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Oxygen radical-mediated oxidation reactions of an alanine peptide motif - density functional theory and transition state theory study

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Abstract

Background: Oxygen-base (O-base) oxidation in protein backbone is important in the protein backbone fragmentation due to the attack from reactive oxygen species (ROS). In this study, an alanine peptide was used model system to investigate this O-base oxidation by employing density functional theory (DFT) calculations combining with continuum solvent model. Detailed reaction steps were analyzed along with their reaction rate constants.

Results: Most of the O-base oxidation reactions for this alanine peptide are exothermic except for the bond-breakage of the C_α-N bond to form hydroperoxy alanine radical. Among the reactions investigated in this study, the activated energy of OH α-H abstraction is the lowest one, while the generation of alkylperoxy peptide radical must overcome the highest energy barrier. The aqueous situation facilitates the oxidation reactions to generate hydroxyl alanine peptide derivatives except for the fragmentations of alkoxyl alanine peptide radical. The C_α-C_β bond of the alkoxyl alanine peptide radical is more labile than the peptide bond.

Conclusion: the rate-determining step of oxidation in protein backbone is the generation of hydroperoxy peptide radical via the reaction of alkylperoxy peptide radical with HO_2. The stabilities of alkylperoxy peptide radical and complex of alkylperoxy peptide radical with HO_2 are crucial in this O-base oxidation reaction.

Introduction

During the past decade, there was a rapidly increasing interest in oxidative damage to proteins, and its relevance to aging and pathological disorders [1-12]. The oxidation mechanism was constructed by identifying the trace of possible reaction intermediates and it is surmised that the mechanism is composed of a series reactions with HO_2 radical, as suggested by Stadtman et al. [2-4]. However, the details of reactions are still undetermined. These reaction mechanisms can provide scientists with some clues to aid in the design of medicines or nutrients to slow down the aging process or to decrease the probability of the age-related diseases. Obviously, an understanding of the processes of the protein oxidation is important, particularly as life expectancy increases. To the best of our knowledge, there are only few articles using ab initio or density functional theory (DFT) methods to study the oxidative damage of proteins through structural factors [13-20]. The main goals of the previous studies were to estimate the stability of carbon-center radicals and the strength of the C-H bond via bond dissociation energy calculations. In our previous study, it was found that the α-H located on a β-sheet is more difficult to abstract than one located on an α-helix [21]. However, this finding contradicts the results reported by Rauk et al. [18] and Owen et al. [22,23]. There were several articles about the OH H-abstraction from several amino acids [24-28]. Huang and Rauk [29] performed a series of theoretical calculations on reactions theoretically starting from an alkylperoxyl radical. Wood et al. [30] studied the C-C backbone fission of four small alkoxy radicals. To our best knowledge, there are no studies regarding the kinetic and thermodynamic aspects of the overall oxidation processes in proteins and therefore it becomes the

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most crucial issue in understanding the protein oxidative damage.

To facilitate the calculation, acetyl(Ala)NH_2, HC(O)$NHCH(CH_3)C(O)NH_2$ denoted as **PA-H**, was chosen to model a simple alanine peptide, as used in the previous studies [13,29], to investigate the oxidation reactions mediated by reactive oxygen species (ROS). In this study, a simple peptide represents a peptide before any oxidation reactions take place. The following reaction scheme is the oxidation reaction process adopted from those suggested by Stadtman, [1-4] but without the metal catalysis in the reactions.

$$\mathbf{PA-H} + \mathrm{OH} \rightarrow \mathbf{PA} + \mathrm{H2O} \quad \text{(a)}$$

$$\mathbf{PA} + \mathrm{O2} \rightarrow \mathbf{PA-O2} \quad \text{(b)}$$

$$\mathbf{PA-O2} + \mathrm{HO2} \rightarrow \mathbf{PA-O2H} + \mathrm{O2} \quad \text{(c)}$$

$$\mathbf{PA-O2H} + \mathrm{HO2} \rightarrow \mathbf{PA-O} + \mathrm{O2} + \mathrm{H2O} \quad \text{(d)}$$

$$\mathbf{PA-O} \rightarrow \mathrm{NHCHO} + \mathrm{NH2COCHO} \quad \text{(e)}$$

$$\mathbf{PA-O} \rightarrow \mathrm{NHCHOCOCH3} + \mathrm{NH2CO} \quad \text{(f)}$$

$$\mathbf{PA-O} + \mathrm{HO2} \rightarrow \mathbf{PA-OH} + \mathrm{O2} \quad \text{(g)}$$

Where **PA-** represents (CHO)$NHC(CH_3)CO(NH_2)$, an α-C center radical of this alanine peptide. Base on the previous theoretical study [31] for CH_3 reacting with HO_2, an association channel of **PA** reacting with HO_2 was also considered in this study.

$$\mathbf{PA} + \mathrm{HO2} \rightarrow \mathbf{PA-O2H} \quad \text{(h)}$$

the rate constants of the above reactions at 298.15 K was calculated using transition state theory to understand their reaction kinetics. However, the effect of tunneling was not considered in this study. The solvent effect was also simulated by using continuum model in order to investigate the influence of an aqueous solution enviroment on the protein oxidation reactions. Through this study, we hope to shed some light on the process of protein oxidation.

