One-Electron Oxidation of Methionine in Peptide Environments: The Effect of Three-Electron Bonding on the Reduction Potential of the Radical Cation

Patrick Brunelle and Arvi Rauk*

Department of Chemistry, University of Calgary, Calgary, AB T2N 1N4, Canada

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The complexes between the radical cation of dimethyl sulfide **2** and models for eight biologically available electron pair donors, :X (:X = H_2O (**2a**), H_2CO (**2b**), $HC(O)NH_2$ (**2c**), $HC(O)NHCH_3$ (**2d**), HCO_2^- (**2e**), HCO_3^- (**2f**), $H_2PO_4^-$ (**2g**), and CH_3NH_2 (**2h**)), were optimized at the B3LYP/6-31G(d) level of theory. S∴X bond dissociation enthalpies (BDEs) were determined by single point calculations at the CBS−RAD level, a method designed for quantitative thermochemistry of free radicals. The effect of solvation was determined by application of a polarizable continuum model. Only the amine complex is predicted to be stable in water. H_2O and $H_2PO_4^-$ make transient complexes, and the remaining complexes are predicted to dissociate spontaneously. The dissociation is driven by entropy and conformationally constrained complexes are predicted to be stable in water. Reduction potentials, $E°$, accurate to ±0.1 V were calculated for the complexes with dimethyl sulfide and for the amino acid, methionine, both as an isolated amino acid and incorporated into a polypeptide at the N- and C-terminals and midchain. Stabilization of the radical cation of Met by three-electron bonding is predicted if an S∴N bond can be formed to a free amino group, as in N-terminal Met or a nearby Lys. Likewise, Met oxidation is facilitated by phosphodiesters, but not by carboxylate groups or amide groups. No lowering of $E°$ is predicted for C-terminal Met or for midchain Met. The implications of the results for the redox chemistry associated with Alzheimer's disease are discussed.

Introduction

Oxidation of methionine sulfur is an important feature of "oxidative stress" in biological systems,[1−7] and may be a key step in the neurotoxicity of the β-amyloid peptide (Aβ) in Alzheimer's disease.[8−11] Two distinct in vitro experiments have shown that a substitution of Met35 (the lone methionine in Aβ) by norleucine eliminated the neurotoxicity of Aβ toward hippocampal neurons.[12,13] Bush and co-workers have shown that Cu^{II} and Fe^{III} are able to oxidize Aβ(1−40).[14,15] However, Aβ-(1−28) lacking Met35 was not able to reduce Cu^{II} to Cu^{I}, but with the addition of methionine (the amino acid), Cu^{II} was reduced. This led to the speculation that Cu^{II} is oxidizing Met35, forming Cu^{I} and a radical cation on the sulfur of the methionine ($MetS^{•+}$).[11]

The proposed redox chemistry involving Cu^{II}/Aβ/Met is problematic in two respects. First, the reduction potential of the Cu^{II}/Aβ complex has been measured as $E° = 0.7$ V (relative to the standard hydrogen electrode),[16] significantly higher than $E°$ of Cu^{II}/Cu^{I} in aqueous solution, $E° = 0.16$ V.[17] While reduction potentials for Cu^{II} in peptide complexes as high as 1.0 V have been reported,[18] the precise binding mode that gives rise to the elevated value in the Cu/Aβ complex is not known. Second, on the face of it, the reduction potential of a typical dialkyl sulfide radical cation appears to be too high for it to be oxidized by Cu^{II}/Aβ (reaction 1) at a reasonable rate.

$$Cu^{II}/A\beta + R_2S \rightarrow Cu^{I}/A\beta + R_2S^{•+} \qquad (1)$$

Oxidation of aqueous methionine is an irreversible process. The peak anodic oxidation potential, $E*$, of Met in its zwitterionic form, is $E* = 1.48$ V.[19] In the case where the reverse reductive wave of the cyclic voltammogram is not seen, $E*$ is an upper limit to the standard reduction potential, $E°$. Thus, $E°$ of zwitterionic Met is somewhat lower than that of a typical dialkyl sulfide radical cation, e.g., dimethyl sulfide, $E° = 1.66$ V.[20] It follows that if the reduction potential of the $MetS^{•+}$ residue in Aβ (or any other polypeptide) were comparable to the amino acid, the oxidation of Met by Cu/Aβ by reaction 1 would be endothermic by 0.78 V, or 75 kJ mol^{-1}.

The radical cation of a dialkyl sulfide can make a two-center three-electron bond with an electron pair donor, :X. We designate such a bond as S∴X. Both S∴O and S∴N bonds in Met derivatives have been proposed[21] and observed spectroscopically,[22,23] in pulse-radiolysis and flash photolysis experiments in aqueous solution. It has been implied that stabilization of $MetS^{•+}$ by such three-electron bonding to accessible carbonyl oxygen atoms can lower the reduction potential sufficiently for reaction 1 to take place.[24] In the case of C-terminal Met, where the carboxylate group is accessible to the S radical cation, the stabilization is expected to be higher still.[12]

In connection with Alzheimer's disease, it has been postulated that the redox chemistry of Met35 in α-helical Aβ is facilitated by an interaction with the oxygen atom of the nearest amide group, that of Ile31.[24] An analogous interaction with a proximal carbonyl group in a β-sheet was found computationally.[25] In either case, three-electron bonding to a carbonyl group occurs partially at the expense of an existing N−H hydrogen bond to the oxygen atom, and the stabilization due to the S∴O bonding is reduced by the partial loss of the H-bond. In the β-sheet case, the S∴O -bonded complex was calculated to be stabilized by only 10 kJ mol^{-1}.[25] In neither study was it attempted to assess quantitatively the extent to which such bonding lowers the

* To whom correspondence should be addressed: rauk@ucalgary.ca

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reduction potential of the complexed sulfide radical cation in an aqueous environment. This study quantifies the effect of this interaction on the reduction potential and compares it with the effect on the reduction potential of other potential complexing species present in biological systems.

We have calculated reduction potentials of two dialkyl sulfides, dimethyl sulfide and methionine. The reduction potential of dimethyl sulfide is known experimentally (E° = 1.66 V).[20] Peak anodic oxidation potentials of the amino acid methionine (Met) have also been measured by cyclic voltammetry in aqueous solution at different pH's.[19] The fully protonated Met (pH = 2.1) has E^* = 1.72 V, the fully deprotonated Met (pH = 12.2) has E^* = 1.40 V and the zwitterionic Met, E^* = 1.48 V (vs the hydrogen electrode).[19] In addition, the reduction potential of oxidized MetGly, which is known to possess an intramolecular S∴N bond, has been determined, 1.42 ± 0.3 V.[26] These systems serve as benchmarks.

Methods

Ab initio calculations were performed with the Gaussian 98 suite of quantum chemistry programs.[27] Geometry optimization and harmonic frequency analysis for all species except zwitterionic Met ($\mathbf{3}^{(+-)}$) were carried out at the B3LYP/6-31G(d) level of theory. Zwitterionic amino acids are unstable at this level of theory in the gaseous phase and revert by proton transfer to species with neutral, but H-bonded, $-NH_2$ and $-COOH$ groups. Structure $\mathbf{3}^{(+-)}$ was optimized in the presence of the solvent reaction field, SCRF=CPCM, and also at the HF/6-31G(d) level for the purpose of obtaining harmonic frequencies. A thorough investigation of the geometries of related three-electron bonded systems in the gaseous phase and in solution found only minor differences in the two geometries.[28] Zero point energies, entropy, and temperature correction to the enthalpy ($H_{298}^\circ - H_0^\circ$) were obtained from frequencies scaled by a factor of 0.9806, with the exception of $\mathbf{3}^{(+-)}$ for which a scale factor of 0.8929 was applied to the HF frequencies.[29] No attempt was made to locate all conformers of all species. The contribution of conformational flexibility to the entropy of each species, i.e., the entropy of mixing, was approximated as $R\ln n$, where n is an estimate of the number of significantly populated conformers.[30,31] Improved enthalpy differences were obtained by the CBS−RAD method[32] applied to the B3LYP/6-31G(d)-optimized geometries. CBS−RAD is a high-level theoretical procedure for obtaining thermochemical values for radicals to experimental accuracy. 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,[33] and the β-scission reactions of alkoxy radicals.[34]

The COSMO procedure, implemented in G98 as SCRF=CPCM,[35,36] was used to model solvation effects. The cavity of the solute was defined by its 0.001 electrons bohr^{-3} gaseous-phase isodensity surface,[37,38] and it was fitted by overlapping atom-centered spheres as required by the G98 CPCM implementation. It is anticipated that calculation of the free energy of solvation in this manner will render the inevitable errors to be more systematic and hence amenable to correction by reference to experiment. Values derived from experiment were adopted for the free energy of solvation of water ($\Delta G_{solv}(H_2O)$ = −26.4 kJ mol^{-1})[39] and of the proton ($\Delta G_{solv}(H^+)$ = −1107 kJ mol^{-1}).[40]

Primary energy data and geometries of all species are listed in Tables S1 and S2 of the Supporting Information.

