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Mediated Electrochemical Measurements of Intracellular Catabolic Activities of Yeast Cells

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Abstracts: Coupling with the dual mediator system menadione/ferricyanide, microelectrode voltammetric measurements were undertaken to detect the ferrocyanide accumulations arising from the mediated reduction of ferricyanide by yeast cells. The results indicate that the dual mediator system menadione/ferricyanide could be used as a probe to detect cellular catabolic activities in yeast cells and the electrochemical response has a positive relationship with the specific growth rate of yeast cells.

Keywords: Catabolism, intracellular, microelectrode, votammetric, mediator, yeast cells.

Mediated intracellular redox activities have been used in several fields, such as BOD measurement[1], viable cell population determinations[2] and single cell imaging[3]. Many oxidant electroactive substances act as mediators such as ferricyanide and menadione, they performed the same functions as shuttling electrons from intracellular reducing equivalent produced by metabolic reactions or directly from the respiratory chain to the cell surface. Double mediator system comprising lipophilic and hydrophilic mediators was often used for the electrochemical detection of intracellular redox activity in eukaryotic cells. The function of the lipophilic mediator menadione was to shuttle electrons from intracellular redox sites to the cell surface for reaction with the hydrophilic mediator ferricyanide (**Figure 1**). Menadione accepts electrons from cytosolic and mitochondrial enzymes catalyzing electron transfer from NAD(P)H to quinone substrates[4]. Menadione mediates the intracellular redox activities relating to all NAD(P)H-dependent dehydrogenases both in cytoplasm (glycolysis) and in mitochondria (citric acid cycle and respiration chain) of living cells, so menadione/ferricyanide system could be used as a probe to detect the global catabolic activity within yeast cells. The selected species were *S. cerevisiae* CBS 1200, *C. shehatae* CBS 2779, hoping that differences in their behavior might be useful to get a deeper insight into the mediated intracellular redox detection and the relationship between electrochemical response and the specific growth rate of yeast cells.

The yeasts were cultured aerobically in shake flasks at 30 ºC and 200 rpm using the growth medium and fermentation conditions as previously described[1]. Growth was monitored gravimetrically[1]. For electrochemical measurements, growth proceeded to

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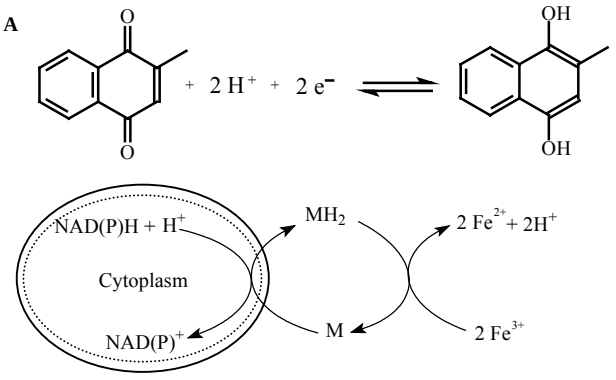
allow the cells growth to their respective stationary phase, after which the broth was centrifuged at 10,000 rpm for 10 min the cell pellet was washed twice in phosphate buffer (PB, 0.05 mol/L K_2HPO_4/KH_2PO_4, pH 6.0) and suspended with phosphate buffered saline solution (PBS, 0.05 mol/L K_2HPO_4, 0.05 mol/L KH_2PO_4 and 0.1 mol/L KCl, pH 7.0). Cells density was adjusted to an optical density at 600 nm (OD_{600}) of 9.0 using a spectrophotometer. The treated cells were used immediately in the following experiments.

Figure 1 Detection of cellular redox reactions with use of menadione to link intra- and extracellular redox pairs.



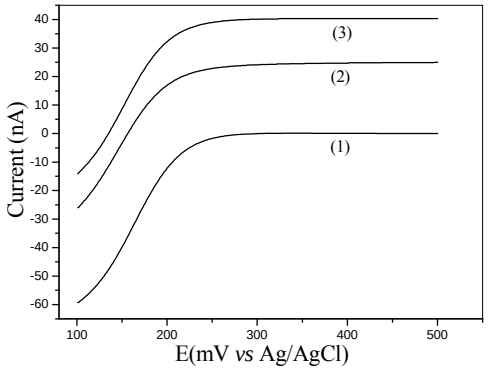
(A) Two-electron reduction of menadione to menadiol. (B) Hydrothetical menadione reaction cycle. Menadione (M) diffuses into a yeast cell, where it is reduced to menadiol (MH_2) then diffuses out of the cell and reacts with ferricyanide, labeled for simplicity Fe^{3+}, converting it into ferrocyanide, labeled Fe^{2+}, and regenerating menadione (M) to repeat the cycle. Ferrocyanide accumulations arising from the above reaction cycle was assayed by voltametric measurements.

Potassium ferricyanide solutions were prepared in phosphate buffered saline to give a concentration of 0.3 mol/L. Glucose solution was prepared in phosphate buffered saline to give a concentration of 18g/L glucose. Menadione was dissolved in 96% ethanol to give a 20 mmol/L solution.

