Oxidative Damage to and by Cysteine in Proteins: An ab Initio Study of the Radical Structures, C−H, S−H, and C−C Bond Dissociation Energies, and Transition Structures for H Abstraction by Thiyl Radicals

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Abstract: Ab initio computations (B3LYP/6-31G(D), coupled with isodesmic reactions) were used to predict bond dissociation energies (BDEs) of $^{\alpha}$C−H (D^{α}_{CH}) and other bonds of cysteine, both as free neutral amino acid (**AH**(Cys)) and as a residue in a model peptide (**PH**(Cys)). The latter was intended to mimic the environment in proteins. Transition structures were located for intermolecular and intramolecular H atom transfer to a thiyl radical (RS$^{\bullet}$) from a sulfhydryl group (RSH) or the $^{\alpha}$C−H bond. The predicted BDEs, at 298 K, in kJ mol^{-1} to an estimated accuracy of 10 kJ mol^{-1} for the fully optimized system are (**AH**(Cys)) $D^{\alpha}_{CH} = 322$, $D^{\beta}_{CH} = 390$, $D^{\alpha}_{CC} = 264$, and $D_{SH} = 373$ and (**PH**(Cys)) $D^{\alpha}_{CH} = 346$, $D^{\beta}_{CH} = 392$, $D^{\alpha}_{CC} = 287$, and $D_{SH} = 367$. In **PH**(Cys) with torsional angles constrained to simulate β-sheet and α-helical secondary structure, D^{α}_{CH} rises to 359 and 376, respectively. Cystine in the peptide environment was modeled by replacing −SH by $-SSCH_3$, **PH**($CysSCH_3$), $D^{\alpha}_{CH} = 330$. Enthalpies of activation for intermolecular H transfer to RS$^{\bullet}$ were found to be low: from RSH, 12 kJ mol^{-1}; from $^{\alpha}$C−H, about 25 kJ mol^{-1}, the latter being consistent with reaction rates on the order of 10^5 M^{-1} s^{-1}. The enthalpic barrier for intramolecular H transfer from $^{\alpha}$C−H to −S$^{\bullet}$ within a single cysteine residue is too high (83−111 kJ mol^{-1}) for this to be a competitive process.

Introduction

The site of radical attack causing oxidative damage to proteins is a matter of great interest, since such damage is implicated in numerous pathological disorders[1] and the process of aging. Oxidation is generally initiated by OH$^{\bullet}$ radicals or other radicals created from enzyme reactions,[1−5] or by ultraviolet and ionizing radiation.[6] The side group of the cysteine residue reacts rather rapidly with OH$^{\bullet}$,[1,7,8] forming the thiyl radical, RS$^{\bullet}$, which, in experiments involving derivatives of cysteine, homocysteine, and glutathione, has been shown to abstract a hydrogen atom intra- and intermolecularly from the α-carbon center of peptide residues.[9,10] The α-carbon center of amino acid residues is also a major point of direct attack.[1,11,12] In the presence of oxygen the α-C-centered radical forms peroxy compounds and the protein is cleaved at that point.[11−13]

The cysteine residue is present in most proteins and is often an integral component of protein tertiary structure (through its dimer, cystine). More importantly, it plays a central role in a number of proteins active in oxidation−reduction reactions where free radicals are abundant. Sulfide groups of cysteine residues typically serve as anchors for metallic redox sites such as [2S−2Fe] and [4S−4Fe] clusters and iron heme (in cytochrome *c*).[14] In thioredoxin reductase and similar enzymes, cysteines appear in pairs of the type Cys−X−X−Cys and reducing power is transferred through electron transfers which effect cycling between disulfide and sulfhydryl forms.[14,15] Cysteine is a component of glutathione (γ-glutamylcysteinylglycine, GSH), a ubiquitous tripeptide which plays a number of vital roles in cell metabolism, including repair of oxidative damage in red blood cells and elsewhere.[14]

In previous work we have established the methodology for the accurate determination of $^{\alpha}$C−H bond dissociation energies (BDEs), D^{α}_{CH}.[16] We showed explicitly in the case of glycine, alanine, serine, and threonine residues,[17] and the anhydrides of

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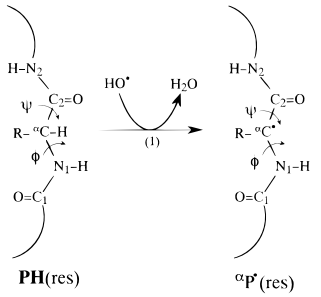


Figure 1. Illustration of the parent peptides, **PH**(res), and $^{\alpha}$C-centered radicals, $^{\alpha}$**P**•(res), considered here, and of the OH• abstraction of H from an $^{\alpha}$C atom. R is $-CH_2SH$ for res = Cys.

glycine and alanine,[18] that the $^{\alpha}$C−H BDEs are lower than RS−H, and therefore potentially damageable by thiyl radicals. We also established that some site specificity of such oxidative damage is expected, namely, that these residues in disordered or β-sheet regions of the protein may be most readily damaged, but that the α-helical secondary structure confers some protection.[17] The present work addresses the potential modes of oxidative damage to cysteine through H atom abstraction and the mechanism of subsequent intermolecular or intramolecular H atom abstraction by thiyl radical.

The initial hydrogen atom abstraction from a $^{\alpha}$C-center, reaction 1, is shown in Figure 1. The peptide unit, designated as **PH**(res), is intended to model an amino acid residue far from an end group of a protein. The unit is truncated by H at each end, replacing the $^{\alpha}$C of the next residue in each direction.[16] The $^{\alpha}$C-centered radical is designated as $^{\alpha}$**P**•(res). In most instances in the present work, (res) will be (Cys). By extension, radical sites at the carbon of the side chain (the β-center) and at the sulfur atom are designated $^{\beta}$**P**• and ^{S}**P**•, respectively. The preferred sites of attack by OH• will be those for which the energy barriers to reaction are smallest. Since these are normally the ones with the most exergonic reactions, a knowledge of the C−H and S−H bond dissociation energies and/or the $^{\alpha}$**P**•/**PH** reduction potentials are of fundamental interest. Indeed for radicals generated on the exterior of a given protein the relative reduction potentials of exposed amino acid residues should provide a first-order prediction of the relative frequencies of attack. In addition, such thermodynamic information will indicate which lesions are potentially repairable by endogenous cellular repair agents, such as glutathione (GSH)[5,6,19−21], viz.

$$^{\alpha}\mathbf{P}^{\bullet}(\text{res}) + \text{GSH} \rightleftharpoons \mathbf{PH}(\text{res}) + \text{GS}^{\bullet} \qquad (2)$$

For the $^{\alpha}$C-centered radicals in proteins the only available information appears to be the theoretical estimates of D^{α}_{CH} and E° for the midchain glycine, alanine, serine, and threonine residues from this laboratory.[17] The predicted low values of D^{α}_{CH} (344−356 kJ mol^{-1}) and E° (0.7−0.9 V) for most species indicate that not only is the $^{\alpha}$C−H highly susceptible to H abstraction by hydroxyl radical, but the damaged site is not likely to be repairable by glutathione!

