Elucidating the Role of the Pyridoxal 5′-Phosphate (PLP)-Dependent β-Lyases in Selenocysteine Se-Conjugates Metabolism: A Density Functional Study

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The selenocysteine Se-conjugates {RSe−Cys, or $RSeCH_2CH[(NH_2)(COOH)]$} metabolism catalyzed by pyridoxal 5′-phosphate (PLP)-dependent β-lyases has been elucidated in the gas-phase at the density functional level. In this study, pyridoxal 5′-phosphate (PLP)-dependent β-lyases has been modeled by the $[(OMe)P(OH)(O)_2]^-$ phosphate. It was shown that the reaction RSe−Cys → RSeH + $CH_2C[(NH_2)(COOH)]$, which is suggested to occur via the β-H elimination, is energetically unfavorable and encounters high activation barriers of 54.0 (54.1), 54.6 (54.6), and 50.7 (50.9) kcal/mol for R = H, Me, and Ph, respectively. The participation of pyridoxal 5′-phosphate (PLP)-dependent β-lyases significantly facilitates this reaction, and reduces the C^3-H^2 (or C^β-H^β) activation barrier by almost three times, from 50.7 (50.9) kcal/mol to 18.0 (18.1) kcal/mol for R = Ph. Amazingly, the presence of enzyme completely changes the suggested mechanism of the metabolism of selenocysteine Se-conjugates: In the absence of β-lyases the reaction (in general) occurs via the direct β-H-transfer mechanism, whereas with the participation of enzyme it may occur via the indirect β-H-transfer mechanism called the "H-atom relay" mechanism which includes: (a) the β-H atom uptake by enzyme from the RSe−Cys and (b) the delivery of another H atom from the phosphate cofactor to the Se center.

I. Introduction

Selenium is an essential micronutrient, which is incorporated into selenoproteins in the form of selenocysteine and selenomethionine.[1] The substantial knowledge gained from epidemiological studies suggests that the decrease in the selenoprotein level and consequent Se deficiency might cause various types of cancer, coronary heart disease, and liver necrosis.[2,3] Therefore, a deeper understanding of the functions of selenoproteins and the mechanisms of their actions are critical for the designing of new drugs, targeting of specific tissues, and Se distribution in the body. Numerous studies[4−8] indicate that the understanding of the metabolism of organoselenium compounds including selenocysteine and selenomethionine is a prerequisite for cancer prevention, targeting of specific tissues, and Se distribution in the body. Indeed, it is well established that selenium metabolites (such as selenols, selenenic acids, selenolates, and glutathione conjugates) rather than parent compounds are responsible for the chemopreventive and/or antitumor activities of these species.[9−11]

Previous studies have shown that the selenium metabolism could be complex and organ (tissue) selective. For example, it was reported[4,11−13] that the enzymatic activation (metabolism) of the selenocysteine Se conjugates (RSe−Cys), which is one of the most promising selenium based anti-cancer species, might occur via several different mechanisms depending on enzyme involved in this process (see Scheme 1). It has been proposed[4,14,15] that pyridoxal 5′-phosphate (PLP)-dependent β-lyases (such as cysteine conjugate β-lyase/glutamine transaminase K) reduce it to chemopreventive selenols, pyruvate, and ammonia via the β-H elimination mechanism (reaction A in Scheme 1). Meanwhile, the flavin-containing monooxygenases activate the RSe−Cys conjugates via an alternative selenoxidation *syn*-elimination mechanism, which leads to the formation of selenenic acid, pyruvate, and ammonia (reaction B in Scheme 1). In addition to PLP-dependent cysteine conjugate β-lyases and flavin-containing monooxygenases, some non-PLP-dependent enzymes (for example amino acid oxidase (AAO)) also appeared to be involved in the β-H elimination of the RSe−Cys conjugates.

