Is Oxidative Damage by β-Amyloid and Prion Peptides Mediated by Hydrogen Atom Transfer from Glycine α-Carbon to Methionine Sulfur within β-Sheets?

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Abstract: Methionine in glycine-rich regions of both β-amyloid peptide and prion peptide is thought to be crucial to their neurotoxic properties. We postulate here a role for methionine in the propagation of oxidative damage. The S−H bond dissociation enthalpies, BDE(S−H)s, of dimethylsulfonium ion $(CH_3)_2SH^+$ and a S-protonated methionine residue of a polypeptide strand are estimated to be 351 and 326−331 kJ mol^{-1}, respectively, by the application of calculations at the B3LYP level with large basis sets. These species are direct products of H atom abstraction by radical cations of sulfides. The reactions between a glycine residue and the radical cations of $(CH_3)_2S$ and Met were investigated and the transition structures for H atom transfer located. The results suggest that it is thermodynamically feasible for the S-ionized form of Met to cause oxidative damage at the $^{\alpha}$C−H site of almost any amino acid residue of a nearby polypeptide strand (BDE($^{\alpha}$C−H) = 330−360 kJ mol^{-1}) or to nearby lipids with a bis(allylic) methylene group (BDE(C−H) = 335 kJ mol^{-1}). However, a key observation is that, when the Met residue is incorporated into an antiparallel β-sheet, only a Gly residue is exposed and susceptible to oxidation at the $^{\alpha}$C−H site. Furthermore, the Gly must lie on a strand of the β-sheet different from that containing Met and must be part of a (5,5) rather than a (3,3) cycle. The same considerations apply to the methyl-deprotonated form of the sulfide radical cation but not the methylene-deprotonated form. These findings suggest a possible mechanism for generating and propagating oxidative damage via a Met residue of the Aβ peptide of Alzheimer's disease and of the prion peptide of Creutzfeldt−Jakob disease. To our knowledge, this is the first proposed mechanism that accounts for the radical damage in either of these diseases and requires peptide β-sheets and amino acids, methionine and glycine.

Introduction

Protein-based radicals on the peptide backbone[1,2] ($^{\alpha}$C-centered radicals) or other types of radicals in the side chains[3−5] of amino acid residues have been of considerable interest in recent years. These may be created by the actions of reactive oxygen species (ROS) produced by toxic chemicals or radiation, and by the detoxification of extraneous chemicals in the liver.[2,6,7] ROS are also byproducts of the normal respiratory chain[7,8] and include mainly superoxide ($O_2^{\bullet -}$), peroxyl (ROO$^{\bullet}$), and hydroxyl ($^{\bullet}$OH) radicals and hydrogen peroxide (H_2O_2). ROS react with cellular membranes, nucleic acids, and proteins and have far-reaching effects on the living cell.[6,8] Indeed, ROS from the side reactions of normal metabolism may be the ultimate cause of aging,[9,10] and ROS damage has been implicated in apoptosis in both animals[11,12] and plants.[13] In a series of studies of the structure and reactivity of $^{\alpha}$C-centered radicals,[14−16] we have demonstrated theoretically the bond dissociation capacity of the $^{\alpha}$C−H bond in all amino acid residues and the possibility that damage to this site may be caused by thiyl radicals (of cysteinyl residues and glutathione).[15] The role of secondary structure in conferring protection to the $^{\alpha}$C-site has also been clarified to some extent. The theoretical studies show that α-helical and β-sheet structures effectively protect the $^{\alpha}$C-site of all residues from damage by ROS or thiyl radicals, with the sole exception of glycyl residues in antiparallel β-sheets.[17]

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Reports that a relatively small peptide, amyloid β-peptide (Aβ), and some fragments of it[18] were capable of generating hydrogen peroxide[19] seemingly without the presence of external agents other than oxygen were of considerable interest to us in the light of our theoretical results. Amyloid β-peptide is the major component of extracellular water-insoluble plaques found in the brains of patients with Alzheimer's disease,[20] and there is now strong evidence for a pathogenic role of Aβ in Alzheimer's disease.[21] The Aβ sequences 1−42, 1−40, 25−35, and 31−35 have all been shown to be neurotoxic.[22] However, the chemical mechanism by which neurotoxicity originates is unknown.

The generation of free radicals by Aβ peptides is somewhat controversial.[23] Early ESR experiments that suggested the generation of radicals by Aβ alone in the presence of aqueous buffers[24] appear to have been compromised by degradation of radical trapping agents.[25,26] On the other hand, there is mounting evidence that metal ions, notably Cu^{2+} and Fe^{3+}, are reduced by Aβ peptides and do generate both peptide radicals and hydrogen peroxide.[27] This evidence comes from the identification of Cu(I) using titration experiments, from the disappearance of the EPR signal for Cu(II) in the presence of Aβ, and from measurements of peroxide evolution in solutions of Aβ1−42 incubated with Cu^{2+} or Fe^{3+}.[26,27] Thus, it would appear that Aβ1−42 reduces Cu^{2+} to Cu^{+} and is presumably oxidized to its radical cation, A$\beta^{+\bullet}$.