Empirical Correction of Systematic Errors in Free Energies of Solvation, ΔG_{solv}. It is known that continuum solvation methods cannot account for cases where specific hydrogen bonding is an important component of the free energy of solvation. In such circumstances, inclusion of a first solvation shell of H-bonded water molecules is desirable. In the present cases, these are expected to be most important for the cationic $-NH_3^+$ group, and the anionic $-CO_2^-$ group. To avoid convergence problems and problems associated with multiple minima, we have opted instead to make empirical corrections to minimize errors that ensue from the treatment of the first solvation shell as a continuum. For acidic species in solution, experimentally determined pK_a values provide a sensitive measure of relative free energies in aqueous solution against which computed free energies can be compared. The pK_a of methylammonium ($CH_3NH_3^+$) and formic acid (HCO_2H) can be calculated from the data in Table S1, yielding values of 14.45 and 8.05, respectively, as compared to the experimental values, 10.64 and 3.75, respectively. For such small species, it may be assumed that errors in the free energy changes in the gaseous phase are negligible. The discrepancies in pK_a values, which correspond to 22 and 25 kJ mol^{-1} for the cationic and anionic reactions are most likely the result of errors in the calculation of the relative free energy of solvation, $\Delta\Delta G_{solv}$, of reactions 2 and 3.

$$(CH_3NH_3^+)_{aq} \rightarrow (CH_3NH_2)_{aq} + (H^+)_{aq} \quad (2)$$

$$(HCO_2H)_{aq} \rightarrow (HCO_2^-)_{aq} + (H^+)_{aq} \quad (3)$$

Free energies of solvation, ΔG_{solv}, of the neutral species, CH_3NH_2 and HCO_2H, are small in absolute magnitude (−5.6 and −22.6 kJ mol^{-1}, respectively), compared to the ionic species, $CH_3NH_3^+$ and HCO_2^- (−318.6 kJ mol^{-1} and −286.9 kJ mol^{-1}, respectively). The calculated and experimental values may be reconciled if one assumes that the systematic procedure we have adopted for the evaluation of ΔG_{solv} *over*estimates the solvation of the ammonium group by 22 kJ mol^{-1}, and *under*estimates the solvation of the carboxylate group by 25 kJ mol^{-1}. Indeed, addition of very similar amounts, +23 kJ mol^{-1} to the CPCM-derived ΔG_{solv} of protonated methionine, $\mathbf{3}^+$ (Figure 3), and −26 kJ mol^{-1} to deprotonated methionine, $\mathbf{3}^-$ (Figure 3), reproduces the experimentally determined solution free energy difference of the two species, as determined from the sum of the pK_as of eqs 4 and 5, where $\mathbf{3}^{(+-)}$ is the zwitterionic form of methionine.

$$(\mathbf{3}^+)_{aq} \rightarrow (\mathbf{3}^{(+-)})_{aq} + (H^+)_{aq} \quad (4)$$

$$(\mathbf{3}^{(+-)})_{aq} \rightarrow (\mathbf{3}^-)_{aq} + (H^+)_{aq} \quad (5)$$

As many of the species of concern to this study involve either a $-NH_3^+$ group or a $-CO_2^-$ group, their calculated ΔG_{solv} values may be improved by the application of these corrections. The ΔG_{solv} values for these species listed in Table S1 include the empirical corrections. The zwitterionic form of methionine, $\mathbf{3}^{(+-)}$, having both groups, is a unique case. Simple additivity of the corrections would imply that the CPCM-derived ΔG_{solv} (= −98.1 kJ mol^{-1}) should be lowered by only 3 kJ mol^{-1}. However, a somewhat larger correction, −10 kJ mol^{-1}, is required to reproduce the experimentally determined pK_as of eqs 4 and 5, 2.1 and 9.3, respectively. The ΔG_{solv} value for $\mathbf{3}^{(+-)}$ that appears in Table S1 and is used in subsequent calculations of reduction potentials includes the −10 kJ mol^{-1} correction.

The solvation of the most important charged species, the sulfide radical cation, may be assessed by comparing the calculated pK_a of $(CH_3)_2S^{\bullet+}$ (eq 6)

$$((CH_3)_2S^{\bullet+})_{aq} \rightarrow (CH_3SCH_2^{\bullet})_{aq} + (H^+)_{aq} \qquad (6)$$

to the value estimated from experiment, $pK_a \approx -1.8$.[20] The calculated value, $pK_a = 0.3$, is in reasonable agreement, given the uncertainty in the experimental estimate, and represents an *overestimate* of ΔG_{solv} by about 12 kJ mol^{-1} if the experimental value were precise. However, no adjustments are applied to ΔG_{solv} of any of the S-oxidized species or their complexes unless they contain $-NH_3^+$ or $-CO_2^-$ groups.

Results and Discussion

An orbital interaction diagram that describes a two-center three-electron ($2\sigma/1\sigma^*$) bond, S∴X, is shown in Figure 1. The singly occupied molecular orbital (SOMO) of the sulfur radical and the highest occupied molecular orbital (HOMO) of an electron-pair donor interact to form a bonding (σ) and antibonding (σ^*) pair of orbitals.[41] The stabilization is greater if the orbital energies are closer together. This may be achieved by substituting a negatively charged O atom for a neutral one, or an N atom for an O atom. Such bonding has been shown to lower the peak anodic oxidation potential, E^*, in a series of conformationally constrained 2-substituted 6-(methylthio)-bicyclo[2.2.1]heptanes in acetonitrile.[42]

Structures of Three-Electron-Bonded Systems (Figure 2). The structures of all of the dimethyl sulfide-derived systems in the present study, are shown in Figure 2. The S∴O bond lengths range from 2.35 to 2.57 Å. The three-electron bonds are substantially longer than typical S−O single bonds, as in sulfoxides (R_2SO, $r_{SO} = 1.47$ Å). Similarly, the S∴N three-electron bond, $r_{SN} = 2.61$ Å, is half again as long as a typical S−N single bond ($RS-NR_2$, $r_{SN} = 1.77$ Å). In each case, the "ligand", :X, is positioned almost perpendicular to the two C−S bonds ($\angle_{CSX} \approx 90°$). This is an indication that it is the singly occupied 3p orbital on the S atom that makes up half of the bond. The orientation of the ligand is that expected for involvement of the doubly occupied lone pair orbital of that species (nonplanar in the case of H_2O and $\angle_{SOC} \approx 120°$ in the case of the carbonyl ligands) as the other orbital involved in the bond.

The spin distribution about the S∴O (or S∴N) linkage is given in italics in Figure 2. As required by the interaction diagram (Figure 1), the spin density is partially localized to the O (or N) atom. The spin delocalization is least in the case of the complex with H_2O, and greatest in the case of the negatively charged ligands. In the case of the H_2O complex (**2a**), 82% of the spin remains on the S atom, while just over half remains in the case of bicarbonate (**2f**) (55%) and dihydrogen phosphate (**2g**) (56%). Formate anion (**2e**), a model for carboxylate groups, is extensively oxidized with only 40% of the spin residing on the S atom. The neutral carbonyl compounds show an intermediate level of spin delocalization: formaldehyde (**2b**), 76% on S; formamide (**2c**), 73% on S; *N*-methylformamide (**2d**), 71% on S. *N*-Methyl formamide is a model for the amide group in polypeptides. The most stable conformation of the radical cationic complex, **2d**, has the S atom situated in the plane of the amide group, a geometry that would compete with H-bonding in protein secondary structure.

Accurate ab initio description of three-electron bonds ($2\sigma/1\sigma^*$) is problematic. As shown by Braïda and co-workers[43−47] and others,[28,48−52] no single broadly applicable theoretical method yields an accurate and reliable description in all cases. In particular, B3LYP has been reported to yield bonds that are up to 0.1 Å too long in the case of symmetrical systems.[43] This appears not to be the case in the complex with water (**2a**, Figure 2) ($r_{SO} = 2.57$ Å). The geometry of **2a** was also optimized at the QCISD/6-31G(d) and MP2/6-31G(d)levels, both of which yielded longer S∴O distances, $r_{SO} = 2.70$ Å and $r_{SO} = 2.67$ Å, respectively. Single-point energy evaluations at the CBS−RAD level on the B3LYP-, QCISD-, and MP2-optimized geometries yielded relative energies of 3.3, 0.2, and 0.0 kJ mol^{-1}, respectively, indicating that the intermediate MP2 value is closest to being correct. Thus, contrary to previous reports, the B3LYP value is *shorter* by about 0.1 Å in this case. As the effect of solvation has been shown computationally to shorten S∴O bonds by about 0.1 Å,[28] we judge the B3LYP/6-31G(d) geometries to be adequate for the present study which is primarily concerned with redox behavior in solution. The B3LYP method has also been shown to yield bond dissociation enthalpies (BDEs) that are too high. Discrepancies from 3 to 30 kJ mol^{-1} compared to MP4 calculations were found for symmetrical three-electron bonds.[43] As we show below, BDEs of S∴O and S∴N bonds calculated from B3LYP/6-311+G-(3df,2p) energies of B3LYP/6-31G(d) structures are within a few kilojoules per mole of, and in general *lower* than, CBS−RAD energies. Therefore, for efficiency, we have adopted the B3LYP/6-31G(d) procedure for the geometry optimization and frequency determination, and employ the CBS−RAD procedure to obtain accurate enthalpy differences. The assumption is that small errors in the long S∴X bonds will affect the energies by only a few kilojoules per mole because of the flatness of the potentials associated with such long bonds.

