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VDAC electronics: 4. Novel electrical mechanism and thermodynamic estimations of glucose repression of yeast respiration

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ABSTRACT

Inhibition of cell respiration by high concentrations of glucose (glucose repression), known as "Crabtree effect", has been demonstrated for various cancerous strains, highly proliferating cells and yeast lines. Although significant progress in understanding metabolic events associated with the glucose repression of cell respiration has been achieved, it is not yet clear whether the Crabtree effect is the result of a limited activity of the respiratory chain, or of some glucose-mediated regulation of mitochondrial metabolic state. In this work we propose an electrical mechanism of glucose repression of the yeast *S. cerevisiae*, resulting from generation of the mitochondrial outer membrane potential (OMP) coupled to the direct oxidation of cytosolic NADH in mitochondria. This yeast-type mechanism of OMP generation is different from the earlier proposed VDAC-hexokinase-mediated voltage generation of cancer-type, associated with the mitochondrial outer membrane. The model was developed assuming that VDAC is more permeable to NADH than to NAD^+. Thermodynamic estimations of OMP, generated as a result of NADH(2-)/NAD^+(1-) turnover through the outer membrane, demonstrated that the values of calculated negative OMP match the known range of VDAC voltage sensitivity, thus suggesting a possibility of OMP-dependent VDAC-mediated regulation of cell energy metabolism. According to the proposed mechanism, we suggest that the yeast-type Crabtree effect is the result of a fast VDAC-mediated electrical repression of mitochondria due to a decrease in the outer membrane permeability to charged metabolites and owing their redistribution between the mitochondrial intermembrane space and the cytosol, both controlled by metabolically-derived OMP.

1. Introduction

For at least half a century, it has been known that yeast and plant mitochondria have a pathway of direct oxidation of external NADH [1–4], in contrast to mammalian mitochondria (see [5] and references therein). The excess of NADH, produced in the cytosol of the yeast *Saccharomyces cerevisiae* under aerobic conditions, is conveyed to the mitochondrial respiratory chain through the two most important channeling systems [6–8]: 1) the inner membrane external NADH dehydrogenase(s) (DH_e), facing the NADH-binding center to the mitochondrial intermembrane space (MIMS), and 2) the glycerol 3-phosphate shuttle, in which cytosolic NADH is used to convert dihydroxyacetone phosphate into glycerol 3-phosphate subsequently oxidizing by corresponding dehydrogenase located on the external surface of the mitochondrial inner membrane. NAD^+, produced during oxidation of cytosolic NADH by DH_e, returns into the cytosol trough the mitochondrial outer membrane (MOM) to recover NADH in a steady state process. This external pathway of NADH oxidation in *S. cerevisiae* mitochondria is additional to the internal oxidation of NADH produced in the matrix, catalyzed by the matrix rotenone-insensitive Ndi1 oxidoreductase transferring electrons to ubiquinone of the respiratory chain [9].

The possibility exists that the turnover of NADH and NAD^+, natural organic anions with the net charges of 2- and 1-, respectively, through the voltage-dependent anion channel (VDAC) of MOM is coupled to the generation of the mitochondrial outer membrane potential (OMP), if VDAC permeability to these pyridine nucleotides is different. It has been reported that NADH, in comparison to NAD^+, generates significantly higher VDAC current noise, which suggests that NADH has higher binding affinity to the adenine nucleotide binding site(s) located inside the channel [10]. According to recent theoretical considerations [11], a molecule should electrostatically and allosterically match a constriction zone in order to pass the entropy barrier inside the channel, facilitating translocation through VDAC. Even weak binding of ATP to several low-affinity adenine nucleotide binding sites inside the channel has been shown to increase VDAC permeability to ATP at least one

Abbreviations: VDAC, the voltage-dependent anion channel; OMP, the mitochondrial outer membrane potential; MOM, the mitochondrial outer membrane; MIMS, the mitochondrial intermembrane space; DH_e, the external NADH dehydrogenase(s) of the mitochondrial inner membrane