During aggregation of Aβ into antiparallel β-sheets, oxygen-dependent generation of free radicals takes place, leading to cross-linking and fibril formation. Contact of the fibrils with cultured neurons leads to radical damage in the membrane and inside the cell.[28,29] Met35 appears to be the key residue required for aggregation, neurotoxicity, and radical generation (which may be responsible for the neurotoxicity).[30,31]

The clear implication is that an oxidized form of Met35 may be involved. We investigate here the possible role of the S-oxidized radical cation of methionine, Met35($S^{+\bullet}$). Such species tend to be very short-lived.[32] Reversible complex formation takes place through three-electron, two-center bonding to water, sulfides, or other Lewis bases. However, under the special circumstances of β-sheet aggregated Aβ, given that Met35 resides in a hydrophobic environment, its oxidized form may be sufficiently long-lived that it can do other chemistry. Such a species may cause oxidative damage directly by electron transfer (reaction 1),

$$[\text{donor}] + \text{Met}(S^{+\bullet}) \rightarrow [\text{donor}]^{+\bullet} + \text{Met}(S) \qquad (1)$$

or hydrogen atom transfer (reaction 2),

$$\text{Met}(S^{\bullet+}) + RH \rightarrow \text{Met}(SH^{+}) + R^{\bullet} \qquad (2)$$

or indirectly, by deprotonation at either carbon next to the sulfur (e.g., reaction 3),

$$\text{Met}(S^{\bullet+}CH_3) + B: \rightarrow \text{Met}(SCH_2^{\bullet}) + BH^{+} \qquad (3)$$

thus generating a reactive C-centered side-chain radical which is itself a strong oxidant by H atom abstraction (reaction 4),

$$\text{Met}(SCH_2^{\bullet}) + RH \rightarrow \text{Met}(SCH_3) + R^{\bullet} \qquad (4)$$

We consider these possibilities separately.

Concerning reaction 1, Aβ itself has only one suitable electron donor, Tyr10, in the hydrophilic region, but this is not present in 25−35 or 31−35, which are also neurotoxic. In addition, to the extent that water may be present, electron transfer is hindered by a substantial free energy barrier since it requires a reorganization of the solvent shell around both the donor and the acceptor.[33] On the other hand, transfer of a neutral H atom to the S atom (reaction) leaves it positively charged, and solvent reorganization is minimized. We therefore do not consider here the possibility of electron transfer but rather focus on hydrogen atom and proton transfer (reactions 2−4). Concerning the formation of the Met($SCH_2^{\bullet}$) radical in reaction 3, the cation radical of a dialkyl sufide is known to be a strong protic acid by proton loss from a carbon α to the oxidized sulfur:[34] for $(CH_3)_2S^{\bullet+}$, $pK_a \cong -2$ has been estimated,[32] and this process is expected to be fast.

The bond dissociation energies (BDEs) of the C−H bonds in CH_3SH, 387 kJ mol^{-1},[35] and CH_3SCH_3, 392 kJ mol^{-1},[36] are sufficiently high that H atom abstraction (reaction 4) from tertiary carbon atoms of Leu, Ile, and Val side chains (BDE = 400 kJ mol^{-1}, based on isobutane),[16] from the $^{\alpha}$C-center of any residue not incorporated in secondary structure (BDE = 330−370 kJ mol^{-1}),[16] or from susceptible sites in lipids (see below) would be an exergonic or nearly thermoneutral process. The feasibility of reaction 4 will then depend on whether the C-centered radical can get close enough to a susceptible C−H bond for the reaction to take place in the local environment in which the radical is formed.

In this paper, we examine the thermodynamic and kinetic feasibility that the methionyl radical cation, Met($S^{\bullet+}$), or one of its deprotonated forms, Met($CH_2SCH_2^{\bullet}$) or Met($CH^{\bullet}SCH_3$), may abstract a H atom from a residue of Aβ or other protein,

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PH, (reactions 5 and 4, respectively),

$$\mathrm{Met(S^{\bullet+})} + \mathbf{PH} \rightarrow \mathrm{Met(SH^{+})} + \mathbf{P}^{\bullet} \quad (5)$$

or from the lipid bilayer (reaction 6)

$$\mathrm{Met(S^{\bullet+})} + \mathbf{LH} \rightarrow \mathrm{Met(SH^{+})} + \mathbf{L}^{\bullet} \quad (6)$$

Lipids which possess the bis(allylic) methylene group, RCH=CHCH$_2$CH=CHR, are particularly susceptible to oxidative damage because of the weak C−H bonds of the central CH$_2$ group, BDE(C−H) = 335 kJ mol^{-1}.[35] Thus, **L**$^{\bullet}$ is probably [RCH=CH−C$^{\bullet}$H−CH=CHR]. The nature of the residue-derived radical, **P**$^{\bullet}$, is identified tentatively as $^{\alpha}$C-centered since the $^{\alpha}$C−H bonds have been shown to be exceptionally weak, in the range 330−370 kJ mol^{-1}, depending upon the nature of the residue and the local H-bonding environment.[14−16] Experimental values exist for the strength of the S−H bonds (in kJ mol^{-1}) of H_3S^+ (397), $CH_3SH_2^+$ (368), and $(CH_3)_2SH^+$ (351).[37] The last is expected to be a reasonable model for protonated methionine, Met(SH$^+$). Thus, on the basis of BDEs, one may conclude that a methioninyl radical cation, Met(S$^{\bullet+}$), is thermodynamically capable of generating an $^{\alpha}$C-centered radical by H abstraction. In the present work, we redetermine the SH BDEs theoretically in order to assess computational procedures and then calculate activation enthalpies for the transfer of a H atom between S and the $^{\alpha}$C-site of two peptide model reactions intended to mimic methionine involvement with glycine residues in random coil and β-sheet environments. We also determine the activation energy for H transfer from the $^{\alpha}$C-site of a glycine residue in a β-sheet environment to the methyl- and methylene-deprotonated forms, Met(CH$_2$SCH$_2^{\bullet}$) and Met(CH$^{\bullet}$SCH$_3$), respectively. While such reactions are expected to be exergonic by about 30 kJ mol^{-1}, the activation barriers within the β-sheet framework are unknown.

Theoretical Methods

Most calculations were carried out at the B3LYP level of theory using the 6-31G(d) basis set as implemented in the Gaussian 94 and 98 suites of programs.[38] Geometry optimizations were carried out to the point that successive steps produced no more than a 10^{-6} hartree (0.03 kJ mol^{-1}) change in the energy. Harmonic vibrational frequency analysis at the B3LYP/6-31G(d) level was performed on the structures optimized with no constraints for the purpose of estimating the zero-point vibrational energy (ZPVE).

