Trans-hyponitrite ($N_2O_2^{2-}$) adopts an unconventional ligand binding mode in *ba*$_3$-type cytochrome *c* oxidase

Elin Hellquist*, Johan Glerup* and Gisela Brändén*

*Department of Chemistry and Molecular Biology, University of Gothenburg

In cellular respiration, cytochrome *c* oxidase (C*c*O) is the terminal electron acceptor of the electron transport chain, catalysing the reduction of molecular oxygen to water. The *ba*$_3$-type C*c*O found in the thermophilic bacterium *Thermus thermophilus* is one of few heme copper oxidases that also exhibits NO reductase activity[1], but the turnover mechanism of this reaction is not well understood. In particular, it is contested within the field whether the reduction of NO to N_2O proceeds via a cis- or trans-hyponitrite ($N_2O_2^{2-}$) intermediate in the *ba*$_3$-type C*c*O and similar enzymes[2]. Here, we have applied UV/VIS spectroscopy to reveal that the hyponitrite intermediate produced by NO turnover generate a different absorption spectrum than the static binding of a trans-hyponitrite species in solution. Furthermore, the trans-hyponitrite/C*c*O complex is spectroscopically distinct and remarkably stable, suggesting an unconventional electronic structure and binding mode.

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(2) Blomberg, M. R. A. Activation of O2 and NO in Heme-Copper Oxidases – Mechanistic Insights from Computational Modelling. *Chem. Soc. Rev.* **2020**, *49* (20), 7301–7330. https://doi.org/10.1039/D0CS00877J.