Intramolecular Addition of Cysteine Thiyl Radical to Phenylalanine and Tyrosine in Model Peptides, Phe (CysS•) and Tyr(CysS•): A Computational Study

Sergej Naumov*,† and Christian Schöneich*,‡

Leibniz-Institut für Oberflächenmodifizierung, e. V., Permoserstrasse 15, D-04318 Leipzig, Germany, and Department of Pharmaceutical Chemistry, The University of Kansas, 2095 Constant Avenue, Lawrence, Kansas 66047

Received: August 27, 2008; Revised Manuscript Received: January 15, 2009

Density Functional Theory (DFT) calculations were carried out to evaluate the potential for *intra*molecular addition of peptide cysteine (Cys) thiyl radicals (CysS•) to aromatic amino acids (Phe and Tyr) in water. These calculations yielded cyclic conformations, in which π-complexes were more stable than cyclohexadienyl radicals in water. In these π-complexes, the C_2-S distances were significantly shorter compared to the C_1-S and C_3-S distances. Comparable results on the relative stabilities were obtained for model calculations for the addition of $HS^•/CH_3S^•$ to toluene and *p*-hydroxytoluene. The adduct of thiyl radicals with Phe was more stable than that with Tyr, and the stabilization energies depended on the C-terminal substituents.

Introduction

Cysteine thiyl radicals (CysS•) are important intermediates in the one-electron oxidation of cysteine (Cys) and one-electron reduction of protein disulfide bonds (reactions 1 and 2).[1] In general, Cys oxidation has been considered a biologic "sink" for redox processes of proteins, where ultimately CysS• radicals will be reduced back to Cys through reaction with endogenous antioxidants, e.g., ascorbate (reaction 3; $k_3 = 6 \times 10^8\ M^{-1}\ s^{-1}$).[1]

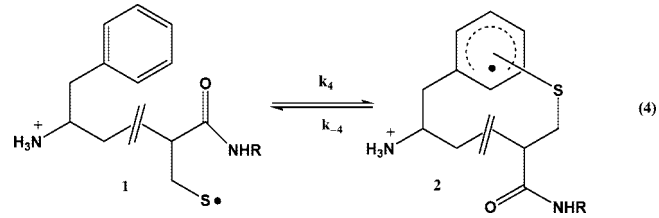
$$CysSH + Ox \rightarrow CysS^• + Ox^{•-} + H^+ \quad (1)$$

$$CysSSCys + e^- + H^+ \rightarrow CysS^• + CysSH \quad (2)$$

$$CysS^• + H^+ + Asc^- \rightarrow CysSH + Asc^• \quad (3)$$

Various biological processes depend on the generation of CysS• radicals. For example, a number of enzymes require CysS• radicals for turnover, such as the ribonucleotide reductases,[2−4] pyruvate-formate lyase,[3,5−8] and benzylsuccinate synthase.[9,10] In addition, a mechanism for *S*-nitrosocysteine generation has been suggested, which involves a radical−radical reaction between CysS• and nitric oxide (•NO).[11] For the most part, the CysS• radical has been considered to be chemically inert toward other amino acids within a protein.[12] However, this paradigm needs to be reevaluated[13] in view of recent results, which indicate that the CysS• radical can efficiently abstract hydrogen atoms from $^{\alpha}C-H$ and/or $^{\beta}C-H$ bonds of amino acid residues.[14−16] In fact, rate constants as high as $10^4-10^5\ s^{-1}$ have been measured for the *intra*molecular hydrogen atom abstraction by CysS• from Ala and Gly residues in model peptides.[17] These hydrogen transfer reactions are reversible, leading to covalent H/D exchange when reactions are performed in D_2O.[15]

By means of covalent H/D exchange, we obtained experimental evidence that CysS• radicals reversibly abstract hydrogen atoms from the $^{\beta}C-H$ bond of Phe,[15] which was not unexpected based on the C−H bond dissociation energies of benzylic C−H bonds. We then designed time-resolved pulse radiolysis experiments to measure absolute rate constants for such reversible hydrogen atom transfer in a CysS• radical-containing dipeptide, Phe-(CysS•), generated through one-electron reduction of the disulfide-containing precursor, $(PheCys)_2$. To our surprise, these time-resolved experiments[18] did not lead to the observation of an intermediary Phe benzyl radical. Instead, within 1−4 μs after one-electron reduction of $(PheCys)_2$, the transient spectra were more reminiscent of a cyclohexadienyl-type radical. Similar observations were made upon one-electron reduction of a slightly larger disulfide-containing model peptide, $(PheGlyCysGly)_2$. On the basis of the absorbance coefficients of various known cyclohexadienyl radicals, we concluded that in these model peptides CysS• radicals **1** may exist in a reversible *intra*molecular addition−elimination equilibrium with thioether-substituted cyclohexadienyl-type radicals **2** (equilibrium 4). Quantitative considerations revealed that within 4 μs after thiyl radical generation, thioether-substituted cyclohexadienyl radicals could account for ca. 20% of the total available thiyl radicals.



