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In silico analysis of catalytic amino acid residues of friedelin synthase

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Abstract:

Quinonamethide triterpenes are derived from the pentacyclic triterpene friedelin and have shown various biological activities such as anti-inflammatory, antitumor, antimicrobial, anti-malarial, spermicide and antioxidant [1]. The catalysis of the triterpenes occurs via carbocation formation in 2,3-oxidosqualene molecule and cyclization followed by rearrengements in the pentacyclic triterpenic cations formed, depending on the product-specific oxidosqualene cyclases [1]. Oxidosqualene cyclase enzymes (OSCs) in plants compete for the same substrate to form tetra or pentacyclic triterpenes [2]. The friedelin is the only pentacyclic triterpene ketone and it has the maximum number of structural rearrangements [1]. To better understand the mechanism of friedelin production by friedelin synthase, we used *in silico* structural analysis of this enzyme to try to predict the residues located at the active site for further mutagenesis analysis. Molecular modelling and refinement of friedelin synthase was performed using Modeller and the human lanosterol synthase (PDB 1W6K) as template (primary sequence identity of 40%). A total of 100 models were built and evaluated for DOPE potential and the three best models were evaluated for their distribution of dihedral angles in the Ramachandran diagram and representativeness residues that constitute the active site by the arrangement of the hydrophobic and polar regions. The best model was submited to AutoDock Vina for lanosterol and friedelin docking in the active site. Table 1 shows the residues in the active site selected for mutation by analysis of the docking with PyMOL. The next steps aim the generation of mutants by site-directed mutagenesis and the analysis of the expression and production of mutants using the heterologous system of *Saccharomyces cerevisiae*. The products of the mutant enzyme versions will be evaluated by gas chromatography-mass spectrometer and modelling of the amino acid residues substituted will be generated to better understand the different products observed.

Table 1. Residues of amino acid in the active site selected for site-directed mutagenesis.

Amino acid	Mutation	Position	Observation
Trp	His	417	Study of the interaction between Trp417 and Tyr736 and between Asp484 and Trp417 on the accommodation of the lanosterol and friedelin in the active site
Phe	Trp	473	Study of the interaction between Phe473 and Trp612 on the accommodation of the lanosterol and friedelin in the active site
Asp	Glu	484	Study of the interaction between Asp484 and Trp417 on the accommodation of the lanosterol and friedelin in the active site
Trp	Tyr	534	Study of the accommodation of the lanosterol and friedelin in the active site
Leu	Phe	552	Study of the accommodation of the lanosterol and friedelin in the active site
Trp	Phe	612	Study of the interaction between Phe473 and Trp612 on the H ligation or pi stacking
Tyr	Phe	736	Study of the interaction between Trp417 and Tyr736 on the loss of the acceptor of the H ligation

References:

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