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Analysis of the Reactivities of Protein C−H Bonds to H Atom Abstraction by OH Radical

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Abstract: Ab initio and density functional theory calculations are used to monitor the process wherein a OH· radical is allowed to approach the various CH groups of a Leu dipeptide, with its $CH_2CH(CH_3)_2$ side chain. After forming an encounter complex, the OH· abstracts the pertinent H atom, and the resulting HOH is then dissociated from the complex. The energy barriers for H· abstraction from the β, γ, and δ CH groups are all less than 8 kcal/mol, but a significantly higher barrier is computed for the $C^{\alpha}H$ removal. This higher barrier is the result of the strong H-bonds formed in the encounter complex between the OH· and the NH and C═O groups of the peptide units that surround the C^{α} atom. This low-energy complex represents a kinetic trap which raises the energy needed to surmount the ensuing H· transfer barrier.

Introduction

The interactions of free radicals with biological molecules are essential components to life. NO·, for example, has been shown in recent years to play a major role in mammalian tissue, e.g. cellular communication.[1] The proper handling of superoxide anion radical too is an essential ingredient in maintaining a proper metabolic balance. The reactions of free radicals with peptides and proteins[2,3] are involved in entire classes of enzymes, such as ribonucleotide reductases, pyruvate formate lyase, cytochrome c reductase, prostaglandin H synthase, galactose oxidase,[4] quinol oxidase[5] and ferredoxin oxidoreductase,[6] as well as lysine 5,6-aminomutase,[7] and proposed for cytochrome P450.[8] Nevertheless, our understanding of these reactions remains much less complete than many other reactions of biochemical importance.

It has been noted recently[9] that free radical reactions with amino acids and peptides are of fundamental importance and indeed ubiquitous. Of possible radicals, hydroxyl (OH·) is of especial significance.[10,11] The primary first step of these reactions[11−13] is the abstraction of a H atom from a C−H bond, either from the protein backbone or from an amino acid side chain. The question of preferred site is important as abstraction of H· from the α-carbon and subsequent oxidation leads directly to cleavage of the peptide backbone, whereas abstraction from the side chain carbons leads primarily to hydroxylation instead of cleavage. The determination of the site of initial abstraction is not always straightforward, as it can be obscured[10] by the multiplicity of products that are typically found.

It was learned some time ago, from study of certain derivatives,[12−14] that abstraction of a H atom from Gly usually occurs from the C^{α} atom. The α radical was thought to be stabilized by a "captodative effect" that combined resonance of electron-withdrawing with the electron-donating capabilities of substituents.[15] This work hypothesized that the reactivity of Gly was due in large part to its lesser steric interactions, since it has no side chain. Extensive work, carried out over a long time frame,[10−13] has shown that the OH radical can remove a H atom from other sites as well, such as from alkane side chains of certain amino acid residues. In fact, at physiological pH, the α-H atoms of the amino acids are less reactive to the OH· than are the side chain C−H bonds. NMR $^1H/^2H$ exchange studies[11] confirmed these ideas and indicated that aliphatic amino acids are the most active. This idea was quantified,[16] in that the reaction of side chains with hydroxyl radicals occurs at rates 10 to 1000 times faster than the abstraction of hydrogen from C^{α}. The lower reactivity of backbone CH groups was reiterated recently,[9] where it was also pointed out that β and γ positions offer more reactive sites for H abstraction and that reactivity increases as one moves further from the backbone.

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There were a series of ab initio calculations that addressed the issue of H· abstraction from protein models. Computations were used to characterize the parent species and C^{α} radicals for Ala, Ser, and Thr, both as free neutral amino acids and as residues in model peptides, intended to mimic the midchain environment in proteins.[17] This work focused its attention on the bond dissociation energies, BDEs, needed to fully remove the H atom from the molecule of interest, with the presumption that the overall thermodynamics of this H removal should mirror the kinetics in the actual chemical process. The calculations indicated that the removal of a $C^{\beta}H$ requires some 13−27 kcal/mol more than that of the corresponding $C^{\alpha}H$. This idea was extended to Cys,[18] with much the same general conclusions, but the work successfully identified a number of transition states for H· abstraction, albeit only for the $C^{\alpha}H$. More extensive consideration of all the amino acids[19] again focused on the BDEs, reiterating that the energy required for $C^{\alpha}H$ removal is less than that of other C−H bonds, attributed to the captodative effect. A later set of calculations[20] considered the effects on the BDE of the occurrence of the amino acid within the context of a β-sheet and identified the transition state for removal of the $C^{\alpha}H$ of Gly. Other groups of workers[21] also focused on BDEs several years later,[22] using higher levels of theory, but unfortunately limited themselves to only the C^{α}−H bonds. The energetics of the removal of a $C^{\alpha}H$ from Gly and Ala were studied by Galano et al.,[23] who demonstrated that a prereactive complex was formed prior to the H· abstraction, work that was later confirmed by Lin et al.[24] that applied higher levels of theory to the Gly amino acid.

These theoretical studies suffered first from the presumption that the BDE is directly correlated with the rate of H· abstraction. In other words, a more exothermic overall reaction must be tied to a lower energy barrier. While there is a certain amount of logic in this idea, it is certainly not guaranteed and can easily be complicated by a number of other issues. A second problem was the assumption that the H atoms that are removed by OH· come only from the C^{α} atom.

Determination of the site of OH· attack is important in understanding[10] the pathological role that the OH radical may play in oxidative stress and to predict[11] sites of oxidative damage to proteins. It is to this question, of assessing the relative reactivities of different CH sites to H· abstraction, not only α but also β, γ, and δ, that the current work applies quantum chemical calculations. One goal is to resolve an apparent contradiction in that, although the $C^{\alpha}H$ bonds are easiest to fully pry apart, yet they seem to be more resistant to abstraction of a H atom by a OH· radical. Moreover, the computed data are analyzed to provide insights into the underlying reasons for the differing susceptibilities to OH· attack and how such principles can be used to better understand the reactions of proteins with free radicals.

Computational Details

The Leu amino acid was chosen for investigation here for a number of reasons. First, it contains a simple alkyl side chain which has generated perhaps the most discussion regarding site of H· abstraction.[10,11,16] The alkyl side chains react most readily with radicals,[9,11] and Leu the most reactive of these, providing an optimal platform from which to attempt to understand the details of the mechanism. It is also helpful for purposes of analysis that the results will not be complicated by functional groups such as −OH or −COOH, or aromatics, which could obscure the natural predispositions of neighboring CH groups to lose a H atom. With specific regard to Leu, not only is it the most reactive residue, but it contains in addition to α, also β, γ, and δ CH groups from which a H· can be extracted in principle. The work will therefore provide an analysis of the relative reactivity of each of these different groups, within an alkyl environment.