(4)

Earlier, radical addition processes to aromatic substrates have been a subject of great interest as part of mechanistic investigations of homolytic aromatic substitutions. Evidence for the bimolecular addition of silyl and germyl radicals to benzene and other aromatic substrates, generating cyclohexadienyl radicals, was obtained by UV and electron spin resonance (EPR) techniques.[19−21] However, no cyclohexadienyl radical was detected during the *bi*molecular reaction of several thiyl radicals with benzene,[19] rationalized by the possibility that the addition reaction is substantially slower compared to the reverse, elimination, reaction. This is consistent with the trend observed

* Corresponding author. Phone: +49 (0)341-235-2046. E-mail: sergej.naumov@iom-leipzig.de. Phone: 1-785-864-4880.
† Leibniz-Institut für Oberflächenmodifizierung.
‡ The University of Kansas.

10.1021/jp8076358 CCC: $40.75
Published on Web 03/23/2009

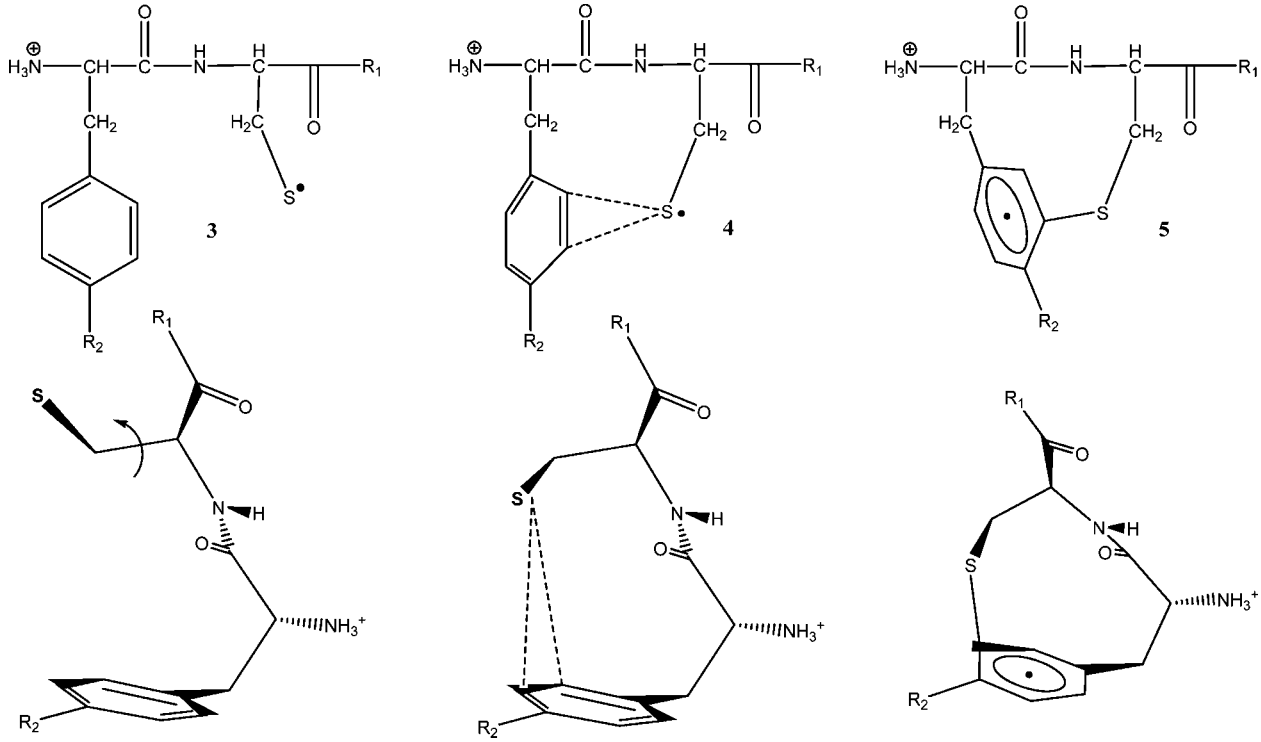


Figure 1. Acyclic structure **3** and cyclic structures **4** and **5** formed via the addition of the Cys thiyl radical to the aromatic ring of either Phe (R_2 = H) or Tyr (R_2 = OH).

