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Oxidation of Methionine Residues in Aqueous Solutions: Free Methionine and Methionine in Granulocyte Colony-Stimulating Factor

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Abstract: The free energy barriers and a mechanism of the oxidation of the amino acid methionine in water and in granulocyte colony-stimulating factor (G-CSF) are analyzed via combined quantum mechanical and molecular mechanical (QM/MM) methods, constrained molecular dynamics, and committor probability calculations. The computed free energy barrier of free methionine amino acid is very close to the measured value (14.7 ± 1.2 versus 15.5 ± 0.02 kcal/mol). The reaction coordinate was found to be the difference between the O−O bond of H_2O_2 and the S−O bond, where the S is the sulfur atom of the methionine residue. It was confirmed by computing the committor probability distribution and the distribution of constrained forces that this coordinate is not coupled to the activation of other degrees of freedom. The computed free energies of the oxidation of methionine residues in G-CSF indicate that the protein environment has insignificant effects on the reaction barriers of oxidation. This result further validates our proposal that the access of solvent to methionine sites, as measured by the two-shell water coordination number, governs the kinetics of the oxidation reaction of methionine groups in a protein molecule. We also found that the number of hydrogen bonds between the distal oxygen of H_2O_2 and the water molecules near the methionine increases along the reaction coordinate as oxidation progresses, indicating that the charge separation developed during the oxidation by H_2O_2 is stabilized by specific interactions with water molecules, such as hydrogen bonding.

Introduction

Oxidation of methionine amino acids is one of the major chemical pathways that degrade protein molecules[1−6] and is also related to the cause of several diseases.[7−9] In a solution environment, peroxides efficiently oxidize the sulfur atom of methionine residues to form methionine sulfoxide, leading to the inactivity of protein pharmaceuticals.[4,5,10] To stabilize protein pharmaceuticals in solution against processes such as oxidation, additives are introduced into the aqueous solutions, and the resulting mixtures are then called protein formulations.[2,4−6] These formulations are typically quite difficult to develop because a detailed mechanistic understanding of processes, such as oxidation, is lacking.

Oxidation of organic sulfides by peroxides has traditionally been conceived of as an acid-catalyzed mechanism,[10−13] in which a general acid facilitates proton transfer. The transfer of protons has also been hypothesized to be the rate-limiting step for the oxidation of organic sulfides by peroxides. However, ab initio studies have led to other mechanisms being proposed.[14−18] Recently, we have demonstrated that only one of the mechanisms in the literature is consistent with all experimental data.[18] In this mechanism that we proposed, water molecules stabilize the developing charge separation during the

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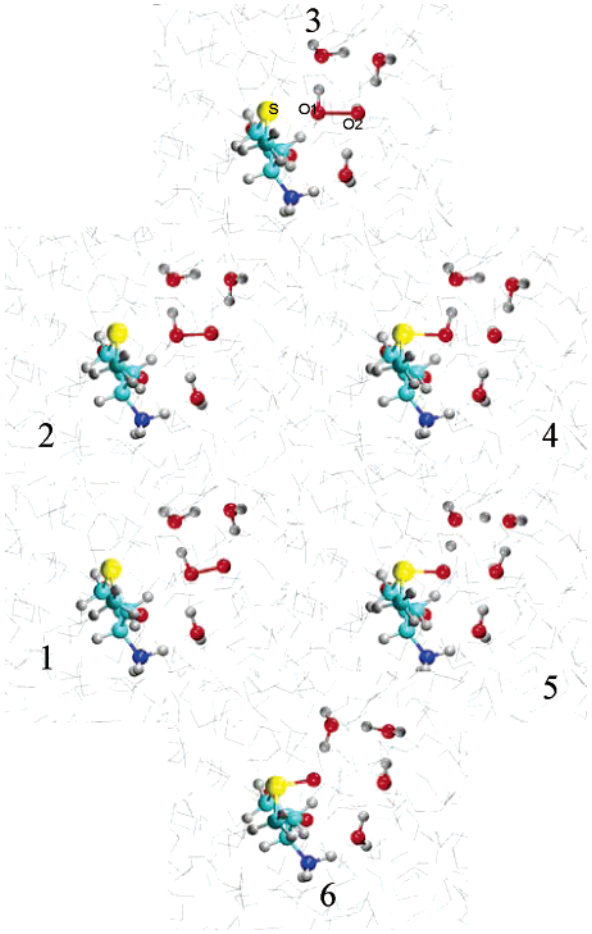