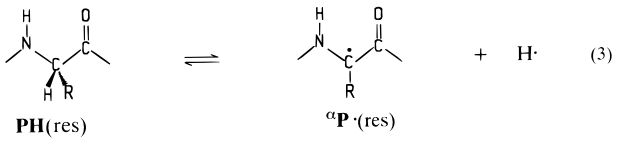
The ultimate objective of our research is to provide quantitative information which can be used in predicting focal points of oxidative damage to proteins in cells. Since the Ramachandran angles, Φ and Ψ, vary widely in different proteins and within a protein, depending on the local secondary structure, a knowledge of the effects of these parameters on the $^{\alpha}$C−H bond dissociation energies of particular amino acid residues (i.e., specific R groups) will eventually be necessary to enable systematic predictions of the sites of free radical-induced oxidative damage and the possibility of propagation of damage from the initiation site to be made. The present study is intended to focus attention on the relative sensitivities of the α- and β-C and S centers of cysteine (and to some extent cystine) to damage by H atom abstraction, and to establish the stereoelectronic requirements of hydrogen atom transfer from carbon to sulfur.

Computational Details

All ab initio calculations presented here were performed with the Gaussian-92/94 molecular orbital packages.[22] The geometry optimizations and frequency calculations were carried out at the B3LYP/6-31G(D) level by the hybrid HF-DFT procedure implemented in the Gaussian molecular orbital packages with the keyword INT=FINEGRID. The vibrational frequencies were scaled by a factor of 0.98 in considering the zero-point energy.[23]

Thermochemistry. The values of $H^{\circ}_{298} - H^{\circ}_{0}$ required to obtain heats of reaction at 298 K and the entropies needed in the evaluation of reduction potentials were calculated by standard statistical thermodynamic methods, based on the rigid rotor-harmonic oscillator model[24] and using the frequencies obtained at the B3LYP/6-31G(D) level. The frequencies were scaled by a factor of 0.98 in the calculation of these thermodynamic functions.[23] In the case of the transition structures for intermolecular H transfer, the lowest real frequency normal mode corresponds to torsion about the line connecting the migrating hydrogen and was treated as a free rigid rotation. In the treatment of bond dissociation energies (BDEs), i.e., bond enthalpies, as discussed below, where temperature is not given explicitly, it should be assumed to be 298 K.

Bond Dissociation Energies (BDEs). The $^{\alpha}$C−H BDEs, D^{α}_{CH}, are defined as the heat of reaction (3), $\Delta H^{\circ}_{(3)}$. If calculated directly from eq 3 at the B3LYP/6-31G(D) level, $^{\alpha}$C−H BDEs are systematically



(3)

underestimated by about 20 kJ mol^{-1}. It is expected that similar errors would be incurred for $^{\beta}$C−H and S−H BDEs. As a means of partially cancelling errors due to basis set and correlation effects, the BDEs were

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derived from the heats of isodesmic reactions.[25] These reactions can be represented by process 4. AH is a reference molecule for which

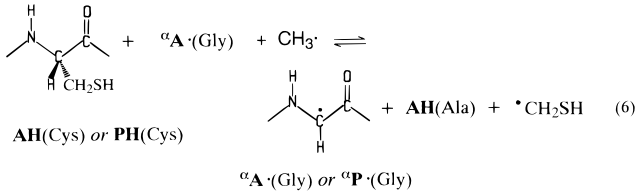
$$^{\alpha}\mathbf{P}^{\bullet}(\text{res}) + \text{AH} \rightleftharpoons \mathbf{PH}(\text{res}) + \text{A}^{\bullet} \quad (4)$$

the BDE, D_{CH}(**AH**), is known accurately. For each **PH**(res) the heat of reaction (4), $\Delta H_{(4)}°$, was evaluated from the energies obtained in the ab initio calculations at the B3LYP/6-31G(D) level, which was shown to give reliable results for the glycine model peptides.[16,17] In the context of bond dissociation energies, D_{CH}(PH) is then given by

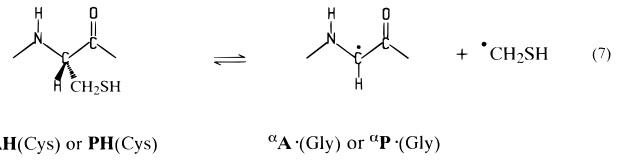
$$D_{CH}(\text{PH}) = D_{CH}(\text{AH}) - \Delta H_{(4)}° \quad (5)$$

In order to obtain the most effective cancellation of residual errors, the structures of the reference molecule and radical used in reaction 4 should be related as closely as possible to those of **PH**(res) and $^{\alpha}$**P**$^{\bullet}$-(res), respectively. Ideally **AH** should have both an adjacent amino group and a carbonyl group so that the special feature of the captodative effect[26] can be taken into account. Previous studies have shown[17] that H_2NCH_2COOH (**AH**(Gly)) is the most suitable reference molecule to give reliable values of D^{α}_{CH}. The magnitude of D^{α}_{CH} for glycine itself (331.0 kJ mol^{-1})[16] was not directly available from experiment. However, it has been derived from a number of isodesmic reactions with heats of reaction based on G2(MP2) calculations.[16] Values of D^{α}_{CH} for the other parent amino acids alanine, serine, and threonine were also obtained using an isodesmic reaction similar to reaction 4 with glycine as reference.[17] Here we apply this procedure to neutral cysteine (**AH**(Cys)), cysteine model peptide (**PH**(Cys)), and "cystine" model peptide (**PH**(CysSCH$_3$)).

The $^{\beta}$C−H bond dissociation energies of the side group, D^{β}_{CH}, and the dissociation energy of the $^{\alpha}$C−C bond associated with removal of the side group, D^{α}_{CC}, were also calculated for the peptide model and the parent amino acid. For D^{β}_{CH}, CH_3SH was selected as the reference molecule in an isodesmic reaction like reaction 4. The D_{CH} in CH_3SH is known experimentally (387 ± 8 kJ mol^{-1}).[27] D^{α}_{CC} was derived from the heat of reaction (6), which is an isodesmic reaction involving neutral



alanine (**AH**(Ala)), for which D^{α}_{CC} = 292 kJ mol^{-1} (298 K) has been derived from experimental data and calculated at a modified G2(MP2) level.[17] D^{α}_{CC} could also be derived directly from the heat of reaction (7), although we know from previous experience with alanine that direct calculation of D^{α}_{CC} will underestimate the strength of the bond by about 20 kJ mol^{-1}.[17]



The value of D_{SH} was derived by isodesmic reaction, using CH_3SH (D_{SH} = 360 ± 3 kJ mol^{-1})[27,28] as isodesmic partner. In the case of CH_3SH itself, direct calculation of D_{SH} yields a value which is 30 kJ mol^{-1} too low. The heats of reaction in each of the above cases were obtained directly from the ab initio energies of the fragments and the parent molecules and corrected to 298 K.