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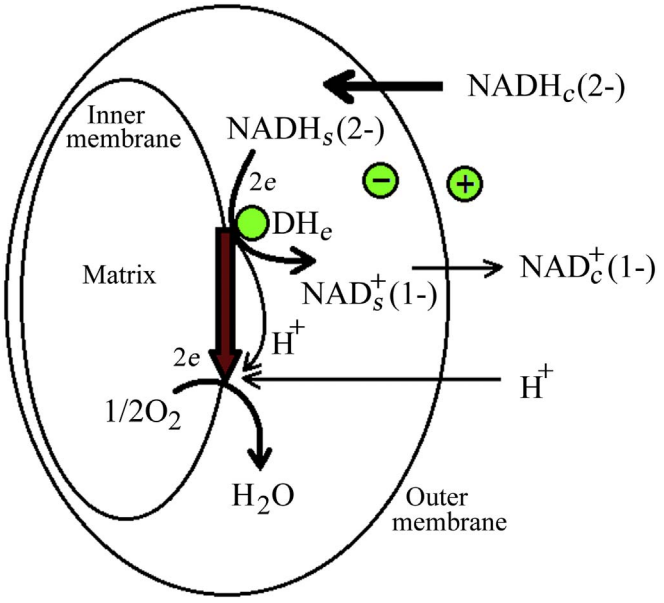

Fig. 1. Possible mechanism of OMP generation in mitochondria having the external pathway of NADH oxidation. DH_e – the inner membrane external NADH dehydrogenase (s), red arrow – the respiratory chain.

order of magnitude [12].

The mentioned above experimental data and theoretical works allow assumption that VDAC permeability to NADH is higher than to NAD^+. In this case, the steady state process of external NADH oxidation in MIMS of yeast *S. cerevisiae* mitochondria could be coupled to generation of negative OMP (Fig. 1), thus directly modulating the flux of other metabolites between the cytosol and mitochondria, such as ATP, ADP, glycerol 3-phosphate, etc.

The mitochondrial VDAC has been earlier assumed to be responsible for the inhibition of yeast respiration by glucose, because aluminum hydroxide, known to prevent electrical closure of VDAC, strongly reduced this effect in cells [13]. Detailed study of *S. cerevisiae* mitochondrial metabolism allowed suggestion that the movement of NADH, ADP and other metabolites between the cytosol and mitochondria in permeabilized cells is limited by a low permeability of MOM to these metabolites, and that this diffusion barrier is due to the opening-closing of VDAC channels [13–15]. Moreover, the ability of NADH to decrease the MOM permeability of potato tuber mitochondria to ADP (due to an interaction of NADH with VDAC, increasing the VDAC voltage sensitivity) has been suggested as a possible factor contributing to the Crabtree effect [16].

On the other hand, according to many authors, the mechanism of the Crabtree effect is not clear yet [17–19], because it is not even known whether this metabolic phenomenon results from a limited respiratory capacity of mitochondria, or from some mechanism of glucose-mediated inhibition of mitochondrial respiratory activity [20–22].

We have suggested earlier a possible explanation of the Crabtree and Warburg effects in cancer cells as a result of electrical suppression of mitochondria via direct metabolically-dependent generation of OMP by the VDAC-hexokinase complexes of MOM [23–27], thus controlling metabolite flux through free (not bound to hexokinase) VDACs. However, such an explanation of the Crabtree effect cannot be applied to yeast *S. cerevisiae*, because yeast mitochondria seem to not bind yeast hexokinase (for review see [28,29]). At least, the hexokinase binding capacity of yeast mitochondria, reported by some authors [30,31], is several orders of magnitude less than that of rat liver mitochondria [28,29] or cancer cell mitochondria with respect to corresponding hexokinases (for references see [24,27]). Moreover, the deletion of hexokinase II does not convert *S. cerevisiae* into a Crabtree-negative cell [32].

