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Application of the liquid-membrane combined tetraphenyphosphonium-selective electrode for permeability assay of yeast *Saccharomyces cerevisiae* cells

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A new liquid membrane combined electrode was designed and applied for an assay of tetraphenylphosphonium cation (TPP^+) accumulation in yeast cells. A combination of reference (Ag/AgCl) and TPP^+ electrode demonstrated an extreme sensitivity (10^{-7} M) and simplicity in use, moreover, a small volume (300 μl) of the test suspension was needed. The method suggested by us was applied to study permeability properties of the yeast *Saccharomyces cerevisiae* strains having a defective cell wall architecture.

Key words: ion-selective electrodes (ISE), lipophilic cations (LC), tetraphenylphosphonium (TPP^+), *Saccharomyces cerevisiae*

Abbreviations: TPP^+, tetraphenylphosphonium ion; TPB, tetraphenyl borate; PVC, polyvinyl chloride; DOF, dioctyl phthalate; DDF, dodecyl phthalate; LC, lipophylic cation; LMCE, liquid membrane combination electrode; ISE, ion selective electrode

INTRODUCTION

The measurement of the electrical potential difference ($\Delta\psi$) is central to the analysis of energy conversion processes in mitochondria, chloroplasts, bacteria cells, yeast and other systems in which proton pumps play a primary role in the energy conversion processes [1].

The polymer membrane ion-selective electrodes (ISE), known for already 30 years, are a well-established tool for determination of many inorganic and organic ions [2]. Potentiometry using an ion-selective electrode sensitive to lipid soluble cations has been applied for the estimation of membrane potential and the permeability of organisms, cells, organelles, membrane vesicles and liposomes [1, 3]. Lipophilic cations (LC) such as tetraphenylphosphonium (TPP^+) or fluorescent dyes are frequently used as probes for the membrane potential ($\Delta\psi$) measuring of prokaryotic and small animal cells [3, 4].

The poly(vinyl chloride) (PVC) based TPP^+ selective membrane electrode was developed by Naoki Kamo [5] in 1979, and since then it has been widely used as a TPP^+ concentration estimation instrument. The construction of such TPP^+ electrode gives a possibility to estimate TPP^+ in the micromolar concentrations in aliquots with the volume exceeding one millilitre.

The last limitation comes with the necessity of the external reference electrode in the estimation system. Moreover, further investigations showed that the TPP^+ concentrations overrunning 1 μM inhibit the functions of mitochondrial and other cells, organelles [6, 7].

The aim of our study was to improve the electrode construction by suggesting a combinative system free from the above-mentioned limitations.

We present here the preparation of the liquid membrane combination electrode (LMCE) with an interchangeable TPP^+ selective module and the properties of this combination. The electrode was applied for measuring the permeability of *Saccharomyces cerevisiae* strains having a defective cell wall architecture.

EXPERIMENTAL

Reagents. All reagents used in the preparation of the electrode and the solutions were of analytical grade. DOF, PVC, TPBNa, THF were of Selectophore grade from Fluka.

Preparation of the electrode. The electrode body was made from a polypropylene tube (Ø6 mm × × 100 mm) with an Ag/AgCl reference electrode placed in the body (Fig. 1). The liquid junction was made as a glass fibre thread over a silicone plastic layer. The polypropylene-bodied ion selective module (ISM) socket had a cone-shaped form.

The copper screw junction was fixed on the top of the cone. The ion-selective module (ISM) had PVC-bodied construction with a screw junction fused in copper. The cone-shaped ISM outer surface was precisely polished to fit the LMCE module socket inner surface in the process of insertion. The ISM inner Ag/Ag element was soldered with a screw junction to contact with the outer back seals. The ISM was filled with a solution of 10^{-2} M TPPCl in glycerol.