Reduction Potentials, *E*°. The reduction potentials of the C-centered radicals were obtained from the standard free energy changes at 298 K, $\Delta G°_{298}$, of reaction 8. $\Delta G°_{298}$ is related to D^{α}_{CH} by expression

$$-\text{NHCR}^{\bullet}\text{C(O)}- + {}^{1}/_{2}\text{H}_{2(g)} \rightleftharpoons -\text{NHCH(R)C(O)}- \quad (8)$$

9, in which $\Delta S°_{298}$ is the entropy change in reaction 8. The difference in entropy of the parent and radical species was derived from the calculated entropies (Table S1 in the Supporting Information). $S°_{298}$ of

$$\Delta G°_{298} = -D_{\alpha\text{CH}} - T\Delta S°_{298} + \text{of } \Delta_f H°_{298} \text{ of } \text{H}^{\bullet}_{(g)} \quad (9)$$

$^{1}/_{2}H_{2(g)}$ (65 J K^{-1} mol^{-1}) and $\Delta_f H°_{298}$ of $H^{\bullet}_{(g)}$ (218 kJ mol^{-1}) were taken from ref 29. The reduction potential for the $-NHCR^{\bullet}C(O)-$ radical versus the standard hydrogen electrode corresponds to $E°_{(10)}$ for the half-cell reaction (10). This is given by $[\Delta G°_{298} + \Delta(\Delta G°_{(soln)})]/F$,

$$-\text{NHC}^{\bullet}\text{RC(O)}- + \text{H}^{+} + \text{e}^{-} = -\text{NHCH(R)C(O)}- \quad (10)$$

where F is the Faraday constant and $\Delta(\Delta G°_{(soln)})$ is the difference in the free energies of solution for the parent molecule and its radical. For large carbon-centered radicals $\Delta(\Delta G°_{(soln)})$ is expected to be negligible.[30] Thus, $E°_{(10)}$ was estimated from $\Delta G°_{298}/F$. A similar approach was used to estimate the standard reduction potentials for the side chain radical systems.

The reduction potentials at pH 7, $E°'$, are of primary interest for biological systems, and can be obtained as follows. All of the radical reduction reactions considered here, including those for OH$^{\bullet}$ and GS$^{\bullet}$ (see below), are of the form

$$\text{D}^{\bullet} + \text{H}^{+} + \text{e}^{-} = \text{DH} \quad (11)$$

The dependence of the reduction potential on $[H^+]$ is therefore given by[31]

$$E = E° + (RT/F)\ln\{[\text{D}^{\bullet}]([\text{H}^{+}] + K_i)[\text{DH}]^{-1}\} \quad (12)$$

where K_i is the acid ionization constant of DH. Since $K_i < 10^{-7}$ for all systems considered, $E°' = E° - 0.41_4$ V.

Transition Structures. The hydrogen atom transfer reactions (13 and 14), were studied at the B3LYP/6-31G(D) level. The methanethiyl system is a model for cysteine or GSH, and (res) = (Gly) is intended to model an arbitrary residue. Transition structures for reactions 13

$$\text{CH}_3\text{S}-\text{H} + {}^{\bullet}\text{SCH}_3 = \text{CH}_3\text{S}^{\bullet} + \text{H}-\text{SCH}_3 \quad (13)$$

$$\mathbf{AH}(\text{res}) \text{ (or } \mathbf{PH}(\text{res})) + {}^{\bullet}\text{SCH}_3 = {}^{\alpha}\mathbf{A}^{\bullet}(\text{res}) \text{ (or } {}^{\alpha}\mathbf{P}^{\bullet}(\text{res})) + \text{H}-\text{SCH}_3 \quad (14)$$

and 14, as well as the intramolecular reaction (15), were located and verified by the presence of a single imaginary frequency. In this case,

$$^{S}\mathbf{A}^{\bullet}(\text{Cys}) \text{ (or } {}^{S}\mathbf{P}^{\bullet}(\text{Cys})) = {}^{\alpha}\mathbf{A}^{\bullet}(\text{Cys}) \text{ (or } {}^{\alpha}\mathbf{P}^{\bullet}(\text{Cys})) \quad (15)$$

the device of isodesmic reactions cannot be used to compensate for errors in the energy of the transition structures (TSs) and computed activation parameters will have a greater uncertainty. There are mixed revues on the applicability of density functional methods for the study

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of radical hydrogen abstractions.[32] However, the B3LYP hybrid functional (among a few others) has been demonstrated to give accurate activation energies for H abstraction from halomethanes by hydroxyl radical,[32] a system with many features in common with the present systems. For confirmation, calculations in the spirit of G2(MP2)[33] were carried out on the methanethiol system. Using geometries and zero point vibrational energies obtained at the B3LYP/6-31G(D) level, we define a G2(MP2)-like energy, E(G2(MP2-B3LYP)), eq 16, where

$$E(\text{G2(MP2-B3LYP)}) = E(\text{QCISD(T)/6-31G(D,P)}) - E(\text{MP2/6-31G(D,P)}) + E(\text{MP2/g-311+G(3DF,2P)}) - 0.005(\text{NVEP}) - 0.00019(\text{NUE}) + \text{ZPE} \quad (16)$$

NVEP and NUE are the number of valence electron pairs and number of unpaired electrons, respectively, and ZPE is the zero point energy scaled by 0.98. The results, discussed below, are supportive of the suitability of the B3LYP/6-31G(D) level for H abstraction by thiyl radicals.

Results

The structures of the cysteine-derived species, optimized at the B3LYP/6-31G(D) level are presented in Figure 2. Bond distances between heavy atoms, important hydrogen bonding distances, and the Ramachandran angles, Φ and Ψ, are given. All optimized structures shown here are local minima, as confirmed by the frequency calculations.

The electronic energies and the vibrational ZPEs calculated at the B3LYP/6-31G(D) level are listed in Table S1 in the Supporting Information. The calculated values of $H^\circ_{298} - H^\circ_0$ and S°_{298} are also given there. It may be noted that $H^\circ_{298} - H^\circ_0$ are very similar for the parent and radical species, which means that in most cases the $H^\circ_{298} - H^\circ_0$ corrections to $\Delta H^\circ_{(4)}$ are less than 1 kJ mol^{-1}. The BDEs, D^{α}_{CH}, D^{β}_{CH}, D^{α}_{CC}, and D_{SH} of the amino acid and the model peptide with opt/opt geometries are reported in Table 1 at 298 K and also as D_0 values (i.e., at 0 K). Values of D^{α}_{CH} are also reported for the case where the conformational freedom of the cysteine residue is constrained by the secondary structure of the protein. Generic values of Φ and Ψ were adopted and fixed for the antiparallel β-sheet and right-handed α-helical environments. Reduction potentials are reported in Table 2. The energies and thermodynamic data for all species required for the isodesmic reactions are given in Table S2.

The transition structures for the H atom transfer reactions (13 and 14) are shown in Figures 4 and 5, respectively, and for the intramolecular reactions of the cysteinyl radicals in Figure 6. Enthalpies, entropies, and free energies of activation for the reactions which transfer H to thiyl radical from S or C are presented in Table 3. Entropies and free energies of activation (at 298 K) are provided for two standard states, 1 atm ($\Delta S_p^{\ddagger}$, suitable for gas phase reactions) and 1 M ($\Delta S_c^{\ddagger}$, suitable for reactions in solution). B3LYP/6-31G(D) energies, ZPEs, and $H^\circ_{298} - H^\circ_0$ and S°_{298} of the transition structures are given in Table S3 of the Supporting Information.

