Toward Site Specificity of Oxidative Damage in Proteins: C−H and C−C Bond Dissociation Energies and Reduction Potentials of the Radicals of Alanine, Serine, and Threonine Residues—An ab Initio Study

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Abstract: High-level ab initio computations were used to characterize the parent species and $^{\alpha}$C radicals for alanine, serine, and threonine, both as free neutral amino acids (AH) and as residues in model peptides (PH) intended to mimic the midchain environment in proteins. The ab initio energies were used in isodesmic reactions to predict bond dissociation energies (BDEs, $D_{^{\alpha}CH}$) at 298 K, in kJ mol^{-1}, to an estimated accuracy of ±10 kJ mol^{-1}. For the fully optimized systems the values of $D_{^{\alpha}CH}$ are AH(Gly), 331; AH(Ala), 317; AH(Ser), 327; AH(Thr), 328; PH(Gly), 348; PH(Ala), 344; PH(Ser), 348; PH(Thr), 356. All of the $D_{^{\alpha}CH}$ values are *less* than the BDE of a typical SH bond (370 kJ mol^{-1}), as in cysteine or glutathione (GSH), a result that suggests that oxidative damage at the $^{\alpha}$C site will not be repaired efficiently by the mechanism of H donation from GSH. Values of $D_{^{\alpha}CH}$ in typical peptide conformations, such as β-sheet and α-helical secondary structure, were estimated by constraining the Ramachandran dihedral angles, Φ and Ψ, to values typical of these structures. Thus $D_{^{\alpha}CH}$ values are estimated as PH(Gly), 361; PH(Ala), 359; PH(Ser), 347; PH(Thr), 356 in the β-sheet conformation, and PH(Gly), 402; PH(Ala), 384; PH(Ser), 381; PH(Thr), 363 in the α-helix conformation. Hence, these residues are also expected to be susceptible to irreparable oxidative damage in β-sheet structures, but Gly, Ala, and Ser residues in α-helical regions should be less susceptible to damage and should be repairable by GSH. A consideration of reduction potentials calculated from the BDEs and entropies derived from the ab initio results leads to the same conclusions and indicates that certain radicals other than OH$^{\bullet}$ that occur in cells (e.g., ROO$^{\bullet}$) may also cause oxidative damage to β-sheet structures. Ab initio calculations were also done for the C-centered radicals formed by removal of H from the side chains. These showed that there is a marked increase in the ease of abstraction of this H in the series Ala, Ser, and Thr.

Introduction

Oxidative damage to proteins has been implicated in pathological disorders, such as protein turnover, cataractgenesis, atherosclerosis, and tissue injury during ischemia−reperfusion.[1] Oxidation is generally initiated by OH$^{\bullet}$ radicals, which may be formed intracellularly by a Fenton type reaction or by other radicals created from enzyme reactions.[1−5] Ultraviolet and ionizing radiation can also produce OH$^{\bullet}$ radicals.[6] The site of radical attack is obviously a matter of great interest, since this will determine how a protein is degraded and whether potentially damaging products may be produced from it. The side groups of some amino acid residues, for example, cysteine, tyrosine, and phenylalanine, react rather rapidly with OH$^{\bullet}$ [7] and, if present, are often affected.[1,6,8] However, the $^{\alpha}$C center of amino acid residues is also a major point of attack.[1,9,10] In the presence of oxygen the $^{\alpha}$C-centered radical forms peroxy compounds, and the protein is cleaved at that point.[1,9−11] It is of considerable interest to identify which amino acid residues are most susceptible to such oxidative damage and whether the predisposition to damage is site dependent.

The initial hydrogen atom abstraction from an $^{\alpha}$C-center, reaction 1, is shown in Figure 1 where the terminology used in this paper is also introduced. The peptide unit is designated as PH(res), where "res" specifies the type of residue, viz., Gly, Ala, etc. The radical is designated as $^{\alpha}$P$^{\bullet}$(res). The preferred sites of attack by OH$^{\bullet}$ 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 bond dissociation energies and/or the $^{\alpha}$P$^{\bullet}$/PH reduction potentials is 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),[5,6,12−14] viz.

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(1) Stadman, E. R. *Annu. Rev. Biochem.* **1993**, *62*, 797.
(2) Sies, H. *Oxygen Stress*; Academic Press: London, 1985.
(3) Sies, H. *Oxidative Stress-Oxidants and Anti-Oxidants*; Academic Press: London, 1991.
(4) Simic, M. G.; Taylor, K. A.; Ward, J. F.; von Sonntag, C. *Oxygen Radicals in Biology and Medicine*; Plenum Press: New York, 1988.
(5) Davies, K. J. A. *Oxidative Damage and Repair: Chemical, Biological and Medical Aspects*; Pergamon Press: New York, 1991.
(6) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.
(7) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.
(8) Sharpatyi, V. A. *High Energy Chem.* **1995**, *29*, 85.
(9) Garrison, W. M. *Curr. Top. Radiat. Res.* **1968**, *4*, 43; *Radiat. Res. Rev.* **1972**, *3*, 305.
(10) Mieden, O. J.; von Sonntag, C. *Z. Naturforsch.* **1989**, *44b*, 959.
(11) Mieden, O. J.; Schuchmann, M. N.; von Sonntag, C. *J. Phys. Chem.* **1993**, *97*, 3783.
(12) von Sonntag, C.; Schuchmann, H.-P. In *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; NATO ASI Series; Plenum Press: New York, 1990; p 409.

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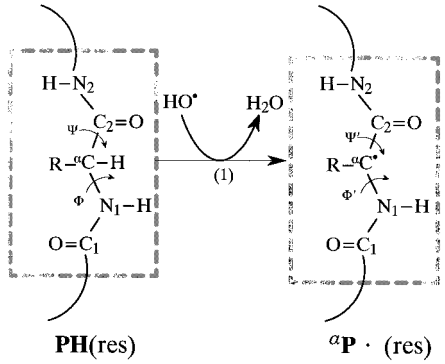


Figure 1. Illustration of the parent peptides, PH(res), and $^{\alpha}$C-centered radicals, $^{\alpha}$P$^{\bullet}$(res), considered here and of the OH$^{\bullet}$ abstraction of H from an α-carbon atom (reaction 1). R is H, CH_3, CH_2OH, and $CH(CH_3)$-OH, respectively, for res = Gly, Ala, Ser, and Thr. Φ and Ψ are the usual Ramachandran angles; see also Figure 2.

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

At the present time reduction potentials are available for the side groups of a number of amino acids.[15,16] However, for the $^{\alpha}$C-centered radicals only theoretical estimates of these parameters for the anions of glycine[17] and alanine[18] appear to exist. For the $^{\alpha}$C-centered radicals in proteins, the only available information appears to be the theoretical estimate of bond dissociation energies (BDEs, $D_{^{\alpha}CH}$) and $E°$ for the midchain glycine residue from this laboratory.[19] On the basis of a nearly quantitative theoretical model, values of $D_{^{\alpha}CH}$ = 348 kJ/mol and $E°$ = 1.2 ± 0.2 V were calculated for the $^{\alpha}$P$^{\bullet}$(Gly)/PH-(Gly) system with Ramachandran angles of Φ = 180° and Ψ = 180°. The predicted low values of $D_{^{\alpha}CH}$ and $E°$ indicate that not only is the $^{\alpha}$C−H bond highly susceptible to H abstraction by hydroxyl radical but the damaged site is not even repairable by glutathione! The Φ,Ψ angles associated with the above values correspond to the fully relaxed glycyl peptide and its radical. Glycine residues with structures close to the relaxed geometry are especially prevalent in glycine-rich proteins with a substantial *β*-sheet content, such as silk fibroins.

Obviously it is important to examine the effects of changing the R group from H to larger substituents, and the objective of the present work is to obtain estimates of $D_{^{\alpha}CH}$ and $E°$ values for the residues of alanine (Ala, R = CH_3−), serine (Ser, R = $HOCH_2$−), and threonine (Thr, R = (*R*)-$HOCH(CH_3)$−). These were chosen for several reasons. Alanine is the simplest amino acid after glycine and is also of high abundance in the fibroins. Serine and threonine, with polar hydroxyl groups, are most often found on the surface of proteins (together with the ionizable amino acids) and so are accessible in the first instance to oxidizing free radicals. Serine is of fundamental importance for its role in proteolytic enzymes like chymotrypsin. Threonine is one of the essential amino acids and one of only two with an additional stereogenic center (isoleucine is the other). Here, in addition to $D_{^{\alpha}CH}$, it will also be of interest to examine the C−H bond dissociation energy at the second chiral site and to compare it with that of the C−H bond adjacent to the hydroxyl group in serine. The free neutral amino acids, $H_2NCH(R)C(O)OH$, are not important in polar solvents and have less biological significance. However, they are important as models and for purposes of reference. The structures of the $H_2NCH(R)C(O)$-OH molecules and their radicals and the BDEs were therefore also calculated.

