Chemico-Biological Interactions 187 (2010) 220–224

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Reaction profiles of the interaction between sarin and acetylcholinesterase and the S203C mutant: Model nucleophiles and QM/MM potential energy surfaces

Jeremy M. Beck, Christopher M. Hadad*

Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Available online 13 February 2010

Keywords:
Nerve agent
Acetylcholinesterase
Phosphonylation
QM/MM

ABSTRACT

The phosphonylation mechanism of AChE and the S203C mutation by sarin (GB) is evaluated using two reaction schemes: a small model nucleophile (ethoxide, $CH_3CH_2O^-$) and quantum mechanical/molecular mechanical (QM/MM) simulations. Calculations utilizing small model nucleophiles indicate that the reaction barrier for addition to GB is the rate-limiting step for both ethoxide and ethyl thiolate ($CH_3CH_2S^-$); moreover, the activation barrier for addition to the phosphorus center of GB by ethyl thiolate is significantly larger (13.2 kcal/mol) than for ethoxide (8.3 kcal/mol). The decomposition transition state for both nucleophiles was determined to be ~1 kcal/mol. QM/MM simulations for AChE suggest a similar reaction mechanism for phosphonylation of the catalytic S203; however, the relative energetics are altered significantly compared to the isolated system. QM/MM results indicate that formation of the penta-coordinate intermediate is the rate-limiting step in the enzymatic system, with an activation barrier of 3.6 kcal/mol. Hydrogen-bonding interactions between the fluoride leaving group of GB with Y124 in AChE are observed throughout the reaction profile. The S203C mutation alters the relative energetics of the reaction, increasing the energy barrier for formation of the penta-coordinate intermediate to a value of 4.5 kcal/mol; moreover, the penta-coordinate intermediate (as product) is stabilized by an additional 6 kcal/mol when compared to wild-type AChE.

1. Introduction

Nerve agents, a subfamily of organophosphorus compounds (OPs), have a history of utilization as chemical weapons and insecticides. There are two main families of chemical weapons, described as the G-series, and V-series nerve agents. The main structural difference between these two families is that G-series nerve agents generally contain a small fluoride leaving group, while the V-series nerve agents possess a larger amino-thiolate leaving group. These compounds exert their toxicity via the inhibition of acetylcholinesterase (AChE), leading to a buildup of acetylcholine and resulting in overstimulation of cholinergic receptors. The active site of acetylcholinesterase is the typical serine hydrolase catalytic triad, comprised of Ser203, His447, and Glu334 (numbering for human AChE) [1]. OPs inhibit AChE through phosphylation of the catalytic serine and the formation of a stable OP-AChE adduct (Fig. 1), thereby resulting in a P–O bond that is resistant to cleavage by water molecules in the gorge. Although enzymatic function can be regenerated by cleaving the P–O(Ser) bond using a stronger nucleophile, most commonly an oxime [2], reactivation of the native serine is complicated by inefficient reactivation by oximes for specific OPs; frequently, the efficacy of an oxime is correlated to a specific OP nerve agent. In addition, the OP adduct can undergo dealkylation (aging) which renders the enzyme unreactivatable by common methods [3].

Due to the difficulty in regenerating cholinesterase activity, a detailed study of the enzymolysis of OPs was undertaken previously with the hopes of creating a mutant enzyme capable of catalyzing the hydrolysis of nerve agents [4–6]. One of the more interesting mutations, a substitution of the catalytic serine for cysteine (S203C) was found to render the protein inactive against CMP [7], an OP with a coumarin leaving group. A chemically similar mutation (Ser/Cys to selenocysteine) in other enzymes has resulted in increases of 100–500× activity over the corresponding wild-type enzyme [8]. It is, therefore, not clear why the substitution of a stronger nucleophile in the form of cysteine resulted in an inactive enzyme for the S203C mutant.

The hydrolysis of OPs has a long history of study, both experimentally and computationally, that suggests the hydrolysis of nerve agents proceeds via an addition–elimination pathway, forming a stable penta-coordinated phosphorus intermediate [9–13]. Along this pathway, there are two main transition state structures: the first corresponds to the association between nucleophile and OP, which forms a penta-coordinate intermediate, and the second transition state corresponds to cleavage of the OP-leaving group bond, followed by dissociation of the complex. Depend-

* Corresponding author. Tel.: +1 614 688 3141; fax: +1 614 292 1685.
E-mail address: hadad.1@osu.edu (C.M. Hadad).

0009-2797/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2010.02.012

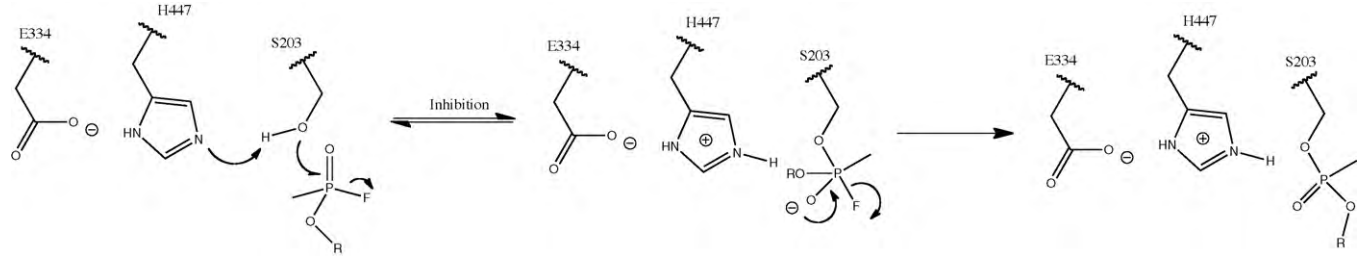

Fig. 1. Proposed mechanism for inhibition of AChE by OPs.