Discussion

Structures of Minima. Since the primary purpose of the present paper is to report the BDEs of the weakest bonds, and to obtain reliable estimates for H transfer activation energies, an exhaustive search of conformational space was not undertaken. In each case, the parent cysteine moieties **AH**(Cys) or **PH**(Cys) have the L (i.e., (*R*)) absolute configuration. Backbone conformation, as defined by (Φ, Ψ) in the case of the residue model, was selected to coincide approximately with the normal orientation of the backbone in disordered or β-sheet regions of proteins. That is, the formyl amide unit has a trans conformation, and the Ramachandran angles Φ and Ψ are near 180°. Each model peptide (**P**) structure has an intramolecular N−H···O═C hydrogen bond as can be seen from the short H···O separation (Figure 2b,c). This attractive interaction can be accommodated by disrotatory deformation of the (Φ, Ψ) angles, yielding a (+, −) or (−, +) pattern of signs. Only the latter gives rise to locally stable conformations independent of the orientation of the side chain. It involves optimized (Φ, Ψ)

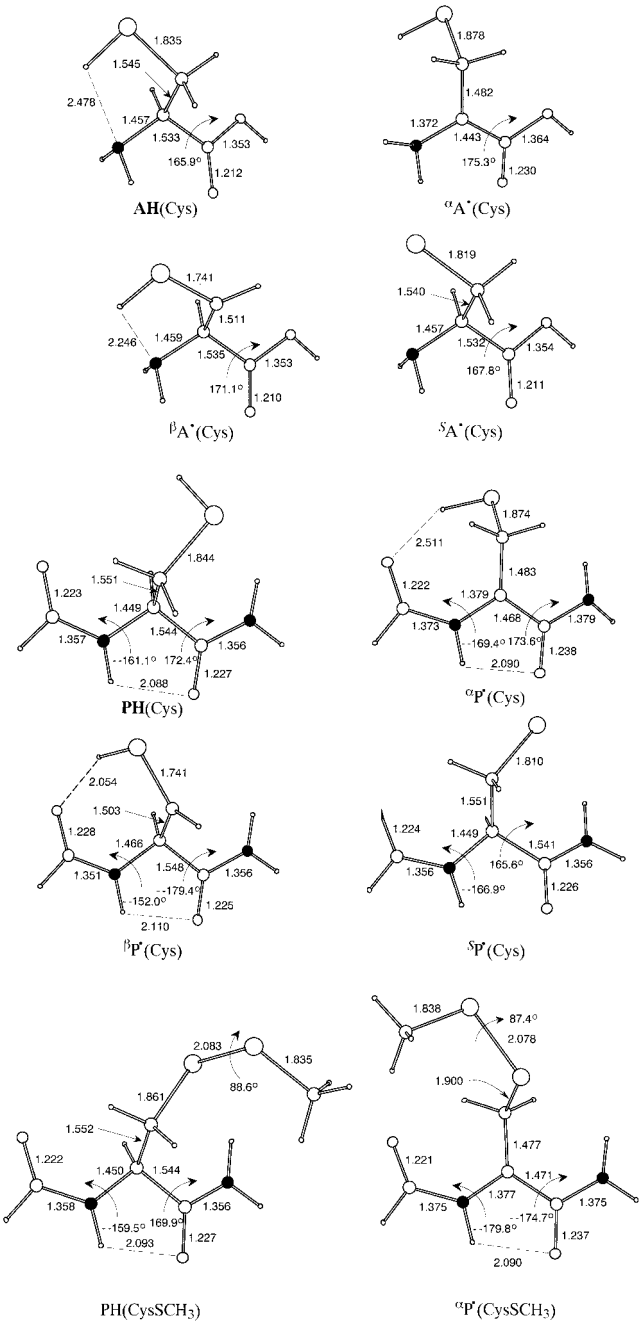

Figure 2. B3LYP/6-31G(D) optimized structures of the parent molecule and radical species of (a, top two rows) cysteine free neutral amino acid, (b, middle two rows) cysteine peptide model, and (c, bottom row) cystine peptide model. The filled circles stand for nitrogen and the shaded ones for oxygen atoms. The small, medium, and large open circles are hydrogen, carbon, and sulfur, respectively. Bond lengths are in angstroms and angles in degrees.

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Table 1. Bond Dissociation Energies at 298 K (kJ mol^{-1})[a]

compound[b]	D^{α}_{CH}[c]	D^{β}_{CH}[d]	D^{α}_{CC}[e]	D_{SH}[f]
AH(Cys) opt/opt	321.7 (315.6)	378.3 (372.9)	264.3 (259.6)	373.1 (367.6)
PH(Cys) opt/opt	345.5 (338.7)	380.9 (374.9)	286.9 (281.1)	367.0 (360.9)
PH(Cys) β-sheet	359.1			
PH(Cys) α-helix	375.7			
PH(CysSCH$_3$) opt/opt	329.8 (324.7)			

[a] Values in parentheses are at 0 K. B3LYP/6-31G(D) + 0.98 × ZPE level calculations and isodesmic reactions. [b] opt/opt designates optimized Φ, Ψ: for β-sheet, $\Phi = -150°$, $\Psi = +150°$; for α-helix, $\Phi = -60°$, $\Psi = -45°$. [c] From isodesmic reaction: $^{\alpha}$**A**$^{\bullet}$ or $^{\alpha}$**P**$^{\bullet}$ + $NH_2CH_2COOH \rightarrow$ **AH** or **PH** + $NH_2CHCOOH^{\bullet}$. [d] From isodesmic reaction: $^{\beta}$**A**$^{\bullet}$ or $^{\beta}$**P**$^{\bullet}$ + $HSCH_3 \rightarrow$ **AH** or **PH** + $HSCH_2^{\bullet}$. [e] From isodesmic reaction with alanine, see the text, eq 6. [f] From isodesmic reaction: **AH**(Cys) or **PH**(Cys) + $CH_3S^{\bullet} \rightarrow$ ^{S}**A**$^{\bullet}$(Cys) or ^{S}**P**$^{\bullet}$(Cys) + CH_3SH.

angles close to those observed for the antiparallel pleated sheet structure. For each species, several orientations of the side chain were sampled. The structures shown in Figure 2 are expected to be the most stable of each species.

