Identification of the peroxy adduct in multicopper oxidases by a combination of computational chemistry and extended X-ray absorption fine-structure measurements

Ryde, Ulf; Hsiao, Ya-Wen; Rulisek, Lubomir; Solomon, Edward I.

Published in:
Journal of the American Chemical Society

DOI:
10.1021/ja062954g

2007

Document Version:
Peer reviewed version (aka post-print)

Link to publication

Citation for published version (APA):

Total number of authors:
4

Creative Commons License:
Unspecified

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Identification of the peroxo adduct in multi-copper oxidases by a combination of computational chemistry and EXAFS measurements

Ulf Ryde, Ya-Wen Hsiao, Lubomír Rulíšek, Edward I. Solomon

Department of Theoretical Chemistry, Lund University, Chemical Center, P.O. Box 124, S-221 00 Lund, Sweden

RECEIVED DATE (automatically inserted by publisher); Ulf.Ryde@teokem.lu.se

The multi-copper oxidases (MCO) are a group of enzymes that couple the four-electron reduction of molecular oxygen to water with four one-electron oxidations of various substrates. Typical examples are laccase, ascorbate oxidase, ceruloplasmin, and CueO. The MCOs contain at least four copper ions: one isolated type 1 Cu near the substrate-binding site and a trinuclear cluster, consisting of a type 2 (T2) Cu ion and a pair of type 3 (T3) Cu ions in a triangular arrangement. Crystal structures of several MCOs are known. They show that the T2 Cu is bound to two His ligands and a solvent molecule, whereas the two T3 Cu ions are bound to three His ligands each. Depending on the oxidation state of the Cu ions, there may be a bridging ligand between the two T3 Cu ions, a solvent molecule, or perhaps a derivative of the O2 substrate.

Extensive kinetic and spectroscopic studies have been performed to deduce the mechanism of the MCOs. Four distinct states have been identified in the mechanism: the fully oxidized resting state, a fully reduced state, the peroxo intermediate (PI), and the native intermediate (NI). The PI arises after the binding of O2 to the reduced state, leading to the two-electron reduction of O2 to the peroxide level. In the NI, O2 is reduced by two additional electrons (to the level of two water molecules) and all Cu ions are oxidized.

It is clear that O2 binds to the trinuclear cluster, but the details of the binding are still unclear. On the basis of the spectroscopic evidence, two possible structures have been suggested for the PI: One with O2 bridged to the three coppers in the center of the cluster (C3), and one with HO bridged to two coppers on the side of the cluster (S3) and with an additional OH bridged to a second pair of coppers. In a recent combined quantum mechanical and molecular mechanics (QM/MM) study based on the crystal structure of CueO (which had the highest resolution, 1.4 Å), these two structures were refined (Figure 1). The results indicated that the C3 structure was 24–130 kJ/mol more stable than the S3 structure, but the multiplicity of the electronic ground state was not in accordance with experimental data (by 6–20 kJ/mol). Both structures fitted the experimental extended X-ray absorption fine structure (EXAFS) data equally well: EXAFS indicates that there is a short Cu–Cu interaction of 3.4 Å in the PI and the best computational structures of the C3 and S3 states had Cu–Cu distances of 3.45–3.57 and 3.42–3.53 Å, respectively.

This illustrates the problem of comparing EXAFS data with structures from other sources: A direct comparison has only a weak discriminatory potential. However, the raw EXAFS spectra contain much more information, including many other Cu–ligand (and Cu–Cu) distances, as well as some angular information, but it is not easy to use all this information, especially considering possible systematic errors in the structures. Recently, we have developed a technique to combine EXAFS refinements with QM calculations (EXAFS/QM). In essence, it is a standard QM geometry optimization in which we also include numerical forces from a standard EXAFS refinement. Thereby, we obtain a structure that is a compromise between EXAFS and QM. This method has successfully been applied to a number of small inorganic complexes and porphyrins in solution. In this paper, we extend this method to QM/MM calculations on a full protein and apply it to the MCOs.

For technical reasons, we have used the EXAFS data from the peroxo adduct (PA), which is the complex of H2O2 with the fully oxidized resting state of the protein. Spectroscopic and computational studies have shown that the structures of the PI and the PA are very similar, although the T2 Cu is reduced in the former, but oxidized in the latter.

To start with, we optimized the two putative structures of PA in a vacuum with QM and made a standard EXAFS fit, in which only Eb (the absorption edge energy) was free to vary. The results of these calculations are collected in Table 1 and show that the C3 model fits the EXAFS data better than the S3 model (χ2 is 76, compared to 113). If three Cu–ligand distances are also fitted (QM+EXAFS fit), the difference between the two structures is reduced (the shortest Cu–Cu distance is 3.41–3.42 Å in both structures), but the C3 structure is still better (χ2 = 72 and 77). Similar results are obtained if a different number of distances are fitted, but when the number is increased, we soon obtained distances that would give unphysical structures (e.g. strongly distorted His imidazole rings).

The EXAFS/QM method avoids this problem: It always ensures that the system is close to a realistic QM structure and also gives a measure of the deviation from the ideal QM structure in energy terms, ΔEQM. Our previous experience indicates that

---

1 Department of Molecular Modeling, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo náměstí 2, 166 10, Praha 6, Czech Republic
2 Department of Chemistry, Stanford University, Stanford, California 94305
realistic structures should have $\Delta E_{\text{QM}} < -10$ kJ/mol. Thus, we use the QM calculations in the same way as MM is used in almost all NMR and crystallographic refinements to supplement the experimental data and ensure that a realistic structure is obtained.

