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PII: S0005-2728(18)30083-5
DOI: doi:10.1016/j.bbabio.2018.04.009
Reference: BBABIO 47908

To appear in:

Received date: 5 October 2017
Revised date: 21 March 2018
Accepted date: 23 April 2018

Please cite this article as: Mariana C. Rocha, Roger Springett , Spectral components of detergent-solubilized bovine cytochrome oxidase. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Bbabio(2018), doi:10.1016/j.bbabio.2018.04.009

Spectral components of detergent-solubilized bovine cytochrome oxidase

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Running title: Spectral components of CytOx

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Highlights

- Redox titrations were performed on the isolated detergent-solubilized bovine enzyme
- The Soret- and α-band was analyzed with precise multiwavelength spectroscopy
- The presence of the 602nm form of heme *a* was verified
- This state was stabilized at high pH and on binding of azide
- Results confirm that redox titrations can be successfully performed in living cells

Keywords Cytochrome oxidase, midpoint potential, proton pumping, heme spectroscopy, redox titration, azide.

Abstract

Cytochrome oxidase is the terminal oxidase of the mitochondrial electron transport chain and pumps 4 protons per oxygen reduced to water. Spectral shifts in the α-band of heme *a* have been observed in multiple studies and these shifts have the potential to shed light on the proton pumping intermediates. Previously we found that heme *a* had two spectral components in the α-band during redox titrations in living RAW 264.7 mouse macrophage cells, the classical 605nm form and a blue-shifted 602nm form. To confirm these spectral changes were not an artifact due to the complex milieu of the living cell, redox titrations were performed in the isolated detergent-solubilized bovine enzyme from both the Soret- and α-band using precise multiwavelength spectroscopy. This data verified the presence of the 602nm form in the α-band, revealed a similar shift of heme *a* in the Soret-band and ruled out the reversal of calcium binding as the origin of the blue shift. The 602nm form was found to be stabilized at high pH or by binding of azide, which is known to blue shift the α-band of heme *a*. Azide also stabilized the 602nm form in the living cells. It is concluded there is a form of cytochrome oxidase in which heme *a* undergoes a blue shift to a 602nm form and that redox titrations can be successfully performed in living cells where the oxidase operates in its authentic environment and in the presence of a proton motive force.