The Leu side chain ($R = CH_2CH(CH_3)_2$) was placed within the context of a $NH_2COCHRNHCHO$ dipeptide so as to mimic the peptide environment by surrounding the side chain by two full peptide units. A OH· radical was allowed to approach the Leu residue from a number of directions, as described below. After identification of a fully optimized minimum on the potential energy surface, in which the OH· forms a H-bond with one of the CH donor groups of Leu, the latter H atom was transferred, leading to a complex involving a HOH molecule and a remaining Leu· radical, whose geometry was also fully optimized. The transition state characterized by roughly a half transfer of the H atom was optimized and identified, allowing extraction of the energy barrier to this transfer.

All calculations were performed using the Gaussian03 code.[25] The initial scans of the potential energy surface, including geometry optimizations, were carried out by the DFT approach, using the BHandHLYP variant,[26−29] as earlier tests[24,28,30] have suggested this particular DFT functional provides the best results for systems of this type, especially for H· abstractions[31] comparable in accuracy[32,33] to the highly accurate CCSD(T), leading to data that are in excellent agreement with experiment.[33,34] The first basis set used was 6-31+G**, a versatile doubly polarized set, containing diffuse functions. Geometries were optimized as described below, and minima and transition states characterized by the number of imaginary frequencies were obtained.

Single-point energies were computed at higher levels of theory, using the geometries optimized at the BHandHLYP/6-31+G** level. The first such calculations involved the correlated UMP2 level[35,36] (inner shells excluded from the correlation), again with the 6-31+G** set. This particular prescription has had good success in mirroring experimental rate information with H· abstraction

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reactions by OH•, for example from CH_3SCH_3.[37] To ensure a low level of sensitivity to the basis set, UMP2 computations were also performed with the aug-cc-pVDZ set, designed specifically[38] for correlated calculations. Prior calculations[39] confirm that the 6-31+G** basis set is capable of supplying geometries quite similar to those obtained with much larger sets, introducing an error of no more than 0.2 kcal/mol into the results in reactions of this type. Analogously, energies computed using DFT geometries are very similar to those of UMP2-optimized geometries in related H• abstraction reactions.[40] In order to provide an even more rigorous check on the computed data, calculations were also carried out using levels of theory more sophisticated than UMP2. CCSD(T)[41−43] has been shown to provide results for reactions of this type quite similar to multireference calculations,[44] especially for single-point calculations of BHandH geometries[45−47] of H• abstractions, the procedure applied here. In most cases, energies are reported as electronic energies, absent vibrational and thermal terms. The former quantities better represent the intrinsic energetic properties of each system. Also, computed vibrational energies would strictly apply only to the model systems undergoing the calculations, and not to the larger proteins which are the real subject of this work.

In order to approximate the effects of moving the system from an isolated environment to one more closely approximating the protein interior, the self-consistent reaction field (SCRF) approach[48−50] was employed in which the polarizable continuum method (PCM)[51−53] embeds the solute in a cavity that reproduces the shape of the molecule by a series of overlapping spheres. The particular variant of this method used here is the conductor polarized continuum model (CPCM)[54] wherein the apparent charges distributed on the cavity surface are such that the total electrostatic potential cancels on the surface. Recent calculations[55] had shown that the CPCM variant provides results that are in good agreement with other approaches, notably PCM and SCIPCM, in treating CH••O as well as conventional H-bonds.

Results

A full geometry optimization of the Leu dipeptide results in the structure illustrated in Figure 1, in which the terminal O and NH_2 groups of the two peptide units engage in an intramolecular H-bond. This geometry has been characterized in the literature as a C7 conformation, as there are seven atoms involved in the ring of which this H-bond is a part. The (φ,ψ) dihedral angles of this C7 structure are (−83.6°,77.7°), enabling the NH proton to approach within 2.141 Å of the terminal O. Figure 1 also labels each relevant C atom with its appropriate label as α, β, etc.; note that there are two C^{δ} atoms in this particular amino acid side chain.

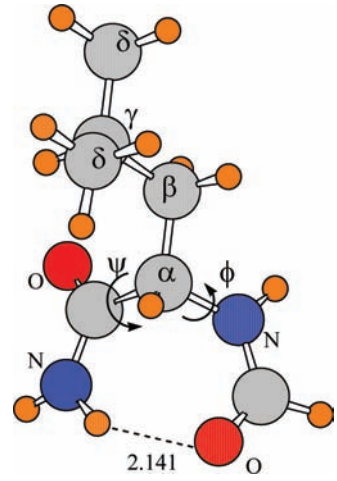

Figure 1. Optimized geometry of Leu dipeptide, illustrating the dihedral angles (φ,ψ) within the backbone, and the appropriate labels of each C atom. R(H••O) in Å.

Table 1. Electronic Energies (kcal/mol) Required To Remove a H Atom from Each of the Four C Atoms of Leu Dipeptide and from HOH

	BHandHLYP/6-31+G**	UMP2/6-31+G**	UMP2/aug-cc-pVDZ
α	94.6	96.7	96.1
β	105.2	105.3	105.1
γ	100.3	101.1	101.4
δ	106.2	105.3	104.7
HOH[a]	117.4	121.5	122.8

[a] Experimental value = 127 kcal/mol, evaluated by correcting ΔH of 118.8 kcal/mol by zero-point and thermal energies.

Removal of a H atom from each of the α, β, γ, and δ C atoms leads to the corresponding radical, each of which was also fully optimized at the BHandHLYP/6-31+G** level. Upon removal of its H atom, the corresponding C atom converts from a tetrahedral geometry to one that is closer to a planar sp^2 structure. Removal of H• from the β, γ, and δ carbons left the (φ,ψ) angles virtually unchanged, but the α radical was quite different in this respect. The planarization of the C^{α} atom caused these two angles to change a good deal, to (−35.9°,+3.3°). The remaining α radical retained the intramolecular C7 NH•••O H-bond, which in fact shortens to 1.865 Å, indicating a strengthening.