To reduce computational errors for bond dissociation reactions, the basis set was expanded to 6-311+G(3df,2p), and single-point calculations were carried out on all species. This level of theory is referred to as B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d). The CBS-RAD[39] and G2(MP2-B3LYP) procedures were applied to species derived from H_3S^+, $CH_3SH_2^+$, and $(CH_3)_2SH^+$ at the B3LYP/6-31G(d) geometry. The CBS-RAD procedure has been developed to yield accurate thermochemistry for radicals.[39] The G2(MP2-B3LYP) procedure is a variation of the G2(MP2) theory,[40] in which B3LYP/6-31G(d) geometries and ZPVEs (scaled by 0.98) are substituted for MP2/6-31G(d) geometries and HF/6-31G(d) ZPVEs.

The S−H BDE for the sulfonium ion is defined as the heat of reaction 7, $\Delta H_{(7)}^{\circ}$.

$$\mathrm{R^1R^2SH^+} \rightarrow \mathrm{R^1R^2S^{\bullet+}} + \mathrm{H^{\bullet}} \quad (7)$$

where R^1 and R^2 are either CH$_3$ or H. Comparison of experimental, CBS-RAD, and G2(MP2-B3LYP) values for $\Delta H_{(7)}^{\circ}$ should yield an accurate value for the S−H BDE of $(CH_3)_2SH^+$, which may then be used in an isodesmic reaction (eq 8) to improve the value of this quantity in the larger complexes for which lower level calculations must be done.

$$\mathrm{R^1R^2SH^+} + \mathrm{(CH_3)_2S^{\bullet+}} \rightarrow \mathrm{R^1R^2S^{\bullet+}} + \mathrm{(CH_3)_2SH^+} \quad (8)$$

The S−H BDE of an unknown sulfonium ion is related to the heat of reaction 8, $\Delta H_{(8)}^{\circ}$, by eq 9.

$$\mathrm{BDE(R^1R^2SH^+)} = \mathrm{BDE((CH_3)_2SH^+)} + \Delta H_{(8)}^{\circ} \quad (9)$$

Transition structures were located for the transfer of an $^{\alpha}$C−H hydrogen atom in reaction 10,

$$\mathbf{PH}(\mathrm{Res}) + \mathrm{R^1R^2S^{\bullet+}} \rightarrow {}^{\alpha}\mathbf{P}^{\bullet}(\mathrm{Res}) + \mathrm{R^1R^2SH^+} \quad (10)$$

from *N*-formylglycine amide, **PH**(Gly), to the S atom of the radical cations of dimethyl sulfide and *N*-formylmethionine amide, **PH**(Met(S$^{\bullet+}$)), at the B3LYP/6-31G(d) level of theory. Transitions structures were also located for reactions 11 and 12,

$$\mathbf{PH}(\mathrm{Gly})\cdots\mathbf{PH}(\mathrm{Met(CH_2SCH_2^{\bullet})}) \rightarrow {}^{\alpha}\mathbf{P}^{\bullet}(\mathrm{Gly})\cdots\mathbf{PH}(\mathrm{Met}) \quad (11)$$

$$\mathbf{PH}(\mathrm{Gly})\cdots\mathbf{PH}(\mathrm{Met(CH^{\bullet}SCH_3)}) \rightarrow {}^{\alpha}\mathbf{P}^{\bullet}(\mathrm{Gly})\cdots\mathbf{PH}(\mathrm{Met}) \quad (12)$$

involving the deprotonated forms of **PH**(Met(S$^{\bullet+}$)). Here, ··· indicates that the two residues are complexed by H-bonding, as discussed below. The device of isodesmic reactions cannot be used to reduce errors in the predicted activation parameters. However, at the B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d) level of theory, the $^{\alpha}$C−H and S−H BDEs are predicted to be too low by nearly the same amount, 16 and 15 kJ mol^{-1} (see below), respectively. In the case of C−H adjacent to divalent sulfur, the error is similar (18 kJ mol^{-1} for CH_3SCH_3). It is therefore likely that the errors in the TS energies will also be similar and that the calculated enthalpies of activation, a relative energy, will be fairly accurate.

Results and Discussion

The feasibility of carrying out reaction 10 hinges on the relative bond strengths of the $^{\alpha}$C−H bonds of amino acid residues and the strength of the S−H bond in dialkylsulfonium ions. In order for this reaction to form part of a viable mechanism of C-centered radical formation, the enthalpy of reaction 10 should be no greater than about +20 kJ mol^{-1}. The reaction would be abetted by the fact that even weak bases can deprotonate the product sulfonium ion (p$K_a \cong -5$),[41] reaction 13, and thereby prevent the reverse reaction.

$$\mathrm{R^1R^2SH^+} + \mathrm{B:} \rightarrow \mathrm{R^1R^2S} + \mathrm{BH^+} \quad (13)$$

In fully relaxed peptide geometries, the BDE($^{\alpha}$C−H) is in the range 330−370 kJ mol^{-1}.[14−16] The strength of the S−H bond in dialkylsulfonium ions is also in this range (351 kJ mol^{-1} reported for $(CH_3)_2SH^+$).[37] As the BDE($^{\alpha}$C−H), at 0 K, of glycine model peptide (**PH**(Gly)) has been established to be

(37) BDE(S−H) at 0 K; see sources given in Table S1.

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345 kJ mol^{-1},[42] the enthalpy change for reaction 14 is about −6 kJ mol^{-1} if the experimental BDE(S−H) of dimethylsulfonium is correct.

$$(CH_3)_2S^{\bullet+} + \mathbf{PH}(\mathrm{Gly}) \rightarrow (CH_3)_2SH^+ + {}^{\alpha}\mathbf{P}^{\bullet}(\mathrm{Gly}) \quad (14)$$

The reaction of the radical cation of methionine residue, **PH**(Met(S$^{\bullet+}$)), with a glycine residue in a disordered region of the peptide chain should have similar thermochemistry.

