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Experimental and computational studies of chalcone synthase

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PURPOSE OF THE ABSTRACT
Polyketide synthases (PKS) are key enzymes for biosynthesis of flavonoid/isoflavonoid pathway in bacteria, fungi, and plants. PKS based on differences in nucleotide sequences, primary structure and catalytic mechanisms fall into three subclasses (type I,II, and III) [1, 2]. Chalcone synthase (CHS) is a member of type III, and are homodimers including two identical domains of 41-44 kDa polypeptide. CHS uses p-coumaroyl-CoA as a starter molecule and three malonyl-CoA as extender molecules to form a tetraketide intermediate that is cyclized into 2',4,4',6'-tetrahydroxychalcone (Chalcone). The chalcone scaffold is an essential for producing a wide range of biologically important compounds, that can act e.g. for antimicrobial, pollen fertility, biotic and abiotic stress management [3, 4]. These secondary metabolites also have a wide range of applications in industry, medicine and pharmacy, e.g. anticancer, antiparasitic, and antioxidant compounds [5]. Furthermore, CHS like other type III PKSs, due to the spacious binding pocket, can accept a wide range of substrates including aromatic and aliphatic CoA, and also thioesters non-physiological substrates. All of these applications make PKS as a target for bioengineering to improve its activity and yield. The binding pocket of CHS consists of substrate binding pocket, catalytic site, and cyclization site [4, 6]. Four residues (Cys167, His306, Asn339, and Phe218), which are highly conserved, form the catalytic center of CHS enzyme [4]. Derailment of intermediates on the path of chalcone production lead to production of side products, which can be a barrier for large-scale in vitro application of CHS. Specific activity of TaCHS was defined as micromole conversion of p-coumaorly-CoA (substrate) per minute under standard conditions per milligram of total protein. Specific activity was determined by conducting a spectrophotometric assay at 410λnm using Ellman reaction. The HPLC was used to determine the main product (chalcone naringenin) as main product and CTAL as by product.
In order to understand the role of key residues in the active site of enzyme, we performed covalent molecular mechanism-based docking with natural substrate (4-coumaroyl-CoA). Molecular docking studies revealed that Asn339 play an important role to stabilize the substrate. In addition, after three steps elongation with malonyl-CoA, the formation of the tetraketide intermediate alters the substrate conformation, thereby facilitating cyclization steps. As the substrate conformation changes, Phe218 and Phe268 act as gatekeepers to prevent the entry of additional malonyl-CoA and further elongation, and also navigate the substrate toward the cyclization residues. To identify the rate-determining step, we performed semi-empirical quantum chemical calculations using the PM7 method [7]. Based on calculations for the first intermediate, we can infer that the nucleophilic attack of Cys167 on the carbonyl with an activation energy of 27 kcal/mol is the rate-determining step.

FIGURES

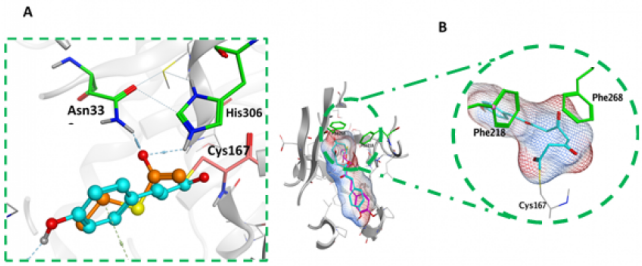

FIGURE 1

Covalent mechanism-based docking of CoA-ester substrates in the TaCHS enzyme

A) Catalytically competent docking pose; 4-coumaroyl-CoA and malonyl-CoA are shown in cyan and golden, respectively, with a ball-stick representation. Catalytic residues are displayed in green, while Cys167, which is covalently bonded to the substrate is

FIGURE 2

KEYWORDS

Polyketide synthases | molecular docking | Naringenin

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