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Effects of Structure on $^{\alpha}$C−H Bond Enthalpies of Amino Acid Residues: Relevance to H Transfers in Enzyme Mechanisms and in Protein Oxidation[†]

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ABSTRACT: The bond dissociation enthalpies (BDE) of all of the amino acid residues, modeled by HC(O)NHCH(R)C(O)NH$_2$ (PH(res)), were determined at the B3LYP/6-31G*//B3LYP/6-31G* level, coupled with isodesmic reactions. The results for neutral side chains with ϕ,ψ angles ~180°,~180° in ascending order, to an expected accuracy of ±10 kJ mol^{-1}, are Asn 326; cystine 330; Asp 332; Gln 334; Trp 337; Arg 340; Lys 340; Met 343; His 344; Phe 344; Tyr 344; Leu 344; Ala 345; Cys 346; Ser 349; Gly 350; Ile 351; Val 352; Glu 354; Thr 357; Pro-*cis* 358; Pro-*trans* 369. BDEs calculated at the ROMP2/6-31G*//B3LYP/6-31G* level exhibit the same trends but are ~7 kJ mol^{-1} higher. All BDEs are smaller than those of typical secondary or tertiary C−H bonds due to the phenomenon of captodative stabilization. The stabilization is reduced by changes in the ϕ,ψ angles. As a result the BDEs increase by about 10 kJ mol^{-1} in β-sheet and 40 kJ mol^{-1} in α-helical environments, respectively. In effect the $^{\alpha}$C−H BDEs can be "tuned" from about 345 to 400 kJ mol^{-1} by adjusting the local environment. Some very significant effects of this are seen in the current literature on H-transfer processes in enzyme mechanisms and in oxidative damage to proteins. These observations are discussed in terms of the findings of the present study.

During the past decade interest in protein-based free radicals has literally exploded. There are two reasons for this. First, it is now well-established that such radicals are involved as intermediates in the mechanisms of reaction of several enzymes (*1*). For example, the class 1 and 2 ribonucleotide reductases involve thiyl (RS$^\bullet$) radicals derived from the side chains of cysteine residues, while pyruvate formate lyase and anaerobic ribonucleotide reductases make use of $^{\alpha}$C-centered radicals, which are part of glycine residues in the backbone structures. The second reason for interest in protein-based radicals arises from the fact that $^{\alpha}$C-centered radicals on the peptide backbone (*2*, *3*) or other types of radicals in the side chains (*4*−*6*) of amino acid residues may be created by the reactions of reactive oxygen species (ROS). ROS can be produced by the actions of toxic chemicals and radiation on cells and by the detoxification of extraneous chemicals in the liver (*3*, *7*, *8*). More important is the fact that they are also byproducts of the normal respiratory chain (*7*, *9*). Although the complex structures of that system are designed to carry out transfers of electrons with a minimum number of errors, there are, nevertheless, side reactions which produce ROS. These are principally superoxide (O$_2^{\bullet-}$), peroxyl (ROO$^\bullet$), and hydroxyl ($^\bullet$OH) radicals and hydrogen peroxide (H$_2$O$_2$). ROS react with membranes and nucleic acids, as well as proteins, and their overall effects on the living cell are quite far-reaching (*7*, *9*). Indeed ROS from the side reactions of normal metabolism may be the ultimate cause of aging (*10*, *11*), and ROS damage has been implicated in apoptosis in both animals (*12*, *13*) and plants (*14*).

The roles of protein radicals in enzyme mechanisms and in the oxidative damage caused by ROS cannot be properly understood in the absence of knowledge of the structures and relative stabilities of radicals derived from different amino acid residues. A considerable body of information relating to side chain radicals, such as those of cysteine, tyrosine, and tryptophan, has been built up from studies of peptides and smaller model compounds (*15*, *16*). Results for backbone $^{\alpha}$C-centered radicals, however, are not extensive. Key studies of their mechanisms of decay and reactions with oxygen have been reported (*2*, *17*), but data relating to the relative stabilities or bond dissociation enthalpies is sparse. One reason for that is the special feature of captodative stabilization which they possess (*18*, *19*). The extent of this varies with the Ramachandran angles ϕ and ψ at the radical site (*20*), and this will complicate determinations of thermodynamic properties by experiment. To date the only relevant experimental thermochemical study has been done with the cyclic anhydrides of glycine and alanine, in which the ϕ and ψ both have values close to 0° (*21*).

