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The Se-S/N interactions as a possible mechanism of δ-aminolevulinic acid dehydratase enzyme inhibition by organoselenium compounds: A computational study

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ABSTRACT

Organoselenium compounds present many pharmacological properties and are promising drugs. However, toxicological effects associated with inhibition of thiol-containing enzymes, such as the δ-aminolevulinic acid dehydratase (δ-AlaD), have been described. The molecular mechanism(s) by which they inhibit thiol-containing enzymes at the atomic level, is still not well known. The use of computational methods to understand the physical-chemical properties and biological activity of chemicals is essential to the rational design of new drugs. In this work, we propose an in silico study to understand the δ-AlaD inhibition mechanism by diphenyl diselenide (DPDS) and its putative metabolite, phenylseleninic acid (PSA), using δ-AlaD enzymes from Homo sapiens (Hs-δ-AlaD), Drosophila melanogaster (Dmδ-AlaD) and Cucumis sativus (Csδ-AlaD). Protein modeling homology, molecular docking, and DFT calculations are combined in this study. According to the molecular docking, DPDS and PSA might bind in the Hsδ-AlaD and Dmδ-AlaD active sites interacting with the cysteine residues by Se-S interactions. On the other hand, the DPDS does not access the active site of the Csδ-AlaD (a non-thiol protein), while the PSA interacts with the amino acids residues from the active site, such as the Lys291. These interactions might lead to the formation of a covalent bond, and consequently, to the enzyme inhibition. In fact, DFT calculations demonstrated that the selenylamide bond formation is energetically favored. The in silico data showed here are in accordance with previous experimental studies, and help us to understand the reactivity and biological activity of organoselenium compounds.

1. Introduction

The utilization of selenium (Se) in organic synthesis has been producing a vast number of organoselenium compounds since the second half of the 19th century. For instance, Ebselen (EBS) was synthesized in 1924, and nowadays is the most investigated of the organoselenium compounds [8–11]. The utilization of selenium (Se) in organic synthesis has been producing a vast number of organoselenium compounds since the second half of the 19th century. For instance, Ebselen (EBS) was synthesized in 1924, and nowadays is the most investigated of the organoselenium compounds [8–11].

The organoselenium derivatives present many pharmacological properties, such as anti-inflammatory, cardioprotective, neuroprotective, and antioxidant, this last one due to their ability to reduce hydrogen peroxide (H₂O₂) to water (H₂O). Therefore, these compounds are considered mimetics of the glutathione peroxidase (GPx) enzyme and are promising drugs [3–6].

In addition, EBS and DPDS can oxidize thiol groups of proteins [3,4,7] as observed in the mammalian enzyme δ-aminolevulinic acid dehydratase (mδ-AlaD) or porphobilinogen synthase (PBGS) (EC 4.2.1.24). Since the δ-AlaD is an important enzyme involved in the porphyrins’ synthesis, its inhibition can have toxicological consequences [8–11]. The δ-AlaD catalyzes the asymmetric condensation of two molecules of 5-aminolevulinic acid (δ-aminolevulinic acid – δ-Ala), forming the porphobilinogen (PBG), which is the precursor of porphyrins’ synthesis (Fig. 1B). In the enzyme active site, each substrate binds at two different subsites (A and P), leading to the regioselective product PBG. The acetic acid and propanoic acid side-chains of PBG originate from the subsites A and P, respectively [12–14]. Porphyrins are essential to living beings, particularly to the aerobic life, due to the heme prosthetic group, which is involved in the transport of oxygen (hemoglobin and myoglobin), xenobiotic metabolism (cytochrome P450), protection against peroxides (peroxidases and catalases), and chlorophyll synthesis [13,15–17]. There are two major classes of δ-AlaD: the Zn-dependent enzymes (that are present in mammals, fungi
and some bacteria, such as *Escherichia coli* [15,18,19], and the *Mg*-dependent enzymes, that are found mainly in plants, protozoa and other bacteria [13,20–22].