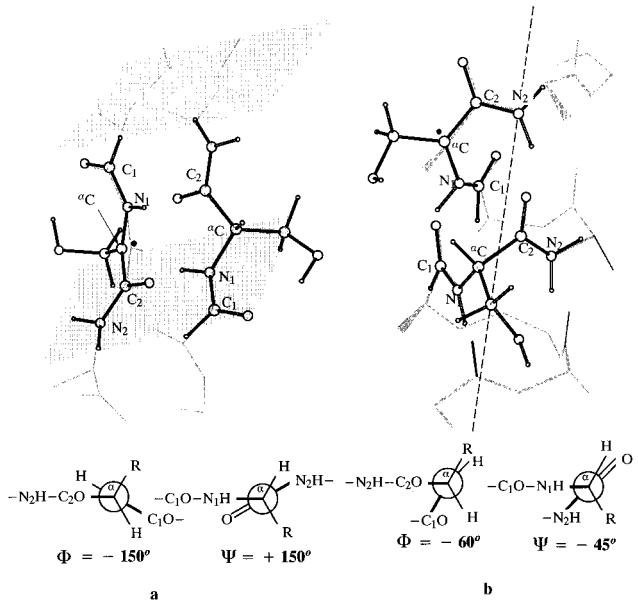


Figure 2. Definition of the average Ramachandran angles for and display of PH(Ser) and $^{\alpha}$P$^{\bullet}$(Ser) superimposed on *β*-sheet (a) and α-helix (b) secondary structures.

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 Ψ and Φ 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 sensitivies of the $^{\alpha}$C centers of glycine, alanine, serine, and threonine in generic α-helical and *β*-sheet regions, i.e., with Φ,Ψ constrained to values representative of the particular secondary structure (Figure 2).

Individual amino acids are of a tractable size for high-level ab initio calculations, and results of previous calculations have been important in predicting microwave or IR spectra, in the analysis of X-ray data on proteins and in studies of the conformations of model peptides. As well as yielding structural information, ab initio calculations carried out at high level can provide valuable thermochemical data. For example, heats of formation determined by the method of isodesmic reactions in conjunction with reliable heats of formation for related systems can be expected to be accurate to within ±10 kJ mol^{-1}.[19−21] These procedures have been used by us in detailed investigations of the structure and thermochemistry of glycine[22] and of its ionic and free radical forms in the gas phase[23] and in solution.[17] Earlier predictions[20] of the captodative stabilizing effect of the H_2N− and −C(O)OH groups on the $^{\alpha}$C−H bond dissociation energy were confirmed,[23] and the stabilization was shown to be

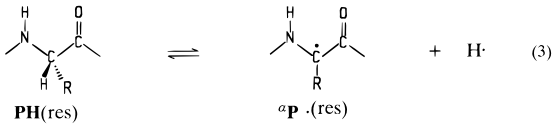
(13) Wardman, P. In *Atmospheric Oxidation and Antioxidants*; Scott, G., Ed.; Elsevier: New York, 1993; Vol. 3, p 101.
(14) Wardman, P.; Von Sonntag, C. *Methods Enzymol*. **1995**, *251*, 31.
(15) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637.
(16) Faraggi, M.; Klapper, M. H. *J. Chim. Phys.* **1993**, *90*, 711.
(17) Armstrong, D. A.; Rauk, A.; Yu, D. *J. Chem. Soc., Perkin Trans. 2* **1995**, 553.
(18) Zhao, R.; Lind, J.; Merényi, G.; Eriksen, T. *J. Am. Chem. Soc.* **1994**, *116*, 12010.
(19) Armstrong, D. A.; Rauk, A.; Yu, D. *Can. J. Chem.* **1996**, *74*, 1192.
(20) Leroy, G.; Sana, M.; Wilante, C. *J. Mol. Struct.* **1991**, *228*, 37.
(21) Hehre, W. J.; Ditchfield, R.; Radom, L.; Pople, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 4796.
(22) Yu, D.; Armstrong, D. A; Rauk, A. *Can. J. Chem.* **1992**, *70*, 1762.
(23) Yu, D.; Rauk, A.; Armstrong, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 1789.

diminished in the $H_2NCH^{\bullet}CO_2^-$ radical anion resulting in a higher $D_{\alpha CH}$. It was found in the study of $D_{\alpha CH}$ of glycine peptide models[19,24] that the minimum structural features needed to reproduce the local environment of the $^{\alpha}$C region at a midchain residue in a protein are defined by the boxed area in Figure 1. The chain extensions outside the box are terminated by hydrogen atoms.

Computational Details

All ab initio calculations presented here were performed with the Gaussian 92/94 molecular orbital packages.[25] 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 0.95 in considering the zero-point energy.

The $^{\alpha}$C−H BDEs, $D_{\alpha CH}$, would normally be obtained from the heat of reaction 3, $\Delta H^{\circ}_{(3)}$:



In the present case, as a means of reducing residual errors due to basis set and correlation effects, they were derived from the heats of isodesmic reactions.[21] These reactions can be represented by process 4:

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

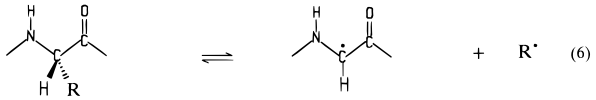
AH is a reference molecule for which the BDE, D_{CH}(AH), is known accurately. For each PH(res) the heat of reaction 4, $\Delta H^{\circ}_{(4)}$, was evaluated from the energies obtained in the ab initio calculations at the B3LYP/6-31G(D) level, which was shown to give reliable results for the glycine model peptides.[19] In the context of BDEs, D_{CH}(PH) is then given by:

$$D_{CH}(PH) = D_{CH}(AH) - \Delta H^{\circ}_{(4)} \qquad (5)$$

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 can be taken into account. Previous studies have shown[19] that H_2NCH_2COOH (glycine) is the most suitable reference molecule to give reliable values of $D_{\alpha CH}$. The magnitude of $D_{\alpha CH}$ for glycine itself (331.0 kJ mol^{-1})[23] 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.[23] Values of $D_{\alpha CH}$ 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. Consistent with the scheme for peptide models, the neutral amino acids and their $^{\alpha}$C radicals are referred to as AH(res) and $^{\alpha}$A$^{\bullet}$(res), respectively, with res = Gly, Ala, Ser, or Thr.

As already stated, the calculations described above for the model peptides and the $^{\alpha}$P$^{\bullet}$(res) radicals were performed with both PH(res) and $^{\alpha}$P$^{\bullet}$(res) in optimized geometries. Since both Φ and Ψ are fully relaxed, we refer to these structures as 'opt/opt'. The electronic energies for these systems are referred to as E(PH) and E($^{\alpha}$P$^{\bullet}$). In order to estimate the values of the BDEs in the protein environment, calculations were also performed on the glycine, alanine, serine, and threonine model peptide systems at two other sets of Ramachandran angles: (a) Φ = −150°, Ψ = +150°, which simulates β-sheet protein, and (b) Φ = −60° and Ψ = −45°, which simulates the α-helical protein (See Figure 2). Geometry optimizations were carried out at the B3LYP/6-31G(D) level with Φ and Ψ fixed as indicated above to obtain the electronic energies E(PH){Φ′/Ψ′} and E($^{\alpha}$P$^{\bullet}$){Φ′/Ψ′} for the parent and radical structures with those angles. From these energies the corrections, δ = [E($^{\alpha}$P$^{\bullet}$){Φ′/Ψ′} − E($^{\alpha}$P$^{\bullet}$)] − [E(PH){Φ′/Ψ′} − E(PH)], which will add to the BDEs in the opt/opt geometries, were obtained. The values of E(PH) and E($^{\alpha}$P$^{\bullet}$) and the structures for the glycyl model were taken from ref 19.

The $^{\beta}$C−H BDEs of the side groups, $D_{\beta CH}$, and the dissociation energy of the $^{\alpha}$C−C bond associated with removal of the side group, $D_{\alpha CC}$, were also calculated for the peptide models and the parent amino acids. For $D_{\beta CH}$ ethane was selected as the reference molecule in an isodesmic reaction like (4). The D_{CH} in C_2H_6 is known experimentally (375.3 kJ mol^{-1}),[26] and the value calculated directly at the B3LYP/6-31G(D) level was in good agreement with it (374.7 kJ mol^{-1}). For all systems the $D_{\alpha CC}$ values were derived from the heat of reaction 6:



where $^{\alpha}$A$^{\bullet}$(Gly) or $^{\alpha}$P$^{\bullet}$(Gly) and R$^{\bullet}$ represent the corresponding C-centered fragments. The heats of reaction were obtained directly from the ab initio energies of these fragments and the parent molecules, with corrections to 298 K.

