From Red to Green: Re-Engineering the Active Site of Ascorbate Peroxidase

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Myeloperoxidase (MPO) is a mammalian heme peroxidase enzyme involved in the catalytic breakdown of cellular H2O2 resulting in the oxidation of chloride ion to hypochlorous acid, an anti-bacterial agent. MPO differs from other heme peroxidase enzymes as novel covalent linkages exist between amino acid residues on the protein and the haem active site. The links modulate the heme redox potential allowing oxidation of high potential substrates such as chloride.

Recently there has been much interest into the mechanism of formation of these linkages, with several different auto-catalytic mechanisms proposed1. In this study we have engineered pea ascorbate peroxidase (rpAPX) to probe the mechanism of formation of these covalent links using a combination of mutagenesis, spectroscopy, kinetics and mass spectrometry. We present evidence for the specific formation of a sulfonium type covalent link in APX, similar to that found in MPO, and show that Compound I formation is an essential intermediate in this process2.

1 Colas, C.; Ortiz de Montellanao, P.R.; Chem. Rev.; 2003; 103(6); 2305-2332

2 Metcalfe CL, Ott M, Patel N, Singh K, Mistry SC, Goff HM, Raven EL.; JACS.;2004; 126(49); 16242-16248