Studies have demonstrated that the DPDS can inhibit the δ-AlaD enzyme from human (*Hsδ-AlaD*) and rodents [10,11,23–28]. The δ-AlaD from *Drosophila melanogaster* (Dmδ-AlaD) can also be inhibited by DPDS [29]. In contrast, DPDS do not inhibit δ-AlaD from cucumber, *Cucumis sativus* (Csδ-AlaD); nevertheless, its putative metabolite, the phenylseleninic acid (PSA), can inhibit the Csδ-AlaD [30]. In fact, the toxicity of organoselenium compounds could be associated with their metabolic oxidation by flavin-containing monooxygenases [4,31,32]. However, the inhibition mechanism(s) involved in these cases has not been established yet.

To complement and better understand the in vivo and in vitro data, in *silico* methods have been used to analyze, simulate, and predict the pharmacology and toxicity of chemicals [33–37]. There are many types of computational methods, where the molecular docking stands out by simulating the interactions between macromolecules (proteins and DNA) and ligands (substrate, inhibitor, and agonist). This method consists in predicting the binding mode of the ligand at the binding site of a given target, in addition to the estimation of affinity for the receptor, by predicting binding free energy (ΔG) [38–41]. Quantum mechanical methods, such as the density functional theory (DFT) approach, are frequently used in the study of structures, reactions, and molecular properties [42–44], but are strictly limited to systems of few hundreds of atoms. In addition, the protein homology modeling has been successfully employed to predict the 3D protein structure, which is essential in many cases when the tertiary or quaternary structure must be studied [45–49].

Different in *silico* methods have been adopted to predict the reactivity, toxicity, and pharmacology of organoselenium compounds and selenoproteins [44,50–58]. Here, to better understand the toxicological effects of organoselenium molecules, and how they interact with target proteins, we propose an in *silico* approach combining protein homology modeling, molecular docking simulations, and DFT calculations (Scheme 1). Based on the difference of DPDS and PSA inhibition behavior on δ-AlaD enzymes, this study aims to compare the intermolecular interactions between the *Hsδ-AlaD*, *Dmδ-AlaD* and Csδ-AlaD enzymes and the DPDS and PSA organoselenium compounds, to gain insight into their mechanisms of inhibition.

2. **Materials and methods**

2.1. **Protein homology modeling**

First, the homology analysis of the primary structure of δ-AlaD enzymes from cucumber (*Cucumis sativus*), fruit fly (*Drosophila melanogaster*), human (*Homo sapiens*), mouse (*Mus musculus*), zebrafish (*Danio rerio*), cockroach (*Blattella germanica*), protozoa (*Toxoplasma gondii*), yeast (*Saccharomyces cerevisiae*), archaean (*Pyrococcus caldiphilis*) and bacteria (*Chlorobaculum parvum*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Wolbachia*) were performed. The FASTA amino acid sequences for δ-AlaD enzymes were obtained from the the National Center for Biotechnology Information – NCBi (https://www.ncbi.nlm.nih.gov/pubmed/), UniProt (http://www.uniprot.org/), and Protein Data Bank – PDB (http://www.rcsb.org/pdb), according to the respective codes: *Blattella germanica*: UniProt (A0A2P8XHW3_BLAAGE); *Chlorobaculum parvum*: PDB (2C1H); *Cucumis sativus*: UniProt (A0A0A01Q9K9_CUCSA); *Danio rerio*: NCBi (NP_001017645.1); *Drosophila melanogaster*: UniProt (Q9VT99_DROME); *Escherichia coli*: PDB (1L6S); *Homo sapiens*: PDB (1ES1); *Mus musculus*: NCBi (NP_001263375.1); *Pseudomonas aeruginosa*: PDB (1GZG); *Pyrococcus caldiphilis*: PDB (5LZL); *Saccharomyces cerevisiae*: PDB (1H7N); *Staphylococcus aureus*: UniProt (HEM2_STAAR); *Toxoplasma gondii*: PDB (3OBK); *Wolbachia*: NCBi (WP_041571452.1). Regarding of FASTA from PDB, it was used the FASTA associated with the corresponding PDB file on the database (we do not extract the FASTA from the PDB file). The Clustal Omega server (http://www.ebi.ac.uk/Tools/msa/clustalo) was used to make the multiple sequence alignment, and the similarity between the δ-AlaD sequences was calculated from the Geneious program (https://www.geneious.com) (Fig. 2, S1, S2 and Table S1).