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[27] and using the frequencies obtained at the B3LYP/6-31G(D) level. The frequencies were scaled by a factor of 0.95 in the calculation of these thermodynamic functions.

The reduction potentials of the C-centered radicals were obtained from the standard free energy changes at 298 K, ΔG°_{298}, of reaction 7:

$$-NHCR^{\bullet}C(O)- + {}^{1}/_{2}H_{2(g)} \rightleftharpoons -NHCH(R)C(O)- \qquad (7)$$

ΔG°_{298} is related to $D_{\alpha CH}$ by expression 8:

$$\Delta G^{\circ}_{298} = -D_{\alpha CH} - T\Delta S^{\circ}_{298} + \Delta_f H^{\circ}_{298} \text{ of } H^{\bullet}_{(g)} \qquad (8)$$

in which ΔS°_{298} is the entropy change in reaction 7. The difference in entropy of the parent and radical species was derived from the calculated entropies (Table 1). S°_{298} of $^{1}/_{2}H_{2(g)}$ (65 J K^{-1} mol^{-1}) and $\Delta_f H^{\circ}_{298}$ of $H^{\bullet}_{(g)}$ (218 kJ mol^{-1}) were taken from ref 28. The reduction potential for the −NHCR$^{\bullet}$C(O)− radical versus the standard hydrogen electrode corresponds to $E^{\circ}_{(9)}$ for the half-cell reaction 9:

$$-NHC^{\bullet}RC(O)- + H^{+} + e^{-} = -NHCH(R)C(O)- \qquad (9)$$

This is given by [ΔG°_{298} + $\Delta(\Delta G^{\circ}_{(soln)})$]/$F$, where F is the Faraday constant and $\Delta(\Delta G^{\circ}_{(soln)})$ is the difference in the free energies of solution for the parent molecule and its radical. For large carbon-centered radicals $\Delta(\Delta G^{\circ}_{(soln)})$ is expected to be negligible.[29] Thus

(24) Barone, V.; Adamo, C.; Grand, A.; Brunel, Y.; Fontecave, M.; Subra, R. *J. Am. Chem. Soc*. **1995**, *117*, 1083.

(25) (a) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *Gaussian 92*, Revision B; Gaussian, Inc.: Pittsburgh, PA, 1992. (b) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, SGI Revision B.3; Gaussian, Inc.: Pittsburgh, PA, 1995.

(26) Berkowitz, J.; Ellison, G. B.; Gutman, D. *J. Phys. Chem*. **1994**, *98*, 2744.

(27) McQuarrie, D. A. *Statistical thermodynamics*; Harper & Row: New York, 1973.

(28) Wagman, D. D.; Evans, W. H.; Parker, V. B.; Schumm, R. H.; Halow, I.; Bailey, S. M.; Churney, K. L.; Nuttal, R. L. *J. Phys. Chem. Ref. Data* **1982**, *11* (Suppl. 2).

Table 1. Electronic and Vibrational Energies Calculated at the B3LYP/6-31G(D) Level and S°_{298} and $H^\circ_{298} - H^\circ_0$

species	E (hartree)	ZPE (kJ mol^{-1})	$H^\circ_{298} - H^\circ_0$ (kJ mol^{-1})	S°_{298} (J K^{-1} mol^{-1})
AH(Ala), $NH_2CH(CH_3)C(O)OH$	−323.739 64	284.7	21.2	345.5
$^{\alpha}$A$^{\bullet}$(Ala), $NH_2C^{\bullet}(CH_3)C(O)OH$	−323.111 76	249.9	21.3	348.4
$^{\beta}$A$^{\bullet}$(Ala), $NH_2CH(C^{\bullet}H_2)C(O)OH$	−323.065 81	244.9	21.8	352.4
PH(Ala), $HCONHCH(CH_3)C(O)NH_2$	−417.218 14	341.9	27.2	397.1
$^{\alpha}$P$^{\bullet}$(Ala), $HCONHC^{\bullet}(CH_3)C(O)NH_2$	−416.580 38	309.0	26.5	395.3
$^{\beta}$P$^{\bullet}$(Ala), $HCONHCH(C^{\bullet}H_2)C(O)NH_2$	−416.544 37	303.2	27.5	406.9
AH(Ser), $NH_2CH(CH_2OH)C(O)OH$	−398.949 05	299.6	23.0	362.2
$^{\alpha}$A$^{\bullet}$(Ser), $NH_2C^{\bullet}(CH_2OH)C(O)OH$	−398.317 41	264.6	23.3	366.3
$^{\beta}$A$^{\bullet}$(Ser), $NH_2CH(C^{\bullet}HOH)C(O)OH$	−398.292 57	263.7	22.8	365.3
PH(Ser), $HCONHCH(CH_2OH)C(O)NH_2$	−492.428 96	357.7	28.3	405.1
$^{\alpha}$P$^{\bullet}$(Ser), $HCONHC^{\bullet}(CH_2OH)C(O)NH_2$	−491.789 98	326.1	27.3	402.8
$^{\beta}$P$^{\bullet}$(Ser), $HCONHCH(C^{\bullet}HOH)C(O)NH_2$	−491.775 44	322.0	27.7	402.5
AH(Thr), $NH_2CH(CH(CH_3)OH)C(O)OH$	−438.267 72	373.5	26.5	388.2
$^{\alpha}$A$^{\bullet}$(Thr), $NH_2C^{\bullet}(CH(CH_3)OH)C(O)OH$	−437.635 62	337.0	27.6	399.4
$^{\beta}$A$^{\bullet}$(Thr), $NH_2CH(C^{\bullet}(CH_3)OH)C(O)OH$	−437.614 47	337.7	26.9	400.1
PH(Thr), $HCONHCH(CH(CH_3)OH)C(O)NH_2$	−531.744 56	432.0	31.6	429.5
$^{\alpha}$P$^{\bullet}$(Thr), $HCONHC^{\bullet}(CH(CH_3)OH)C(O)NH_2$	−531.102 56	400.0	30.8	427.5
$^{\beta}$P$^{\bullet}$(Thr), $HCONHCH(C^{\bullet}(CH_3)OH)C(O)NH_2$	−531.096 47	396.2	31.8	434.2

$E^\circ_{(9)}$ was estimated from $\Delta G^\circ_{298}/F$. A similar approach was used to estimate the standard reduction potentials for the side chain radical systems.

The reduction potentials at pH = 7, $E^{\circ\prime}$, 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:

$$D^{\bullet} + H^+ + e^- = DH \qquad (10)$$

The dependence of reduction potential on [H$^+$] is therefore given by:[30]

$$E = E^\circ + (RT/F)\ln\{[D^{\bullet}]([H^+] + K_i)[DH]^{-1}\} \qquad (11)$$

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

Results

All species discussed here are explicitly identified in figures and tables by the scheme noted in the Introduction. The parent molecules are either the free neutral amino acids, designated as AH(res) (for example AH(Ala)), or the corresponding peptide models, PH(res). The radical products are designated as $^{\alpha}$A$^{\bullet}$(res) or $^{\alpha}$P$^{\bullet}$(res) for $^{\alpha}$C-centered species and $^{\beta}$A$^{\bullet}$(res) or $^{\beta}$P$^{\bullet}$(res) for $^{\beta}$C-centered species.

The structures of the alanine-, serine-, and threonine-derived species, optimized at B3LYP/6-31G(D) level, are presented in Figure 3. Bond distances between heavy atoms, important hydrogen-bonding distances, and the Ramachandran angles Φ and Ψ are given. All optimized structures are local minima, as confirmed by the frequency calculations, and have C_1 symmetry. Except for Ψ of AH(Thr) and PH(Thr), all of the optimized Φ and Ψ angles differ from 180° by less than 20°.

The electronic energies and the vibrational ZPEs calculated at the B3LYP/6-31G(D) level are listed in Table 1. The calculated values of $H^\circ_{298} - H^\circ_0$ and S°_{298} are also given. It may be noted that $H^\circ_{298} - H^\circ_0$ values 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, $D_{\alpha CH}$ and $D_{\beta CH}$, of the amino acids and the model peptides with opt/opt geometries are reported in Table 2. The effects of changing the Ramachandran angles Φ and Ψ for PH(Gly), PH(Ala), PH(Ser), and PH(Thr) are also given in Table 2.