The energies required to remove a H• atom from each of the four C atoms are reported in Table 1 where it may be seen first that the α radical is lower in energy than the others by several kcal/mol. Of the other three, the γ radical is most stable, with β and δ very close to one another. It is of interest to note that the C^{γ} atom is tertiary, surrounded by three other C atoms, while C^{β} is secondary and C^{δ} primary. The latter factor must be weighed in with the proximity of the C−H bond of interest to the electronegative peptide groups. It is worth stressing that the results and patterns are affected very little by inclusion of correlation via the UMP2 procedure, or by changing the basis set from 6-31+G** to aug-cc-pVDZ, adding some confidence in the validity of BHandHLYP/6-31+G** data. While there are no experimental values available for these properties with which

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to compare directly, quantities derived from related systems are available. C−H bond dissociation enthalpies of $EtOCOCH_2$-NMe_2 and $MeCOCH_2NR_2$, and for the Gly and Ala cyclic anhydrides, all corresponding roughly to $C^{\alpha}H$ dissociation, have been determined[56−58] to lie in the 76−83 kcal/mol range. By adding a vibrational and thermal correction of −8.5 kcal/mol to the electronic energies in Table 1, the BHandHLYP/6-31+G** value for the $C^{\alpha}H$ dissociation enthalpy is 86 kcal/mol, quite close to experimental analogues.

The last row of Table 1 contains the corresponding bond dissociation energy of the water molecule, which would take it to the OH· radical. This quantity is a bit larger than those reported above, indicating that the abstraction of a H· atom from any of these sites in leucine would be exothermic, as discussed below. The computed dissociation energies of HOH are several kcal/mol smaller than the experimental value of 127 kcal/mol, obtained by correcting the experimental ΔH[59,60] by zero-point and thermal terms. This small discrepancy is apparently not an artifact caused by the levels of theory applied here, since higher levels yield very similar values. For example, when applying the augmented, polarized, triple-valence 6-311++G** set, the HOH bond dissociation energies computed at the UMP2, UMP3, UMP4SDQ, CCSD, and CCSD(T) levels are, respectively, 122.3, 117.6, 118.0, 119.3, and 119.3 kcal/mol, all of which fall right within the range of computed values in the last row of Table 1. Nor would higher levels of theory significantly affect the bond dissociation energies of the various CH sites of leucine. Again considering the 6-311++G** basis, the CH^{α} bond dissociation energies computed at the various correlated levels listed above are all in the narrow range of 95.5−96.6 kcal/mol, again quite similar to the values in the first row of Table 1. Similar insensitivity to the level of correlation, with a polarized, augmented, triple-valence basis set, is exhibited also by the β, γ, and δ H atom dissociations, wherein the values computed at the higher levels of correlation fall right into the ranges reported in Table 1. It is not unusual for ab initio calculations of bond dissociation energies, even at very high levels, to deviate to a certain extent from experimental measurements. For example, a recent set of G3 computations of the C−S bond dissociation energy in the amino acid cysteine[61] differed from the experimental value by several kcal/mol.

The H· abstraction reaction was presumed to proceed by the attack of a OH· radical which first forms a CH···OH H-bonded complex with the H that is to be removed, a supposition supported by a good deal of prior data on related systems.[62−66] It should first be noted that the NH groups of the peptide units offer a much stronger proton donating site than do the various CH groups. As a second point, the two O atoms of the peptide units are both strong proton acceptors and will tend to attract the H end of the OH· radical. Bearing this in mind, it is thus no surprise that when placed in the vicinity of the H^{α} atom, the OH· radical moves toward the stronger H-bonding sites and forms a complex in which the $C^{\alpha}H\cdots O$ is longer, and presumably weaker, than are the two other H-bonds illustrated in Figure 2a. Following the full transfer of the H^{α} atom to OH, the complex between the α-radical and HOH is depicted in Figure 2c, where the two primary H-bonds again involve the two peptide units. The transition state for this H^{α} abstraction is displayed in Figure 2b, where it may be seen that the H atom lies slightly closer to C^{α} than to the hydroxyl O atom, consistent with an overall exothermic process (see below).

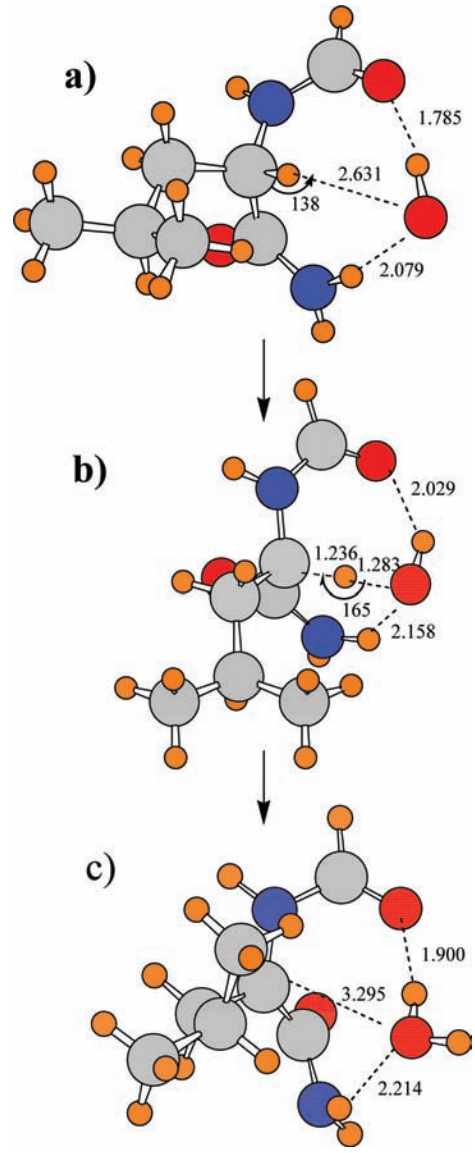

Figure 2. Optimized structures of (a) Leu dipeptide + OH·, (b) transition state for transfer of H^{α}, and (c) complex pairing α-radical with HOH. All distances in Å, angles in deg.