ISM membranes were prepared by mixing 650 mg dodecyl phthalate and 350 mg PVC in 4 ml of freshly distilled tetrahydrofuran (THF) saturated with a TPP^+TPB^- ion-pair complex. The THF was slowly evaporated at room temperature on a flat Petri dish. A piece of ion selective membrane was glued on the tip of ISM with tetrahydrofuran.

Yeast strains and cultivation. The following *Saccharomyces cerevisiae* strains were used in this study: SEY6210 (*MATa, leu2-3, ura3-52, his3-Δ200, lys2-801, trp-Δ901, suc2-Δ9*) as a parental strain, SEY6210 ΔKre1 (*MATa, leu2-3, ura3-52, his3- Δ200, lys2-801, trp-Δ901, suc2-Δ9, ΔKre1::HIS*), XCY42-30D (*MATa, ade2-101, adex, ura3, trp1, lys2, leu2-3,112, Dmnn1::LEU2).* The strains were kindly gifted by A. Meškauskas, Vilnius. The yeast cells were grown in a complete YEPD medium (1% yeast extract, 2% peptone (Difco, USA), 2% glucose) at 30 °C on a reciprocal shaker at 150 rpm to the logarithmic growth phase.

Tetraphenylphosphonium accumulation measurements. The yeast cells were washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.8) two times, concentrated 200 times in the same buffer, TPPCl was added to the final concentration 3.10^{-7} M. After 30 min of incubation at 30 °C the yeast cells were precipitated and the supernatant was used for measuring the residual TPP^+ concentration. 100 µl of the supernatant was added to 200 µl of TE buffer (with $3{\cdot}10^{-7}$ M TPPCl), where a TPP^+ selective combined electrode was immersed. The electrode potential drift was estimated with a Hanna pH213 ion meter in magnetically stirred solution and the yeast-absorbed quantity of TPP^+ was calculated. The protein concentration was determined by the Lowry method [8].

RESULTS AND DISCUSSION

We prepared a number of TPP^+ ISM where the composition of the ion-selective membrane varied. The best result was obtained with the membrane described in Materials and Methods. The TPP^+ selective ISM with the glycerol-based internal solution was more stable. Besides, we preferred dodecyl phthalate (DDP) in the preparation of TPP^+ selective electrodes instead of usually used dioctyl phthalate. The prepared membrane was transparent and 0.3 mm thick. The ISM with the membrane proposed by us showed an electrode response of 56 mV/decade in the range 10^{-7}–10^{-5} M TPP^+ concentration (Fig. 2).

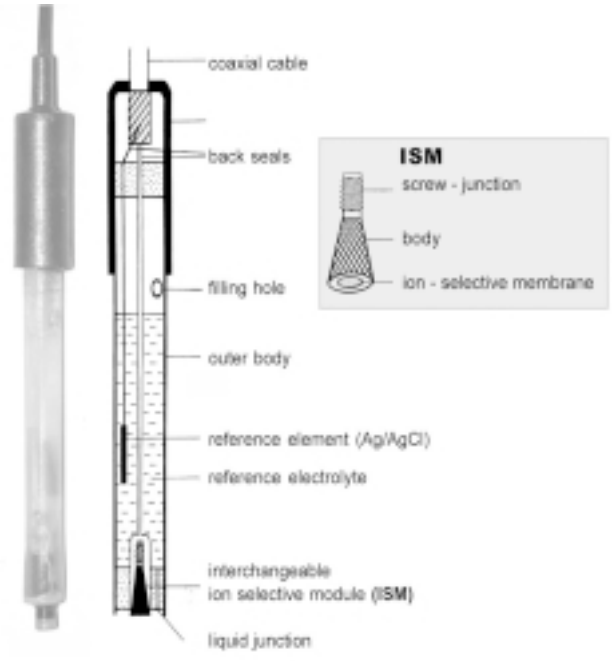

Fig. 1. Schematic view of the LMCE and ISM

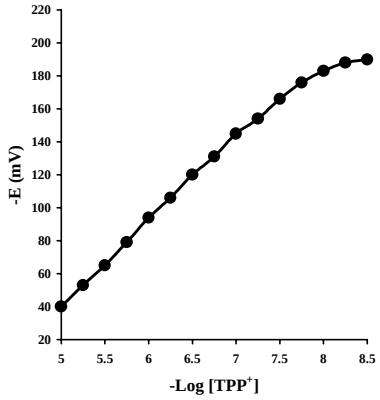

Fig. 2. Calibration graph for LMCE with TPP^+ selective module in a solution containing 0.9% NaCl. Electromotive force (E) is plotted against logarithmic ion concentration