Figure 1. Minimum energy path (MEP) for the oxidation of methionine solvated in a water sphere with a radius of 18 Å. QM atoms are shown in ball-and-stick representation, and classical water molecules are shown as gray lines. Box 1 is the reactant cluster consisting of a free methionine amino acid, hydrogen peroxide, three QM water molecules, and 787 classical water molecules. Box 3 is the transition state, and box 6 is the product. The MEP was obtained using the nudged elastic band method (refs 19 and 20).

breaking of the O−O bond of H_2O_2 and the formation of the S−O bond via specific interactions, such as hydrogen bonding.[18] An example of the reaction path of the new mechanism for the oxidation of methionine is shown in Figure 1. It can be seen that breaking of the O−O bond of H_2O_2 and the formation of the S−O bond is the rate-limiting step, not the transfer of a proton as previously thought, since proton transfer occurs after the transition state, as shown in Figure 1. We have also shown that the dominant solvent effects are specific interactions, such as hydrogen bonding, but not dielectric polarization, and that 2−3 water molecules are enough to stabilize the transition state of oxidation so that additional water molecules do not lower the barrier of reaction significantly.[18] Although our mechanism satisfies all available experimental data on oxidation, such as the measured activation energies and the pH dependence of the rates of oxidation in the solution, it was developed for small molecules, not proteins, and it does not treat the dynamics effects of the solvent and protein environment.

We have also performed a combined experimental and classical molecular dynamics study of a protein with sites that are susceptible to oxidation, granulocyte colony-stimulating factor (G-CSF) (Figure 2), and demonstrated that even sites that appear in X-ray structures to be buried can be accessed by

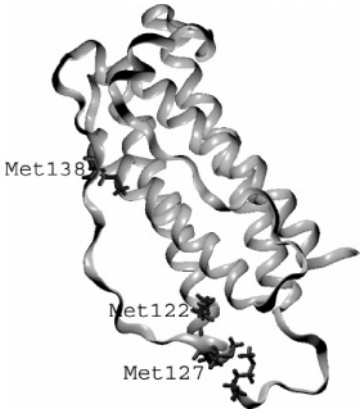

Figure 2. Methionine residues of G-CSF.

water.[21] Furthermore, we found that the measured rate constants correlate well with the water coordination number, averaged over two solvation shells. This together with our results on organic sulfides implies that the access of solvent molecules to methionine sites governs the oxidation process in proteins. However, it leaves many fundamental questions unanswered, such as the following:

(1) What are the governing degrees of freedom of the oxidation process in solution for organic sulfides and in proteins?

(2) What role does water dynamics play in the oxidation process?

(3) What is the mechanism of oxidation of methionine sites in proteins?

This study addresses those questions by applying sophisticated theoretical and computational methods to an important therapeutic protein, G-CSF.

The effects of water and the protein molecule on the oxidation of methionine sites are quantified via employing a combined quantum mechanical and molecular mechanical (QM/MM) Hamiltonian.[22,23] To compute the free energy barrier, constrained MD simulations[24] are performed along a reaction coordinate that was chosen from minimum energy paths (MEP).[19,20] The committor probabilities of the configurations at the maximum of the free energy profile are then calculated to examine rigorously whether our hypothesized reaction coordinates are indeed the correct ones.[25] In addition to broadening the current knowledge of the mechanism of oxidation of methionine residues in solution and in proteins, the results in this study add to our understanding of the application of the concepts of transition path sampling methods for studying complex chemical processes in solution.[26]

Computational Details

QM/MM Models for the Oxidation of a Methionine Amino Acid and of Methionine Residues in G-CSF. To study the oxidation

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reaction in an aqueous environment, we performed combined quantum mechanical and molecular mechanical (QM/MM) calculations using all atom models, with at least five solvation shells (15 Å) of water molecules to solvate the methionine amino acid and G-CSF. The local region around and including the site being oxidized is treated via quantum mechanics, and the rest of the system is treated using classical force fields.

