

P020 Construction and characterisation of horseradish peroxidase mutants that mimic some of the properties of the cytochromes P450

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Plant peroxidases catalyse the production of radical products at a discrete haem-edge site. A relatively closed haem architecture, which includes the residues normally responsible for highly efficient O-O bond cleavage, sterically restricts direct access of substrates to the ferryl intermediate. Hence, plant peroxidases do not normally catalyse direct oxygen atom transfer to substrates. In part, by mimicking the more open hydrophobic architecture of chloroperoxidase, variants of horseradish peroxidase have been engineered which have some of the functional properties of cytochromes P450. Several variants have proved to be very efficient peroxygenases (17s^{-1}) and are highly enantioselective. They also undergo a low spin to high spin transition on substrate binding (with sub micro molar K_d 's). Their optical features suggest that all variants remain high-spin, unless the haem pocket is both very open and a His residue is located at position 70 in a strained but flexible loop region immediately above the haem pocket. Some of the variants showed evidence of a concerted mechanism in which prior binding of substrate activates the enzyme for reaction with hydrogen peroxide. The tight binding of substrates to the engineered cavity displaces the labile low spin ligand, returning the enzyme to the high spin form, presumably by expulsion of water from the engineered haem cavity.