Reactions of One-Electron-Oxidized Methionine with Oxygen: An ab Initio Study

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A one-electron oxidation of a methionine residue is thought to be a key step in the neurotoxicity of the beta amyloid peptide of Alzheimer's disease. The chemistry of the radical cation of *N*-formylmethioninamide (**11**$^{\bullet+}$) and two model systems, dimethyl sulfide (**1**$^{\bullet+}$) and ethyl methyl sulfide (**6**$^{\bullet+}$), in the presence of oxygen have been studied by B3LYP/6-31G(d) and CBS-RAD calculations. The stable form of **11**$^{\bullet+}$ has a three-electron bond between the sulfur radical cation and the carbonyl oxygen atom of the $i-1$ residue. The radical cation may lose a proton from the methyl or methylene groups flanking the oxidized sulfur. Both **11**$^{\bullet+}$ and the resultant C-centered radicals may add oxygen to form peroxy radicals. The calculations indicate that unlike C-centered radicals the sulfur radical cation does not form a covalent bond to oxygen but rather forms a loose ion-induced dipole complex with an S−O separation of about 2.7 Å, and is bound by about 13 kJ mol^{-1} (on the basis of **1**$^{\bullet+}$ + O_2). Direct intramolecular abstraction of an H atom from the $^{\alpha}$C site is unlikely. It is endothermic by more than 20 kJ mol^{-1} and involves a high barrier ($\Delta G = 79$ kJ mol^{-1}). The α-to-S C-centered radicals will add oxygen to form peroxy radicals. The OH BDEs of the parent hydroperoxides are in the range of 352−355 kJ mol^{-1}, similar to SH BDEs (360 kJ mol^{-1}) and $^{\alpha}$C−H BDEs (345−350 kJ mol^{-1}). Thus, the peroxy radicals are oxidizing species comparable in strength to thiyl radicals and peptide backbone $^{\alpha}$C-centered radicals. Each peroxy radical can abstract a hydrogen atom from the backbone $^{\alpha}$C site of the Met residue to yield the corresponding $^{\alpha}$C-centered radical/hydroperoxide in a weakly exothermic process with modest barriers in the range of 64−92 kJ mol^{-1}.

Introduction

Protein oxidation caused by free radicals is a key feature of cardiovascular diseases, neurodegenerative diseases, and numerous other disease states, and it may be the principal cause of aging.[1,2] It occurs naturally as a byproduct of normal metabolism but may be exacerbated by environmental pollutants. In Alzheimer's disease (AD),[3−8] the most common form of dementia among older people, the brain is subjected to extensive oxidative damage. It has been reported that amyloid β-peptide (Aβ)[9] is central to the pathogenesis of AD.[10,11] Although the mechanism of the neurotoxicity, free radical oxidative stress, is unknown, the oxidation of a single residue, methionine 35, has been reported to play a key role in it.[12,13] Methionine likely serves as an electron donor for the reduction of Aβ-bound Cu^{2+} to Cu^{1+}. In the process, the Met residue is oxidized to its radical cation, MetS$^{\bullet+}$. The reaction of reduced copper with molecular oxygen regenerates Cu^{2+} and produces hydrogen peroxide,[14,15] a strong oxidizing agent that may be a source of hydroxyl radicals or may cause further Met oxidation by nucleophilic processes by itself[16] or in combination with CO_2.[17] Under normal circumstances, the MetS$^{\bullet+}$ is reduced back to Met by endogenous antioxidants such as vitamin E, vitamin C, or glutathione.[18] In the Radical Model of AD,[19] the MetS$^{\bullet+}$ may also be reduced by the abstraction of a hydrogen atom from a peptide backbone $^{\alpha}$C site, yielding a stable $^{\alpha}$C-centered radical.[20] It has been argued on the basis of computations that this must be at a glycine residue if the abstraction occurs within a β-sheet secondary structure.[21,22] Such an $^{\alpha}$C-centered radical may react with molecular oxygen[23] and be carried into a lipid bilayer to initiate lipid peroxidation. Although both the thermodynamics and kinetics of the interstrand H-abstraction reaction by MetS$^{\bullet+}$ have been shown to be feasible by theoretical calculations,[20] little information is available on the direct reaction of the oxidized Met residue with molecular oxygen. The reaction of simple dialkyl sulfur radical cations, $R_2S^{\bullet+}$, or the dimeric form, $(R_2S)_2^{\bullet+}$, with O_2 was not detected in pulse radiolysis experiments.[24,25] The purpose of the present work is to carry out an investigation of the possible reactions of MetS$^{\bullet+}$ with O_2, limiting the discussion to the case of a single Met residue as occurs in Aβ.

Simple alkyl free radicals, and $^{\alpha}$C-centered radicals of peptides[23] and sulfides,[24] react with molecular oxygen essentially without activation to yield peroxy radicals. We will attempt to ascertain why this is apparently not true of sulfide radical cations (eq 1).[24]

$$\mathrm{MetS}^{\bullet+} + \mathrm{O_2} \rightarrow \mathrm{MetS}^{+}\mathrm{OO}^{\bullet} \qquad (1)$$

A key feature of a radical cation of a dialkyl sulfide is the acidity of the C−H bonds adjacent to the oxidized sulfur atom.[26] The pK_a of oxidized dimethyl sulfide ($(CH_3)_2S^{\bullet+}$, **1**$^{\bullet+}$) has been estimated to be −2.[27] Thus, such radical cations will deprotonate readily in the presence of water or other bases to yield α-C-centered radicals. In particular, MetS$^{\bullet+}$ will deprotonate at either the $^{\gamma}$C (methylene) or the $^{\epsilon}$C site (methyl) of the Met side chain. The resulting α-C-centered radicals will rapidly react with

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TABLE 1: C−O Bond Dissociation Enthalpies of Peroxy Radicals at 298 K (kJ mol^{-1})

compounds	C−O B3LYP/6-31G*	C−O CBS-RAD
3•, $CH_3SCH_2OO^•$	101.3	111.4
8′•, $CH_3CH_2SCH_2OO^•$	100.6	113.1
8″•, $CH_3CH(OO^•)SCH_3$	106.2	124.5
13′•, $HCONHCH(CH_2CHSCH_2(OO^•)C(O)NH_2$	112.8	[125.3][a]
(**R**)-**13″•**, $HCONHCH(CH_2CH(OO^•)SCH_2)C(O)NH_2$	107.0	[125.3][b]
(**S**)-**13″•**, $HCONHCH(CH_2CH(OO^•)SCH_2)C(O)NH_2$	102.7	[121.0][b]
19•, $HCONHCOO^•(CH_2CH_2SCH_3)C(O)NH_2$	47.3	[70.7][c]

[a] $\Delta D_{CO} = 12.5$ kJ mol^{-1}, from **8′•**. [b] $\Delta D_{CO} = 18.3$ kJ mol^{-1}, from **8″•**. [c] $\Delta D_{CO} = 23.4$ kJ mol^{-1}, from ref 23.

TABLE 2: O−H Bond Dissociation Enthalpies of Hydroperoxides at 298 K (kJ mol^{-1})

compounds	O−H B3LYP/6-31G(d)	O−H CBS-RAD
5, $CH_3SCH_2OO\cdots H$	321.4	357.1
10′, $CH_3CH_2SCH_2OO\cdots H$	321.7	353.8
10″, $CH_3CH(OO\cdots H)SCH_3$	318.9	355.6
15′, $HCONHCH(CH_2CHSCH_2(OO\cdots H)C(O)NH_2$	332.9	[355.0][a]
(*R*)-**15″**, $HCONHCH(CH_2CH(OO\cdots H)SCH_2)C(O)NH_2$	334.7	[352.4][b]
(*S*)-**15″**, $HCONHCH(CH_2CH(OO\cdots H)SCH_2)C(O)NH_2$	334.2	[351.9][b]
20, $HCONHCOO\cdots H(CH_2CH_2SCH_3)C(O)NH_2$	354.9	[386.9][c]

[a] $\Delta D_{CO} = 32.1$ kJ mol^{-1}, from **10′**. [b] $\Delta D_{CO} = 36.7$ kJ mol^{-1}, from **10″**. [c] $\Delta D_{CO} = 32.0$ kJ mol^{-1}, from ref 23.