S−H Bond Dissociation Enthalpy (BDE) of Sulfonium Ions, R_2SH^+: Calculation of BDE(S−H) of $(CH_3)_2SH^+$. In Table S1 of the Supporting Information are shown the results of calculations at various levels of theory, including CBS-RAD and G2(MP2-B3LYP), on the parent, ionized, and protonated forms of H_2S, CH_3SH, and $(CH_3)_2S$ and the interrelated values of ionization potential (IP), proton affinity (PA), and BDE(S−H). The experimental values[37] of these quantities are also given. Specifically, the experimental BDE(S−H) of the three sulfonium ions are H_3S^+, 397 kJ mol^{-1}; $CH_3SH_2^+$, 368 kJ mol^{-1}; $(CH_3)_2SH^+$, 351 kJ mol^{-1}. Both CBS-RAD[39] and G2(MP2-B3LYP) procedures yield values for BDE(S−H) which are in good agreement with each other and with the experimental values. As a result, we will adopt the experimental value, BDE(S−H) = 351 kJ mol^{-1}, as the reference value for use in isodesmic reactions involving dimethylsulfonium (reaction 8). It is not feasible to carry out calculations at such a high level on the larger species which are of principal interest here. On the basis of the data in Table S1, it is apparent that the B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d) level of theory provides reliable BDE(S−H) results which may be further corrected by application of the isodesmic reaction scheme, eqs 8 and 9. At the B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d) level of theory, the isodesmic correction for BDE(S−H) of dimethylsulfonium is +15 kJ mol^{-1}, which is very similar to the isodesmic correction for BDE($^{\alpha}$C−H), +13 kJ mol^{-1} (from data in Table S2, footnote *c*).

Reaction between $(CH_3)_2S^{\bullet+}$ and PH(Gly) (Reaction 4). The energetics of the interaction between $(CH_3)_2S^{\bullet+}$ and **PH**(Gly), a model for interstrand reaction between glycine and oxidized methionine residues (reaction), are listed in Table S2. $(CH_3)_2S^{\bullet+}$ and **PH**(Gly) form a reactant complex, **RC(O∴S$^{+\bullet}$)**, shown in Figure 1, which is characterized by a three-electron, two-center bond formed from the half-filled 3p orbital of the sulfur atom and the occupied nonbonded in-plane orbital of the oxygen atom of the N-terminal amide group. Substantial polarization of the amide group is evident in the structural distortions: the C=O bond is lengthened by 0.03 Å and the C−N bond shortened by the same amount, providing evidence for increased importance of the amide group resonance structure, $^-$O−CR=N$^+$HR. The expected increase in acidity of the N−H bond is seen in the short H···O= separation, 1.967 Å. **RC(O∴S$^{+\bullet}$)** is calculated to be bound by 95 kJ mol^{-1}.[43]

From **RC(O∴S$^{+\bullet}$)**, 68 kJ mol^{-1} is required to reach the transition structure for H atom transfer, **TS($^{\alpha}$C···H···S)** (Figure 1). It should be noted that, while there is a modest enthalpy of activation from **RC(O∴S$^{+\bullet}$)**, **TS($^{\alpha}$C···H···S)** is actually 27 kJ mol^{-1} *below* the separated species. The H atom being transferred is almost on a line between the S and $^{\alpha}$C ($\angle$SHC = 174°), and the S−H distance, 1.510 Å, is increased moderately from its normal value, 1.353 Å. The H−C separation is proportionally much more elongated, by 0.5 Å, indicating a late transition structure. From the viewpoint of the attacking sulfide radical cation, the vector of approach is approximately perpendicular to the C−S−C plane. This approach is readily achieved for the dimethyl sulfide radical cation. However, it may be more difficult in the case of methionine sulfide radical cation, where one of the C atoms is attached to the peptide backbone by a short tether, the intervening CH_2 group. We address this point further below.

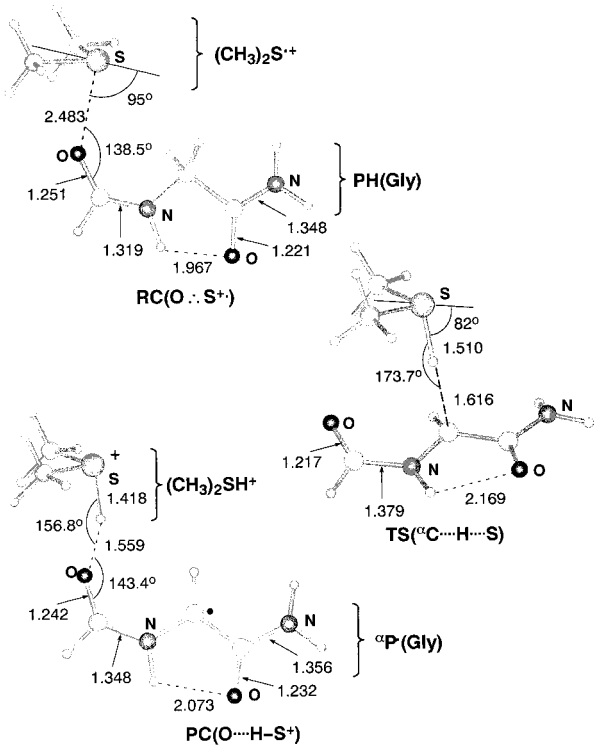

Figure 1. B3LYP/6-31G(d) structures: **RC(O∴S$^{+\bullet}$)** = reactant complex with three-electron S−O bond between $(CH_3)_2S^{\bullet+}$ and *N*-formylglycine amide, **PH**(Gly); **TS($^{\alpha}$C···H···S)** = transition structure for H atom transfer between $^{\alpha}$C site and $(CH_3)_2S^{\bullet+}$; **PC(O···H−S$^+$)** = product complex with S−H to O hydrogen bond between $(CH_3)_2SH^+$ and the $^{\alpha}$C-centered radical of *N*-formylglycine amide, $^{\alpha}$**P**$^{\bullet}$(Gly).