Since there is no available three-dimensional structure of *Dmδ-AlaD* and *Csδ-AlaD*, the Swiss-Model (https://swissmodel.expasy.org) [59], Phyre2 [60], and Geno3D servers [61] were used to obtain their structures, using the amino acid sequence of the *Cucumis sativus* and *Drosophila melanogaster* δ-AlaD, taken from UniProt with the codes A0A0A01K9Q9_CUCSA and Q9VT99_DROME, respectively. The 3D structures of the *Chlorobaculum parvum* (PDB: 2C1H [62]), *Pseudomonas aeruginosa* (PDB: 1GZG [63]), and *Toxoplasma gondii* (PDB: 3OBK [21]) were used as template to build the Csδ-AlaD models, while the *Escherichia coli* (PDB: 1L6S [64]), *Pyrococcus caldiphilis* (PDB: 5LZL [18]), and *Saccharomyces cerevisiae* (PDB: 1H7N [19]) structures where used as template to build the Dmδ-AlaD models. The validation of the protein models were carried out by the programs: Verify 3D [65,66], ProSA [67], PROCHECK [68,69], and ERRAT [70]. The Ramachandran plot was made by the PDbsum server (www.ebi.ac.uk/pdbsum/) [71]. More details can be found in the Supporting information.

2.2. **Molecular docking**

To carry out the docking simulations, the *Hsδ-AlaD* was obtained from PDB with the code 1ES1 [72], and the *Dmδ-AlaD*-1L6S and *Csδ-AlaD*-3OBK models were obtained from protein homology modeling by the Swiss-model program (as described above). The CHIMERA 1.8 program [73] was used to add the hydrogen atoms to the proteins. The Lys199/195/291 and Lys252/258/344 residues were considered neutral (deprotonated) [14], which was confirmed by H++ analysis (http://biophysics.cs.vt.edu). The ligands (PBG and the organoselenium compounds) were built in the Avogadro 1.1.1 software [74], followed by a geometric optimization using the MOPAC program (http://openmopac.net/MOPAC2012.html) with the semi-empirical method PM6 (with the water dielectric constant) [75]. The PSA was considered deprotonated (pka = 4.79) [76] during the docking simulations. The protein and ligands were converted to the pdbqt format with the AutoDockTools [77], with the Gasteiger and MOPAC charges, respectively. The partial charge (0.302) of the Zn²⁺ ion from *Hsδ-AlaD* and *Dmδ-AlaD* were obtained from a previous study [51].

The AutoDockVina 1.1.1 software [78] was used for the docking simulations, with exhaustiveness of 100. The best docking protocol was obtained using the ligands and the side chain of Arg209 and Lys252 residues from *Hsδ-AlaD* (Arg205 and Lys248 from *Dmδ-AlaD*-1L6S, and Arg301 and Lys344 from *Csδ-AlaD*-3OBK) flexible. The grid boxes (with spacing of 1 Å) were centered in the active site of the enzymes Hsδ-AlaD (coordinates: x = 31.63; y = 73.65; z = 57.08), Dmδ-AlaD-1L6S

![Fig. 1](image-url)
(coordinates: x = 19.72; y = 83.35; z = 52.14), and Csδ-AlaD-3OBK (coordinates: x = -64.60; y = -77.40; z = 28.05), with a size of 25 × 25 × 25 Å, in both cases. The Discovery Studio Visualizer 17.2.0. (DSV) program (https://www.3dsbiovia.com/) was used to analyze the results, where the conformers of lowest binding free energy (ΔG) were selected as the best model. The molecular docking protocols were validated by the RMSD (root-mean-square deviation) values from the PBG molecules, which give the relationship between the experimental and the theoretical data in a receptor-ligand complex. RMSD values lower than 2.0 Å indicate good quality of data reproduction (Fig. S4) [41,79,80] (details can be found in the Supporting Information).