Table 2. BDEs (kJ mol^{-1}, at 298 K)[a]

compd	$D_{\alpha CH}$[b] opt/opt[e]	β-sheet[f]	α-helix[g]	$D_{\beta CH}$[c] opt/opt[e]	$D_{\alpha CC}$[d] opt/opt[e]
		Amino Acids			
AH(Gly)	331.0[h]				
AH(Ala)	317.4 (325.3)[i]			427.3	269.0 (292)[i] (292)[j]
AH(Ser)	327.2			384.6	261.4
AH(Thr)	327.8			376.8	250.3
		Peptide Residues			
PH(Gly)	348.0[k]	361	402		
PH(Ala)	344.3	359	384	427.8	287.6
PH(Ser)	348.4	347	381	376.6	283.5
PH(Thr)	356.2	356	363	363.1	264.1

[a] From B3LYP/6-31G(D)+0.95×ZPE level calculations and isodesmic reactions. [b] From isodesmic reaction: $^{\alpha}$A$^{\bullet}$ or $^{\alpha}$P$^{\bullet}$ + NH_2CH_2COOH → AH or PH + $NH_2CHCOOH^{\bullet}$. [c] From isodesmic reaction: $^{\beta}$A$^{\bullet}$ or $^{\beta}$P$^{\bullet}$ + CH_3CH_3 → AH or PH + $CH_3CH_2^{\bullet}$. [d] Unless otherwise indicated, by direct calculation based on the reaction: AH or PH → $^{\beta}$A$^{\bullet}$(Gly) or $^{\beta}$P$^{\bullet}$(Gly) + R$^{\bullet}$ (see text). [e] Optimized Φ, Ψ. [f] $\Phi = -150°$, $\Psi = +150°$ (see Figure 2). [g] $\Phi = -60°$, $\Psi = -45°$ (see Figure 2). [h] From ref 23. [i] From G2(MP2)′ energies (see ref 19 for the method). [j] From experimental heats of formation of alanine (ref 31) and $CH_3^{\bullet}$ (ref 26) and our theoretical value for glycyl radical (ref 23). [k] From ref 19.

The $^{\alpha}$C−C bond dissociation energy in the case of alanine ($H_2NCH(CH_3)C(O)OH$) was obtained from the heat of reaction 6 based on the ab initio energies at both the B3LYP/6-31G(D) and G2(MP2)′ levels. It could also be determined from the experimental heats of formation of alanine and methyl radical and from the theoretical estimate of the heat of formation of glycyl radical.[31] As can be seen from column 6 of Table 2, the $D_{\alpha CC}$ values for AH(Ala) from the G2(MP2)′ energies and experiment are identical, and the one from B3LYP/6-31G(D) energies is 23 kJ mol^{-1} smaller.

Reduction potentials at pH = 7, $E^{\circ\prime}$, for the $^{\alpha}$C- and $^{\beta}$C-centered radicals of the peptide models are given in Figure 4. The results for the neutral acids, $H_2NCH(R)C(O)OH$, have not been included but can be calculated from the data in Tables 1 and 2 if desired.

(29) Kanabus-Kaminska, J. M.; Gilbert, B. C.; Griller, D. *J. Am. Chem. Soc.* **1989**, *111*, 3311.

(30) See ref 15, p 1644, for derivation.

(31) There are three consistent values (ref 47) of the heat of combustion of D-alanine which average to 1620 ± 1 kJ mol^{-1}. Taking this and the values of the heats of formation of H_2O and CO_2 (ref 28), one finds $\Delta_f H^\circ_{(c)} = -560.6 \pm 1.5$ kJ mol^{-1}. The average of three experimental values of $\Delta_f H^\circ_{(sub)}$ at ca. 430 K (ref 48) corrected (ref 49) to 298 K is 135.7 ± 3.3 kJ mol^{-1}, which gives $\Delta_f H^\circ_{(g)} = -425 \pm 2$ kJ mol^{-1} at 298 K. The C−C bond energy was calculated from that and $\Delta_f H^\circ_{(g)}$ at 298 K for $CH_3^{\bullet}$ (146 kJ mol^{-1} (ref 26)) and Gly$^{\bullet}$ (−279 kJ mol^{-1} (ref 23)). The result is 292 kJ mol^{-1}.

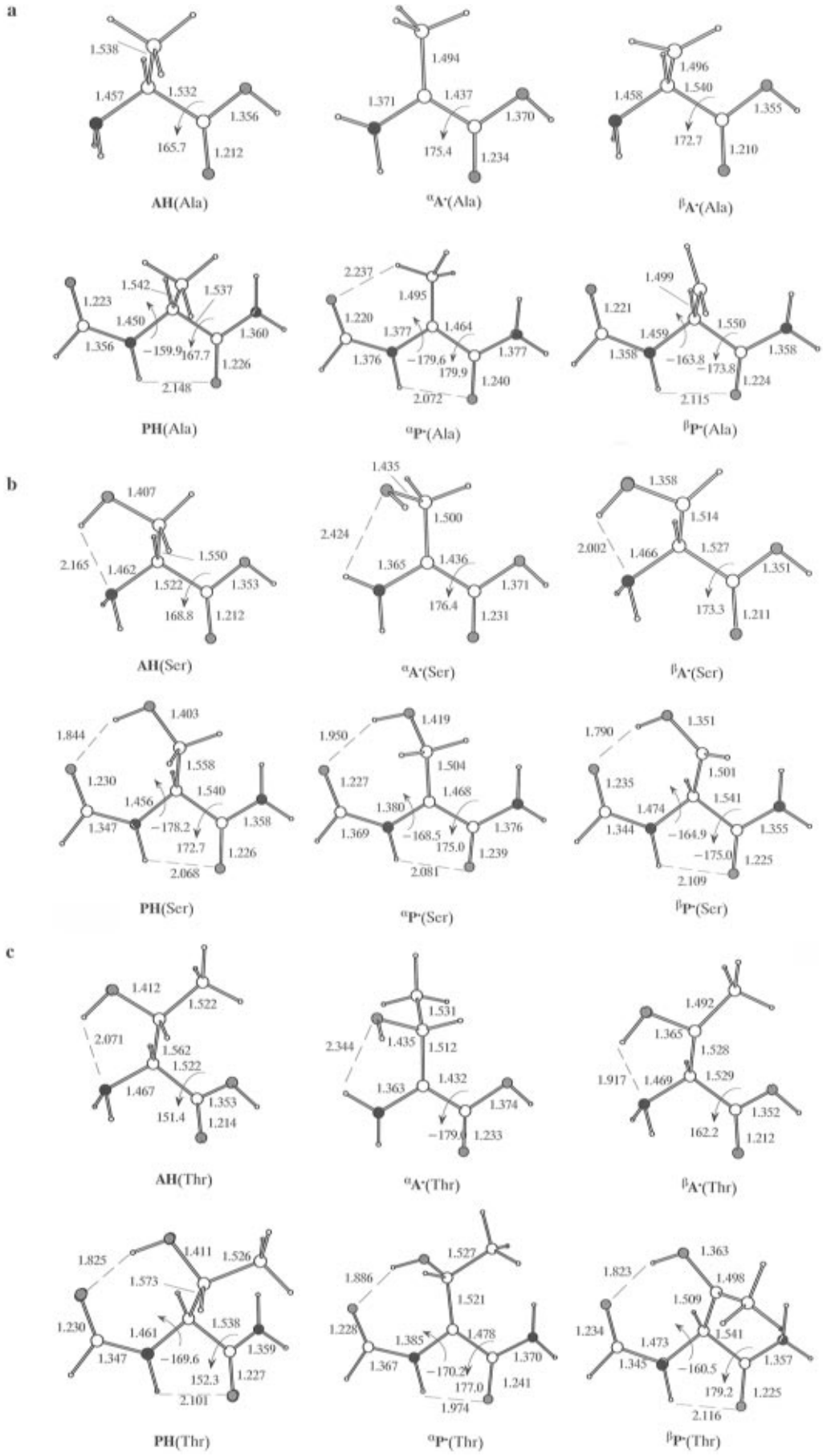


Figure 3. B3LYP/6-31G(D)-optimized structures of the parent molecule and radical species of (a) alanine, (b) serine, and (c) threonine. The filled circles stand for nitrogen, open ones for carbon, and shielded ones for oxygen atoms, respectively. The small circles represent the hydrogen atoms. Bond lengths are in angstroms and angles in degrees.