TABLE 3: α-C−H Bond Dissociation Enthalpies of the Hydroperoxides at 298 K (kJ mol^{-1})

compounds	B3LYP/6-31G(d)	CBS-RAD
5 → **4•**, $H\cdot\cdot CH_2SCH_2OOH$	388.6	389.1
10′ → **9′•**, $CH_3CH\cdot\cdot HSCH_2OOH$	379.1	380.9
10″ → **9″•**, $CH_3CH(OOH)SCH_2\cdot\cdot H$	389.6	392.1
15′→**14′B**, $HCONHCH(CH_2CHSCH_2(OO\cdots H)C(O)NH_2$	377.0	[378.8][a]
(*R*)-**15″** → (**R**)-**14″A**, $HCONHCH(CH_2CH(OOH)SCH_2\cdot\cdot H)C(O)NH_2$	398.2	[400.7][b]
(*S*)-**15″** → (**S**)-**14″A**, $HCONHCH(CH_2CH(OOH)SCC_2\cdot\cdot H)C(O)NH_2$	391.2	[393.7][b]
15′→**14′C**, $HCONHC\cdot\cdot H(CH_2CH_2SCH_2OOH)C(O)NH_2$	335.3	[350.4][c]
(*R*)-**15″** → (**R**)-**14″C**, $HCONHC\cdot\cdot H(CH_2CH(OOH)SCH_3)C(O)NH_2$	327.9	[343.0][c]
(*S*)-**15″** → (**R**)-**14″C**, $HCONHC\cdot\cdot H(CH_2CH(OOH)SCH_3)C(O)NH_2$	330.6	[345.7][c]

[a] $\Delta D_{CO} = 1.8$ kJ mol^{-1}, from **10′** → **9′•**. [b] $\Delta D_{CO} = 2.5$ kJ mol^{-1}, from **10″** → **9″•**. [c] $\Delta D_{CO} = 15.1$ kJ mol^{-1}, from ref 32.

molecular oxygen to generate peroxy radicals (eqs 2, 3, and 4).[24]

$$MetS^{•+} \rightarrow Met(CH_2SCH_2^•) + Met(CH^•SCH_3) + H^+ \quad (2)$$

$$Met(CH_2SCH_2^•) + O_2 \rightarrow Met(CH_2SCH_2OO^•) \quad (3)$$

$$Met(CH^•SCH_3) + O_2 \rightarrow Met(CH(OO^•)SCH_3) \quad (4)$$

We examine here whether the resulting S- or C-centered peroxy radicals (eqs 1, 3, and 4) can undergo intramolecular H-transfer reactions. Starting conformations of the model peptide are extended, that is, close to β-sheet secondary structure.[21]

Method

The systems examined are shown in Tables 1, 2 and 3. Except as noted below, the geometries of all species were optimized at the B3LYP/6-31G(d) level of theory as implemented in the Gaussian 98 program suite. Transition structures (TSs) were generally located using the QST2 algorithm. Harmonic vibrational analysis was carried out to determine the nature of the structures as minima (all real frequencies) or TSs (exactly one imaginary frequency). The vibrational zero-point energy was scaled by a factor of 0.9806.[28] CBS-RAD calculations[29] were carried out on the B3LYP/6-31G(d)-optimized structures of all systems derived from dimethyl sulfide (**1**) and ethyl methyl sulfide (**6**). CBS-RAD is a high-level theoretical procedure for obtaining thermochemical values for radicals to experimental accuracy.[29] The CBS-RAD(B3LYP) combination employed here has been found to yield good reaction enthalpies and barriers for the addition of C-centered radicals to alkenes[30] and the β-scission reactions of alkoxy radicals.[31]

In the case of the L-methionine (**11**) derivatives (Tables 2 and 3), relative energies and activation energies calculated at the B3LYP/6-31G(d) level were corrected by the application of isodesmic reactions.[32] Briefly, for each process that breaks an X−Y bond, an isodesmic correction, ΔD_{X-Y}, is determined as the difference between the "correct" value and the value calculated at the B3LYP/6-31G(d) level for the equivalent bond in a model system. Normally, the correct value would be the most reliable value determined experimentally. However, there are almost no experimental data pertaining to the present systems. Instead, we have used the CBS-RAD values from ethyl methyl sulfide (**6**) derivatives (Table 1) to obtain the ΔD_{X-Y} corrections to B3LYP/6-31G(d) results. The corrections are given in the footnotes to Tables 1−3. In the case of a reaction involving a radical and a closed-shell species, the principal error is assumed to occur in the radical, and a ΔD_{X-H} value is applied to each side of the equation, appropriate for the radical appearing on that side. A simple average of the two ΔD_{X-H} values is applied to the TS. Thus for reaction 5

$$X^• + Y-Z \rightarrow X-Y + Z^• \quad (5)$$

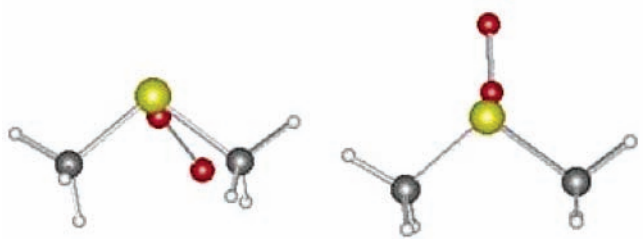


Figure 1. Minimum-energy conformations of $\mathbf{1}^{\bullet+}$ **OO** (the adduct of $\mathbf{1}^{\bullet+} + O_2$). The C_s structure is on the right.

the energy of reaction, ΔE, and energy of activation, $\Delta E^{\ddagger}$, are obtained as

$$\Delta E = \Delta E(\text{B3LYP/6-31G(d)}) + \Delta D_{\text{Z-H}} - \Delta D_{\text{X-H}} \quad (6)$$

$$\Delta E^{\ddagger} = \Delta E^{\ddagger}(\text{B3LYP/6-31G(d)}) + \frac{1}{2}(\Delta D_{\text{Z-H}} - \Delta D_{\text{X-H}}) \quad (7)$$

ΔE and $\Delta E^{\ddagger}$ were identified as enthalpy changes at 0 K. Enthalpy and free energy changes at 298 K were obtained from thermochemical quantities reported by Gaussian 98[33] at the B3LYP/6-31G(d) level, except that entropies (and therefore free energies) were adjusted to the standard state appropriate for solution, 1 mol dm^{-3}. This entails the subtraction of 26.6 J K^{-1} mol^{-1} from the entropy values reported by Gaussian 98 (which are for 1 atm of pressure).

Results and Discussion

Absolute energies and thermochemical properties at 298 K of all species considered here are listed in Table S1 of Supporting Information. Structures and structure labels are given in Tables 1−3.

Reactions of Dimethyl Sulfide Derivatives (Table 1). *Oxygen Addition to the Sulfide Radical Cation,* $\mathbf{1}^{\bullet+}$ (*eq 3*). Optimization of the $CH_3S^+(OO^{\bullet})CH_3$ radical cation at the B3LYP/6-31G(d) level gave rise to two minimum structures shown in Figure 1. One of them, $\mathbf{1}^{\bullet+}$**OO** (C_1), has C_1 symmetry, in which the orientation of the O−O bond is almost eclipsed by an S−C bond (dihedral angle is 11°). The other, $\mathbf{1}^{\bullet+}$**OO** (C_s), with C_s symmetry, is lower in energy by 0.8 kJ mol^{-1}. Optimization with a larger basis set, 6-311+G(d,p), increased the difference between two conformers to 3.2 kJ mol^{-1}. The large S−O separation, (2.67 Å) suggests that the structures are ion-induced dipole complexes. At the B3LYP/6-311+G(d,p) level of theory, $\mathbf{1}^{\bullet+}$**OO** (C_s) is bound by about 18 kJ mol^{-1} relative to $\mathbf{1}^{\bullet+} + O_2$. In sharp contrast, both UHF/CBSB3 and UMP2/CBSB3 calculations yielded complexes with substantially shorter S−O separations, 1.67 and 1.74 Å, respectively, but which were higher in energy than the separated products by 78 and 23 kJ mol^{-1}, respectively. Optimization at the B3LYP/6-311+G(d,p) level from either the UHF or UMP2 minimum invariably led to $\mathbf{1}^{\bullet+}$**OO** (C_1) or $\mathbf{1}^{\bullet+}$**OO** (C_s). In an attempt to locate a minimum at shorter S−O distances, the S−O distance in $\mathbf{1}^{\bullet+}$**OO** (C_s) was scanned at the B3LYP/6-311+G(d,p) level in 0.05-Å increments between 1.65 and 2.90 Å with full geometry optimization at each scan point. The resulting potential energy curve, which decreased monotonically, is shown in Figure 2 (black squares). UHF/CBSB3, UMP2/CBSB3, and CCSD(T)/6-31+G(d) energies were evaluated as single-point calculations at each point, using the wave function of the previous point as an initial guess. Failure to follow this practice resulted in sporadic convergence to several higher-energy surfaces, all of the same symmetry as that of $\mathbf{1}^{\bullet+}$**OO** (C_s), $^2A''$. The smooth curves of the UHF, UMP2, and CCSD(T) potential

