Computational and Theoretical Chemistry 980 (2012) 85–91

Contents lists available at SciVerse ScienceDirect

Computational and Theoretical Chemistry

journal homepage: www.elsevier.com/locate/comptc

A general acid–general base reaction mechanism for human brain aspartoacylase: A QM/MM study

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ARTICLE INFO

Article history:
Received 25 October 2011
Received in revised form 9 November 2011
Accepted 9 November 2011
Available online 25 November 2011

Keywords:
Human brain aspartoacylase
QM/MM
Reaction path
Potential of mean force
Electrostatic interaction

ABSTRACT

Aspartoacylase is a crucial enzyme for the Canavan disease, which is a fatal autosomal recessive disorder that affects the central nervous system. In this work, we employed a hybrid quantum mechanical/molecular mechanical (QM/MM) approach to investigate the reaction mechanism of human brain aspartoacylase. Two possible pathways were considered: the promoted-water and the anhydride mechanisms. The calculated results indicate that the enzymatic reaction proceeds favorably through the promoted-water process with Glu178 serving as the general base and general acid. The free-energy calculations suggest that the first nucleophilic attack step is rate-determining with a barrier of 15.9 kcal/mol, which is consistent with experimental k_{cat} value. During the formation of the tetrahedral intermediate, the Zn^{2+} ion activates the zinc-bound water nucleophile and stabilizes the transition state by interacting with the substrate carbonyl oxygen atom. In addition, the Arg63 plays a key role in the transition state stabilization.

1. Introduction

Aspartoacylase (ASPA) belongs to the zinc-carboxypeptidase family and binds one zinc ion per monomer [1]. ASPA releases acetate and L-aspartate by deacylation of *N*-acetyl-L-aspartate (NAA) (Scheme 1), a highly abundant amino acid in the brain [2]. NAA is synthesized from L-aspartate and acetyl-CoA in the neuronal mitochondria of brain gray matter [3]. and it can react with glutamate to produce N-acetylaspartyl-glutamate (NAAG) in neuronal cytosol. NAA is transported to oligodendrocytes before being released from neurons to the cerebrospinal fluid [4,5]. The defect of the NAA degradative enzyme ASPA has been identified as the cause of Canavan disease (CD), which is an autosomal recessive neurodegenerative disorder that affects the central nervous system, characterized by dysmyelination and spongiform degeneration of white matter in children [3,6–8].

The high resolution structures of the rat form and the human form of ASPA have been determined in the apo form [9]. These structures confirm the presence of zinc in the enzyme and the identity of the zinc binding ligands. The zinc cofactor is coordinated by three protein ligands, namely His21, Glu24 and His116, mutation either of these three residues to Ala resulting in a no detectable activity [10]. Recently, the structure of hASPA in complex with a stable tetrahedral intermediate analog, N-phosphonomethyl-L-aspartate, has been determined by Le Coq et al. [11]. It was observed that, in this structure, the phosphonamidate oxygens of the intermediate analog coordinate directly with the catalytic zinc. In addition, one of the oxygens of the phosphonamidate interacts with Arg63, while the other oxygen forms hydrogen bond with Glu178. The nitrogen, the α-carboxylic group and the β-carboxylic group form an extensive network of interactions with the enzyme. These interactions are believed to play an important role in both substrate binding and catalysis and mutations of any of these substrate binding groups have a significant impact on the catalytic efficiency of hASPA [11]. On the basis of the observed enzyme-intermediate analog complex and the remarkable similarity of the active site of hASPA (Scheme 2) to those of thermolysin (TLN) [12] and carboxypeptidase A (CPA) [13], Le Coq et al. proposed a promoted-water enzymatic reaction mechanism to hASPA, in which the zinc-bound water is activated by the essential Glu178 served as the general base and attacks the acetyl carbonyl carbon to generate the tetrahedral intermediate (TI). Protonation of the amino group by Glu178 leads to collapse of the TI and cleavage of the C–N bond, then form of the acetate and L-aspartate [11].

As no structure about any complexes of hASPA with bound substrates or products was reported in literature, our previous molecular dynamics (MD) simulation [14] predicted the Michaelis complex of hASPA with its substrate NAA and the putative nucleophilic water molecule by using the hybrid quantum mechanical/molecular mechanical (QM/MM) approach based on the self-consistent charge-density functional tight-binding (SCC-DFTB) model,

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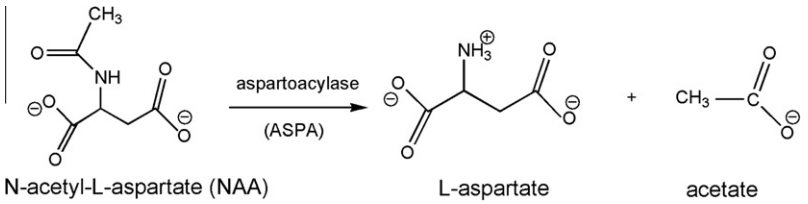
doi:10.1016/j.comptc.2011.11.023



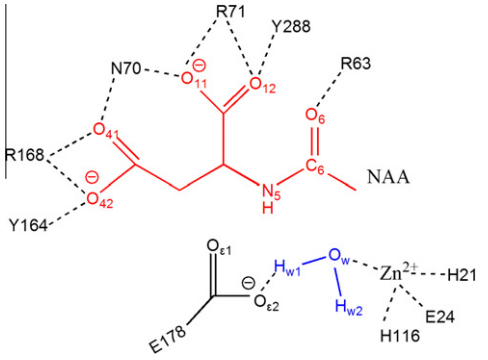
Scheme 1. Deacetylation of *N*-acetyl-L-aspartate (NAA) catalyzed by aspartoacylase (ASPA).