A second complex, **PC(O···H−S$^+$)** (Figure 1), is found on the product side, corresponding to an association of $(CH_3)_2SH^+$ with $^{\alpha}$**P**$^{\bullet}$(Gly) through H-bonding to the same amide carbonyl as in **RC(O∴S$^{+\bullet}$)**. **PC(O···H−S$^+$)** is bound by 79 kJ mol^{-1} relative to the separated reactants and 52 kJ mol^{-1} below **TS($^{\alpha}$C···H···S)**. The strength of the H bond is apparent in the unusually close approach of the H to the O=, 1.559 Å, and a substantial elongation, by 0.065 Å, of the S−H bond.

It should be noted that both the three-electron bond of **RC(O∴S$^{+\bullet}$)** and the H bond of **PC(O···H−S$^+$)** lie in the direction normally occupied by an existing H bond in secondary structure. Neither **RC(O∴S$^{+\bullet}$)** nor **PC(O···H−S$^+$)** could be established without considerable disruption to secondary structure. However, the direction of approach of the dialkyl sulfur in **TS($^{\alpha}$C···H···S)** is along the $^{\alpha}$C−H bond vector, one of which, in the case of glycine, lies perpendicular to the average plane of the β-sheet secondary structure. If dimethyl sulfide is a suitable model for methionine, the present results imply that H atom abstraction to form an $^{\alpha}$C-centered radical at a glycyl residue in an antiparallel β-sheet may occur *without activation* if a free (i.e., external to the β-sheet) Met S-centered radical cation could reach within striking distance. We examine below

(42) Armstrong, D. A.; Yu, D.; Rauk, A. *Can. J. Chem.* **1996**, *76*, 1192−1199.

(43) The B3LYP procedure tends to overestimate the strength of three-electron bonds by 12−30 kJ mol^{-1} for related compounds; see: Braida, B.; Hiberty, P. C.; Savin, A. *J. Phys. Chem. A* **1998**, *102*, 7872−7877.

the appropriateness of dimethyl sulfide as a model for the methionine residue.

S−H Bond Dissociation Enthalpy (BDE) of PH(Met-(SH$^+$)). Table S1 also lists the IP, PA, and BDE(S−H) values for the parent, ionized, and protonated forms of **PH**(Met). The BDE(S−H) value of the S-protonated form, after isodesmic correction, is 331 kJ mol^{-1}. Thus, the sulfonium ion derivative of the methionine residue is predicted to have a weaker S−H bond than the prototype, dimethylsulfonium ion. The reduction of BDE(S−H) by 20−25 kJ mol^{-1} in the Met-derived species relative to that of dimethylsulfonium is attributable mainly to additional stabilization of the radical cation by the vicinal C−C bond and internal "solvation" of the positive charge.

The reduced value of the BDE(S−H) will affect the energetics of the proposed interstrand reaction between the S radical cation, **PH**(Met(S)), and glycine residues, modeled by **PH**(Gly). Thus, assuming that the $-CH_2SCH_3$ moiety of **PH**(Met) can adopt the same TS orientation as was found for CH_3SCH_3 (Figure 1, **TS(**$^{\alpha}$**C···H···S)**), the H atom transfer reaction (reaction 15) is predicted to be *endo*thermic by about 20 kJ mol^{-1}.

$$\mathbf{PH}(\mathrm{Met}(\mathrm{S}^{\bullet+})) + \mathbf{PH}(\mathrm{Gly}) \rightarrow \mathbf{PH}(\mathrm{Met}(\mathrm{SH}^{+})) + {}^{\alpha}\mathbf{P}^{\bullet}(\mathrm{Gly}) \quad (15)$$

As mentioned earlier, an endothermicity of this magnitude would not preclude rapid H abstraction from the peptide backbone because rapid exergonic deprotonation of **PH**(Met(SH$^+$)) would prevent the reverse reaction.

Role of Secondary Structure. Incorporation of Gly in secondary structure increases the strength of the $^{\alpha}$C−H bond: α-helix, 402 kJ mol^{-1};[14] parallel β-sheet, 404 kJ mol^{-1};[17] antiparallel β-sheet, 361 kJ mol^{-1}.[14,17] In general, only in the case of antiparallel β-sheet secondary structure is the $^{\alpha}$C−H bond weak enough for reaction with sulfide radical cations to be energetically feasible. There is a second consequence of the presence of secondary structure. A residue embedded in any of these three kinds of secondary structures has its side chain oriented outward in the sterically less crowded environment, and consequently its $^{\alpha}$C−H bond lies in a sterically inaccessible or protected region of the structure. The unique exception is glycine, where the "side chain" is also an $^{\alpha}$C−H bond which is consequently exposed to oxidants. One may conclude from these considerations that a weak oxidant like **PH**(Met(S$^{\bullet+}$)) may abstract an $^{\alpha}$C−H bond in principle from almost any amino acid residue in a random coil region of a protein. However, the presence of secondary structure offers protection to $^{\alpha}$C−H bonds for all residues except glycine, and then only in an antiparallel β-sheet.[17]

Methionine in an Antiparallel β-Sheet. We consider here the case when the strand containing Met, modeled by **PH**(Met), is part of an antiparallel β-sheet. Two consequences may ensue. First, the denser solvent-excluded "medium" may affect the properties of the sulfide side chain through electrostatic or steric interactions. In Table S1 are shown the computed results for the IP, PA, and BDE(S−H) of a model of a β-sheet-embedded Met, **PH**(Gly)···**PH**(Met), and its ionized and protonated forms. For the purpose of examining the effect of complexation on the sulfide properties, the conformations of the side chain (Figure 2) were chosen so as to avoid complications due to intramolecular H-bonding or three-electron bonding. Compared to uncomplexed single strand Met(SH$^+$), i.e., **PH**(Met(SH$^+$)), the BDE(S−H) of **PH**(Gly)···**PH**(Met(SH$^+$)) is reduced by a negligible amount to 326 kJ mol^{-1}. For all intents and purposes, the properties of the sulfide moiety are not affected by the long-range H-bonding interactions.