Modern theoretical/computational techniques are now able to provide structures and energies of model peptides, which are large enough to reflect the protein environment. We have previously used these methods to obtain values of D_{CH}, the enthalpy of bond dissociation (BDE) at 298 K, for the residues of glycine (*19*) and the amino acids with smaller aliphatic side chains: alanine, serine, threonine (*20*), proline (*22*), and cysteine (*23*). Application of the same methods to the anhydrides of glycine and alanine gave good agreement with experiment (*21*). Here we apply this procedure to model

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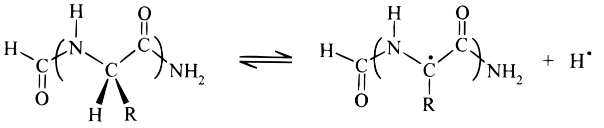
peptides of all of the remaining amino acids. The main objective was to determine how the larger amino acid side chains, including those with aromatic groups, affect the captodative stabilization. The procedure uses neutral glycine in an isodesmic reaction, and the $^{\alpha}$C−H BDEs and the stabilities of the radicals are all determined relative to this same standard. Captodative stabilization was found in all of the amino acid residues. Results from the literature show that it has important consequences. These are discussed in terms of the present results.

METHODS

The $^{\alpha}$C−H BDE for the model PH(res) is defined as the heat of reaction (eq 1), $\Delta H_{(1)}°$, where the specific amino acid residue can be indicated by inserting the conventional abbreviation (Gly, Ala, etc.) in place of "res" in parentheses.



(1)

PH(res) **$^{\alpha}$P$^{\bullet}$(res)**

If calculated directly, $\Delta H_{(1)}°$ is subject to substantial computational errors. Instead, as a means of reducing errors due to basis set and correlation effects, BDEs were derived from the heats of isodesmic reactions (*24*). Here reaction 2 was used with H_2NCH_2COOH (= 331.0 kJ mol^{-1}) (*19*) as the reference molecule, AH.

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

Then one has

$$D_{\mathrm{CH}}(\mathrm{PH}) = D_{\mathrm{CH}}(\mathrm{AH}) - \Delta H_{(2)}° \qquad (3)$$

where $\Delta H_{(2)}°$ was calculated from the energies of the four species in eq 2 each computed at the B3LYP/6-31G* level of theory. This procedure has been shown to yield D_{CH}(PH) values with an accuracy within 10 kJ mol^{-1} (*19*, *21*). Structures of several of the most stable conformations of the parent residue model peptides and the derived $^{\alpha}$C-centered radicals were obtained by full optimization at this level of theory, as implemented in the Gaussian 94 suite of quantum chemistry codes (*25*). In a study of radical stabilization energies of a number of multiply substituted C-centered radicals, it was demonstrated that energies calculated at the level of restricted open shell Moller−Plesset perturbation theory up to second order on the B3LYP-optimized geometries (ROMP2/6-31G*//B3LYP/6-31G*) are closer in a absolute sense to higher level theoretical results than B3LYP itself (*26*). In this study the BDEs of a representative number of residues were also derived at this level within the isodesmic reaction scheme explained above.

Only those structures were considered in which the "backbone" peptide chain conformation is extended, as shown by the examples in Figures 2−4. This corresponds to ϕ,ψ angles in the vicinity of 180°,180°, the predominant conformation in disordered regions of a protein and not far from typical values for an antiparallel β-sheet (−150°,+150°). The extended conformation permits an intraresidue H-bond between the carbonyl oxygen and the vicinal N−H. The side chains of Lys, Arg, His, Asp, and Glu were examined in both their neutral and charged forms. In the latter case, the extended conformation of the side chain was adopted as representative of the structure in aqueous solution.

RESULTS

In Table 1 are listed the $^{\alpha}$C−H BDEs for all of the amino acid residues at both the B3LYP and ROMP2 levels of theory, based on the present model system which adequately incorporates the local internal stereoelectronic effects experienced by a midchain residue (*19*). The data are shown graphically in Figure 1, arranged from left to right in order of increasing BDE (by B3LYP) of the neutral amino acid residue. The vertical scale is in kJ mol^{-1}, and the horizontal bold line denotes the BDE of the S−H bond of glutathione (GSH) or cysteine, 367 kJ mol^{-1} (*23*). Figure 1 also orders in a visual way the relative stabilities of the $^{\alpha}$C radical species, from most stable (Asn) to least (*trans*-Pro).

Although this was not done in the present instance, one may note that the BDEs can be used to estimate the reduction potentials for the $^{\alpha}$C-centered radicals in reaction 4 (*20*):

$$^{\alpha}P^{\bullet}(\mathrm{res}) + e^{-} + H^{+} \rightleftharpoons \mathrm{PH(res)} \qquad (4)$$

The $E°$ ($^{\alpha}$P$^{\bullet}$(res),H^{+}/PH(res)) values are measures of relative stabilities of the radicals or ease of oxidation of their parents in solution.