At the BHandHLYP/6-31+G** level, the overall energetics for this reaction are as follows. The prereaction complex in Figure 2a lies 10.5 kcal/mol lower in energy than the fully separated Leu + OH· reactants, due primarily to the H-bonds that are present. From this point, an energy barrier of 13.4 kcal/mol must be surmounted, in order to reach the complex between the α-radical and HOH, which in turn lies 16.6 kcal/mol lower

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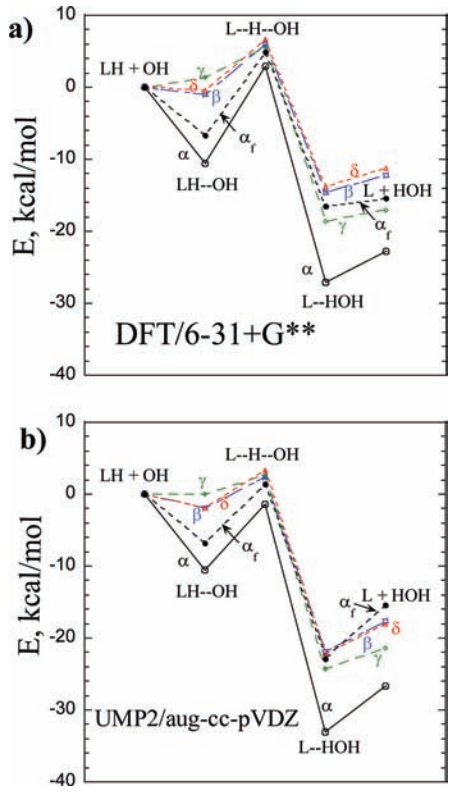

Figure 3. Energetics of H• abstraction reaction at (a) BHandHLYP/6-31+G** and (b) UMP2/aug-cc-pVDZ levels. LH refers to Leucine dipeptide, and L to its radical. The α_f designation signifies a freezing of the (φ,ψ) angles of the dipeptide. No vibrational terms have been added to electronic quantities.

in energy than the original complex between Leu and OH•. The final dissociation to the separated α-radical and HOH requires 4.3 kcal/mol. The energetics may be pictured by the lowest-most solid curve in Figure 3a, where the separated Leu + OH• is taken as the arbitrary zero for the energy.

The β group is far enough removed from the two peptide units that it can form a $C^{\beta}H\cdots OH$ H-bond that is not influenced by the stronger NH and C═O groups of the former. As may be seen in Figure 4a, the H-bond is linear, albeit rather long with $R(H\cdots O) = 2.62$ Å, typical of such CH••O H-bonds. The situation is more problematic, however, subsequent to the transfer of the H^{β} atom to OH. The trigonal C^{β} atom is a very poor proton acceptor indeed, a finding common to other work,[67] so the HOH moves down toward the two peptide groups, leaving the distance between C^{β} and the H of HOH at 3.0 Å, as compared to 1.9 Å for the distance between this same H and the carbonyl O of a peptide unit. On the other hand, if the HOH is held up near the C^{β} atom in a linear $(OH\cdots C^{\beta})$ configuration, it is possible to obtain an appropriate minimum for the H-transferred state. The relevant geometries are illustrated in Figure 4, which includes the transition state for the transfer of H^{β}. As in the α case, the transition state occurs with nearly equal $r(C\cdot\cdot H)$ and $r(H\cdot\cdot O)$ distances, with the former slightly shorter than the latter.

Due to the presence of only a weak $C^{\beta}H\cdot\cdot O$ H-bond in the initial association complex, the latter lies only 0.9 kcal/mol lower in energy than the fully separated species. The barrier to the transfer is 6.9 kcal/mol, leading to a reaction product (Figure

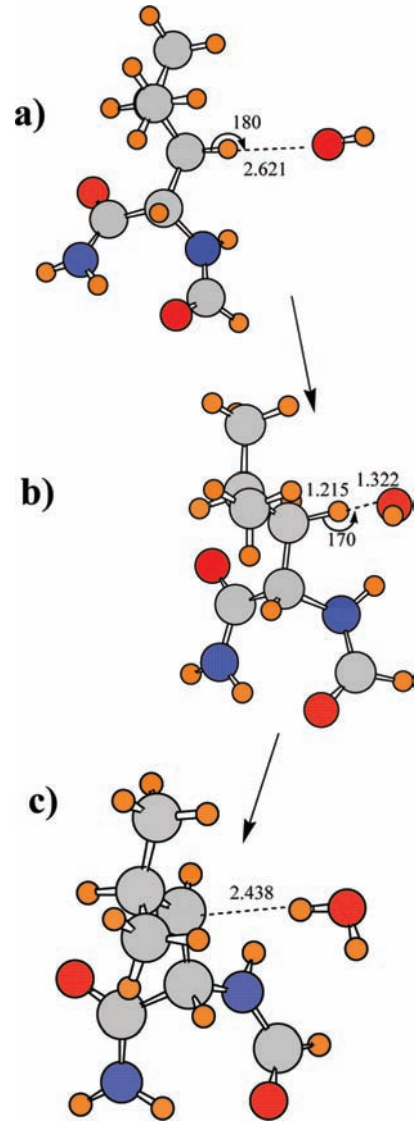

Figure 4. Optimized structures involving transfer of β H atom showing (a) Leu dipeptide···OH•, (b) transition state, and (c) complex pairing β-radical with HOH.

4c) that is 13.6 kcal/mol lower in energy than the initial encounter complex. Due to the weakness of the $OH\cdots C^{\beta}$ H-bond in the latter, the removal of the water molecule requires only 2.3 kcal/mol. The full reaction profile for this β H• extraction complex is illustrated by the broken blue curve in Figure 3a.

As one last point of information, if the restriction that the water in Figure 4c lie directly perpendicular to the C^{β} atom is removed, the water moves down closer to the superior H-bonding atoms of the two peptide units. The energetic result of this relaxation is to reduce the energy barrier for the H• transfer from 6.9 to 3.1 kcal/mol and to enhance the exothermicity of the $LH\cdots OH \rightarrow L\cdots HOH$ process from −13.6 to −22.7 kcal. In summary, such a relaxation would facilitate this entire process.

Turning next to the CH^{γ} removal, the process looks much like the β reaction. Specifically, the OH• radical forms a weak $CH^{\gamma}\cdot\cdot O$ H-bond, with $R(H^{\gamma}\cdot\cdot O) = 2.64$ Å. Subsequent to H^{γ} removal, the HOH remains engaged with the now-planar C^{γ}, with $R(H^{\gamma}\cdot\cdot C) = 3.2$ Å, but also forms what is a shorter H-bond with one of the C^{β} H atoms, with $R(O\cdot\cdot H^{\beta}) = 2.65$ Å. As is

(67) Mardyukov, A.; Crespo-Otero, R.; Sanchez-Garcia, E.; Sander, W. *Angew. Chem., Int. Ed.* **2010**, *16*, 8679–8689.

evident from the green broken curve in Figure 3a, the γ energetics are much like those for the β removal, with nearly coinciding transition state L--H--OH energies. Likewise is the case for the δ H atom removal, wherein the red broken line in Figure 3a very nearly overlaps the β energetics.