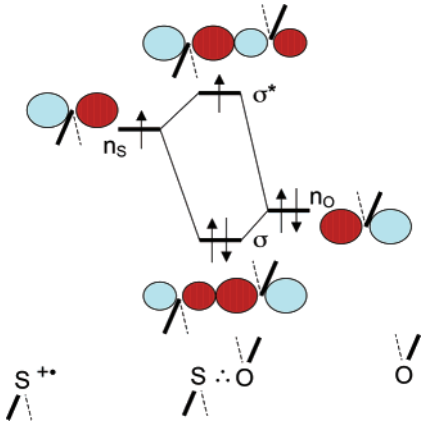

Figure 1. Orbital interaction diagram showing S∴X bond formation between a dialkyl sulfide radical cation and an electron-pair donor, :X.

S∴X Complexation Energies for $(CH_3)_2S$∴X^+ (Table 1). The primary computed energies, enthalpies, temperature corrections, entropies, and free energies of solvation for all species are listed in Table S1 of the Supporting Information. The gaseous phase enthalpy and free energy changes for the dissociation reaction, eq 7, are shown in Table 1.

$$(CH_3)_2S\therefore X^+ \rightarrow (CH_3)_2S^{\bullet+} + :X \qquad (7)$$

The most accurate estimates for the BDEs of the S∴O and S∴N three-electron bonds are given in column 4 (ΔH(298K)(g)) of Table 1. The weakest bond, 46.4 kJ mol^{-1}, is to H_2O. A similar value is expected for the OH group of Ser and Thr residues in proteins. Formamide and *N*-methyl formamide serve as models for the amide carbonyl group of peptides. The bonds between the amide carbonyl oxygen and the S radical cation are considerably stronger, 101 and 109 kJ mol^{-1}, respectively, than the divalent oxygen of water, or a simple carbonyl, represented by formaldehyde. Bonds to the negatively charged species are

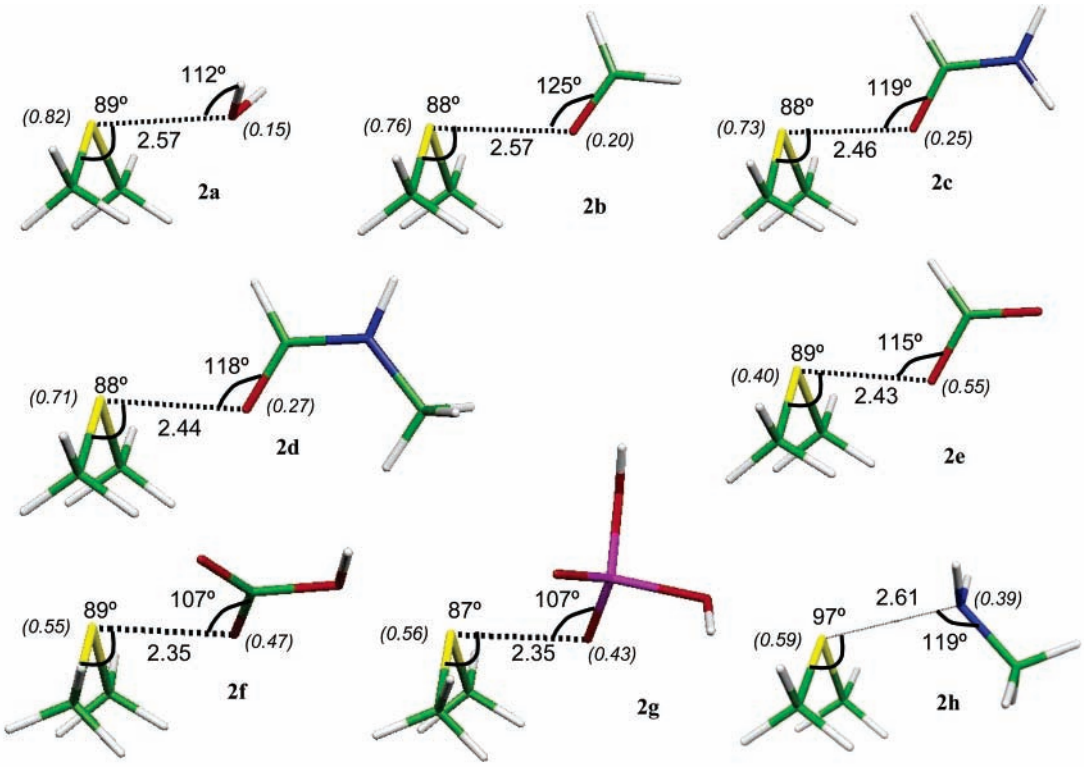

Figure 2. Representative B3LYP/6-31G(d)-optimized structures of complexes between the dimethyl sulfide radical cation and H_2O (**2a**), H_2CO (**2b**), $HC(O)NH_2$ (**2c**), $HC(O)NHCH_3$ (**2d**), HCO_2^- (**2e**), HCO_3^- (**2f**), $H_2PO_4^-$ (**2g**), and CH_3NH_2 (**2h**).

TABLE 1: Energy Changes (kJ mol^{-1}) for $(CH_3)_2S\therefore X^+ \rightarrow (CH_3)_2S^{\bullet+}$ (2) + :X (reaction 7)[a]

:X (species)	ΔH(0K)(g) B3LYP[b]	ΔH(0K)(g) CBS−RAD	ΔH(298K)(g)	$-T\Delta S$(g)	ΔG(g)	$\Delta\Delta G_{solv}$	ΔG(aq)	$-T\Delta S_v$[c]	$\Delta G(aq)_v$[c]
H_2O (**2a**)	42.0	44.2	46.4	−28.4	18.0	−35.3	−7.3		
OCH_2 (**2b**)	61.8	60.4	60.8	−32.7	28.2	−58.0	−29.8		
$OCHNH_2$ (**2c**)	97.5	99.9	101.3	−41.1	60.2	−79.1	−18.9	16.4	38.6
$OCHNHCH_3$ (**2d**)	108.4	111.2	109.3	−29.5	79.8	−96.4	−16.7	28.1	41.0
HCO_2^- (**2e**)	552.1	556.1	558.9	−40.8	518.1	−554.6	−36.5	14.7	18.9
HCO_3^- (**2f**)	530.7	547.3	547.7	−36.7	511.1	−544.1	−33.0	21.3	25.0
$H_2PO_4^-$ (**2g**)	504.7	523.3	523.4	−39.9	483.5	−488.6	−5.1	30.6	65.4
CH_3NH_2 (**2h**)	104.7	104.0	104.3	−33.6	70.8	−63.7	7.0	19.9	60.5

[a] The standard state for all entropies and free energies is 1 M at 298 K. To convert the gaseous-phase results to a standard state of 1 atm at 298 K, 7.9 kJ mol^{-1} must be subtracted from $-T\Delta S$(g) and ΔG(g). Free energy changes are derived from the CBS−RAD enthalpies. [b] B3LYP/6-311+G(3df,2p) energies of B3LYP/6-31G(d)-optimized structures. [c] Translational and rotational components of the entropy removed—see text for an explanation.

much higher still (>500 kJ mol^{-1}), as they entail heterolysis into oppositely charged species. Bicarbonate is a ubiquitous species in biological systems. The formate anion is a model for the carboxylate group of Asp and Glu residues and the C-terminal end of proteins. Dihydrogen phosphate is a model for phosphodiesters as occur in nucleic acids, in the headgroups of phospholipids, and elsewhere. Methylamine, a model for the amino group of Lys residues and the N-terminal end of polypeptides, has a gaseous-phase binding energy (104 kJ mol^{-1}) that is comparable to the amide carbonyl group.

The second and third columns of Table 1, labeled ΔH(0K)-(g), compare the BDEs at the B3LYP/6-311+G(3df,2p) level with the more accurate CBS−RAD-level values. Contrary to the previously noted trend, the B3LYP values for the most part are within 3 kJ mol^{-1} of the most accurate values in the case of the neutral ligands, or *lower* by up to 19 kJ mol^{-1} in the case of the negatively charged ligands.

Stability of Three-Electron-Bonded Complexes in Water. The effect of aqueous solvation on the dissociation of the complexes according to eq 7, is given in columns 7 ($\Delta\Delta G_{solv}$) and 8 (ΔG(aq)) of Table 1. In each case, the separated products are solvated more strongly than the complex and solvation favors the dissociation process ($\Delta\Delta G_{solv} < 0$). Indeed, with the exception of the amine complex, all of the complexes are predicted to dissociate spontaneously (ΔG(aq) < 0). It is apparent that it is the entropy change associated with the dissociation that leads to this result. The binding *enthalpies* calculated in the gaseous phase for all of the complexes are greater in magnitude than $|\Delta\Delta G_{solv}|$, but the *free energies* of dissociation, with the exception of that for the amine complex **2h**, are less. The calculated results are consistent with experiments involving photochemical oxidation of thiaalcohols.[22] The presence of an intramolecularly S∴O-bonded species was detected spectroscopically, but only in the case of a five-membered ring.

Perhaps most surprising is the result that the cation−anion pairs, **2e**, **2f** and **2g**, are also predicted to dissociate in water. In these cases also, the large binding enthalpy in the gaseous phase is greater than the difference in combined ΔG_{solv} of the ionic products and the relatively low ΔG_{solv} of the neutral complexes. It is the entropic component that tips the balance in the direction of dissociation.

