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First principles model calculations of the biosynthetic pathway in selinadiene synthase

Susanta Das, Mudit Dixit, Dan Thomas Major *

Department of Chemistry and the Lise Meitner-Minerva Center of Computational Quantum Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel

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ABSTRACT

Terpenes comprise the largest class of natural products currently known. These ubiquitous molecules are synthesized by terpene synthases via complex carbocationic reactions, incorporating highly reactive intermediates. In the current study, we present a mechanistic investigation of the biosynthetic pathway for the formation of selina-4(15),7(11)-diene. We employ density functional theory to study a model carbocation system in the gas-phase, and delineate the energetic feasibility of a plausible reaction path. Our results suggests that during formation of selina-4(15),7(11)-diene, the substrate is likely folded in a conformation conducive to sequential cyclizations. We propose that a required proton transfer cannot occur intramolecularly in the gas-phase due to a high free energy barrier, and that enzyme assistance is essential for this step. Hybrid quantum mechanics-molecular mechanics docking studies suggest that enzyme intervention could be realized through electrostatic guidance.

1. Introduction

Terpenes constitute a ubiquitous class of natural molecules that are synthesized by terpene cyclases. Terpene synthases catalyze what are arguably the most complex chemical reactions in nature. Cyclic terpenoid compounds are generated from acyclic isoprenoid diphosphate substrates via intricate carbocation cyclization reactions. The terpenoid synthase chemical repertoire includes regio- and stereospecific ring formations, deprotonations to form double bonds, quenching of carbocations by water to form alcohols, and stereospecific hydride, proton, methyl, and methylene migrations.[1] Currently, more than 60,000 isoprenoids have been identified in terrestrial and marine plants, fungi, and bacteria, and their chemistry has been studied comprehensively.[2]

One of the first computational investigations into biosynthetic carbocation chemistry was performed by Jenson and Jorgensen.[3,4] In this pioneering work, the authors adopted a combined gas-phase quantum mechanics (QM) and molecular mechanics (MM) approach to model sterol biosynthesis. The reaction path in the gas-phase was mapped out employing ab-initio and density functional theory (DFT) calculations, while the effect of solvation was accounted for via MM Monte Carlo simulations. One of the main conclusions of this study was that ring formation in terpenes might be concerted processes. Another important conclusion was that the effect of non-polar solvents, mimicking the hydrophobic active site environment in terpenoid cyclases, did not significantly influence the gas-phase reaction energy profiles. The authors noted, however, that selective placement of nucleophilic groups and indole rings originating from the enzyme matrix, could readily shift the carbocation equilibrium. These conclusions, which rather remarkably were drawn in the absence of a crystal structure, remain largely true today, and constitute important aspects of control elements in terpene cyclases.[5] Later important work in the area of gas-phase modeling of terpenes includes that of Hess and co-workers on sesquiterpenes[6] and triterpenes,[7] and the extensive multifaceted work of Tantillo and co-workers.[8–11]

In the current work we focus on selina-4(15),7(11)-diene (Sd), which is synthesized by Sd synthase (SdS).[12] Selinenes are sesquiterpenes[13] found in a wide variety of plant sources, such as hops,[14,15] oranges,[16] and mango.[17] The proposed mechanism for selina-4(15),7(11)-diene, **3**, formation is shown in Scheme 1.

Following initial C–O bond cleavage of farnesyl diphosphate (FPP), **1**, to yield allyl cation **A**, a C1–C10 carbon bond formation ensues to give cation **B**. A subsequent deprotonation gives germacrene, **2**, which is a minor side-product in the enzymatic synthesis.[12] An additional carbocation, **C**, is formed via a protonation at C6, followed by a carbon bond formation between C2 and C7 to yield intermediate **D**. The final product selina-4(15),7(11)-diene, **3**, is obtained by deprotonation at the exocyclic C3 position.

An important ingredient of the above mechanism is an acid-base pair that can carry out the required proton shuffling between

* Corresponding author.
E-mail address: majort@biu.ac.il (D.T. Major).

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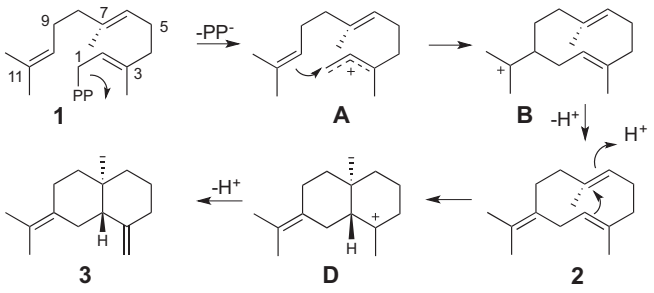


Scheme 1. Proposed biosynthetic pathway for selina-4(15),7(11)-diene, **3**.

positions C10 and C6. Inspection of the active site of SdS does not suggest a clear-cut candidate for this role. Hence, a compelling question regarding this proposed mechanism is the possibility of a direct proton transfer between C10 and C6. In the current work we perform model gas-phase QM calculations within the framework of DFT to shed light on this mechanistic question, as a prelude to a complete in-enzyme simulation study. Additionally, we perform hybrid QM/MM docking to elucidate the possible pose of the carbocation moiety during catalysis.

