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IN SILICO STRUCTURAL ANALYSIS OF PEROXIDASE IN *THEOBROMA CACAO*, A POTENTIAL ALTERNATIVE OF *RHAPANUS SATIVUS*

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Abstract

Peroxidase obtained from *Rhapanus sativus*, is the main source for this enzyme, and has been studied for its key role protecting cells from the adverse effects of free radicals. Since the yield of this enzyme is relatively low, we performed an *in silico* structural analysis of *Theobroma cacao* peroxidase, a plant that is widely distributed in Guatemala. In this study, the two enzymes were compared using homology modeling, RSMD and percentage of identity. We ascertain from this work that there are structural and activity similarities between the two peroxidases, suggesting that *Theobroma cacao* class III peroxidase, could be used as an alternative to *Rhapanus sativus* peroxidase as an antioxidant and anti-aging enzyme either in phytotherapeutics and phytocosmetics.

Keywords: *Peroxidase, Rhapanus sativus, Theobroma cacao, Homology model, in silico*

Introduction

Class III peroxidases are widely distributed and conserved as catalytic enzymes in plants.[1] They belong to the category of oxidoreductases, and catalyze redox-type bisubstrate reactions.[2] *Rhapanus sativus* peroxidase has been extensively studied, mainly due to its antioxidant properties, great stability, commercial use, applications in immunochemical techniques and simplicity to detect it.[3] However, the extraction process yields low active protein, so there is a need to search for alternative peroxidases that could be used in natural antioxidant products and enzymes for cosmetic uses.[4] *Theobroma cacao* is an abundant native crop in Guatemala, and it could be of commercial and phytocosmetic interest for a more stable peroxidase, with similar or better catalytic properties than *Rhapanus sativus* peroxidase.

Cacao, a widely distributed plant species in Latin America, presents little variability in the region, the sequence used in this study responds to the most widely cultivated variety. [5]

Cacao has been investigated for its multiple benefits, however it has not been approached with respect to its peroxidase.

Multiple sequence alignments and 3D analysis are a common tool in many molecular biology projects, and are often the result of extensive theoretical and experimental exploration of a certain family of proteins. The homology modeling allows to compare the structural differences and similarities of the two proteins, given an amino acid alignment. [6]Peroxidase is a very small protein with a hemoproteic center that facilitates its modeling and analysis [7].

Raphanus sativus peroxidase is extensively used for its antioxidant properties, great stability, and commercial applications in immunochemistry. Cacao peroxidase could be used in natural and cosmetic products as an antioxidant enzyme. *Theobroma cocoa* peroxidase may provide similar or better catalytic properties than those from *Raphanus sativus*.

Materials and Methods

The peroxidase sequence of *T. cacao* was retrieved from the UniProt Knowledgebase (UniprotKB-A0A061ET), a public domain for the collection of functional information on proteins. An extensive search on Protein Data Bank, using ModBase and ModIDB m was performed to obtain the 3-dimensional structure of *R. sativus*[5]. Swiss-Model, a protein structure homology-modelling server, was used with Software ProMod3 Version 1.0.1, to obtain a 4A5G-PDB template corresponding to *R. sativus*. [8,9,10]. A PDB file, was then created for *T. cacao* , and the software UCSF Chimera (Ver. 11.2) [12,13,14] was used to obtain the percentage of identity between the two peroxidases, and root- square-mean deviation (RSMD) was calculated. An alignment with the Smith-Waterman algorithm was also performed with the existing databases in NCBI [5].

A 4A5G-PDB template corresponding to *Rhapanus Sativus* was usted to create a PDB file.

Smith-Waterman algorithm was used to compare both aminoacid sequences with the existing databases in NCBI.

Results and Discussion

The homology (QMEAN -3.24 and 0.65 QMQE) and the identity (RMSD of 0.33) values are consistent with a small, conserved structure of the class III peroxidases. Figure 1 A, B and C. Both peroxidases share similarities in their glycosylation sites and the central position of the Hemo group especially in the Residue His 170 that is present equally in both structures coordinating forces of van of Waals with the iron. However, we found significant differences at the level of Arg 38 and His 42, which are key in the stabilization of the complex formed by the heme group in its catalytic function, Figure 2.

The molecular model was performed by homology using ProMod3 Version 1.0.1. The peroxidase obtained from the sequence of *Theobroma cacao*, showed values for QMEAN -3.24 and 0.65 QMQE. By using USFC Chimera Software, we obtained a 0.33 RMSD and a 33.67% of identity between the two enzymes.

