

The taming of the bleach: enzymatically formed hypochlorite as a halogenating agent in regioselective biological halogenation

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During the last 10 years our knowledge about biological halogenation has changed enormously. Until 1995 haloperoxidases and perhydrolases were considered the only types of enzymes known to catalyse the incorporation of halogen atoms into organic compounds. They use hydrogen peroxide and halide ions (chloride, bromide, and iodide) for the formation of free hypohalous acid which then reacts with electron-rich compounds to form halogenated products in a non-enzymatic reaction [1]. Thus their involvement in the biosynthesis of complex halogenated metabolites can only be very limited. During the investigation of the biosynthetic pathway of the antifungal antibiotic pyrrolnitrin, two halogenating enzymes were detected which showed no similarity to haloperoxidases at all. One of these enzymes was found to catalyse the first step in pyrrolnitrin biosynthesis, the regioselective chlorination of tryptophan to 7-chlorotryptophan and was thus named tryptophan 7-halogenase (PrnA). PrnA showed hardly any sequence similarity to the second halogenating enzyme (PrnC) involved in pyrrolnitrin biosynthesis. However, both halogenases contain a conserved motif (GxGxxG) known to be part of a nucleotide binding site which turned out to be required for the binding of reduced FAD. The reduction of FAD is accomplished by a flavin reductase as the second component of the two-component halogenase system. The three-dimensional structure of PrnA showed that the binding site of the flavin cofactor was separated from the substrate tryptophan by a tunnel of ~10 Å. Thus direct contact between the flavin and the substrate was not possible. Instead a diffusible halogenating agent is formed at the active site and guided along the tunnel towards the substrate. This is achieved by reaction of the reduced flavin with oxygen leading to the formation of a flavin hydroperoxide which then reacts with a chloride ion bound close to the FAD resulting in the formation of hypochlorite. In contrast to haloperoxidases, where enzymatically formed hypochlorite was released from the enzyme, this hypochlorite cannot leave the active site but is guided along the tunnel towards the substrate by a lysine residue [1]. However, since the substrate of PrnA, tryptophan, cannot be chlorinated at the 7-position by hypochlorite, the electrophilicity of the chlorine species must be increased and must be correctly positioned for regioselective incorporation into the 7-position. This is achieved via involvement of a glutamate residue which interacts with the chlorine species of hypochlorite. Exchanging this glutamate residue against an aspartate residue results in inactive enzyme. The lysine and the glutamate residue are also conserved in the tryptophan 6-halogenase Thal from thienodolin biosynthesis and in the tryptophan 5-halogenase PyrH from pyrroindomycin biosynthesis. Thus, for regioselective chlorination at position 5 or 6, the substrate must be positioned in a way that the 6- and 5-positions become located at the same place as the 7-position in PrnA. The three-dimensional structure of the tryptophan 5-halogenase PyrH revealed how this is achieved and which amino acids are responsible for the exact positioning of the substrate in the active site without changing the overall mechanism and the architecture of the active site including two tryptophan residues which are also absolutely conserved in all flavin-dependent halogenases known so far.

References

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