2. Computational details

2.1. Model electronic structure calculations in the gas-phase

In earlier studies, we have performed extensive benchmark calculations to establish a reliable, yet practical approach to terpene synthase modeling.[5,18–20] Based on these investigations of model carbocation reactions, we employ the meta-hybrid, M06-2X density functional[21,22] with a 6-31+G(d,p) basis set.[23] All integrations employed an ultrafine grid size. Optimization of reactants and transition states were performed using the Berny algorithm as implemented in the Gaussian 09 package.[24] Normal modes of vibration of the optimized molecule were carefully inspected to verify the nature of the stationary points. All the reported free energy profiles were computed using the electronic energy combined with zero-point and thermal corrections using standard statistical mechanics expressions within the harmonic approximation and with a vibrational scaling factor of 0.967.[25] Intrinsic reaction coordinate (IRC) calculations were performed to confirm that the transition structures connect reactants and products along the positive and negative directions of the chemical reaction coordinate.[26,27] In cases where different conformations were feasible, these were constructed and evaluated.

2.2. Hybrid classical and QM/MM docking studies

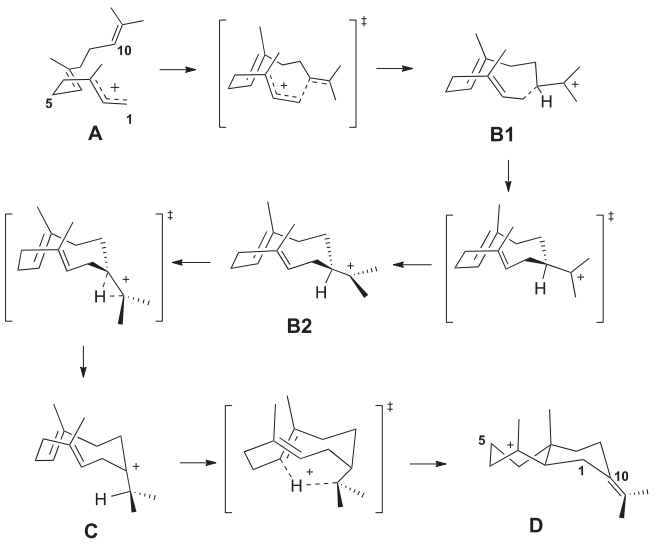
To estimate the bound conformation of the intermediate structure, **D**, we performed classical and hybrid QM/MM[28] docking studies. Initially, we performed classical docking using the CDocker method,[29] as implemented in Discovery Studio (Biovia, Inc.). Subsequently, the most favorable poses were subjected to QM/MM MD simulations using the CHARMM program.[30,31] Initially, 1 ns of QM/MM Molecular Dynamics simulations were performed, wherein the QM region was treated using the AM1 Hamiltonian[32] and consisted of the carbocation moiety only. The remaining enzyme-cofactor-solvent system was treated by the CHARMM force field.[33–35] Following this step, an additional 1 ps of QM/MM MD simulations were carried out, wherein the QM region was described by the M06-2X functional and included the carbocation, pyrophosphate moiety, and three Mg-ions. The remaining enzyme-solvent system was treated by the CHARMM force field. The former QM/MM simulations employed semi-empirical code embedded in the CHARMM package, whereas the latter simulations used CHARMM interfaced with the Q-Chem program.[36] In all the enzyme studies, the 4OKZ crystal structure was employed,[12] which corresponds to the closed form of the enzyme, and the system setup followed standard procedures.[5,18,19] Briefly, the enzyme was embedded in a water droplet of 24 Å of TIP3P water.[37] Water molecules that were within 2.6 Å of any enzyme, carbocation, crystal water heavy atom, PP or Mg^{2+} ions were deleted. All crystal water molecules beyond 24 Å of the reaction center were deleted. All protein atoms beyond the 24 Å sphere were fixed throughout the simulations, the atoms that were within a shell of 20–24 Å were treated by Langevin dynamics, whereas all atoms within a 20 Å sphere were treated by Newtonian MD.[38,39] In the Langevin region, the protein atoms were assigned a friction coefficient of 200 ps^{-1}, while for the water molecules the friction coefficient was set to 62 ps^{-1}. The temperature of the simulations was 298 K. The simulations employed the Leap-Frog integration scheme with a time step of 1 fs.[40] TIP3P water hydrogens were constrained using the SHAKE algorithm.[41] The non-bonded interactions were set to zero at distances beyond 14 Å. The electrostatic forces were shifted to zero from a distance of 12 Å, while the vdW interaction energy was switched to zero at 12 Å.

3. Results

3.1. Gas phase reaction

To gain an in-depth understanding of the reaction mechanism for the formation of Sd, **3**, by SdS, it is important to understand the inherent energetics of the chemistry involved.[42] Hence, we performed first-principles DFT gas phase calculations that can shed light on the possible reaction pathways in SdS biosynthesis.[8,18] Scheme 2 compiles the carbocation intermediates and associated transition states along the path from cation **A** to cation **D**, which is the immediate precursor to Sd, **3**.

The calculations commenced with a folded conformation of **A** (Fig. 1). In this state the C1–C10 distance is 6.32 Å, and the C1–C2 and C10–C11 π-systems are not fully aligned for reaction. The carbocation **B1** may then be formed by a C1–C10 single bond formation, and this step is endergonic by 3.08 kcal/mol. The C1–C10 bond distance is 1.71 Å and the C1–C11 distance is 2.27 Å, suggesting hyperconjugation in this intermediate carbocation.[43] The formation of **B1** proceeds via a transition state with a low free



Scheme 2. Reaction steps along the biosynthetic pathway for selina-4(15),7(11)-diene, **3**, investigated using DFT calculations in the gas-phase.