Nano-Osmotic Coupling in Active Cell Membrane Water Permeability

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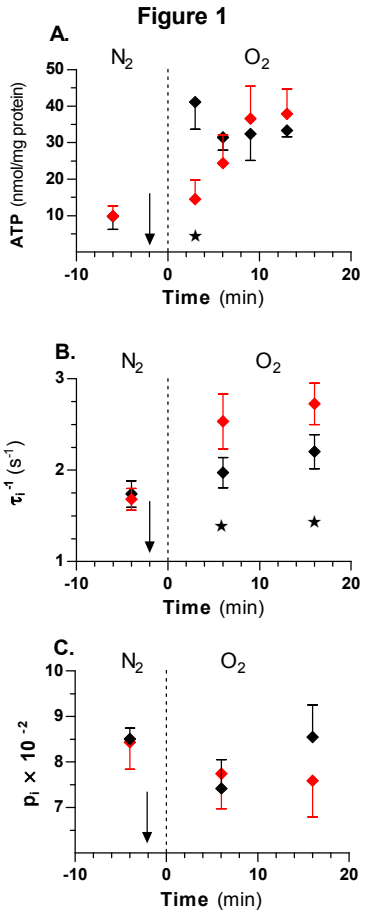
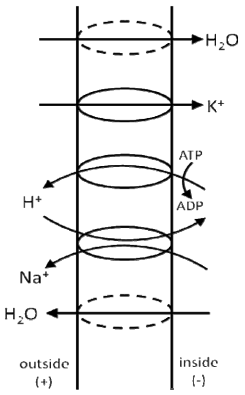
Introduction: It is possible to estimate the mean tissue intracellular water molecule lifetime (τ_i) *in vivo* from minimally invasive (Dynamic-Contrast-Enhanced) DCE-MRI data [1, 2]. The reciprocal, τ_i^{-1}, is the (pseudo) first-order rate constant for (reversible, equilibrium) cellular water efflux, k_{io}. This parameter is averaged over a region-of-interest or, if S/N permits, an image voxel. Parametric τ_i maps of a human MS lesion [3], osteosarcoma [3], malignant breast [3, 4], and prostate (*submitted*) tumors have been reported. The τ_i can be measured with greater precision in cell suspension NMR experiments (reviewed in [1]), which also allow manipulations to elucidate the factors determining the τ_i magnitude. Studies of yeast cells have shown that τ_i is inversely dependent on the cellular ATP level [5]: *i.e.*, equilibrium water exchange may have an active component. The τ_i quantity is inversely related to the cell membrane water permeability coefficient, P_W; $P_W = \tau_i^{-1}$ (V/A), where V and A are the individual cell volume and surface area values [1, 2]. Thus, a component of P_W may be active [5]. Net trans-cell membrane water transport is driven by osmotic gradients. Many osmolytes are ions. The major active ion transport protein of the yeast cell membrane is the P-type H^+-ATPase (PMA). It pumps H^+ out of the cell and establishes the membrane potential using ATP hydrolysis. Since much other membrane transport is driven directly or indirectly by PMA activity, this could explain a τ_i correlation with cellular ATP concentration. To test whether PMA activity is linked to τ_i^{-1}, anaerobic (N_2) yeast cell suspensions were treated with ebselen ((2-phenyl-1,2-benzisoselenazol-3(2H)-one), **EBS**), a PMA inhibitor [6], and then energized by changing to aerobic (O_2) conditions.

Methods: *Saccharomyces cerevisiae* cells were suspended in 4.04 mM $MgSO_4$, 13.4 mM KCl (pH = 6.24), and 9.3 mM $GdDTPA^{2-}$, a contrast reagent, at 30% cell density. ^1H_2O NMR inversion recovery (IR) data sets comprising 64 delay measurements were conducted at 9.4T (Varian Inova). The IR time-courses were fitted using a two-site-exchange (2SX) model [1, 7] with two adjusted parameters: τ_i and p_i, the mole fraction of water that is intracellular. ATP was measured in separate studies by HPLC. Switching yeast suspension bubbling gas from N_2 to O_2 causes a cellular ATP increase and a τ_i decrease [5]. EBS in the solvent DMSO (EBS) or DMSO (Control) was added as indicated; the final EBS concentration was 3 mM.

Results: **Figure 1** displays the averaged results (error bars; SDs) from suspensions of **Control** (n = 4; **red**) yeast or yeast with **EBS** (n = 5; **black**) added 2 min (arrow) before O_2 (dashed vertical line at t = 0). The ATP content of the Control and EBS treated yeasts was equal at all times (panel A), except immediately after the change to O_2 (t = 3 min) when EBS treated yeast ATP was increased relative to that of the Control yeast. The Control yeast suspension τ_i^{-1} increased 6 min after O_2 (panel B). The equilibrium water exchange rate increased. In the EBS treated yeast suspensions τ_i^{-1} did not increase as much. It did not increase at 6 min (p = 0.65), but did by 16 min (relative to N_2, t = –4 min). Control yeast τ_i^{-1} values were greater than those of the EBS group at both times (★). Thus, EBS treatment suppressed the τ_i^{-1} increase with increased ATP (O_2). We examined p_i (panel C). The p_i initially decreased after O_2 in the EBS cells but it was not different from Control cells at 6 min. Although p_i trended up at 16 min in the EBS cells, again it was not different in Control and EBS cells. Thus, p_i in Control and EBS cells was similar while τ_i^{-1} increased in Control but not EBS cells at these times. Since V/A will follow p_i, we conclude that P_W increases with initial O_2 in Control but not EBS yeast. Although there is a suggestion of a p_i decrease (Fig. 1C), the changes in Control and EBS cells are comparable immediately following O_2. This indicates that the net H_2O efflux (p_i decrease), which occurs with the onset of respiratory activity, is independent of PMA activity and may reflect other osmolyte movement (perhaps HCO_3^- or H_2CO_3).





Discussion: Interestingly, it appears that EBS PMA inhibition reduces ATP utilization enough to transiently increase (at 3 min) cellular ATP (Fig. 1A). However, within 6 minutes after the change to O_2, ATP was equal in Control and EBS yeast. Inhibition of PMA activity inhibits the O_2-induced τ_i^{-1} increase (Fig. 1B) but not the ATP increase (Fig. 1A). This correlation of membrane ion pumping activity with equilibrium water exchange may reflect that water cycling is osmotically-coupled to equilibrium ion transport processes (ion cycling) that are net non-osmogenic [8]. The **cartoon** suggests how the known PMA, Na^+/H^+ exchanger, and unidirectional K^+ channel transporters function together to pump Na^+ ions out of and K^+ ions into the cell against their electrochemical potentials. It is known that there is water flux associated with the selective ion flux of individual transporters because of nano-scale osmotic coupling [8]. Putative water transporters (dashed ovals) are drawn near the K^+ channel and the Na^+/H^+ exchanger. These can represent: a) water transport through the actual cation channels, b) water channel proteins directly associated with the ion channel proteins, and/or c) independent water channel proteins that are near the ion channel proteins on the nano-scale. These results therefore implicate PMA in these processes, and help elucidate the molecular contributions to yeast cell membrane P_W. It may be that τ_i^{-1} reflects such cellular membrane metabolic activity in many physiological systems and could change in pathophysiology.

Grant Support: NIH: R01-HL078634, R01-EB00422, R01-NS40801.

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