DALTON COMMUNICATION

The catalytic activity of xanthine oxidase: mechanistic insights through computer modelling

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The catalytic cycle for substrate oxidation at the molybdenum centre of xanthine oxidase has been modelled with density functional theory; using our previously developed active-site model plus formaldehyde as substrate, the structures and relative energies of intermediate and transition-state species have been employed to examine the key issues of active-site conformation, the role of Mo–C interactions and the identity of the transferred oxygen.

The outstanding mechanistic question for the molybdoenzyme xanthine oxidase (XnO) is the nature of the catalytically active oxygen ligand.[1] Its resolution is well suited to computer modelling which currently represents perhaps the only viable technique for obtaining definitive transition-state structures and energies. As a basis for this, our theoretical investigation of the active site structure of XnO[2] established that the Mo centre is five-co-ordinate and supported the contention[3–6] that the fifth ligand is probably hydroxide. The single-crystal X-ray diffraction study of a closely related enzyme, aldehyde oxidoreductase (AOR),[7,8] has also shown a five-co-ordinate Mo site confirming that our theoretical active-site model is reasonable. We now address the issue of which oxygen (oxo or hydroxy) is catalytically labile. In addition, we consider the role of Mo–C interactions in the catalytic cycle as well as the significance of the active-site conformation.

Taking the last topic first, the active-site structure previously presented by us was a distorted square-based pyramid with an apical oxo group [Fig. 1(*a*)].[2] New calculations† show that the analogous structure with an apical thio group is equally stable and has similar bond lengths [Fig. 1(*b*)]. Structures with apical hydroxyl groups were found to be unstable. Based on the density functional theory (DFT) results, the ground-state apical oxo and apical thio conformations of the oxidised resting state are intrinsically equally likely and although the AOR structure suggests the latter, the crystals were obtained by diffusing sulfur into the inactive desulfo form which has the equatorial oxo group in a protected environment and thus only the apical position is susceptible to substitution by S. In the absence both of definitive structural data for the native form of AOR and for XnO itself, further calculations on the mechanism were carried out with the (marginally more stable) apical oxo conformer.

† The Amsterdam density functional suite (ADF version 2.0.1)[9,11] was used for all DFT calculations. Unconstrained geometry optimisations employed the local density approximation (LDA) with the correlation potential due to Vosko *et al.*,[10] STO (Slater orbital) triple-ζ-plus polarisation basis sets[11] and the frozen core approximation[12] (up to and including 3d for Mo, 2p for S, and 1s for C and O). DFT binding energies were calculated at the LDA geometries using the gradient corrected functionals of Becke[13] and Perdew.[14] Transition states were confirmed by frequency calculations. Solvation corrections for charged species were computed as described in ref. 15 assuming water as the bulk solvent.

It is well known that the catalytically active oxygen is ultimately derived from the solvent,[16] and that it is transferred from the enzyme itself.[5,17] Formaldehyde is a substrate for XnO and has the advantage of being more computationally tractable than xanthine yet thought to react by the same mechanism.

The traditional reaction pathway[18] suggests that the thio group abstracts a C–H proton from the substrate and the oxo group is attacked directly by the carbon atom. However, no transition state for this reaction has been located to date. In fact, the calculation suggested an Mo–C interaction and intermediates with this feature were duly found (Fig. 2). The orientation of the bound CHO fragments seems ideally set up for a subsequent oxo-transfer reaction.

For the alternative, OH-transfer pathway, Mo–C bonded species appeared again (Fig. 3). In contrast to the oxo-transfer intermediates, these structures are first-order transition states. Fig. 3(*a*) suggests that the proton transfer to the thio group and Mo–C binding may occur stepwise while Fig. 3(*b*) suggests a concerted process. However, for the latter, the energy of the subsequent transition state is a few kJ mol^{-1} higher than for the stepwise process. From either point, the computed mechanism suggests H loss from the hydroxyl group ultimately yielding co-ordinated formate consistent with EXAFS measurements on alloxanthine[3a,b] and violopterin.[3a] Such a mechanism does not necessarily involve hydride transfer and agrees with a recent suggestion by Howes *et al.*[5]