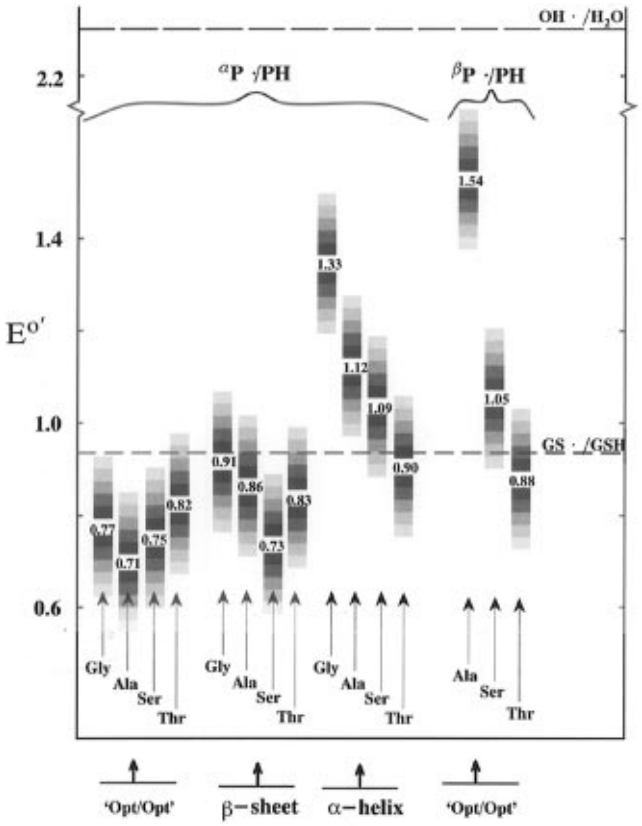


Figure 4. Reduction potentials at pH = 7, $E^{\circ\prime}$, of α- and β-centered radicals of the model peptides. The $E^{\circ\prime}$ values in volts are given above the abbreviations for the residues.

The electronic energies and the vibrational ZPEs calculated at the B3LYP/6-31G(D) level, as well as the $H^{\circ}_{298} - H^{\circ}_{0}$ and S°_{298} values, for species involved in the isodesmic reactions, or the direct calculations of the C−C BDEs, are given in Table 3.

Discussion

This section begins with a discussion of the structures of the radicals and their parent species. The bond dissociation energies are considered next and related to different structural features. Finally, in the section on biological significance the relative stabilities and the reduction potentials of the radicals of the model peptides are discussed.

Structures. 1. Alanine Species. AH(Ala), $^{\alpha}$A•(Ala), and $^{\beta}$A•(Ala). There are a number of previous theoretical and experimental investigations of alanine. O'Hair et al.[32] measured the gas phase acidity of alanine and carried out ab initio calculations at the HF/3-21G(D) level for alanine and at the HF/3-21+G(D) level for alanine carboxylate. Several possible conformers were examined. The minimum energy conformer has two NH bonds which are symmetrically disposed to the carbonyl oxygen atom, as in the case of glycine.[22] More recently Gronert and O'Hair[33] examined 36 possible conformers of alanine at the AM1 level and optimized the 10 most stable ones at the HF/6-31G(D) level. The conformer of lowest energy was the same as found previously.[32] At the MP2/6-31+G(D)//HF/6-31G(D) level, the second most stable conformer was only 3.8 kJ mol^{-1} above, and there were another five conformers in an energy gap of 2.5 (3.8−6.3) kJ mol^{-1}. Csaszar has recently published detailed calculations which confirm the structure of lowest energy.[34] The most stable structure of alanine (AH(Ala)) obtained in this study at the B3LYP/6-31G(D) level (see Figure 3a) corresponds to that previously found.[32−34]

As indicated in Figure 3a, formation of the $^{\alpha}$C-centered radical, $^{\alpha}$A•(Ala), leads to extensive changes. The N−C(−CH_3)−C frame approaches planarity, and in order to maximize the captodative stabilization of the radical, the H_2N- group also becomes nearly coplanar. The bond between the $^{\alpha}$C and the carboxyl group becomes shorter, but the geometry of the latter is changed relatively little. The structure of this radical is similar to that of the $H_2NC{\cdot}HC(O)OH$ radical ($^{\alpha}$A•(Gly)).[23] The deprotonated (anionic) $^{\alpha}$A•(Ala) radical is well known from EPR studies,[35] but the structure of that form was not investigated here.

In the optimized structure of the $^{\beta}$C-centered radical, $^{\beta}$A•(Ala), most features of the AH(Ala) $H_2N-C-C(O)OH$ framework are maintained. However, the removal of the H atom from the $-CH_3$ group causes a shortening of the C−C bond in the $^{\alpha}C-CH_2$ unit. The $-CH_2$ radical center is almost planar. The dihedral angles formed by the H atoms of the $-CH_2$ group and the $^{\alpha}$C−H bond (−75.0° and 95.3°) suggest an alignment of the singly occupied p orbital with that bond.

PH(Ala), $^{\alpha}$P•(Ala), and $^{\beta}$P•(Ala). In the alanyl peptide model, PH(Ala), Figure 3a, the H atom of the −NH group lies almost in the plane of the N−$^{\alpha}$C−C(O)− framework in a position to form a hydrogen-bonding interaction with the carbonyl group. The H atoms of the second $-NH_2$ group also lie almost in this plane. The geometry of the main chain is very similar to that of the glycine model peptide $HCONHCH_2$-$CONH_2$ (PH(Gly)) discussed previously.[19]

The structure of the $^{\alpha}$C-centered radical, $^{\alpha}$P•(Ala), is essentially planar. The values of Φ and Ψ are close to 180° for this species, having increased in magnitude from −159.9° and +167.7°, respectively, for the parent, PH(Ala). The relatively modest flattening of the peptide chain skeleton is accompanied by approximately a 55° swing of the methyl group into the common plane. As a consequence, one of the H atoms of the methyl side group now exhibits a repulsive interaction with the O atom of the formyl group, the separation of 2.237 Å (Figure 3a) being smaller than the sum of the van der Waals radii. However, the subsequent distortion of the affected peptide link results in an increased N−H···O hydrogen-bonding interaction, as judged by the shortened H···O separation.

For the side chain radical $^{\beta}$P•(Ala), the H−C(O)−N−$^{\alpha}$C−$C(O)-NH_2$ geometry in the optimized structure is almost unchanged from that of PH(Ala) (Figure 3a). The $-CH_2$ group is nearly planar, lying perpendicular to the long axis of the molecule. This is in contrast to the situation in $^{\beta}$A•(Ala) where the plane of the $-CH_2$ group is effectively parallel to the plane containing the long axis of the molecule. There is no obvious steric explanation for the change in conformation. The change may reflect enhanced $^{\alpha}$C−N bond and/or decreased $^{\alpha}$C−H bond involvement in stabilizing the β-radical upon peptide formation. The orientation of the $-CH_2$ group suggests that the single-electron-containing p orbital is 20° out of alignment with the $^{\alpha}$C−N bond.

2. Serine Species. AH(Ser), $^{\alpha}$A•(Ser), and $^{\beta}$A•(Ser). Serine (AH(Ser)) (Figure 3b) differs from alanine in the substitution of an OH group for one of the H atoms of the methyl side chain. The presence of this group adds two 3-fold rotators, and it has the potential to form hydrogen bonds. Thus the number of possible conformations increases dramatically, and this leads to a level of complexity not found in glycine or alanine. Van

(32) O'Hair, R. A. J.; Bowie, J. H.; Gronert, S. *Int. J. Mass Ion Proc.* **1992**, *117*, 23.
(33) Gronert, S.; O'Hair, R. A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2071.
(34) Csaszar, A. G. *J. Phys. Chem.* **1996**, *100*, 3541.
(35) Ciesielski, B.; Wielopolski, L. *Radiat. Res.* **1994**, *140*, 105.

Alsenoy et al. carried out one of the earliest ab initio studies of serine in 1981[36] and refined the investigation in 1988.[37] They examined 14 conformations at the HF/4-21G level. As in alanine and glycine, the two NH bonds in the most stable conformer were found to be almost symmetrically disposed to the carbonyl oxygen atom, while the side chain −OH formed a hydrogen bond with the $-NH_2$ lone pair. Following their mass spectrometric investigation and a brief ab initio calculation,[32] Gronert and O'Hair carried out an extensive study of serine conformations.[33] At a correlated level, the three most stable conformers were found to lie within 1 kJ mol^{-1} of each other. At least 15 other conformers existed in a 10 kJ mol^{-1} interval above this.

We reoptimized the global minimum structure of refs 33 and 37 at the B3LYP/6-31G(D) level (Figure 3b). The distance between the H atom of the −OH in the side group and the N atom of the $-NH_2$ group is 2.165 Å, well within the van der Waals separation and indicating a strong hydrogen-bonding interaction.