TABLE 2: Energy Changes (kJ mol^{-1}) and Reduction Potentials (V) for [$(CH_3)_2S\therefore X^+$](aq) + $^1/_2H_2$(g) → $(CH_3)_2S$(aq) + :X(aq) + H^+(aq) (Reaction 8)[a]

:X	ΔH(298K)(g)	$-T\Delta S$(g)	ΔG(g)	$\Delta\Delta G_{solv}$	ΔG(aq)	$E°$	$-T\Delta S_v$[b]	$\Delta G(aq)_v$[b]	$E°$ [b]
no ligand (**2**)	692.5	−2.8	689.7	−843.6	−153.9	**1.60**			
H_2O (**2a**)	738.9	−25.5	713.4	−878.9	−155.5	**1.61**			
OCH_2 (**2b**)	753.4	−29.8	723.5	−901.6	−178.1	**1.85**			
$OCHNH_2$ (**2c**)	793.6	−36.1	757.5	−925.9	−168.4	**1.75**	17.9	−114.4	**1.19**
$OCHNHCH_3$ (**2d**)	801.8	−26.7	775.2	−940.1	−164.9	**1.71**	29.3	−108.9	**1.13**
HCO_2^- (**2e**)	1257.1	−37.9	1219.2	−1396.2	−179.0	**1.86**	15.9	−125.2	**1.30**
HCO_3^- (**2f**)	1240.3	−33.9	1206.4	−1385.7	−181.5	**1.88**	22.5	−124.8	**1.29**
$H_2PO_4^-$ (**2g**)	1215.8	−34.8	1181.0	−1330.7	−149.7	**1.55**	31.8	−83.1	**0.86**
CH_3NH_2 (**2h**)	796.9	−30.7	766.1	−907.3	−141.2	**1.46**	21.1	−89.3	**0.93**

[a] The standard state for all entropies and free energies is 1 M at 298 K. To convert the tabulated gaseous-phase results to a standard state of 1 atm at 298 K, −7.9 kJ mol^{-1} must be added to $-T\Delta S$(g) and ΔG(g). Free energy changes are derived from the CBS−RAD enthalpies. [b] Translational and rotational components of the entropy removed—see text for an explanation.

In the case of dihydrogen phosphate, the free energy for dissociation of the complex **2g** is close to zero (ΔG(aq) = −5.1 kJ mol^{-1}). This result is consistent with the observation that high concentrations of added phosphate effectively prolonged the lifetime of oxidized Met species.[21]

The nature of the sulfide radical cation (**2**) in water is of some interest. As the first row of Table 1 indicates, a discrete 1:1 complex between **2** and a water molecule, namely **2a**, is predicted to have transient existence at best. We return to this point below in the discussion of the reduction potentials.

Modeling Intramolecular Stabilization of Sulfur Radical Cations. In the systems discussed above, the sulfur radical cation and the ligand, :X, are independent species in solution. Dissociation is favored by entropy, as seen in column 5 of Table 1. However, the situation may be radically different in a biological system where the sulfide radical cation (of Met) and the ligand may be constrained into close proximity by the tertiary structure of the protein, protein/membrane, or protein/nucleic acid complex. The last two columns of Table 1 model the case where the sulfur radical cation and the ligand, :X, are *not* free to drift apart. We simulate this situation by removing all the translational and rotational contributions to the entropies of all species in eq 7 (except H_2O, H_2CO, and HCO_3^-) and retaining only the vibrational and electronic components. Thus, the entropy change for eq 7, ΔS_v, is negative and its contribution to the free energy change is positive. The last column of Table 1 gives the free energy change, $\Delta G(aq)_v$, of reaction 7 with the "intramolecular" constraints. For all of the ligands, $\Delta G(aq)_v$ is positive and falls in the range, 19−65 kJ mol^{-1}. This range represents the amount of stabilization of the S radical cation by nearby electron pair donors and it will be reflected in a lowering of the reduction potentials, $E°$, for such systems, as seen below.

Dimethyl Sulfide Oxidation. The reduction potential, $E°$, of $(CH_3)_2S^{\bullet+}$ in the presence of electron pair donors, :X

The reduction potential of dimethyl sulfide radical cation in the presence of different lone pair donors, relative to the standard hydrogen electrode, is calculated from the free energy change of reaction 8

$$[(CH_3)_2S\therefore X^+](aq) + {}^1/_2H_2(g) \rightarrow (CH_3)_2S(aq) + :X(aq) + H^+(aq) \quad (8)$$

by the relation (eq 9)

$$E°((CH_3)_2S\therefore X^+/(CH_3)_2S) = -\Delta G(aq)/F \quad (9)$$

where F is the Faraday constant, F = 96.485 kJ mol^{-1} V^{-1}.

The computed data for reaction 8 are collected in Table 2. The first two rows compare the effect of continuum solvation for dimethyl sulfide radical cation without and with explicit solvation of the cation by one water molecule. The predicted $E°$ values, 1.60 and 1.61 V, respectively, are close to the experimental value, $E°$ = 1.66 V.[20] The similar values of $E°$ with and without explicit complexation with water suggest at least partial complexation, consistent with the observation of a solvent isotope effect in the photochemical oxidation of dimethyl sulfide.[22]

The reduction potentials for all three-electron-bonded complexes are given in column 7 of Table 2. As anticipated from the aqueous dissociation data in Table 1, a modest lowering of $E°$ is seen only in the case of the amine and the phosphate ligands. As the data up to column 7 represent the situation where the ligand and cation radical are free *not* to associate, a reduction potential higher than that of the aquated radical cation **2** is not expected for a solution containing **2** and any of carboxylate or bicarbonate salts, or amide groups. By extension, one must conclude that the mere presence of electron-pair donors in the vicinity of a Met residue is not a sufficient condition for facilitating its oxidation.

The Reduction Potential, $E°$, of $(CH_3)_2S^{\bullet+}$ in Close Proximity to Intramolecular Electron Pair Donors, :X. The situation discussed in connection with the complexation data in the last two columns of Table 1 is repeated in the last three columns of Table 2. If the Met (modeled by $(CH_3)_2S$) and electron-pair donor are constrained into close proximity by the secondary structure of the protein or protein/membrane or protein/nucleic acid complex, then a substantial reduction of $E°$ is predicted due to the reversal of the sign of the entropic components of the free energy change. $E°$ values (last column of Table 2) are predicted to fall into the narrow range 0.9−1.3 V. The largest effects are predicted for alkylamino groups (modeled by CH_3NH_2) and for phosphodiesters (modeled by $H_2PO_4^-$). It must be emphasized that modeling of constrained systems by the devise of removing translational and rotational contributions to the entropy is a very crude approximation. A more realistic treatment is described below in the case of Met derivatives.

Oxidation of Methionine Derivatives. The modeling of various S∴X systems discussed above has indicated that the reduction potential, and therefore the barrier for the reverse oxidation, may be lowered significantly (cf. columns 7 and 10 of Table 2) if one removes all conformational flexibility from the oxidized and reduced forms. This represents a limiting case. We consider below the more realistic case of the redox chemistry of a Met residue in isolation, or in a polypeptide environment, while taking into account the conformational flexibility of the

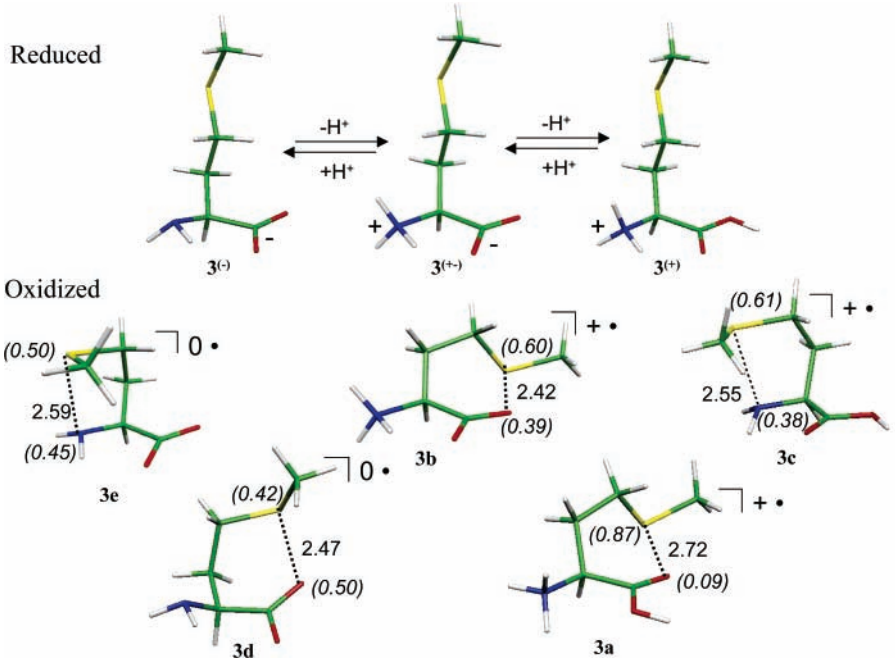

Figure 3. Species involved in redox chemistry of aqueous methionine.

Met and its immediate environment. $E°$ for each of the cases listed in Table 3 and shown in Figures 3−5 is calculated (using eq 9) from the free energy change for reaction 10 or 11, as appropriate.

$$\text{-S}\therefore\text{X}]_{(aq)} + \tfrac{1}{2}\,H_{2(g)} \longrightarrow \text{-S:}\quad\text{:X}]_{(aq)} + H^+_{(aq)} \qquad (10)$$

$$\text{-S}\therefore\text{X}]_{(aq)} + \tfrac{1}{2}\,H_{2(g)} \longrightarrow \text{-S:}\quad\text{:XH}]_{(aq)} \qquad (11)$$

Here the "S" represents the S atom of the Met side chain, and X represents an O or N atom of an amide group, or the $-NH_2$ and $-CO_2^-$ groups of the N and C terminals, respectively. Charges are understood.

