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A unified mechanism for plant polyketide biosynthesis derived from in silico modeling

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ABSTRACT

The polyketide synthases found in a variety of plants and fungi provide a varied source of biologically active compounds of pharmacological and medicinal interest. Stilbene synthase and chalcone synthase catalyze the formation of a common tetraketide intermediate, but use different cyclization mechanisms to produce distinct and separate natural products. While key structural differences have been identified to explain this functional diversity, a fuller explication of the factors responsible for this mechanistic disparity is required. Based on the energetics of our models of the bound tetraketides, and our structural analysis of the active sites we propose that a key tautomeric conversion provides a mechanistic framework common to both cyclizations. A previously unidentified active water molecule facilitates cyclization in chalcone synthase through a Claisen mechanism. Such a "Claisen switch" is comparable to the previously characterized "aldol switch" mechanism proposed for the biosynthesis of resveratrol in stilbene synthase.

1. Introduction

Stilbene synthase (STS) and chalcone synthase (CHS) are plant polyketide synthases that catalyze the formation of a tetraketide intermediate (Fig. 1A) through successive additions of acetyl groups from malonyl-CoA to a p-coumaryl starter fragment. While STS and CHS share over 75% sequence identity STS cyclizes the tetraketide via an aldol condensation mechanism (Fig. 1B), yielding the hydroxystilbene resveratrol (RSVL, Fig. 1C), but CHS utilizes a Claisen condensation (Fig. 1D) of the same intermediate to generate chalcone (Fig. 1E), which can then be converted to the flavanone naringenin (Fig. 1F). In both enzymes the conserved residues Cys_{164}, Phe $_{215}$, His_{303} and Asp_{336} define an *active site* where the cysteine functions as an attachment site for the polyketide intermediate, while the asparagine and histidine facilitate polyketide extension through nucleophilic attack by the malonyl fragment. The residues Ser_{133}, Glu_{192}, Thr_{194}, Thr_{197} and Ser_{338} define a *binding pocket* that surrounds the p-coumaryl portion of the tetraketide. Finally Thr_{132}, Phe_{215}, Ile_{254}, Gly_{256}, Phe_{265} and Pro_{375} define the *cyclization site*, which facilitates the proper folding of the tetraketide either through the aldol (STS) or Claisen (CHS) condensation mechanisms. This site is also bounded by the Met_{137} residue from an adjoining monomer. Together these domains provide a cavity to facilitate and control chain elongation through successive acetylations of the bound coumaryl ligand. The growing polyketide is alternately attached and detached from the Cys_{164} residue by successive nucleophilic substitutions, and ultimately cyclized to the natural product.

Given that STS and CHS share substantial sequence identity without any significant insertions or deletions it is not unexpected that their mechanisms of product formation should be very similar. But while they do share a common method for ketide elongation, cyclization of the final tetraketide occurs using different mechanisms, namely the aldol condensation for the production of RSVL (Fig. 1B) and a Claisen condensation in CHS (Fig. 1D) that results in the production of either chalcone or nargenin. Several structural features have been identified that correlate with the observed selectivities. The most notable of these is what is termed an "aldol switch", a modulation of the cyclization mechanism mediated by an active water molecule unique to STS crystal structures [1]. Through hydrolysis of the thioesterase this active water facilitates the base catalysis necessary for the aldol mechanism. However no equivalent base capable of catalyzing the Claisen condensation has been identified, and in the absence of a discrete proton abstraction step the current mechanism for cyclization in CHS requires that "the intermediate itself provide the driving force for carbanion

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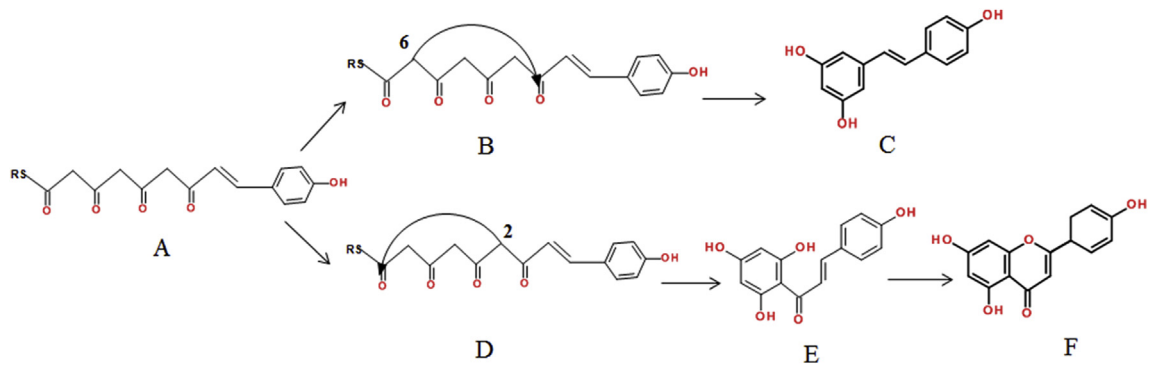