Computational method

The geometry optimizations and frequency calculations of all species in this study, such as reactants, intermediates, transition states (**TS**) and products, were carried out at B3LYP/6-31 G(d) level. The final energy values were calculated at BHandHLYP/6-31 + G(d, p) level with ZPE correction. This BHandHLYP calculated level is rather computationally inexpensive and sufficient to obtain the relative energies as described in the previous studies [24] about OH α-H abstraction from alanine and glycine, in spite of its small basis set. The conductor-like polarizable continuum model (CPCM) using UAHF cavities at B3LYP/6-31 + G(d, p) level was suggested as a proper method to obtain the free energies of aqueous solvation [32]. Therefore, in this study CPCM was used to estimate the aqueous solvation free energy of all species. The rate constants were calculated as the following equation

$$k^{TST}(T) = \frac{k_b}{h}\frac{Q^{\neq}}{Q_A Q_B}\exp\left(\frac{-E_0}{k_b T}\right) \quad (1)$$

where Q is partition function, which can be obtained in Gaussian output file; E_0 is the energy with zero-point correction; k_b is Boltzmann constant; h is Planck constant and T is temperature. The transition states were verified by harmonic vibrational frequency analysis with only one negative frequency and checked their reaction pathways with intrinsic reaction coordinate (IRC) analysis by connecting the associated reactants and products. As to the ΔH_{rxn} value, we just used those reported in Gaussian output. We used the structures optimized in the gas phase to do single point calculation with CPCM at B3LYP/6-31 G(d, p) level without zero-point correction. There are several issues are worth noting for the proposed oxidation mechanism before entering the discussion section. In **Reaction *b***, as mentioned in the introduction section, alkylperoxy peptide radical is an important intermediate in the oxidation chain reactions of many chemical and biological systems due to its stability [33-37]. An alkylperoxy peptide radical can be generated easily from an α-C peptide radical surrounded by oxygen molecules. There are rotational isomers for this radical, which can be located by rotating the oxygen molecule with respect to the α-C-C bond, followed by structural optimization. After locating the stable structures, their heat of formation values can be calculated. In **Reaction *d***, although **PA-O_2H**s are rather difficult to obtaine via **Reaction *c***, as stated in previous subsection, it can be generated without energy barrier by attaching the terminal O of HO_2 directly to the carbon radical site of **PA**, Reaction h. Therefore, it is important to study the reaction between **PA** and HO_2 in present study. Apart from **PA-O_2H**s, the related isomers can be classified according to the rotation of the C_α-O or O-O bond. The H-migration reactions involved in **Reaction *c* and *g*** are crucial for the oxidation mechanism. Without them, the reactions involve higher energetic barriers. In **Reaction *c***, the spin densities of hydroperoxyl radical (HO_2) [38,39] and alkylperoxy alanine peptide radical (**PA-O_2**s) are mostly located on the terminal oxygen. From literature [40,41], the most

stable structure of HO_2 dimer is HOO-OOH. This implies that the first step of **Reaction *c*** is to generate **PA-OOOOH** by attaching a HO_2 to the terminal O of **PA-O_2s**. After three consecutive H-migration reactions, the products of the **Reaction *c*** can be obtained. **Reaction *g*** is a reaction involving an HO_2 radical attacking an alkoxyl alanine peptide radical (**PA-O**), generating a hydroxyl alanine peptide derivative, i.e., **PA-OH**, and O_2. Similarly, owing to the dominant spin density of **PA-O** located on O, the pre-reactive species **PA-OOOH** are formed first and then **Reaction *g*** occurs via three consecutive H-migrations. All calculations were performed with Gaussian03 package [42].

Result and discussion

The focus of the present study is the oxidation reactions taking place at protein backbone, and therefore, no other intermediates, such as those generated via the rotation of the bonds in backbone, were considered. The relative energies were shown under the molecular labels in Figures 1,2,3,4,5,6,7 and the values without parentheses were in gas phase, while those in parenthesis were taken the free energies of aqueous solvation into consideration. The following discussion was organized according to the reaction types of the previously mentioned reaction scheme.

(a) Reaction *a*: the generation of an α-C center peptide radical by OH α-H abstraction

The mechanism of the α-H abstraction reaction by OH radical from alanine peptide -**PA-H** was presented in Figure 1. A pre-reactive complex **IN1-a-PA** is formed first, followed by H_2O elimination to generate an α-C center radical peptide intermediate, **PA**. The energy barrier of the OH α-H abstraction from **PA-H**, is 22.9 kJ mol^{-1} in gas phase compared with 24.6 kJ mol^{-1} in an aqueous environment. Their corresponding rate coefficients of these two reactions, without tunnel effect consideration are 4.38 × 0^7 M^{-1} s^{-1} and 2.24 × 10^7 M^{-1} s^{-1}, respectively. The rate constant we found is one order lower than that for oligopeptides [8,43] (k ~10^8) and two order lower than that for cyclic peptides [8,44] (k ~10^9) in aqueous solutions. However, they are close to the rate constant [9] for free alanine, 7.7 × 10^7 M^{-1} s^{-1}, at pH ca. 7.

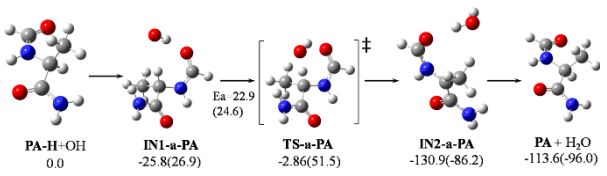

Figure 1 The mechanism and structures of the OH α-H abstraction reaction from PA-H, with the relative energy in kJ mol^{-1} at 0 K.

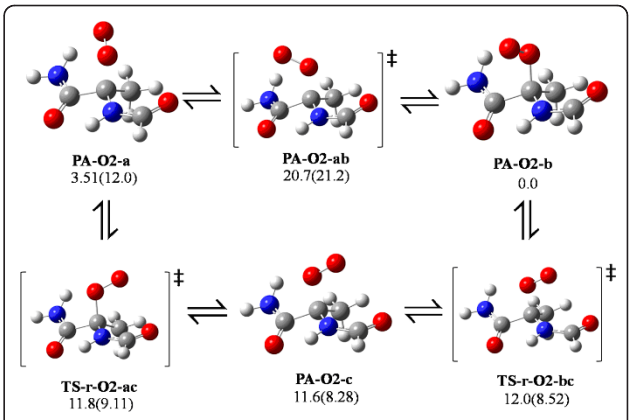

Figure 2 The conversion mechanism between the PA-O_2 isomers and their corresponding structures with the relative energy in kJ mol^{-1} at 0 K.