DISCUSSION

Assessment of Theoretical Reliability. The B3LYP and ROMP2 BDEs of the residues in Table 1 and Figure 1 follow the same trends, with the largest BDEs occurring for Pro and Thr and the weakest for Asn and Asp. The results for Gly by the two methods are identical. For all others the ROMP2 BDEs are higher than those obtained with B3LYP, the average difference being 7.5 kJ mol^{-1}. However, the differences are within the ±10 kJ mol^{-1} uncertainties of the two procedures. Both sets of results are based on isodesmic reactions with H_2NCH_2COOH, taking a BDE of 331 kJ mol^{-1} for that molecule. There are as yet no experimental values for this species, but methylene C−H BDEs for R_2NCH_2-COR compounds determined from pK_{HA} and oxidation potential data are 320 kJ mol^{-1} (*27*), and the value for Me_2-NCH_2COOEt has been shown by photoacoustic calorimetry to lie in the range 331−347 kJ mol^{-1} (*28*). The only experimental BDEs, which are available in systems related to the residues, are those for the cyclic anhydrides of glycine and alanine, 340 and 325 kJ mol^{-1}, respectively (*21*). These are shown by the black hexagons in Figure 1. The values have an absolute error of ±15 kJ mol^{-1}, and agreement with the B3LYP results (glycine, 351 kJ mol^{-1}; alanine, 335 kJ mol^{-1}) is satisfactory. More importantly, the B3LYP values reproduce the experimental difference of 15 kJ mol^{-1} between the two systems, which is much more accurate than the absolute values. The ROMP2 values for the anhydrides, 350 and 340 kJ mol^{-1} for glycine and alanine, respectively, do not reproduce the experimental difference quite as well but are also in accord with the measured BDEs. Thus, in an overall sense the theoretical results are consistent with available experimental data. Since the ROMP2 and B3LYP

Table 1: $^{\alpha}$C−H Bond Dissociation Energies of Amino Acid Residues in the Peptide Model

	E(PH) (hartree)	ZPE (kJ mol^{-1})	*E*($^{\alpha}$P$^{\bullet}$) (hartree)	ZPE (kJ mol^{-1})	BDE^{a} (kJ mol^{-1})	BDE^{b} (kJ mol^{-1})
Nonpolar Aliphatic R groups						
glycine	−377.90145	266	−377.26202	235	350^{c}	350
alanine	−417.21814	342	−416.58038	309	345^{d}	352
valine	−495.84487		−495.20400		352	
leucine	−535.15898	567	−534.52174	535	344	352
isoleucine	−535.15696	567	−534.51748	536	351	360
proline^{e} *cis*	−533.95219		−533.30896		358^{f}	366
trans	−533.95938		−533.31201		369^{f}	
Polar Uncharged R groups						
serine	−492.42896	356	−491.78998	326	349^{d}	352
threonine	−531.74456	432	−531.10256	400	357^{d}	364
cysteine	−815.40336	343	−814.76563	310	346^{g}	353
cystine^{h}	−1252.90711	424	−1252.27536	393	330^{g}	338
methionine	−894.02856		−893.39101		343	
asparagine	−585.92249		−585.29148		326	331
glutamine	−625.22897		−624.59461		334	342
Aromatic R Groups						
phenylalanine	−648.26675		−647.62876		344	
tyrosine	−723.48279		−722.84487		344	356
tryptophan	−779.83622		−779.20100		337	
Positively Charged R Groups						
lysine (0)	−590.49829		−589.86182		340	
(+)	−590.86959		−590.23383		338	
arginine (0)	−700.00074		−699.36422		340	
(+)	−700.40863		−699.77169		341	
histidine (0)	−642.24515		−641.60646		346	353
(+)	−642.63173		−641.99381		344	356
Negatively Charged R Groups						
aspartate (0)	−605.78119		−605.14768		332	
(−)	−605.23293		−604.60547		316	
glutamate (0)	−645.09727		−644.46028		341	
(−)	−644.52919		−643.89605		331	

[a] B3LYP. [b] ROMP2. [c] Reference *19.* [d] Reference *20.* [e] *N*-Acetyl rather than *N*-formyl. [f] Reference *22.* [g] Reference *23.* [h] PH(CysSSCH$_3$), i.e., SH of PH(Cys) replaced by SSCH$_3$.

