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A bacterial oxidase like no other?

"A phenomenon can't be unique and universal at the same time." Lewis Thomas (The Medusa and the Snail)

By Gregory M Cook and Robert K Poole

Living organisms use many different enzymatic mechanisms for reducing oxygen. However, about 90% of biological oxygen consumption is carried out by membrane-integrated oxidases that terminate a sequence of redox reactions conveying electrons from a reductant to oxygen, the terminal electron acceptor in aerobic respiration. Membrane localization of these reactions offers, not only access to the enhanced solubility of oxygen in the membrane phospholipid bilayer, but also provide opportunities for vectorial proton separation across the membrane, thereby contributing to the generation of a protonmotive force (1). The most extensively studied superfamily of respiratory oxygen reductases comprise the heme-copper oxidases (HCO) of mitochondria and many bacteria (2). These have a binuclear oxygen reduction site comprising a heme (a-, o- or b-type) plus a copper atom that achieve virtually complete and rapid reduction of oxygen to water, without release of toxic partial reduction products, like superoxide anion or hydrogen peroxide. These are energetically efficient since the reduction chemistry is coupled to direct proton translocation via a pump mechanism. A second distinct class comprises the ‘alternative’ oxidases (AOX) found in certain plants, fungi and bacteria, notably Vibrio fischeri where its haem-less, Fe-Fe reactive site (3) confers nitric oxide-resistant respiration (4). The third unique class comprise the bacterial cytochrome bd-type oxidases (5), to date the only class for which no atomic resolution structure has been available. These oxidases are lacking from all eukaryotic cells.

On page XXXX of this issue, Safarian et al. REF provide the first atomic resolution structure of a cytochrome bd-type oxidase from Geobacillus thermodenitrificans. This is a long-awaited structure for several reasons. First, the primary sequence of these oxidases and the derived models based on hydrophobicity profiles and biochemical approaches (5), have long been interpreted as showing that this class of oxidase has no structural resemblance to the heme-copper super-family of oxidases. Second, oxidases in this class have a distinctive heme composition (two hemes b, one heme d) and the unique spectral signatures of heme d have made the identification of this oxidase in numerous types of bacteria straightforward (indeed,

such an oxidase was first described in the 1930s (6)). Third, the oxidase has extraordinary ligand-binding activities: in particular, respiration via cytochrome bd exhibits nanomolar affinity for oxygen (7) even though oxygen is able to form a unique stable oxygenated complex (8) (analogous to the oxygenated globins) that is the first intermediate in the accepted oxygen reduction mechanism (5). Fourth, there is ample evidence that cytochrome bd plays special roles in pathogenic bacteria (9). For example, Brucella species - the causative agents of brucellosis in swine and cattle – depend on cytochrome bd for replication and survival in macrophages. This may be in part a consequence of the insensitivity of cytochrome bd to nitric oxide (10), hydrogen peroxide (11), hydrogen sulfide (Forte et al., in the press) and other toxic species. These and other experiments in Salmonella and Mycobacterium tuberculosis strongly suggest that this oxidase may be an attractive therapeutic target. Indeed, mutants of M. tuberculosis and Mycobacterium smegmatis lacking cytochrome bd are hypersensitive to bedaquiline, a first in class tuberculosis drug that targets the F_1F_o-ATP synthase (12, 13). Furthermore, cydAB mutants of M. smegmatis are hypersusceptible to clofazimine (14), a frontline drug for leprosy, and respiration mediated by cytochrome bd is essential for the killing of Escherichia coli by many antimicrobials (15). There is every reason to expect that the new crystal structure will facilitate targeted and rational drug development against cytochrome bd.

The new structure shows that the oxidase comprises 19 helices that span the bacterial cytoplasmic membrane (Fig. 1A). CydA and CydB each have nine helices and the last is the small peptide called CydS by Safarian et al. In E. coli, the two major subunits have appeared to have 9 and 8 helices, respectively, but in G. denitrificans CydA and CydB share the same fold, most likely the result of a gene duplication of a single ancestral gene that encoded a homo-dimeric oxidase. However, the three hemes are associated only with CydA, which also houses the Q-loop, or site of ubiquinol oxidation. CydS is a single transmembrane helix of 33 amino acid residues and may stabilise the b_{558} heme that is located adjacent to the Q-loop. A third subunit is now also recognised in the E. coli oxidase, CydX (12), and it is perhaps significant that, despite numerous attempts over many years to crystallise the E. coli enzyme, no success was obtained with two-subunit preparations.

A surprising feature of the structure is the triad arrangement of the hemes (Fig. 1B) and not the linear arrangement that might be predicted from models of electron transfer from quinol to heme b_{558} and then to b_{595} and d, where oxygen is reduced to water. Instead the distance

between the two b-type hemes is greater than that between b_{558} and d, suggesting that electron transfer proceeds from b_{558} to d, followed by equilibration with heme b_{595}. Thus there is no clear analogy to the binuclear centre (heme-Cu_B) in which these two centres are only 4-5 Å apart (13), characteristic of the mitochondrial family, or of the non-heme di-iron carboxylate found in AOX (3). These mechanistic details of electron transfer are critical although the extraordinary affinity of (some) bd-type oxidases and the stability of the oxygenated form even at room temperature remain currently unexplained. Whatever the details of the oxygen reduction mechanism, which are not significantly informed by this manuscript, cytochrome bd must achieve a concerted four-electron reduction of oxygen to avoid deleterious release of partially reduced oxygen intermediates.

Unlike all members of the hem-copper superfamily, cytochrome bd does not pump protons across the membrane. However, it does result in net proton translocation as a result of proton extrusion from quinol oxidation and the uptake of protons from the cytoplasm for oxygen reduction. In the Geobacillus oxidase there appear to be two probable channels, one in each major subunit, that converge on cytochrome b_{595} within the bilayer (Fig. 1A). An onward route to haem d has not been identified.

Further detailed experimental approaches, informed by the present structure, and in time the structures of other members of the cytochrome bd class, will be required. But for the present, the structural framework provided by Safarian and co-workers will lead the way in our understanding of this fascinating and important enzyme.

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