The comparative sequences of *Rhapanus Sativus* peroxidase and *Theobroma cacao* differ in a high percentage; however a low RSMD explains a conserved structure of the class III peroxidases. Both peroxidases share similarities in their glycosylation sites and the central position of the Hemo group especially in the Residue His 170 that is present equally in both structures coordinating forces of van of Waals with the iron, reason why both structures could have similar roles in respect of the reduction of hydrogen peroxide.

The QMEAN and QMQE values confirm the quality of the model, as well as the Ramachandrand plot shows an acceptable model with residues within the defined theoretical spaces.

The sequence of peroxidases class III, is very short, which makes more acceptable its low percentage of identity, in addition to its 300 amino acids, the group Hemo in the small structure and its low standard deviation between the compared structure, explains that its operation is Similar in spite of their mutations found in the sequence.

For modeling by homologation, the potential structures for template did not exceed the template of radish so its similarity may be greater than with other peroxidases obtained in format PDB.

However, the main differences between both structures are in Arg 38 and His 42, which are key aminoacids for the stabilization of the complex formed by the heme group, and its activity against hydrogen peroxide.

Conclusions

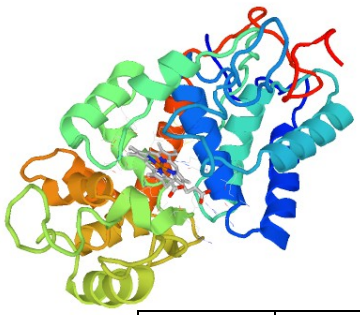
The *Theobroma cacao* peroxidase is very similar to Rhapanus Sativus RSMD 0.33A. Particularly the 4A5G.PDB with model homology created in this study. This warrants further research to verify if the in vitro activity of this enzyme is similar to or superior to *Rhapanus Sativus*.

References

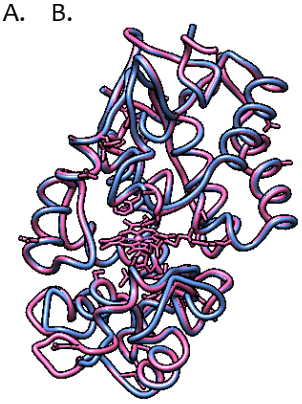
1. Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., & Matsui, H. (2001). A large family of class III plant peroxidases. *Plant and Cell Physiology*,*42*(5), 462-468.
2. Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A., & Pedreno, M. A. (2008). Class III peroxidases in plant defence reactions. *Journal of experimental botany*, *60*(2), 377-390.
3. Satar, R., & Husain, Q. (2009). Applications of Celite-adsorbed white radish (Raphanus sativus) peroxidase in batch process and continuous reactor for the degradation of reactive dyes.*Biochemical Engineering Journal*, *46*(2), 96-104.
4. McEldoon, J. P., & Dordick, J. S. (1996). Unusual thermal stability of soybean peroxidase. *Biotechnology progress*, *12*(4), 555-558.
5. Motamayor, J. C., Mockaitis, K., Schmutz, J., Haiminen, N., Livingstone III, D., Cornejo, O & Saski, C. (2013). The genome sequence of the most widely cultivated cacao type and its use to identify candidate genes regulating pod color. *Genome biology*,*14*(6), r53.
6. Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*, *22*(2), 195-201.
7. Bernardes, A., Textor, L. C., Santos, J. C., Cuadrado, N. H., Kostetsky, E. Y., Roig, M. G. & Polikarpov, I. (2015). Crystal structure analysis of peroxidase from the palm tree Chamaerops excelsa. *Biochimie*, *111*, 58-69.
8. Kopp, J., & Schwede, T. (2006). The SWISS-MODEL Repository: new features and functionalities. *Nucleic acids research*,*34*(suppl_1), D315-D318.
9. Kiefer, F., Arnold, K., Künzli, M., Bordoli, L., & Schwede, T. (2008). The SWISS-MODEL Repository and associated resources. *Nucleic acids research*, *37*(suppl_1), D387-D392.
10. Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*, *22*(2), 195-201.
11. Crystallographic Structure of the Raphanus Sativus Anionic Peroxidase, Gil-Rodriguez, P., Jimenez-Arroyo, N., Rojas-Trejo, S.P., Rudino-Pinera, E., Valderrama, B. Protein data bank doi 10.2210/pdb4a5g/pdb
12. Molecular graphics and analyses were performed with the UCSF Chimera package. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).
13. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.
14. Vargas RJ, Higueros RA, Saldaña DG, Xajil LY. DPP-IV Structural similarities in rat and human- Journal of Chemical and Pharmaceutical Research. 2015, 7(1):294-297