(b) Reaction *b*: Alkylperoxy peptide radical generation through molecular oxygen molecular addition to α-C center peptide radical

Three different conformers of alkylperoxy alanine peptide radical can be found, i.e., **PA-O_2-a, PA-O_2-b** and **PA-O_2-c**. There is no energy barrier for this oxygen addition reaction. Interestingly, the rate constants of cyclic peptides with oxygen were measured, ca. 10^9 M^{-1} s^{-1} [44], which indicates that it could be barrierless and controlled by diffusion. In the gas phase, the most stable conformer is **PA-O_2-b**, consistent with the previous study [29], followed by **PA-O_2-a** and **PA-O_2-c** is the least stable one among these three. The optimized structures and the interconversion mechanisms among these three isomers were shown in Figure 2. However,

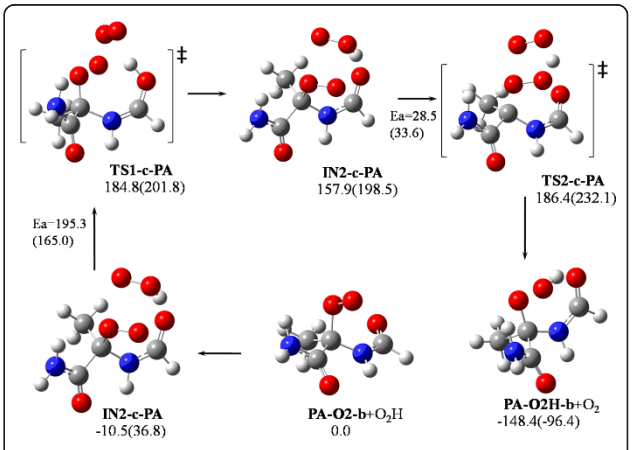

Figure 3 The most probable mechanism of Reaction *c*, PA-O_2 + O_2H → PA-O_2H + O_2, with the lowest reaction barriers and the relative energy in kJ mol^{-1} at 0 K.

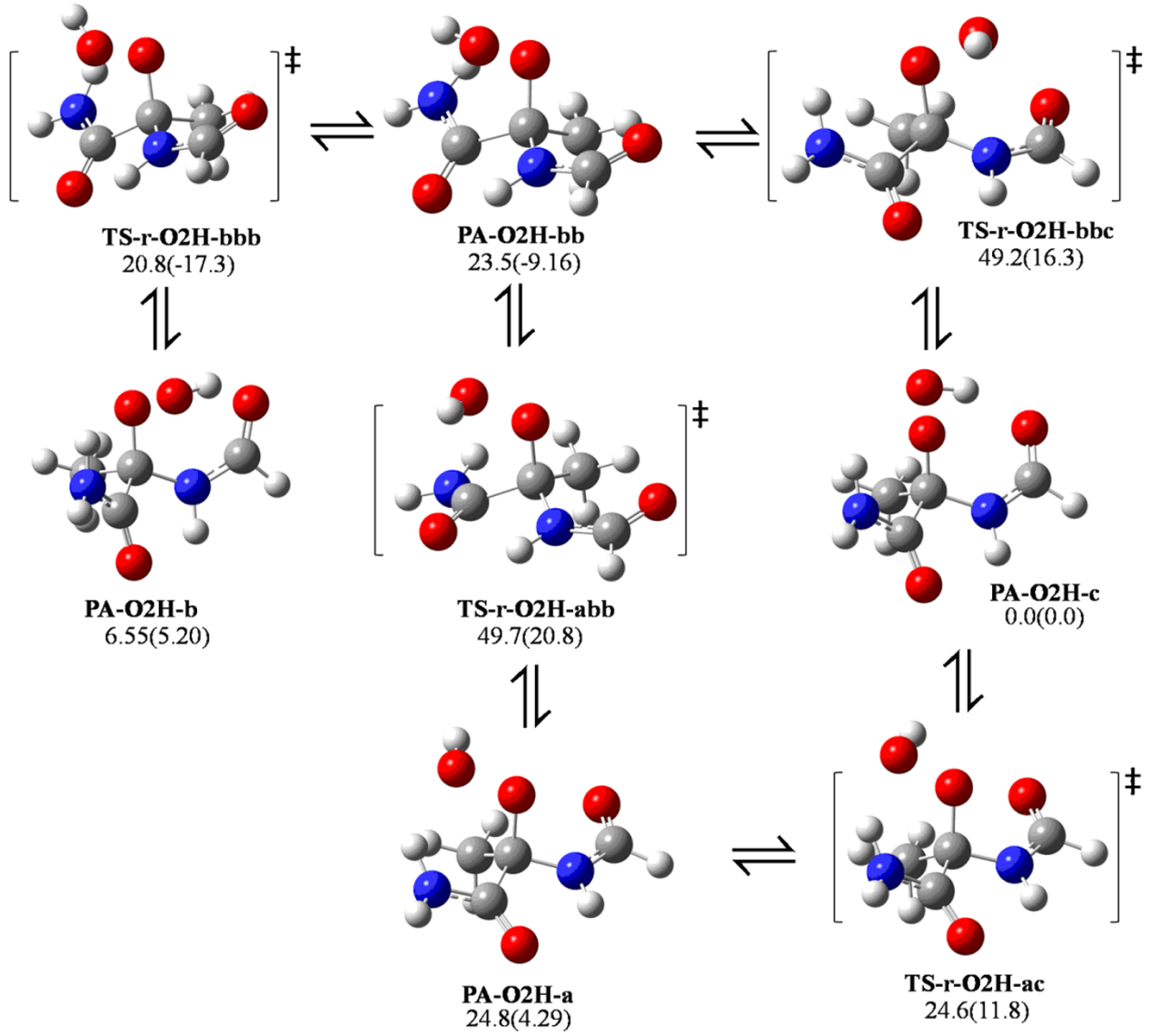

Figure 4 The conversion mechanism between the PA-O_2H isomers and their corresponding structures with the relative energy in kJ mol^{-1} at 0 K.

including solvent effect, **PA-O_2-c** becomes more stable than **PA-O_2-a**. **PA-O_2-b** is a stable intermediate since its heat formation is not very large, 55.7 kJ mol^{-1} in gas phase and 64.7 kJ mol^{-1} in aqueous phase. All the energy barriers of the interconversion among **PA-O_2s**, by rotating the C_α-O bond, are pretty small and lower than the energy required for dissociating the oxygen molecule directly.