The EXAFS/QM results in Table 1 show that the two structures give a similar Cu–Cu distance (3.42–3.44 Å). They are both strongly improved by the optimization, but the C$_3$ structure is still best ($\chi_{\text{red}}^2 = 48$ and 50). The $\Delta E_{\text{QM}}$ energies were 4 and 11 kJ/mol, respectively.

Next, we made a similar investigation of the QM/MM structures obtained in our previous study. Interestingly, the original structures fitted the EXAFS data quite poorly and the C$_3$ structure gave worse results than the S$_2$ structure ($\chi_{\text{red}}^2 = 218$ and 134), although the shortest Cu–Cu distance, 3.52 Å, is closer to 3.4 Å than that in the S$_2$ structure, 3.72 Å. This illustrates the problem of comparing only distances in QM calculations with those obtained in EXAFS fits. However, a standard EXAFS fit of three distances (QM/MM+EXAFS fit) made the C$_3$ structure better than the S$_2$ structure again.

Of course, the ideal procedure would be to optimize the copper site inside the protein with both QM and EXAFS data. We have therefore implemented such an EXAFS/QM/MM procedure by combining our EXAFS/QM and QM/MM software. The results of this approach (last two rows in Table 1) show that the C$_3$ structure is strongly improved, much more than the S$_2$ structure ($\chi_{\text{red}}^2 = 38$ and 74). The resulting structure (Figure 2) has a Cu–Cu distance of 3.41 Å, i.e. close to what was found in all the other structures which included EXAFS data in the fits. However, the other two Cu–Cu distances are quite far from what was obtained in the EXAFS/QM structure (4.02 and 4.44 Å compared to 3.71 and 4.60 Å). This shows that both the EXAFS and the QM energies are insensitive to these distances. The $\Delta E_{\text{QM}}$ energy is 10 kJ/mol. Closely similar results are obtained with other density functional methods (cf. Table S2 in the supporting information).

In conclusion, we have implemented a combination of EXAFS refinement and QM/MM calculations, which allows for the refinement of an EXAFS structure inside a protein, keeping the structure chemically reasonable. By this method, we show that the C$_3$ structure in Figure 2 fits the experimental EXAFS data better than the alternative S$_2$ structure. Thus, this investigation provides an independent confirmation of our previous energetic results that O$_1$ binds in an all-bridged mode to the center of the trinuclear cluster in the MCOs. This is also in accordance with a recent QM investigation of other spectroscopic properties of the two tentative models of the PI in the MCOs. \(^{13}\)

Acknowledgment. This work has been supported by grants from the Swedish research council, NIHDK 31450, and LC512 (MSMT CR). It has also been supported by computational recourses from Lunarc at Lund University. We thank Profs. K. O. Hodgson and B. Hedman, Stanford University, for making the EXAFS data available to us.

Supporting Information Available: A detailed description of the EXAFS/QM/MM interface; a more detailed account of the quantum calculations and EXAFS fits; calibration of the EXAFS/QM/MM method on the oxidized state of the MCOs; influence of the QM method on the results; the final C$_3$ EXAFS/QM/MM structure of PA in CueO; a comparison of the experimental and calculated EXAFS for the C$_3$ and S$_2$ structures in both k and R space; and a description of the considered EXAFS paths.

References

(10) QM calculations were performed with the Turbomole software (Schäfer, A.; Horn, H.; Ahlrichs, R. J. Chem. Phys. 1992, 97, 2571-2577) using the Becke-Perdew86 (BP86) density functional, the DZP basis set (Schäfer, A.; Huber, C.; Ahlrichs, R. J. Chem. Phys. 1994, 100, 5829-5835) on Cu and 6-31G* on the other atoms.
(11) EXAFS fits were performed with the IFEFFIT software (Newville, M. J., Synchrotron Rad. 2001, 8, 322-324), using theoretical scattering amplitudes and phase shifts calculated with the FEFF program (Ankudinov, A.; Bouladin, C.; Rehr, J. J.; Sims, J.; Hung, H. Phys. Rev. B 2002, 65, 104107; up to four scattering legs and a maximum effective path length of 10 Å). Debye-Waller factors were calculated by FEFF with the equation-of-motion method using force constants obtained from a frequency calculation on vacuum-optimized structures of the C$_3$ and S$_2$ models of the PA. The fitting range was the same as in the original EXAFS investigation, $k = 3.5-12.5$ Å$^{-1}$ and $R' = 0.8-4.0$ Å.
We have developed a computational method that combines EXAFS refinements with the integrated quantum mechanical and molecular mechanics (QM/MM) method. This method allows us to obtain a structure of a metal site inside a protein that is compatible with both EXAFS data and QM calculations (i.e. that is chemically reasonable). Thereby, the QM calculations play the same role as MM in nearly all NMR and crystallographic refinements – EXAFS ensures that the metal–ligand distances are accurate and QM/MM fills in all the other structural data. We have used this method to show that a structure with peroxide ion in the center of the trinuclear cluster fits experimental EXAFS data better than a structure with the peroxide ion on the side of the cluster for the peroxide adduct of multi-copper oxidases.