The QM region has 25 QM atoms, including hydrogen peroxide, three surrounding water molecules, the side chain of the methionine residue being oxidized, and a QM link atom. The choice of three quantum mechanical waters is based on our results in gas phase models that show that going from 3 to 5 QM waters did not significantly change the activation energy of oxidation (<1 kcal/mol). The quantum subsystem is described by the B3LYP density functional and the 6-31G basis set, using the GAMESS-UK program.[27] This choice of method is convenient because of the high cost of running dynamics on quantum mechanical systems. It is also quite accurate compared with higher level ab initio theories as tested on a model reaction, such as oxidation of dimethyl sulfide in the gas phase. The difference in activation energies between an MP4//B3LYP/6-31++G(d,p) calculation and a B3LYP/6-31G calculation is only 1.4 kcal/mol.

The classical region is treated using the CHARMM22 all-atom force field.[28] Although a QM/MM boundary between the β-carbon and the γ-carbon has been shown to reproduce full QM geometry and activation energy in gas-phase models, we still choose the QM/MM interface to be between the α-carbon and the β-carbon of the methionine residue in order to minimize the effects of the QM/MM interface. The double-link atom (DLA) method is used to divide the QM/MM interface; that is, a QM link atom (hydrogen) is added to saturate the valence electrons in the QM region, and a classical link atom is also added to endeavor a net charge of zero in the classical region.[22] All electrostatic interactions between quantum particles, classical particles, and link atoms at the interface are included with the use of a Gaussian blur function with a width of 2 Å on the classical link atom to avoid short-distance singularities. This method has been shown to remove the undesired artifacts related to the electrostatic properties of a chemical system, such as proton affinity induced by using only one link atom and by ignoring arbitrarily the electrostatic interactions between the link atoms and classical particles at the QM/MM interface.[22] We also compared the QM/MM system with a full quantum treatment of the oxidation of a methionine amino acid with three water molecules in the gas phase and found that the DLA method is able to reproduce the reaction activation energy within 0.6 kcal/mol. Choice of the van der Waals parameters of QM water and H_2O_2 are described in the Supporting Information.

The initial configuration of the free methionine amino acid in the zwitterion form with hydrogen peroxide and three water molecules is obtained from geometry optimizations of gas-phase models at 0 K. These configurations are then solvated by placing them in the center of a pre-equilibrated droplet of TIP3P water with a radius of 18 Å. Overlapping TIP3P water molecules are removed. The final system contains 787 water molecules and 2459 atoms in total. All nonbound electrostatic, QM/MM, and van der Waals interactions are calculated explicitly without using a cutoff.

The initial configuration of G-CSF is obtained from the X-ray structure, PDB entry 1CD9.[29] From the 2.95 ns trajectory in an earlier study,[21] a representative frame is chosen so that the two-shell water coordination numbers (2SWCNs) of methionine residues of the chosen frame are equal to the averaged value of this property along the trajectory. The protein molecule is then solvated with a shell of 15 Å. The system contains 5280 water molecules and, in total, 18 495 atoms, including G-CSF. To make the calculations feasible, a 17 Å cutoff is applied to nonbound interactions with a smooth switch function started at 15 Å, and the nonbound list is updated heuristically during energy minimizations and MD simulations.

Although fairly thick water layers are used in our simulations to mimic solvation, the inevitable finite-size effects need to be examined. The use of a finite system in calculating Columbic interactions neglects the effects of long-range electrostatics on the oxidation reaction. For the oxidation of organic sulfides, we have examined the effects of solvent polarization using the polarizable continuum model[46] and found that solvent polarization has insignificant effects on the activation barriers.[18] These results of cluster models suggest that neglecting long-range electrostatics in the solvated system would not significantly affect the reaction barriers. Moreover, finite-size models without periodic boundary conditions introduce pressure gradient in the system due to the existence of the interface. Because the activation volume of oxidation is quite small ($\Delta V^{\ddagger} = 20$ Å^3), the most significant pressure effect on the activation enthalpy (in the case of solvated freeMet) is <0.5 kcal/mol.

Minimum Energy Paths of Methionine Oxidation. A useful approach to gaining insight into chemical and physical processes occurring in complicated systems is to find a minimum energy path (MEP) connecting two local minima on the potential energy hypersurface of a system. In addition to snapshots of a chemical process, the activation energy at 0 K can be obtained from an MEP. However, for reactions occurring in the liquid state, the dynamics of the solvent can be important, even governing, and free energies are the meaningful quantities to describe the processes. Nevertheless, knowledge of the MEP may still be useful in getting an indication of the underlying reaction coordinate. Moreover, the geometries along the MEP can also be used as the starting configuration for dynamic simulations and for free energy calculations.