However, detailed mechanisms and factors controlling these processes still need comprehensive investigations. Therefore, in this paper, we investigate the β-H elimination mechanism of selenocysteine Se-conjugates (RSe−Cys) metabolism and elucidate the role of the pyridoxal 5′-phosphate (PLP)-dependent β-lyases in this process. For these purposes, mechanisms of the following reactions have been investigated in detail:

$$RSeCH_2CH[(NH_2)(COOH)] \rightarrow RSeH + CH_2C[(NH_2)(COOH)] \quad (1)$$

and

$$\text{PLP-dependent } \beta\text{-lyases} + RSeCH_2CH[(NH_2)(COOH)] \rightarrow RSeH + CH_2C[(NH_2)(COOH)] + \text{PLP-dependent } \beta\text{-lyases} \quad (2)$$

In the studies of reaction 2, we use $[(OMe)P(OH)(O)_2]^-$ as a model of pyridoxal 5′-phosphate (PLP)-dependent β-lyases, which was chosen on the basis of knowledge accumulated in the literature. Indeed, X-ray crystallographic studies[16] clearly show the existence of a PO_4 group at the "active-site" of pyridoxal 5′-phosphate (PLP)-dependent β-lyases (see Scheme 2). Furthermore, numerous studies have established that, at the physiological pH, a phosphate group can exist in two, mono-

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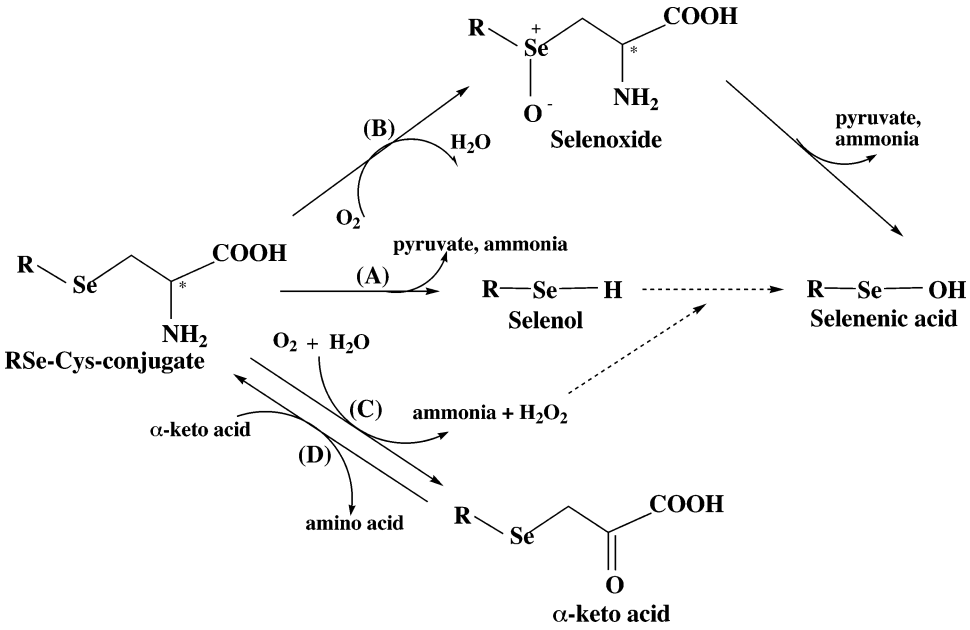
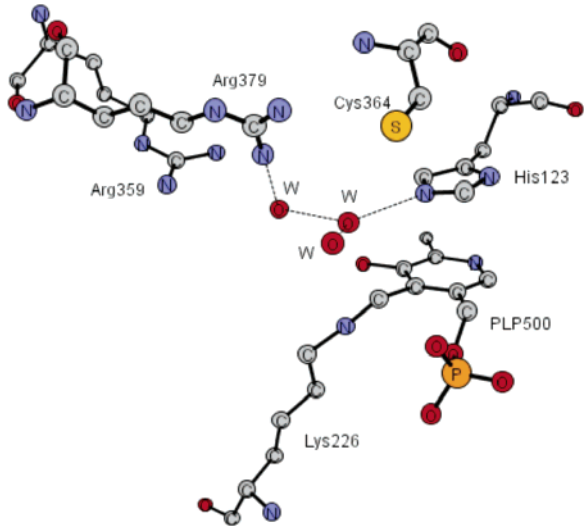
SCHEME 1: Several Proposed Mechanisms for Selenocysteine Se-Conjugates (RSe−Cys) Metabolism[a]