2.3. Density functional theory calculations

All quantum chemistry calculations have been performed using density functional theory (DFT) approach as implemented in Gaussian 09 rev. E.01 program [81]. mPW1PW91 (Perdew-Wang hybrid functional) [82] was used, in combination with the def2TZVP (Triple zeta quality with polarization functions) basis set for all the atoms [83,84]. Full geometry optimizations were carried out in gas phase; solvation (water) effects were taken into account in subsequent single point calculations at the same level of theory using PCM approximation [85].

### 3. Results and discussion

#### 3.1. Protein sequence comparison and homology modeling

Considering that DPDS inhibits the Hsδ-AlaD [11] and Dmδ-AlaD [29] and does not inhibit Csδ-AlaD [30], we initially compared the primary structure of the δ-AlaD enzymes (including other different species) through multiple sequence alignment (Fig. 2, Fig. S1/S2 and Table S1). The analysis of the sequence alignment data demonstrated that are two groups of proteins, i.e. Group A, which includes the species that present Cys residues in the active site (Saccharomyces cerevisiae, Drosophila melanogaster, Danio rerio, Homo sapiens, Mus musculus, Escherichia coli, Pyrobaculum caldifontis and Staphylococcus aureus), and Group B, which includes the species that have Asp residues (Toxoplasma gondii, Cucumis sativus, Wolbachia, Pseudomonas aeruginosa and Chlorobaculum parvum) (Fig. S2). Interestingly, the Blattella germanica δ-AlaD is small when compared to the other species (146 vs ~ 330 residues) and has not the Cys region of the active site; however, the catalytic Lys residues are conserved (Fig. S1). According to the phylogenetic tree (Fig. S2) it belongs to Group A.

In general, the three cysteine residues from the active site of Group A δ-AlaD were replaced by two aspartate residues and one alanine residue in Group B (Fig. 2 and Fig. S1/S2) indicating a significant change in the nature of the active site. In addition, the Arg221 (in the human protein) were replaced by a Lys residue in the δ-AlaD from Group B (Lys313 in Csδ-AlaD). As the Lys and Arg are basic and positively charged residues, practically, the same physical–chemical properties are conserved.

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**Scheme 1.** Overview of all the steps involved in this study.

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**Fig. 2.** Multiple alignments of the δ-AlaD amino acids sequence of different organisms. Only a fragment from the active site of the proteins are shown. The residues from the active site are highlighted: Cys (yellow); residues that remain conserved (cyan), residues that are not conserved when compared to the human enzyme (green and pink). The complete alignment is shown in Fig. S1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
are conserved. These observations are in accordance with previous studies of Kervinen et al. (2001) [86] where five δ-AlaD enzymes (from *Pisum sativum*, *Pseudomonas aeruginosa*, *Bradyrhizobium japonicum*, *Escherichia coli*, and *H. sapiens*) sequences were analysed, and the metal-binding region determined.

Here, based on these observations, we can suppose that DPDPS does not inhibit the Csδ-AlaD because this enzyme does not present Cy5 residues in its active site. However, it does not explain why PSA inhibits the Csδ-AlaD. For a better understanding of the interactions between inhibitors and enzymes, the molecular docking simulations were performed. Taking into account that there are no Csδ-AlaD and δMsp-AlaD structures available, the 3D model of these enzymes were built using protein homology modelling.

Homology modelling is the most accurate method to build protein structure models [87–89]. Among the different programs developed for this purpose, in this study we have chosen the Swiss-Model [59], Phyre2 [60], and Geno3D [61] to create the δMsp-AlaD and Csδ-AlaD structures. Taking in account the primary structure similarity between the δ-AlaD enzymes (Fig. 2, Figs. S1–S2 and Table S1), three templates were selected for δMsp-AlaD (PDB ID: 1H7N, 1L6S and 5ZL2) and three for Csδ-AlaD (PDB ID: 1GZG, 2C1H, and 30BK). Each template was used in the protein homology modeling with the three programs above cited, to find the best protein model. The 3D structure models of δMsp-AlaD and Csδ-AlaD built were validated using the programs: Verify 3D [65,66], ProSA [67], PROCHECK [68,69], and ERRAT [70] (Tables S1–S2).