The MEPs for the oxidation of free methionine and methionine sites in G-CSF are thus obtained using the nudged elastic band method[19] and a superlinear minimization scheme based on the adapted basis Newton−Raphson method in CHARMM.[20,30] The details of calculations are reported in the Supporting Information. As shown below, it turns out that the MEPs describe the mechanism correctly (Figure 1), and $\xi = d(\mathrm{O-O}) - d(\mathrm{S-O})$ is the reaction coordinate of oxidation.

Constrained MD Simulations and Free Energy Calculations. The free energy barriers of the oxidation of methionine residues are

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calculated by integrating the mean force required to constrain the system at specific values along the reaction coordinate, $\xi = d(O-O) - d(S-O)$. We have implemented the constraint in CHARMM[30] using the SHAKE algorithm, and the formula developed by Sprik et al.[24] is used for the correction of the constraint MD ensemble ($\xi = \xi'$, $\dot{\xi} = 0$) to the blue-moon ensemble ($\xi = \xi'$).

From the MEPs of oxidation, the reactants have ξ values between -1.8 and -1.6 Å, and the transition states have ξ values around -0.05 Å. Because we are interested in oxidation, only the forward reaction barrier is calculated; for example, the constrained MD simulations are stopped after the transition state is reached. Constrained MD simulations were performed at 9−10 values of ξ so that the free energy barriers could be computed via integration; the details are reported in the Supporting Information.

The starting structures for the MD simulations are chosen from the replicas on the MEPs, as described in the Supporting Information. The leapfrog[31] integration scheme is used to propagate MD trajectories of the QM/MM Hamiltonian, and a time step of 0.001 ps is employed. For each simulation, the system is first heated to 300 K by randomly assigning velocities from the Boltzmann distribution at an increasing rate of 30 K/0.05 ps. Next, the system is equilibrated for 0.5 ps by scaling velocities every 0.02 ps if the average temperature is beyond a 300 ± 2 K window. The equilibrated system is then run for 10 ps, at the chosen value of ξ at 300 K, and the constraint forces are recorded. For the freeMet simulation containing 787 water molecules and 2459 atoms, the water molecules with a distance to the origin >9 Å are propagated by Langevin dynamics,[32,33] with a collision frequency of 50 ps^{-1} and a bath temperature at 300 K. For the protein system, the velocity scaling method is applied at a frequency of every 0.5 ps if the averaged temperature is beyond a 300 ± 2 K window. Statistical uncertainties of each simulation are estimated by using the standard asymptotic block-averaging method.[31,34]

Committor Probability and Transmission Coefficient Calculations. To examine the effects of the solvent on the oxidation reaction and whether the chosen ξ is indeed the reaction coordinate, the committor probability distribution[25,35,36] of the ensemble defined by the value of ξ, at which there is a maximum in the free energy profile, is computed. Ten structures are randomly selected from the 10 ps trajectory, and 20 0.1 ps trajectories are shot from each structure, with velocities randomly assigned from the Boltzmann distribution at 300 K. In this way, the probability of the structure going to the product state is obtained. If the ensemble chosen is indeed the transition state ensemble, the histogram of the committor probabilities should peak at 0.5.[25,35,36] This also means that the chosen reaction coordinate is correct. The transmission coefficient used for correcting dynamic recrossing events is also obtained, according to the methods of Carter et al.[37]

Results and Discussion

Oxidation of Free Methionine in Water. The free energy profile for the oxidation of a methionine amino acid along ξ is shown in Figure 3. The calculated barrier is 14.7 ± 1.2 kcal/mol, close to the experimentally measured value of 15.5 ± 0.02 kcal/mol.[38] Compared to the maximum of the MEP, $\xi = -0.05$ Å, the maximum of the free energy surface at 300 K is located at -0.2 Å. Although the hydrogen atoms of surrounding quantum waters are hydrogen bonded to the O2 atom of H_2O_2 (see panel 3 in Figure 1), the proton transfer does not occur during the 10 ps simulation period at this point. On the other hand, proton transfer is observed during the constrained MD simulation at $\xi = -0.1$ Å, after the transition state ($\xi = -0.2$ Å), during which the constrained forces are predominantly negative. This observation provides evidence that proton transfer occurs after the system passes the transition state in an aqueous solution.[18] As mentioned earlier, the activation energy of oxidation as a function of the number of water molecules in the gas phase converges at three. We anticipate that treating additional water molecules quantum mechanically in the solvated system would merely introduce different proton-transfer pathways after the transition state, but would not change the reaction coordinate, ξ, or the free energy barrier. In fact, our results on DMS oxidation indeed showed that various proton-transfer pathways can occur after the transition state has passed.[18]

