Structural analysis of peroxidase from the palm tree *Chamaerops excelsa*

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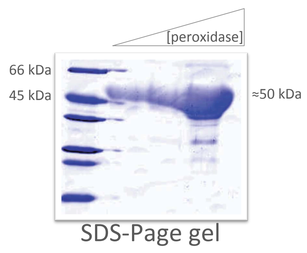
Abstract

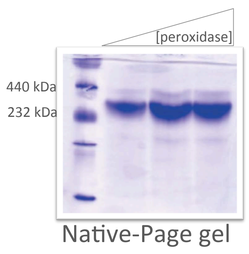
The diversification of energy production through the use of renewable resources is a major goal of the contemporary society. Although Brazil has exponent advances in ethanol production, the utilization of residual lignocellulosic biomass could ensure better energy efficiency within the second-generation ethanol production. As part of the biomass composition, lignin is the most recalcitrant substance for biochemical conversion to biofuels, and it cleavage require synergistic action of various ligninolytic enzymes, including those with high redox potential ligninolytic: oxidases, peroxidases and laccases. Peroxidases are involved in several physiological processes and many biotechnological applications, since they are able to oxidize a broad variety of organic and inorganic substrates. This group of enzymes, especially those from palm trees, is known to be very stable enzymes. To date, the structural and molecular reasons for such biochemical behavior have not been extensively explored. In order to identify the structural characteristics accountable for the high stability of palm tree peroxidases, we solved and refined X-ray structure of native peroxidase from the *Chamaerops excelsa* (CEP).

Enzyme purification

- CEP was purified from leaves of the palm tree *C. excelsa*
- Leaves were milled and homogenized in water
- Purification by phase separation and pheynil-Sepharose column
- Final buffer: 50 mM TRIS pH 8.0



SDS-Page gel



Native-Page gel

Oligomer

DLS studies:
- protein MW 99 ± 15 kDa
- monodisperse distribution

Crystallization, data collection and refinement

- Crystal were obtained using the hanging-drop vapor diffusion technique
- Crystalization condition: 170 mM ammonium sulfate; 85 mM TRIS pH 8.0; 17% PEG MME 2000; 15% glycerol
- The diffraction data collected at MX2 beamline (LNLS, Brazil)
- Structure resolution with molecular replacement, Phaser program (model PDB 3HDL, 83% of sequence identity)
- Refinement with Coot and Refmac 5

Parameters	Data statistics
Wavelength	1.4586
Space group	$P2_12_12_1$
Unit cell dimensions (Å)	70.18; 100.65; 132.31
Resolution (Å)	80.1-2.6 (2.7-2.6)
Nº of unique reflections	29,525 (5,779)
Mosaicity (º)	0.5
Multiplicity	4.3 (4.3)
Completeness (∞)	90.1 (91.8)
R_{merge} (%)	10.1 (55.3)
Mean I/σ(I)	10.5 (3.8)
R_{work}/R_{free} (%)	21.8/24.6
R.m.s.d. bond lenghts (Å)	0.021
R.m.s.d. bond angles (º)	1.760
Ramachandran outliers (%)	0
PDB ID	4USC

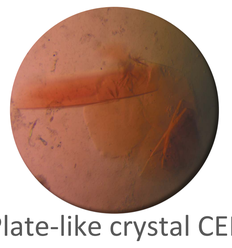
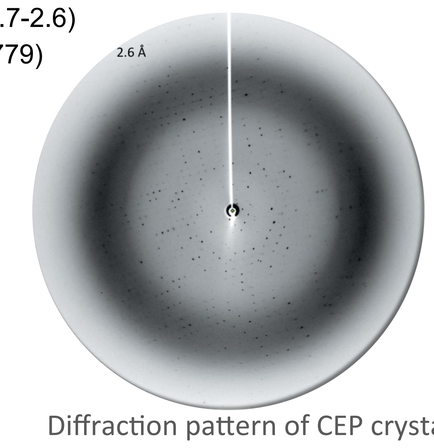
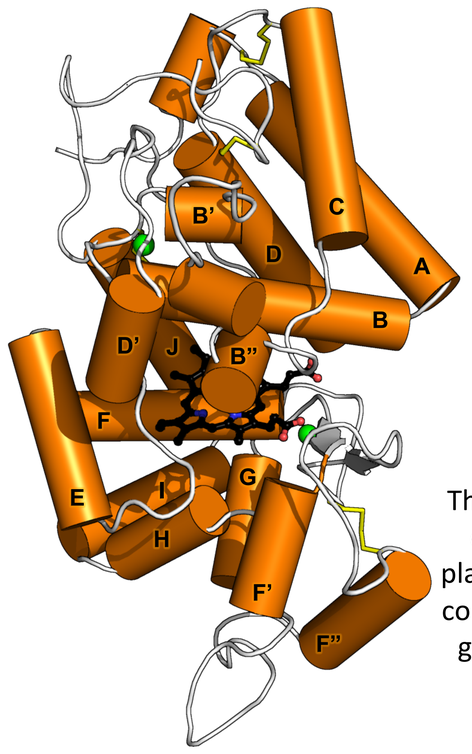


Plate-like crystal CEP



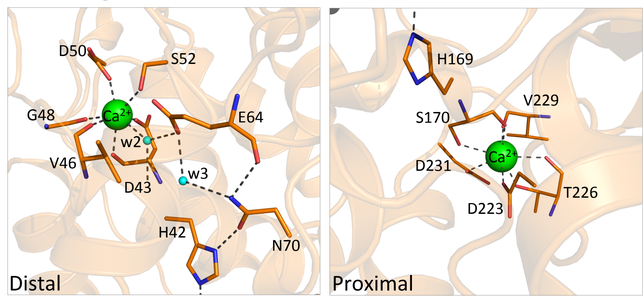
Diffraction pattern of CEP crystal extending to a resolution of 2.6 Å