[a] B-β-H-Elimination mechanism initiated by pyridoxial 5′-phosphate(PLP)-dependent β-lyases; B-selenoxidation via *syn*-elimination mechanism initiated by flavin-containing monooxygenases; C-oxidative deamination by amino acid oxidases, and D-transamination by PLP-dependent cysteine conjugate β-lyases (see ref 4).

SCHEME 2: Schematic Presentation of the "active-site" of *E Coli* NifS CsdB Protein, which Is a Member of the Homodimeric Pyridoxal 5′-phosphate (PLP)-Dependent β-Lyases (See Ref 16).



and dianionic, forms out of which the monoanionic form is the most attractive one.[17]

In this study, we determine the structure and energetics of all possible intermediates and transition states for the reactions 1 and 2 involving various R-substitutents (such as H, Me, and Ph). We hope that the comparison of the structure and energetics of all possible intermediates and transition states, as well as the different reaction pathways of reactions 1 and 2 will allow us to elucidate the role of pyridoxal 5′-phosphate (PLP)-dependent β-lyases in the selenocysteine Se-conjugates (RSe−Cys) metabolism.

This paper is organized as follows: In section II, the computational details are presented. In section III.1, we discuss the mechanisms of reaction 1 for different R groups (R = H, Me, and Ph). In section III.2, we discuss the mechanism of reaction 2 and elucidate the role of the pyridoxal 5′-phosphate (PLP)-dependent β-lyases in this process. In section IV, we summarize our findings.

II. Computational Details

In the present study, all calculations were performed using the quantum chemical package Gaussian 2003.[18] The geometries, vibrational frequencies, and energetics of all reactants, intermediates, transition states, and products were calculated at the hybrid density functional theory B3LYP level[19] using the split-valence 6-311G(d,p) basis sets without imposing any geometry constraints. In the discussions below, the enthalpy values were used, and the Gibbs free energies (in parentheses) are also presented. We have shown previously that the B3LYP/6-311G-(d,p) approach provides very close agreement with the more sophisticated approaches, such as CCSD(T), G2, and CBS-Q using the 6-311G(d,p) basis sets for the geometries of the optimized structures.[20] It is also well-known that the B3LYP/6-311G(d,p) approach underestimates the calculated activation barriers by a few kcal/mol in comparison to the more sophisticated methods.[21] Since in this paper we will mainly discuss the trends, any underestimation of the barriers by the B3LYP method should not affect our conclusions.

III. Results and Discussions

III.1. Mechanism of Reaction 1. In Figure 1, we present the calculated reactants, intermediates, transition states and products of reaction 1 for R = H, Me, and Ph, along with their important geometrical parameters. Table 1 includes the calculated relative energies of these structures.

Reaction 1 starts with the H^2 transfer from the C^3 to Se that occurs via the transition state TS1, **2**, where the broken C^3-H^2 and $Se-C^1$ bond distances are elongated by 0.271, 0.333, and 0.215 Å and 0.700, 0.597, and 0.768 Å, for R= H, Me, and Ph, respectively, relative to those in the reactant, **1**. Meanwhile, the $Se-H^2$ bond formed is nascent with the $Se-H^2$ distance of 1.878, 1.806, and 1.830 Å, respectively. A comparison of the C^1-C^2 bond distances in the reactants and transition states