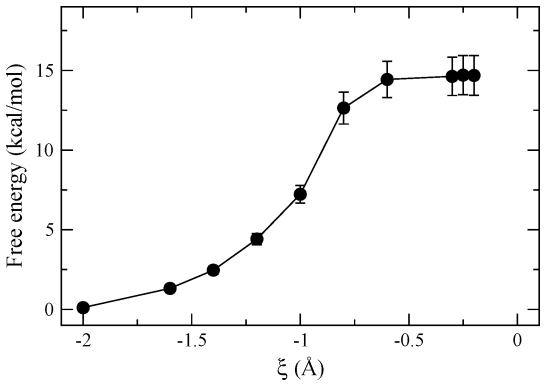

Figure 3. Free energy profile of the oxidation reaction of a methionine amino acid solvated in water molecules. The *x*-axis is the reaction coordinate, $\xi = d(O-O) - d(S-O)$ (in Å), and the *y*-axis is free energy (in kcal/mol), obtained by integrating the mean force along the reaction coordinate from the last 5 ps of the constrained MD simulations.

The committor probability distribution at $\xi = -0.2$ Å is shown in Figure 4. It indeed peaks at 0.5, suggesting that the chosen reaction coordinate has not coupled to the activation of other degrees of freedom. In other words, $\xi = d(O-O) - d(S-O)$ is the representative reaction coordinate to describe the oxidation reaction. Moreover, the transmission coefficient is calculated to be 0.7 from the shooting trajectories, indicating that recrossing due to the interaction with the solvent environment has only a small effect on the reaction barrier.

In addition to the committor probability distribution, the constrained forces during the dynamics also contain information about the coupling of the constrained degree of freedom to the surrounding environment.[39−43] The distribution of constraint forces during the 10 ps simulation is shown in Figure 4. It can be seen that the constraint force has a symmetric, Gaussian distribution centered at zero. Although the forces acting on the reaction coordinate at the transition state depend on the dynamics of the surrounding molecules, the distribution in Figure 4 suggests that the activation of variables other than ξ is not involved in the oxidation process. If the activation of a different coordinate was involved, a different type of distribution of constraint forces would likely result, for example, a distribution with a minimum centered at zero.[35] Our analysis of Figure 4 leads to the idea that the distribution of constrained forces in an MD simulation can be used as a very quick way of testing whether degrees of freedom other than the chosen constrained coordinate are likely to play a role in the reaction. Note, however, that if the transmission coefficient is desired, trajectories from the transition state are still necessary.

As indicated in our previous study,[18] the charge separation developed during the breaking of the O−O bond of H_2O_2 and the formation of the S−O bond is to a large degree responsible for the reaction barrier for the oxidation of methionine by H_2O_2. For example, in the transition state shown in Figure 1, the distal oxygen atom (denoted as O2 in Figure 1) becomes more