Peroxidase three-dimensional structure

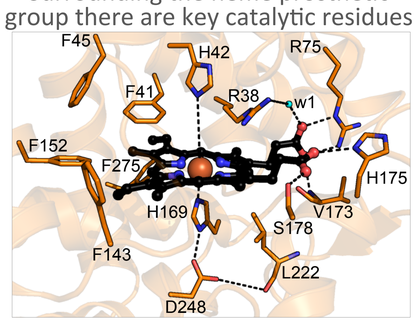


The CEP structure has an overall fold typical of plant peroxidases and the conservation of the heme group and calcium ions

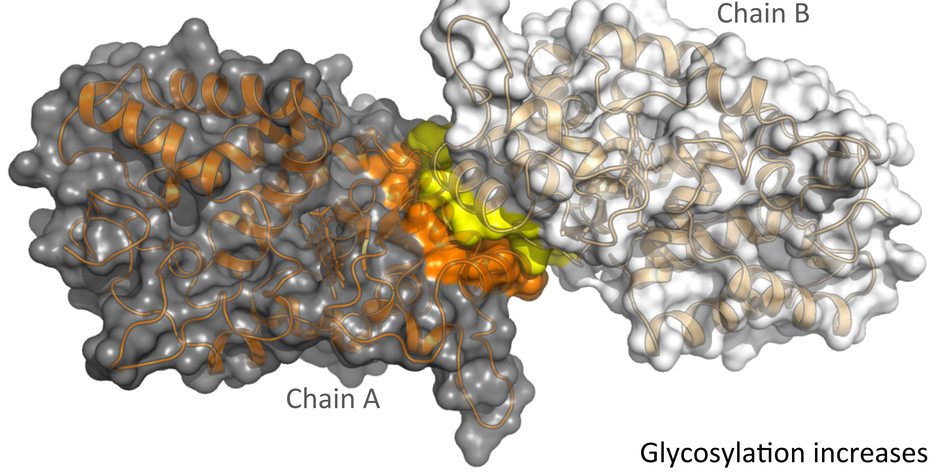
Ca^{2+} ions – coordination through extensive hydrogen-bonding network – formation of a functional active site



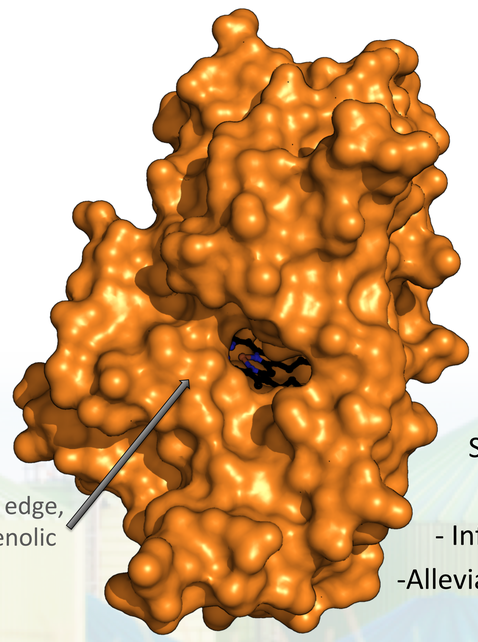
Surrounding the heme prosthetic group there are key catalytic residues



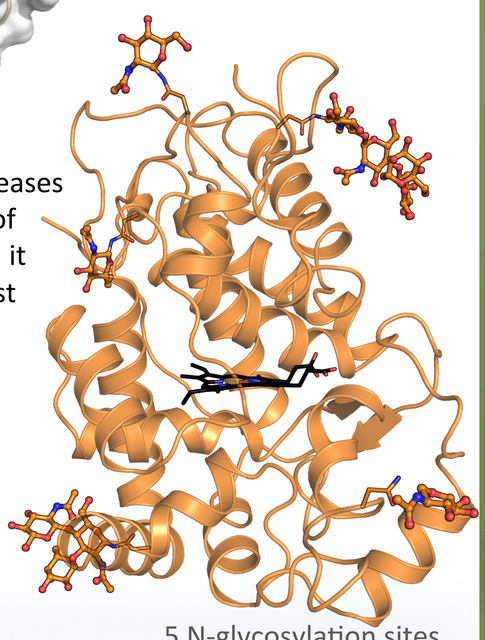
CEP is a highly stable enzyme over a pH-range and high temperature



This dimeric arrangement results in a more stable protein quaternary structure through stabilization of the regions that are highly dynamic in other peroxidases


The exposed heme edge, binding-site of phenolic substrates

Glycosylation increases the solubility of peroxidase and it stability against proteolysis


5 N-glycosylation sites

Several residues modifications in the vicinity of binding-site:
- Influence in the enzyme specificity
-Alleviate the enzymatic activity inhibition

Conclusions

- Native peroxidase was successfully extracted, purified, crystallized and it structure solved by protein crystallography
- Structure analysis revealed a possible dimeric assembly and several glycosylation sites that might explain the improved CEP stability
- Differences in the morphology of active site and heme edge access is related to substrate specificity and protection of the enzyme from inactivation
- Our results provide new insights into the structure-function relationships of plant peroxidases and their quaternary structures

References

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