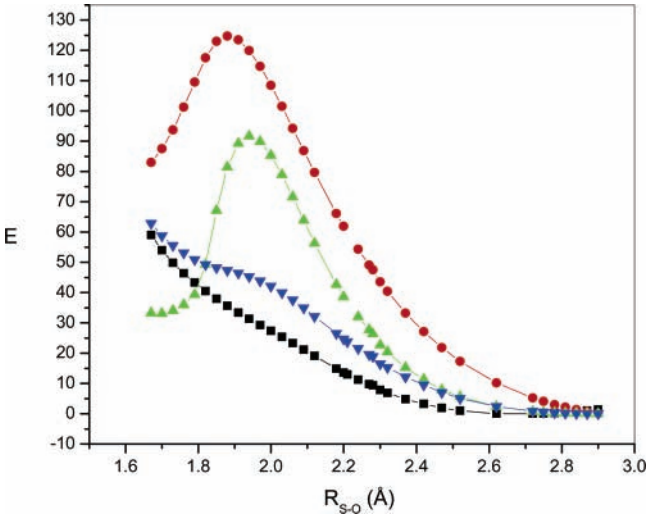


Figure 2. Potential curve of the S−O dissociation of $\mathbf{1}^{\bullet+}$ **OO** (C_s) (kJ mol^{-1}): ■ B3LYP/6-311+G**($\mathbf{1}^{\bullet+} + O_2$ relative energy compared with the saddle point is 17.6 kJ mol^{-1}); ▼ CCSD(T)/6-31+G*($\mathbf{1}^{\bullet+}$ O_2 relative energy is 13.5 kJ mol^{-1}); ▲ MP2/CBSB3 ($\mathbf{1}^{\bullet+} + O_2$ relative energy is 11.3 kJ mol^{-}); ● HF/CBSB3($\mathbf{1}^{\bullet+} + O_2$ relative energy is 5.4 kJ mol^{-1}).

surfaces are plotted in Figure 2. Both the UHF and UMP2 curves show deep metastable minima at short distances, 1.65 and 1.70 Å, respectively. The CCSD(T)/6-31+G(d) surface (inverted triangles in Figure 2) may be regarded as being the most reliable. It follows reasonably closely the B3LYP/6-311+G(d,p) surface, reaching the same shallow minimum as $\mathbf{1}^{\bullet+}$**OO** (C_s), 13.5 kJ mol^{-1} lower than $\mathbf{1}^{\bullet+} + O_2$.

In the case of $\mathbf{1}^{\bullet+}$**OO** (C_s), one could take advantage of symmetry to carry out calculations on the $^2A'$ potential energy surface. Two minima were located along the S−O stretching coordinate, at $r_{SO} = 1.67$ and 2.84 Å The covalently bonded complex ($r_{SO} = 1.67$ Å) is lower in energy relative to the ion-induced dipole bound complex by 60 kJ mol^{-1} at the B3LYP/6-31G(d) level of theory. The $^2A'$ structures are derived from the interaction of $\mathbf{1}^{\bullet+}$ with singlet oxygen. The energy separation of $\mathbf{1}^{\bullet+} + {}^1O_2$ and the $^2A''$ ion-induced dipole complexes is calculated to be 164 kJ mol^{-1}, somewhat greater than the experimental separation between singlet and triplet oxygen, 92 kJ mol^{-1}.[34]

The conclusion of the above study is that $\mathbf{1}^{\bullet+}$ does not form a covalently bonded complex with molecular oxygen in its triplet ground state. A loose ion-induced dipole complex is found in which the two species are separated by slightly less than the sum of the van der Waals radii of sulfur and oxygen atoms and bound by 13.5 kJ mol^{-1}.

Reactions of α*-Peroxydimethyl Sulfide (3*$^{\bullet}$*).* As mentioned in the Introduction, the C−H bonds of $\mathbf{1}^{\bullet+}$ are unusually acidic, p$K_a \approx -2$. Rapid deprotonation in the presence of water or other bases is expected, yielding the C-centered radical, $CH_3SCH_2^{\bullet}$ (**2**$^{\bullet}$), that will react rapidly with molecular oxygen.[24] The structure and stability of the peroxy product, $CH_3SCH_2OO^{\bullet}$ (**3**$^{\bullet}$), have been studied by McKee by using high-level calculations, (QCISD(T)/6-31+G(2df, p)//MP2/6-31G(d)).[35] The O_2 binding energy was reported to be 112 kJ mol^{-1}. We have determined the structures of **2**$^{\bullet}$, **3**$^{\bullet}$, $^{\bullet}CH_2SCH_2OOH$ (**4**$^{\bullet}$, the product of intramolecular H-atom abstraction,), **TS**[**3**$^{\bullet}$→**4**$^{\bullet}$] (the transition structure for the process), and the hydroperoxide CH_3SCH_2-OOH (**5**) at the B3LYP/6-31G(d) level of theory. Energies of each of the structures were evaluated at the CBS-RAD level to determine accurate values for the C−O bond dissociation enthalpy (BDE) (Table 1), the O−H BDE (Table 2), and the

TABLE 4: Enthalpies[a], Entropies[b], and Free Energies[a] of Activation and Total Reaction for the Intramolecular H Transfer at 298 K

	activation			total reaction		
species	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta G^{\ddagger}$	ΔH	ΔS	ΔG
3•→ 4•, H···CH2SCH2OO•	89.0 (73.6)[c]	−30.0	98.0 (82.6)	68.6 (33.4)	4.5	67.3 (32.1)
8•′ → 9•′, $CH_3CH\cdots HSCH_2OO^{\bullet}$	81.3 (67.1)[c]	−27.9	89.6 (75.5)	58.8 (28.5)	5.3	57.2 (26.9)
8•″ → 9•″,$CH_3CH(OO^{\bullet})SCH_2\cdots H$	85.6 (70.7)[c]	−29.0	94.3 (70.4)	70.8 (36.6)	−5.9	72.6 (38.4)
13•′ → 14•′ B,$HCONHCH(OO^{\bullet})SCH_2\cdots H$	96.5 [81][d]	12.3	92.9 [77]	44.1 [14]	13.6	40.0 [10]
13•′ → 14•′ C, $HCONHC\cdots H(CH_2CH_2SCH_2(OO^{\bullet})C(O)NH_2$	59.6 [51][d]	−25.9	67.3 [59]	2.4 [−15]	−1.8	3.0 [−14]
(*R*)-**13•″ → (*R*)-14•″ A**, $HCONHCH(CH_2CH(OO^{\bullet})SCH_2\cdots H)C(O)NH_2$	97.4 [80][d]	−20.4	103.4 [86]	63.6 [29]	−24.0	70.7 [36]
(*S*)-**13•″ → (*S*)-14•″ A**, $HCONHCH(CH_2CH(OO^{\bullet})SCH_2\cdots H)C(O)NH_2$	91.5 [74][d]	−23.2	98.4 [81]	56.9 [22]	−16.7	61.9 [27]
(*R*)-**13•″ → (*R*)-14•″ C**, $HCONHC\cdots H(CH_2CH(OO^{\bullet})SCH_3\cdots H)C(O)NH_2$	63.6 [52][d]	−26.3	71.4 [60]	−6.8 [−18]	−14.8	−2.4 [−14]
(*S*)-**13•″ →(*S*)-14•″**C, $HCONHC\cdots H(CH_2CH(OO^{\bullet})SCH_3)C(O)NH_2$	79.7 [68][d]	−47.5	93.8 [82]	−3.6 [−15]	−17.1	1.5 [−10]
TS[**11**•+ (*S*)-**16**+], $HCONH\ C\cdots H(CH_2CH_2S^{\bullet+}CH_3)C(O)NH_2$[e]	81.9 [78][d]	−2.7	82.7 [79]	69.2 [61]	8.8	66.6 [58]
TS[**11**•+ (*R*)-**16**+], $HCONH\ C\cdots H(CH_2CH_2S^{\bullet+}CH_3)C(O)NH_2$[f]	65.8 [62][d]	−7.4	68.0 [64]	51.2 [43]	−5.3	52.8 [45]

[a] In kJ mol^{-1}. [b] In J K^{-1} mol^{-1}. [c] CBS-RAD values in parentheses. [d] Corrected values from isodesmic reactions in brackets. [e] pro-S face of sulfur. [f] pro-R face of sulfur.