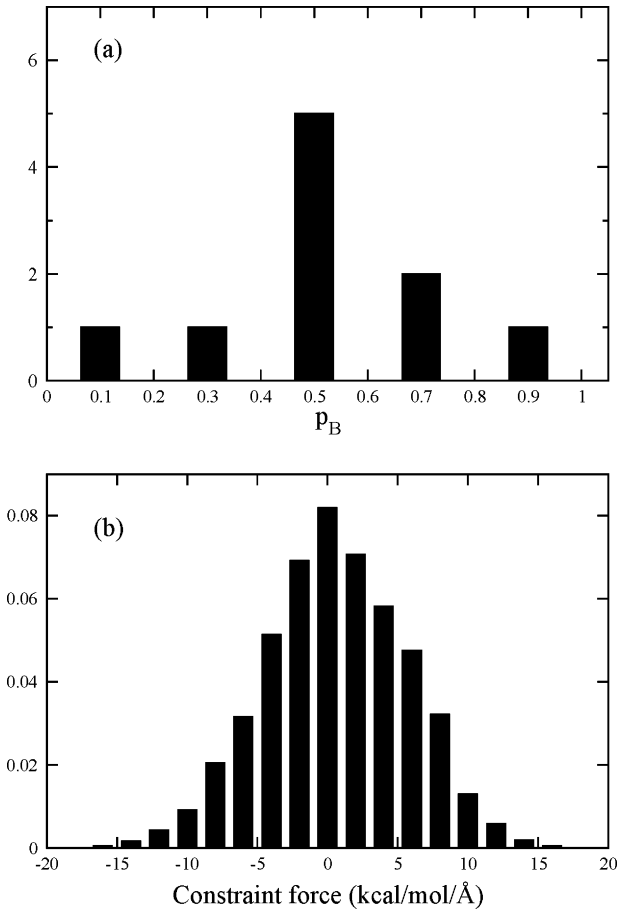

Figure 4. Statistics of the transition-state ensemble obtained by constrained MD simulations at $\xi = -0.2$ Å. (a) Probability distribution of the committor probability of going to the product, p_B. To compute the committor probability distribution, 10 configurations are randomly chosen from the transition-state ensemble. Twenty 0.1 ps trajectories are then generated for each configuration by randomly assigning the velocities of particles from the Boltzmann distribution at 300 K. From the 20 shooting trajectories, p_B is calculated. The p_B distribution is then calculated from the p_B values of the 10 configurations and is coarse-grained with a bin size of 0.25. (b) Probability distribution of the constrained forces at $\xi = -0.2$ Å.

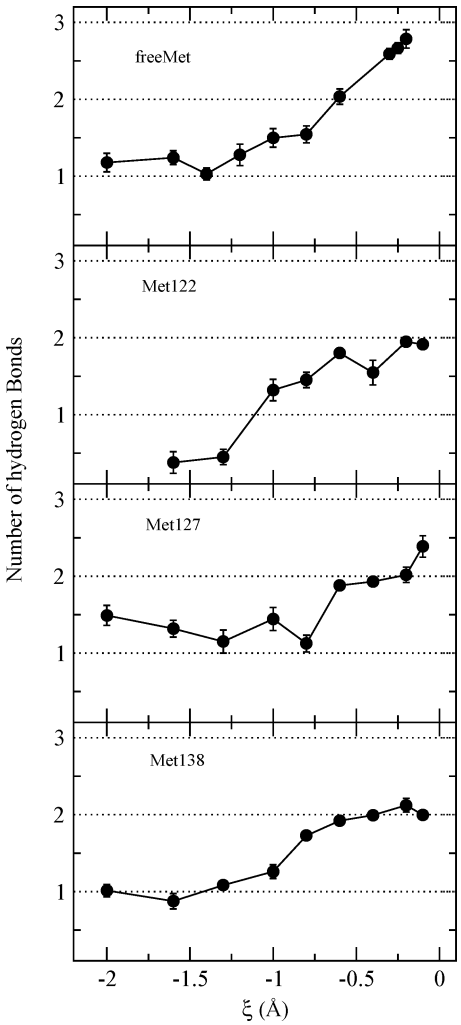

Figure 5. Average number of hydrogen bonds (HBs) between the O2 atom of H_2O_2 (Figure 1) and the hydrogen atoms of water molecules along the oxidation reaction. The *x*-axis is the reaction coordinate, $\xi = d(\mathrm{O-O}) - d(\mathrm{S-O})$, and the *y*-axis is the average number of HBs calculated from the 10 ps trajectory.

negative, and the transferring oxygen (denoted as O1 Figure 1) becomes less negative than the oxygen atoms of H_2O_2 in the reactant state.[18] The major role of solvent molecules is to stabilize the charge separation via specific interactions, such as hydrogen bonding.