The reduction potential derived from eq 10 (or eq 8) does not depend on pH. However, in the case of eq 11, for which the half-reaction is of the form of eq 12

$$S\therefore X + e^- + H^+ \rightarrow S{:}\_XH \qquad (12)$$

the measured reduction potential, $E°'$, will be dependent on pH by eq 13

$$E°' = E° + (RT/F)\ln([H^+] + K_a) \qquad (13)$$

where K_a is the acid ionization constant of S:_XH and the stoichiometric quantities of oxidized and reduced species are equal. At pH = 7, if $K_a < 10^{-7}$, then $E°' = E° - 0.41$ V. The potential, $E°'$, is a more meaningful measure of redox activity in biological systems. In the few cases where eq 11 applies, we will give both the calculated $E°$ and $E°'$ values.

Reduction Potentials of Simple Met Derivatives. As discussed in the Introduction, the redox chemistry of the amino acid, methionine **3**, has been studied under acidic, neutral, and alkaline conditions by cyclic voltammetry. The measured peak anodic potentials, $E*$, cannot be equated directly with $E°$ values but provide a measure of the change expected as a function of pH or substitution pattern. For Met, they are as follows: pH = 2.1, $E*$ = 1.73 V; pH = 7, $E*$ = 1.48 V; pH = 12.2, $E*$ = 1.40 V.[19] The variation of $E*$, and presumably $E°'$, with pH was attributed to the possibility of complex formation between the sulfur radical cation and either the $-NH_2$ or COO(H) group, as verified by a parallel experiment on a Co(III)-coordination complex in which both groups (but not S) were coordinated to the metal and therefore not available for cation radical stabilization. In this case, the peak anodic potential was greater than 1.82 V.

The corresponding three-electron bonded complexes of oxidized **3** are shown in Figure 3. Five distinct modes may be considered for intramolecular coordination of the S radical cation: (1) to the carboxylic acid group via S∴O bonding to the carbonyl oxygen (**3a**); (2) via S∴O bonding to the carboxylate group in its deprotonated state with the amino group protonated (**3b**); (3) to the deprotonated amino group via S∴N bonding with the carboxylate group protonated (**3c**); (4) via S∴O bonding to the carboxylate group in its deprotonated state with the amino group also deprotonated (**3d**); (5) to the deprotonated amino group via S∴N bonding with the carboxylate group also deprotonated (**3e**). B3LYP/6-31G(d)-optimized structures were located for **3a**−**d**. Attempts to optimize structure **3e** were difficult due to a tendency toward spontaneous decarboxylation to $CH_3SCH_2CH_2C{\cdot}HNH_2 + CO_2$. We were, however, unable to obtain the CBS−RAD energy since some of the intermediate steps converged to an oxy-centered radical. In **3a**, the S∴O bond distance is the longest of the present study, r_{SO} = 2.72 Å, and only 9% of the spin is transferred to the oxygen end of the three-electron bond. The S∴O bond distance in **3d**, r_{SO} = 2.47 Å, is 0.04 Å longer than found in the formate complex (Figure 2) discussed above. As in that case, substantial oxidation of the carboxylate group is evident, as only 42% of the spin remains on the S atom.

The relative free energies of **3a**, **3b** + H^+, **3c** + H^+, and **3d** + $2H^+$, derived from the data in Table S1 and given in Table 3, are (in kJ mol^{-1}) 39.5, 45.3, 0.0, and 86.9, respectively. Thus, the most stable structure for oxidized Met in aqueous solution is **3c** (Figure 3), which has an intramolecular S∴N bond. The energy of the other S∴N-bonded species **3e** + H^+ relative to **3c** is estimated to be 17 kJ mol^{-1} based on the assumption that $pK_a \approx 3$ for **3c**, i.e., intermediate to that of $\mathbf{3}^+$ (pK_a = 2.1, eq 4) and HCO_2H (pK_a = 3.74, eq 3). Thus, at pH < 3 the dominant form of the oxidized Met is **3c**, while at pH > 3, structure **3e**

TABLE 3: Energy Changes (kJ mol^{-1}) and Reduction Potentials (V) of Met Derivatives for [$(CH_3)_2S\therefore X^+$](aq) + $^1/_2H_2$(g) → $(CH_3)_2S$(aq) + :X(aq) + H^+(aq) (Reaction 8)[a]

reaction	ΔH(298K)(g)	$-T\Delta S$(g)	ΔG(g)	$\Delta\Delta G_{solv}$	ΔG(aq)	pK_a	$E°$ ($E°'$)
			Methionine Amino Acid **3**[b]				
$\mathbf{3}^+ \rightarrow \mathbf{3}^{(+-)} + H^+$	991.9	−24.9	967.0	−954.1	12.8	2.25	
$\mathbf{3}^{(+-)} \rightarrow \mathbf{3}^- + H^+$	1336.4	−22.3	1314.0	−1259.5	54.6	9.56	
3a → **3b** + H^+	572.2	−17.7	554.5	−546.3	8.3	1.45	
3b → **3c**	73.4	1.6	75.0	−27.7	47.3		
3c → **3d** + H^+	1037.9	−29.3	1008.6	−919.2	89.4	15.66	
			Low pH (pH = 2)				
3a + $^1/_2H_2$ → $\mathbf{3}^+ + H^+$	378.2	−11.4	366.8	−563.1	−196.3		2.03
3b + $^1/_2H_2$ → $\mathbf{3}^+$	−191.5	6.2	−185.3	−16.8	−202.1		2.09 (1.87)
3c + $^1/_2H_2$ → $\mathbf{3}^+$	−118.1	7.8	−110.3	−44.5	−154.8		1.60 (1.50)
			Neutral pH (pH = 7)				
3b + $^1/_2H_2$ → $\mathbf{3}^{(+-)} + H^+$	800.3	−18.6	781.7	−971.0	−189.3		1.96
3c + $^1/_2H_2$ → $\mathbf{3}^{(+-)} + H^+$	873.7	−17.0	856.7	−998.7	−142.0		1.47
3d + $^1/_2H_2$ → $\mathbf{3}^{(+-)}$	−164.2	12.3	−151.9	−79.5	−231.4		2.40 (1.99)
3e + $^1/_2H_2$ → $\mathbf{3}^{(+-)}$							1.66[f](1.25)
			High pH (pH = 12)				
3c + $^1/_2H_2$ → $\mathbf{3}^- + 2H^+$	2210.1	−39.3	2170.7	−2258.2	−87.4		0.91
3d + $^1/_2H_2$ → $\mathbf{3}^- + H^+$	1172.2	−10.0	1162.1	−1336.9	−176.8		1.83
3e + $^1/_2H_2$ → $\mathbf{3}^- + H^+$							1.09[f]
			N-Terminal Met **4**[c]				
$\mathbf{4}^+ \rightarrow \mathbf{4} + H^+$	947.5	−23.7	923.8	−892.1	31.7	5.55	
4a + $^1/_2H_2$ → **4** + H^+	824.0	−17.3	806.8	−922.1	−115.3		1.20
4a + $^1/_2H_2$ → $4^{(+)}$	−123.5	6.5	−117.0	−30.0	−147.0	1.52	(1.11)
			C-Terminal Met **5**[d]				
5a + $^1/_2H_2$ → 5 + H^+	1135.0	−10.5	1124.5	−1316.4	−191.9		1.99
			Midchain Met **6**[e]				
$\mathbf{6O}^{(i-1)} \rightarrow \mathbf{6O}^{(i)}$	7.2	−0.3	6.9	−1.8	5.1		
$\mathbf{6O}^{(i-1)} \rightarrow \mathbf{6N}^{(i)}$	9.3	−0.6	8.6	−6.1	2.5		
$\mathbf{6O}^{(i-1)} \rightarrow \mathbf{6N}^{(i+1)}$	52.4	−4.4	48.0	−30.7	17.3		
$\mathbf{6O}^{(i-1)} + {}^1/_2H_2 \rightarrow 6 + H^+$	784.5	−18.8	765.6	−933.4	−169.8		1.74
$\mathbf{6O}^{(i)} + {}^1/_2H_2 \rightarrow 6 + H^+$	777.3	−18.5	758.8	−931.6	−172.8		1.79
$\mathbf{6N}^{(i)} + {}^1/_2H_2 \rightarrow 6 + H^+$	775.2	−18.2	757.0	−927.3	−170.3		1.76
$\mathbf{6N}^{(i+1)} + {}^1/_2H_2 \rightarrow 6 + H^+$	732.1	−14.4	717.7	−902.7	−185.0		1.92

[a] The standard state for all entropies and free energies is 1 M at 298 K. To convert the tabulated gaseous-phase results to a standard state of 1 atm at 298 K, −7.9 kJ mol^{-1} must be added to $-T\Delta S$(g) and ΔG(g). Free energy changes are derived from the CBS−RAD enthalpies. [b] Refer to Figure 3 for structures. [c] Refer to Figure 4 for structures. [d] Refer to Figure 5 for structures. [e] Refer to Figure 6 for structures. [f] Derived from reduction of **3c** by eq 14, assuming pK_a = 3.