In the α-radical, $^{\alpha}$A$^{\bullet}$(Ser), the $-NH_2$ group has a nearly planar geometry, as was the case with $^{\alpha}$A$^{\bullet}$(Ala). In this instance the planar structure receives stabilization from an H-bonding interaction between the NH_2 group and the O atom of the hydroxide group (Figure 3b). However, the interatomic distance indicates that this is a relatively weak bond. Other aspects of the $^{\alpha}$A$^{\bullet}$(Ser) structure resemble those of $^{\alpha}$A$^{\bullet}$(Ala).

In $^{\beta}$A$^{\bullet}$(Ser), the N···H−O interaction is strengthened, as judged by the shortened H to N distance. As in the corresponding alanine system, the geometry of the $H_2N-C-C(O)-OH$ framework of the β-radical is similar to that of the parent molecule, and the orientation of the radical center is similar to that of $^{\beta}$A$^{\bullet}$(Ala).

PH(Ser), $^{\alpha}$P$^{\bullet}$(Ser), and $^{\beta}$P$^{\bullet}$(Ser). The optimized structures of peptide model systems, PH(Ser), $^{\alpha}$P$^{\bullet}$(Ser), and $^{\beta}$P$^{\bullet}$(Ser), all show strong hydrogen-bonding interactions between the −OH of the side group and the O atom of the HCO− group (Figure 3b). $^{\beta}$P$^{\bullet}$(Ser) has the shortest O···H distance (1.790 Å) and $^{\alpha}$P$^{\bullet}$(Ser) the longest (1.950 Å). The strong interaction in $^{\beta}$P$^{\bullet}$(Ser) distorts the structure so that the orientation of the H−C−OH group is not perpendicular to the long axis of the molecule as it is in $^{\beta}$P$^{\bullet}$(Ala). In the $^{\alpha}$P$^{\bullet}$(Ser) radical, maintenance of the strong hydrogen-bonding interaction is associated with a larger deviation from planarity of the peptide backbone and is at the expense of some captodative stabilization of the radical from the N donor. Since the C−O bond is nearly perpendicular to the plane of the $^{\alpha}$C radical site, some additional stabilization from the acceptor σ_{CO}* orbital may be in effect.

3. Threonine Species. AH(Thr), $^{\alpha}$A$^{\bullet}$(Thr), and $^{\beta}$A$^{\bullet}$(Thr). Threonine has been studied by Schafer et al. in their series of ab initio calculations on amino acids.[38] At the HF/4-21G level, 10 conformations of threonine were optimized. Although internal rotations produced 10 conformers with widely different geometrical parameters, all of them fell in an energy gap of 17 kJ mol^{-1}. The B3LYP/6-31G(D)-optimized threonine structure obtained in this work (see Figure 3c) corresponds to the global minimum reported by Schafer et al.[38] As pointed out earlier, the magnitude of Ψ for AH(Thr) (+151.4°) is smaller than observed in the case of AH(Ser) and AH(Ala). This can be attributed to interference between the extra $-CH_3$ group in the side chain and the −OH of the carboxyl. Rotation of the side group partially relieves the effect in the $^{\beta}$A$^{\bullet}$(Thr) radical and effectively eliminates it in $^{\alpha}$A$^{\bullet}$(Thr), where Ψ is −179.0°. Apart from this, the geometries in the $H_2N-C-C(O)OH$ framework of the parent molecule and the threonine radicals, as well as the H-bonding interactions, are very similar to those of the corresponding species of serine. The additional methyl group poses no steric impediment for the peptide skeleton to achieve a planar geometry.

PH(Thr), $^{\alpha}$P$^{\bullet}$(Thr), and $^{\beta}$P$^{\bullet}$(Thr). The general features in the optimized structures of PH(Thr), $^{\alpha}$P$^{\bullet}$(Thr), and $^{\beta}$P$^{\bullet}$(Thr) (Figure 3c) are very similar to those of the corresponding serine species. In each case a strong C═O···H−O hydrogen bond is maintained. In the parent, PH(Thr), both skeletal dihedral angles, Φ and Ψ, indicate a further distortion from planarity, the latter by 20°, in the direction which minimizes steric interaction with the methyl group.

Bond Dissociation Energies. The BDEs for the parent amino acids and the model peptides with opt/opt geometries are considered first. These have been summarized in columns 2, 5, and 6 of Table 2 under the headings $D_{\alpha CH}$(opt/opt), $D_{\beta CH}$, and $D_{\alpha CC}$. The literature values of $D_{\alpha CH}$ for the $^{\alpha}$C−H bonds of glycine (H_2NCH_2COOH) and the glycine model peptide, PH(Gly), have been included in column two for comparison with those of the present systems. The different types of bonds are discussed in turn below. Since the C−H bonds were all obtained from isodesmic reactions, the errors are expected to be ≤10 kJ mol^{-1}; a larger error is expected for C−C bonds since suitable isodesmic reactions are not available for these (see below).

1. $D_{\alpha CH}$. The BDE for neutral alanine ((AH(Ala)) is $D_{\alpha CH}$ = 317 kJ mol^{-1}. The value predicted from G2(MP2)′ energies is 325 kJ mol^{-1} (Table 2). Thus $D_{\alpha CH}$ of AH(Ala) is lower by 14 kJ mol^{-1} than for AH(Gly)[23] at the B3LYP/6-31G(D) level and 6 kJ mol^{-1} at G2(MP2)′. This indicates a small net increase in the radical stability due to the methyl group. Additional stabilization due to the methyl group acting as a second electron pair donor is expected.

As seen from Table 2, $D_{\alpha CH}$ is almost the same for serine (AH(Ser)) and threonine (AH(Thr)), i.e., there is little effect on altering the side chain from $-CH_2OH$ to $-CH(CH_3)OH$. The 14 kJ mol^{-1} lowering of $D_{\alpha CH}$ seen for AH(Ala) relative to AH(Gly) is largely lost due to additional stabilization of the parent by H-bonding (Figure 3b,c). The value of $D_{\alpha CH}$ for serine and threonine (∼327 kJ mol^{-1}) differs little from that for glycine (331 kJ mol^{-1}).

For the model peptide series, PH(Gly), PH(Ala), PH(Ser), and PH(Thr), relative to the corresponding neutral amino acids, conversion to amide groups reduces the electron-donating ability of the −NH group as well the electron-withdrawing power of the −C(O)− group. Thus one expects to see a diminution in the stabilization of the $^{\alpha}$C-centered radical, and $D_{\alpha CH}$ should increase. This is observed in all cases, the magnitude of the increase ranging from 17 to 29 kJ mol^{-1}. Thus, if the amino acid residue in the peptide and the $^{\alpha}$C-centered radical derived from it were both able to adopt fully relaxed geometries, the predicted $^{\alpha}$C−H BDEs at 298 K are (kJ mol^{-1}) PH(Gly), 348; PH(Ala), 344; PH(Ser), 348; PH(Thr), 356.

Attention is now directed to the simulation of constraints imposed by protein secondary structure by fixing the Ramachandran angles, Φ and Ψ, to average values for the α-helix and β-sheet structures (Figure 2). Although, in principal, $D_{\alpha CH}$ may increase or decrease if nonoptimum structures are involved, we expect in the present case that such constraints will result in an increase in $D_{\alpha CH}$, especially for the α-helix deformations. This is because imposition of constraints should have a greater

(36) van Alsenoy, C.; Scarsdale, J. N.; Sellers, H. L.; Schafer, L. *Chem. Phys. Lett.* **1981**, *80*, 124.
(37) van Alsenoy, C.; Kulp, S.; Siam, K.; Klimkowski, V. J.; Ewbank, J. D.; Schafer, L. *J. Mol. Struct.* **1988**, *181*, 169.
(38) Schafer, L.; Kulp-Newton, S. Q.; Siam, K.; Klimkowski, V. J.; van Alsenoy, C. *J. Mol. Struct.* **1990**, *209*, 373.

destabilizing effect on the free radical since the inability of the local geometry to achieve a quasi-planar geometry will result in loss of the special captodative stabilization. In other words, the free radical has more to lose. An additional factor, which we are unable to explore at this time, is the effect of the hydrogen-bonding network which holds the secondary structure in place. One might anticipate that this will have a rather small effect on the free radical stability, since the decreased donor ability of the hydrogen-bonded amide nitrogen atom may be compensated by increased acceptor ability of the hydrogen-bonded acyl group.