We may wish to ask then how the number of HBs changes along the reaction coordinate. The average number of HBs between the distal oxygen atom (the O2 atom, as shown in Figure 1) and the hydrogen atoms of surrounding water molecules are plotted along the reaction coordinate in Figure 5. The criterion for an HB is that the hydrogen atom of a water molecule must be within 2.0 Å of the distal oxygen atom (O2). As expected, HB increases from the reactant state to the transition state along the reaction coordinate by about 1.5 HBs. Note that the surrounding QM water molecules form only at most 2 HBs with O2 in all of simulations; an HB value >2 indicates that classical water molecules are also involved in the specific interactions with O2. The MD trajectories themselves also indicate that mobile classical water molecules can interact specifically with the H_2O_2 molecule and can also exchange with one of the three QM water molecules (the one at the cis side to methionine in Figure 1) surrounding H_2O_2. This result also provides additional evidence that using three QM waters is enough. Although ξ is the governing degree of freedom, solvent molecules still participate in the reaction and respond to the change of electronic properties of the system.

Oxidation of Methionine Residues of G-CSF. Experimental data show that the four methionine residues of G-CSF are oxidized with different rates in the following order: Met1 > Met138 > Met127 > Met122.[21,44] The locations of the methionine residues in G-CSF are shown in Figure 2. Among the three methionine residues studied in this work, Met122 is the most buried, being located in one of the four helices of G-CSF (see Figure 2), and has the lowest rate of oxidation. Met127 is the first residue of the helix in which Met122 resides and is thus more exposed than Met122. It is oxidized 2.4 times faster than Met122. Met138, on the other hand, is located in a loop connecting two helices and is the second most exposed residue among the four Met groups of G-CSF, being oxidized 2 times faster than Met127. We have demonstrated from classical MD simulations and from experiments that the relative rates of oxidation of methionine residues by H_2O_2 in G-CSF can be quantified accurately by a two-shell water coordination number, 2SWCN.[21] The correspondence between a conformational

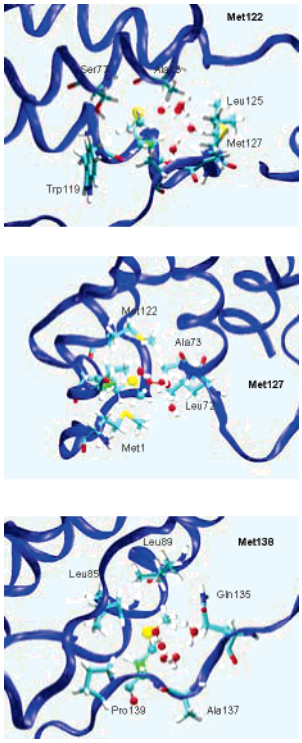

Figure 6. Local environment of Met122, Met127, and Met138 of G-CSF. The methionine residue, hydrogen peroxide, and QM water molecules are shown in a ball-and-stick representation. The residues within 6 Å of a methionine residue are shown in a licorice-like representation.

property that measures the degree to which solvent accesses a methionine site and the rate of oxidation of that site suggests that the major factor that determines the rates of oxidation of methionine is the extent of exposure to solvent molecules, but not the protein environment. This observation is also consistent with the oxidation mechanism that we proposed; that 2−3 water molecules are needed to stabilize the transition state of the oxidation reaction.[21] This hypothesis is now examined by computing the free energy barriers of Met122, Met127, and Met138 in G-CSF.

Details of the local environment of Met122, Met127, and Met138 are shown in Figure 6. Met122 is located in a hydrophobic core composed of Ser77, Ala78, Trp119, Leu125, and Met127. Met127 is at the beginning of a 20 residue helix and is surrounded by Met1, Leu72, Ala73, and Met122. Met138 is surrounded by Leu85, Leu89, and Gln135, and the loop region in which it resides has high flexibility.[21] Generally speaking, the local environments of methionine residues in G-CSF are hydrophobic and provide various degrees of spatial restriction. Concepts of spatial restriction have been used to address the enzymatic activity of proteins.[45] To our knowledge, our study is the first attempt to examine the effects of spatial restriction of a protein molecule on the barriers of chemical reactions of the protein itself.

The free energy profiles of the oxidation of different methionine residues in G-CSF obtained by constrained MD simulations are shown in Figure 7, and the free energy barriers are listed in Table 1. The activation free energies of the three methionine residues are equivalent within statistical uncertainties, indicating that the reaction barriers are indeed not sensitive to the different environments around different methionine sites, once the reactants are near each other.