Electrode characteristics. All characteristics of this electrode are summarised in Tables 1 and 2. The electrode was stored in 10^{-6} M of TPPCl solution through all studies.

Table 1. **Selectivity coefficients measured by the fixed interference method [10] (expressed as -log $_{TPP, X}$ =pk$_{TPP, X}$)**

Interference (X)	pk $_{TPP, X}$
TrisHCl	–6.6
ATPNa	–5.3
ADPNa	–4.6
EDTANa	–5.2
Na_2HPO_4	–5.6
$MgCl_2$	–5.6
Sucrose	–5.0
SuccinateK	–5.7
TricineHCl	–6.6

Table 2. **Specifications of TPP+ selective module (ISM) for LMCE**

Parameter	Range
TPP^+ measurement range	10^{-5}–10^{-7} (M)
Temperature range	0–60 °C
pH range	4–10
Approx. slope at 20 °C	56 mV/decade
Electric resistance	20 Mohm
Response time	1.5 min (for 10^{-7} M TPP^+)
	1 min (for $5 \cdot 10^{-7}$ M TPP^+)
Minimal volume	0.2 ml

The space-saving construction of the electrode made it possible to decrease the estimation probe volume to 300 µl. The electrode response time, temperature and pH ranges corresponded to the previously described parameters [5, 9].

The presence of any substances in a sample solution can interfere detection of changes in TPP^+ concentration. The selectivity coefficients of the electrode were measured by the fixed interference method [10] and are presented in Table 1. It can be concluded that the electrode was highly selective for various substances usually contained in the incubation medium of cells and mitochondria.

Yeast permeability estimation. LMCE with TPP^+ ISM was applied to evaluate the permeability properties of yeast *S. cerevisiae* strains with a defective wall structure. According to Slayman, TPP^+ do not distribute to equilibrium with plasma membrane voltage in intervals of minutes in intact *S. cerevisiae* cells. To reach the steady-state, from 0.5 up to 2 hours for some strains are needed [7]. We observed a maximum of accumulated TPP^+ after 30 min of incubation; continuous incubation did not cause a significant increase in the amount of accumulated TPP^+.

The concentration of TPP^+ was estimated in the supernatant where yeast cells were incubated (Fig. 3).

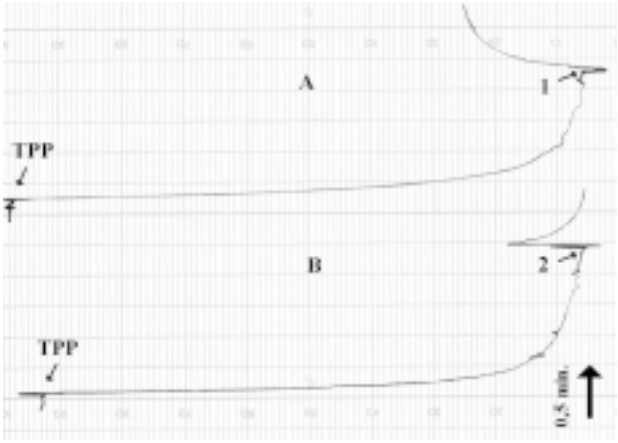

Fig. 3. TPP^+ uptake by *S. cerevisiae* cells represented as decrease in TPP^+ concentration after addition of cell incubation medium.
Arrows 1 and 2 indicate the time when 100 µl of samples were added.
A: supernatant of yeast cells after 30 min incubation with TPP^+;
B: control aliquot of the medium without incubation with yeast cells.
Calibration was performed by increasing TPP^+ concentration from $2 \cdot 10^{-7}$ M to $3 \cdot 10^{-7}$ M.