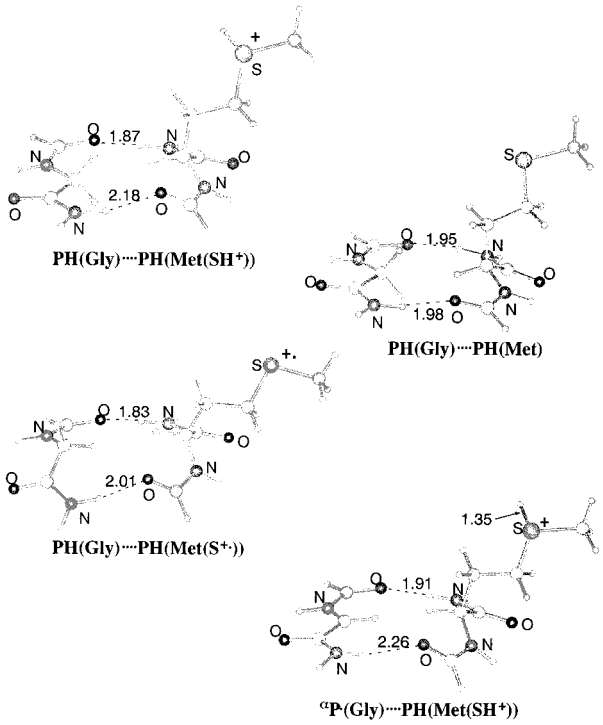

Figure 2. B3LYP/6-31G(d) structures of the H-bonded (5,5) cyclic complexes of **PH**(Gly) with **PH**(Met), and derivatives discussed in the text.

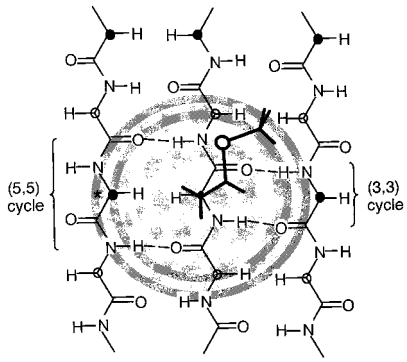

Figure 3. Hypothetical antiparallel β-sheet structure with a methionine residue surrounded by glycine residues; small filled and open circles indicate $^{\alpha}$C−H bonds on the same side and opposite side, respectively, of the β-sheet relative to the methionine side chain. Gray bulls-eye denotes striking radius of the S (larger open circle) and neighboring C atoms, and * denotes site of reaction.

A second consequence of incorporation of Met into a β-sheet is that the reduced mobility of the backbone due to H-bonding may make it more difficult to achieve the required geometry of the transition structure for H abstraction. Simplistic modeling (Figure 3) suggests that if the methionine is embedded in an antiparallel β-sheet and its nearest-neighbor residues were glycines, the ionized sulfur atom can only reach a single $^{\alpha}$C−H bond, namely that of the glycine residue of a different peptide strand and across a (5,5) cycle. The "side chain" $^{\alpha}$C−H bonds of the $i + 1$ and $i - 1$ residues from the same strand are close enough but are on the opposite side of the β-sheet, and the $^{\alpha}$C−H bond across the (3,3) cycle appears to be too far away.

The peptide model of the methionine residue hydrogen bonded in a (5,5) cycle to a glycine residue in an antiparallel fashion, **PH**(Gly)···**PH**(Met), is shown in Figure 2. The corresponding S-protonated (**PH**(Gly)···**PH**(Met(SH$^+$))) and S

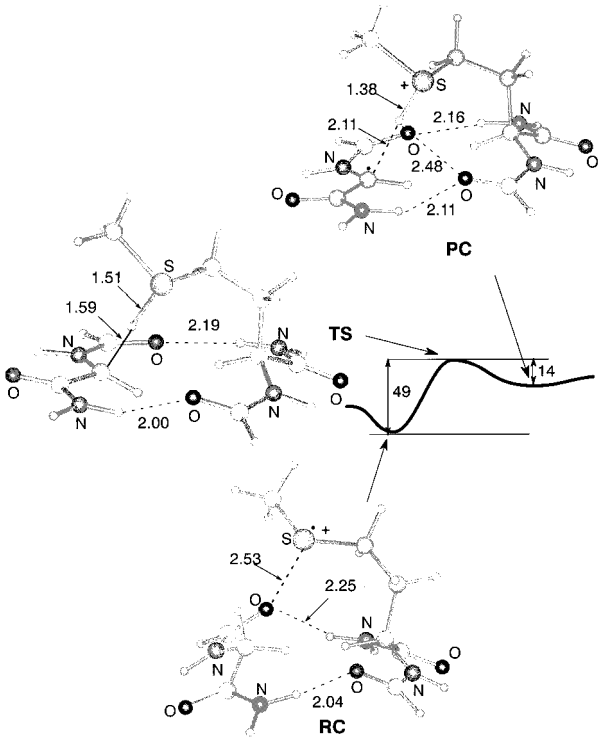

Figure 4. B3LYP/6-31G(d) structures of reactant and product complexes, and transition structure of the **PH**(Gly)···**PH**(Met) system, analogous to those shown in Figure 1.

