The structure of coniferaldehyde dehydrogenase from maize (Zea mays L) as a target for lowering the biomass lignification.

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INTRODUCTION. Residues of ferulic acid (FA) play an important role in the architecture of the cell wall, so that small reductions in its amount can significantly increase the digestibility of lignocellulosic biomass, favoring the production of cellulosic ethanol. The enzyme coniferaldehyde dehydrogenase (CALDH, E.C. 1.2.1.68) plays a key feature in the FA production. The objective of this work was to solve the structure of maize CALDH (ZmaysCALDH) and use it as a target to find a selective inhibitor which can be used to reduce the FA production in plants.

MATERIAL AND METHODS: Homology modeling was used to solve the monomeric Apo form of ZmaysCALDH. Docking procedures from a library of known ALDH inhibitors was performed with Autodock and Molegro softwares. Energy minimization with CALDH-Ligand complex was performed with NAMD followed by NPT Molecular Dynamics simulations from the docked monomer and homotetramer. In vitro enzymatic assays were performed with crude extract in the presence of substrate and inhibitor. The ferulate production was monitored by HPLC. RESULTS AND DISCUSSION: The amino acid sequence of ZmaysCALDH was confirmed by pBLAST and InterPro Scan. The sequence shares 53% identity with the human mitochondrial ALDH used as template. Docking assays selected Daidzin (CID107971) as the best candidate with a $\Delta G_{\text{binding}}$ of -9.3 Kcal/mol, lower than substrate coniferaldehyde, suggesting a competitive inhibition. Redocking of daidzin at minimized complex shows an rmsd < 0.6 Å indicating a well defined docking mechanism. Protein complex was stable during 20ns MD simulation and analysis strongly suggests that the tetramer is more stable than monomer. Enzymatic assays confirm the competitive inhibition of daidzin with $K_M$ 6.9µM and $K_I$ 2.4µM.

CONCLUSION: Computational approach was efficiently in the discovering of an inhibitor of ZmaysCALDH, and these results can have a direct biotechnological application in the field of biofuels.

Word keys: Coniferaldehyde dehydrogenase, biofuel, molecular docking
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