METALLOENZYMES

Thiolate gates superoxo states

The FGE active site is different to those of well-known Cu oxidase enzymes

Sulfatases are hydrolytic enzymes that prevent the build up of sulfate esters in our bodies, an excess of which can spell death for brain, skeletal and skin cells. Sulfate esters are cleaved by a sulfatase at its geminal diol active site, which is derived from a formylglycine residue (fGly). fGly, in turn, originates from a Cys residue in a reaction catalysed by the formylglycine-generating enzyme (FGE), whose structure and mechanism has long remained unknown. However, a team led by John Tainer, Edward Solomon and Carolyn Bertozzi now describe in the *Proceedings of the National Academy of Sciences* how the Cu centre in FGE binds a Cys residue in a peptide to trigger O₂ attack and the formation of fGly.

Making a geminal diol –CH(OH)₂ from an aldehyde –CHO is simply a matter of hydration, but how FGE produces –CHO from the –CH₂SH side chain of Cys is less clear. Being an oxidation reaction, one might suspect a redox cofactor to be involved, and indeed FGE features Cu at its active site. Earlier structural studies of apo-FGE and its inactive Ag^I complex had identified two Cys residues as a potential Cu binding site. Tainer, Solomon, Bertozzi and colleagues go a step further and characterize the holoenzyme — FGE complete with its native Cu^I cofactor. Prepared from apo-FGE, Cu^{II} and the reductant dithiothreitol, the holoenzyme has a linear Cu^I(SCys)₂ site that is similar to its Ag^I congener and typical of d¹⁰ metal complexes. With Keith Hodgson and Britt Hedman, the team performed X-ray absorption spectroscopy and extended X-ray absorption fine structure to confirm that the S–Cu^I–S motif also persists in solution. “The FGE active site is different to those of well-known Cu oxidase enzymes, which feature higher coordination numbers and N-donor and O-donor ligands,” says Bertozzi.

Having developed a reliable method to reconstitute the enzyme, the team investigated its binding to Cys-containing peptides. Tainer, Solomon, Bertozzi and co-workers appended a Tyr residue to a Cys-containing sequence in sulfatase to afford a peptide that is a realistic model for the native substrates. To the team’s delight, and in contrast to previous studies, this peptide bound FGE strongly enough to afford a stable enzyme–substrate complex under anaerobic conditions. Again, X-ray spectroscopy came to the

fore and revealed direct substrate coordination to give the trigonal trithiolate Cu^I(SCys)₂(peptide–CH₂S). In contrast to the linear dithiolate Cu^I(SCys)₂, this is sufficiently electron-rich to activate O₂ and give the superoxo Cu^{II}(SCys)₂(peptide–CH₂S)(O₂[•]). “Substrate binding is likely lowering the redox potential of the Cu^{III/II} couple, thereby enabling the difficult one-electron reduction of dioxygen to give a bound superoxo that can abstract a H atom from Cys in the substrate,” notes Solomon. Intramolecular H atom abstraction gives the tautomer Cu^{II}(SCys)₂(peptide–[•]CHS)(OOH), a peroxo that eventually liberates thioaldehyde peptide–CHS, which undergoes hydrolysis to give the fGly product peptide–CHO.

That O₂ activation only occurs when Cu^I(SCys)₂ binds the peptide reflects nature’s careful control over O₂ reactivity. For example, if Cu^I(SCys)₂ itself were to first bind O₂, then FGE might engage in promiscuous reactions and cause oxidative damage. What remains unclear is how, after product formation, the resulting peroxo Cu^I(SCys)₂(OOH) undergoes reduction to Cu^I(SCys)₂ to complete the cycle. Although there is still work to be done, the study of Tainer, Solomon, Bertozzi and colleagues arms us with the knowledge to prepare antibodies with fGly functional handles and better understand diseases such as multiple sulfatase deficiency.

David Schilter

ORIGINAL ARTICLE Appel, M. J. et al. Formylglycine-generating enzyme binds substrate directly at a mononuclear Cu(I) center to initiate O₂ activation. *Proc. Natl Acad. Sci. USA*, <https://doi.org/10.1073/pnas.1818274116> (2019)