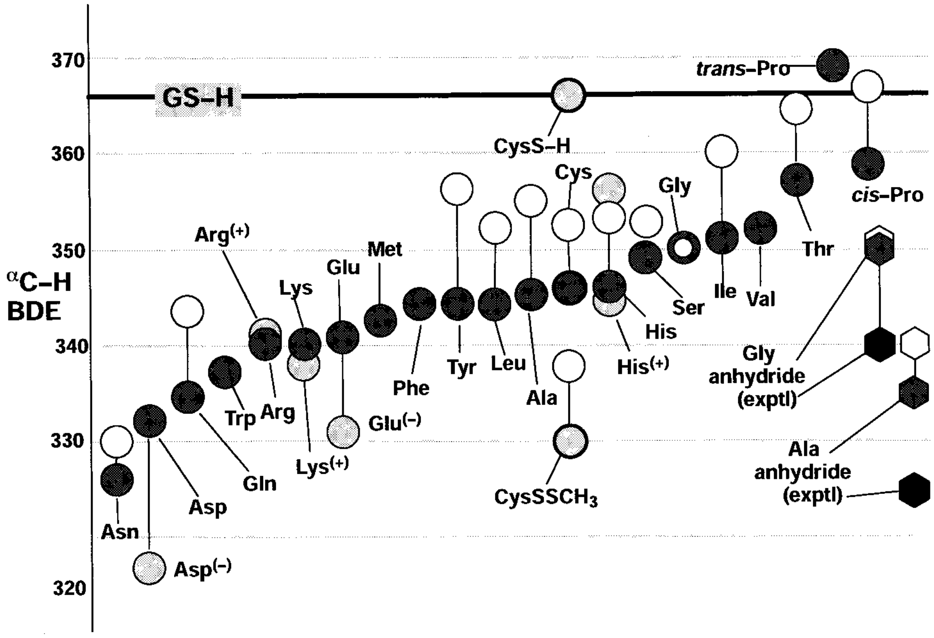


FIGURE 1: $^{\alpha}$C−H bond dissociation energies of the amino acid residues in a fully relaxed model peptide (see text). CysSSCH$_3$ is a model for the cystine residue. The $^{\alpha}$C−H BDEs of residues with ionized side chains are designated with (+) and (−). All results are at the B3LYP level, except white circles and hexagons are at ROMP2. Results for Gly and Ala anhydride are from ref *21*.

BDEs show the same trends, the remaining discussion is based on the B3LYP data. However, the minor differences are noted where relevant. As already mentioned, for Gly residues the ROMP2 and B3LYP results are identical.

Factors That Affect the Stability of the $^{\alpha}$C Radical. All of the $^{\alpha}$C radical species are exceptionally stable compared to most C-centered radicals. For example, the glycine residue has a $^{\alpha}$C−H BDE of 350 kJ mol^{-1}. This value is to be

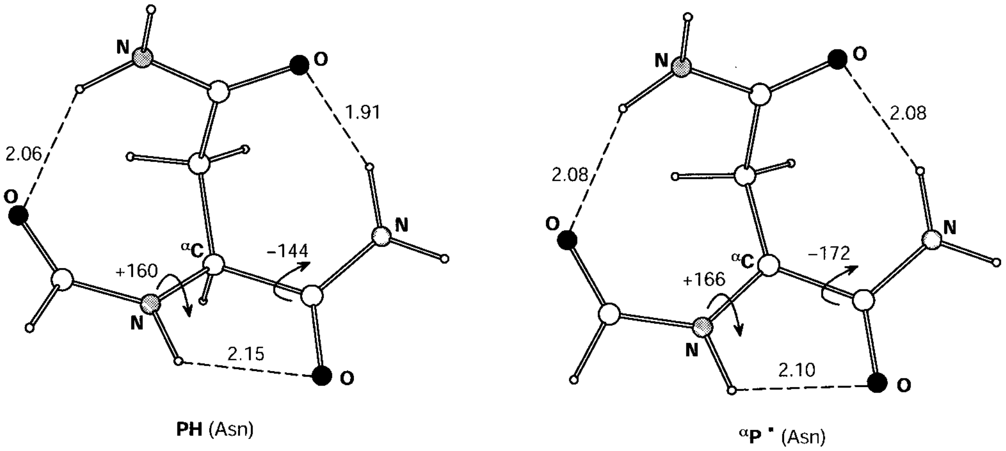


FIGURE 2: Asparagine model peptide and $^{\alpha}$C radical showing the H-bonding network. Hydrogen bond lengths are in angstroms and dihedral angles in degrees.