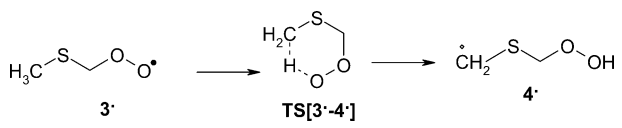
C−H BDE (Table 3). The energetics of the intramolecular H-atom abstraction reaction is summarized in Table 4.

At the CBS-RAD level, the O_2 binding energy is 111.4 kJ mol^{-1}, in excellent agreement with the value obtained by McKee.[35] The direct B3LYP/6-31G(d) calculation underestimates the BDE value by10 kJ mol^{-1} compared to the CBS-RAD results. It was previously noted that the strengths of C−O bonds in alkyl peroxy radicals increase as the product radical stability increases (kJ mol^{-1}): $CH_3OO^{\bullet}$, 137; $CH_3CH_2OO^{\bullet}$, 147; $(CH_3)_2CHOO^{\bullet}$, 158; $(CH_3)_3COO^{\bullet}$, 162.[23] This unexpected trend was attributed to higher stabilization of the peroxy radical as the electron donor ability of the substituents on C increases. In this context, the methylthio group behaves similarly to a methyl group.

The O−H BDE of CH_3SCH_2OOH (**5**), 357.1 kJ mol^{-1}, is essentially the same as in CH_3OOH, 359.8 kJ mol^{-1}, at the CBS-RAD level. However, the B3LYP/6-31G(d) value is 35.7 kJ mol^{-1} too low(Table 2).

From the CBS-RAD energies of **4•** and **5**, the C−H BDE of the distal methyl group is calculated to be 389.1 kJ mol^{-1} (Table 3). This value is almost identical to that found in $(CH_3)_2S$ (**1**) at the same level, 387.5 kJ mol^{-1}. However, unlike case **1**, where the B3LYP/6-31G(d) calculation yields a value that is19.4 kJ mol^{-1} too low, there is almost no discrepancy in the case of **5** (C−H BDE = 388.6 kJ mol^{-1}). It appears that B3LYP/6-31G-(d) calculations attribute the correct amount of stabilization of the α-C-centered radical by the lone pair on S if the lone pair is competitively involved in delocalization into the adjacent σ_{CO}* orbital in **5** but overestimate the stabilization if the lone pair is free as in **1**.

Intramolecular H-Atom Transfer: 3• → 4•. The oxygen atom of the peroxy radical, **3•**, may abstract a hydrogen atom from the other methyl group through a six-membered ring transition state yielding the α-C-centered radical hydroperoxide, **4•.**



3• **TS[3•-4•]** **4•**

From the comparison of the O−H and α-C−H BDEs of **5** (Tables 2 and 3, respectively), this process will be endothermic by 32 kJ mol^{-1} at 298 K. The activation parameters are listed in Table 4. The transition structure, TS[**3•→4•**], is 74 kJ mol^{-1} above **3•**. The free-energy profile in the gaseous phase at 298 K is shown in Figure 3 along with the structures of the lowest energy conformer of the reactant, TS and product. The barrier is higher than on the enthalpy surface, 83 kJ mol^{-1}. Although the three species differ only by the position of an H atom, one might expect an aqueous environment to favor the product side because the more polar O−H bond of **4•** is formed. Thus, the profile shown in Figure 3 should be more or less applicable to aqueous solution with the proviso that the TS and product may be lower.

*Reactions of the Radical Cation of Ethyl Methyl Sulfide (**6**•+) with Oxygen.* Computational experiments analogous to those described above in the case of **1** and its derivatives were also carried out at the CBS−RAD//B3LYP/6-31G(d) level on ethyl methyl sulfide, **6**, and its oxidized (**6**•+), deprotonated (**7•′**, **7•″**) and oxygenated (**8•′**, **8•″**) derivatives (Table 1). Compound **6** has a methylene group as well as a methyl group flanking the sulfur atom and therefore serves as a better model for the side chain of methionine. The difference in the free energy of primary (**7•′**) and secondary (**7•″**) α-C radicals is 8.3 kJ mol^{-1} at 298 K (Supporting Information, Table S1). Assuming that this difference is the same in solution and that the pK_a of the α-methyl C−H group is −2 as in **1**•+, one derives p$K_a \approx -3.4$ for the CH_2 group of **6**•+. As in the case of **1**•+, rapid deprotonation should occur from both α-C atoms of **6**•+ to yield the two α-C-centered radicals, **7•′** and **7•″**.The two C-centered radicals, **7•′** and **7•″**, will add oxygen essentially without activation to yield the primary and secondary peroxy radicals, **8•′** and **8•″**, respectively. The C−O BDEs are 113.1 and 124.5 kJ mol^{-1}, respectively (Table 1). Thus, as in the case of the simple alkyl peroxy radicals, the order of the BDEs is contrary to the order of stability of the product radicals. This fact is reflected at the B3LYP/6-31G(d) level although the difference in BDEs is only half as large as at the CBS-RAD level.

The O−H BDEs of the parent primary and secondary hydroperoxides, **10′** and **10″**, are 353.8 and 355.6 kJ mol^{-1}, respectively, slightly lower than in **5**. The B3LYP/6-31G(d) values are 32.1 and 36.7 kJ mol^{-1}too low, respectively. Thus, in reactions involving peroxy radicals of the Met side chain, values calculated at the B3LYP/6-31(d) level must be corrected by these amounts as explained above, that is, $\Delta D_{O-H} = 32.1$ and 36.7 for methyl- and methylene-substituted peroxy radicals, respectively.

The energetic parameters for the intramolecular abstraction of an H atom in both peroxy radicals are given in Table 4, and the gas-phase free-energy profiles for

8•′ → 9•′ and **8•″ → 9•″** are shown in Figures 4 and 5, respectively. As expected, the H abstraction by the primary peroxy radical to yield the secondary α-C-centered radical (**8•′ → 9•′**) is less endothermic and involves a lower barrier than the converse process (**8•″ → 9•″**). The chirality of **8•″** has no

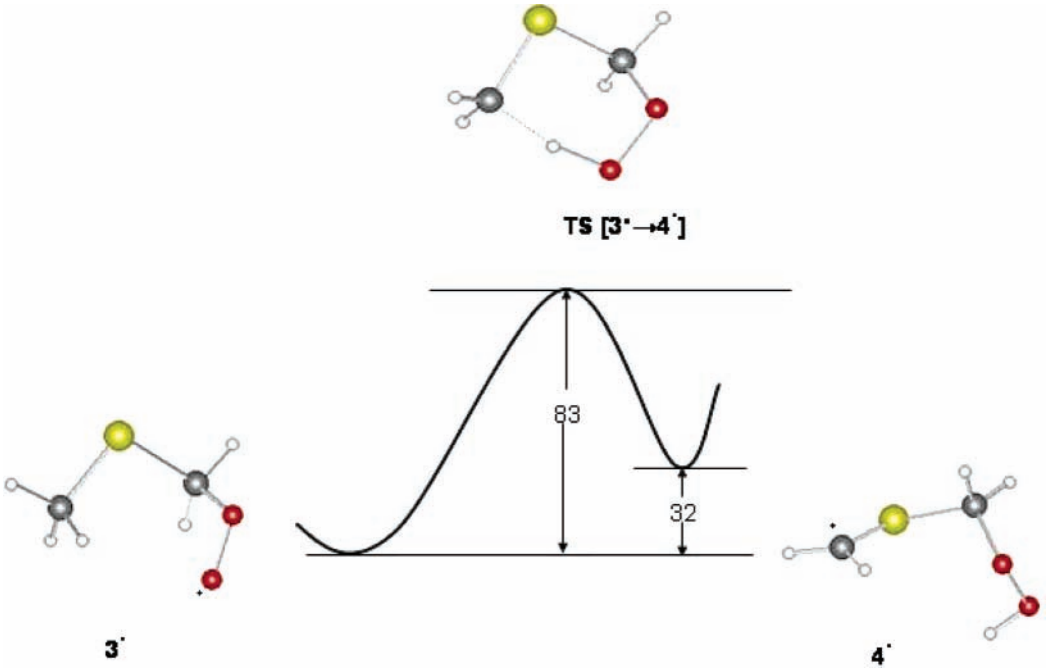


Figure 3. Intramolecular H abstraction reaction of peroxy radical dimethyl sulfide (**1**).

