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Modeling catalytic mechanism of nitrile hydratase by semi-empirical quantum mechanical calculation

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ABSTRACT

Nitrile hydratase (NHase) is an important industrial enzyme capable of converting nitriles to corresponding amides. Utilizing the method of semi-empirical quantum mechanical (QM) calculation by TRITON, the bioconversion process of acrylonitrile to acrylamide catalyzed by NHase was successfully performed on a computer. Crystal structure of a Co-type NHase from *Pseudonocardia thermophila* JCM 3095 (PDB code 1IRE) was selected as the target for acrylonitrile autodock. *In silico* calculations were performed on the NHase–acrylonitrile complex to simulate the enzyme catalysis mechanism by quantitatively comparing energy changes of each reaction pathway. Simulation results showed that active site activation is the first step of NHase catalysis, in which the Co^{2+} coordinated to a water molecule forms a Co–OH complex mediated by the oxidized α-CEA113. Then the oxygen atom in the Co–OH attacks the C atom in the –CN triple bond of acrylonitrile, forming a precursor of acrylamide. Consequently, proton rearrangement happens transforming the precursor into the final product of acrylamide, under the assistance of the hydrogen atom in the –OH group of α-SER112. Gibbs energy changes of three steps corresponding to the active center activation, nucleophilic attack and proton rearrangement are around −31, 23 and −12 kcal/mol, respectively.

1. Introduction

Nitrile hydratase (NHase) [EC 4.2.1.84], an important industrial enzyme involved in microbial hydration of nitriles to corresponding amides, is a metalloenzyme containing either cobalt or iron in its catalytic center [1,2]. Based on this, NHases can be classified into two broad groups: Fe-type NHases and Co-type NHases. The representative Fe-type NHases existing in *Rhodococcus* sp. N-774 and *Pseudomonas chlororaphis* B23 were used for industrial acrylamide production as the first and the second generation catalyst, respectively. These NHases showed unique reactivity to light and nitric oxide (NO) [1,3]. To date, the third generation biocatalyst from *Rhodococcus rhodochrous* J1 has been developed for acrylamide production which contains a cobalt ion at its active center [4].

NHase consists of two subunits, α and β, without homology in amino acid sequence. All NHases contain a highly conserved active center of C–(T/S)–L–C–S–C in the α subunit, in which the Co-type NHases have threonine, whereas the Fe-type NHases have serine residue. Researchers have found that the tertiary structure of the NHase-active center forms an octahedron structure with either Fe or Co ion, mainly composed of four amino acid residues of the α subunit (αCys108, αCys111, αSer112 and αCys113) and two residues of the β subunit (βArg52 and βArg157) [5–7]. Generally, the ligand environments of the metal-ions in both Fe-type and Co-type NHases are similar. In the conserved active center with the common motif of CYS–(THR/SER)–LEU–CSD–SER–CEA, CYS was post-translationally modified into the oxidized forms of CSD, cysteine sulfuric acid (αCys111-SO_2H) and CEA, cysteine sulfenic acid (αCys113-SOH), respectively [8–10]. Site-directed mutagenesis confirmed that these three cysteine residues are essential for active expression of the cobalt-containing H-NHase (high molecular mass-nitrile hydratase) [11].

However, the mechanism of NHase catalysis remains unknown till now. Researchers have suggested three plausible mechanisms of catalysis which are designated as the inner-sphere mechanism, the outer-sphere mechanism and the second outer-sphere mechanism, respectively [3,5]. In the postulated inner-sphere mechanism, the nitrile binds to the metal ion directly and the metal-bound nitrile undergoes hydrolysis by a water molecule. In the outer-sphere mechanism, a hydroxide ion coordinated to the metal ion activates nitriles and attacks on the nitrile substrate. The second outer-sphere mechanism presents that the metal-bound hydroxide will activate another free water molecule from the

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second coordination shell, and this second water attacks the substrate nitrile. Most recently, Peplowski et al. performed auto-docking studies of nitriles and amides into a Co-type NHase [12]. Their analyses of relative positions of crystallographic waters and the best-docked acrylonitrile indicated that the outer-sphere mechanism is more probable [12]. Theoretical investigation by Hopmann et al. showed that based on the first-shell mechanism in which the nitrile substrate binds directly to the low-spin iron in the sixth coordination site, the generally suggested role of the Fe(III) center as Lewis acid, activating the substrate toward nucleophilic attack, is shown to be unlikely [13].

In this work, quantum mechanical calculation was performed to simulate the detailed procedure of nitrile hydratase catalysis by TRITON [14], a graphical software package for modeling enzymatic reactions, analyzing interactions between the active site residues and the substrate, and *in silico* constructing protein mutants. TRITON had been successfully used for the catalytic mechanism study of a haloalkane dehalogenase [15,16]. A novel reasoned catalysis mechanism of NHases was finally constructed including three major steps assigned as the active site activation, nucleophilic attack and proton rearrangement, respectively.

2. Methodology

The crystal structure used for generating the enzyme–substrate complex was obtained from Protein Data Bank (code 1IRE), which is a Co-type NHase from *Pseudonocardia thermophila* JCM 3095 [7].