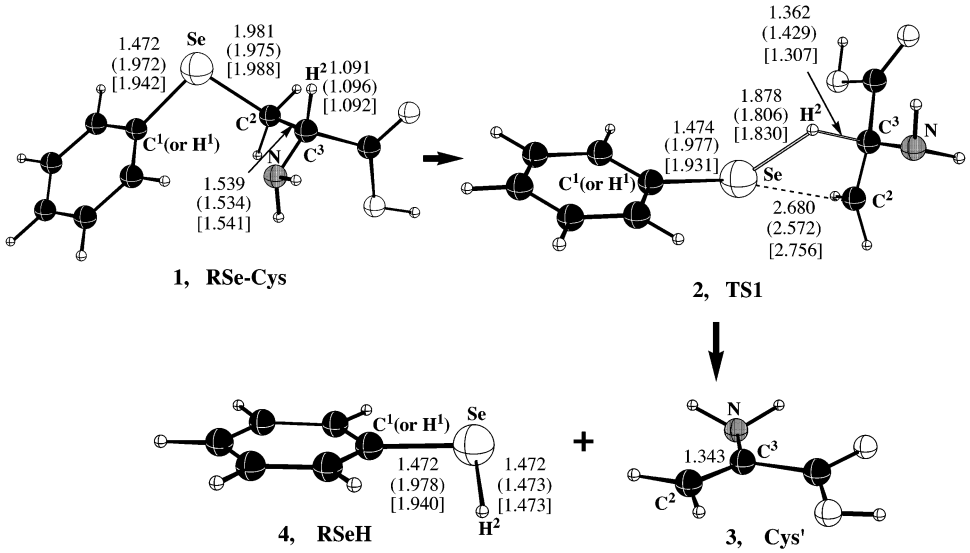


Figure 1. Calculated (distances in Å, angles in deg.) important geometries of the reactants, transition states, and products of reaction 1. Numbers without parenthesis, with parenthesis, and with brackets are for R = H, Me, and Ph, respectively.

TABLE 1: Calculated Energetics (Relative to the Reactants, in kcal/mol) of the Transition States and Products of Reaction 1 for R = H, Me, and Ph

structures	ΔE^b	ΔH^c	ΔG^d
	R = H		
RSe−Cys, **1**	0.0	0.0	0.0
TS1, **2**	59.4	54.0	54.1
products[a]	8.1	3.7	−6.6
	R = Me		
RSe−Cys, **1**	0.0	0.0	0.0
TS1, **2**	54.1	59.9	54.6
products[a]	8.4	4.3	−7.0
	R = Ph		
RSe−Cys, **1**	0.0	0.0	0.0
TS1, **2**	55.8	50.7	50.9
products[a]	8.1	3.9	−7.2

[a] Products are RSeH, **3**, + Cys′, **4**. [b] Electronic energy. [c] Enthalpy. [d] Gibbs Free energy.

shows that in the transition state **2** this bond changes its single C−C bond character to C═C double bond character. All these geometry changes are consistent with the nature of **2**.

Overcoming the barrier at TS1 leads to the formation of the product, $H_2C{=}C(NH_2)(COOH)$, **3**, (here and below will be called as Cys′) and RSeH, **4**. In **3**, the $C^1{=}C^2$ double bond is effectively formed with the bond distance of 1.343 Å. Also, in the structure **4**, the Se−H bond is formed with the bond distance of 1.473 Å.

As shown in Table 1, reaction 1 occurs with a large H^2-transfer barrier: 54.0 (54.1), 54.6 (54.6), and 50.7 (50.9) kcal/mol for R = H, Me, and Ph, respectively. The overall reaction is calculated to be slightly endothermic (exothermic at the free energy level): 3.7 (−6.6), 4.3 (−7.0), and 3.9 (−7.2) kcal/mol for R = H, Me, and Ph, respectively. From the above-presented data it is clear that the metabolism of the RSe−Cys via the β-H-elimination mechanism without the participation of enzyme is both energetically and kinetically unfavorable process, and cannot occur at the moderated conditions.

These findings also show that the nature of the R ligand has insignificant effect on the calculated energetics of the reaction 1. Therefore, in the studies of the mechanism of the reaction 2 below, we will use only R = Ph.

Now, let us discuss the mechanism of the reaction 2 and elucidate the reasons how the enzyme facilitates this kinetically unfavorable process.

