

P006 Engineering a covalent link in horseradish peroxidase: formation of a sulphonium link between the haem and an introduced methionine

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There are two well-defined haem peroxidase superfamilies: the plant and the mammalian. Mammalian peroxidases share a unique feature, namely the formation of covalent bonds between the protein and the prosthetic haem group, something that does not naturally occur in plant peroxidases. Mostly this takes the form of two ester links, however myeloperoxidase (MPO) contains an additional sulphonium linkage, between a non-conserved Met residue and the β -carbon of the 2-vinyl haem substituent. Ser167 in horseradish peroxidase C (HRPC) was identified as equivalent to Met243 in MPO, the methionine residue responsible for the formation of the sulphonium linkage. The S167M HRPC* variant has been produced and has a red-shifted UV/visible absorbance spectrum. Its substrate binding and peroxidase activity have also been severely compromised.

A covalent linkage was found to be autocatalytically generated by treatment of the methionine substituted variant with 10 equivalents of hydrogen peroxide. The nature of the covalent bond has been investigated using a variety of kinetic, spectroscopic and structural techniques and appears to be quite labile. The linkage can also form spontaneously without peroxide treatment.