under our *uni*molecular reaction conditions with Phe(CysS•),[18] where $k_{-4} > k_4$; however, for Phe(CysS•) the ratio k_{-4}/k_4 appears sufficiently small that the cyclohexadienyl-type radical is experimentally detectable.

The addition of CysS• radicals to aromatic amino acids in proteins may have significant biological consequences. For example, suitable electron acceptors could oxidize cyclohexadienyl-type radicals, which would rearomatize via subsequent deprotonation, generating a stable Cys-Phe cross-link. Analogous cross-links have been detected between Cys and Tyr in proteins, for example, galactose oxidase,[22] where mechanisms of formation are still under investigation. In addition, a reaction of CysS• radicals with Phe was recently postulated as part of the NO-mediated guanine nucleotide dissociation of the Ras oncogene.[23] Therefore, because of the potential significance of our experimental results on CysS• radical addition to Phe (and eventually to other aromatic amino acids), we felt that a theoretical analysis of such an addition reaction was warranted. Such calculations are described in the present paper, which will show that indeed cyclic adducts of CysS• to Phe in Phe(CysS•) and of CysS• to Tyr in Tyr(CysS•) are energetically slightly more stable compared to the acyclic, free CysS• radicals, specifically in an aqueous environment.

Computational Methods

Quantum chemical calculations were carried out on thiyl radical **3** (Figure 1) by using different Density Functional Theory (DFT) methods, namely B3LYP,[24,25] BH&HLYP,[26] MPW1K,[27] and X3LYP[28] with standard 6-311+G** basis set, as implemented in the Jaguar (version 7.0) program.[29] The BH&HLYP method leads[26b] to stronger localization of the unpaired electron than B3LYP. The MPW1K method is optimized against a database of 20 forward barrier heights, 20 reverse barrier heights, and 20 energies of reaction. It reduces the mean unsigned error in the reaction barrier height by a factor of 3 compared to the B3LYP method. The X3LYP method improves the accuracy in heats of formation, ionization potential, electron affinities, and total atomic energies compared relative to the B3LYP method. The interactions between the molecule and the solvent were evaluated by Jaguar's Poisson−Boltzmann solver, which fits the field produced by the solvent dielectric continuum to another set of point charges.[30] The molecular geometries, energies, and frequencies were calculated both in the gas phase and in water at the same B3LYP/6-311+G** level of theory. The frequency analysis was used to obtain thermochemical parameters such as zero point energy (ZP), entropy (S), and Gibbs free energy (ΔG) at 298 K. The comparison of the calculated relative stabilities obtained with different DFT methods (given in Table 1) revealed very similar results. We note that a stable cyclohexadienyl radical could not be located with the B3LYP method.

Results

1. Peptide Structures. By geometry optimization of the cysteine thiyl radical **3**, different stable structures were obtained in water (Figure 1). Here, the π-complex **4** is more stable compared with the open structure **3** and, with one exception (see below), also than cyclohexadienyl radical **5**, where the Cys sulfur is connected to C_3 of the aromatic moiety. For PheCysS•, a stable cyclohexadienyl radical with the Cys sulfur connected to C_2 of the aromatic moiety could not be located at the BH&HLYP/6-311+G(d,p)/PBF level of theory; such structure being easily converted into the π-complex **4**, likely because of conformational restrictions of the peptide. However, model calculations for the addition of HS•/CH_3S• to toluol located a stable cyclohexadienyl radical with bond formation between sulfur and C_2.