III.2. Mechanism of Reaction 2 and Elucidation of the Role of Pyridoxal 5′-Phosphate (PLP)-Dependent β-Lyases in the Metabolism of the Selenocysteine Se Conjugates. Our studies of the mechanism of reaction 2 using $[(OMe)P(OH)(O)_2]^-$ model **5** for the enzyme show that, at the first stage of the reaction, the PhSe−Cys coordinates to the phosphate cofactor by forming multiple H-bonds and generates the pre-reaction complex $[Se{-}Cys][(OMe)P(OH)(O)_2]^-$, **6**, shown in Figure 2.

As shown in Figure 2, in structure **6**, there are three H-bonds between the PhSe−Cys and phosphate: H^1−Se, $O^2{-}H^2$, and $O^3{-}H^3$ with the bond distances of 2.764, 2.036, and 2.062 Å, respectively. It has to be noted that the [PhSe−Cys]−$[(OMe)P(OH)(O)_2]^-$ interaction only slightly affects the geometries of the fragments.

Complex **6** is calculated to be 20.5 (7.8) kcal/mol stable relative to the [PhSe−Cys] + $[(OMe)P(OH)(O)_2]^-$ dissociation limit.

From complex **6**, the reaction may proceed via two different pathways, stepwise or concerted. In the concerted mechanism, the H^1 atom is transferred to the Se center with the simultaneous transfer of the H^2 atom to the O^2 center. This step not only leads to the final products but also regenerates the catalyst in the same step. However, all our attempts to localize the concerted transition state failed and converged to the transition state TS1E, which corresponds to the stepwise mechanism.

In the first step of the stepwise mechanism, the H^2 atom transferred from the C^3 to the O^2 center via the transition state TS1E, **7**. As shown in Figure 2, in this transition state, the $C^3{-}H^2$ bond length has elongated from 1.100 Å in **6** to 1.669 Å, and the $O^2{-}H^2$ bond distance has shortened from 2.036 Å in **6** to 1.068 Å. The $Se{-}C^2$ bond distance is elongated by 0.173 Å, whereas the $C^2{-}C^3$ bond is shortened from 1.541 to 1.462 Å. Among the other geometrical parameters, only the $Se{-}H^1$ and the $O^3{-}H^3$ bonds distances are changed significantly. All of these geometry changes at the TS1E are consistent with the nature of the process that leads to the intermediate $[OP(OH)_2(OMe)][PhSe][Cys']^-$, **8**, where the PhSe and Cys′ are bound to $[OP(OH)_2(OMe)]$ via the H bonds.