dominates. Structure **3e** is very prone to decarboxylation computationally and probably is the precursor species that is responsible for the experimentally observed rapid decarboxylation of Met upon one-electron oxidation.[21,26]

Low pH. At sufficiently low pH, both the amino and carboxylate groups of the parent Met are protonated, $\mathbf{3}^+$ (Figure 3). However, at pH = 2.1, at which the peak of the anodic oxidation potential was measured, Met exists as a 1:1 mixture of $\mathbf{3}^{(+-)}$ and $\mathbf{3}^+$. As mentioned above, the dominant form of the oxidized Met is the S∴N species, **3c**. The calculated reduction potentials from this form are $E°(\mathbf{3c}+\mathbf{H}^+/\mathbf{3}^+) = 1.60$ V ($E°'(\mathbf{3c}+H^+/\mathbf{3}^+) = 1.50$ V at pH = 2.1) and $E°(\mathbf{3c}/\mathbf{3}^{(+-)}) = 1.47$ V, both of which are somewhat lower than E* observed, 1.70 V.[19]

High pH. At high pH, pH > 10, both the amino and carboxylate groups of the parent Met are deprotonated, $\mathbf{3}^-$. As discussed above, the dominant form of the oxidized Met is the deprotonated S∴N species, **3e**, a structure for which we were unable to obtain the CBS−RAD energy. The reduction potential of **3e**, $E°(\mathbf{3e}/\mathbf{3}^-)$, is related to $E°(\mathbf{3c}/\mathbf{3}^-)$ and the acid ionization constant of **3c**, K_a, by the relation 14

$$E°(\mathbf{3e}/\mathbf{3}^-) = E°(\mathbf{3c}/\mathbf{3}^-) - RT\ln K_a/F \qquad (14)$$

where $E°(\mathbf{3c}/\mathbf{3}^-) = 0.91$ V from Table 3, and we have assumed the value $K_a = 10^{-3}$. Thus, $E°(\mathbf{3e}/\mathbf{3}^-) \approx 1.1$ V, which is lower than E* observed, 1.40 V at pH = 12.2.[19]

Neutral pH. At pH = 7, the parent Met is in its zwitterionic form, $\mathbf{3}^{(+-)}$ (Figure 3). As mentioned above, the dominant form of the oxidized Met is the S∴N species, **3e**. The calculated reduction potential of this form, $E°(\mathbf{3e}/\mathbf{3}^{(+-)})$, may be derived from $E°(\mathbf{3c}/\mathbf{3}^{(+-)})$ in a manner analogous to eq 12. From Table 3, $E°(\mathbf{3c}/\mathbf{3}^{(+-)}) = 1.47$ V, from which one obtains $E°(\mathbf{3e}+H^+/\mathbf{3}^{(+-)}) \approx 1.66$ V and $E°'(\mathbf{3e}+H^+/\mathbf{3}^{(+-)}) \approx 1.25$ V. The $E°'$ value is somewhat lower than E* observed, 1.48 V[19] as expected.

Reduction Potentials of N- and C-Terminal Met Residues. N-Terminal Methionine. An N-terminal Met residue is modeled by the *N*-methylamide of methionine in its deprotonated form, **4**, and in its protonated form, $\mathbf{4}^+$ (Figure 4). The acid ionization of $\mathbf{4}^+ \rightarrow \mathbf{4} + H^+$ is calculated as pK_a = 5.55 (Table 3). Interestingly, without the empirical correction for the $-NH_3^+$ group mentioned above, one finds pK_a = 9.49, i.e., in the range expected for a free amino group. This suggests that the empirical correction may not be warranted in this case.

The structure of its intramolecularly coordinated radical cation **4a** has a short S∴N separation, r_{SN} = 2.55 Å. Coordination of the N to the *pro-S* face of the S atom is shown in Figure 4. The structure of the diastereomeric form with coordination to the *pro-R* face was not located. It is assumed to have similar energy to **4a** and its influence on the free energy of **4a** is estimated as an entropy of mixing (Rln 2). Structures analogous to **4a** were proposed as intermediates in the formation of azasulfonium salts through the reaction of photooxidized *N*-methionyl peptides with oxygen or superoxide.[53]

Table 3 collects the data associated with the calculation of the reduction potentials, $E°(\mathbf{4a}/\mathbf{4}) = 1.20$ V and $E°(\mathbf{4a}+H^+/\mathbf{4}^+) = 1.52$ V ($E°'(\mathbf{4a}+H^+/\mathbf{4}^+) = 1.11$ V at pH = 7). In the

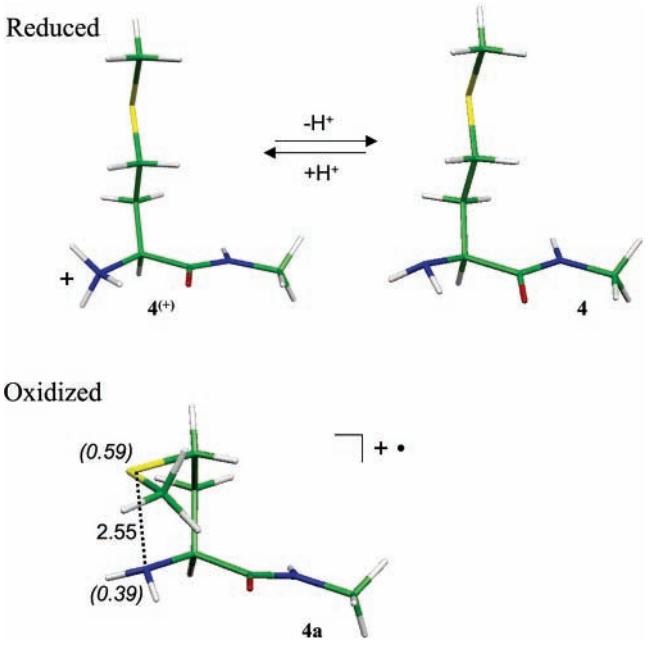

Figure 4. Models for the reduction of N-terminal Met.

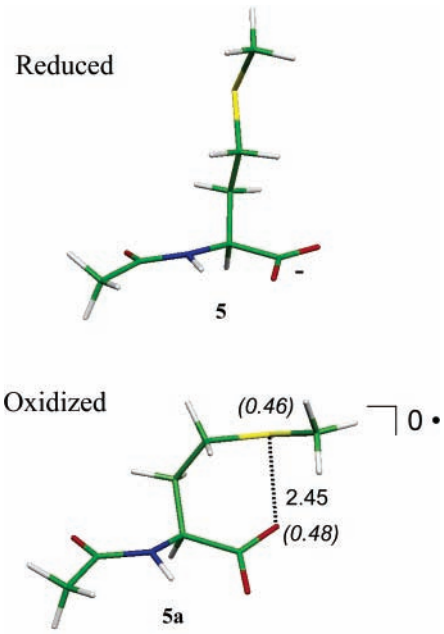

Figure 5. Models for the reduction of C-terminal Met.

case of $E°$(**4a**/**4**), a single factor is mainly responsible for the low value namely the large difference in solvation of the radical cation compared to the neutral product. The difference is substantially decreased in the case of $E°$(**4a**+H^+/**4**$^+$), and even more so if one uses the "uncorrected" ΔG_{solv} value, in which case $E°$(**4a**+H^+/**4**$^+$) = 1.74 V and $E°'$(**4a**+H^+/**4**$^+$) = 1.33 V. Both the corrected and uncorrected $E°'$ values fall within the error bars of the experimental value determined for MetGly which differs from **4**$^+$ only by the presence of a carboxylate group, $E°'$(MetGly$^{\bullet+}$+H^+/MetGly) = 1.42 ± 0.3 V.[26]

C-Terminal Methionine. A C-terminal Met residue is modeled by *N*-acetylmethionine anion, **5** (Figure 5). The structure of one of its intramolecularly coordinated oxidized forms, **5a**, is also shown in Figure 5. The diastereomeric form, involving the *pro-R* face of the S atom was not located. It is assumed to have similar energy and, as above, its presence is accommodated through a contribution to the entropy of mixing of the *pro-S* form. Table 3 collects the data associated with the calculation of the reduction potential, $E°$(**5a**/**5**) = 1.99 V. A value, $E°$(**5a**/**5**) = 1.75 V, is obtained if the empirical correction for the carboxylate group of **5** is not included. The high value of the reduction potential is remarkable but not unexpected based on the model data presented in Tables 1 and 2. The high neurotoxicity of the 11-residue fragment, GSNKGAIIGLM (Aβ-(25−35)), has been attributed to the ready oxidation of the C-terminal Met due to the CO_2^--group stabilization of the oxidized form (although the nature of the oxidizing species was not identified).[12] The present results suggest that this unlikely to be the explanation. However, as the extensive amount of spin delocalization (Figure 5) suggests, **5a** is also prone to irreversible decarboxylation, and this factor may be responsible for the apparent ease of oxidation.

It is evident from the calculations that the intrinsic stabilization due to the S∴O bond formation (see data in Table 1) is more than balanced by lower solvation of the neutral oxidized species, **5a** (ΔG_{solv} = −30 kJ mol^{-1}), compared to the reduced parent, **5** (ΔG_{solv} = −239 kJ mol^{-1}), with the result that the reduction potential is actually raised. In reality, as in the case of the carboxylate complex, **2e** (Figure 2 and Tables 1 and 2), the intramolecularly bonded three-electron species **5a** would not be stable in solution relative to a ring-opened form, and the observed reduction potential would be that of the solvent-separated Met S radical cation, namely $E° \approx 1.6$ V.