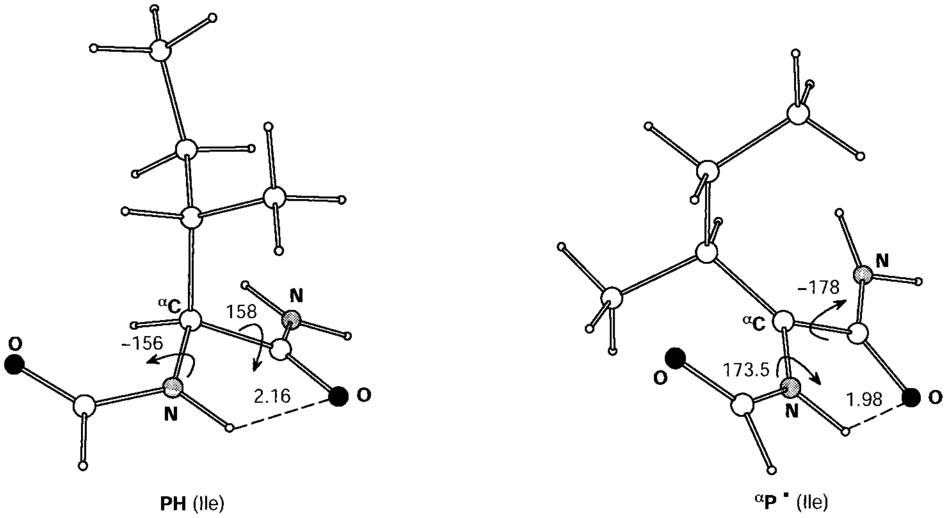


FIGURE 3: Isoleucine model peptide and $^{\alpha}$C radical showing steric crowding. Dimensions as in Figure 2.

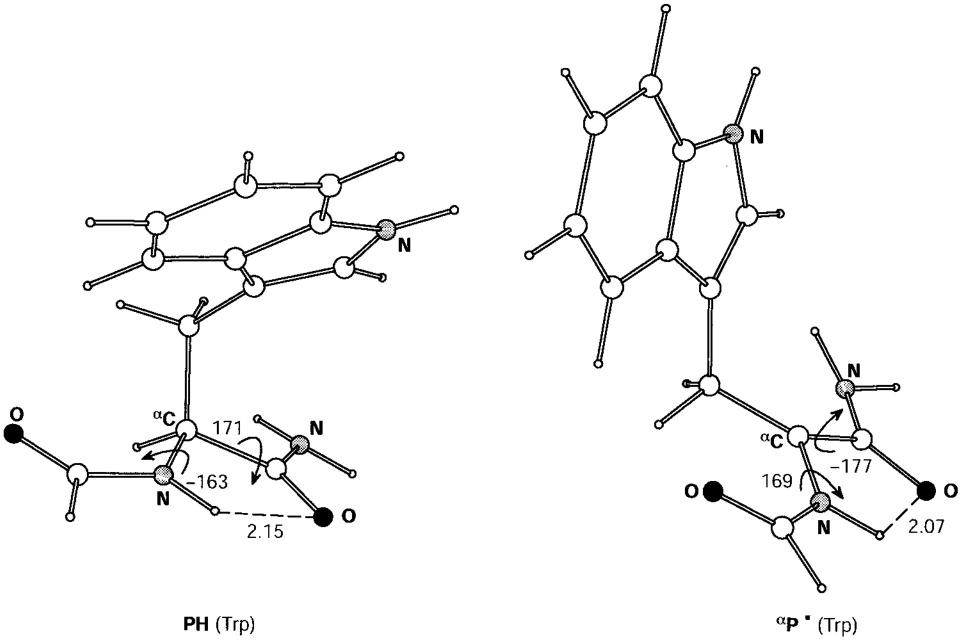


FIGURE 4: Trytophan model peptide and $^{\alpha}$C radical showing steric crowding. Dimensions as in Figure 2.

compared with a secondary C−H BDE of 412 kJ mol^{-1} in C_3H_8 (*29*), 396 kJ mol^{-1} for C−H next to OH in CH_3CH_2-OH (*30*), and 388 kJ mol^{-1} for CH next to N (CH_3NH_2) (*31*). The underlying reason has been called *captodative* stabilization (*18*), a special feature of radicals which can enter into conjugative delocalization with at least one π-acceptor

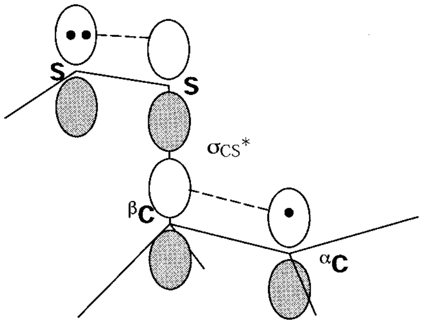


FIGURE 5: Alignment of the nonbonded sulfur lone pair, the antibonding σ orbital of the $^{\beta}$C−S bond, and the singly occupied 2p orbital at $^{\alpha}$C which lead to additional stabilization of the radical in cystine.