In order to feel comfortable that the energetics in Figure 3a were not an artifact of the use of a DFT method, in this case BHandHLYP, the energies of all stationary states in the figure were recomputed at the UMP2 level. The UMP2/6-31+G** results were quite similar to BHandHLYP/6-31+G** in all respects. As a further check, this time upon the basis set, UMP2 calculations were repeated using the aug-cc-pVDZ basis set. The data are depicted in Figure 3b which may be seen to mimic the lower level data in Figure 3a to a high degree.

It was noted earlier that removal of the β, γ, and δ H atoms left the (φ,ψ) dihedral angles of the dipeptide virtually unchanged. This same immutability applies also to the complexes with OH and HOH, as well as the transition states for H· transfer. The φ angle remains within the very narrow range of $-86° < \varphi < -83°$, while there is a little more flexibility in the other angle: $68° < \psi < 87°$. But it is the H^{α} removal that causes by far the biggest changes in these angles, not only when the H· is fully removed but also in the various stages. Specifically, the φ angle changes from $-105°$ when the OH· is initially complexed with the Leu dipeptide, to $-68°$ in the transition state, and then to $-53°$ after H^{α} has been extracted to HOH. The changes in ψ are just as dramatic, going from 113° to 130° and finally to 152°. In other words, these angles must each change by some 40°−50° to follow the reaction energetics illustrated by the solid α curves in Figure 3.

Recalling that the (φ,ψ) angles involve the polypeptide backbone, it follows that changes of these angles by this magnitude would correspond to substantial alterations in the folding pattern of the protein, locally if not globally. The α energetics in Figure 3 thus presuppose a high degree of flexibility in the protein's backbone, which may in fact not be present. In order to more fully probe this issue, the process of extraction of the H^{α} atom was recomputed, only this time the (φ,ψ) angles were held to their values in the fully optimized Leu dipeptide, prior to any association with the OH·.

As before, the initial site of OH· association is aligned with the C═O H-bonding position of a peptide unit, much like that pictured in Figure 2a. The enforcement of the aforementioned restrictions on φ and ψ forces the HOH to abandon any association with C^{α} after H· extraction, which would result in a very high transfer barrier. One might thus conclude that enforcing a certain amount of rigidity into the polypeptide backbone would effectively preclude extraction of the H^{α} atom by OH·.

On the other hand, one can pursue this idea further by insisting that the HOH remain associated with C^{α}. This task was carried out here by forcing the HOH to lie along the approximate perpendicular of the C^{α} atom. The energetic result of this restriction is illustrated by the broken black curve labeled α_f in Figure 3. The frozen energetics differ overall in being higher in energy throughout. Both the initial LH--OH and final L--HOH complexes are less stable, as is the transition state for H· transfer. The energy barrier for passing from the initial complex to the transition state, however, is only slightly smaller in the frozen case, still higher than the barriers for the β, γ, and δ cases.

The latter statement is true not only at the DFT level but also for UMP2 with either basis set. More specifically, Table 2 shows that, at all levels of theory, the barrier for H^{γ} removal is the lowest, followed closely by H^{β} and then by H^{δ}. These barriers lie in the range of 2.7−5.2 kcal/mol at the UMP2/aug-cc-pVDZ level and 4.1−7.1 kcal/mol for DFT. The barrier for H^{α} extraction is two to four times higher. The latter distinction is ameliorated only a little if the φ and ψ backbone angles are frozen. Indeed, even higher levels of theory reveal precisely the same trends. The H· abstraction barriers were calculated with the triple-valence polarized 6-311++G** basis set at a range of different levels of incorporation of electron correlation. As displayed in Table 3, these levels varied from UMP2 to UMP4SDQ, as well as CCSD and CCSD(T). In every case, the barrier for abstraction of the H^{α} atom is highest, appreciably higher than any of the other three H atoms. Also in common with the trends in Table 2 which were limited to UMP2, the lowest barrier is associated with H^{γ} abstraction, followed by H^{β} and H^{δ} in that order. One may conclude then that the trends in H· abstraction barrier computed and displayed in Table 2 and Figure 3 are independent of the level of theory chosen.

Table 2. Energy Barriers[a] (kcal/mol) for the Extraction of a H Atom from Each of the Four C Atoms of Leu Dipeptide, from the Initial Encounter Complex LH--OH

	BHandHLYP/6-31+G**	UMP2/6-31+G**	UMP2/aug-cc-pVDZ
α	13.4	12.1	9.3
α_f	11.6	10.7	8.2
β	6.9	7.0	4.3
γ	4.1	4.9	2.7
δ	7.1	7.9	5.2

[a] Electronic energies, without addition of vibrational terms.

Table 3. Energy Barriers[a] (kcal/mol) for the Extraction of a H Atom from Each of the Four C Atoms of Leu Dipeptide, from the Initial Encounter Complex LH--OH, All Computed with the 6-311++G** Basis Set

	UMP2	UMP3	MP4SDQ	CCSD	CCSD(T)
α	10.9	15.0	12.7	11.1	8.0
β	6.2	10.0	8.1	7.0	4.2
γ	4.3	7.5	5.7	4.4	2.1
δ	7.1	10.9	8.9	8.0	5.7

[a] Electronic energies, without addition of vibrational terms.

One may wonder how much the higher barrier of the α H· extraction might slow the process down, relative to the other sites. If one simply incorporates the energy barriers listed in Table 2 into the Arrhenius expression, wherein the rate is proportional to $\exp(-E^{\dagger}/RT)$, with $T = 298$ K, then the H· abstraction rate of 13.4 kcal/mol for the H^{α} extraction at the DFT level is estimated to be 2.3×10^{-5} times slower than the rate for H^{δ} extraction, with a barrier of 7.1 kcal/mol. This ratio is even smaller, 1.4×10^{-7}, if one compares H^{α} with H^{γ}. Considering the UMP2/aug-cc-pVDZ barriers in the last column of Table 2, the ratios for H^{α} to H^{β}, H^{γ}, and H^{δ} are respectively 3.8×10^{-5}, 2.5×10^{-6}, and 1.8×10^{-4}. These relative rates are qualitatively similar to experimental estimates[16] that the reaction of side chains with hydroxyl radicals occurs at rates 10 to 1000 times faster than the abstraction of hydrogen from the C^{α} carbon, and that little OH-catalyzed $^2H/^1H$ exchange occurs in Gly, which contains only $C^{\alpha}H$ protons.[11]

Of course, the aforementioned values are only approximations. For one thing, the quantitative effects of quantum mechanical tunneling have not been included. Also, the energetics applied refer only to the electronic energies, without inclusion of vibrational contributions. It is problematic to apply the vibrational energies computed for these systems, in which a

dipeptide segment of a full protein interacts only with OH•, to the broader picture of a larger protein molecule, where all vibrational motions would be heavily influenced by the entire biomolecular system. But having said that, there is a broad uniformity in that the total vibrational energy of the LH--OH system exceeds that of the L--H--OH transition state by 4 kcal/mol for all systems, whether α, β, γ, or δ. Hence, introduction of vibrational energies would have the effect of reducing all energy barriers uniformly by this amount. This consideration would thus not influence the relative quantities in Table 2, leaving all trends unchanged. Nor would this uniform barrier reduction affect the reaction rate ratios reported above, although all rates would be faster by a factor of some 900.