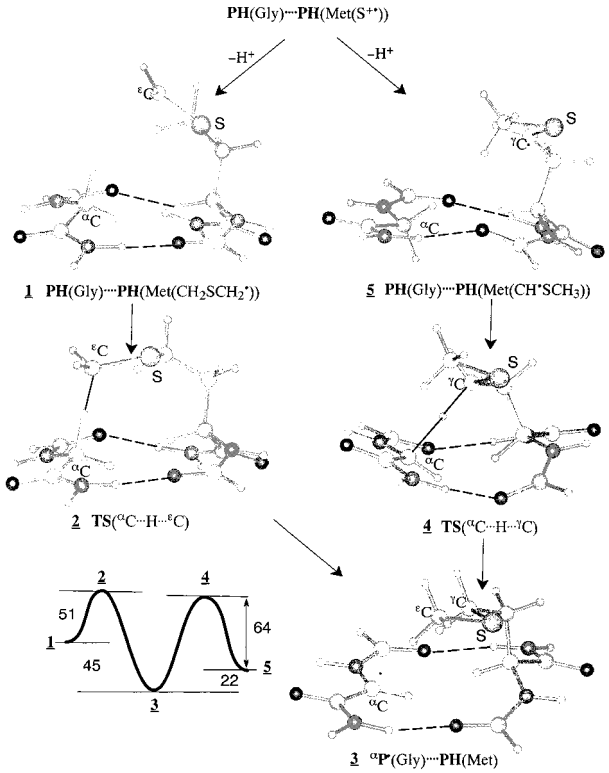

Figure 5. B3LYP/6-31G(d) structures of species involved in H atom transfer between $^{\alpha}$C of Gly and $^{\gamma}$C or $^{\epsilon}$C of Met, after deprotonation of **PH**(Gly)···**PH**(Met($S^{+\bullet}$)).

radical cationic (**PH**(Gly)···**PH**(Met($S^{\bullet+}$))) forms are also shown in Figure 2, as is the H-atom-transferred form ($^{\alpha}$**P**$^{\bullet}$(Gly)··· **PH**(Met(SH^{+}))). Reorientation of the side chains of these species toward the $^{\alpha}$C site across the (5,5) cycle yields the structures shown in Figure 4. The energies are listed in Table S3. **PH**(Gly)···**PH**(Met($S^{\bullet+}$)) forms a reactant complex, **RC**, analogous to **RC(O**$\therefore$**S**$^{\bullet+}$**)** shown in Figure 1. **RC** is only 10 kJ mol^{-1} more stable than the extended structure. Thus, the advantage of forming a three-electron bond is largely offset by disruption to the H-bonding network of the (5,5) cycle. As shown in Figure 4, the transition structure for H transfer, **TS**, is 49 kJ mol^{-1} above **RC**, and 39 kJ mol^{-1} higher than the extended structure, **PH**(Gly)···**PH**(Met($S^{\bullet+}$)). The product-side complex, **PC** (analogous to **PC(O**···**H**−**S**$^{+}$**)**, Figure 1), lies 14 kJ mol^{-1} below **TS**. The difference between **PC** and the extended form, $^{\alpha}$**P**$^{\bullet}$(Gly)···**PH**(Met(SH^{+})), is only 5 kJ mol^{-1}. Thus, a rapid equilibrium between **PC** and $^{\alpha}$**P**$^{\bullet}$(Gly)··· **PH**(Met(SH^{+})) can carry the S−H bond away from the $^{\alpha}$C site into the solvent, resulting in irreversible loss of the proton from the sulfur atom.

Radical Properties of PH(Gly)···PH(Met($CH_2SCH_2^{\bullet}$)) and PH(Gly)···PH(Met($CH^{\bullet}SCH_3$)). There is no question that the strengths of the C−H bonds adjacent to the S atom are considerably higher than that of the $^{\alpha}$C−H bond of an amino acid residue in a peptide. We address here directly whether such a species, generated by loss of a proton from S-oxidized Met residue in an antiparallel β-sheet environment, can abstract a H atom from the adjacent Gly residue in an adjoining strand. The simplistic modeling discussed in connection with Figure 3 suggests that a glycyl $^{\alpha}$C−H bond of the (3,3) ring is out of reach of either C-centered radical, but that the methyl-derived radical can also reach the $^{\alpha}$C−H bond of a glycine residue of the (5,5) cycle. Such a situation is modeled by **PH**(Gly)··· **PH**(Met($CH_2SCH_2^{\bullet}$)) shown in Figure 5 in a conformation suitable for H abstraction. Although it appears less likely from Figure 3, we also examine the reaction of the methylene-derived radical, **PH**(Gly)···**PH**(Met($CH^{\bullet}SCH_3$)) (Figure 5). The computational data are collected in Table S4, and the structure of the common product, $^{\alpha}$**P**$^{\bullet}$(Gly)···**PH**(Met), as well as the respective transition structures and their relative energies, are also shown in Figure 5.

From Table S4 and Figure 5, it is apparent that the Met $^{\epsilon}$C- and $^{\gamma}$C-centered radicals, **PH**(Gly)···**PH**(Met($CH_2SCH_2^{\bullet}$)) and **PH**(Gly)···**PH**(Met($CH^{\bullet}SCH_3$)), respectively, are less stable than the Gly $^{\alpha}$C-centered radical, $^{\alpha}$**P**$^{\bullet}$(Gly)···**PH**(Met), by 45 and 22 kJ mol^{-1}, respectively. In a hydrous environment, the pK_a of the methyl proton of **PH**(Gly)···**PH**(Met($S^{\bullet+}$)) should be similar to $(CH_3)_2S^{(\bullet+)}$, namely p$K_a \cong -2$.[32] If the enthalpy difference of 23 kJ mol^{-1} between **PH**(Gly)···**PH**(Met($CH_2SCH_2^{\bullet}$)) and **PH**(Gly)···**PH**(Met($CH^{\bullet}SCH_3$)) were reflected in their relative free energies of solution, one would expect for the latter, pK_a $\cong -6$. It follows that if conditions suitable for deprotonation by solvent exist in β-sheet aggregated Aβ, one would expect quantitative formation of the $^{\gamma}$C-centered radical at the expense of the $^{\epsilon}$C-centered radical.

The transition structure for H abstraction by the methyl-derived radical, **TS**($^{\alpha}$C···H···$^{\epsilon}$C), lies 96 kJ mol^{-1} above $^{\alpha}$**P**$^{\bullet}$(Gly)···**PH**(Met), corresponding to an enthalpy of activation of 51 kJ mol^{-1} for reaction 11. Thus, H-abstraction by methyl radical, once it is formed by deprotonation of the initially formed sulfide radical cation, should be competitive with the direct reaction 15 (Figure 4).