Reduction Potentials of Midchain Met Residues. A Met residue in the middle of a polypeptide chain is modeled by *N*-acetylmethioninamide **6** (Figure 6). The side chain is long enough to permit the S radical cation to make intramolecular three-electron bonds to the amide group on either side. Coordination may be to either the *pro-R* or *pro-S* face of the −CH_2−S−CH_3 plane, and each amide group may be either a σ donor through the carbonyl O atom, or a π donor, principally through the amide N atom. We find that oxygen-coordinated structures are similar in stability to the amide N-coordinated ones. Examples of the B3LYP-optimized structures of all four coordination motifs of the radical cation are shown in Figure 6.

The most stable structure, **6O**$^{(i-1)}$, has a three-electron bond between the S and the carbonyl oxygen atom of the preceding (i−1) residue, forming a seven-membered ring. The S∴O distance, r_{SO} = 2.47 Å, is only slightly longer than found for the amide complexes discussed above (**2c**, **2d** in Figure 2). Extensive spin delocalization is evident with 67% of the spin on S and 31% on O. The other S∴O structure, **6O**$^{(i)}$, is 5 kJ mol^{-1} higher in energy than **6O**$^{(i-1)}$. The six-membered ring of **6O**$^{(i)}$ permits a shorter S∴O separation, r_{SO} = 2.40 Å. The principal difference between the structure of **6O**$^{(i)}$ and all of the others shown in Figure 6, is that the backbone has folded away from the extended structure (with an internal H bond between the =O and H−N of the same residue) into one with an H bond between the =O and H−N of the i−1 and i+1 residues, respectively. Structures analogous to **6O**$^{(i-1)}$ and **6O**$^{(i)}$ have been discussed previously by Pogocki, Serdiuk, and Schöneich[28] who found the six-membered ring structure to be more stable than the seven-membered-ring structure, contrary to the present results. However, their structures were diastereomeric to, and less stable than, both of **6O**$^{(i)}$ and **6O**$^{(i-1)}$.

One of the S∴N coordinated structures, **6N**$^{(i)}$, is only 2.5 kJ mol^{-1} less stable than **6O**$^{(i-1)}$. The other, **6N**$^{(i+1)}$, is the least stable of the four, 17.3 kJ mol^{-1} higher than **6O**$^{(i-1)}$. The amide-incorporated N atom is not as good an electron pair donor as that of a free amino group. In the more stable form, the S∴N

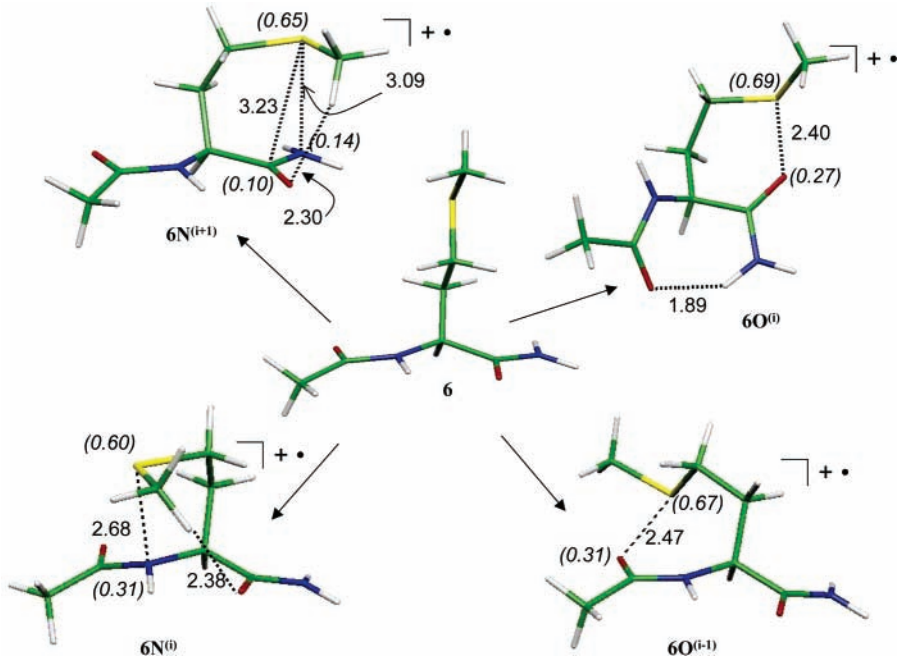

Figure 6. Models for the reduction of midchain Met.

distance, $r_{SN} = 2.68$ Å, is somewhat longer than $r_{SN} = 2.61$ Å in **2h** and $r_{SN} = 2.55$ Å in **4a**. *N*-deprotonated forms of $\mathbf{6N}^{(i)}$ and $\mathbf{6N}^{(i+1)}$ have been observed through time-resolved conductivity measurements.[23] Such structures and the mechanism of their formation are beyond the scope of the present investigation.

The predicted reduction potentials are as follows: $E°(\mathbf{6O}^{(i-1)}/\mathbf{6}) = 1.74$ V; $E°(\mathbf{6O}^{(i)}/\mathbf{6}) = 1.79$ V; $E°(\mathbf{6N}^{(i)}/\mathbf{6}) = 1.76$ V; $E°(\mathbf{6N}^{(i+1)}/\mathbf{6}) = 1.92$ V. Thus, in an aqueous environment, no reduction in the $E°$ of Met is expected by its incorporation into a protein strand if the only electron pair donors available for S∴O or S∴N bond formation were nearby amide groups. This conclusion is expected to extend to the case discussed by Pogocki and Schöneich in which stabilization of the radical cation by the carbonyl of the Ile31 residue of helical Aβ was deemed responsible for the neurotoxicity of Aβ.[24]

Conclusions

Three-electron bonding between sulfide radical cation **2** and various biologically available oxygen and nitrogen electron-pair donors was studied in aqueous solution with a view to quantifying its effect on the reduction potential of dialkyl sulfides and methionine. The gaseous-phase S∴X bond dissociation enthalpy (BDE) between dimethyl sulfide radical cation and water is weak, 46 kJ mol^{-1}, less than half of the value for amines, 104 kJ mol^{-1} (:X = CH_3NH_2), or amide carbonyl, 109 kJ mol^{-1} (:X = $OCHNHCH_3$). The gaseous-phase BDE of cation−anion complexes is much higher (:X, kJ mol^{-1}): HCO_2^-, 559; HCO_3^-, 548; $H_2PO_4^-$, 523. With the exception of the amine complex, and possibly the water and dihydrogen phosphate complexes, the three-electron bond is predicted to dissociate spontaneously in water. In every case, the BDE is higher than the free energy of solvation of the dissociated products but the difference is small and overwhelmed by the entropy-derived part of the free energy of dissociation. For oxidized dimethyl sulfide **2**, the standard reduction potential is accurately predicted by the CPCM continuum model employed: $E°$(calc) = 1.60 V; $E°$(exptl) = 1.66 V. We estimate that the uncertainty in $E°$(calc) in the present study is ±0.1 V.

In a conformationally constrained environment, where the entropic driving force for dissociation is less, the S∴X bond is predicted to be stable in water in the model systems. $E°$ values as low as 0.9 V may arise as a result of stabilization by amines (e.g., the side chain of Lys) or phosphodiesters (nucleic acids, phospholipid bilayers).

The pH dependence of the electrochemistry of methionine in water is well reproduced: pH = 2, $E°(\mathbf{3c}+H^+/\mathbf{3}^+) = 1.63$ V, $E°'(\mathbf{3c}+H^+/\mathbf{3}^+) = 1.51$ V; pH = 7, $E°(\mathbf{3e}+H^+/\mathbf{3}^{(+-)}) = 1.64$ V, $E°'(\mathbf{3e}+H^+/\mathbf{3}^{(+-)}) = 1.23$ V; pH = 12, $E°(\mathbf{3e}/\mathbf{3}^-) = 1.12$ V. At neutral and high pH, the stable form of the oxidized Met, **3e**, has an intramolecular S∴N bond and a deprotonated carboxylic acid group.

The product radical cation **4a** of one-electron-oxidized N-terminal Met **4** is predicted to be strongly stabilized by an intramolecular S∴N bond and to have a low reduction potential, $E°(\mathbf{4a}+H^+/\mathbf{4}^+) = 1.54$ V, $E°'(\mathbf{4a}+H^+/\mathbf{4}^+) = 1.13$ V (1.35 V uncorrected) at pH = 7. Similarly, a one-electron-oxidized C-terminal Met forms a strong intramolecular S∴O bond with the free carboxylate group. However, the neutral complex **5a** is predicted not to be stable in water. Thus, in an aqueous environment, no stabilization of the oxidized sulfur by the carboxylate group is expected. If the gaseous-phase geometry of **5a** is maintained in solution, $E°(\mathbf{5a}/\mathbf{5}) = 1.99$ V. In the case of a midchain Met **6**, the most stable oxidized isomer in the present study, $\mathbf{6O}^{(i-1)}$, has an intramolecular S∴O bond with the carbonyl group of the $i - 1$ residue in the gaseous phase. However, the predicted high reduction potential, $E°(\mathbf{6O}^{(i-1)}/\mathbf{6}) = 1.76$ V, suggests that the cyclic structure is unlikely to be stable in solution. The possible involvement of experimentally observed S∴N species involving a deprotonated backbone amide[23] are not part of the present study but are under investigation.