The relative stabilities, ΔE, of **4** and **5** in water are summarized in Table 1, which displays reasonably good agreements between various density functional methods. The stabilization energies ΔE of **4** vary depending on the nature of

TABLE 1: Stabilities ΔE (kcal mol^{-1}) of the Cyclic Structures 4 and 5 (Displayed in Figure 1) Relative to the Acyclic Conformers 3 As a Function of Substituents R_1 and R_2, Calculated in Water (B3LYP/6-311+G/PBF, Jaguar 7.0 program), Spin Delocalization ΔS from the Sulfur Atom to the Phenyl Ring through Cyclization, and *Intra*molecular Bond Lengths L between Sulfur and the C Atoms of the Aromatic Ring (Å)**

R_1	CH_3	CH_3	$O^{(-)}$	$O^{(-)}$	NH_2	NH_2
R_2	H	OH	H	OH	H	OH
ΔE(**3**−**4**)	−2.2	−1.6	−7.7	−3.2	−5.6	−3.5
	−2.8[a]	−1.9[a]	−7.6[a]	−4.0[a]	−4.7[a]	−3.0[a]
	−2.0[b]	−1.9[b]	−8.8[b]	−1.6[b]	−3.8[b]	−2.9[b]
	−8.9[c]	−4.4[c]	−9.1[c]	−0.5[c]	−6.1[c]	−4.0[c]
ΔE(**3**−**5**)	+2.5[c]	+3.3[c]	0.0[c]	+1.4[c]	+7.7[c]	+3.2[c]
$\Delta E_0(E + ZP)$, **3**−**4**	−1.4	−1.2	−11.8	−4.5	−4.0	−2.1
$\Delta G_{tot}(E_0 - T^*S)$, **3**−**4**	−1.8	−1.8	−10.1	−4.8	−4.7	−2.6
ΔS, **4**	0.048	0.046	0.027	0.049	0.032	0.038
L(S−C_1) (**4**)	3.551	4.033	3.156	3.862	3.719	3.732
L(S−C_2) (**4**)	3.186	3.327	3.097	3.218	3.556	3.441
L(S−C_3) (**4**)	3.515	3.444	3.637	3.480	3.888	3.754

[a] Calculated in water with X3LYP/6-311+G**/PBF. [b] Calculated in water with MPW1K/6-311+G**/PBF. [c] Calculated in water with BH&HLYP/6-311+G**/PBF DFT methods.

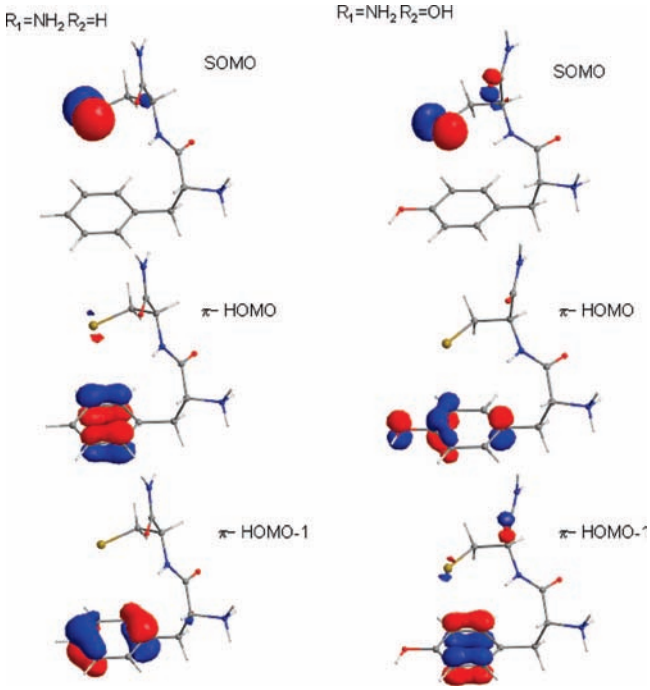


Figure 2. SOMO and π-HOMO of thiyl radicals **3** from PheCys and TyrCys derivatives.

the substituents R_1 and R_2. For Phe (R_2 = H), the relative stability of **4** increases in the order (R_1 = H) < (R_1 = NH_2) < (R_1 = O^-). The combination R_1 = O^-/R_2 = H represent the model peptide for which our time-resolved pulse radiolysis experiments have demonstrated the association of CysS$^\bullet$ with Phe.[18] Importantly, this peptide (R_1 = O^-/R_2 = H) shows the most stable cyclohexadienyl radical **5**, which is isoenergetic with the open structure **3**.