Considerable variability is found in the C−S bond length. In the parents, **AH**(Cys) and **PH**(Cys), r(C−S) = 1.84 Å, the value also found in CH_3SH at the same level of theory, and about 0.02 Å longer than experimentally measured in paraffinic thiols and thioethers and crystal X-ray data on L-cysteine.[34] Substitution of the −SH by $-SSCH_3$ (**PH**(CysSCH$_3$), Figure 2c) results in a slight lengthening of the $^{\alpha}$C−S bond but not the S−CH_3 bond. Loss of the hydrogen atom from sulfur results in a 0.02−0.03 Å shortening of the C−S bond, and formation of the $^{\beta}$C-centered radical has a more profound effect. Formation of the $^{\alpha}$C radical has an opposite effect. This radical can derive some additional benefit from a stabilizing one-electron interaction between the 2p orbital of the radical site and the antibonding σ orbital (σ^*_{CS}) of the C−S bond; in the most stable structure ($^{\alpha}$**A**$^{\bullet}$(Cys), $^{\alpha}$**P**$^{\bullet}$(Cys), or $^{\alpha}$**P**$^{\bullet}$(CysSCH$_3$), Figure 2), these orbitals are parallel as can be seen from the perpendicular arrangement of the C−S bond relative to the plane at the radical site. As this interaction involves some electron transfer into σ^*_{CS}, a lengthening of the bond is expected (0.03−0.06 Å calculated). Conversely, the singly occupied 2p orbital of the $^{\beta}$C-centered radical partakes directly in an attractive three-electron interaction with the occupied 3p orbital of the S atom. The increase in double bond character of the C−S bond is reflected in a shortening by 0.1 Å of the bond to a value similar to that found in thiophene. This interaction involves charge transfer away from sulfur and a concomitant acidification of the S−H bond. The latter is evident in the shorter hydrogen bonds in those species where H-bonding occurs (i.e., in $^{\beta}$**A**$^{\bullet}$(Cys) vs **AH**(Cys), and $^{\beta}$**P**$^{\bullet}$(Cys) vs $^{\alpha}$**P**$^{\bullet}$(Cys)).

Bond Dissociation Energies (BDEs) at 298 K. Table 1. D_{SH}**.** The S−H BDE of **AH**(Cys) is found to be 373.1 kJ mol^{-1} by isodesmic reaction with CH_3SH (D_{SH} = 365.5 ± 2.4 kJ mol^{-1}).[28] The corresponding value in **PH**(Cys) is 367.0 kJ mol^{-1}, nearly the same as for CH_3SH. The slightly higher value in **AH**(Cys) probably arises from stabilization of the parent by the N···H−S hydrogen bond (Figure 2a), and the difference, 6 kJ mol^{-1}, may be taken as an indication of the strength of such a bond. The most stable conformation of the parent **PH**(Cys) does not have a hydrogen bond although there is a weak S−H···O= hydrogen bond in the radical, $^{\alpha}$**P**$^{\bullet}$(Cys). Direct calculation of D_{SH} by use of eq 17 at the B3LYP/6-31G(D) level yields

$$R{-}H = R^{\bullet} + H^{\bullet} \qquad (17)$$

values which are too low by about 24 kJ mol^{-1}, i.e., 341.2, 348.8, and 342.6 for CH_3SH, **AH**(Cys), and **PH**(Cys), respectively.

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D_{CS}**.** Bond dissociation energies (not shown in Table 1) of the C−S bonds in **AH**(Cys) (D_{CS} = 301.2 kJ mol^{-1}) and **PH**(Cys) (D_{CS} = 304.3 kJ mol^{-1}) are calculated by isodesmic reaction with CH_3SH, for which D_{CS} = 303.8 kJ mol^{-1}.[28] Energies for $^{\beta}$**A**$^{\bullet}$(Ala) and $^{\beta}$**P**$^{\bullet}$(Ala) required for the isodesmic reaction were previously published.[17]

D^{α}_{CC}**.** Dissociation of the $^{\alpha}$C−C bond was previously discussed for the case of **AH**(Ala) and **PH**(Ala). As it yields $^{\alpha}$**A**$^{\bullet}$(Gly) and $^{\alpha}$**P**$^{\bullet}$(Gly), respectively, no suitable isodesmic partner existed. It was shown that the present method, B3LYP/6-31G(D), applied to the equation for direct dissociation of **AH**(Ala) (analogous to eq 7) underestimates D^{α}_{CC} by 23 kJ mol^{-1} relative to higher level calculations (G2(MP2)′) and a value derived from experimental heats of formation.[17] However, **HA**(Ala) (D^{α}_{CC} = 292.0 kJ mol^{-1}) may now be used as an isodesmic partner to determine D^{α}_{CC} by eq 6. For **AH**(Cys) and **PH**(Cys), the values 264.3 and 286.9 kJ mol^{-1}, respectively, are obtained. The difference reflects the relative stabilities of the $^{\alpha}$**A**$^{\bullet}$(Gly) and $^{\alpha}$**P**$^{\bullet}$(Gly) radicals and is also seen in the D^{α}_{CH} values of the glycine species.[16] It should be noted that direct application of eq 7 yields 240.3 and 262.8 kJ mol^{-1}, respectively, which are about 25 kJ mol^{-1} too low as expected.

D^{β}_{CH}**.** Removal of one of the hydrogen atoms from $^{\beta}$C yields an alkyl radical adjacent to sulfur. The experimental value (at 0 K)[27] for D_{CH} of CH_3SH, 387 ± kJ mol^{-1}, is lower than a value derived at the G2 level, 397.5 kJ mol^{-1}.[35] Direct calculation by the B3LYP/6-31G(D) method yields an intermediate value, 393.2 kJ mol^{-1} (=399.9 at 298 K). In Table 1 are reported the BDEs of **AH**(Cys) and **PH**(Cys), obtained by isodesmic reaction with CH_3SH. The values obtained are (at 298 K) (**AH**(Cys)) D^{β}_{CH} = 378.3 kJ mol^{-1} and (**PH**(Cys)) D^{β}_{CH} = 380.9 kJ mol^{-1}. Because of the large experimental uncertainty in the methanethiol value, the uncertainties in the computed values for **AH**(Cys) and **PH**(Cys) are correspondingly larger. The C−H BDE of ethane is known to greater precision (D_{CH} = 423.0 ± 1.7 kJ mol^{-1} (298 K)).[36] The values obtained using ethane as isodesmic partner are (**AH**(Cys)) D^{β}_{CH} = 389.7 kJ mol^{-1} and (**PH**(Cys)) D^{β}_{CH} = 392.2 kJ mol^{-1}. While these values are just inside the expected uncertainty, we believe the lower values of Table 1 to be the more realistic. The C−H bond of CH_3SH is slightly weaker than that of CH_3OH (D_{CH} = 395 kJ mol^{-1}),[36] and this should be reflected to some extent in the $^{\beta}$C−H bonds of **AH**(Cys) and **PH**(Cys) compared to **AH**(Ser), D^{β}_{CH} = 385 kJ mol^{-1}, and **PH**(Ser), D^{β}_{CH} = 377 kJ mol^{-1}.[17]

D^{α}_{CH}**.** Of seminal interest are the strengths of the $^{\alpha}$C−H bonds, since, together with the S−H bonds, these are likely to be the primary sites of oxidative damage to cysteine in proteins. The results shown in Table 1 indicate that, in the case of neutral