Consideration of Figure 3 and Table 2 leads to the conclusion that the β, γ, and δ H• abstraction processes are energetically similar to one another, but all have some important distinctions with the H^{α} removal. The entire process, beginning with the initial approach of the OH• radical to the dipeptide, and then the final product of dipeptide radical + HOH, is exothermic in all cases, but substantially more so for H^{α} abstraction. This distinction can easily be traced to the lower energy requirement to fully dissociate a H atom from the C^{α}, as opposed to the β, γ, or δ carbons, as reported in Table 1.

But consideration of energy barriers leads to a different story. The barrier that the system must surmount in order to pass beyond the LH--OH encounter complex (Table 2) is notably higher for the α H• abstraction than for the other H atom removals. A glance at Figure 3 reveals the major reason for the higher barrier in the α case lies in the lower energy of its LH--OH complex, which is due in turn to the stronger H-bonds formed when the OH• radical interacts with the C═O and NH groups of the peptides which surround the α C. This stable complex acts as a kinetic trap of sorts, slowing down the H• transfer to the OH radical. In other words, the lesser predilection of a OH• radical to abstract a H^{α} atom is due not to any intrinsic properties of the various C−H bonds, but rather to the proximity of the peptide groups which form strong H-bonds to OH•, which stabilize the system, impeding progress toward the transition state for H^{α} transfer.

Discussion

This work agrees with some of the most recent findings[9−11,16] that the backbone (i.e., C^{α}) is deactivated toward H• abstraction and that reactivity is enhanced as the site is further removed from the backbone. This trend results from the fact that the increased distance from the backbone diminishes the chance of the OH• engaging in H-bonds with the peptide groups, and thus falling into a kinetic trap. The important role played by the NH and CO groups that surround the C^{α} atom is supported by a recent study[24] wherein a OH• radical was allowed to interact with a simple glycine amino acid NH_2CH_2COOH. Of course, this system is quite different from our Leu dipeptide in that, first, it contains only H^{α}, with no β, γ, or δ H atoms. Second, the C^{α} is surrounded by the smaller NH_2 and COOH, rather than the more complete peptides of our model. Nonetheless, two prereactive complexes were identified: the OH formed a strong H-bond with the NH in one and with the C═O group in the other.

It was stressed above that although it requires less energy to completely remove a H• atom from the α C atom than from the other sites, this low bond dissociation energy does not imply that the H^{α} abstraction by a radical represents the most facile process when the entire process is considered. This distinction was attributed to the proximity of H-bonding segments of the neighboring peptide units. This behavior is to be contrasted with a number of simpler systems, where direct correlations have been found between energy barrier and C−H bond dissociation energy. In butanol, for example, Moc and Simmie found that the −OH group does not interfere with the association of the incoming OH• radical with the appropriate C−H group.[68] Consequently, the extraction of a H atom from the position adjacent to the −OH group by the OH• radical is associated with the lowest energy barrier, just as the C^{α}−H bond has the lowest dissociation energy. There is a strong correlation also between these energy barriers and bond dissociation energies for the other three C positions in butanol. It is relevant as well to note that when the OH• is replaced by OOH•, high level calculations with large extended basis sets[69] find an order of ease of H• abstraction from the various C atoms of butanol identical to that noted with OH•, suggesting that our conclusions here for OH• are likely more general, applicable to other small radicals as well.

In terms of the quantitative values obtained for the barriers of H• abstraction, the values in Table 2 are consistent with other calculations of related systems. The barrier height for the abstraction of a H atom from a saturated C atom, within the context of the morpholine ring,[70] was computed to lie between 7.8 and 9.4 kcal/mol by a MP2/6-311+G(d,p) set of calculations. Comparable values, in the 5−10 kcal/mol range, were computed for abstraction of H• from a series of hydroxy ethers[33] and fluorinated ethers[71] with a similar basis set. Highly accurate multireference calculations with an aug-cc-pVTZ basis set, involving H• abstraction from C−H bonds of ethenol,[72] yielded values of 7 to 11 kcal/mol for the barrier. Other work has estimated CH abstraction barriers of 7 kcal/mol for the methyl hydrogens of methyl glyoxal[45] and 4−5 kcal/mol for the methylene hydrogens of glycolaldehyde[46] and hydroxyacetone,[47] values which agree nicely with experiment for these systems; lower barriers on the order of 2 kcal/mol are found for aldehydic CHO hydrogens in these systems. Further confidence in the data is achieved by consideration of adding in progressively higher levels of correlation.

As mentioned above, and reported in Table 3, as one progresses from UMP2 to UMP3 and then to UMP4, and then considering CCSD and CCSD(T), there are some small oscillations in the barriers. But most importantly, all means of computation, from UMP2 to CCSD(T), agree that the H^{α} atom must traverse the highest barrier, by a significant amount. It may also be worth noting that the spin contamination of each calculation was checked and found to be minimal in all cases. Before correction, S^2 was always less than 0.79 and came down to 0.75 following correction, confirming the doublet state for complexes and transition states. Use of the spin-projected energies results in only small change in the calculated quantities and has no effect on the trends reported above.

This work has drawn a parallel between the height of each calculated energy barrier and the rate of reaction. While it is not justified to expect the calculated H• abstraction energy barriers to simply and easily translate to highly accurate rate

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constants, there is ample evidence that the barriers will indeed correlate nicely with reaction rates. Calculations of the OH· reaction with CF_3CHFCH_2F, for example, obtained rate constants in very good agreement with experiment[73] using a variational transition state theory formalism, in the context of the same 6-31+G** basis set employed here. In different, but related, radical reactions, the rate constants for H· abstraction from $CF_3CHFOCF_3$ by OH·[74] were calculated in good agreement with experiment, using transition state theory applied to BH&HLYP computation of the energetics; likewise is the case for the reaction of the hydroxyl radical with fluorinated propanes,[75] $CF_3CH_2CH_3$[65] and $CF_3CH_2CF_3$,[76] or with ethers,[64] saturated aldehydes,[77] CH_3NHNH_2,[78] or propene[79] and 4-picoline,[80] or the reaction of OOH· with propenesulfenic acid.[81] And transition state computation of the rates derived from the quantum calculations of the energetics of the aforementioned glyoxal derivatives[45−47] also yielded excellent agreement with experiment.

