Current Topics in Biophysics (Zagadnienia Biofizyki Współczesnej) vol. 18 (1), 1994, 29-38

Spin-label oximetry in dense cell suspensions: problems in closed- and open-chamber methods

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The EPR spin-label oximetry has employed both: the closed- and open-chamber method; the former to measure the rate of cellular respiration and the latter to study the gradients of oxygen concentration across the cell plasma membrane. Both approaches require a high density of cell suspension. In the present work we measured the changes of the average oxygen concentration in the dense Chinese hamster ovary (CHO) cell suspension in a closed- and open-chamber using 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxy (CTPO) as an oxygen sensitive spin-label probe. Additionally we followed the loss of the electron paramagnetic resonance (EPR) signal of CTPO during measurements. In the closed-chamber the loss of the EPR signal is observed only after all oxygen in the sample is consumed. In the open-chamber the loss of the EPR signal is observed also in the presence of oxygen, after oxygen concentration in the sample reaches minimum. In the open-chamber for very dense cell suspension ($6x10^7$ cells ml^{-1}) and oxygen partial pressure outside the capillary equaling 0.1 atm, the average oxygen concentration inside the capillary drops to zero within ~5 minutes. At low cell density ($2x10^7$ cells ml^{-1}) the oxygen concentration drops to zero within ~10 minutes but after ~50 minutes increases again. For cell density 10^7 cells ml^{-1} oxygen concentration reaches minimum, but does not stabilize at this level and increases again reaching, after ~3 hours, the value of 105 μM which is the concentration of oxygen in water equilibrated with the oxygen partial pressure outside the open-chamber. These results indicate that the metabolism of the CHO cells changes during measurements. We discuss obtained results in terms of exhausting the substrates for cell respiration and in terms of gradients of oxygen concentration created across the plastic wall of the open-chamber and inside the sample. All of these considerations were not discussed in earlier works and should be taken into account during measurements with the open-chamber systems, during averaging of the oxygen concentrations, and during processing of the EPR data.