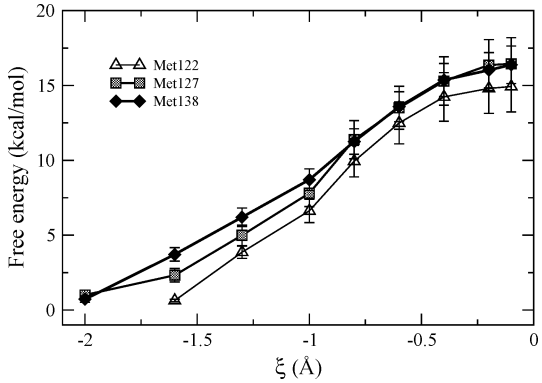

Figure 7. Free energy profiles of the oxidation of methionine residues in G-CSF. The *x*-axis is the reaction coordinate, $\xi = d(\mathrm{O-O}) - d(\mathrm{S-O})$ (in Å), and the *y*-axis is free energy (in kcal/mol), obtained by integrating the mean force along the reaction coordinate from the last 5 ps of the constrained MD simulations.

Table 1. Activation Free Energies of Free Methionine in Water and Methionine in G-CSF

methionine	$\Delta F^{\ddagger}$ (kcal/mol)
freeMet	14.70 ± 1.22
Met122	14.93 ± 1.70
Met127	16.45 ± 1.75
Met138	16.38 ± 1.25

The HBs between the distal oxygen and the hydrogen atoms of water molecules along the reaction coordinate are shown in Figure 5 for the different methionine residues. For Met122, the distal oxygen of H_2O_2 has almost no HBs with water molecules in the reactant state, an indication of the restricted access to its environment. As the reaction progresses, the number of HBs on the distal oxygen increases, reaching a value of 1.8 at the transition state. Both QM and MM water molecules are involved in the hydrogen bonding with H_2O_2.

For Met127 and Met138, the access to solvent molecules is not restricted as much as it is for Met122, and therefore, the number of HBs on the distal oxygen of H_2O_2 in the reactant state of each of these is higher than that for Met122. Along the reaction coordinate, the number of HBs on the distal oxygen also increases, reaching a value of about 2.0 at the transition state, slightly less than that of the freeMet case, but enough such that the reaction barriers are stabilized to the reaction barrier of freeMet.

The above results show that as long as 2−3 water molecules are present around buried methionine sites, the reaction barrier is similar to that of free methionine. The solvent accesses spatially restricted residues via thermo fluctuations, explaining the excellent correlation between 2SWCNs and oxidation rate constants.[21] If different environments of methionine sites in the protein molecule affected the reaction barrier in a specific manner, such a correlation would not occur. The key element of the oxidation mechanism is that the ensuing charge separation of the transition-state complex during oxidation is stabilized by 2−3 water molecules. Our results do not rule out the possibility that polar atoms of protein molecules could play the role of stabilization via specific interactions if they are close to the sulfur atom in methionine. For G-CSF, however, this arrangement was not observed, and in fact, such a possibility would seem to be rare, at best, because of the tendency for hydrophobic residues to be present in protein cores.

Summary and Conclusions

In this work, QM/MM and constrained MD simulations are employed to study the oxidation of the amino acid methionine by H_2O_2 in an aqueous environment and in G-CSF. The computed reaction free energy of free methionine is very close to the value that we measured. The reaction coordinate, governing the degree of freedom, was found to be $\xi = d(O-O) - d(S-O)$. That this is truly the reaction coordinate was verified by computing the committor probability distribution and the distribution of constrained forces at the maximum of the free energy profile, as shown in Figure 4.

This study is the first to explore the effects of both solvent and protein environment on the oxidation reaction of the amino acid methionine using detailed molecular theoretical methods. We found that the reaction barriers of oxidation do not depend on different protein environments surrounding each methionine site in G-CSF. This is consistent with the observation in an earlier study that the measured rates of oxidation correlate with the two-shell water coordination number of different methionine sites.[21] The hydrogen bonds between the distal oxygen of H_2O_2 and the hydrogen atoms of water molecules are shown to increase along the reaction coordinate, indicating that the charge separation developed during the S_N2 oxidation of methionine by H_2O_2 is stabilized by specific interactions, such as hydrogen bonding, with water molecules, even for the most spatially restricted residue of G-CSF, Met122.

The results of this study lead to an enhanced mechanistic understanding of the oxidation of proteins and should be useful in designing ways to control protein oxidation by peroxides. They also adds to our knowledge of how complex environments affect chemical reactions.

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Supporting Information Available: Additional experimental details and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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