Molecular characterisation of a peroxidase from a *Bjerkandera* sp.

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Introduction

A novel class of ligninolytic peroxidases, named versatile peroxidases (VP), with high affinity for manganese and dyes, has been described; these enzymes can also oxidise 2,6-dimethoxyphenol (DMP) and veratryl alcohol (VA) in a manganese-independent reaction; until now, they have only been isolated from *Pleurotus* and *Bjerkandera* strains (Mester et al. 1998; Camarero et al. 2000; Ruiz-Duenas et al. 2001; Martinez 2002). A novel versatile peroxidase was isolated from a novel *Bjerkandera* strain in our lab and named RBP.

The objective of this research effort was the characterisation of the *rbpa* gene coding for RBP from this *Bjerkandera* sp, as well as the construction of a 3D model for the versatile peroxidase.

Materials and Methods

A dendogram was built from PAM distances between mature proteins using PHYLIP software package after multiple alignment of 38 fungal peroxidase sequences and UPGMA Method from PHYLIP software package.

Molecular modeling of *Bjerkandera* sp. versatile peroxidase (without signal peptides), by sequence homology was performed using PROMODII program. The geometry of the whole system was optimized at the molecular mechanics (MM) level with the amber force field and using the program Discover running on a SGI Indy workstation. A dielectric constant equal to 2 was chosen and the threshold for convergence was taken to be the maximum force fixed at 0.02 kcal mol^{-1} $Å^{-1}$.

Results and Discussion

In this research effort is reported the characterisation of the *rbpa* gene coding for RBP from *Bjerkandera* sp. strain B33/3. The 1777 bp—isolated fragment contained a 1698 bp—peroxidase encoding gene, interrupted by 11 introns. The 367-amino acid deduced sequence includes a 27-amino acid signal peptide. A dendogram illustrating the amino acid sequence relationships between 38 fungal peroxidases is further discussed.

The molecular model built showed high structural similarity with *P. chrysosporium* LiP, presenting the smaller root mean square (RMS) distance with LIPH2, and also a high structural similarity with *Pleurotus eryngii* versatile peroxidase PS1. This peroxidase includes both a putative manganese binding site (E41, D183 and perhaps E37) and two potential long-range electron transfer (LRET) pathways to heme from exposed histidine H83 and tryptophan W172 (Fig 1.).

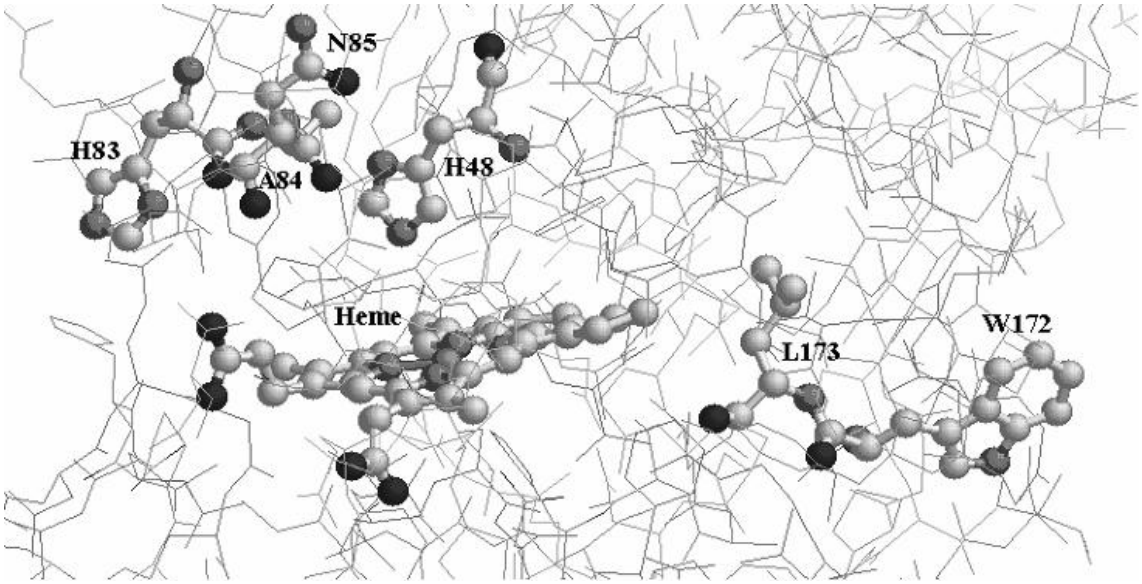

Figure 1: Close up views of LRET pathways from H83 (proceeding *via* A84 and N85, H-bonded to distal histidine, H48) and W172 (proceeding *via* L173, H-bonded to the porphyrin ring). Atomic coordinates correspond to PDB entry 1YGQ. Highlighted residues are represented as balls and sticks with CPK colours.

The LRET might be involved in the catalytic cycle of RBP and its ability to oxidise aromatic substrates.

References

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