The results of the β-sheet and α-helix model calculations are given in columns 3 and 4 of Table 2. For PH(Gly) and PH(Ala), imposition of β-sheet skeletal structure leads to a 13−15 kJ mol^{-1} increase in $D_{^{\alpha}CH}$ relative to the opt/opt geometry. The more severe distortions associated with the α-helix geometry cause a further increase in $D_{^{\alpha}CH}$, 41 and 25 kJ mol^{-1}, respectively. The BDE of PH(Gly) in α-helix conformation, 402 kJ mol^{-1}, is close to the value in propane (412 kJ mol^{-1}),[26] indicating that the captodative stabilization of the radical is largely lost. The smaller value of the BDE in α-helix PH(Ala), 384 kJ mol^{-1}, may be due to compensatory stabilization by the methyl group through a combination of electronic stabilization of the radical and relief of steric repulsion in the parent. Notably, the BDE is significantly less than for a tertiary alkyl C−H bond (404 kJ mol^{-1}).[26]

As the results in Table 2 indicate, unlike for glycine and alanine, constraining serine or threonine residues into the β-sheet configuration has little or no effect on $D_{^{\alpha}CH}$ compared to the opt/opt conformations. A serine residue and its $^{\alpha}$C radical are shown superimposed on a β-sheet framework in Figure 2a. A close scrutiny of Figure 2a suggests that the calculated $D_{^{\alpha}CH}$ values for the 'β-sheet' skeletal deformation may be *underestimates* of the actual values, since the side chain must move from a relatively uncrowded position perpendicular to the plane more or less into the plane upon formation of the free radical. One may conclude that such free radical formation would be energetically easiest on the edges rather than in the interior of multistrand sheets.

In the α-helix environment (Figure 2b; column 4 of Table 2), formation of the radical site is not accompanied by increased steric repulsion due to the helical structure, as it may be in the β-sheet environment, since the side chain moves outward from the helix axis. Whether the space is occupied by residues from the rest of the structure depends on the particular protein. In the first instance we expect that the calculated values are reasonable estimates in a real protein context. The BDE of PH(Ser) in the α-helix conformation (shown in Figure 2b) is predicted to be virtually the same as that of PH(Ala), but a substantial decrease is expected for PH(Thr), such that the threonine peptide model $D_{^{\alpha}CH}$ values are very similar in all three circumstances.

2. $D_{^{\beta}CH}$. The calculated value for the $^{\beta}$C−H(methyl) BDE, $D_{^{\beta}CH}$, of neutral alanine, AH(Ala), 427.3 kJ mol^{-1}, is comparable to the experimental C−H BDE of C_2H_6 (423.6 kJ mol^{-1})[26] and typical of a primary C−H. The value in PH(Ala) is virtually the same, 427.8 kJ mol^{-1}.

Reference to Table 2 shows that the $D_{^{\beta}CH}$ values become progressively smaller for the alanine, serine, and threonine systems and more so in the peptide models, PH, than in the neutral amino acids, AH. Two factors likely contribute to this decline: (a) the effects of substitution at the radical site, i.e., an −OH group in serine and −OH plus methyl group in threonine, and (b) the H to O hydrogen-bonding interactions discussed above, which from the distances shown in Figure 3b,c generally seem stronger in the radicals than in the parent molecules. The difference in D_{CH} between CH_3CH_2−H (423 kJ mol^{-1})[26] and $CH_3C(OH)H$−H (405 kJ mol^{-1})[39] is 18 kJ mol^{-1}, and one may expect a drop of about this magnitude to be produced as a result of the substitution of one H atom by OH. The decrease in $D_{^{\beta}CH}$ from the alanine to the serine systems is more than 2 times as large, 43 kJ mol^{-1} for the amino acid and 51 kJ mol^{-1} for the model peptide. That these are significantly larger than 20 kJ mol^{-1} suggests that the hydrogen-bonding interactions *do* make definite contributions, the radicals being more highly stabilized than the molecules. The contribution may be slightly greater in the model peptides than in the amino acids.

The decrease in $D_{^{\beta}CH}$ in going from the serine to the threonine systems in Table 2 is relatively small, ∼10 kJ mol^{-1}, and comparable to that between $CH_3CH(OH)$−H and $(CH_3)_2C(OH)$−H.[39] Thus the contributions of the H bonds are probably about the same in the threonine as in the serine systems. Relief of steric crowding in PH(Thr) upon abstraction of the β-hydrogen probably also plays a role since a 27° change is observed in Ψ.

3. $D_{^{\alpha}CC}$. There does not appear to be any mechanism, biochemical or otherwise, that would lead to $^{\alpha}$C−C bond dissociation, although these bonds are expected to be weak for the same reasons as the $^{\alpha}$C−H bonds. We include here a brief discussion of the predicted $D_{^{\alpha}CC}$ values for fundamental reasons. The $^{\alpha}$C-centered radical of glycine, $^{\alpha}$A$^{\bullet}$(Gly) ($H_2NC^{\bullet}HCOOH$) is produced in the $^{\alpha}$C−C bond dissociation (reaction 6) from each of the three species AH(Ala), AH(Ser), and AH(Thr). This radical has been shown by previous work to be strongly stabilized.[23] For the case of AH(Ala), the $D_{^{\alpha}CC}$ value in Table 2 (292 kJ mol^{-1}), estimated[31] from the experimental heats of formation of alanine and $CH_3^{\bullet}$ and the best value for glycyl,[23] clearly reflects this, being about 80 kJ mol^{-1} smaller than the C−C BDE of C_2H_6 (375.3 kJ mol^{-1}).[26] The direct B3LYP/6-31G(D) value underestimates $D_{^{\alpha}CC}$ by about 23 kJ mol^{-1}. The G2(MP2)′[19] value is identical with the experimental value. Since there are no suitable isodesmic reactions for $^{\alpha}$C−C bonds in amino acids, these cannot be estimated as accurately as $^{\alpha}$C−H bonds, and one can anticipate a systematic error of up to 30 kJ mol^{-1}.

Some trends may be discerned among the B3LYP/6-31G(D) values. The $^{\alpha}$C−C bond becomes progressively weaker in AH(Ser) and AH(Thr), being 8 and 19 kJ mol^{-1} lower, respectively, in these molecules. This feature is best attributed to increasing stabilization of the R$^{\bullet}$ radical, as one goes from CH_3 to CH_2OH to $CH(CH_3)OH$, and to increasing repulsion between these groups and the $H_2NCHC(O)OH$ fragment within the parent molecules. The stabilization of the parent molecules by the $-O-H\cdots NH_2-$ hydrogen bonds shown in Figure 3b,c would act to *increase* $D_{^{\alpha}CC}$ in AH(Ser) and AH(Thr). Evidently the effects due to these interactions are outweighed by stabilization of the radical center and relief of steric repulsion in the parent. The trend in the series PH(Ala), PH(Ser), and PH(Thr) is similar. The larger difference between PH(Thr) and PH(Ser) lends additional emphasis to the effects of PH destabilization as a factor in determining the bond dissociation energy.

Biological Significance. The BDEs in Table 2 and reduction potentials calculated from them with the aid of eq 8 make it possible to estimate the heats and free energies of reactions 1

(39) Schwarz, H. A.; Dodson, R. W. *J. Phys. Chem.* **1989**, *93*, 409. One may note that, while the absolute values are not the same, similar differences in D_{CH} are obtained from the earlier data of: McMillen, D. F.; Golden, D. M. *Annu. Rev. Phys. Chem.* **1982**, *33*, 493.

and 2 for different peptides and to find which are most favored. Figure 4 displays the values of $E^{\circ\prime}$ (reduction potentials at pH = 7) for the radicals of the peptide models in the half-cell reactions:

$$^{\alpha}P^{\bullet}(\text{res}) + H^{+} + e^{-} = PH(\text{res}) \quad (12)$$

$$^{\beta}P^{\bullet}(\text{res}) + H^{+} + e^{-} = PH(\text{res}) \quad (13)$$

For ease of reference, processes 12 and 13 have been identified by the notations $^{\alpha}P^{\bullet}$/PH and $^{\beta}P^{\bullet}$/PH, respectively. Notations on the bottom of the figure distinguish the values of $E^{\circ\prime}(^{\alpha}P^{\bullet}/PH)$ for opt/opt, β-sheet, and α-helix type conformations of the different residues. The horizontal dashed lines denote the potentials for the half-reactions 14[15] and 15:[40]

$$OH^{\bullet} + H^{+} + e^{-} = H_2O \quad (14)$$

$$GS^{\bullet} + H^{+} + e^{-} = GSH \quad (15)$$

The absolute uncertainties in these quantities are ≤0.04 V. The uncertainties for the values estimated here for the peptide models are much larger, ≲0.15 V, the range indicated by the graduated error bars.

