Journal of Molecular Graphics and Modelling 29 (2011) 1039–1046

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Probing the reactivation process of sarin-inhibited acetylcholinesterase with α-nucleophiles: Hydroxylamine anion is predicted to be a better antidote with DFT calculations

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ARTICLE INFO

Article history:
Received 8 February 2011
Received in revised form 22 April 2011
Accepted 27 April 2011
Available online 6 May 2011

Keywords:
Reactivation
OP-inihited AChE
Alpha-nucleophiles
Density functional calculations
Hydroxylamine anion

ABSTRACT

Inactivation of acetylcholinesterase (AChE) due to inhibition by organophosphorus (OP) compounds is a major threat to human since AChE is a key enzyme in neurotransmission process. Oximes are used as potential reactivators of OP-inhibited AChE due to their α-effect nucleophilic reactivity. In search of more effective reactivating agents, model studies have shown that α-effect is not so important for dephosphylation reactions. We report the importance of α-effect of nucleophilic reactivity towards the reactivation of OP-inhibited AChE with hydroxylamine anion. We have demonstrated with DFT [B3LYP/6-311G(d,p)] calculations that the reactivation process of sarin–serine adduct 2 with hydroxylamine anion is more efficient than the other nucleophiles reported. The superiority of hydroxylamine anion to reactivate the sarin-inhibited AChE with sarin–serine adducts 3 and 4 compared to formoximate anion was observed in the presence and absence of hydrogen bonding interactions of Gly121 and Gly122. The calculated results show that the rates of reactivation process of adduct 4 with hydroxylamine anion are 261 and 223 times faster than the formoximate anion in the absence and presence of such hydrogen bonding interactions. The DFT calculated results shed light on the importance of the adjacent carbonyl group of Glu202 for the reactivation of sarin–serine adduct, in particular with formoximate anion. The reverse reactivation reaction between hydroxylamine anion and sarin–serine adduct was found to be higher in energy compared to the other nucleophiles, which suggests that this α-nucleophile can be a good antidote agent for the reactivation process.

1. Introduction

Many persistent chemicals such as paraoxon, parathion and chemical warfare compounds such as VX, sarin, etc. are hydrophobic phosphorus (V) esters or phosphylating agents. They can irreversibly react with the enzyme acetylcholinesterase (AChE), inhibiting its control over the central nervous system [1–3]. AChE catalyzes the ester hydrolysis process of the neurotransmitter acetylcholine (ACh) to terminate synaptic transmission [4–6]. Inhibition of AChE occurs as a result of the phosphylation of the active serine residue with organophosphorus compounds [7–9]. Such AChE inhibition results in acetylcholine accumulation at cholinergic receptor sites, thereby excessively stimulating the cholinergic receptors. This can lead to various clinical disorders sometimes causes death. Therefore, reactivation of organophosphorus compound inhibited AChE is necessary to get back its catalytic activity towards the hydrolysis of ACh. The inhibited AChE may further undergo "aging" process that normally involves dealkylation or deamidation depending upon the nature of organophosphorus compounds attacked and is irreversible in nature [10–12]. Therefore, there is a need to develop efficient reactivators for the OP-inhibited AChE. Computational methods offer an ability to explore these types of reactions [3,12–24] while avoiding exposure to these deadly agents. Hence, it is quite useful in suggesting new nucleophiles with greater efficiency for the reactivation of inhibited AChE. It has been reported that α-nucleophiles such as oximes are able to reactivate organophosphate–cholinesterase conjugates, giving rise to free enzyme [22]. In our previous studies, we have reported that hydroxylamine anion (NH_2O^-) is an efficient α-nucleophile for the detoxification of the organophosphorus compounds such as VX and sarin [20]. In this article, we report the efficacy of NH_2O^- towards the reactivation of sarin-inhibited AChE adduct.

2. Computational methodology

The following protocol was used to generate conformations of modeled sarin–serine adduct. (i) An exhaustive conformational

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doi:10.1016/j.jmgm.2011.04.009

search was performed with the molecular modeling program Macromodel [25] using MMFFs force field [26–30]. Energy minimizations were performed with the Polak–Ribiere conjugate gradient (PRCG) method [31], which involves the use of first derivatives with convergence criterion set to 0.05 kJ/Å-mol. Conformational search was performed with the random variation of all of the rotatable bonds and combining the Monte Carlo conformational search (MCMM) algorithm [32,33] using 5000 Monte Carlo steps. (ii) Sort all found conformations according to energy. (iii) Stored conformations whose relative energy was within 50 kJ/mol of the lowest energy structure. The resulting conformations were clustered based on torsional RMS using XCluster approach [34]. Based on the minimum separation ratio, we have selected clustering level with two clusters. Minimum separation ratio was employed to find the clustering level at which the distance between members of the clusters is much smaller than the distance between the clusters [35]. Two representative adducts were selected from this clusterization process. The selected conformations from the conformational families were stored for further higher level DFT calculations.

All geometries were optimized using the B3LYP [36–38] density functional and the 6-311G(d,p) basis set. Harmonic frequency calculations at the same level were used to confirm the stationary points and to calculate thermodynamic corrections. Single-point calculations were performed at the B3LYP/6-311 + G(d,p) level to get accurate energies using B3LYP/6-311G(d,p) geometries. Aqueous free energies of solvation of the gas-phase structures were determined with the polarizable continuum model (PCM) [39–43]. Intrinsic reaction coordinate (IRC) calculations were performed to connect all the transition states with their corresponding minima [44,45]. All quantum chemical calculations were performed using Gaussian 03, Revision E.01 program [46].