Alternatively, the possibility exists that the yeast *S. cerevisiae* might have another mechanism of the Crabtree effect, taking into account that NADH is produced in excess in the cytosol of these cells at high levels of glucose [8]. The direct oxidation of cytosolic NADH by mitochondria might be coupled to the generation of OMP, thus modulating the VDAC-mediated MOM permeability. In this case, OMP generated due to the NADH(2-)/NAD + (1-) turnover through MOM might represent a new metabolically-dependent electrical mechanism controlling the metabolic state of mitochondria and cells. That is why thermodynamic estimations of such a possibility represent certain interest.

In the present work, we propose the mechanism of OMP generation coupled to the direct steady-state oxidation of cytosolic NADH in yeast mitochondria. This concept was supported by thermodynamic estimations performed on the basis of a mathematical model and is consistent with many experimental observations reported in the literature. The calculated values of metabolically-derived OMP match the known range of the VDAC voltage sensitivity, thus showing a high probability of OMP-dependent VDAC-mediated regulation of the cell energy metabolism. The model also suggests explanations of various metabolic effects caused by VDAC knockdown, VDAC deficiency or inhibition by natural or artificial modulators of the VDAC permeability and VDAC voltage sensitivity. Specifically, we suggest that at least the "short-term" Crabtree effect in the yeast *S. cerevisiae* cells results from an electrical repression of mitochondrial respiration by glucose due to the NADH-dependent generation of OMP. This hypothetical mechanism is different from the recently proposed generation of OMP by the VDAC-hexokinase complexes of MOM in cancer cells [24,25,27], although cancer-type and yeast-type Crabtree effects might both have an electrical origin.

2. Materials and methods

The known external pathway of NADH oxidation in yeast mitochondria is schematically presented in Fig. 1, showing possible charge separation through MOM. The activity of DH_e, catalyzing irreversible reaction of oxidation of external NADH in mitochondria, depends on the concentration of NADH in MIMS as:

$$v = \frac{v_m \cdot [NADH]_s}{K_{m,NADH} + [NADH]_s}, \quad (1)$$

where, v_m is the maximum activity of DH_e, $[NADH]_s$ is the MIMS concentration of NADH. The Michaelis-Menten constant of DH_e was taken at $K_{m,NADH}$ = 50 μM NADH [33,34]. The rates of oxidations v_m and v, as well as the metabolite flux NADH/NAD^+ through MOM were presented in arbitrary units (a.u.).

The flux of NADH(2-) through MOM (J_{NH}) may be described by Goldman equation:

$$J_{NH} = P_{NH} \cdot \frac{\Delta\psi_o \cdot 2F}{RT} \cdot \frac{[NADH]_c - [NADH]_s \cdot \exp\left(\frac{-\Delta\psi_o \cdot 2F}{RT}\right)}{1 - \exp\left(\frac{-\Delta\psi_o \cdot 2F}{RT}\right)}, \quad (2)$$

Here, $\Delta\psi_o$ is OMP, P_{NH} is the VDAC-mediated permeability of MOM to NADH(2-), F is the Faraday constant, R is the universal gas constant, $T = 298\,K$ is the incubation medium temperature, $[NADH]_c$ and $[NADH]_s$ are NADH concentrations in the cytosol and MIMS, respectively.

Similarly, the flux of NAD^+(1-) through MOM (J_N) is presented as:

$$J_N = P_N \cdot \frac{\Delta\psi_o \cdot F}{RT} \cdot \frac{[NAD^+]_c - [NAD^+]_s \cdot \exp\left(\frac{-\Delta\psi_o \cdot F}{RT}\right)}{1 - \exp\left(\frac{-\Delta\psi_o \cdot F}{RT}\right)}, \quad (3)$$

where P_N is the VDAC-mediated permeability of MOM to NAD^+(1-); $[NAD^+]_c$ and $[NAD^+]_s$ are NAD^+ concentrations in the cytosol and MIMS, respectively.

The dependence of P_{NH} on OMP ($\Delta\psi_o$) was presented