Table 2: Bond Dissociation Energies (in kJ mol^{-1} and at 298 K)[a]

	D α_{CH}[b]		
compound	'opt/opt' [c]	β-sheet[d]	α-helix[e]
PH(Gly)	350	361	402
PH(Ala)	345	359	384
PH(Ser)	349	347	381
PH(Thr)	357	356	363
PH(Cys)	346	357	376
PH(Met)	343	359	376
PH(Val)	352	364	379

[a] From B3LYP/6-31G(D) + 0.98 × ZPE level calculations and isodesmic reaction: $^{\alpha}$P$^{\bullet}$ + NH_2CH_2COOH → PH + $NH_2CHCOOH^{\bullet}$. [b] Values for Gly, Ala, Ser, and Thr are from ref *20.* [c] Optimized. Φ,Ψ. [d] $\Phi = -150°, \Psi = +150°$; see ref *20.* [e] $\Phi = -60°, \Psi = -45°$; see ref *20.*

group (in this case, the carbonyl of the amide group) and at least one π-donor group (the N atom of the amide link). In order for maximum effectiveness of the captodative effect, the radical must be able to achieve coplanarity of the $^{\alpha}$C center and the donor and acceptor groups. In the peptide context, this means that ϕ,ψ must be able to approach 180°,-180°. The nature of the side chain poses additional restrictions. If the $^{\alpha}$C center is to become planar, the leading atom of the side chain must also be able to fit into the plane. This is most difficult for the highly substituted aliphatic side chains of Ile, Val, and Thr and for Pro, due to the constraints imposed by the five-membered ring. The situation for the first three is illustrated for Ile in Figure 3, and Pro was discussed in ref *22*. As a consequence, these four residues have less stable radicals and consequently higher BDEs (Table 1 and Figure 1). The side chains of all of the remaining amino acid residues lead off with a CH_2 group, which is not too large in a space-filling sense to fit into the plane. As shown by the example of Trp in Figure 4, this is true even when large aromatic groups are attached. The side chains can act as additional weak π-donors, leading to additional stabilization. Thus most of these remaining amino acid residues have similar C−H bonds to Gly, falling in the range 345 ± 5 kJ mol^{-1} (B3LYP, dark circles in Figure 1) or 353 ± 3 kJ mol^{-1} (ROMP2, white circles in Figure 1). In the cases of Asn, Asp, Gln, and His, the side chains are in a position to make additional H-bonded interactions with both the parent and radical, evidently favoring the radical in most cases. The intramolecular H-bonding network of Asn is illustrated in Figure 2. In the case of Asp$^{(-)}$, where the carboxylate group of the side chain is deprotonated, H-bonding in the radical is especially effective. However, this is unlikely to be the case in aqueous solution where the side chain carboxylate group will be hydrogen-bonded to water or chelated to a metal ion. One may expect a similar situation for the deprotonated side chain of Glu (Glu$^{(-)}$).

The significantly lower value of the $^{\alpha}$C−H BDE of the cystine residue (modeled by PH($CysSSCH_3$)) as compared to cysteine requires some comment. In this case, the interaction of one of the occupied lone pairs (Figure 5) of the second S atom with the $^{\beta}$C−S σ bond lowers the energy of the antibonding σ orbital of this bond, making it a better "π-like" acceptor for stabilizing the $^{\alpha}$C radical, than in cysteine itself. The optimized geometry of cystine radical has the $^{\beta}$C−S bond perpendicular to the average plane of the radical site and parallel to the singly occupied 2p orbital at $^{\alpha}$C (*23*). The alignment of the orbitals is shown in Figure 5.

Secondary Structure Considerations: Effect of Variation in ϕ,ψ. The BDE values given in Table 1 for extended chain conformations are suitable for random coil regions of the protein in a nonaqueous environment as occurs in a lipid bilayer. The chain geometry, defined by ϕ,ψ, local to a particular residue, which is determined by the secondary structure imposed by intramolecular interactions or hydrophilic or -phobic interactions with the aqueous medium, poses limitations on the stabilization of the $^{\alpha}$C radical and hence the BDE. The $^{\alpha}$C−H BDEs for a few amino acid residues whose geometries are constrained to that imposed by β-sheet or α-helical secondary structure are presented in Table 2. Typically, the β-sheet environment leads to about a 10 kJ mol^{-1} increase in BDE, while the severely distorted (from planarity) α-helical environment adds about 40 kJ mol^{-1}.