The transition structure for H abstraction by the methylene-derived radical, **TS**($^{\alpha}$C···H···$^{\gamma}$C), lies 90 kJ mol^{-1} above $^{\alpha}$**P**$^{\bullet}$(Gly)···**PH**(Met), corresponding to an enthalpy of activation of 64 kJ mol^{-1} for reaction 12. This value should be regarded as a lower estimate since the substantial distortion in the H-bonded (5,5) ring which is evident in **TS**($^{\alpha}$C···H···$^{\gamma}$C) (Figure 5) would be more difficult to achieve in an actual β-sheet. Thus,

H-abstraction by methylene radical is not expected to be competitive with either reaction 11 or reaction 15.

Methionine-Containing β-Sheet Peptides with Radical Properties.

sequence	protein	residues
GSNKGAIIGLMVG	Aβ peptide	25−37
GAVVGGLGGYMLG	prion peptide	111−123

It is of some note that both Aβ and the prion peptide, which are implicated in neurodegeneration, have a common repeating glycine sequence at *i*, *i* + 4, *i* + 8, and *i* + 12 residues, as well as a common methionine residue at the *i* + 10 position.[44] Both of these peptides form amyloid via β-sheet structures, and methionine appears to be critical for their radical-based neurobiology.[45] Another common feature of these two hydrophobic peptides is that the *i* to *i* + 12 sequence mentioned above lies in a region that can be α-helical under some, including membrane-simulating, conditions.[46] Presumably the *i*, *i* + 4 repeating Gly residues help to destabilize the helix in solution and promote conformational switching to a β-sheet that seems important for peptide aggregation and amyloid properties. The analysis described above is certainly consistent with the requirements of an antiparallel β-sheet, methionine, and glycine residues and provides a framework for understanding the radical chemistry and biology of these cytotoxic peptides.

Conclusions

It must be borne in mind that the neurotoxicity exhibited by Aβ and prion protein is the result of an accumulation of unusual circumstances; otherwise, any Met-containing proteins would be lethal. We have delineated here, and summarize below, some of these circumstances.

The BDE(S−H) of a S-protonated methionine residue of a polypeptide strand is estimated to be 326−331 kJ mol^{-1}. Thus, it is thermodynamically feasible that the S-ionized form may cause oxidative damage to nearby lipids with a bis(allylic) methylene group (BDE(C−H) = 335 kJ mol^{-1}) or at the $^{\alpha}$C−H site of almost any residue of a nearby polypeptide strand with a random coil geometry (BDE($^{\alpha}$C−H) = 330−360 kJ mol^{-1}). However, in the special case where a Met residue is incorporated into an antiparallel β-sheet, the only residue of the same β-sheet that is susceptible to oxidation at the $^{\alpha}$C−H site is a glycine, and only if it is in a different strand than Met and across a (5,5) cycle from Met. If prior deprotonation of the radical cation occurs, the resulting methyl-derived radical can react in a similar fashion with similar activation energy. These findings suggest a possible mechanism for generating and propagating oxidative damage via a Met of β-sheet-forming Aβ and prion peptides of neurodegenerative diseases. A more detailed investigation that specifically addresses this oxidation process in the context of Met35 and the β-amyloid peptide of Alzheimer's disease will follow in a subsequent publication.[47]

Acknowledgment. Partial support for this work was provided by the Natural Sciences and Engineering Research Council of Canada, the Australian Research Council, and the National Health and Medical Research Council of Australia. We also thank Joel Tyndall for conducting the sequence search.[44]

Supporting Information Available: Calculated total energies, zero point vibrational energies, ionization potentials, proton affinities, and bond dissociation energies (Table S1), and relative energies for all species discussed in the text (Tables S2−S4) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA994436U

(44) The Swiss Protein database (http://www.motif.genome.ad.jp/MOTIF2.html) was used to search for sequence matches to GxxxGxxxGxMxG corresponding to Aβ(25−37). Although some 50 distinct proteins were found to contain this sequence, there is currently no information on the effects of selective methionine oxidation or the influence of these sequences on conformation. However, like Aβ1−42, several of the proteins containing this sequence are involved in redox or electron-transfer processes (e.g., amyloid precursor protein APP 696−708; major prion protein precursor PrP 111−123; photosystem I protein PSI 136−148; NADH-ubiquinone oxidoreductase 299−311; mitochondrial import protein 3 108−120; copper resistance protein A precursor 421−433), bind to lipid membranes (e.g., Annexin VII binds to Apolipoprotein like Aβ1−42), or are membrane transport proteins, and a large proportion are heat shock proteins for which conformational changes may be important. Indeed, many of these proteins are up or down regulated by environmental stresses encountered in cells (e.g., heat, oxidation, acid pH, metal ions). Alzheimer's disease has been attributed to oxidative stress mediated by β-amyloid peptide and free radicals. A number of studies suggest that prion proteins, their selective oxidation at methionine residues (ref 45), and possibly copper may be involved in cellular responses to oxidative stress leading to several neurodegenerative diseases (Brown, D. R.; Schulz-Schaeffer, W. J.; Schmidt, B.; Kretzschmar, H. A. *Exp. Neurol.* **1997**, *146*, 104−112).

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(46) (a) Coles, M.; Bicknell, W.; Watson, A. A.; Fairlie, D. P.; Craik, D. J. *Biochemistry* **1998**, *37*, 11064−11077. (b) Riek, R.; Hornemann, S.; Wider, G.; Billeter, M.; Glockshuber, R.; Wurthrich, K. *Nature* **1996**, *382*, 180−182.

(47) Tyndall, J. D. A.; Fairlie, D. P.; Armstrong, D. A.; Rauk, A., manuscript in preparation.