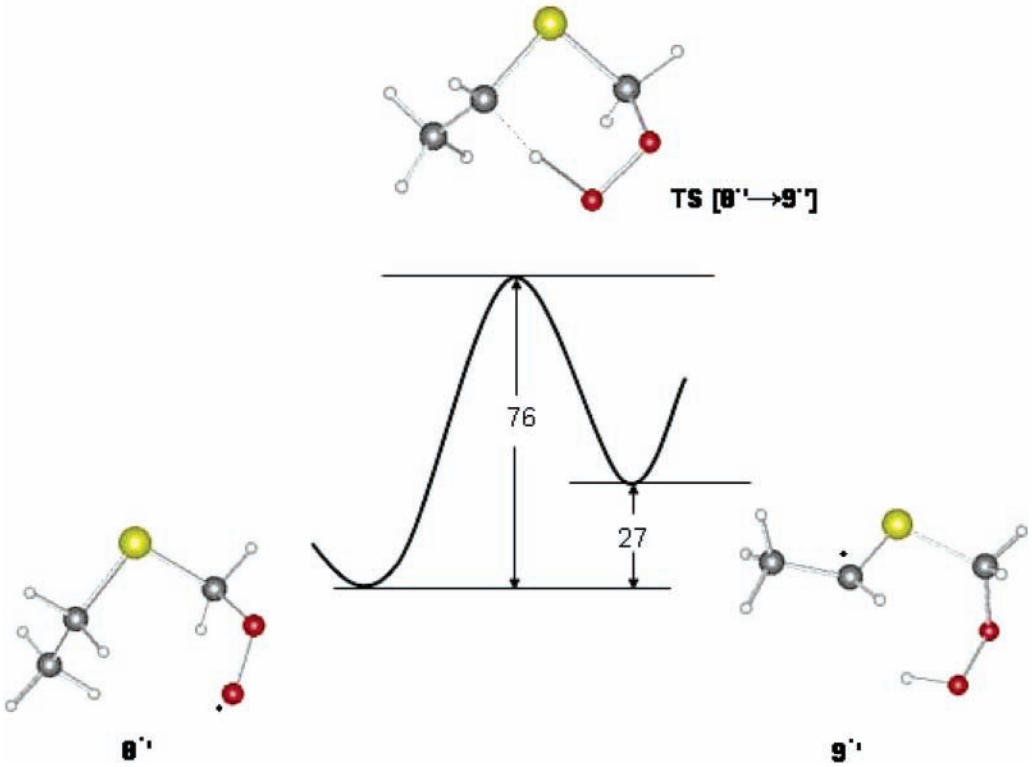


Figure 4. Intramolecular H abstraction reaction of methylperoxy radical ethyl methyl sulfide (**6**).

significance in the reactions of **6** but will play a role in the reactions of the methionine residue (**11**).

*Reactions of the Radical Cation of L-Methionine (**11**$^{\bullet+}$) with Oxygen.* The present model for a methionine residue in a protein is L-*N*-formylmethioninamide (**11**). The formyl group corresponds to the carbonyl group of the $i - 1$ residue. It is not practical to carry out CBS-RAD calculations on systems the size of **11**. However, CBS-RAD-like results can be obtained by correcting the B3LYP/6-31G(d) energies as discussed in the Methods section using the ethyl methyl sulfide-based systems described above. The corrected enthalpies and free energies are reported in square brackets in Table 4. Unless stated otherwise, the discussion that follows is based on the corrected energies.

The most stable structure of the *S*-oxidized form (**11**$^{\bullet+}$) has a three-electron bond with the $i - 1$ carbonyl group (see Figure 9). The S and O atoms are separated by only 2.47 Å. The stabilization of **11**$^{\bullet+}$ by three-electron bonding will reduce the acidity of the α-C hydrogen atoms relative to that of **1**$^{\bullet+}$ and **6**$^{\bullet+}$, as discussed in the next section. In the last section, we consider the possibility that **11**$^{\bullet+}$ may undergo intramolecular H-atom abstraction from its own $^{\alpha}$C site analogous to the intermolecular process shown previously.[20]

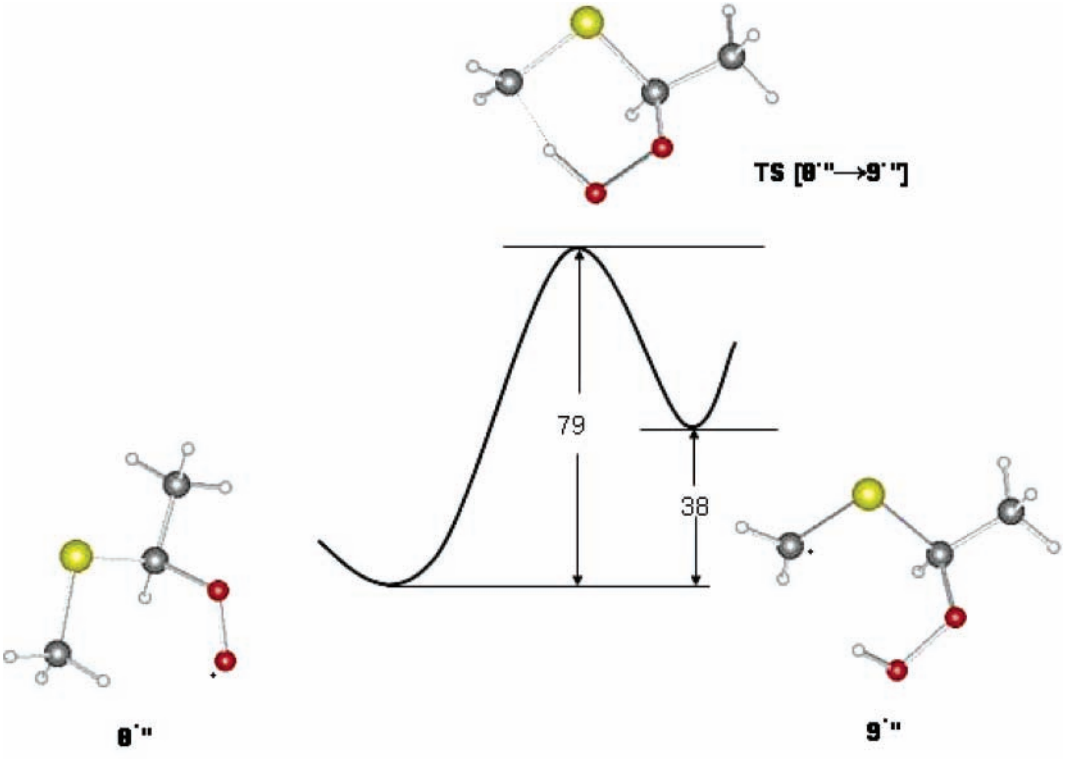


Figure 5. Intramolecular H abstraction reaction of the 1-ethylperoxy radical ethyl methyl sulfide (**1**).

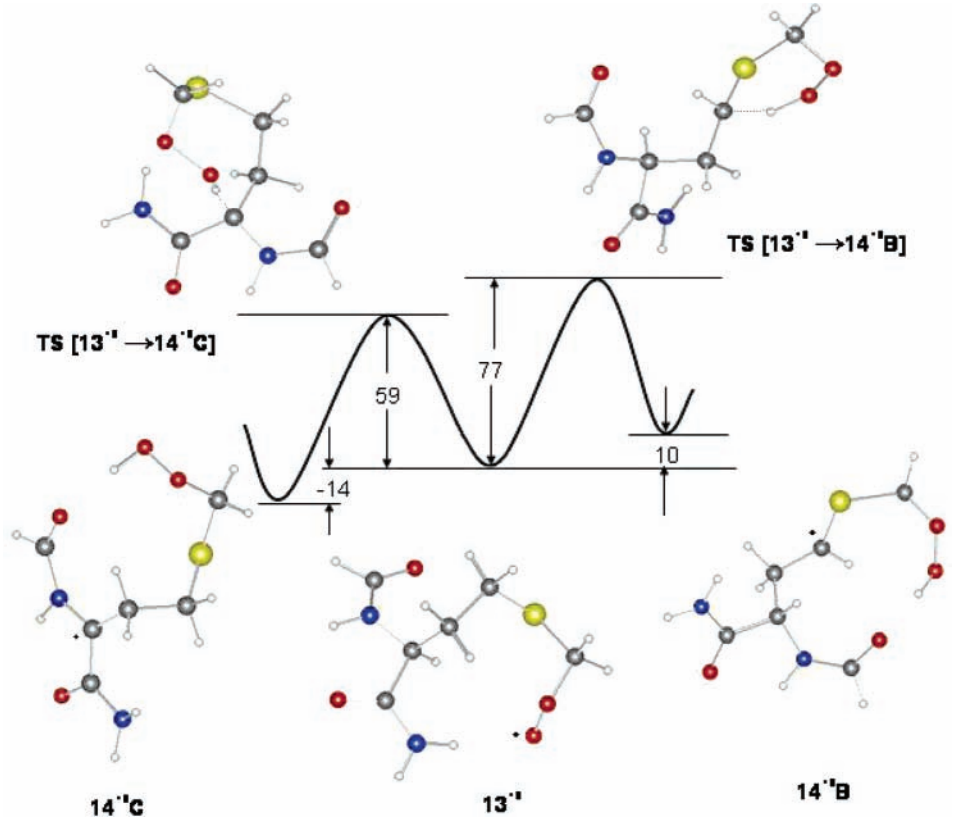


Figure 6. Intramolecular H abstraction reactions of the methylperoxy radical of methionine peptide (**11**): abstraction from $^{\gamma}$C and $^{\alpha}$C.