According to the data in Tables S2 and S3, the best δMsp-AlaD and Csδ-AlaD models were obtained from the PDB ID 1L6S and PDB ID 30BK templates, respectively, using the Swiss-Model program, which turned out to be the most performant program for this task. δMsp-AlaD-1L6S and Csδ-AlaD-30BK models showed a satisfactory protein structure, because the validation parameters are in the range of native protein structure (see the Supporting information), and they were used for the molecular docking simulations.

Despite the differences in the primary structure between the *Homo sapiens* δ-AlaD, δMsp-AlaD and Csδ-AlaD, the comparison of the tertiary structure of the three enzymes exhibited a very similar organization of the residues, with the simulated PBG binding pose presenting practically the same conformation and interactions (Fig. 3). Here, we highlighted the major difference in the active site of both enzymes. As shown in Fig. 3A, in the model of δAlaD and Csδ-AlaD the thiolates of the Cy5 residues are coordinated to a zinc ion (Zn$^{2+}$), whereas this metal nucleus acts as a Lewis acid and Zn-N coordination with the amino moiety (Lewis base) from PBG is formed. This Zn-N interaction is essential to the catalysis of the δ-AlaD, because it specifically guides one molecule of 5-Ala substrate in subsite A, before the cyclization to pyrrole ring [86,90]. In fact, the Cy5 mutations cause a dramatic reduction in the activity [12]. On the other hand, according to the docking simulation between the PBG and Cy5-AlaD, the orientation of one molecule of 5-Ala substrate is likely driven by the H-bonds between the amino moiety from 5-Ala and the carboxylate groups of Asp217 and Asp225 residues (Fig. 3C). The PBG binding pose obtained by the docking in Cy5-AlaD is very similar to the crystallographic data collected from *T. gondii* δ-AlaD [21]. Interestingly, Asp217 and Asp225 are the residues that correspond to Cy5124 and Cy5132 residues in the human enzyme, respectively (Figs. 2 and 3).

In addition, in the case of Csδ-AlaD, the Mg$^{2+}$ ion is not present in the active site (Fig. 3C), and does not participate directly in the catalysis. However, the Mg$^{2+}$ is essential to enzyme function, as observed in *E. coli*, *Bradyrhizobium japonicum*, *Pseudomonas aeruginosa*, and *P. sativum*, due to the Mg$^{2+}$ bound network around this metal ion maintaining the quaternary structure of δ-AlaD [13–15,86]. This difference in the active site of δ-AlaD from different species must be taken into account in the design of selective inhibitors with useful applications, such as in the case of δ-AlaD from *Wolbachia* [91–93] and *Staphylococcus aureus* [94]. Moreover, due to the similarity of the active site from δ-AlaD of the group B, the use of plant δ-AlaD (such as cucumber) can provide a simple, practical and cheap in vitro assay to find new selective inhibitors.

### 3.2. Organoselenium molecular docking study

Molecular docking simulations were carried out to understand the δ-Ala inhibition by DPDPS and PSA. According to the docking between the δ-Hs-AlaD and DPDPS, this latter interacts with the enzyme active site mainly by hydrophobic interactions (π-π stacking with Phe79, Tyr205 and Phe208 residues and alkyl-π with Pro125). The selenium atoms of DPDPS interact with the carboxyl group of Asp120 and with the Zn$^{2+}$ ion, besides the thiolate group from Cy5124 (Fig. 4A). The putative DPDPS metabolite, PSA, also interacts in the δ-Hs-AlaD active site, by π-π stacking with Tyr205 and Phe208, H-bond with Ser168, and interactions with Tyr196 (anion-π interaction between the seleninate and the phenyl moieties), Asp120 (repulsive electrostatic interaction between the seleninate and carboxyl groups), and zinc ion (coordination). In addition, Se-S interaction with Cy5124 is observed (Fig. 4B).

