Contributions to structural characterisation of fungal versatile peroxidases

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INTRODUCTION

Fungal versatile peroxidases were first described in 1992 in a *Bjerkandera* sp. strain [1]. Their ability to oxidise high molecular and high redox potential compounds (such as dyes) as well as high substrate versatility, make versatile peroxidases of biotechnological interest. The first fully described versatile peroxidase was isolated from a *Pleurotus eryngii* strain [2]. A versatile peroxidase from a novel Bj*erkandera* sp., *Bjerkandera paranensis* [3], was isolated, characterised [4] and its encoding gene duly sequenced [5].

The objective of this research effort was construction of a 3D model for *Bjerkandera paranensis* versatile peroxidase, as well as structural characterisation thereof.

MATERIALS AND METHODS

Molecular modeling

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 $mol^{-1}.Å^{-1}$.

Analysis and comparison of 3D models

The Swiss-PDBViewer v 3.7 SP5, Rasmol v 2.7.2.1 and PyMOL programs were used for model analysis, comparison and presentation.

RESULTS AND DISCUSSION

A three-dimensional model for the mature RBPA protein (PDB entry 1YGQ) was obtained by homology modeling (Fig. 1), taking advantage of the high identity with *Phanerochaete chrysosporium* LiP and MnP. The molecular model built showed high structural similarity with *Pleurotus eryngii* versatile peroxidase PS1 (Fig. 2).

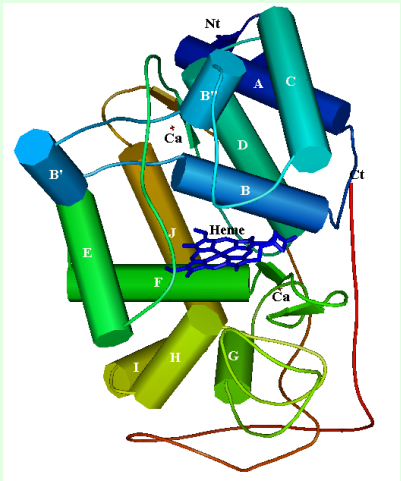
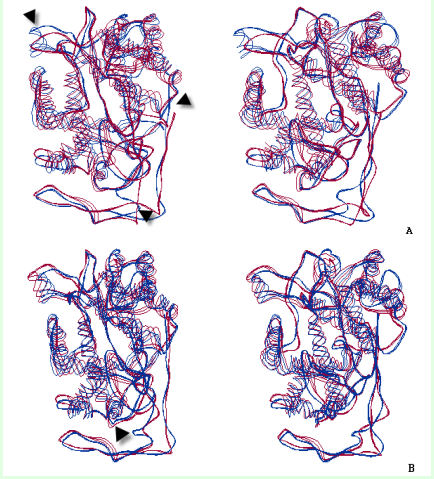


Figure 1: Schematic molecular representation of RBP peroxidase from *Bjerkandera paranensis*. The positions of α-helices (A to J), heme group and C- and N-termini are indicated (Ct and Nt, respectively). Atomic coordinates correspond to PDB entry 1YGQ.

Four putative disulphide bonds (C3:C16, C15:C286, C35:C121 and C250:C316) are also present (but are not shown for simplicity).



RBP presents RMS distances of 2.00 Å (328 residues computed), after superimposition with MnPL1 (PDB 1A20), and 1.81 Å (337 residues computed), with PS1 (PDB 1QJR).

Figure 2: Two stereo views showing superimposition of ribbon-type models of RBP peroxidase (coloured blue in A and B), obtained by homology modeling with MNPL1 (coloured red in A), as well as PS1 (coloured red in B) from *Pleurotus eryngii*. The stereo angle between the views is 4°. Arrows indicate positions of significant differences in the global architecture.

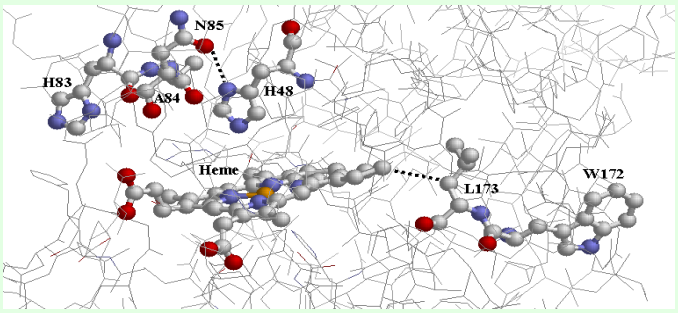


Figure 3: Close up views of LRET pathways from H83 (proceeding *via* A84 and N85, H-bonded to distal histidine, H48) and W172 (proceeding *via* L173, H-bonded to porphyrin ring). Highlighted residues are represented as balls and sticks, with CPK colours.

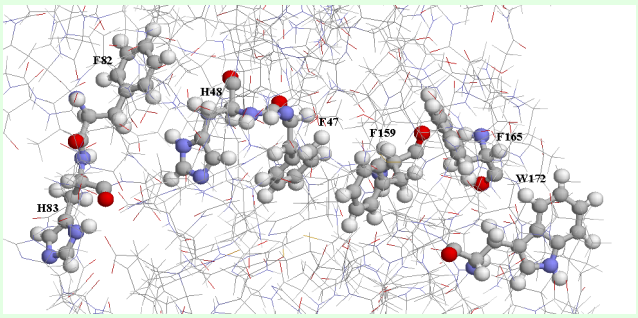


Figure 4: Close up views of proposed pathway from W172 (proceeding *via* F165, F159, F47, H48 and F82) to H83. Highlighted residues are represented as balls and sticks, with CPK colours.

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. 3). The LRET might be involved in the catalytic cycle of RBP, and determine its ability to oxidise aromatic substrates. Two distinct pathways of electron transfer residues (phenylalanines and histidines), connecting W172 to H83, are also proposed (one of which is depicted in Fig. 4). Structural comparison with versatile peroxidases from *Pleurotus eryngii* suggests that two subgroups within the versatile peroxidase group should be established.

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