revealing the well organized active site of hASPA. Furthermore, we used the density functional theory B3LYP/6-31G(d) method and the truncated active-site model to obtain the energy profile for the promoted-water mechanism of hASPA [14]. However, in that model study, the calculated energy barrier is 28.8 kcal/mol [14], being much higher than that of the experimental observation [1]. So, it is necessary to perform the further investigation for the catalytic deacylation mechanism of hASPA including the enzyme environment. On the other hand, the QM/MM studies about the hydrolysis reaction catalyzed by CPA that has the similar active site to hASPA were reported, where an alternative anhydride mechanism was examined [15,16]. These investigations inspire us to question whether the anhydride pathway also takes place in hASPA system, besides the promoted-water mechanism? And if they are compatible, what is the preference of the two possible ways?

In this study, we combined QM/MM MD simulations and potential of mean force (PMF) calculations to explore the atomic details and energetics of the catalytic mechanism of hASPA. Two proposed mechanisms: the promoted-water and the anhydride pathways were tested (see Scheme 3). Molecular dynamics simulation of biomolecules provided the detailed atomistic information about the effects of the protein environment on the energy profile of the NAA hydrolysis reaction of hASPA that is often difficult to obtain directly from experiments. The result of the free energy calculation agrees with the experimental study [1]. In addition, an electrostatic interaction analysis was performed to estimate the key role played by Arg63.

2. Methods

The combined QM/MM approach is considered as a useful tool to understand characteristics of the enzyme systems [17–22] and it is a simulation approach which combines accuracy of QM and efficiency of MM. In this work, the QM region was treated by the recently developed approximate density functional method, namely the self-consistent charge-density functional tight binding (SCC-DFTB) method [23] and the MM region by the CHARMM all atom force field [24]. The SCC-DFTB method is an approximate DFT method and it is much more efficient than ab initio QM/MM approaches. The SCC-DFTB model has been extensively tested [25–28] and applied successfully to several zinc enzymes including cytidine deaminase [29], carbonic anhydrase [30,31], lethal factor (LF) [32] and CPA [15,16]. To ensure the accuracy of the SCC-DFTB/MM results, we have also performed single point calculations or minimizations at the DFT/MM level. The DFT/MM calculations were performed using CHARMM interfaced with GAMESS-US package [33], and the DFT level is B3LYP/6-31G(d).



Scheme 2. Arrangement of the active site for the ES complex of hASPA and atom definition.

Our model is based on the recent X-ray structure of hASPA complexed with the methylphosphonamidate inhibitor bound in the active site (PDB code 2O4H) [11]. Since our previous MD simulation of Michaelis complex supported the promoted-water mechanism, for studying this pathway, the starting configuration was adopted from our previous MD simulation [14]. For studying the anhydride pathway, the procedure of building model is the same as that our previous constructing Michaelis complex [14], but the water nucleophile was removed. In short, the model structure was solvated in a pre-equilibrated sphere of TIP3P waters [34] with 25 Å radius centered at the zinc atom, subjected to stochastic boundary conditions [35]. The reaction region was a sphere with radius R of 22 Å, and the buffer region had R equal to 22 Å $\leqslant R \leqslant$ 25 Å. The atoms outside the 25 Å were deleted.

Unless stated otherwise, all simulations were performed with CHARMM with a SCC-DFTB interface [36]. The QM subsystem includes the zinc atom, the coordinated ligands (His21, Glu24, His116), and the chemically active moieties (Glu178, the water nucleophile, substrate NAA). For the anhydride pathway, there is no water nucleophile in the QM region. The interface between the QM and MM regions was approximated using link atoms [17] which were added to C_β atoms of the protein residues.

The QM/MM MD simulation of the enzyme substrate (ES) complex for the anhydride pathway was carried out for 500 ps after 100 ps equilibration, in which the starting system was brought to 300 K. The integration step for MD trajectories was 1 fs, and the SHAKE algorithm [37] was applied to restrain the covalent bonds involving hydrogen atoms.

Minimal energy paths (MEP) were calculated using adiabatic mapping along putative reaction coordinates. For the promoted-water pathway, the reaction coordinate for the nucleophilic addition (NA) of the water nucleophile is given by the distance between the water oxygen (O_w) and the substrate carbonyl carbon (C_6): $r1 = d_{O_w-C_6}$ .The corresponding reaction coordinate for the elimination (E) of the leaving group is given by a combination of N_5–H_{w1} and C_6–N_5 distances: $r2 = d_{N_5-H_{w1}} - d_{C_6-N_5}$. For the anhydride pathway, the NA reaction coordinate is given by the distance between a Glu178 oxygen ($O_{\varepsilon 2}$) and the substrate carbonyl carbon (C_6): $r1' = d_{O_{\varepsilon 2}-C_6}$.

The potential of mean force (PMF) was computed using umbrella sampling [38] with the MEP structures along a putative reaction coordinate as the initial structures. The harmonic force constant ranged from 100 to 350 kcal/(mol Å^2). 12 and 17 windows were employed for the NA and E steps for the promoted-water pathway, respectively. On the other hand, for the anhydride pathway, the