The π-complex **4** is less stable for Tyr (R_2 = OH) compared to Phe, and displays a smaller effect of R_1 on ΔE. This lower stability of **4** for Tyr can be rationalized by the lower π-electron density on the aromatic ring, which is displayed in Figure 2 for the open structure **3** (R_1 = NH_2, R_2= H, OH).

In the π-complex **4**, the S−C distance to the C_2 carbon of the aromatic ring is shorter than distances of the sulfur to C_1 and C_3. The C−S distances are given in Table 1. Except for the combinations R_1 = CH_3/R_2 = H and R_1 = NH_2/R_2 = OH, there are significant differences in the distances S−C_1 and S−C_3, so that the sulfur atom appears to complex to the aromatic moiety in a bridged structure (a η_2−π complex, representatively shown for C_2 and C_3 in structure **4**).

For all thiyl radicals **3**, the formation of the π-complex **4** appears thermodynamically possible, because of a negative change of total Gibbs free energy (ΔG_{tot}) at 298 K. Due to the rather weak interactions between the sulfur atoms and the phenyl rings only small delocalizations of the spin densities, ΔS, were calculated (Table 1), which in structures **4** are localized largely on the sulfur atom. Figure 3 displays the spin densities representatively for structures **3**, **4**, and **5** from the model peptide with R_1= CH_3 and R_2 = H.

We have computed the trends in relative energies of structures **3**, **4**, and **5** from PheCysS$^\bullet$ (representatively for R_1 = CH_3 and R_2 = H) with an ab initio method, UHF/6-311++G(3df,3dp)/PBF. At this level of theory, **4** is 6 kcal/mol more stable than **3**, while **3** is 9.2 kcal/mol more stable than **5**, i.e., the stability decreases in the order of **4** > **3** > **5**, which is qualitatively in agreement with the results derived with density functional theory for the structure with R_1 = CH_3 and R_2 = H.

To assess the potential influence of peptide conformation alone on the formation of the cyclic structures **4** and **5**, we optimized species **6** (^+H_3N-CH(CH_3)-CONH-CH($CH_2S^\bullet$)-CH_3) at the BH&HLYP/6-311+G**/PBF level of theory. Species **6** is derived from **3** (R_1 = CH_3, R_2 = H) by replacement of the benzyl side chain of Phe through a methyl side chain (of Ala). The two most stable conformers of **6**, an open (**6-o**) and a closed (**6-c**) structure (Figure 4), have a frame analogous to the acyclic and cyclic structures **3** and **4** where structure **6-c** is merely 0.9 kcal mol^{-1} more stable in water compared to **6-o** (0.5 kcal mol^{-1} less stable in the gas phase). This value is significantly lower compared to the stabilization of **4** (R_1 = CH_3, R_2 = H), which amounts to 8.9 kcal/mol at the same level of theory. In addition, the spin density on the sulfur does not change upon going from **6-o** to **6-c**, supporting the absence of any interaction between the thiyl radical and the side chain methyl group of the Ala residue.

2. Calculations of Model Systems. To eliminate any conformational restrictions imposed by the peptide skeleton on the addition of the thiyl radical to the aromatic residue, we calculated the adduct of HS$^\bullet$/$CH_3S^\bullet$ to both toluene ($PhCH_3$) and *p*-hydroxytoluene (HO-Ph-CH_3) (Figure 5). For the C_3-cyclohexadienyl radical and the π-complex, initial conformers were selected, in which the coordinates of the aromatic residues and thiyl radicals were identical with those for which stable structures had been calculated for PheCysS$^\bullet$ and TyrCysS$^\bullet$, respectively. Subsequently, full translational and rotational freedom was given to all adducts during the optimization of the respective conformations. The results are summarized in Table 2.

For HS$^\bullet$/$CH_3S^\bullet$ adducts to toluene (R_2 = H), we derive qualitatively the same picture as for the *intra*molecular addition within PheCysS$^\bullet$, i.e., a more stable π-complex compared to a C_3-cyclohexadienyl radical. However, formation of the C_2-cyclohexadienyl radical is more favorable (by 2.4 kcal/mol for HS$^\bullet$) than formation of a C_3-cyclohexadienyl radical, which is expected based on the presence of a methyl substituent in the ortho position. This result underlines that the absence of a C_2-cyclohexadienyl radical for PheCysS$^\bullet$ is best rationalized by conformational restrictions of the peptide skeleton. This picture changes for cyclohexadienyl radical formation from *p*-hydroxy-