The tertiary structure of acrylonitrile substrate was generated by software Discovery Studio 2.0 (Accelrys, Inc.). The docking of acrylonitrile into 1IRE was performed by AutoDock3.0.5, the most classical autodock software [17]. Hydrogen atoms were added into the structure file of 1IRE with water molecules remaining. Define the degree of freedom for acrylonitrile as zero, add standard Gasteiger charges as implemented in AutoDock, and generate the calculating grid with size of 80 Å × 80 Å × 80 Å. Distance of the grid node was 0.375 Å. The searching algorithm was LGA (Lamarckian genetic algorithm) with increments of 2.0 Å distance step-size and 10° angle step-size, 50 of searching individuals and 0.02 of mutating rate. Four pre-files, i.e. nitrile hydratase structure, acrylonitrile molecule structure, calculating grid and searching parameters, were used for docking to generate the nitrile hydratase-acrylonitrile complex. A series of binding-energy data were obtained and the conformation with the lowest binding energy was selected as the starting enzyme–substrate complex for following catalysis reaction calculations.

Enzymatic reaction modeling was performed using the TRITON software freely supported by the National Center of Biomolecular Research (http://ncbr.chemi.muni.cz/triton/) consisting of three modules of MOPAC, MODELLER and DRIVER [14]. To include the important Co^{2+} into the calculations, MOPAC7.0 in the original TRITON was renewed as MOPAC2006 [18] to perform the semi-empirical quantum mechanical (QM) calculations. Detailed simulation process was carried out as follows:

Step 1: Load into the structure model of the enzyme–substrate complex. Step 2: Specify the catalysis substrate including the acrylonitrile molecule, water molecule and the cobalt ion. Step 3: Specify functional groups composing the catalysis "cavity" based on the X-ray structure of the enzyme. We divided these active site groups into two types, i.e. the structural amino acids and the reactant amino acids. The structural amino acids assured the correctness of the key residues' conformations, while the reactant amino acids satisfy three constraints as follows: (1) belong to nucleophiles containing –OH group; (2) close to the active site; (3) locating in highly conserved region of NHases. The "cavity" scope was limited as 10 Å distance around the active site. Within this region, the functional groups were finally designated as α-CYS[108], α-CSD[111], α-SER[112], α-CEA[113] and β-TYR[69] which either involving in the octahedron formation of the active site or containing a hydroxyl group. Step 4: Search the reaction pathway of atoms taking part in catalysis. For a single reaction step, we need to specify two interacted atoms, interacting step length and final distance. In a real reaction, substrate will exhibit a trend moving toward the direction with preferable free-energy changing to transform into product, although all atoms are in the state of random thermal movement. For every postulated reaction pathway, there will generate an activation energy result indicating its feasibility. By comparing these energy results, we can revise the reaction pathway approaching to the real mechanism. Step 5: Specify the main chain atoms involving in the calculating "cavity" as immobilized groups to ensure the correct conformation of the catalysis center. Step 6: Determine the QM calculating parameters in MOPAC. The calculation method was chosen as the semi-empirical modified neglect of diatomic overlap (MNDO, the type of Hamiltonian used), the self-consistent field (SCF) equation was selected as conventional mode, the optimization method for the heat of formation minimization was BFGS, the increment step-size was 0.05 Å and the maximum running time was 10,000,000 s. The control parameters were set as GEO-OK, MMOK and PRECISE.

3. Results and discussion

3.1. Active site structure of NHase and docking of acrylonitrile

From the X-ray-observed crystal structure of cobalt-containing nitrile hydratase [7], it can be seen that there is a water molecule involved in the formation of the octahedron structure of the active center, together with amino acids of α-CYS[108], α-CSD[111], α-SER[112] and α-CEA[113], as shown in Fig. 1.

To start the enzyme reaction of NHases, the catalysis substrate (acrylonitrile) needs to be docked into the active center first, adjacent to the metal ion and the octahedron structure as illustrated in Fig. 1. In the docking process of the acrylonitrile molecule into the 3D structure of 1IRE, a suite of binding energy data were obtained ranging from −2.69 to −3.86 kcal/mol which corresponding to 10 distinct conformations of enzyme–substrate complexes. Consequently, the conformation with the lowest binding energy of −3.86 kcal/mol was selected as the starting

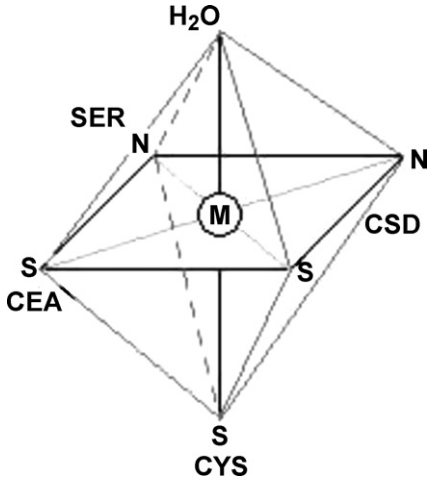

Fig. 1. Schematic octahedron structure of the active site of NHases [7]. The ligands to the cobalt atom (M) include a water oxygen atom, two main chain amide nitrogen atoms (N) (α-SER[112] and α-CEA[113]) and three sulfur atoms (S) of the α-CYS[108], α-CSD[111], and α-CEA[113], where CSD is the post-translationally modified cysteine-sulfunic acid and CEA is the post-translationally modified cysteine sulfenic acid.