(c) The generation of hydroperoxy analine peptide intermediate (PA-O_2H) via Reactions *c*

The products of the **Reaction *c*** require three consecutive H-migration reactions as shown in Equation (2).

$$HO2+PA-O2 \rightarrow PA-OOOOH \rightarrow PA-OOOHO \rightarrow PA-OOHOO \rightarrow PA-O2H+O2 \quad (2)$$

According to the Equation (2), the possible conformations of **PA-OOOOH** were searched based on the orientation between **PA-O_2s** and HO_2 and the corresponding transition states and intermediate (**PA-OOO(H)O**) of the two consecutive H-migration reactions were found. Figure 3 showed the least energy pathway of **Reaction *c*** found in our calculation. The adduct (**IN1-c-PA**) of HO_2 with **PA-O_2-b** is only stable in gas phase (10.5 kJ mol^{-1}) but is unstable in aqueous environment. The first H-migration, via **TS1-c-PA**, is the most difficult step in **Reaction *c*** and its energy barrier

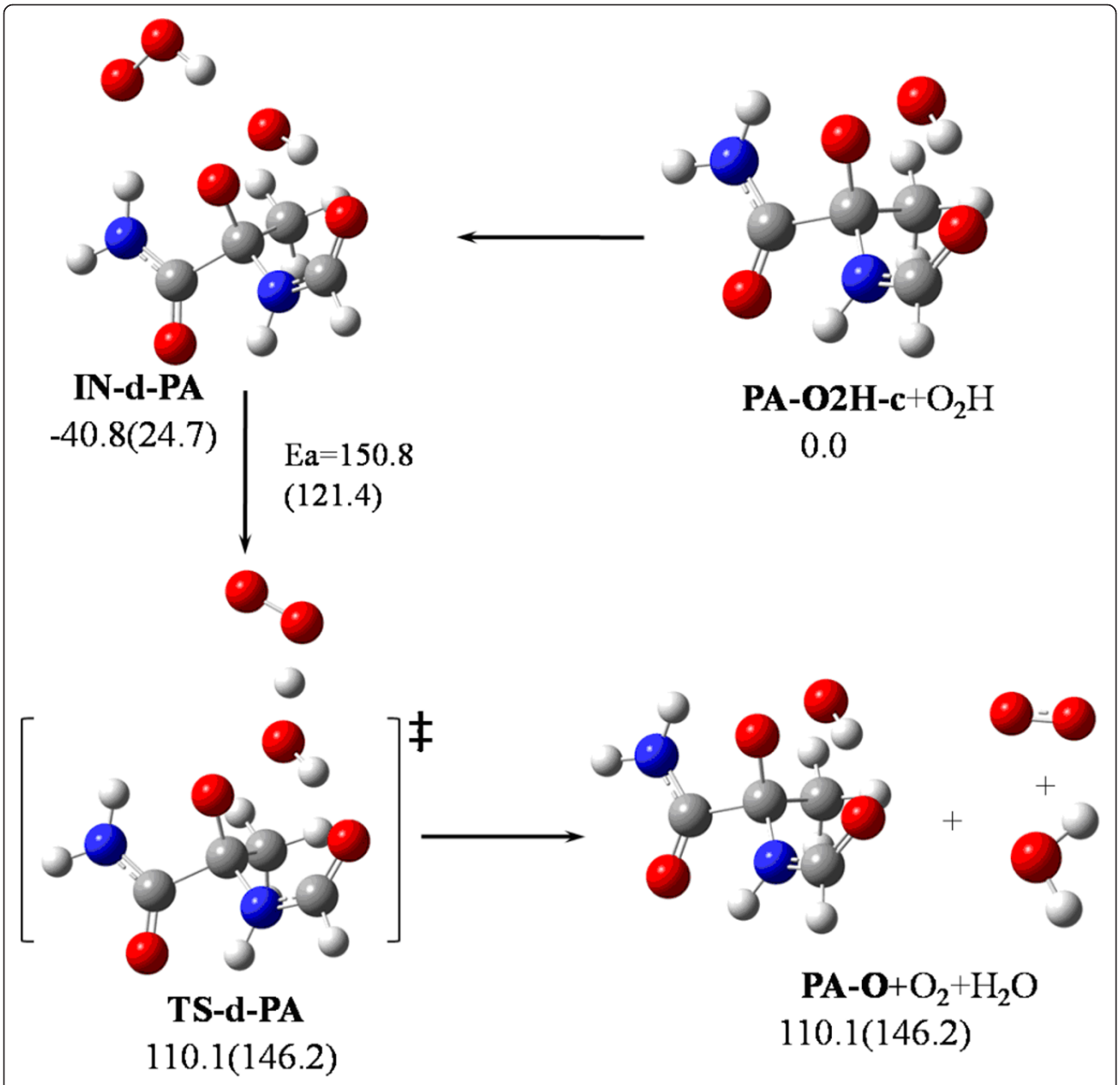

Figure 5 The most probable mechanism of Reaction *d*, PA-O_2H + O_2H → PA-O + H_2O + O_2, with the lowest reaction barriers and the relative energy in kJ mol^{-1} at 0 K.

in kJ mol^{-1} are 195.3 in gas phase, and 165.0 in an aqueous phase. Their corresponding rate constants were 5.38 × 10^{-22} and 1.09 × 10^{-16} s^{-1} M^{-1}, respectively. Unstable as the intermediate **IN2-c-PA** is, it easily undergoes the second H-migration via **TS2-c-PA** and subsequently forms the product by losing O_2. Therefore, to estimate the energy barrier and rate constant of **Reaction *c*** in the present study, only the first H-migration reaction was taken into consideration. The energy barriers were found in gas and in aqueous phases as 195.3 and 165.0 kJ mol^{-1}, respectively. Their corresponding rate constants in sec^{-1} M^{-1} were 5.38 × 10^{-22} and 1.09 × 10^{-16}, respectively. **Reaction *c*** can also take place via a direct abstraction channel, as shown in following equation

$$PA - O2 + HO2 \rightarrow PA - OO \ldots HOO \rightarrow PA - O2H + O2 \quad (3)$$