Acidity of the α*-C−H Bonds of* ***11***$^{\bullet+}$. The added three-electron stabilization of the cation radical will reduce the acidity of the methyl C−H bond relative to that in **1**$^{\bullet\prime}$ or **5**$^{\bullet\prime}$ because the resultant radical (**12**$^{\bullet\prime}$) is not similarly stabilized. The stabilization due to the three-electron bond has been estimated to be 17 kJ mol^{-1} in water.[36] Assuming that this free-energy difference is reflected in the ionization constant, we estimate p$K_a \approx 1$ for the methyl group of **11**$^{\bullet+}$. The free-energy difference between **12**$^{\bullet\prime}$ and secondary α-C radical **12**$^{\bullet\prime\prime}$ is estimated to be 13.3 kJ mol^{-1} from the gas-phase free-energy difference (Supporting Information, Table S1). This implies that p$K_a \approx -1.7$ for the CH_2 group of **11**$^{\bullet+}$. Thus, significant deprotonation is expected from the methylene group and, to a lesser extent, the methyl group to give α-C radicals, **12**$^{\bullet\prime\prime}$ and **12**$^{\bullet\prime}$, respectively. These

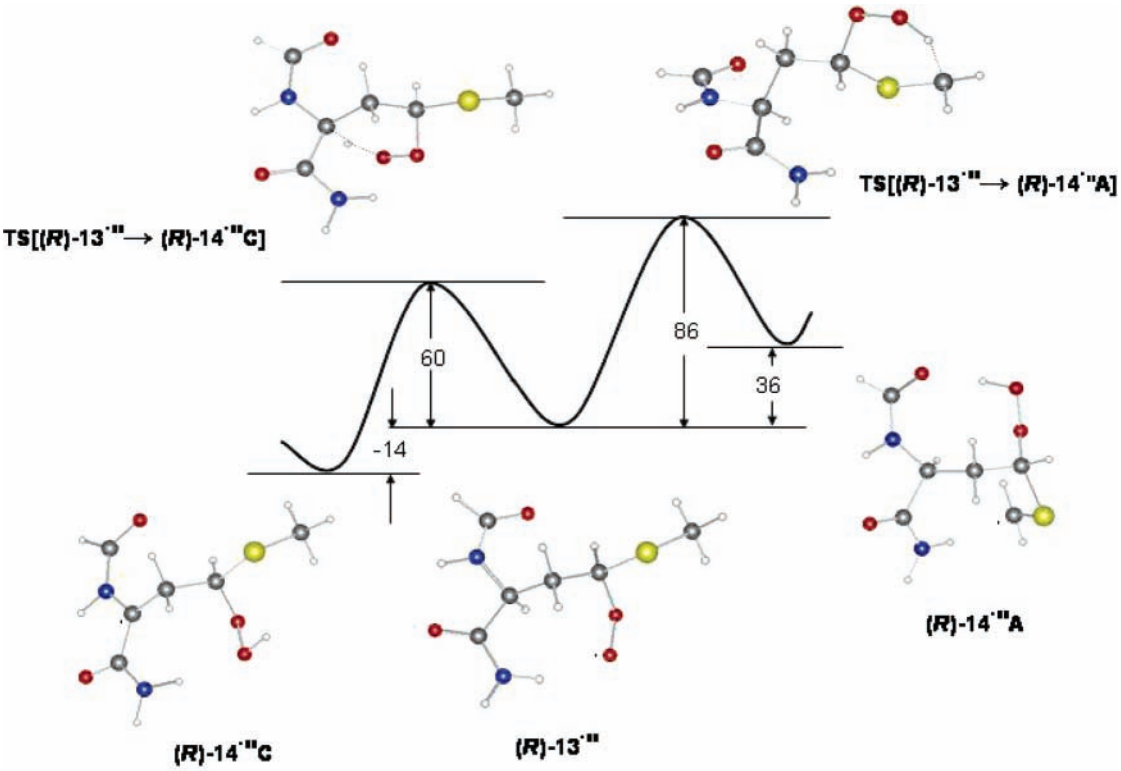


Figure 7. Intramolecular H abstraction reactions of the (R^{γ})-γ-peroxyl radical of L-methionine peptide (**11**): abstraction from $^{\epsilon}$C and $^{\alpha}$C.

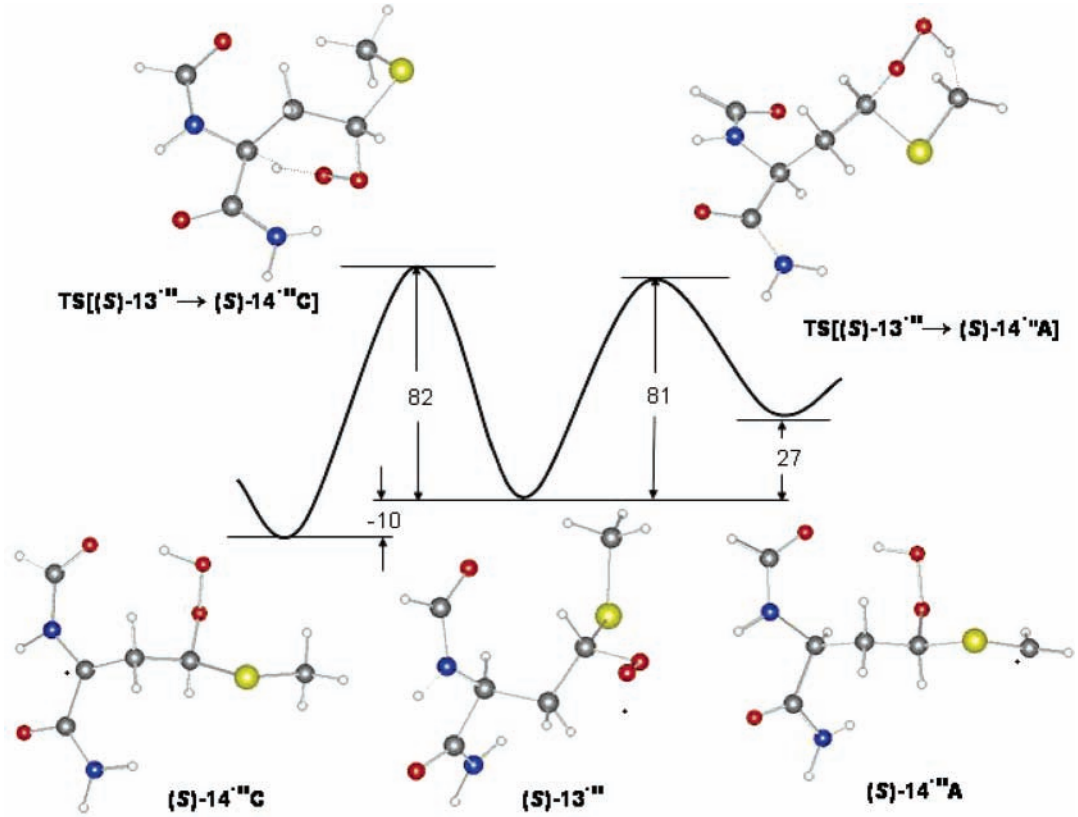


Figure 8. Intramolecular H abstraction reactions of the (S^{γ})-γ-peroxyl radical of L-methionine peptide (**11**): abstraction from $^{\epsilon}$C and $^{\alpha}$C.

in turn will add O_2 rapidly[24] to give the two diastereomeric secondary peroxy radicals ((*R*)-**13**$^{\bullet\prime\prime}$ and (*S*)-**13**$^{\bullet\prime\prime}$) and the primary peroxy radical (**13**$^{\bullet\prime}$) (Table 2).