Fig. 1. The common tetraketide intermediate (A) cyclized via an aldol condensation mechanism (B), yielding resveratrol (C), and cyclized via a Claisen condensation (D) to generate chalcone (E), which can then be converted to the flavanone naringenin (F).

formation" [2]. The Phe_{256} residue, positioned between the binding pocket and the cyclization site, is thought to function as a "steric gate" [2], mediating polyketide elongation and cyclization. This residue is disorderd in the ligand-free STS crystal structure, but adopts a well-defined conformation when RSVL is bound, a conformation however distinct from that characterized in CHS structures [3]. Most dramatically, whereas in CHS the cyclization mechanism results in cleavage of the Cys_{16}-tetraketide thioester linkage, in STS the biosynthesis of RSVL involves separate thioester cleavage followed by cyclization and subsequent decarboxylation [1].

Because of its antioxidant and biomedical properties resveratrol has been co-crystallized with a variety of enzymes including transthyretin [4], quinone reductase [5], leukotriene-A4-hyrolyase [6], the cardiac regulatory protein troponin C [7] and, not surprisingly, stilbene synthase [3]. In all of these structures RSVL is planar or just slightly distorted from planarity. The notable exception is STS itself, where the dihedral angle between the π-bond and the aromatic rings is 60° (Fig. 2A), despite the fact that both NMR data and theoretical calculation confirm that the planar conformation is an energy minimum. Even a mutant chalcone synthase crystallized with a bound RSVL yields a conformation for RSVL with only a marginal, less than 3°, deviation from planarity [1]. Using the STS-bound RSVL ligand as a template we have developed a model for the STS-bound tetraketide intermediate. Quantum mechanical (QM) and quantum mechanical-molecular mechanical (QM-MM) calculations on this, and an analogously derived CHS-Bound tetraketide model has allowed us to develop a common mechanistic framework for the production of both the hydroxystilbenes and flavanones. Our analysis points to an energetically favored enol-keto tautomerism as a common element in both cyclizations, with the isomerism catalyzed by the previously characterized

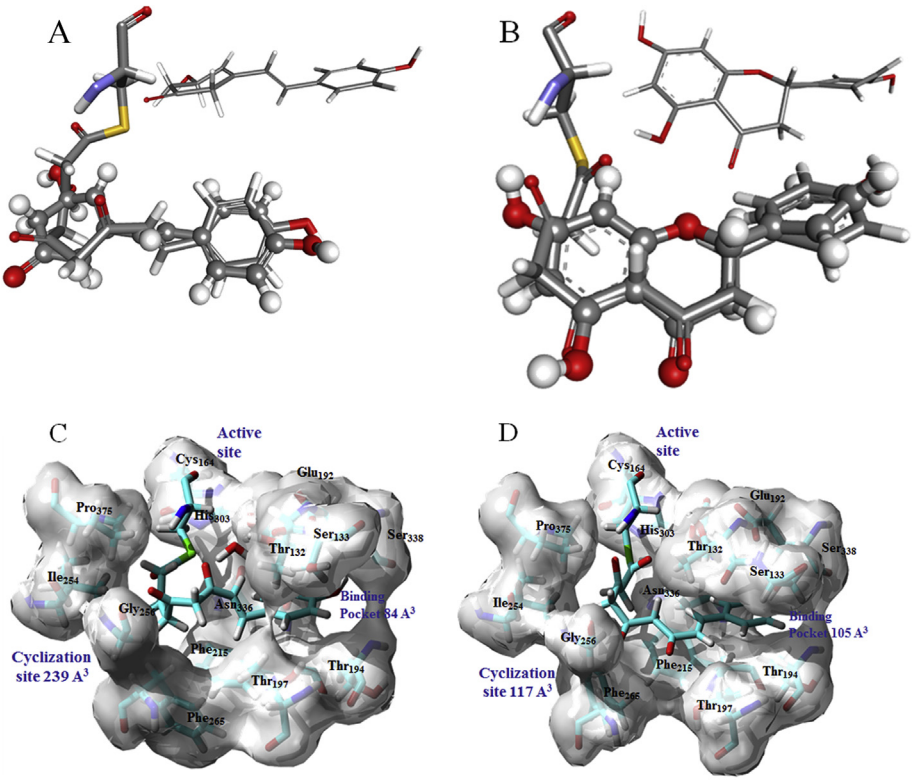

Fig. 2. Modeled structures for the bound tetraketide in STS (A) and CHS (B) are shown overlaid with the bound resveratrol and naringenin ligands, with the crystal structures for the ligands shown in inset; binding pockets, active sites and cyclization sites in STS (C) and CHS (D), showing the calculated cavity volumes for the cyclization sites and binding pockets.

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