The crucial aspect of these calculations is the computed

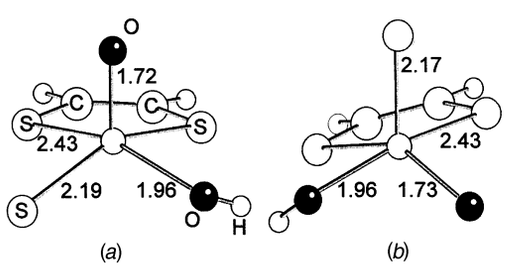

Fig. 1 Calculated structures with selected bond lengths (Å) of active-site-model conformers. (*a*) Apical oxo group, (*b*) apical thio group

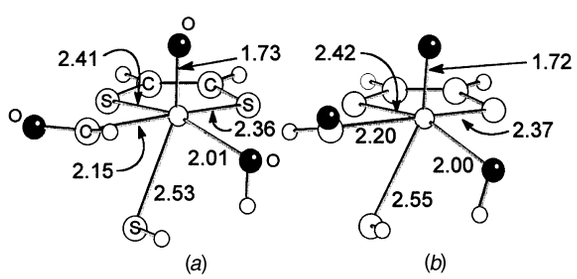

Fig. 2 Calculated structures with selected bond lengths (Å) of potential intermediates on oxo-transfer pathway, (*a*) and (*b*) differ only in the orientation of the co-ordinated CHO fragment

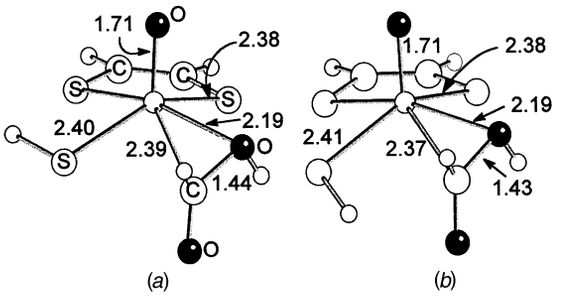

Fig. 3 Calculated structures with selected bond lengths (Å) of transition states on hydroxy-transfer pathway, (*a*) and (*b*) differ only in the orientation of the co-ordinated SH fragment

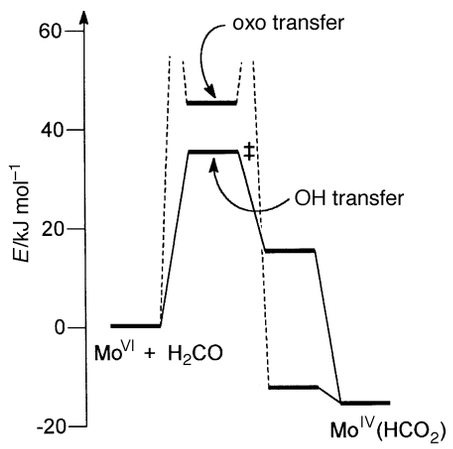

Fig. 4 Simplified schematic reaction profile for the Mo reduction cycle

energy profile. A simplified scheme is shown in Fig. 4. The barrier to hydroxyl transfer is in the region of 35 kJ mol^{-1} while the intermediates for oxo transfer are 5–20 kJ mol^{-1} higher depending on the particular choice of oxo-transfer intermediate and hydroxy-transfer transition state. Since the former presumably have transition states of still higher energy on either side, we conclude that the (frequently assumed) oxo-transfer mechanism is less likely than OH transfer. Some important points on the reaction profile are still missing [*e.g.* the oxo-transfer transition state(s) and species associated with a starting apical thio active-site conformation] and we use formaldehyde and a model dithiolene ligand rather than xanthine and the pterin cofactor. However, assuming the theoretical results are at least qualitatively correct, we conclude that (*i*) apical oxo and apical thio ground-state conformations of the active site are about equally stable, (*ii*) Mo–C interactions play a definite role and appear to be preferred over direct attack on an O atom by the substrate and (*iii*) OH transfer is at least as competitive as oxo transfer and probably the dominant process.

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