The C−O BDEs of the Peroxy Radicals, ***13***$^{\bullet\prime}$*,* ***(R)-13***$^{\bullet\prime\prime}$*, and* ***(S)-13***$^{\bullet\prime\prime}$. The C−O BDEs of the peroxy radicals are (Table 1, in kJ mol^{-1}) **13**$^{\bullet\prime}$, 125.3; (*R*)-**13**$^{\bullet\prime\prime}$, 125.3; and (*S*)-**13**$^{\bullet\prime\prime}$, 121.0. Thus, the C−O bonds of all three isomeric side chain peroxy derivatives of Met are essentially the same and the same as for the secondary peroxy derivative of ethyl methyl sulfide, **8**$^{\bullet\prime}$. The most stable conformation of each of the peroxy radicals has a close approach to the H atom of the amide NH bond corresponding to the N atom of the $i + 1$ residue. The one-electron

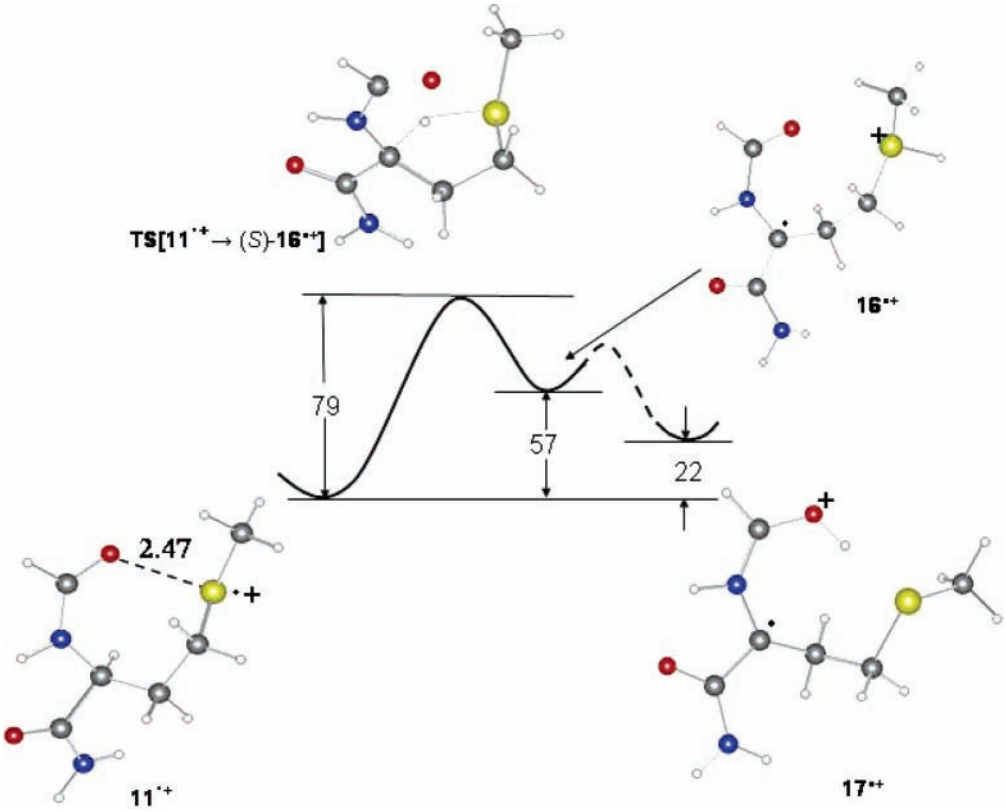


Figure 9. Intramolecular H abstraction reaction of $\mathbf{11}^{\bullet+}$.

$n_O \rightarrow \sigma_{NH}^*$ interaction apparently provides enough stabilization to overcome the distinction between primary and secondary points of attachment of the O−O moiety.

Intramolecular H abstractions: ***13•′ → 14•′B*** and ***13•′ → 14•′C*** *(Figure 6).* The primary peroxy radical, **13•′**, has three available sites for intramolecular H abstraction, two of which are considered here. As in the simpler model systems, it may attack the CH_2 group next to the S atom via a six-membered ring TS to yield **14•′B**, or it can reach the ᵅC−H bond via an eight-membered ring TS to yield ᵅC-centered radical **14•′C**. Free-energy profiles for the two pathways are shown in Figure 6, and the energetics are listed in Table 4. The abstraction of the H atom that is next to the S atom is endothermic by 10 kJ mol^{-1} and involves a high barrier, $\Delta G^{\ddagger} = 77$ kJ mol^{-1}. The abstraction of the ᵅC−H atom is exothermic by 14 kJ mol^{-1} and involves a lower barrier, $\Delta G^{\ddagger} = 59$ kJ mol^{-1}. An attack at the other (β) side-chain CH_2 group would yield an unstabilized alkyl radical and was not considered.

Intramolecular H Abstractions: ***(R)- and (S)-13•″ → 14•″A*** *and* ***(R)- and (S)-13•″ → 14•″C*** *(Figures 7 and 8).* The secondary peroxy radicals, (*R*)- and (*S*)-**13•″**, also have two available sites for intramolecular H-abstraction. They may attack the methyl group next to the S atom via a six-membered ring TS to yield **14•″A**, or they can reach the ᵅC−H bond via six-membered ring TSs to yield the ᵅC-centered radicals, **14•″C**. Free-energy profiles for the two pathways are shown in Figures 7 and 8, and the energy differences are listed in Table 4. The abstraction of the H atom that is next to the S atom by (*R*)-**13•″** is endothermic by 36 kJ mol^{-1} and involves a high barrier, $\Delta G^{\ddagger} = 86$ kJ mol^{-1}. An attack at the same site by (*S*)-**13•″** is slightly less endothermic, $\Delta G = 27$ kJ mol^{-1} and involves a slightly lower barrier, $\Delta G^{\ddagger} = 81$ kJ mol^{-1}. The abstraction of the ᵅC−H atom by (*R*)-**13•″** is exothermic by 14 kJ mol^{-1} and involves a lower barrier, $\Delta G^{\ddagger} = 60$ kJ mol^{-1}. An attack at the ᵅC−H site by (*S*)-**13•″** is also exothermic, $\Delta G = -10$ kJ mol^{-1}, but involves a higher barrier, $\Delta G^{\ddagger} = 82$ kJ mol^{-1}. The increase in the activation barrier is largely enthalpic in nature (Table 4) and originates from a more crowded orientation of the thiomethyl group.

Intramolecular H Abstraction: ***11•+ → 16•+ → 17•+*** *(Figure 9).* Finally, we consider the possibility that the initially oxidized L-methionine peptide $\mathbf{11}^{\bullet+}$ might react by intramolecular H-atom abstraction prior to its deprotonation and subsequent addition of oxygen. The more stable form of $\mathbf{11}^{\bullet+}$ (Figure 9) involves a three-electron bond to the pro-S face of the CH_2-S-CH_3 plane. The diastereomeric form with the three-electron bond to the pro-R face is less stable, $\Delta G = 18.3$ kJ mol^{-1} (Supporting Information, Table S1). The free-energy profile for the reaction involving the more stable form is shown in Figure 9. It leads to the lower-energy TS, although the difference in energy from the diastereomeric TS is less, $\Delta G = 3.5$ kJ mol^{-1}. The free energy of activation is 79 kJ mol^{-1}. The product sulfonium ion, $\mathbf{16}^{\bullet+}$, is substantially less stable than $\mathbf{11}^{\bullet+}$, $\Delta G = 57$ kJ mol^{-1}. However, in the presence of bases, including water, the proton would be quickly lost to yield the ᵅC-centered radical of methionine, **18•**, because $pK_a \approx -5$.[37] Indeed, our attempt to optimize $\mathbf{16}^{\bullet+}$ in a conformation in which the S−H could form a hydrogen bond to the $i-1$ carbonyl led to the exothermic transfer of the proton to the oxygen atom and yielded distal radical cation, $\mathbf{17}^{\bullet+}$, 35 kJ mol^{-1} lower in energy (Figure 9). Deprotonation of the carbonyl of $\mathbf{17}^{\bullet+}$ also results in **18•**.

