Enzymatic reduction of oxygen by small laccase. A rapid freeze-quench EPR study

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Stellingen

behorend bij het proefschrift

Enzymatic reduction of oxygen by small laccase.
A rapid freeze-quench EPR study

1. High-frequency EPR experiments would highly benefit from an improvement of the packing density of rapid freeze-quench samples. *Chapter 3 of this thesis*
2. Commercializing the sucking method for preparation of rapid freeze-quench samples would facilitate the application of multifrequency EPR in the study of a reaction mechanism. *Chapter 2 and 3 of this thesis*
3. Unlike the common three-domain laccases, the two-domain small laccase from *Streptomyces coelicolor* utilizes a tyrosine residue to complete the reduction of O_2 when an insufficient number of electrons is available. *Chapter 4 and 6 of this thesis*
4. A single spectroscopic technique is not sufficient to determine the role of tyrosine 108 in the reduction of O_2 by wild-type small laccase. *Chapter 5 of this thesis*
5. In contrast to the suggestion by Jones and Solomon, it is unlikely that the same mechanism of O_2 reduction applies to all subfamilies of multicopper oxidases. *Chapter 6 of this thesis and S.M. Jones and E.I. Solomon, Cell. Mol. Life Sci. 72, 869 (2015)*
6. The flow optical spectrometer designed by Mitic *et al.* can be applied to detect the peroxide intermediate in a multicopper enzyme on the nanosecond time scale. *S. Mitic et al. Anal. Biochem. 469,19 (2015)*
7. In the mechanism proposed by Kuchenreuther *et al.* for a hydrogenase, the effect of an excess of the reductant on the intermediates is not taken into account. *J.M. Kuchenreuther et al, Science 342, 472 (2013)*
8. The addition of an internal standard improves the accuracy of kinetic studies based on spin quantification. *C.H. Kjaergaard et al.*

Biochemistry 52, 3702 (2013); Y. Lin et al. Anal. Chem. 75, 5381 (2003)

9. Combining knowledge of biology, chemistry and physics is of great help for determining the mechanism of an enzymatic reaction.

10. For women to achieve their desired career, self-imposed barriers are often as discouraging as external factors.

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