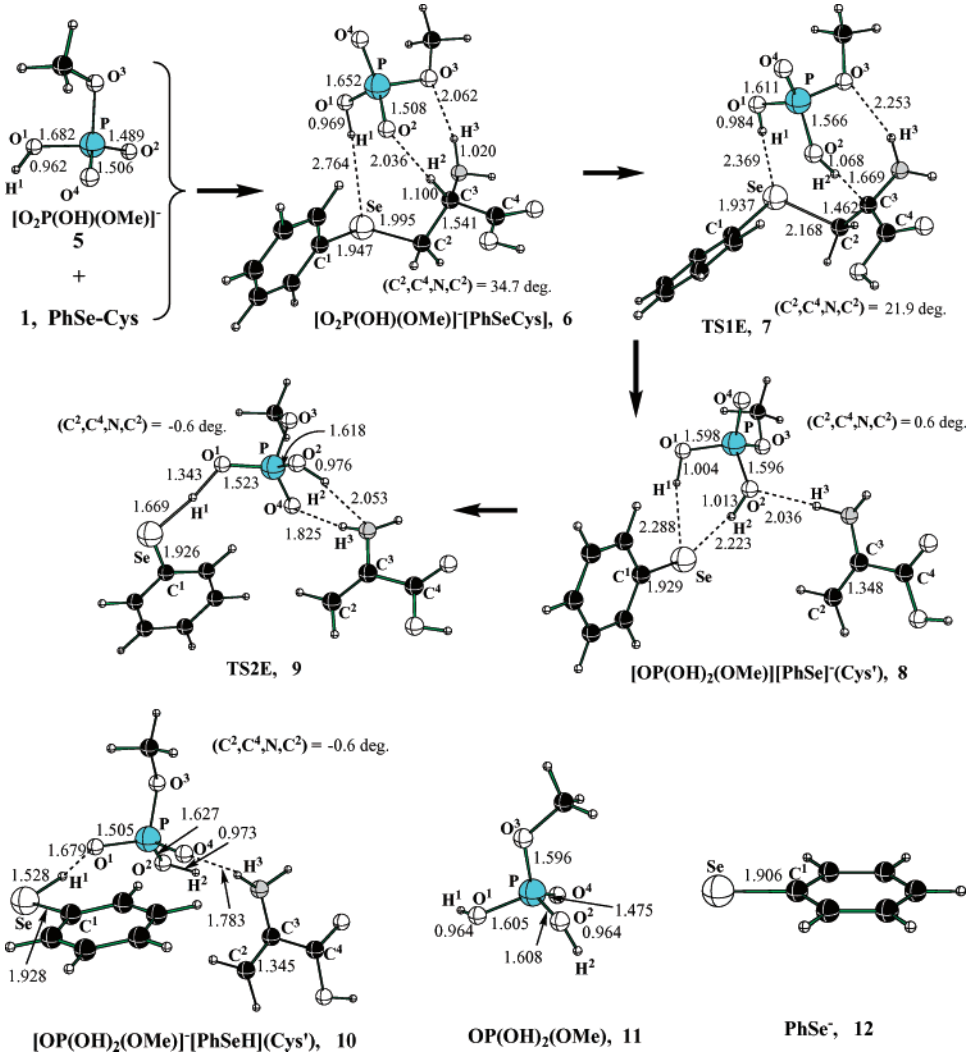


Figure 2. Calculated (distances in Å, angles in deg.) important geometries of the reactants, intermediates, transition states, and products of reaction 2.

TABLE 2: Calculated Relative Energies (Relative to the Reactants, in kcal/mol) of the Intermediates, Transition States and Products of Reaction 2[a]

structures	ΔE	ΔH	ΔG
$[O_2P(OH)(OR)]^-$, **5** + PhSe−Cys, **1**	0.0	0.0	0.0
$[O_2P(OH)(OR)]^-$[PhSeCys], **6**	−21.9	−20.5	−7.8
TS1E, **7**	−0.6	−2.5	10.3
$[OP(OH)_2(OR)][PhSe]^-$[Cys′], **8**	−29.3	−29.6	−22.8
TS2E, **9**	−25.0	−28.4	−19.2
$[O_2P(OH)(OR)]^-$[PhSeH][Cys′], **10**	−25.6	−27.3	−18.7
$[OP(OH)_2(OR)]$, **11** + $[PhSe]^-$, **12** + Cys′, **4**	9.3	7.1	−4.2
$[O_2P(OH)(OR)]^-$, **5** + [PhSeH], **3** + Cys′, **4**	8.1	3.9	−7.2

[a] See footnote for Table 1 for description of *E*, *H*, and *G*. Also, see Figures 1 and 2 for structures.

Analysis of the atomic Mulliken changes shows that about 0.69 e negative charge is located at the PhSe fragment.

As shown in Table 2 and Figure 3, the process **6** → **7** → **8** occurs with a barrier of 18.0 (18.1) kcal/mol and is exothermic by 9.1 (15.0) kcal/mol.

From intermediate **8**, the reaction may split into two different pathways. In the first process, intermediate **8** could be dissociated into three different fragments, $[OP(OH)_2(OMe)]$ + $[PhSe]^-$ + [Cys′]. The DFT calculations show that this process is endothermic by 36.7 (18.6) kcal/mol.