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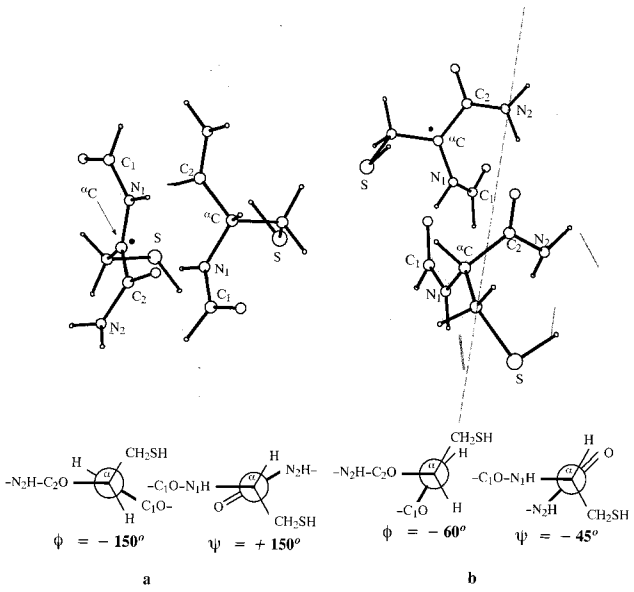

Figure 3. Structures of **PH**(Cys) and $^{\alpha}$**P**$^{\bullet}$(Cys) superimposed on (a) antiparallel β-sheet and (b) right-handed α-helix.

cysteine, **AH**(Cys), $D^{\alpha}_{CH} = 321.7$ kJ mol^{-1}, approximately 51 kJ mol^{-1} weaker than the S−H bond. In the peptide model, **PH**(Cys), the BDE rises due to reduced captodative stabilization, $D^{\alpha}_{CH} = 345.3$ kJ mol^{-1}, approximately 22 kJ mol^{-1} weaker than the S−H bond. The reduction in captodative stabilization arises from the fact that conversion of both the free $-NH_2$ group and the −COOH group to amides reduces their ability to act as donor and acceptor, respectively. The captodative stabilization is also affected by the ability of the $^{\alpha}$C radical to achieve planarity of the heavy atom skeleton. The optimized structures of **PH**(Cys) and $^{\alpha}$**P**$^{\bullet}$(Cys) are shown in Figure 2. As the skeletal dihedral angles, Φ and Ψ, reveal, the structure of $^{\alpha}$**P**$^{\bullet}$(Cys) differs from planarity by only 10.6° and 6.4°, and only a small structural change in the backbone of **PH**(Cys) is required to achieve these optimum values after H atom abstraction. In the case of the cystine model, **PH**(CysSCH$_3$) (Figure 2c), $D^{\alpha}_{CH} =$ 329.8 kJ mol^{-1}. The lower value of $D^{\alpha}C_H$ in **PH**(CysSCH$_3$) compared to **PH**(Cys) is a consequence of additional stabilization of the $^{\alpha}$C radical by the $^{\beta}$C−S bond which is unusually long (1.900 Å). The antibonding orbital of this bond receives electrons from the radical site as well as the nonbonding lone pair of the distal sulfur atom which is almost perpendicular to the first. The latter interaction is evident in the slight shortening of the S−S bond.

It is reasonable that the local structures and structural changes suggested by the model peptide systems shown in Figure 2 are indeed realizable in unstructured regions of real proteins in which cysteine (or its dimeric form, cystine) is more commonly found. Nevertheless, the tertiary and especially the secondary structures of proteins impose limitations on the values of Φ and Ψ that any particular residue can adopt. In Figure 3 are shown **PH**(Cys) and $^{\alpha}$**P**$^{\bullet}$(Cys) superimposed on the antiparallel β-sheet and right-handed α-helix secondary structural units. The backbone geometry is defined by the constraints (Φ = −150, Ψ = +150) and (Φ = −60, Ψ = −45), respectively. By restricting Φ and Ψ to these values, one can model the effect of electronic factors on the bond dissociation energy. For the cysteine residue in the "β-sheet" configuration, $D^{\alpha}_{CH} = 359$ kJ mol^{-1}, about 15 kJ mol^{-1} higher than in a more flexible region modeled by the opt/opt geometries. Interstrand steric effects are not taken into account here. These are minor in the case of the parent **PH**(Cys) because the residue side chain projects out of the sheet. However, formation of the $^{\alpha}$C radical is accompanied by movement of the side chain toward the plane of the sheet. While the local steric bulk of the $-CH_2SH$ group is similar to a simple methyl group, movement may additionally be restricted if the sulfur atom is involved in cross-linking (as in cystine). Therefore, the value 359 kJ mol^{-1} should be regarded as a *lower* estimate.

Table 2. Reduction Potentials, $E^{\circ\prime}$(Radical$^{\bullet}$ + H$^+$ + e$^-$ = Parent), at 298 K at pH 7 (V)

parent\radical	$^{\alpha}$C$^{\bullet}$	$^{\beta}$C$^{\bullet}$	S$^{\bullet}$
PH(Cys) opt/opt	0.70	1.17	0.93 (0.93)[a]
PH(Cys) β-sheet	0.84		
PH(Cys) α-helix	1.01		
PH(CysSCH$_3$) opt/opt	0.57		

[a] GS$^{\bullet}$/GSH, ref 37.

For the cysteine residue in the α-helical environment, $D^{\alpha}_{CH} = 376$ kJ mol^{-1}. The higher value is due to substantial loss of captodative stabilization in the far from planar geometry. The difference from the opt/opt geometry, 30 kJ mol^{-1}, is a lower estimate for the loss of captodative stabilization since some relief of steric strain also ensues from radical formation. The steric situation of a protein α-helical environment is more accurately modeled in this case, since steric hindrance is more local and taken into account in the optimization of the parent. Formation of the $^{\alpha}$C radical causes the side chain to project radially away from the cylinder of the helix. Therefore, the predicted value, $D^{\alpha}_{CH} = 376$ kJ mol^{-1}, should be representative of the actual strength of the $^{\alpha}$C−H bond. Since it is higher than D_{SH}, oxidative abstraction of a hydrogen atom (by OH radical, for instance) would be repairable by S−H, or should occur preferentially at S in the first place.

Reduction Potentials, $E^{\circ\prime}$. Standard reduction potentials at pH 7, $E^{\circ\prime}$, for the peptide mimics are shown in Table 2. $E^{\circ\prime}$ for the couple $^{\alpha}$**P**$^{\bullet}$(Cys)/**PH**(Cys) is compared with GS$^{\bullet}$/GSH in the last column. The electrochemical properties at S should be similar in the two, and the excellent agreement of the computed and experimental values lends support to the present procedures. The free energy change for the H transfer process shown in reaction 2 is directly given by $-F\{E^{\circ\prime}(^{\alpha}\mathbf{P}^{\bullet}/\mathbf{PH}) - E^{\circ\prime}(\mathrm{GS}^{\bullet}/\mathrm{GSH})\}$ and is expected from Table 2 to be positive (favoring the $^{\alpha}$C radical) for both cysteine (**PH**(Cys)) and cystine (modeled by **PH**(CysSCH$_3$)) residues, in disordered or β-sheet regions of the protein. Thus, cysteine and cystine, like glycine, alanine, serine, and threonine residues,[17] are liable to be damaged by thiyl radicals (GS$^{\bullet}$, $E^{\circ\prime} = 0.93$ V),[37] as well as other mild oxidizing agents like peroxy ($E^{\circ\prime} = 1.06$ V)[38] and tyrosyl ($-C_6H_4O^{\bullet}$, $E^{\circ\prime} = 0.97$ V).[39] On the other hand, as was the case with Gly, Ala, Ser, and Thr,[17] the α-helical environment would confer some protection to Cys residues. As expected on the basis of its higher BDE, the $^{\beta}$C−H bond ($^{\beta}$**P**$^{\bullet}$(Cys), $E^{\circ\prime} = 1.17$ V) is not susceptible to damage by the milder oxidizing radicals. We now address the probable *rates* of the thermodynamically favorable free radical H abstraction reactions involving RS$^{\bullet}$.