The simulation of DPDPS with the δMsp-AlaD demonstrated that this organoselenium compound could access the active site making hydrophobic interactions with Arg205, Pro212 (alkyl and phenyl groups), Phe204 and Tyr201 (phenyl and phenyl moieties), besides interacting with Arg217 via H-bond (selenyl and guanidinyl groups) (95,96)) (Fig. 4C). In addition, the DPDPS showed a Se-S interaction with Cy5122. The PSA molecule also binds in the δMsp-AlaD active site, through hydrophobic π-π stacking with Phe204 and Tyr201 (phenyl and phenyl moieties), through H-bonds with Ser165, Lys195, and Gln221 (selenolate and OH, NH and C=O groups, respectively), and Zn-O coordination. Similarly to DPDPS, the PSA also showed Se-S interaction with Cy5122 (Fig. 4D).

On the other hand, the docking simulations between the Csδ-AlaD and Cy5-AlaD demonstrated that it does not enter in the δ-AlaD active site. In fact, DPDPS binds in the superdomain of the enzyme, close to the entrance of the active site, interacting with the Lys313 (phenyl and carbon chain) and presenting an intramolecular π-π stacking (phenyl-phenyl) (Fig. 4E). In contrast, PSA can access the active site of Csδ-AlaD (Fig. 4F), making H-bonds with Arg301 and Lys291 residues, stabilized by electrostatic interaction with Asp217, and π-π stacking with Phe330 (phenyl and phenyl moieties).

Finally, we simulated the interactions of other putative oxidized organoselenium forms [97] (Fig. S5) to verify if these molecules are able to interact with the δ-AlaD enzymes, and its binding partner. For δ-Hs-AlaD, all organoselenium molecules show Se-S interaction (3.1–5 Å) with the Cy5124 residue (Fig. S6), except R,R-DPDS(O). Conversely, for δMsp-AlaD, only S,R-DPDS(O), R,DPS(O) and PhSeOH show Se-S interaction (4–4.4 Å) (Fig. S7). In relation of the Csδ-AlaD, we verified that all the selenoxide forms of DPDS do not bind in the active site (Fig. S8), as observed with DPDPS. However, like PSA, PhSeOH enters in the active site and interacts with Lys291. These data suggest that for Csδ-AlaD small organoselenium electrophilic moieties can indeed inhibit the enzyme. In addition, the stereochemistry of the compounds play an essential role in the binding mode in the enzyme.

The predicted binding free energy ($\Delta G_{\text{bind}}$) for the δ-Hs-AlaD indicates that the interaction of DPDS with the enzyme is energetically more favored than the interaction PSA-enzyme (Table 1). In contrast, $\Delta G_{\text{bind}}$ for δMsp-AlaD suggests a more favorable PSA-enzyme than DPDS enzyme binding. Similarly, in Csδ-AlaD, PSA showed (negatively) larger binding energy than DPDS. Finally, the presence of oxygen atoms in the oxidized forms of DPDS enabled the formation of H-bonds facilitating thermodynamically the binding.

In the δ-Hs-AlaD and δMsp-AlaD enzymes, both PSA and DPDPS presented similar binding pose, interacting with amino acid residues from the active site. Notably, Cy5124 and Cy5122 (δ-Hs-AlaD and δMsp-AlaD, respectively), stabilization occurs via Se-S interaction (Fig. 4A–D). However, for Csδ-AlaD, only PSA binds in the active site, and no Se-S
interaction is present because the Csδ-AlaD does not have Cys residues in the active site (Fig. 4E–F). These outcomes strongly suggest that organoselenium compounds binding in the active sites could prevent the entrance of the substrate 5-Ala, thus inhibiting the enzymes.