3. Results and discussion

Solvolysis of sarin and VX with hydroxylamine anion was found to be a very efficient process towards the detoxification of such organophosphorus compounds [20,21]. Generally, it follows the addition–elimination pathway involving a trigonal bipyramidal intermediate. Recent studies have shown that the reactivation process of sarin-inhibited AChE adduct with nucleophiles also involves addition–elimination pathways [22,24]. The reaction energy profile generated for hydroxylamine anion with the sarin-inhibited AChE adduct also follows the addition–elimination pathway involving a trigonal bipyramidal intermediate. The formation of sarin–serine adduct is generally take place by two-step addition–elimination mechanism where serine moiety replaces fluoride ion (Scheme S1 of the supplementary data) [8]. Initially, we have performed an extensive conformational search on sarin–serine adduct as a model for sarin-inhibited AChE due to its flexibility. Two unique conformers of sarin–serine adduct were identified in the conformational search process using MMFFs force field in aqueous phase (GB/SA) after clusterization of the conformations. The computed energy difference between these two adducts is 0.8 kcal/mol. These adducts were further optimized at B3LYP/6-311G(d,p) level of theory, which leads to a larger energy difference of 2.4 kcal/mol (Fig. 1). The conformational difference between these two adducts is due to the orientation of –NHCHO group of serine moiety. In adduct 1, there is a strong intramolecular hydrogen bond between the hydrogen of the –NH group and the oxygen atom of the phosphoryl group (Fig. 1), which leads to a greater stabilization than the adduct 2. The crystal structure of sarin inhibited AChE reported in the literature shows that the intramolecular hydrogen bonding is not present between sarin and serine moiety [47]. Therefore, both adducts 1 and 2 have been considered to examine the efficacy of hydroxylamine anion for the reactivation process.

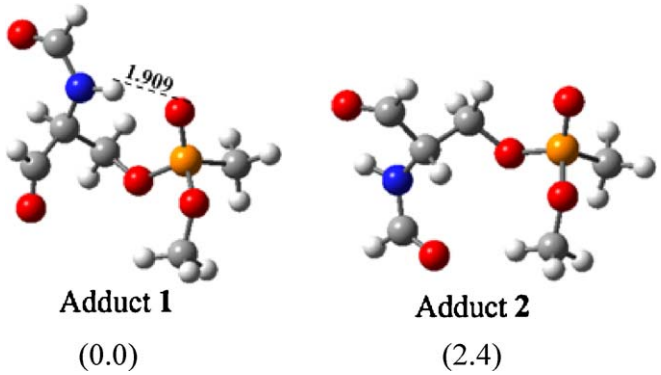

Fig. 1. B3LYP/6-311G(d,p) optimized geometries of two unique conformers of sarin–serine adduct and their relative energies (kcal/mol) (grey = carbon, red = oxygen, blue = nitrogen, white = hydrogen and orange = phosphorus). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The Gibbs free energy with B3LYP/6-311G(d,p) for the reaction between sarin–serine adduct 1 and hydroxylamine anion has been shown in Fig. 2a and the corresponding geometries of stationary points in Fig. 3a. For sarin–serine adduct, the isopropyl group of the sarin moiety was modeled by a methyl group as reported in previous studies [22,24]. Two complexes and two intermediates have been located as local minima on the potential energy surface. Three corresponding transition state structures that link these minima have also been located as first-order saddle points. The intrinsic reaction coordinate (IRC) calculations connect the transition states to the respective minima. The sarin–serine adduct 1 and the hydroxylamine anion forms a complex (C1a), which is energetically stable by 37.6 kcal/mol than the separated reactants. The anionic nucleophile and sarin adduct stabilizes through charge dipole type interactions besides two C–H···O type hydrogen bonds in the complex C1a (Fig. 3a). The free energy of activation computed for the attack of NH_2O^- to the sarin phosphorus atom is 3.2 kcal/mol compared to the complex C1a. NH_2O^- approaches opposite to the oxygen atom of serine moiety in a slightly nonlinear fashion ($\angle$O–P–O = 165.4°) and the P···ONH_2 bond distance is 3.020 Å (TS1a) (Fig. 3a). The Wiberg bond index calculated for the P···ONH_2 distance of TS1a has been found to be 0.05 au, which is about 0.03 au higher than that of C1a signifying a stronger interaction in the former case. In this transition state, N–H···O hydrogen bonding (2.528 Å) has been observed between the nucleophile and the P=O bond of sarin–serine adduct 1 (Fig. 3a) besides the C–H···O type interactions. After TS1a, TBP intermediate IN1a has been found to be 14.5 kcal/mol stable than complex C1a. The –P–ONH_2 bond distance and the bond distance between P and oxygen atom of serine are 1.798 Å and 1.815 Å, respectively in IN1a with the corresponding bond indexes of 0.53 and 0.48 reveals the strengthening and weakening of the corresponding bonds compared to TS1a. The hydrogen-bonding interaction between the nucleophile and the P=O bond of sarin–serine adduct 1 becomes stronger with the shortening of H-bond distance (2.008 Å) in IN1a (Fig. 3a).

To stabilize the leaving group, methoxy group rotates towards the serine moiety of sarin–serine adduct through a rotational transition state TS2a of imaginary frequency 86i cm^{-1} and forms another TBP intermediate IN2a (Fig. 2a). The elimination of the leaving serine group is exergonic in nature and requires only 3.7 kcal/mol free energy of activation (TS3a) from the intermediate IN2a. The Wiberg bond index of 0.66 au for P–ONH_2 further suggests the stronger interaction and the smaller bond index of 0.08 au for the distance between P and the oxygen atom of serine indicates the