Structures of Transition States. In the present context, thiyl radicals (cysteinyl and/or glutathiyl) may abstract hydrogen atoms from other sulfhydryl species (S to S transfer) or from

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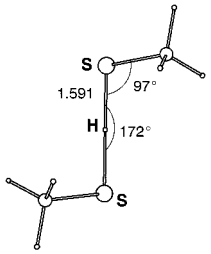

Figure 4. B3LYP/6-31G(D) optimized transition structure for H atom transfer from CH_3SH to $^{\bullet}SCH_3$.

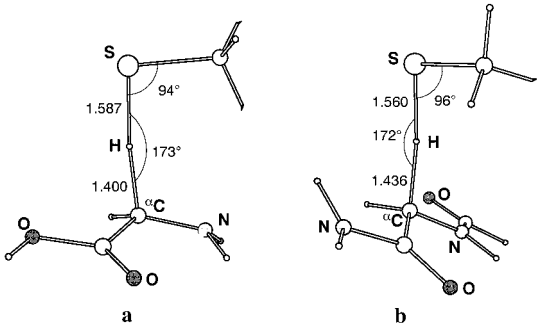

Figure 5. B3LYP/6-31G(D) optimized transition structure for H atom transfer from (a) **AH**(Gly) and (b) **PH**(Gly) to $^{\bullet}SCH_3$.

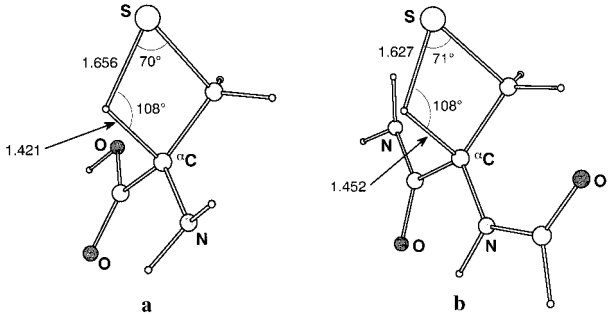

Figure 6. B3LYP/6-31G(D) optimized transition structure for intramolecular H atom transfer within (a) **AH**(Cys) and (b) **PH**(Cys).

the weak $^{\alpha}$C−H bonds (C to S transfer). The transition structures for these two intermolecular processes are well represented by the methanethiyl−methanethiol and methanethiyl−glycine systems shown in Figures 4 and 5, respectively. These unconstrained intermolecular reactions define the ideal stereochemical requirements for H atom transfers at the S atom. In the symmetrical S to S case (Figure 4), the S−H−S geometry is almost linear ($\angle$SHS = 172°), and S−C bonds are oriented nearly perpendicular to the line of the target S−H bond ($\angle$CSH = 97°). The S−H distance is 1.591 Å, about 0.24 Å longer than in methanethiol itself. Similar geometric parameters are found in the TSs for H abstraction from **AH**(Gly) and **PH**(Gly) (Figure 5)—$\angle^{\alpha}$CHS = 173° and 172°, respectively, and $\angle$HSC = 94° and 96°, respectively. The S−H distance is similar to the S to S case, 1.587 Å for abstraction from **AH**(Gly), but shorter, 1.560 Å, for **PH**(Gly). The $^{\alpha}$C−H bond is significantly stretched, to 1.400 Å in the case of **AH**(Gly), and even more so, to 1.436 Å for **PH**(Gly), and the direction of departure of the H from the $^{\alpha}$C center is similar to the direction of the original $^{\alpha}$C−H bond.

The transition structures for intramolecular H transfer in ^{S}**A**$^{\bullet}$(Cys) and ^{S}**P**$^{\bullet}$(Cys) (Figure 6) differ substantially from the intermolecular case (Figure 5). The four-membered ring geometry forces significant deviations from the optimum collinearity at the migrating H ($\angle^{\alpha}$CHS = 108°) and the perpendicular geometry at S ($\angle$HSC = 70°). In addition, both the $^{\alpha}$C−H and H−S distances are longer than optimal, by about 0.05 Å). As discussed below, these deviations represent increased strain and a substantial increase in the activation energy for H atom transfer.

Table 3. Enthalpies, Entropies, and Free Energies of Activation for the Forward Reaction RH + $^{\bullet}$SR′ = R$^{\bullet}$ + HSR′

species	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ [a] (J K^{-1} mol^{-1})	$\Delta G^{\ddagger}$ [a] (kJ mol^{-1})
^{S}**A**$^{\bullet}$(Cys) → $^{\alpha}$**A**$^{\bullet}$(Cys)	82.9	−14.4	87.2
^{S}**P**$^{\bullet}$(Cys) → $^{\alpha}$**P**$^{\bullet}$(Cys)	110.6	−15.5	115.2
$[CH_3S\cdots H\cdots SCH_3]^{\bullet}$	11.9[b]	−118.3 (−91.7)	47.2 (39.2)
$^{\alpha}$**A**$^{\bullet}$(Gly)···H···SCH_3	24.2	−146.7 (−120.1)	67.9 (60.0)
$^{\alpha}$**P**$^{\bullet}$(Gly)···H···SCH_3	26.8	−148.5 (−121.9)	71.1 (63.1)

[a] Standard state = 1 atm; numbers in parentheses correspond to standard state = 1 M. The entropies are related by $\Delta S(1\text{ M}) = \Delta S(1\text{ atm}) - \Delta nR\ln(R'T)$, for a unit change in molarity, $\Delta n = -1$, and $R = 8.3145$ J K^{-1} mol^{-1}, $R' = 0.082\,06$ dm^3 atm K^{-1} M^{-1}, $T = 298$ K. [b] At the G2(MP2-B3LYP) level, $\Delta H^{\ddagger} = 15.8$ kJ mol^{-1} (see the text).

