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EFFECT OF Ca^{2+} ON THE REDOX POTENTIAL OF HEME *a* IN CYTOCHROME *c* OXIDASE

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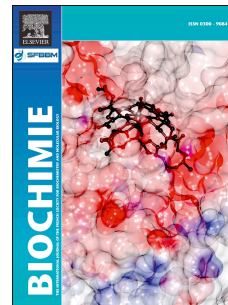
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Subunit I of cytochrome *c* oxidase (CcO) from mitochondria and many bacteria contains a cation binding site (CBS) located at the outer positively charged (*P*-) aqueous phase not far from heme *a*. Binding of Ca^{2+} with the CBS in bovine CcO inhibits activity of the enzyme 2-3 -fold [Vygodina, T., Kirichenko, A. & Konstantinov A.A. (2013) Direct Regulation of Cytochrome *c* Oxidase by Calcium Ions, *PLoS One*. **8** e74436]. Here we show that binding of Ca^{2+} at CBS of bovine CcO shifts E_m of heme *a* to the positive by 15-20 mV. Na^+ ions that bind to the same site and compete with Ca^{2+} do not affect E_m of heme *a* and also prevent and reverse the effect of Ca^{2+} . No effect of Ca^{2+} or EGTA is observed on E_m of heme *a* with the wild type bacterial oxidases from *R.sphaeroides* or *P.denitrificans* that contain tightly-bound calcium at the site. In the D477A mutant CcO from *P. denitrificans* that binds Ca^{2+} reversibly like the mitochondrial CcO, calcium shifts redox titration curve of heme *a* to the positive by ~35-50 mV that is in good agreement with the results of electrostatic calculations; however, as shown earlier, it does not inhibit CcO activity of the mutant enzyme. Therefore the data do not support the proposal that the inhibitory effect of Ca^{2+} on CcO activity may be explained by the Ca^{2+} -induced shift of E_m of heme *a*. Rather, Ca^{2+} retards electron transfer by inhibition of charge dislocation in the exit part of the proton channel H in mammalian CcO, that is absent in the bacterial oxidases.

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