The previous in vitro assays have indicated that the mechanism of Hsδ-AlaD (or mammalian δ-AlaD) and Dmδ-AlaD inhibition by organoselenium compounds involves Cys oxidation because dithiothreitol (DTTred) could reactivate the enzyme from these sources [10,11,24,29,30,98]. The Se−S interaction could lead to the formation of the selenenyl sulfide bond (Se=S) [99,100], an adduct between the protein and the selenium compound, by means of a nucleophilic attack of the thiolate moiety of Cys124(122) to the Se atom of either DPDS or PSA. In fact, previous experimental as well as theoretical studies have indicated that Se-S bound can be easily formed between reduced thiol-containing molecules and diselenide- (Se-Se) and seleninic acid (R-SeO2H)-containing molecules [99,101,110,102–109].

In the next step, a vicinal thiol group – from Cys122(120) and/or Cys132(130) – could perform a nucleophilic attack to the electrophilic S
The active site reacts with this intermediate leading the disulfide bridge, denaturing the active site [51,114]. In fact, the distances between the S atoms are 3.7–4.6 Å for both Hsδ-AlaD and Dmδ-AlaD. Previous studies suggested that the cysteine oxidation (S–S) in the Hsδ-AlaD active site involves Cys124 and Cys132 residues. The Cys124 residue is the first thiolate that reacts with diselenides or seleninamides, forming the Se–S intermediate; then, Cys132 reacts with this intermediate leading the disulfide bridge, denaturing the active site [51,114].

Csδ-AlaD has no Cys residues in the active site and consequently, the Cys oxidation mechanism is not possible. PSA, likely due to its polarity, has a better affinity for the active site of Csδ-AlaD (where polar and basic residues are present). PSA has a highly electrophilic Se atom [30,115,116]. Its Hirshfeld partial charge is higher than the one computed for Se in DPDS and in the other selenium compounds of this study, indicating a deficiency of electrons (Table S4). In addition, due to the short distance between the Se atom and the amino group from Lys291 (Se–N = 3.8 Å, Fig. 4F), a nucleophilic attack from the Lys291 on PSA could occur, forming a seleninamide moiety (Ph-Se(O)NH-Lys), i.e., an adduct between the enzyme and the organoselenium moiety, which might inhibit the Csδ-AlaD. The seleninamide formation from seleninic acid has already been reported in the literature [115,117].

Table 1
Predicted ΔGbind (kcal/mol) from molecular docking.

| Enzyme | Hsδ-AlaD | Dmδ-AlaD | Csδ-AlaD |
|--------|----------|----------|----------|
| DPDS   | −6.2     | −5.9     | −5.2     |
| PSA    | −5.1     | −6.1     | −5.9     |
| R,R-DPDS(S) | −7.0   | −8.0 | −5.1     |
| δ,S-DPDS(O) | −7.0   | −7.9 | −6.7     |
| δ,S-DPDS(O) | −6.1   | −6.1 | −4.8     |
| δ-DPDS(O) | −7.0   | −6.5 | −5.2     |
| PhSeOH | −6.3     | −6.0     | −5.1     |
| Csδ-AlaD | −4.6     | −5.8     | −4.8     |

atom of the Se–S bond, leading to the disulfide bridge (S–S) formation, i.e., thiol oxidation, and the release of zinc ion [7,99,108,111–113]. In fact, the distances between the S atoms are 3.7–4.6 Å for both Hsδ-AlaD and Dmδ-AlaD. Previous studies suggested that the cysteine oxidation (S–S) in the Hsδ-AlaD active site involves Cys124 and Cys132 residues. The Cys124 residue is the first thiolate that reacts with diselenides or seleninamides, forming the Se–S intermediate; then, Cys132 reacts with this intermediate leading the disulfide bridge, denaturing the active site [51,114].

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The formation of seleninamide could prevent the reaction between the Lys291 residue and the S-Ala substrate (the Schiff base formation, which is an essential step in the δ-AlaD catalytic cycle [14,15]). The in vitro study of Farina et al. (2002) [30] showed that in the presence of DTTred the Csδ-AlaD is not inhibited. A possible explanation is that the sulfur atom from DTT could react with the seleninamide adduct, forming a thioselenolate moiety (Ph-Se(O)S-DTT) releasing the free Lys291 and consequently reactivating the enzyme (Ph-Se(O)S-DTT + Lys-NH2). In fact, the thioselenolate intermediate can be formed via a reaction between seleninamide and thiol molecules [5,100,118,119].