Relevance to Radicals in Enzyme Mechanisms. It has been pointed out that the active site of pyruvate formate lyase (PFL) contains an $^{\alpha}$C radical at Gly 734 (*32*), and it is in equilibrium with an S-centered radical of Cys 419 (*33*). This requires that the C−H and S−H BDEs are fairly close. A similar situation exists in class III ribonucleotide reductase (RNR) (*1*). The requirement that a C−H BDE be close to the 367 kJ mol^{-1} value of the Cys S−H (*23*) would of course never occur in the absence of substantial captodative stabilization. The present results for the Gly $^{\alpha}$C−H BDE show that it would be in the range covered by ϕ,ψ angles in Table 2. A fully stabilized (planar) glycyl radical may even be *too* stable for effective equilibration, since the discrepancy in BDEs of the glycine parent $^{\alpha}$C−H and S−H bonds would then be 17 kJ mol^{-1}, yielding a theoretical $^{\alpha}$C$^{\bullet}$:CysS$^{\bullet}$ ratio of several hundred. A distortion of the local glycine geometry away from planarity by the enzyme secondary structure would narrow the gap. Indeed one may predict that such a distortion is essential for effective functioning of the enzyme. The question of the local geometry of the glycyl radical has been addressed by ab initio DFT computation of the hyperfine coupling constants as a function of torsion angles and comparison with experimental observations (*34*). In the case of *E. coli* PFL and *E. coli* anaerobic RNR, the conclusions supported a planar glycyl radical, while in the case of bacteriophage T4 anaerobic RNR, a backbone structure which is nonplanar by up to 50° was suggested.

It may be noted that for the case of H atom transfer from $^{\alpha}$C to S the ideal structure of the transition state requires that the S radical be able to approach along the $^{\alpha}$C−H bond vector and has a near linear geometry at the migrating H atom and an H···S−R angle of about 95° (*23*). These requirements are achievable in a random coil region of a protein, and H atom transfer can take place with an activation free energy in the range 60−70 kJ mol^{-1} (*23*).

In mouse class I RNR, an S-centered radical of Cys 429 is involved in the active site on the R1 subunit. Remarkably, the originating radical source is a Tyr phenoxyl type radical on the R2 subunit. The radical center is evidently transferred over a distance of 35 Å to the cysteinyl $-CH_2SH$ via a hydrogen-bonded pathway consisting of His, Asp, Trp, and three Tyr residues (*35*). Substitution of Asn for Asp in this pathway renders the enzyme inactive (*36*), but details of the transfer steps are not yet clear. In this case, to assist in determining the path of minimum energy and whether electron/proton transfer or H atom transfers or both are involved, a knowledge of side chain BDEs and redox potentials is needed, as well as the present data.

Relevance to Oxidative Damage in Proteins

Oxidative damage is an extremely complex process, frequently involving a cascade of separate events. Here literature results for protein damage by hydroxyl, peroxyl, carbon-centered, and sulfur-centered radicals are discussed in terms of the findings of this study.

Damage by $^{\bullet}$*OH*. Because of the high BDE of the O−H in H_2O (499.2 kJ mol^{-1} *(29)*), H abstractions by $^{\bullet}$OH from virtually all biological molecules are strongly exergonic. The reactions of this radical are usually fast (*37*), and primary damage to large proteins occurs mainly at sites on the solvent-exposed surface, particularly at side chains and turns, which make the transition between secondary structural elements (*5*, *6*, *10*, *38*, *39*). In aerated systems the C-centered radicals formed by $^{\bullet}$OH on side chains are found to produce long-lived protein-based peroxide (ROOH) groups (*5*, *6*, *10*). It appears that the primary protein radicals add O_2, and the resulting peroxyl (ROO$^{\bullet}$) radicals abstract H atoms from other sites.

Damage by Peroxyl Radicals. Peroxyl radicals may be formed by other mechanisms than that noted above *(3*), and a knowledge of their reactivity is important. ROO−H BDEs are relatively weak (368 kJ mol^{-1} (*40*)), and H abstractions must be from sites with similar or weaker R−H BDEs, such as the $^{\alpha}$C−H groups studied here (Tables 1 and 2). Experimental studies of $^{\alpha}$C radicals of small peptides showed that in the presence of oxygen addition to the $^{\alpha}$C center occurs. However, unlike side chain ROO$^{\bullet}$ radicals mentioned above, the $^{\alpha}$C center peroxy radicals are short-lived. They give rise to $O_2^{\bullet-}$ and imine via base hydrolysis (*17*). Subsequent hydrolysis of the imine leads to chain rupture and ketone formation (*2*, *3*, *4*, *17*), and the extent of oxidative damage of this type may be assessed by measurements of terminal amino groups or ketonic derivatives (*4*). A recent report of ROO$^{\bullet}$ radical damage to red blood cells showed that protein fragmentation occurred prior to significant hemolysis or lipid damage (*41*). This indicates a surprising sensitivity of the protein backbone and supports the view that sites of weak BDE like the $^{\alpha}$C−H are involved.

