NO's way out

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REASONS

In respiration electrons are passed down the proteins of the respiratory chain until they are ultimately used to reduce a terminal electron acceptor. In eukaryotes this is always oxygen, which is reduced to water, however lower organisms can use other molecules. A number of bacteria including *Thiosphera pantotropha* can use either oxygen or nitrite as the terminal electron acceptor, the reduction of both being catalyzed by the same enzyme, cytochrome cd_1. Now after thirty years and many attempts from numerous groups, the structure of this bifunctional enzyme, in its oxidized form, has finally been determined by X-ray crystallography (Fülöp,V., Moir, J.W.B., Ferguson, S.J. & Hajdu, J. *Cell*, **in the press**, figure reproduced with permission).

The enzyme consists of a dimer, each subunit of which contains two domains (in the figure the chain is coloured blue to red from amino to carboxy terminus). The N-terminal domain (top) contains a haem group covalently bound to the protein (the c-haem) while the C-terminal domain (bottom) contains an unlinked haem (d_1-haem). The d_1-haem is the site of nitrite and oxygen reduction while the c-haem receives electrons from other electron transport proteins further up the respiratory chain.

The c-haem is contained within an α-helical environment which, although it resembles the Class 1 cytochrome *c* domains, has a different helical topology. In addition, spectroscopic evidence suggests that the axial iron ligands (here two histidines) may be variable between cd_1 cytochromes from different organisms, violating the dogma that within a group of homologous proteins key functional residues are conserved. This variability is also present for the d_1-haem ligands one of which, Tyr 25, is provided by the N- rather than the C-terminal domain. This may be crucial for the expulsion of nitric oxide (NO) during the reaction cycle.

The iron atom of the haem group converts from the Fe(II) to the Fe(III) oxidation state during the reduction of nitrite. NO binds extremely tightly to Fe(II) so it is important that this product leaves the active site before the Fe(II) state is regenerated. It appears that this is achieved by a conformational change in the enzyme. The substrate nitrite ligates to the haem iron but following its reduction to NO the orientation of the two domains alters bringing Tyr 25 into position to ligate the haem iron (as in this structure of the oxidized enzyme) thus kicking the NO out of the site. A similar mechanism can also be proposed for the reduction of oxygen although here the product, water, can exit the active site by diffusion.

The structure of this enzyme has implications beyond the respiratory metabolism of bacteria. Not only does it violate a long held dogma of protein chemistry but it may provide an evolutionary link between denitrifying enzymes and the cytochrome oxidases of higher organisms, fulfilling as it does both functions. In addition NO has recently been implicated in a number of cellular processes not least of which is its activity as a neurotransmitter. The manner by which cytochrome cd_1 expels nitric oxide may well help us to understand the release of NO from cellular receptors in eukaryotes. CS