$D_{\text{HO-H}}$ is 499 kJ mol^{-1},[26] and obviously all of the C−H bonds studied in Table 2 are susceptible to H abstraction (Figure 1, reaction 1). The difference between $E^{\circ\prime}$ ($OH^{\bullet}/H_2O$) and the $E^{\circ\prime}$ value for a particular peptide radical in Figure 4 is a measure of the driving force for the production of that radical in reaction 1. As shown by the $E^{\circ\prime}$s for the $^{\alpha}P^{\bullet}$/PH systems, the sensitivity to $^{\alpha}$C−H abstraction in α-helix geometry will increase in the order Gly, Ala, Ser, Thr. Secondly, all four residues should be more reactive to $OH^{\bullet}$ in β-sheet geometry. However, the difference in $E^{\circ\prime}$ for Thr in β-sheet and α-helix geometry is small (0.07 V), and that residue should show minimal dependence of damage on conformation. For Gly and Ala there is a further decrease in $E^{\circ\prime}$ on going from β-sheet to the opt/opt conformations. There is no apparent change for Ser and Thr.

A second point is that all of the $D_{^{\alpha}\text{CH}}$ values for opt/opt systems in Table 2 are less than the BDE of an alkyl SH bond, such as occurs in GSH, viz., 370 kJ mol^{-1}.[41] This indicates that these $^{\alpha}$C-centered radicals will not be repaired quantitatively by GSH in reaction 2. The overall picture for the feasibility of this process is best examined in terms of Figure 4.

The free energy change for reaction 2 is given by $-F\{E^{\circ\prime}(^{\alpha}P^{\bullet}/PH) - E^{\circ\prime}(GS^{\bullet}/GSH)\}$, and it is evident from Figure 4 that this quantity is likely to be positive or near zero for β-sheet conformations of all four residues and for Thr radicals in α-helix structures. Thus repair of the $^{\alpha}$C-centered radicals in those systems is likely to be negligible or at best very inefficient. For example, it will be unlikely to compete with the reaction of the $^{\alpha}$C-centered radicals with oxygen and the consequent permanent damage.[1,9,10,11] In addition the fact that $E^{\circ\prime}$ values tend to be lower for the opt/opt systems means that any relaxation of the $^{\alpha}$C-centered radicals from β-sheet to more favorable geometries will decrease the efficiency of repair by GSH. This is also true for Gly, Ala, and Ser radicals which are formed in α-helix structures and which in principle should be repairable if conformation were retained.[42]

An even more important point is that, according to the present data, $GS^{\bullet}$ radicals may have the propensity to *create* $^{\alpha}$C-centered radical lesions at Gly, Ala, Ser, or Thr residues in β-sheet structures and for Thr in α-helix structures. While experimental evidence for these specific processes is at present lacking in proteins, $^{\alpha}$C−H to $^{\bullet}$S−hydrogen transfer has been reported for related model systems.[18,43,44] Peroxy ($ROO^{\bullet}$, $E^{\circ\prime}$ = 1.06 V[45]) and tyrosyl ($-C_6H_4O^{\bullet}$, $E^{\circ\prime}$ = 0.97 V[16,46]) radicals are examples of other species which, like $GS^{\bullet}$, may occur in biological systems and have values of $E^{\circ\prime}$ in excess of those for all four residues in β-sheet geometry (Figure 4). It should be noted, on the other hand, that H abstraction by $GS^{\bullet}$, $ROO^{\bullet}$, and $-C_6H_4O^{\bullet}$ from Gly, Ala, and Ser $^{\alpha}$C-centers in α-helix structures in principle should not be important because their $E^{\circ\prime}$ values will be about 0.3 V larger (Figure 4).

The results in Figure 4 for the $^{\beta}P^{\bullet}$/PH systems show that the sensitivity to β-radical formation will increase dramatically from Ala to Ser, with a further increase from Ser to Thr. The radicals of Ala and Ser should be repairable by GSH. At the same time, only the side chain of Thr should be susceptible to attack by $ROO^{\bullet}$ and $-C_6H_4O^{\bullet}$ or to difficulty of repair by GSH in reaction 1. These systems should not be subject to conformational changes.

Summary

High-level ab initio computations were used to investigate the structures of parent compounds and $^{\alpha}$C radicals of alanine, serine, and threonine, both as free neutral amino acids and as residues in model peptides, intended to mimic the environment in proteins. The $^{\beta}$C radicals of the side chains were also examined. The C−H bond dissociation energies for formation of the $^{\alpha}$C and $^{\beta}$C radicals from the parents, with both in fully optimized geometries, were obtained from the absolute energies using isodesmic reactions. Along with our earlier work on glycine,[19] these appear to be the first C−H BDEs for peptide systems. The effects of more restricted peptide environments such as in β-sheet and α-helical secondary structure on $D_{^{\alpha}\text{CH}}$ were estimated by constraining the Ramachandran dihedral angles, Φ and Ψ, to values typical of these structures. Distortion of the opt/opt geometries increases the BDEs with the effect being small for β-sheet and much larger for α-helical structure. Also the effect of the enforced distortion decreased with increasing substitution, i.e., in the order Gly, Ala, Ser, Thr.

The BDEs and $E^{\circ\prime}$ values estimated from them can be used to predict the susceptibility of the four residues (Gly, Ala, Ser, and Thr) to oxidative damage via reaction 1 (Figure 1) and to repair of the radicals through reaction 2. (a) The residues in β-sheet conformation are more sensitive to creation of $^{\alpha}$C radicals by $OH^{\bullet}$ attack; (b) in this conformation the $^{\alpha}$C radicals are unlikely to be repairable by glutathione; and (c) the same radicals may be created by H abstraction from β-sheet residues

(40) (a) Surdhar, P. S.; Armstrong, D. A. *J. Phys. Chem.* **1986**, *90*, 5915. (b) Surdhar, P. S.; Armstrong, D. A. *J. Phys. Chem.* **1987**, *91*, 6532.

(41) Griller, D.; Simões, J. A. M.; Wayner, D. D. M. Thermochemistry of Sulfur-Centered Intermediates. In *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; NATO ASI Series; Plenum Press: New York, 1990; pp 37−52.

(42) Actually it should be noted that for true repair to occur maintenance of the stereochemistry is mandatory. Akhlaq, M. S.; Schuchmann, H.-P.; von Sonntag, C. *Int. J. Radiat. Biol.* **1987**, *51*, 91.

(43) Eriksen, T. E.; Fransson, G. *Radiat. Phys. Chem.* **1988**, *32*, 163.

(44) Grierson, L.; Hildenbrand, K.; Bothe, E. *Int. J. Radiat. Biol.* **1992**, *62*, 265.

(45) Merényi, G.; Lind, J.; Engman, L. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2551.

(46) Lind, J.; Shen, X.; Ericksen, T.; Merényi, G. *J. Am. Chem. Soc.* **1990**, *112*, 479.

(47) Ngauv, S. N.; Sabbah, R.; Lafette, M. *Thermochim. Acta* **1977**, *20*, 371.

(48) de Kruif, C. G.; Voogd, J.; Offringa, J. C. A. *J. Chem. Thermodyn.* **1979**, *11*, 651.

(49) Corrected using gas phase heat capacity data from the present ab initio results and solid phase experimental data from: Hutchens, T. O.; Cole, A. G.; Stout, J. W. *J. Am. Chem. Soc.* **1960**, *82*, 4813.

Table 3. Vibrational and Electronic Energies at the B3LYP/6-31G(D) Level, $H^\circ_{298} - H^\circ_0$ and S°_{298} for Species Involved in the Isodesmic Reactions or Direct Calculations, and Bond Dissociation Energies of Some Parent Molecules

species	*E* (hartree)	ZPE (kJ mol^{-1})	$H^\circ_{298} - H^\circ_0$ (kJ mol^{-1})	S°_{298} (J K^{-1} mol^{-1})	D_{CH} (kJ mol^{-1})	D_{CC} (kJ mol^{-1})
HCONHC•HCONH$_2$ ($^\alpha$P•(Gly))	−377.262 02[a]	234.6	22.3	359.6		
NH$_2$CH$_2$COOH (AH(Gly))	−284.423 45	210.2	17.6	316.6	331[b]	
NH$_2$C•HCOOH ($^\alpha$A•(Gly))	−283.790 39	176.1	17.0	310.2		
CH$_3$C•HOH	−154.375 36	174.0	14.2	277.3		
C•H$_2$OH	−115.052 03	98.5	11.2	240.0		
C$_2$H$_6$	−79.830 42	197.5	11.8	228.4	423.6[c]	375.3[c]
C$_2$•H$_5$	−79.157 85	156.6	13.1	257.7		
C•H$_3$	−39.838 29	78.3	10.7	195.7		

[a] From ref 19. [b] From ref 23. [c] From ref 26.

by radicals other than OH occurring in cells. The residues in α-helical structures should be more resistant to H abstraction and, except for $^\alpha$P•(Thr), should be repairable by glutathione. Susceptibility to $^\beta$P•(res) formation increases in the order Ala, Ser, Thr and should not be strongly influenced by peptide conformation.

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