Activation Parameters and Reaction Rates for H Atom Transfers to Thiyl Radicals. Activation parameters may be derived from the data in Tables 3, S1, and S2, and are shown in Table 3. The calculated enthalpy of activation of the identity reaction (13) for S to S transfer is 11.9 kJ mol^{-1}. The G2(MP2-B3LYP) value is very similar, 15.8 kJ mol^{-1}, and suggests that the B3LYP/6-31G(D) may be on the low side. The reaction has a large negative entropy of activation, −118.3 J K^{-1} mol^{-1}, and consequently, a substantially larger free energy of activation at 298 K, 47.2 kJ mol^{-1}, in the gas phase at 1 atm of pressure. When adjusted for a standard concentration of 1 M, the entropy and free energy of activation become $\Delta S_c^{\ddagger} = -91.7$ J K^{-1} mol^{-1} and $\Delta G_c^{\ddagger} = 39.2$ kJ mol^{-1}, respectively.

The intermolecular process of a thiyl radical abstracting a hydrogen atom from the $^{\alpha}$C position of an amino acid or amino acid residue is modeled by the reaction of methanethiyl with glycine (AH(Gly)) and glycine model peptide (PH(Gly)). The former reaction is exergonic by the difference in the S−H BDE of CH_3SH and the $^{\alpha}$C−H BDE of **AH**(Gly), namely, 35 kJ mol^{-1}. The reaction with **PH**(Gly) is exergonic by 18 kJ mol^{-1}. The predicted enthalpies of activation for the two reactions are very similar, 24.2 and 26.8 kJ mol^{-1}, respectively, as are the entropy changes, −146.7 and −148.5 J K^{-1} mol^{-1}. The Arrhenius activation energy for the reaction of methyl radical with methanethiol has been measured.[40,41] This reaction is exergonic by 69 kJ mol^{-1} in the direction opposite that of the present case. The measured activation energy, $E_a = 17$ kJ mol^{-1} in the temperature range 403−473 K,[40] and $E_a = 21$ kJ mol^{-1} at 303 K,[41] is in the same range as the present values of $\Delta H^{\ddagger}$. Rate constants for the intermolecular abstraction of $^{\alpha}$C−H of glycine and alanine by the thiyl radicals (of cysteine, homocysteine, and glutathione) have been measured in aqueous solution at pH 10.5.[9] The values found were 3.2×10^5 and 7.7×10^5 M^{-1} s^{-1}, respectively. The E_a values implied by these rates, $E_a = 26 \pm 6$ and 24 ± 6 kJ mol^{-1} (assuming $A = 10^{10\pm1}$), are numerically the same as our $\Delta H^{\ddagger}$ values for H abstraction from **AH**(Gly) and **PH**(Gly). In order to compare the theoretical $\Delta H^{\ddagger}$ and experimental E_a values more directly, one would need to know the net change in free energies of solution, and to convert $\Delta H^{\ddagger}$ to E_a.

The rate constant for the intramolecular conversion of glutathione thiyl (GS$^{\bullet}$) to the $^{\alpha}$C radical derived by loss of the glutamyl $^{\alpha}$C−H bond (GC$^{\bullet}$) has also been measured under the same conditions and found to be similar to intermolecular values,

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(41) Kerr, J. A.; Trotman-Dickensen, A. F. *J. Chem. Soc.* **1957**, 3322.

$k_r = 1.8 \times 10^5$ s^{-1}, implying a free energy of activation of 43.0 kJ mol^{-1} (from $\Delta G^{\ddagger} = RT[\ln((k/h)T) - \ln(k_r)]$. If the entropy change for this intramolecular process were in the vicinity of -60 kJ mol^{-1} (a value intermediate between the large intermolecular entropy changes and the predicted intramolecular values for the cysteine derivatives which have less flexibility (Table 3, see below)), then $\Delta H^{\ddagger}$ for this process also is about 25 kJ mol^{-1}. The similarity between the intermolecular processes and the intramolecular reaction in GSH is reasonable because the nine-membered ring TS of the latter would be relatively strain-free. The equilibrium constant for GS$^{\bullet}$ = GC$^{\bullet}$ is larger than 10^4, indicating $\Delta G^{\circ} > 23$ kJ mol^{-1} in favor of the $^{\alpha}$C-centered radical.[10] Assuming that $\Delta S^{\circ} \cong 0$, this value which represents the difference in $^{\alpha}$C−H and S−H BDEs is consistent with the present system, using the BDE of **AH**(Ala), 317 kJ mol^{-1}, to represent the glutamine moiety of glutathione.

The case of GS$^{\bullet}$ = GC$^{\bullet}$ is in sharp contrast to ^{S}**A**$^{\bullet}$(Cys) = $^{\alpha}$**A**$^{\bullet}$(Cys) and ^{S}**P**$^{\bullet}$(Cys) = $^{\alpha}$**P**$^{\bullet}$(Cys), for which the transition structures (Figure 6) are predicted to be at $\Delta H^{\ddagger} = 82.9$ and 110.6 kJ mol^{-1}, relative to the corresponding thiyl radicals. The large calculated activation enthalpy is inconsistent with the high rate ($k_r = 2.4 \times 10^4$ s^{-1}) of intramolecular S to C transfer of H in **AH**(Cys) at pH 10.5 as reported by Zhao and co-workers[9] since this would imply an activation energy of about 48 kJ mol^{-1}.

Conclusions

The weakest bond to hydrogen in the cysteine residue is the $^{\alpha}$C−H bond. The value of D^{α}_{CH} depends on the local peptide environment: 346 kJ mol^{-1} if the radical structure can relax to its optimum value, 359 kJ mol^{-1} if the residue is part of an antiparallel β-sheet secondary structure, and 376 kJ mol^{-1} if it is part of a right-handed α-helix. The increase in BDE mirrors a corresponding decrease in the stability of the $^{\alpha}$C radical due to loss of captodative stabilization as a result of increasing nonplanarity of the peptide backbone. Nevertheless, the low values suggest that $^{\alpha}$C radicals in the first two circumstances will not be repaired by glutathione and in fact may be generated by nearby −S$^{\bullet}$ radicals since $D_{SH} = 367$ kJ mol^{-1} and the predicted reduction potentials are less than that of thiyl radicals. Calculations on the model system for cystine suggest that additional stabilization of the $^{\alpha}$C radical of cystine arises from increased acceptor ability of the adjacent C−S bond, further deceasing the strength of the $^{\alpha}$C−H bond to 330 kJ mol^{-1}.

Intermolecular S to S transfer of a hydrogen atom is predicted to have an activation enthalpy of only 11.9 kJ mol^{-1}. Low enthalpies of activation, about 25 kJ mol^{-1}, are also predicted for intermolecular C to S transfer in cysteine and cysteine residues in peptides. In each case, the transition structure is close to linear in the centers directly involved and the orientation of the S atom is close to perpendicular. These conditions cannot be met in the four-membered ring cyclic transition structure for intramolecular C to S transfer, and as a consequence the computed enthalpy of activation is substantially higher, 83 and 111 kJ mol^{-1}, for neutral cysteine and cysteine residues, respectively.

Since cysteine/cystine residues are naturally found near the redox sites of many enzymes, where free radical species are generated, damage to the $^{\alpha}$C site of neighboring cysteine or other residues may be a primary step in oxidative damage to the proteins involved.

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Supporting Information Available: A listing of calculated energies in hartrees, ZPEs, and S°_{298} and $H^{\circ}_{298} - H^{\circ}_{0}$ values for all species (Tables S1−S3) (3 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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