Evidence for Interactions between the Energy-Dependent Transport of Sugars and the Membrane Potential in the Yeast *Rhodotorula gracilis* (*Rhodosporidium toruloides*)

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Received 20 March 1978; revised 5 June, 1978

Summary. A membrane potential (inside negative) across the plasma membrane of the obligatory aerobic yeast *Rhodotorula gracilis* is indicated by the intracellular accumulation of the lipid-soluble cations tetraphenylphosphonium and triphenylmethylphosphonium. The uptake of these ions is inhibited by anaerobic conditions, by uncouplers, by addition of diffusible ions, or by increase of the leakiness of the membrane caused by the polyene antibiotic nystatin. The membrane potential is strongly pH-dependent, its value increasing with decreasing extracellular proton concentration. Addition of transportable monosaccharides causes a depolarization of the electrical potential difference, indicating that the H\(^+\)-sugar cotransport is electrogenic. The effect on the membrane potential is enhanced by increasing the sugar concentration. The half-saturation constants of depolarization for D-xylose and D-galactose were comparable to those of the corresponding transport system for the two sugars. All agents that depressed the membrane potential inhibited monosaccharide transport; hence the membrane potential provides energy for active sugar transport in this strain of yeast.

Monosaccharides are actively transported into cells of *Rhodotorula gracilis* (Kotyk & Höfer, 1965; Höfer & Kotyk, 1968). Uptake is coupled to an influx of protons (Misra & Höfer, 1975), which always exhibits a stoichiometry of 1:1 (Höfer & Misra, 1978). Similar observations have been reported for bacteria (West & Mitchell, 1973; Deshusses & Reber, 1977; Lagarde & Haddock, 1977), algae (Komor & Tanner, 1976), fungi (Seaston, Inkson & Eddy, 1973; Slayman & Slayman, 1974), and higher plants (Giacquinta, 1977; Komor, Rotter & Tanner, 1977; Racusen & Galston, 1977). Hence, as West and Mitchell (1972) have suggested, the uptake of protons coupled to the transport of nonelectrolytes may be the means by which energy is usually supplied for transporting uncharged substrates. According to Mitchell, the free energy produced by
the transport of protons along the gradient of their electrochemical potential drives the transport of substrates uphill. So the symport of sugars with protons would tap the energy stored (i) in the pH gradient and (ii) in the membrane potential. Such a pH gradient (inside alkaline) across the plasmalemma of *Rh. gracilis* and its utilization by monosaccharide transport has been found by Höfer & Misra (1978). The second component of the electrochemical proton gradient, the membrane potential, is shown here to exist across the plasmalemma of *Rh. gracilis* and to be used in transporting monosaccharides.

**Materials and Methods**

**Growth**

The obligatory aerobic yeast *Rhodotorula gracilis* (*glutinis*) ATCC 26194 and CBS 6681 (*Rhodosporidium toruloides*, mating type a) was grown as described previously (Misra & Höfer, 1975). The cells (inoculum 2 x 10⁶ cells/ml of growth medium) were harvested in the stationary phase after 24 hr (yield approximately 10⁸ cells/ml) and aerated for 6 hr before use as a 5% suspension (wt/wt/vol). In all experiments involving uptake of lipid-soluble cations, the suspension was centrifuged at 5000 x g after aeration for 6 hr and the pellet was stored at 4 °C overnight. After resuspension, the cells were aerated for 2 hr before use.

**Uptake of Sugars**

Transport of D-xylose was measured by the membrane filter technique as described by Heller & Höfer (1975). D-galactose transport was studied by monitoring the activity of ¹⁴C-labeled substrate in the medium as for the lipid-soluble cations (see below).

**Uptake of Lipid-Soluble Cations**

For all experiments, the yeast was suspended in Tris/citric acid buffer (appropriate amounts of 0.3 M Tris with 0.3 M citric acid), to give 4% wet wt of yeast/vol. The experiment was started by adding ³H-tetraphenylphosphonium (TPP⁺) or ³H-triphenylmethylphosphonium (TPMP⁺). Samples of 0.4 ml were withdrawn and centrifuged for 12 sec at 15,000 x g in an ECOO-Quick centrifuge (Collatz, Berlin, Germany). 0.2 ml of the supernatant was mixed with 10 ml toluene/Triton X 100/ethanol scintillation fluid (32:16:3), containing 0.6% 2,5 diphenyloxazole and 0.06% 1,4-bis-(5-phenyloxazol-2-yl)benzene and counted in a Packard 3380 liquid scintillation counter. All cpm values were corrected for quenching effects by the use of an external standard and the channel ratio method (Brewer, Pesce & Ashworth, 1974). The decrease of radioactivity in the supernatant was used as a measure of cations taken up by the cell.

**Calculation of the Membrane Potential**

The membrane potential was estimated by inserting the intra- and extracellular concentrations of the lipid-soluble cations into the Nernst equation. The intracellular concentration...