Another factor in the lesser reactivity of the CH^{α} site resides in the number of H atoms. As there are two H^{β} and six H^{δ} atoms which may be abstracted by a OH· radical, the likelihood of such an occurrence may be multiplied by these factors, relative to the single H^{α} and H^{γ} sites. Indeed, this reasoning led Kislov and Mebel to their conclusion[44] that abstraction of a H atom from the terminal site of 1,2-butadiene by a phenyl radical is more likely than a similar process from a central C atom, despite a slightly higher barrier for the former atoms.

In principle, it would be possible for a radical such as OH· to attack not only the C−H groups but also the peptide units, particularly in view of the fact that the C═O and NH groups represent a strong attractant for initial complexation. Nonetheless, a multitude of prior experiments had previously shown that the C−H groups are the primary target of attack, a finding that was recently confirmed by computations involving model amides.[82]

In model studies of this sort, there is always the question as to how much the truncation of the large protein into a segment, small enough so as to be tractable for accurate quantum calculations, affects the conclusions. It may first be pointed out that an earlier work[18] was encouraging in that the energy barrier required by a CH_3S· radical to remove a H^{α} atom from a Gly dipeptide, similar to the Leu dipeptide examined here, was changed very little if the neighboring full peptide groups were shortened to simply NH_2 and COOH, suggesting a certain degree of insensitivity to chain length. Moreover, the barriers computed there were somewhat higher than our values (Table 2), consistent with the replacement of our OH· radical by CH_3S·.

The calculations described above had centered on a Leu dipeptide removed from the environment of a full protein. Based upon a wealth of prior work, one can expect the major effects of the full protein to arise from a number of principal factors. In the first place, the protein represents a polarizable environment which can help to stabilize any developing charge separations in the system of interest. To a first approximation, one can consider the dipole moments of the transition states for H· transfer as measures of such charge separation. However, these quantities are fairly similar from one transition state to the next. At the UMP2/aug-cc-pVDZ level, for example, they vary from 1.6 D for the H^{γ} abstraction to 2.93 D for the H^{β} abstraction, with the H^{α} value intermediate at 2.47 D. One would thus not expect immersion in a polarizable medium to affect the central conclusion of a higher barrier for H^{α} abstraction. One can take this analysis beyond the simple dipole approximation and quantitatively evaluate the barriers obtained when the systems are immersed in a polarizable medium using an SCRF formalism. Taking a dielectric constant of 4 as the value that is commonly presumed to represent a protein interior,[83−88] one finds only a small effect upon the H· abstraction barriers reported in Table 2, changing most of the barriers by less than 1.6 kcal/mol. (The exception is the β site which sees a rise in its barrier of 3 kcal/mol.) Most importantly, the inclusion of a polarizable surrounding does not affect the relative ordering of the abstraction barriers, leaving the H^{α} site with the highest barrier.

A polarizable medium model does not accurately or completely take into account specific interactions such as particular H-bonds. The peptide units that surround the Leu side chain would probably be engaged in H-bonds with other parts of the protein, in the NH···O═C H-bonds that are so common. The incoming OH· radical would thus have some competition in forming the H-bonds with the peptides that inhibit the H^{α} extraction. There is some question as to how much such peripheral H-bonds might affect the process. On the other hand, given the known ability of the C═O oxygen atom to participate in multiple H-bonds simultaneously, it seems likely that even if engaged in an interpeptide NH···O═C H-bond, the carbonyl O would still present a strong site for binding the approaching OH· radical and thus help to form the kinetic trap for H^{α} abstraction. As another factor to consider, these interpeptide H-bonds might obstruct the accessibility of the $C^{\alpha}H$ group to a radical, which would also tend to diminish its reactivity.

Indeed, the issue of accessibility is an important one that raises other questions. In β-sheets, for example, it has been suggested[89] that the location of $C^{\alpha}H$ groups internal to the sheet protects them from oxidative attack, leaving only the side chains exposed. The effective accessibility of various CH groups will be a function of not only the X-ray structure of the protein but also its degree of flexibility at each site. While the calculations

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presented here argue for greater reactivity of the alkyl side chain CH groups, rigidity which prevents OH· from approaching any such group would of course diminish its ultimate reactivity. And it should be reiterated that our calculations found that resistance of the local backbone to conformational change, via (φ,ψ) modification, would act to further retard the ability of OH· to abstract a H^{α} atom.

A potential limitation of this study is the use of fully optimized geometries. As discussed above, the fully optimized structures generally contain C7-like H-bonds connecting the CO group of one peptide unit with the NH of the other. As such, the (φ,ψ) dihedral angles tend to fall in a comparatively narrow range, which facilitates this H-bond. In the case of a larger protein, the possibility of each peptide unit to form H-bonds with other, nonadjacent, units allows these angles to occupy much broader segments of Ramachandran space. It is certainly not inconceivable that the relative energy barriers of the various C−H sites might be different in other regimes of φ and ψ. Calculations[17,19] indicate a 2−10% variation in the C^{α}−H bond dissociation energies of seven different amino acids in changing from (φ,ψ) characteristic of an α-helix to a β-sheet, so there is clearly at least some small sensitivity of this parameter. On the other hand, the basic principle that the approach of a OH· radical toward the α position is likely to fall into a kinetic trap when it binds to the proximate peptide OH or NH groups would seem to be a general expectation, regardless of these backbone dihedral angles.

The region in Ramachandran space around the C7 conformation considered above, roughly (φ,ψ) ≈ (−80°,80°), is rather well represented in statistical analyses of protein conformations[90] and appears to represent a minimum in the potential energy surface of the dipeptide not only in vacuo but also in water.[91−93] Nonetheless, it would be useful to consider another, fundamentally different, region as well. Some sample calculations were thus performed in the area around (−150°,150°), an even more populated region of (φ,ψ) space of proteins, and a section which contains the extended conformations characteristic of β-sheets. Like the C7 structure, this conformation also represents a minimum within the context of aqueous solution.[83,92,94,95] Despite this rather drastic change in internal conformation, the barriers computed for H· abstraction at the various sites were quite similar to those reported in Table 1, changing by less than 1 kcal/mol. One may conclude that the salient finding of a considerably higher barrier for H^{α} abstraction is not unique to the C7 conformers extensively discussed above.