In summary, we find no three-electron bond assistance for the oxidation of a C-terminal or midchain Met residue. Likewise, neither the carboxylate groups of Glu or Asp side chains, nor the amide groups of Gln or Asn side chains, will facilitate oxidation of the S atom in an aqueous environment. However, accessibility to an amino group, either intramolecularly as in an N-terminal Met, or intermolecularly by, e.g., a lysine side chain, can result in substantial lowering of the reduction potential. Similarly, proximity of a phosphodiester group as in

a protein-nucleic acid complex, or a phospholipid membrane-embedded protein, may facilitate the oxidation of the Met.

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Supporting Information Available: Tables of computed data for all individual compounds discussed in the text and Gaussian 98 archive entries for all species discussed in the paper. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Vogt, W. *Free Radical Biol. Med.* **1995**, *18*, 93−105.
(2) Levine, R. L.; Mosoni, L.; Berlett, B. S.; Stadtman, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15036−15040.
(3) Chao, C. C.; Ma, Y. S.; Stadtman, E. R. *Proc. Nat. Acad. Sci. U.S.A.* **1997**, *94*, 2969−2974.
(4) Moskovitz, J.; Berlett, B. S.; Poston, J. M.; Stadtman, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9585−9589.
(5) Moskovitz, J.; Flescher, E.; Berlett, B. S.; Azare, J.; Poston, J. M.; Stadtman, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14071−14075.
(6) Hoshi, T.; Heinemann, S. H. *J. Physiol.* **2001**, *531.1*, 1−11.
(7) Stadtman, E. R.; Moskovitz, J.; Berlett, B. S.; Levine, R. L. *Mol. Cell. Biochem.* **2002**, *234/235*, 3−9.
(8) Butterfield, A. D. *Chem. Res. Toxicol.* **1997**, *10*, 495−506.
(9) Sayre, L. M.; Zagorski, M. G.; Surewicz, W. K.; Krafft, G. A.; Perry, G. *Chem. Res. Toxicol.* **1997**, *10*, 518−526.
(10) Butterfield, D. A.; Kanski, J. *Peptides* **2002**, *23*, 1299−1309.
(11) Schöneich, C. *Arch. Biochem. Biophys.* **2002**, *397*, 370−376.
(12) Varadarajan, S.; Kanski, J.; Aksenova, M.; Lauderback, C.; Butterfield, D. A. *J. Am. Chem. Soc.* **2001**, *123*, 5625−5631.
(13) Comment on: Manelli, A. M.; Puttfarcken, P. S. *Brain Res. Bull.* **1995**, *38*, 569−576.
(14) Curtain, C. C.; Ali, F.; Volitakis, I.; Cherny, R. A.; Norton, R. S.; Beyreuther, K.; Barrow, C. J.; Masters, C. L.; Bush, A. I.; Barnham, K. J. *J. Biol. Chem.* **2001**, *276*, 20466−20473.
(15) 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.
(16) Huang, X.; Cuajungco, M. P.; Atwood, C. S.; Hartshorn, M. A.; Tyndall, J. D. A.; 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.
(17) Handbook of Chemistry and Physics, 58th ed.; Weast, R. C.; Ed.; CRC Press: Boca Raton, FL, 1977−1978.
(18) Olsson, M. H. M.; Ryde, U. *J. Biol. Inorg. Chem.* **1999**, *4*, 654−663.
(19) Sanaullah, G. S. W.; Glass, R. S. *J. Inorg. Biochem.* **1994**, *55*, 87−99.
(20) Armstrong, D. A. In S−Centered Radicals; Alfassi, Z. B., Ed.; John Wiley & Sons: New York, 1999; pp 27−61.
(21) Hiller, K.-O.; Masloch, B.; Göbl, M,; Asmus, K.-D. *J. Am. Chem. Soc.* **1981**, *103*, 2734−2743.
(22) Bobrowski, K.; Hug, G. L.; Marciniak, B.; Miller, B.; Schöneich, C. *J. Am. Chem. Soc.* **1997**, *119*, 8000−8011.
(23) Schöneich, C.; Pogocki, D.; Hug, G. L.; Bobrowski, K, *J. Am. Chem. Soc.* **2003**, *125*, 13700−13713.
(24) Pogocki, D.; Schöneich, C. *Chem. Res. Toxicol.* **2002**, *15*, 408−418.
(25) Rauk, A.; Armstrong, D. A.; Fairlie, D. P. *J. Am. Chem. Soc.* **2000**, *122*, 9761−9767.
(26) Prütz, W, A,; Butler, J.; Land, E. J.; Swallow, A. J. *Int. J. Radiat. Biol.* **1989**, *55*, 539−556. The reduction potential is identified as $E°$, but it is pH-dependent and should be labeled $E^{\circ\prime}$ in the context of the present work.
(27) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11; Gaussian, Inc.: Pittsburgh, PA, 1998.
(28) Pogocki, D.; Serdiuk, K.; Schöneich, C. *J. Phys. Chem. A* **2003**, *107*, 7032−7042.
(29) Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502−16513.
(30) Guthrie, J. P. *J. Phys. Chem. A* **2001**, *105*, 8495−8499.
(31) In the case of Met derivatives, the number of low-lying conformers was estimated on the basis of molecular mechanics calculations using the MMFF force field as implemented in SPARTAN: Wavefunction, Inc.: Irvine, CA; www.wavefun.com.
(32) Mayer, P. M.; Parkinson, C. J.; Smith, D. M.; Radom, L. *J. Chem. Phys.* **1998**, *108*, 604−615.
(33) (a) Fischer, H.; Radom, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 1340−1371. (b) Wong, M. W.; Radom. L. *J. Phys. Chem.* **1995**, *99*, 8582−8588. (c) Wong, M. W.; Radom, L. *J. Phys. Chem. A*, **1998**, *102*, 2237−2245.
(34) Rauk, A.; Boyd, R. J.; Boyd, S. L.; Henry, D. J.; Radom, L. *Can. J. Chem.* **2003**, *81*, 1−12.
(35) Klamt, A.; Schurmann, G. *J. Chem. Soc., Perkin Trans.* **1993**, *2*, 799−805.
(36) Andzelm, J.; Kolmel, C.; Klamt, A. *J. Chem. Phys.* **1995**, *103*, 9312−9320.
(37) Zhan, C.; Bentley, J.; Chipman, D. M. *J. Chem. Phys.* **1998**, *108*, 177−192.
(38) Leung, B. O.; Reid, D. L.; Armstrong, D. A.; Rauk, A. *J. Chem. Phys. A* **2004**, *108*, 2720−2725.
(39) Calculated as the difference between $\Delta_f G(g)(H_2O)$ and $\Delta_f G(l)(H_2O)$, both corrected to a standard state of 1 M.; ΔG_{solv} of H_2O. The CPCM procedure adopted here yields a very similar value, $\Delta G_{solv}(H_2O) = -28.1$ kJ mol$^{-1.}$
(40) Liptak, M. D.; Gross, K. C.; Seybold, P. G.; Feldgus, S.; Shields, G. C. *J. Am. Chem. Soc.* **2002**, *124*, 6421−6427. See discussion and references therein.
(41) Rauk, A. *Orbital Interaction Theory of Organic Chemistry*, 2nd ed.; Wiley-Interscience: New York, 2001; pp 44−49.
(42) Glass, R. S.; Petsom, A.; Hojjatie, M.; Coleman, B. R.; Duchek, J. R.; Klug, J.; Wilson, G. S. *J. Am. Chem. Soc.* **1988**, *110*, 4772−4778.
(43) Braïda, B.; Hiberty, P. C.; Savin, A. *J. Phys. Chem. A* **1998**, *102*, 7872−7877.
(44) Braïda, B.; Hiberty, P. C. *J. Phys. Chem. A* **2000**, *104*, 4618−4628.
(45) Braïda, B.; Lauvergnat, D.; Hiberty, P. C. *J. Chem. Phys.* **2001**, *115*, 90−102.
(46) Braïda, B.; Hazebroucq, S.; Hiberty, P. C. *J. Am. Chem. Soc.* **2002**, *124*, 2371−2378.
(47) Braïda, B.; Thogersen, L.; Wu. W.; Hiberty, P. C. *J. Am. Chem. Soc.* **2002**, *124*, 11781−11790.
(48) Gill, P. M. W.; Radom, L. *J. Am. Chem. Soc.* **1988**, *110*, 4931−4941.
(49) Aagaard, O. M.; de Waal, B. F. M.; Cabbolet, M. J. T. F.; Janssen, R. A. J. *J. Phys. (Paris), Chem.* **1992**, *96*, 614−623.
(50) Deng, Y.; Illies, A. J.; James, M. A.; McKee, M. L.; Peschke, M. *J. Am. Chem. Soc.* **1995**, *117*, 420−428.
(51) Carmichael, I. *Nukleonika* **2000**, *45*, 11−17.
(52) Fourré, I.; Bergès, J. *J. Phys. Chem. A* **2004**, *108*, 898−906.
(53) Miller, B. L.; Kuczera, K.; Schöneich, C. *J. Am. Chem. Soc.* **1998**, *120*, 3345−3356.