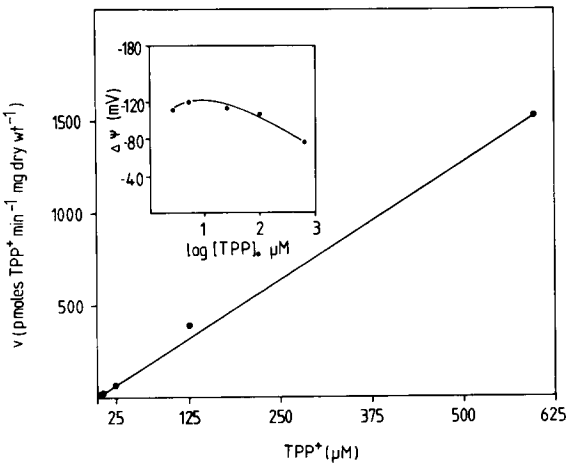

Fig. 3. Uptake of TPP^+ at different external concentrations. The conditions for TPP^+ uptake were as in Fig. 1 except that the uptake of TPP^+ was followed during its linear period of the first 10 min, at varying concentrations of unlabelled TPP^+. The inset shows the relationship between calculated $\Delta\psi$ and TPP^+ concentrations.

massive influx of positive charges at higher external TPP^+ concentrations was compensated for by a stoichiometric efflux of K^+ (data not shown).

It is concluded that TPP^+ accumulation is a quantitative measure of negative membrane potential in *C. albicans*, since: (1) It is accumulated only in energized cells. (2) Agents known to depolarize the membrane also caused rapid efflux of TPP^+ from preloaded cells. (3) TPP^+ accumulation is not mediated via a carrier. (4) TPP^+ is not intracellularly compartmentalized, e.g. in mitochondria, because agents, such as nystatin or low pH of medium caused complete efflux of the accumulated TPP^+. The mitochondrial membranes are insensitive to nystatin [20]. The calculated $\Delta\psi$ values, about -120 mV at pH 7.5 are comparable to those calculated for other yeasts by using both, direct and indirect methods [2–4,21]. In view of the controversy regarding the suitability of TPP^+ as a membrane potential probe, this report provides a further support of its usefulness in at least some eukaryotic microorganisms. It appears that the use of TPP^+ as a $\Delta\psi$ probe, greatly depends upon the permeability properties of the particular plasma membrane under investigation.

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