This is similar to the reaction of HO_2 with CH_3 and RO_2 as described in previous theoretical works [31,45,46]. However, we did not find any intermediate **PA-OO...HOO** that existed at singlet state, which is consistent with the previous study. Due to its high

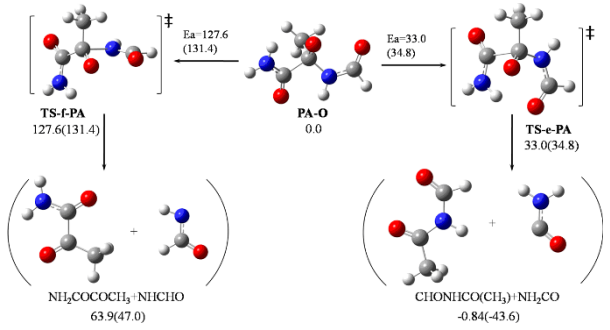

Figure 6 The mechanism of Reaction *e* and Reaction *f* and the related fragmentations of PA-O, with the relative energy in kJ mol^{-1} at 0 K.

energy, triplet state **PA-OO...HOO** was not considered in the present study [45].

(d) The generation of hydroperoxy alanine peptide by a HO_2 addition to PA

The relative energy of **TS-r-O2H-bbb**, which is the activated complex of the conversion reaction between **PA-O_2H-b** and **PA-O_2H-bb**, is lower than that of **PA-O_2H-bb** in gas phase. In aqueous phase, the strong solvation of **TS-r-O2H-bbb** makes its relative energy even lower than those of both reactant and product for the interconversion reaction. These results indicate that the rotation of the O-O bond has a small or no energy barrier, therefore, the conformers formed by rotating the O-O bond were ignored and only the conformers formed by rotation of the C_α-O bond were considered in this study. Two conformers were found, **PA-O_2H-a** and **PA-O_2H-c**. The related optimized structures and conversion mechanism were presented in Figure 4.

In general, the conversion by rotation across C_α-N bond is difficult in gas phase but is rather easy in aqueous phase due to the strong solvation in its **TS, TS-r-O_2H-bbc**. Moreover, the conversions among **PA-O_2Hs** are even harder than the conversions among **PA-O_2s**. The most stable conformer of **PA-O_2Hs** in gas phase is **PA-O_2H-c** because the H in HO_2 can serve as a hydrogen bond (HB) donor [38-41,47,48], interacting with carbonyl O, which is consistent with the previous study [29,30]. It also can explain why **PA-O_2H-b** is more stable than **PA-O_2H-bb** in gas phase. However, **PA-O_2H-bb** becomes the most stable conformer among **PA-O_2Hs** when the aqueous solvation is taken into consideration. The dominant species of **PA-O_2Hs** in gas and in aqueous phases are **PA-O_2H-c** and **PA-O_2H-bb**, respectively. Their corresponding formation heats are -210.7 and -175.6 kJ mol^{-1}, highly exothermic.

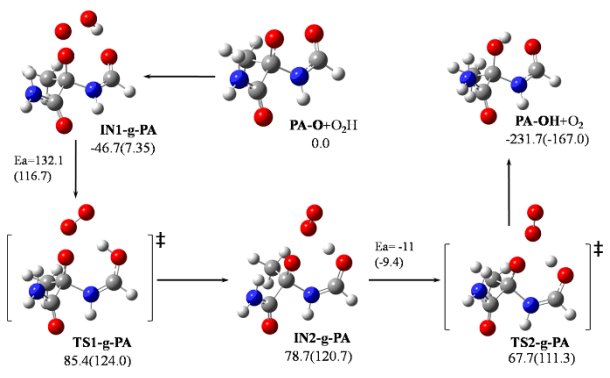

Figure 7 The most probable mechanism of Reaction *g*, PA-O + O_2H → PA-OH + O_2, with the lowest reaction barriers and the relative energy in kJ mol^{-1} at 0 K.

(e) Reaction *d*: PA-O_2H + HO_2 → PA-O + H_2O + O_2

The alkoxyl radical peptide intermediate (**PA-O**) can be generated by the hydroperoxy alanine peptide intermediate (**PA-O_2H**) first reacted with HO_2 and then followed by the loss of H_2O and O_2. The reaction mechanism of **Reaction *d*** can be proposed as the following:

$$PA-O2H + HO2 \rightarrow PA-O2H2 \rightarrow PA-O + H2O + O2 \quad (4)$$

There exists a pre-reactive species, **PA-$(O_2H)_2$**, before the generation of products, i.e., **PA-O**, H_2O and O_2. Therefore, all probable conformations of **PA-$(O_2H)2$** s were searched by taking into account all conformers of **PA-O_2Hs** interacting with HO_2, as found in the previous subsection. Through the above process, the generated **PA-$(O_2H)2$** s were selected as the pre-reactive of **Reaction *d*** and their corresponding **TS**s were found. Meanwhile, the possible **TS**s of **Reaction *d*** were also searched by considering all conformers of hydroperoxy peptide intermediate (**PA-O_2Hs**) attacked by HO_2 directly from all probable directions. The found **TS**s were verified by tracing along the reaction pathway to find the related pre-reactive species. Finally, the lowest energy pathway among those we found were listed in Figure 5.

The structure of **IN-d-PA**, also a pre-reactive specie of **Reaction *d***, has the terminal O of HO_2 connected to the amino H and the H of HO_2 close to the terminal O of the other HO_2 bonded to C_α. Consistent with previous studies [38-41,47,48] about the reaction with HO_2, its terminal O can serve as a HB acceptor and the H as a HB donor. These HB interactions stabilize the complex about 40.8 kJ mol^{-1} in gas phase. Via **TS-d-PA, IN-d-PA** can generate **PA-O**, O_2 and H_2O, with the energy barrier in gas phase and in aqueous phase of 150.8 and 121.4 kJ mol^{-1}, respectively. Their corresponding rate constants are 1.56×10^{-13} and 2.20×10^{-8} s^{-1} M^{-1}, respectively.