The second process involving the same intermediate **8** is the H atom (in our case H^1) transfer from the $[OP(OH)_2(OMe)]$ fragment to the Se center. This process occurs through the transition state TS2E, **9**, with a very small barrier of 1.2 (3.6) kcal/mol and leads to the intermediate, **10**. As shown in Table 2 and Figure 3, the process **8** → **9** → **10** is only slightly, 0.7 (3.1) kcal/mol, endothermic.

The dissociation of complex **10** into three different fragments, $[(OH)P(O)_2(OMe)]^-$ + [PhSeH] + [Cys′], is calculated to be endothermic by 32.8 (26.9) kcal/mol. Since the reverse process **10** → **8** occurs with an extremely small, 0.5 (0.5) kcal/mol, energy barrier and is slightly exothermic, one should estimate the overall enegetics of the process $[OP(OH)_2(OMe)][PhSe]$-$[Cys']^-$ → $[(OH)P(O)_2(OMe)]^-$ + [PhSeH] + [Cys′] from the most stable complex **8**. The product elimination step of reaction 2, i.e., $[OP(OH)_2(OMe)][PhSe][Cys']^-$ → $[(OH)P(O)_2(OMe)]^-$ + [PhSeH] + [Cys′] is found to be endothermic by 33.5 (29.9) kcal/mol. A comparison of the endothermicity of this step with the energetics of the other steps of reaction 2 shows that the former (product elimination step) is the energetically most demanding step of reaction 2. However, we are confident that this step of reaction 2 will be significantly facilitated by the inclusion of the protein environment and solvent effects into the calculations. Indeed, single-point PCM (polarizable-continuum-

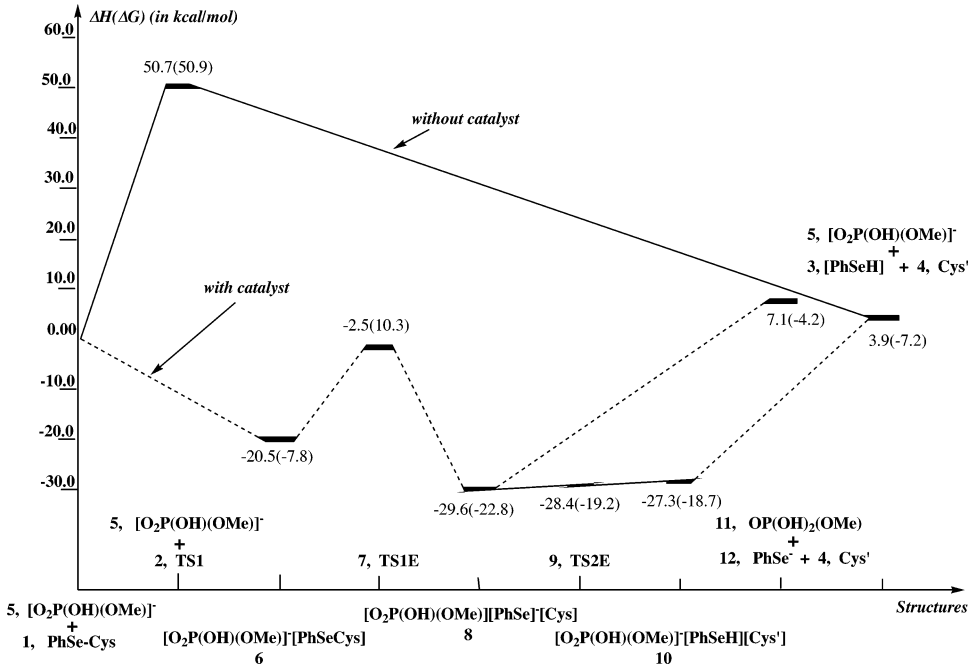


Figure 3. Calculated potential energy surfaces (scaled for the entalphy values) of reactions 1 and 2 for R = Ph.

model)[22] calculations using the water as a solvent reduce the endothermicity of the product elimination steps of reaction 2, e.g. steps of

$$[OP(OH)_2(OMe)][PhSe][Cys']^- \rightarrow [OP(OH)_2(OMe)] + [PhSe]^- + [Cys']$$

$$[OP(OH)_2(OMe)][PhSe][Cys']^- \rightarrow [(OH)P(O)_2(OMe)]^- + [PhSeH] + [Cys']$$

from the $\Delta E = 38.6$ and 37.3 kcal/mol to $\Delta G_{sol} = 0.2$ and 5.8 kcal/mol, respectively. The detailed theoretical investigations of the protein environment and solvent effects on the energetics of the reaction 2 are in progress, and will be reported elsewhere.