But perhaps most importantly, when all is said and done, there is a very relevant finding that the incorporation of an amino acid into a peptide does not significantly alter its reactivity with OH·,[10] unless of course the sites of potential H· removal are excluded from the solvent-accessible surface. This finding argues for at least a certain amount of validity of the data computed here for this dipeptide.

Another line of inquiry into the nature and energetics of transition states derives from curve-crossing ideas within the context of Pross−Shaik valence bond theory.[96] In this picture, the height of the barrier is influenced by a number of factors, beginning with a promotion energy, G, separating the ground and excited states of the reactants. For a H atom transfer of the type under consideration here, G can be equated with twice the energy of the relevant C−H bond.[96] The data reported in Table 1 indicate that all C−H bond energies fall within a fairly narrow range of 96−105 kcal/mol, so this difference would be expected to exert only a minor influence upon the H· abstraction barriers. A second quantity, f, related to the curvature of the individual crossing curves, can be presumed to be nearly uniform from one C−H extraction to the next.[97] Thus, the principal factor anticipated to affect the H transfer barrier in the case of leucine, according to these ideas, would be the C−H bond energy. In accord with the data in Table 1, H· abstraction from C^{β} and C^{δ} would be predicted to have the highest barriers, nearly equal to one another, followed by C^{γ} and then C^{α}. The abstraction barriers in Table 2 uphold this expectation with one notable exception, the surprisingly high barrier for the $C^{\alpha}H$ bond. While a low barrier might occur in a highly idealized context, the external H-bonds that stabilize the CH··O reactant more than they do the C··H··O transition state appear to be the dominating factor here, and to outweigh the suppositions of curve crossing alone.

Analysis of ab initio data for H transfer from alkanes to H or Cl atoms[98] in the language of curve crossing brought up the possibility that the barrier may be influenced by a charge-transfer configuration in those cases where the extracting group is highly electronegative, e.g. Cl·.[97] Such an expectation has been confirmed in Cl abstractions of H atoms from methyl groups,[39] where low-lying charge transfer states are associated with reduced H· abstraction barriers. The degree to which charge transfer states may lower the H· transfer barrier has been connected[39] with the difference between the vertical ionization energy of the Leu radical and the electron affinity of OH·. This difference has been calculated here to vary from a minimum of 8.35 eV for the γ position to higher values of 9.26 and 9.52 eV for β and δ, respectively. This order is consistent with the H· transfer barriers reported in Table 2, also lowest for $C^{\gamma}H$. And again, the α position fails to obey this trend, as its IE-EA value of 8.96 eV is intermediate between the two sets of extremes above, but the transfer barrier is highest.

With regard to the structure of the transition state, and in particular its position along the H transfer coordinate, the r(C··H) and r(H··O) distances are fairly constant from one position to the next. The former varies from 1.19 Å for γ abstraction to 1.24 Å for the α position; the latter is between 1.28 and 1.38 Å. If one defines the percentage H· transfer at the transition state as r(C··H)/[r(C··H) + r(H··O)], this quantity is just below 0.50 for all abstractions, varying between 0.46 and 0.49. There is a certain degree of correlation between this quantity and the overall ΔE of the transfer process, in that the smallest value is associated with the γ abstraction, which is more exothermic than β or δ.

This approximate midpoint location of the TS is consistent with calculations of other OH· abstractions of H from C−H

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bonds, for example, in the case of morpholine[70] wherein the fractional transfer is 0.47. As another example, where OH• extracts a H atom from various CH groups of a number of hydroxyl ethers,[33] the *r*(C••H) and *r*(H••O) distances are quite similar to those obtained here for leucine; further, the fractional transfer parameter, encompassing fully 32 different configurations, also lies in the 0.46−0.50 range. Highly accurate multireference calculations with an aug-cc-pVTZ basis set, involving H• abstraction from ethenol,[72] yielded values of 0.46 to 0.48 for this quantity. And in those cases where a Cl• atom extracts a H atom from a series of alkanes, earlier computations[98] had placed this transfer parameter in the same range of 0.47−0.49.[97] More recent, and higher level, calculations[39] had removed H atoms to a Cl atom from the methyl carbons bonded to various functional groups such as COOH and CHO, with similar results.

A thorough and quantitative kinetic analysis of this system would require the evaluation of the rate constant for abstraction of each and every H atom. The evaluation of each rate constant would require an accurate quantification of the tunneling of the light protons, as well as incorporation of various entropic effects. Dynamic trajectories for H• abstraction would explore a number of different minima, including the deepest minimum in which the OH• radical approaches the H^{α} atom, as well as the more shallow minima. Such a thorough analysis of the kinetics is beyond the scope of the present work, but it is hoped the principles enunciated here will spur future work in this direction. But at the same time, past work[60,99] lends confidence to the idea that the fairly deep minimum in the prereaction complex for H^{α} abstraction will indeed slow the rate of this particular process, relative to the other H removals. For example, this same notion was used recently[100] to explain the experimental observation of a complex that precedes the addition reaction of OH•, despite a very low nominal barrier to an exothermic process that would otherwise preclude any buildup of such a complex. Similar reasoning that the stability of the reaction complex is an important factor in reaction rates arises from work involving H• abstraction from glyoxal and related molecules[45−47,71] where rates computed on this basis were in excellent accord with experiment.

In conclusion, the work presented here argues that the lesser reactivity of α H atoms to abstraction by OH• radicals is due not to intrinsic aspects of various sorts of C−H bonds or to bond dissociation energies but is rather a product of the kinetic trap posed by the NH and C═O groups that lie close to C^{α} that can form strong H-bonds with the OH•. With regard to the various alkyl C−H bonds, the calculations do not find a clear distinction between β, γ, and δ sites with respect to reactivity. The experimental finding[9,11] of increasing reactivity with greater distance from the backbone would thus lead one to hypothesize this distinction arises from the greater flexibility of the alkyl chain as the site of interest is located increasingly distant from the more rigid region of the backbone, thus better allowing accessibility of the approaching radical.

Supporting Information Available: Supporting Information contains full ref 25. It also contains the Cartesian coordinates of isolated subunits, minima, and transition states described in this work, as well as their UMP2/aug-cc-pVDZ energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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