(f) The fragmentations of alkoxyl radical peptide intermediate (PA-O): Reaction e and f

With a rather long C-C_α bond (1.607 Å), **PA-O** can break the C-C_α bond rather easily. Our calculated results also support this statement with the energy barrier, the reaction energies and corresponding rate constant in gas phase are 0.48 kJ mol^{-1}, 33.0 kJ mol^{-1} and 1.05 × 10^7 s^{-1} M^{-1}, respectively. Their counterparts in aqueous phase are 43.6 kJ mol^{-1}, 34.8 kJ mol^{-1} and 5.06 × 10^6 s^{-1} M^{-1}, respectively. These imply the probability for an alkoxy peptide radical to yield peptide fragmentation. The breakage of C_α-N bond in **PA-O** is more difficult than that of the C_α-C bond. As to the C_α-N bond breakage, the energy barrier, reaction energy and the corresponding rate constant in gas phase are 127.6 kJ mol^{-1}, 63.9 kJ mol^{-1} and 7.50 × 10^{-10} s^{-1} M^{-1}, respectively. Their corresponding values in aqueous phase are 131.4 kJ mol^{-1}, 47.0 kJ mol^{-1} and 1.62 × 10^{-10} s^{-1} M^{-1}, respectively. This data were also listed in Table 1 for comparison. The trend is in agreement with the previous theoretical study [29]. The optimized structures of their transition states and fragments for both C_α-N and C_α-C bond-breakage were presented in Figure 6. Because the backbone breaking reaction of the peptide was the main issue in this study, our products were all TS-like species but not the lowest-energy products. Therefore, the energy released in the studied reactions is lower than that found in the previous studies [29,30]. Interestingly, the backbone of the above transition states, **TS-e-PA** and **TS-f-PA**, are almost folded, and therefore, it will be rather difficult for these reactions to take place in proteins with restriction from the whole structure, as observed in this alanine peptide motif.

(g) Reaction *g*: it is a generation of a hydroxyl derivative through an alkoxyl radical with HO_2

In order to generate the products, **Reaction *g*** needs to undergo three consecutive H-migrations as indicated in Equation (4).

$$PA-O+HO2 \rightarrow PA-OOOH \rightarrow PA-OOHO \rightarrow PA-OHOO \rightarrow PA-OH+O2 \quad (5)$$

Table 1 The energy barriers, reaction energies and rate constants of the two fragmentation reactions in Reaction *f* as investigated in the current study, with energy in kJ mol-1 and rate constant in sec-1 mol-1

Bond to be fragmentated	Gas phase			Aqueous phase		
	Ea	ΔH*rxn*	*k*	*Ea*	ΔH*rxn*	*k*
C-Cα	0.48	33.0	1.05 × 10^7	43.6	34.8	5.06 × 10^7
Cα-N	127.6	63.9	7.5 × 10^{-10}	131.4	47.0	1.62 × 10^{-10}

Similar to **Reaction *c***, we searched all possible **PA-OOOHs**, then tried to find the corresponding **PA-OO(H)O** and the **TS**s for the two corresponding H-migration based on Equation (5). Figure 7 lists the lowest energy pathway of **Reaction *g*** that we have found. The association energy of **IN1-g-PA** to form the isolated **PA-O** and HO_2, is 46.7 kJ mol^{-1} in gas phase. The terminal H of HO_2 interacts with carbonyl O of backbone. Similarly, the first H-migration via **TS1-g-PA** is the rate determining step and the second H-migration is almost barrierless since the relative energy of **TS2-g-PA** is lower than that of **IN2-g-PA**. Therefore, only the first H-migration from **IN1-g-PA** to **IN2-g-PA** via **TS1-g-PA** was considered to represent **Reaction *g***. And, the energy barriers were found to be 127.6 kJ mol^{-1} in gas phase and 131.4 kJ mol^{-1} in aqueous phase, respectively. Their corresponding rate constants in were 7.50 × 10^{-10} and 1.62 × 10^{-10} s^{-1} M^{-1}, respectively.

(h) The overview from reaction *a* to reaction *g*

Although, all the oxidation reactions for the alanine peptide considered in the present study are exothermic, except for the breakage of C_α-N bond in **PA-O**. Table 2 lists the energies and rate constants of all the mentioned reactions with barriers in both gas and aqueous phases. It indicates that OH α-H abstraction is the easiest step and the generation of hydroperoxy alanine peptide radical **PA-O2H** via **Reaction *c*** is the most difficult one. Except for those reactions involved the fragmentations of **PA-O**, the oxidation reactions are facilitated by the aqueous solvation, especially for the two most difficult steps, **Reaction *c*** and ***d***. To generate the hydroperoxy peptide intermediate via **Reaction *c*** is rather difficult and should go through the HO_2 addition reaction to form α-C center radical

Table 2 The energy barriers, reaction energies and rate constants of the oxidation reactions for the simple alanine peptide as investigated in the current study, with energy in kJ mol-1 and rate constant in sec-1 mol-1

Reactions	Gas phase			Aqueous phase		
	Ea	ΔH_{rxn}	*k*	*Ea*	ΔH_{rxn}	*k*
(a)	22.9	-113.6	4.38 × 10^7	24.6	-103.3	2.24 × 10^7
(c)	195.3	-148.4	5.38 × 10^{-22}	165.0	-96.4	1.09 × 10^{-16}
(d)	150.8	-129.3	1.56 × 10^{-13}	121.4	-125.9	2.20 × 10^{-8}
(e)	33.0	-0.84	1.05 × 10^7	34.8	-43.6	5.06 × 10^6
(f)	127.6	63.9	7.50 × 10^{-10}	131.4	47.0	1.62 × 10^{-10}
(g)	132.1	-231.7	1.46 × 10^{-10}	116.7	-167.0	8.19 × 10^{-8}

peptide intermediate. With the participation of HO_2, in **Reaction *c*, *d*** and ***g***, pre-reactive peptide intermediates, **IN1-c-PA, IN1-d-PA** and **IN1-g-PA**, were formed first, respectively. Their dissociation energies to separate into reactants were 10.5, 40.8 and 46.7 kJ mol^{-1} in gas phase, respectively. However they all become unstable in aqueous environment. Because **IN1-c-PA** is the most unstable one, the effects that increase its stability will be the key factors to enhance the oxidation processes tha involving it. The generation of alkoxyl alanine peptide radical is a critical step since it can break the C_α-C_β bond easily to yield the peptide backbone fragmentation.