Damage by C-Centered Radicals. Because of their strong propensity to react with oxygen, other reactions of C-centered radicals are only seen under anaerobic conditions. Anaerobic conditions are relevant to some food sterilization processes, and several ESR studies have been made of peptides irradiated at low temperature in air-free conditions (*42*, *43*). The primary radicals in those cases, the positive "hole" and electron, both produce primary or secondary C-centered radicals. These have C−H BDEs much larger than the captodatively stabilized $^{\alpha}$C radicals and may cause damge to the protein backbone by H abstraction. Experimentally this is observed to occur in secondary reactions as the matrix is warmed. $^{\alpha}$C-Centered radicals are also observed as the major species in muscle fibers in air-free systems after other radical species have decayed into them, and they are quite long-lived (*44*).

Role of GSH. Living cells contain hydrogen donor molecules (DH), which are able to repair free radical sites, B$^{\bullet}$, created by H abstraction from biological (BH) molecules (*3*, *7*, *45*, *46*). The requirement for this is that the repair reaction, viz:

$$B^{\bullet} + DH \rightleftharpoons BH + D^{\bullet} \tag{5}$$

be energetically favorable, i.e., that $D_{B-H} > D_{D-H}$. Glutathione is one of the more abundant of these hydrogen donors, often being reported in millimolar concentrations. The horizontal bold line in Figure 1 denotes the S−H BDE of that donor, 367 kJ mol^{-1} (*23*). It is evident that, with the possible exceptions of the prolines and Thr (if the ROMP2 results are preferred), $^{\alpha}$C-centered protein radicals with random coil geometries will not be repairable by GSH. A corollary is that, with the same exceptions, $^{\alpha}$C−H bonds of residues in random coil regions *are susceptible to H abstraction by GS*$^{\bullet}$ *radicals*. This possibility has been demonstrated theoretically (*23*) and experimentally in vitro for intermolecular reactions between thiyl radicals of cysteine, homocysteine, and GSH and the $^{\alpha}$C sites of the anions of glycine and alanine (*47*) and for the intramolecular reaction of GSH (*48*). Notably the rate of reaction of RS$^{\bullet}$ with alanine is twice as large as with glycine. On a per H-atom-available basis, this represents a factor of 4 difference. Thus methyl substitution has a significant effect on the lability of the $^{\alpha}$C H atoms.

Damage and Repair in β-Sheet and α-Helical Regions. Reference to the BDEs in Table 2 shows that purely on the basis of thermochemical considerations $^{\alpha}$C−H sites in *β*-sheet regions would also be susceptible to damage by ROO$^{\bullet}$ and GS$^{\bullet}$ radicals. Because of the more extensive loss of the captodative stabilization, those in the α-helical structure would not be. Also, on formation by more energetic reactants such as $^{\bullet}$OH, $^{\alpha}$C radicals which retained α-helical structures should be repairable by GSH. However, for both the *β*-sheet and α-helical secondary structures, steric factors may be quite important. Inspection of models suggests that in both types the $^{\alpha}$C−H bonds of all residues *except Gly* may be sheltered from labile free radicals. Additionally, generation of a $^{\alpha}$C free radical in a *β*-sheet would require that the side chain move toward the plane of the sheet. The *β*-sheet BDE values listed in Table 2 should therefore be regarded as lower limits to the actual values. The observed propensity to damage at residues with smaller side groups, like glycine and alanine (*49*), may be due to these factors.

CONCLUSIONS

Captodatively stabilized radicals are produced by removal of the $^{\alpha}$C−H bond of amino acid residues in proteins. In random coil regions, where the ϕ,ψ angles can aproach 180°,-180°, the stabilization is at a maximum, and most amino acid residues are predicted to have $^{\alpha}$C−H BDEs in the range 345 ± 10 kJ mol^{-1}. Stronger bonds occur in threonine (357) and proline (*cis* 358, *trans* 369). Exceptionally weak $^{\alpha}$C−H bonds occur in glutamine (334), aspartate (332), cystine (330), and asparagine (326). The stabilization is reduced by changes in the ϕ,ψ angles, and the BDEs increase by about 10 kJ mol^{-1} in β-sheet and 40 kJ mol^{-1} in α-helical environments, respectively.

The existence of the captodative stabilization and its dependence on ϕ,ψ means that the $^{\alpha}$C−H BDEs can be "tuned" from about 345 to 400 kJ mol^{-1} by adjusting the local environment. This has important consequences for H-transfer processes in enzyme mechanisms and in oxidative damage to proteins. In the latter case, for instance, it makes $^{\alpha}$C−H sites in random coil regions susceptible to H abstraction by relatively weak oxidants, like GS$^{\bullet}$ and ROO$^{\bullet}$, as well as the highly damaging $^{\bullet}$OH.

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