The reaction between the PSA and the active site in Csδ-AlaD was investigated by means of DFT calculations. For this purpose, we set up a model reaction, using EtNH2 as a model of the Lys residue and PSA in the protonated form (PhSeOOH), as it should be due to its proximity to Arg301 (Fig. 4F) and because water is a better leaving group than hydroxyl anion. Our results (Fig. 5) indicate that the seleninamide formation is energetically favored, both in the gas and water phase. The reactant complex (PhSeOOHEtNH2) is characterized by an H-bond between the hydroxyl and amino groups and by a short distance Se–N (3.8 Å), promoting the release of a water molecule and the formation of the Se–N bond in the product complex (PhSeONH2EtH2O) (Fig. 5A).

The proximity between electrophilic forms of organoselenium molecules and nucleophilic moieties from critical amino acids residues (in this case Se–S/N interactions from Cys124, Cys132, and Lys291, from Hsδ-AlaD, Dmδ-AlaD, and Csδ-AlaD, respectively) could lead to covalent bonds formation, and consequently, these adducts can impair the functions of enzymes, inhibiting them. This mechanism could justify the toxicity of some organoselenium compounds.

The understanding of the mechanism of organoselenium compounds toxicity will be crucial in the designing of new molecules less toxic and more selective in relation to pharmacological targets. In this sense, the
potential role of metabolites of a given drug can also be informative, as suggested by our present study. Organoselenium molecules have promising biological activity, and Ebselen is under clinical trials as potential lithium mimetic for bipolar disorder [120]. Of particular importance, Ebselen has been recently used against SARS-CoV-2 in vitro and presented antiviral activity possibly by inhibiting the main protease (Mpro) enzyme from the virus of COVID-19 [121]. Selenothioninidines (selenium-containing AZT derivatives) are potential pharmacological agents against cancer [122]. DPDS presents many therapeutics properties (anxiolytic, antidepressant-like, anticancer, neuroprotective, and others) and its mechanism of action involves the modulation of the cellular redox status [123]. DPDS could modulate any protein having reactive thiol groups due to the lack of specific molecular targets. In this way, new DPDS derivatives with higher selectivity for specific protein targets still need to be developed.

4. Conclusion

The present work, entirely performed in silico and combining multiscale approaches, provides an efficient explanation to experimental in vitro data, giving evidence that DPDS inhibits Hsδ-AlaD and Dmδ-AlaD enzymes, but does not inhibit Csδ-AlaD [11,29,30]. The molecular docking simulations between the selected organoselenium molecules and δ-AlaD could provide a possible explanation for this observation. The homology modeling showed that Csδ-AlaD does not present Cys residues in the active site, and consequently, DPDS has not a substrate to oxidize. On the other hand, the putative metabolite PSA could access the active site, interacting with the Lys291 residue (Se–N), preventing the entrance of the 5-Ala substrate, and consequently inhibiting the Csδ-AlaD. By DFT calculations, we have demonstrated that the reaction between PSA and Lys is indeed energetically favored. In Hsδ-AlaD and Dmδ-AlaD enzymes, both DPDS and PSA can access the active site, interacting with Cys124 (122), by Se–S interaction, which could lead to Cys oxidation, and, consequently, protein denaturation and enzyme inhibition. This type of study is essential to understand the reactivity and selectivity of organoselenium compounds in biological systems and can lead to better rational drug design. On the basis of these promising computational results, further studies are prompted. In addition, due to its protein similarity and organoselenium binding pose, Dmδ-AlaD rather than Hsδ-AlaD could be used as a model to test the toxicity of new organoselenium molecules.

CRediT authorship contribution statement

Pablo Andrei Nogara: Conceptualization, Methodology, Investigation, Validation, Writing - original draft. Laura Orian: Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition. Joao Batista Teixeira Rocha: Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comtox.2020.100127.

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