Conclusion

Theoretical O-base oxidation in protein backbone was performed, using an alanine peptide as a model, and focused on the peptide backbone. The solvent participating in oxidation procedure was not considering this study, however, continuum model was used to estimate the influence of the aqueous phase. Several important features were found as shown in the following. Most of the oxidation reactions in this alanine peptide are exothermic, except for the breakage of the C_α-N bond from hydroperoxy alanine peptide radical. The OH α-H abstraction is the easiest step and the generation of alkylperoxy peptide radical is the most difficult one. The aqueous environment facilitates the oxidation processes, except for the fragmentations of alkoxyl alanine peptide radical. The C_α-C_β bond of the alkoxyl alanine peptide radical is more labile than the peptide bond. Generating a hydroxyl alanine peptide derivative from the alkoxyl alanine peptide radical is feasible. The C_α-C_β fragmentation takes place easily and causes large structural deformation by yielding alkoxyl peptide radical. Therefore, the rate determining step of oxidation in protein backbones is the generation of hydroperoxy peptide radical via the HO_2 addition reaction to form the alkylperoxy peptide radical. The stabilities of alkylperoxy peptide radical and the alkylperoxy peptide radical and HO_2 complex are important factors that influence the reaction rate.

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Authors' contributions

HYC and JYC performed all the calcuations. SJ and TRJ analyzed the data. HFL and FYL initiated and designed the study and finalized the manuscript. All authors have read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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References

1. Stadtman ER: **Protein oxidation and aging.** *Science* 1992, **257**:1220.
2. Berlett BS, Stadtman ER: **Protein oxidation in aging, disease, and oxidative stress.** *J Biol Chem* 1997, **272**:20313.
3. Stadtman ER: **Importance of individuality in oxidative stress and aging 1, 2.** *Free Radic Biol Med* 2002, **33**:597-604.
4. Stadtman E, Levine R: **Free radical-mediated oxidation of free amino acids and amino acid residues in proteins.** *Amino acids* 2003, **25**:207-218.
5. Garrison WM: **Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins.** *Chem Rev* 1987, **87**:381-398.
6. Smith MA, Sayre LM, Monnier VM, Perry G: **Radical AGEing in Alzheimer's disease.** *Trends Neurosci* 1995, **18**:172-176.
7. Remmen HV, Richardson A: **Oxidative damage to mitochondria and aging.** *Exp Gerontol* 2001, **36**:957-968.
8. Hawkins CL, Davies MJ: **Generation and propagation of radical reactions on proteins.** *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 2001, **1504**:196-219.
9. Davies MJ: **The oxidative environment and protein damage.** *Biochimica et Biophysica Acta (BBA)-Proteins & Proteomics* 2005, **1703**:93-109.
10. Mariani E, Polidori M, Cherubini A, Mecocci P: **Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview.** *J Chromatogr B* 2005, **827**:65-75.
11. Brennan ML, Hazen S: **Amino acid and protein oxidation in cardiovascular disease.** *Amino acids* 2003, **25**:365-374.
12. Lin MT, Flint Beal M: **The oxidative damage theory of aging.** *Clin Neurosci Res* 2003, **2**:305-315.
13. Armstrong D, Yu D, Rauk A: **Oxidative damage to the glycyl α-carbon site in proteins: an ab initio study of the CH bond dissociation energy and the reduction potential of the C-centered radical.** *Can J Chem* 1996, **74**:1192-1199.
14. Rauk A, Yu D, Armstrong DA: **Toward Site Specificity of Oxidative Damage in Proteins: C - H and C - C Bond Dissociation Energies and Reduction Potentials of the Radicals of Alanine, Serine, and Threonine ResiduesAn ab Initio Study.** *J Am Chem Soc* 1997, **119**:208-217.
15. Block D, Yu D, Armstrong D, Rauk A: **On the influence of secondary structure on the α-C ⊠ H bond dissociation energy of proline residues in proteins: a theoretical study.** *Can J Chem* 1998, **76**:1042-1049.
16. Jonsson M, Wayner DDM, Armstrong DA, Yu D, Rauk A: **On the thermodynamics of peptide oxidation: anhydrides of glycine and alanine1.** *Journal of the Chemical Society, Perkin Transactions 2* 1998, 1967-1972.
17. Rauk A, Yu D, Armstrong D: **Oxidative damage to and by cysteine in proteins: An ab initio study of the radical structures, CH, SH, and CC bond dissociation energies, and transition structures for H abstraction by thiyl radicals.** *J Am Chem Soc* 1998, **120**:8848-8855.
18. Rauk A, Yu D, Taylor J, Shustov G, Block D, Armstrong D: **Effects of structure onα-C ⊠ H bond enthalpies of amino acid residues: relevance to H transfers in enzyme mechanisms and in protein oxidation.** *Biochemistry* 1999, **38**:9089-9096.
19. Rauk A, Armstrong DA: **Influence of α-Sheet Structure on the Susceptibility of Proteins to Backbone Oxidative Damage: Preference for £\C-Centered Radical Formation at Glycine Residues of Antiparallel £]-Sheets.** *J Am Chem Soc* 2000, **122**:4185-4192.
20. Rauk A, Armstrong DA, Fairlie DP: **Is Oxidative Damage by α-Amyloid and Prion Peptides Mediated by Hydrogen Atom Transfer from Glycine £ \-Carbon to Methionine Sulfur within α-Sheets?** *J Am Chem Soc* 2000, **122**:9761-9767.
21. Lu H-F, Li F-Y, Lin SH: **Site specificity of α-H abstraction reaction among secondary structure motif–An ab initio study.** *J Comput Chem* 2007, **28**:783-794.