Figure 1: Quality of the model and comparison in three dimensions

A. B.

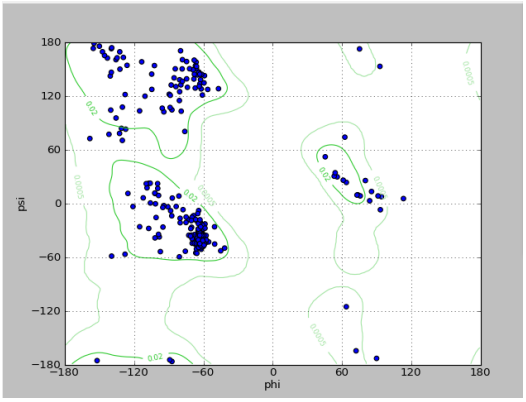


QMEAN	-3.24
QMQE	0.65

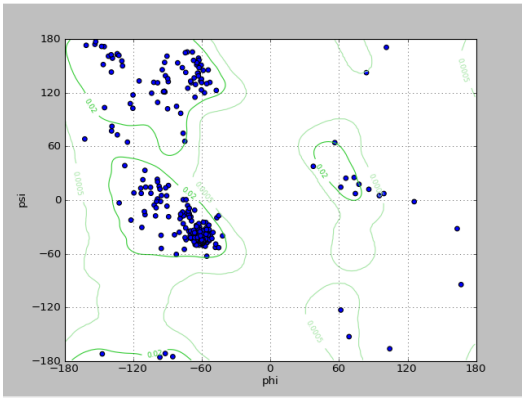


% Identidad	33.67
RMSD	0.33

C.

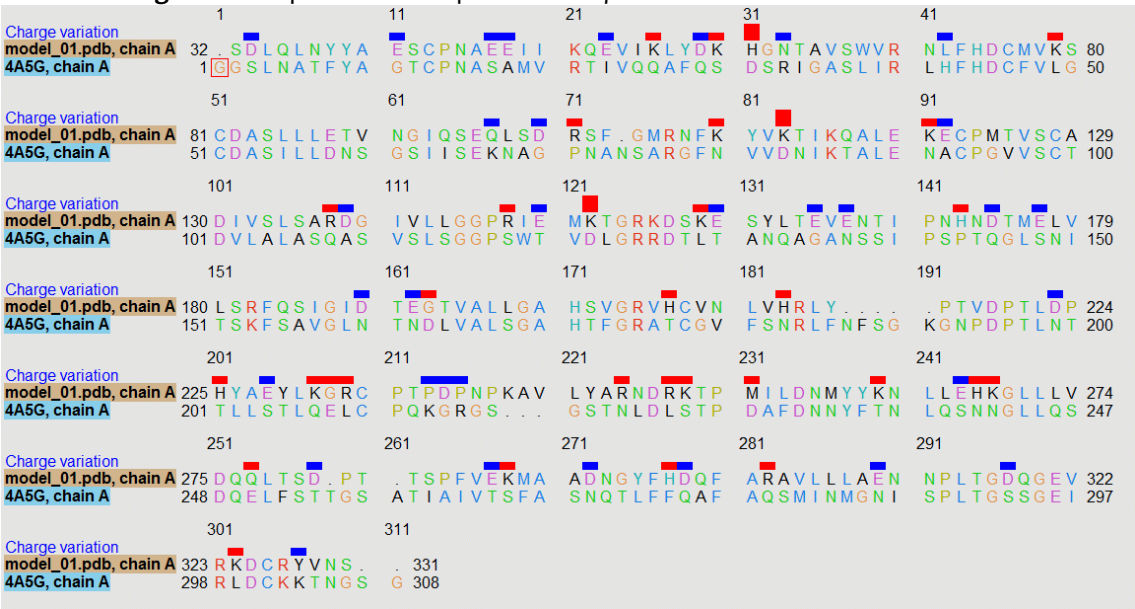


D.



A. The molecular model by homology with ProMod3 Version 1.0.1 of the peroxidase obtained from the *Theobroma cacao* sequence, and its values for QMEAN and QMQE. B. Alignment of 4A5G.PDB with the modeled peroxidase, it was observed that its three-dimensional conformation is similar with an RMSD of 0.33. C. The Ramachandrand plot for 4A5G.PDB. D. The Ramachandrand plot for the peroxidase modeled from the sequence of *Theobroma cacao* is observed.

Figura 2: Comparison the sequences of *Raphanus sativus* and *Theobrma cacao*



The alignment of the primary structure for 4A5G.PDB was observed. PDB with the peroxidase sequence of *Theobroma cacao.*