Expression and structure-function analysis of a versatile peroxidase

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INTRODUCTION

White rot fungi are a family of basidiomycetes and degrade plant cell wall lignin. They produced ligninolytic enzymes, laccase (Lac), lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP). VP is produced by *Pleurotus* and *Bjerkandera* species [1,2] and has enzyme properties of both MnP and LiP, namely, oxidation of Mn^{2+} and high redox-potential aromatic compounds. Moreover, *P. ostreatus* MnP2, one of the VPs, can even oxidize directly high molecular-weight substrates such as RNase A and Poly R-478 [3]. In contrast, *Phanerochaete chrysosporium* LiP H8, a typical LiP, oxidizes directly veratryl alcohol (VA) at an exposed tryptophane residue (W171), however, it required appropriate mediators to oxidize high molecular-weight substrates with high redox potential. In MnP2, W170 was suggested to be the redox active site for VA, RNase A and Poly R-478 by a chemical modification of the enzyme [3].

In this study, we expressed four mutants of MnP2 (W170A, R263N, Q266F and V166/168L) using a homologous expression system and structure-function analyses were done.

MATERIALS and METHODS

Wild type (WT) and four mutants were expressed in *P. ostreatus* homologous expression system [4] and purified from the culture fluid. Purified proteins were analyzed in view of isoelectric point and molecular weight using IEF and SDS-PEGE. WT and four mutants were measured catalytic activity for low molecular-weight substrates (Mn^{2+}, H_2O_2 and VA) and K_m and V_{max} values were determined. Oxidizing activity for high molecular-weight substrate was evaluated by dimerization activity of RNase A (13.7 kDa).

RESULTS and DISCUSSION

Except for R263N, the molecular weight and isoelectric point of the mutants were the same as those of WT. While, possibly caused by a new N-glycosylation site was added, the molecular weight and isoelectric point were slightly different in R263N.

W170A was demonstrated to lose oxidizing activity for VA but the other low molecular-weight substrates was oxidized as did WT. Catalytic activity of Q266F and V166/168L was decreased only for high molecular-weight substrate RNase A. Comparing of a three-dimensional structure model of the two mutants (Q266F and V166/168L) with that of WT and LiP H8, there were steric hindrances around W170 of the mutants, which are similar to LiP H8. Therefore, we concluded that, in wild-type *P. ostreatus* MnP2, high molecular-weight substrates can access W170 where one electron is abstracted *via* long length electron transfer pathway, and that the access was blocked by the surrounding steric hindrances in Q266F, V166/168L and *Ph. chrysosporium* LiP H8.

REFERENCE

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