22. Owen MC, Viskolcz B, Csizmadia IG: **Quantum chemical analysis of the unfolding of a penta-glycyl 3[sub 10]-helix initiated by HO[sup [bullet]], HO[sub 2][sup [bullet]], and O[sub 2][sup - [bullet].** *J Chem Phys* 2011, **135**:035101-035109.
23. Owen MC, Viskolcz B, Csizmadia IG: **Quantum Chemical Analysis of the Unfolding of a Penta-alanyl 310-Helix Initiated by HO•, HO2• and O2-•.** *The Journal of Physical Chemistry B* 2011, **115**:8014-8023.
24. Galano A, Alvarez-Idaboy JR, Montero LA, Vivier-Bunge A: **OH hydrogen abstraction reactions from alanine and glycine: A quantum mechanical approach.** *J Comput Chem* 2001, **22**:1138-1153.
25. Galano A, Alvarez-Idaboy JR, Bravo-Pérez G, Ruiz-Santoyo ME: **Mechanism and rate coefficients of the gas phase OH hydrogen abstraction reaction from asparagine: a quantum mechanical approach.** *J Mol Struct (THEOCHEM)* 2002, **617**:77-86.
26. Galano A, Alvarez Idaboy JR, Cruz Torres A, Ruiz Santoyo M: **Kinetics and mechanism of the gas phase OH hydrogen abstraction reaction from methionine: A quantum mechanical approach.** *Int J Chem Kinet* 2003, **35**:212-221.
27. Galano A, Alvarez-Idaboy JR, Cruz-Torres A, Ruiz-Santoyo ME: **Rate coefficients and mechanism of the gas phase OH hydrogen abstraction reaction from serine: a quantum mechanical approach.** *J Mol Struct (THEOCHEM)* 2003, **629**:165-174.
28. Galano A, Alvarez-Idaboy JR, Agacino-Valdés E, Ruiz-Santoyo ME: **Quantum mechanical approach to isoleucine+OH gas phase reaction. Mechanism and kinetics.** *J Mol Struct (THEOCHEM)* 2004, **676**:97-103.
29. Huang ML, Rauk A: **Structure and reactions of the peroxy radicals of glycine and alanine in peptides: an ab initio study.** *J Phys Org Chem* 2004, **17**:777-786.
30. Wood GPF, Rauk A, Radom L: **Modeling β-Scission Reactions of Peptide Backbone Alkoxy Radicals: Backbone C - C Bond Fission.** *Journal of Chemical Theory and Computation* 2005, **1**:889-899.
31. Zhu R, Lin M: **The CH3+ HO2 reaction: First-principles prediction of its rate constant and product branching probabilities.** *J Phys Chem A* 2001, **105**:6243-6248.
32. Takano Y, Houk K: **Benchmarking the conductor-like polarizable continuum model (CPCM) for aqueous solvation free energies of neutral and ionic organic molecules.** *Journal of Chemical Theory and Computation* 2005, **1**:70-77.
33. Ingold KU: **Peroxy radicals.** *Accounts of Chemical Research* 1969, **2**:1-9.
34. Porter NA: **Mechanisms for the autoxidation of polyunsaturated lipids.** *Accounts of Chemical Research* 1986, **19**:262-268.
35. Bowry VW, Ingold K: **The Unexpected Role of Vitamin E (α-Tocopherol) in the Peroxidation of Human Low-Density Lipoprotein 1.** *Accounts of Chemical Research* 1999, **32**:27-34.
36. Tallman KA, Pratt DA, Porter NA: **Kinetic Products of Linoleate Peroxidation: Rapid β-Fragmentation of Nonconjugated Peroxyls.** *J Am Chem Soc* 2001, **123**:11827-11828.
37. Pratt DA, Porter NA: **Role of Hyperconjugation in Determining Carbon - Oxygen Bond Dissociation Enthalpies in Alkylperoxyl Radicals.** *Org Lett* 2003, **5**:387-390.
38. Jackels CF, Phillips DH: **An ab initio investigation of possible intermediates in the reaction of the hydroxyl and hydroperoxyl radicals.** *J Chem Phys* 1986, **84**:5013.
39. Parreira RLT, Galembeck SE: **Characterization of Hydrogen Bonds in the Interactions between the Hydroperoxyl Radical and Organic Acids.** *J Am Chem Soc* 2003, **125**:15614-15622.
40. Fitzgerald G: **The cyclic, two-hydrogen bond form of the HO2 dimer.** *J Chem Phys* 1984, **81**:362.
41. Fitzgerald G: **The open chain or chemically bonded structure of H2O4: The hydroperoxyl radical dimer.** *J Chem Phys* 1985, **83**:6275.
42. Gaussian 03 RC, Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA: Gaussian, Inc., Wallingford CT; 2004.
43. Rao PS, Hayon E: **Reaction of hydroxyl radicals with oligopeptides in aqueous solutions. Pulse radiolysis study.** *J Phys Chem* 1975, **79**:109-115.
44. Hayon E, Simic M: **Pulse radiolysis study of cyclic peptides in aqueous solution. Absorption spectrum of the peptide radical -NHCHCO.** *J Am Chem Soc* 1971, **93**:6781-6786.
45. Hou H, Wang B: **A Systematic Computational Study on the Reactions of HO2 with RO2: The HO2 + CH3O2(CD3O2) and HO2 + CH2FO2 Reactions.** *J Phys Chem A* 2004, **109**:451-460.
46. Hou H, Li J, Song X, Wang B: **A Systematic Computational Study of the Reactions of HO2 with RO2: The HO2 + C2H5O2 Reaction.** *J Phys Chem A* 2005, **109**:11206-11212.
47. Aloisio S, Francisco JS: **Complexes of Hydroperoxyl Radical with Glyoxal, Methylglyoxal, Methylvinyl Ketone, Acrolein, and Methacrolein: Possible New Sinks for HO2 in the Atmosphere?** *J Phys Chem A* 2003, **107**:2492-2496.
48. Shi Q, Belair SD, Francisco JS, Kais S: **On the interactions between atmospheric radicals and cloud droplets: A molecular picture of the interface.** *Proc Natl Acad Sci* 2003, **100**:9686.

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