A total volume of 18 mL incubation suspension was prepared for each trial. The standard incubation suspension comprised: 12 mL cell suspension, 3.0 mL ferricyanide solution, 100 μL menadione solution, 2.0 mL sugar solution, cell suspensions were adjusted with 2 mol/L HCl, the final volume of cell suspensions was fulfilled with sterile distilled water to 18 mL. Incubation of cells with mediators and substrate was for 1.5 h at 30 ºC under oxygen-free nitrogen sparging. At the completion of incubation, the cells were pelleted by centrifugation (10,000 rpm, 4 ºC, 15 min) and the supernatant removed for analysis. Unless stated otherwise, all trials were performed in triplicate.

Steady-state voltammetry was conducted using an electrochemical working station (CHI 900, USA). Steady-state voltammograms were obtained at a scan rate of 10 mVs^{-1} scanning from 100 to 500 mV *versus* Ag/AgCl reference electrode. A 15 μm diameter Pt disk electrode was used as the working electrode and a Pt gauze auxiliary

Figure 2 Steady-state voltammograms recorded at a 15 μm Pt microelectrode



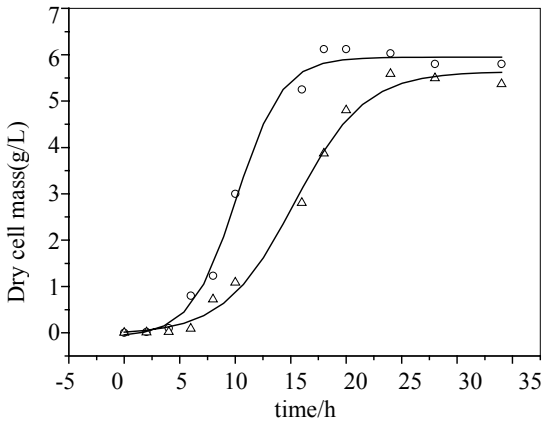
Before (1), and after a 1.5 h incubation of *C. shehatae* CBS 2779 (2), *S. cerevisiae* CBS 1200 (3), yeast cells absorbance (OD_{600}), 6.0; ferricyanide concentration, 50 mmol/L; menadione concentration, 100 μmol/L; glucose concentration, 2g/L; initial pH, 6.0; Scan rate, 10 mV s^{-1}.

electrode was used to complete the three-electrode electrochemical cell. The microelectrode was polished with 0.05 μm alumina/water slurry on a flocked twill polishing cloth. The steady-state current at 500 mV was measured and the mean current from three repeat voltammograms was calculated.

The use of voltammetric microelectrodes has been shown to be a simple, reliable and rapid method for determing the concentration of electroactive species such as ferrocyanide, diffusion limiting currents (quantitatively related to concentration) can be established in the second time scale with virtually zero destruction of analyte[6]. In this work, ferricyanide was converted to ferrocyanide by yeast catabolism. The ferrocyanide accumulations arising from menadione mediated reduction of ferricyanide indicate the intracellular NADH levels in the yeast cells. **Figure 2** (curve 1) shows that before incubation, the mediator is fully in the oxidized form the voltammogram shows only cathodic current due to reduction of ferricyanide. After incubation for 1.5 h with yeast, the voltammogram has shifted up the current axis and there is both anodic current (arising from oxidation of ferrocyanide) and cathodic current (arising from reduction of ferricyanide). The steady-state anodic plateau current measured at E=500 mV was used as a relative measure of the amount of ferrocyanide produced. The oxidative current of the ferrocyanide accumulation arising from the catabolism of glucose by *S. cerevisiae* CBS 1200 was 40.3 nA, which was larger than that (25.0 nA) arising from the catabolism of xylose by *C. shehatae* CBS 2779 (**Figure 2**).

The growth curves of *S. cerevisiae* CBS 1200 and *C. shehatae* CBS 2779 are shown in **Figure 3**. It took 17.5 h and 23 h for *S. cerevisiae* CBS 1200 and *C. shehatae* CBS 2779, respectively, to reach stationary phase. The specific growth rates of *S. cerevisiae* CBS 1200 and *C. shehatae* CBS 2779 were 0.33 g biomass·L^{-1}·h^{-1} and 0.23 g biomass·L^{-1}·h^{-1}, respectively. These data indicate that the catabolic activities of *S. cerevisiae* CBS 1200 were more than that of *C. shehatae* CBS 2779, which are consistent with the menadione mediated intracellular NAD(P)H levels depicted in **Figure 2**.

Figure 3 Growth curve of *S. cerevisiae* CBS 1200 (○), *C. shehatae* CBS 2779 (Δ) obtained by dry cell mass. 30 ºC 160 r/min.



In conclusion, the dual mediator system menadione/ferricyanide could be used as a probe to detect cellular catabolic activities in yeast cells and the electrochemical response has a positive relationship with the specific growth rate of yeast cells.

Acknowledgments

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