ing on the OP, there may also be additional transition states that involve reorientations of the *O*-alkyl substituent. Predicted solvent effects on the potential energy surface vary between studies, with some studies suggesting solvation increases the association barrier while decreasing the decomposition barrier [9], and another predicting solvation will decrease both barriers [10]. The majority of these studies have not optimized the OP in an implicit polarizable continuum model (PCM) solvent representation; instead, they have computed single-point energy evaluations with implicit solvation using the gas-phase geometries, which may be the cause of this disagreement. However, the majority of computational studies indicate that the association between nucleophile and OP is the rate-limiting step, and have accurately reproduced the observed experimental ~9 kcal/mol energy barrier of GB [14].

Several studies have expanded beyond aqueous hydrolysis in order to study the reaction at the catalytic site, using small fragments of the active site [15], as well as the full enzymatic environment through quantum mechanical, molecular mechanical (QM/MM) calculations [16–18]. These studies predict the presence of a stable penta-coordinate intermediate, similar to the isolated systems. However, studies that utilize a full enzymatic approach suggest that, in the active site, decomposition of the intermediate is the rate-limiting step, at least for the acylation/deacylation of AChE [16].

This study aims to determine the potential energy surface for phosphonylation of the AChE active site by GB, as well as to clarify the roles that solvation and active-site residues play in this reaction. Results will be compared to the potential energy surface for aqueous hydrolysis of GB by both ethoxide ($CH_3CH_2O^-$) and ethyl thiolate ($CH_3CH_2S^-$). In addition to wild-type (wt) AChE, the previously studied S203C mutant [7] is investigated in order to elucidate the cause of the experimentally observed inactivity.

2. Computational methods

Calculations were carried out using ethoxide as a model nucleophile. Calculations were completed with the Gaussian 03 [19] suite of programs for the gas-phase optimizations, and in Gaussian 09 [20] for the condensed-phase optimizations. The B3LYP hybrid density functional [21] and a 6-31+G* basis set [22] were utilized for all geometry optimizations, as double zeta basis sets have been shown to be sufficient for geometry optimizations along the OP hydrolysis reaction coordinate [12]. For condensed-phase optimizations, Tomasi's polarizable continuum model [23] was utilized, with atomic radii taken from Cramer's and Truhlar's SMD solvation method [24]. Transition state geometries were optimized, and vibrational frequency calculations confirmed each structure was a single-order saddle point on the potential energy surface. Each transition state was propagated by 10% along the imaginary vibrational frequency in both directions, and the resulting geometries were minimized to obtain the local minima connected by the transition state. Single-point energy evaluations were carried out on relevant structures using the B3LYP method with a larger 6-311+G** basis set for more accurate energetics. Energies presented herein will be ΔG (298 K) with thermal and entropic corrections to the free energy as obtained from the B3LYP/6-31+G* vibrational frequency calculations.

For the QM/MM simulations, the structure for human acetylcholinesterase was constructed using the crystal structure 1B41 [25]. Missing loops in the protein were modeled based on homology to electric eel AChE [26]. Missing sidechain atoms were replaced using the xLEaP module of AMBER [27], and the structure was then minimized. The two crystallographic waters bridging Glu202 were retained in the structure. While the role of these two waters appears to be structural, in that they stabilize the orientation of the Glu202 sidechain, the two water molecules may play an indirect role in the potential energy surface. Glu202 has previously been shown to greatly destabilize the acylation transition state for acetylcholine hydrolysis by 7.4 kcal/mol through electrostatic interactions [28]. To corroborate this possibility, initial testing revealed that the relative energetics are largely unchanged between treating the two waters using QM and MM treatments, suggesting that their roles in the studied potential energy surface are mainly structural, and therefore the MM treatment is suitable. Protonation states of titratable residues were determined for a pH of 7.0 using the program PDB2PQR [29]. The orientation of the GB-AChE intermediate was added to the catalytic site based on the orientation of GB in the GB-AChE product from crystal structure 2JGG [30], and by comparison to our model system's geometries.

QM/MM optimizations were then carried out using ChemShell [31] as an interface to Turbomole (v5.10) [32] for the QM calculations, and ChemShell's internal version of DL_POLY [33] for the MM treatment using the CHARMM force field [34]. Relaxed potential energy surface scans were carried out by varying the reaction coordinate, defined as the difference between the P–O(Ser) and P–F bonds, and optimizing all other degrees of freedom at each step of the fixed reaction coordinate. The QM layer was selected to include Glu202, Ser203, His447, Glu334, and the OP, and was treated using RI-BLYP [35] with an SV(P) basis set, which is comparable to the 6-31+G* basis set used in calculations for the isolated system [36]. All atoms within 15 Å around the GB-Ser203 adduct were allowed to optimize in the MM treatment. The rest of the protein was held rigid. An electrostatic embedding scheme coupled the MM layer to the QM system, allowing the QM system to polarize due to electrostatic influences of the protein environment. Single-point energy calculations were carried out on each point using BLYP with a TZVP basis set. An identical method was utilized to determine the potential energy surface of the S203C mutant, after replacing the Ser203 with a cysteine residue. Due to the inherent difficulty of calculating vibrational frequencies of an enzyme, the QM/MM energetics will be presented as ΔE values in units of kcal/mol.

3. Results and discussion

3.1. Model systems

A three-step reaction profile was determined for the hydrolysis of GB by ethoxide, with transition states corresponding to the asso-