The Protein Redesign Approach to Modeling of Manganese Peroxidase

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Fungal degradation of lignin is an important process in the global carbon cycle.[1] Lignin is a polymer comprised of both phenolic and nonphenolic fragments. Manganese peroxidase (MnP) is one of two extracellular enzymes secreted by the white-rot fungus *P. chrysosporium*.[2] MnP catalyzes the initial one-electron oxidation of lignin and phenolic substrates by hydrogen peroxide. The ability of *P. chrysosporium* to degrade lignin and aromatic pollutants provided the impetus for extensive research into the structure↔function relationships of MnP.[3-13]

One method for studying structure↔function relationships in enzyme systems is protein redesign.[14] In protein redesign, the three-dimensional structure of an existing protein is used as a scaffold for the creation of a new enzyme. The protein model is created by loop- or site-directed mutagenesis. The protein redesign approach is particularly useful for proteins containing multiple metal centers and allows for characterization under physiological conditions.

Protein redesign was applied to the study of MnP. The scaffold for these studies was cytochrome *c* peroxidase.[15-18] C*c*P is easily overexpressed in *E. coli* and large quantities of C*c*P can be purified in a short time. The first protein model of MnP was created by engineering a Mn(II)-binding site into C*c*P.[15] A second protein model displaying similar characteristics was subsequently published.[16]

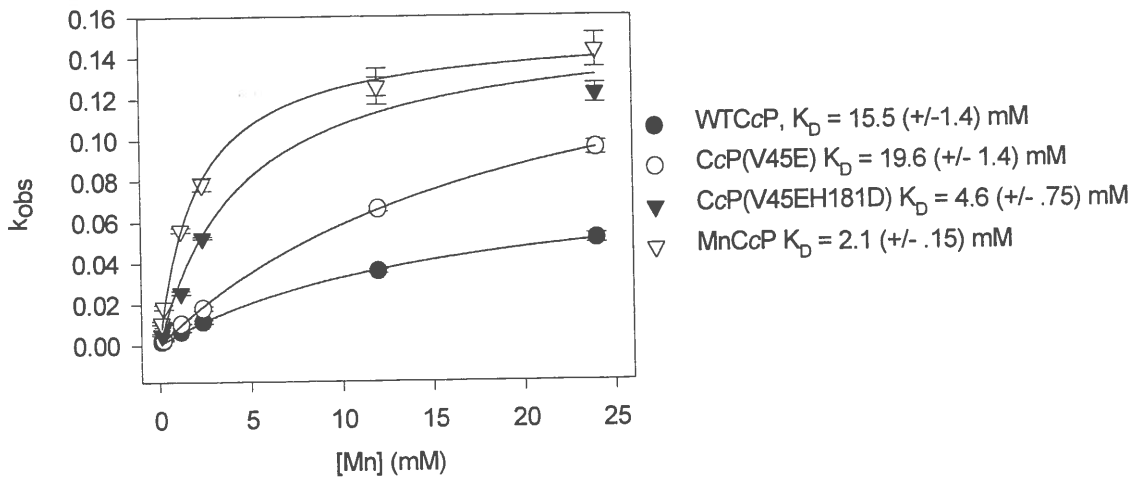

Figure 1.

The Mn(II)-binding site was created adjacent to the heme cofactor by incorporating three point mutations into CcP. The three mutations, G41E, V45E and H181D, replaced residues in C*c*P with the corresponding residues in MnP. MnC*c*P bound Mn(II) near the heme and facilitated the oxidation of Mn(II). A series of C*c*P mutants was constructed to determine the effect of the individual mutations used to create MnC*c*P. C*c*P(V45E), C*c*P(H181D) and

C*c*P(V45E,H181D) were constructed and kinetically characterized. The rate of compound II reduction by Mn(II) for various mutants is shown in Figure 1. The MnP activity of C*c*P increases in the following order: WTC*c*P ≅ C*c*P(H181D) < C*c*P(V45E) < C*c*P(V45E,H181D) < MnC*c*P. These results suggest each mutation used to create MnC*c*P plays a role in improving the MnP activity of C*c*P.

MnC*c*P provides a model through which the importance of structural characteristics of MnP can be addressed. One notable difference between C*c*P and MnP is the presence of redox-active amino acids in the active site. C*c*P contains Trp51 and Trp191 near the heme while the corresponding residues in MnP are phenylalanines.[3,19] Trp191 is the locus of the second oxidizing equivalent in C*c*P compound I'.[20] For MnP and other peroxidases, the second oxidizing equivalent in compound I is located on the heme as a porphyrin π-cation radical.

The active site tryptophans were removed from MnC*c*P in order to investigate the roles of Trp51 and Trp191 in MnP activity.[17] In C*c*P mutants without the Mn(II)-binding site, Trp191 and Trp51 greatly influence the lifetime of the porphyrin π-cation radical. The W51F, W191F, and W51F/W191F double mutations were incorporated along with the binding-site mutations (G41E,V45E,H181D) to create MnC*c*P(W51F), MnC*c*P(W191F) and MnC*c*P(W51F, W191F). Lineweaver-Burk plots for the steady-state oxidation of Mn(II) in 500 mM malonate, pH 5 are shown in Figure 2. The MnP activity observed varied between mutants and increased activity was observed for MnC*c*P(W51F,W191F) and MnC*c*P(W51F). The trend in activity reflects the extent of compound II stabilization present for the various mutants. MnC*c*P(W51F,W191F) is the most active protein model of MnP constructed to date.

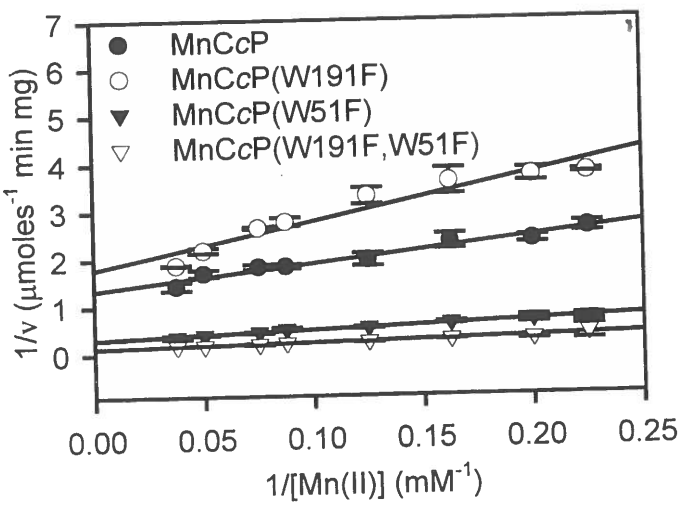

Figure 2.

References

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