For completeness, we examined the oxygen adduct of **18•**, namely, the peroxy radical, **19•**, and its hydroperoxide form, **20**. The C−O BDE of **19•** for loss of O_2 is 71 kJ mol^{-1} (Table 1). This value is about 15 kJ mol^{-1} lower than that calculated at the CBS-RAD level for the ᵅC-peroxy radicals of glycine and alanine peptides.[23] The O−H BDE of **20** is predicted to be 387 kJ mol^{-1} (Table 2). This value is only 4 kJ mol^{-1} higher than that calculated by CBS-RAD theory for the ᵅC-hydroperoxide of alanine peptide.[23]

Conclusions

The stable form of **11**$^{\bullet+}$ has a three-electron bond between the sulfur radical cation and the carbonyl oxygen atom of the *i* − 1 residue. In the absence of oxygen, the radical cation may abstract a hydrogen atom from a suitable donor or may lose a proton from the methyl or methylene groups flanking the oxidized sulfur. The pK_a values for the CH_3 and α-CH_2 groups are estimated to be 1 and −2, respectively. The resultant C-centered radical (**12**$^{\bullet\prime}$ or **12**$^{\bullet\prime\prime}$) may also be quenched by suitable H-atom donors in the absence of oxygen. We have examined the reactions of oxidized methionine residues, modeled by **11**$^{\bullet+}$ and **12**$^{\bullet\prime}$ or **12**$^{\bullet\prime\prime}$, in the presence of dissolved oxygen. Unlike C-centered radicals, the sulfur radical cation does not form a covalent bond to oxygen but rather forms a loose ion-induced dipole complex with an S−O separation of about 2.7 Å and is bound by about 13 kJ mol^{-1} (on the basis of **1**$^{\bullet+}$ + O_2). A direct intramolecular abstraction of an H atom from the $^{\alpha}$C site is unlikely. It is endothermic by more than 20 kJ mol^{-1} and involves a high barrier (ΔG = 79 kJ mol^{-1}).

The C-centered radicals, **12**$^{\bullet\prime}$ and **12**$^{\bullet\prime\prime}$, will add oxygen to form the peroxy radicals, **13**$^{\bullet\prime}$ and **13**$^{\bullet\prime\prime}$, respectively. The OH BDEs of the parent hydroperoxides are in the range of 365−371 kJ mol^{-1}, similar to SH BDEs (360 kJ mol^{-1}) and $^{\alpha}$C−H BDEs (345−350 kJ mol^{-1}). Thus, the peroxy radicals are oxidizing species comparable in strength to thiyl radicals and peptide backbone $^{\alpha}$C-centered radicals. Each peroxy radical can abstract a hydrogen atom from the backbone $^{\alpha}$C site of the Met residue to yield the corresponding $^{\alpha}$C-centered radical/hydroperoxide in a weakly exothermic process with modest barriers ($\Delta G^{\ddagger}$, kJ mol^{-1}): **13**$^{\bullet\prime}$, 59; (*S*)-**13**$^{\bullet\prime\prime}$, 60; and (*R*)-**13**$^{\bullet\prime\prime}$, 82.

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Supporting Information Available: B3LYP/6-31G(d) and CBS-RAD energies, vibrational energies, and thermal corrections, $S°_{298}$ and $H°_{298}-H°_0$ for all species, Cartesian coordinates for the B3LYP/6-31G(d)-optimized structures of all species. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Stadtman, E. R.; Oliver, C. N. *J. Biol. Chem.* **1991**, *266*, 2005−2008.
(2) Stadtman, E. R. *Science* **1992**, *257*, 1220−1224.
(3) Barcikowska, M. *Neurol. Neurochir. Pol*. **1999**, Suppl 1, 29.
(4) Blain, H, Jeandel, C. *Presse Med.* **1998**, *27*, 725.
(5) Gabryelewicz, T. *Neurol. Neurochir. Pol.* **1999**, Suppl 1, 11.
(6) Henderson, V. W.; Finch, C. E. *J. Neurosurg*. **1989**, *70*, 335.
(7) McDowell, I. *Aging (Milano)* **2001**, *13*, 143.
(8) Rowan, M. J. *Proc. Nutr. Soc*. **1993**, *52*, 255.
(9) Näslund, J.; Haroutunian, V.; Mosh. R.; Davis, K. L.; Davis, P.; Greengard, P.; Buxbaum, J. D. *JAMA* **2000**, *283*, 1571.
(10) Dragunow, M.; MacGibbon, G. A.; Lawlor, P.; Butterworth, N.; Connor, B.; Henderson, C.; Walton, M.; Woodgate, A.; Hughes, P.; Faull, R. L. *Rev. Neurosci.* **1997**, *8*, 223.
(11) Pogocki, D. *Acta Neruobiol. Exp*. **2003**, *63*, 131.
(12) Butterfield, D. A.; Kanski, J. *Peptides* **2002**, *23*, 1299.
(13) Schöneich, C. *Arch. Biochem. Biophys*. **2002**, *397*, 370.
(14) Huang, X.; Atwood, C. S.; Hartshorn, M. A.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Cuajungco, M. P.; Gray, D. N.; Lim, J.; Moir, R. D.; Tanzi, R. E.; Bush, A. I. *Biochemistry* **1999**, *38*, 7609−7616.
(15) Huang, X.; Cuajungco, M. P.; Atwood, C. S.; Hartshorn, M. A.; Tyndall, J. D.; Hanson, G. R.; Stokes, K. C.; Leopold, M.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Saunders; A. J.; Lim, J.; Moir, R. D.; Glabe, C.; Bowden, E. F.; Masters, C. L.; Fairlie, D. P.; Tanzi, R. E.; Bush, A. I. *J. Biol. Chem*. **1999**, *274*, 37111−37116.
(16) See, for example, Chu, J.-W.; Yin, J.; Wang, D. I. C.; Trout, B. L. *Biochemistry* **2004**, *43*, 1019−1029.
(17) Richardson, D. E.; Regino, C. A. S.; Yao, H.; Johnson, J. V. *Free Rad. Biol. Med.* **2003**, *35*, 1538−1550.
(18) Opazo, C.; Huang, X.; Cherny, R. A.; Moir, R. D.; Roher, A. E.; White, A. R.; Cappai, R.; Masters, C. L.; Tanzi, R. E.; Inestrosa, N. C.; Bush, A. I. *J. Biol. Chem.* **2002**, *277*, 40302−40308.
(19) Brunelle, P.; Rauk, A. *J. Alzheimer's Dis.* **2002**, *4*, 283−289.
(20) Rauk, A.; Armstrong, D. A.; Fairlie, D. P. *J. Am. Chem. Soc*. **2000**, *122*, 9761−9767.
(21) Rauk, A.; Armstrong, D. A. *J. Am. Chem. Soc*. **2000**, *122*, 4185−4192.
(22) The direct generation of glycyl α-C-centered radicals by one-electron-oxidized Met in polypeptides of the form, *N*-Ac-GGGMGGG have been shown not to occur (Schöneich, C.; Pogocki, D.; Hug, G. L.; Bobrowski, K. *J. Am. Chem. Soc*. **2003**, *125*, 13700−13713). Thus the reaction of a three-electron bond-stabilized Met radical cation does not react with adjacent glycines on the same strand in a random coil arrangement within the expected millisecond lifetime of the cation. It was not possible to test the role of the *β*-sheet secondary structure directly.
(23) Huang, M. L.; Rauk, A. *J. Phys. Org. Chem.*, in press.
(24) Schäfer, K.; Bonifacic, M.; Bahnemann, D.; Asmus, K.-D. *J. Phys. Chem.* **1978**, *82*, 2777−2780.
(25) However, $(R_2S)_2^{\bullet+}$, generated by reaction with the hydroxyl radical in the presence of excess R_2S, reacts rapidly with O_2 after the prior addition of hydroxide, yielding sulfoxides: Schöneich, C.; Aced, A.; Asmus, K.-D. *J. Am. Chem. Soc*. **1993**, *115*, 11376−11383.
(26) Asmus, K.-D. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; John Wiley & Sons: New York, 1999; pp 146−191.
(27) Armstrong, D. A. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; John Wiley & Sons: New York, 1999; Chapter 2.
(28) Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502−16513.
(29) Mayer, P. M.; Parkinson, C. J.; Smith, D. M.; Radom, L. *J. Chem. Phys.* **1998**, *108*, 604.
(30) (a) Fischer, H.; Radom, L. *Angew. Chem., Int. Ed*. **2001**, *40*, 1340. (b) Wong, M. W.; Radom. L. *J. Phys. Chem*. **1995**, *99*, 8582. (c) Wong, M. W.; Radom, L. *J. Phys. Chem. A* **1998**, *102*, 2237.
(31) Rauk, A.; Boyd, R. J.; Boyd, S. L.; Henry, D. J.; Radom, L. *Can. J. Chem.* **2003**, *81*, 1−12.
(32) Armstrong, D. A.; Yu, D.; Rauk, A. *Can. J. Chem*. **1996**, *74*, 1192−1199.
(33) Ochterski, J. W. *Thermochemistry in Gaussian*; April 19, 2000: http://www.Gaussian.com/g_whitepap/thermo.htm.
(34) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley-Interscience: New York, 1988; p 452.
(35) McKee, M. L. *Chem. Phys. Lett.* **1994**, *231*, 257.
(36) Brunelle, P.; Rauk, A. Submitted for publication.
(37) Arnett, E. M. *Prog. Phys. Org. Chem.* **1963**, *1*, 223−403.