The amount of the accumulated TPP was different and related to the properties of the cell wall structure. In *S. cerevisiae*, the cell wall contains β(1–3)-D-glucan, β(1–6)-D-glucan, chitin, and mannoproteins. All the four major components are linked together [11, 12]. On the external surface of the wall there are mannoproteins, which are extensively O- and N-glycolysated. They are densely packed and influence wall permeability. The layered structure of the cell wall, being a general phenomenon in yeast, modifies the surface properties such as hydrophobicity, electrical charge, sexual agglutinability, and porosity [13, 14].

Table 3. **Accumulation of TPP^+ by different *Saccharomyces cerevisiae* strains**
Results presented as moles of TPP^+ per milligram of yeast protein · 10^{-12}. The values are presented with the average of standard errors of five independent experiments

Strains	Accumulated TPP^+ per milligram of protein · 10^{-12}	Per cent to SEY6210 strain
SEY6210 (control)	7.1 ± 0.4	100
SEY6210 (*kre1*)	12.4 ± 1.3	173
XCY42–30D (*mnn1*)	8.1 ± 0.6	113

The results presented in Table 3 show that the yeast strain SEY6210*(kre1)* absorbed TPP^+ 1.7 times more effectively than the parental strain. Strain SEY6210*(kre1)* has a reduced (60%) level of alkali-insoluble cell wall β(1–6)-glucan [12]. Thus, β(1–6)-glucan is the central molecule that keeps together the other components of the cell wall, including β(1–3)-glucan, mannoprotein, and part of chitin. It is not surprising that defects in β(1–6)-glucan formation can interfere with cell wall assembly and have severe effects on cell permeability and accumulation of TPP cations. A moderate increase in TPP^+ accumulation was observed in the XCY42–30D(*mnn1*) strain with a mutation involved in N- and O-glycosylation of mannoproteins. Our data are in good agreement with the data of De Nobel et al. showing that the external protein layer, the N-linked side-chains of mannoproteins in particular, determines the permeability of the yeast cell wall [14].

CONCLUSIONS

High selectivity coefficients and rather low concentrations (<1 μM) of LC are particularly actual in the estimation of the permeability and membrane potential of biological objects. The results presented above have shown that the proposed LMCE selective for TPP^+ can be successfully introduced for evaluation of the structure of yeast cell envelope. We think that the advantages of the compact construction of the electrode presented by us will allow for its extensive use for the estimation of membrane potential and for medicinal investigations as well, when human biopsy tissue mitochondria will be the estimation target.

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KOMBINUOTO SKYSTAMEMBRANINIO TETRAFENILFOSFONIUI SELEKTYVAUS ELEKTRODO PANAUDOJIMAS TIRIANT MIELIŲ *SACCHAROMYCES CEREVISIAE* LĄSTELIŲ LAIDUMĄ

S a n t r a u k a

Pristatoma naujo skystamembraninio kombinuoto elektrodo konstrukcija. Kombinuotas elektrodas, susidedantis iš palyginamojo (Ag/AgCl) ir lipofiliniams tetrafenilfosfonio (TPP^+) jonams jautraus potenciometrinio elektrodo, tyrimų metu buvo jautrus ir patogus. Šitokia konstrukcija įgalino atlikti TPP^+ koncentracijos matavimus mažuose mėginio tūriuose (300 mikrolitrų) naudojant mažas skvarbaus jono koncentracijas ($3 \cdot 10^{-7}$ M). Šio elektrodo dėka buvo registruotas įvairių mielių *Saccharomyces cerevisiae* kamienų gebėjimas absorbuoti TPP^+ jonus. Nustatyta, kad sukaupto TPP^+ kiekis priklauso nuo ląstelės sienelėje esančių defektų.