Thus, the above presented results clearly show that the pyridoxal 5′-phosphate (PLP)-dependent β-lyases significantly facilitates the metabolism of the selenocysteine Se conjugates (RSe−Cys), which may proceed via the "H atom relay" mechanism: in the first step of this mechanism, the enzyme uptakes a β-H atom from the selenocysteine Se conjugates, and in the following step, it delivers another H atom from the phosphate cofactor of enzyme to the Se and completes the formation of the selenol. It has to be mentioned that, only under the conditions used in this study, the "H atom relay" was found to occur in the stepwise manner, but it may occur simultaneously under different conditions. At this stage, we will not strongly emphasize its stepwise or/and simultaneous nature, which could be altered in the protein environment.

A comparison of the mechanisms of reactions 1 and 2 shows (see Figure 3) that the final products of the selenocysteine Se-conjugates (RSe−Cys) metabolism remain the same without and with the participation of the enzyme and consistent with the β-H-elimination mechanism, i.e., H-atom transfer from C^3 (or C^β) to the Se center. However, the formation of the products is achieved via two different pathways without and with the participation of the enzyme: In the absence of the enzyme, products were formed via the direct β-H-transfer mechanism from the C^3 (or C^β) to the Se center, whereas in the presence of the enzyme, they were formed via the "H atom relay" pathway.

IV. Conclusions

From the above-presented findings one can draw the following conclusions:

1. Without the participation of the pyridoxal 5′-phosphate (PLP)-dependent β-lyases, the metabolism of the selenocysteine Se conjugates (RSe−Cys) via the β-H-elimination mechanism is an energetically and kinetically unfavorable process. It has high activation barriers of 54.0 (54.1), 54.6 (54.6), and 50.7 (50.9) kcal/mol for R= H, Me, and Ph, respectively. The nature of the R ligand has an insignificant effect on the calculated energetics of this reaction.

2. The participation of the pyridoxal 5′-phosphate (PLP)-dependent β-lyases significantly facilitates the metabolism of the selenocysteine Se conjugates (RSe−Cys) and reduces the C^3-H^2 (or $C^\beta-H^\beta$) activation barrier by almost three times, from 50.7 (50.9) kcal/mol to 18.0 (18.1) kcal/mol for R = Ph.

3. In the presence of the model of the (PLP)-dependent β-lyases, the metabolism of the selenocysteine Se conjugates (RSe−Cys) proceeds via the "H atom relay" pathway: in the first step of this pathway, th eenzyme uptakes the β-H atom from the selenocysteine Se conjugates, and in the following step, it delivers another H atom from the phosphate group of PLP cofactor of enzyme to the Se, which completes the formation of the selenol.

4. A comparison of the mechanisms of reactions 1 and 2 shows that the final products of the selenocysteine Se-conjugates (RSe−Cys) metabolism remain the same, whereas the formation of the products is achieved via two different pathways in the absence and presence of the β-lyases. In the absence of the enzyme, products were formed via the direct β-H-transfer mechanism, whereas in the presence of the enzyme, they were formed via the in-direct β-H-transfer mechanism, the "H atom relay" pathway.

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Supporting Information Available: Includes the Cartesian coordinates of all reactants, intermediates, transition states, and products of reactions 1 and 2 (Table S1) and their energetics (Table S2 and Table S3 for reaction 1 and 2, respectively). This material is available free of charge via the Internet at http://pubs.acs.org.

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