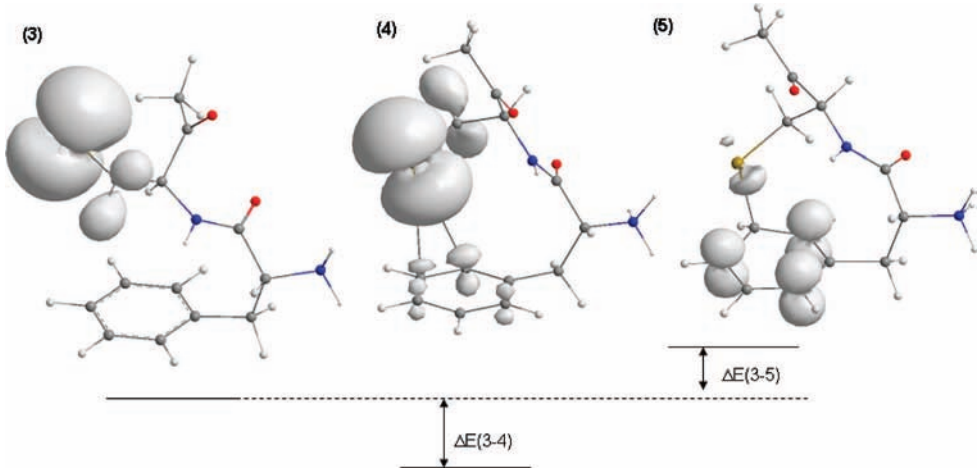


Figure 3. Distribution of spin densities in **3**, **4**, and **5** ($R_1 = CH_3$, $R_2 = H$).

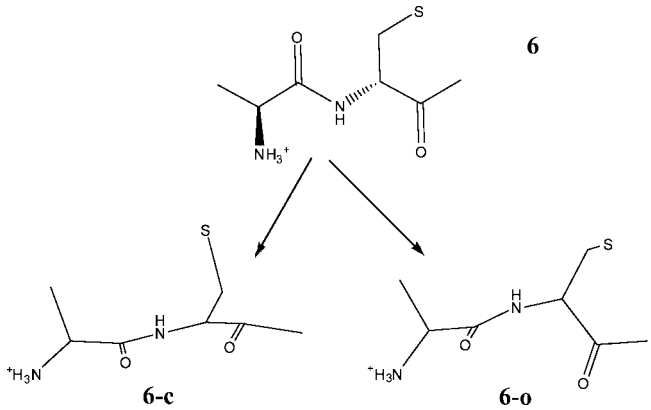


Figure 4. The two most stable conformations of **6** calculated at the BH&HLYP/6-311+G**/PBF level of theory.

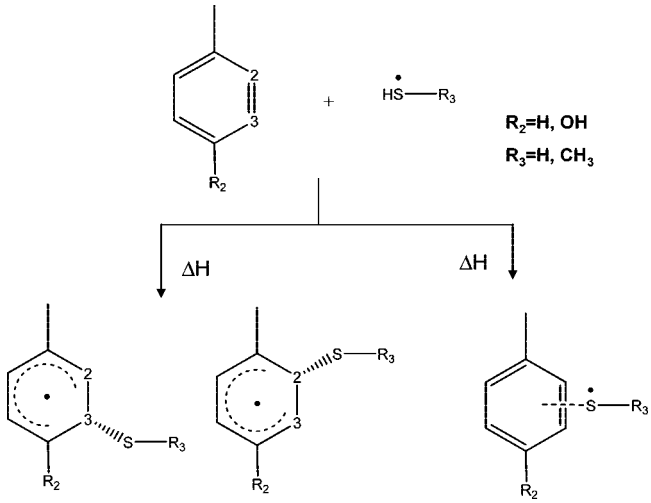


Figure 5. Reaction of HS•/CH_3S• with toluene ($R_2 = H$) and *p*-hydroxytoluene ($R_2 = OH$).

toluene ($R_2 = OH$). Here, the ortho position of the hydroxyl substituent leads to a more favorable addition of the thiyl radical to the C_3 position. Interestingly, the addition of HS• to the C_3 position of *p*-hydroxytoluene in water to form a cyclohexadienyl radical is slightly more favorable compared to π-complex formation, whereas the opposite is true for the addition of CH_3S• to *p*-hydroxytoluene.

Because of the potential relevance for transitions between cyclohexadienyl radicals and π-complexes in PheCysS•, we have representatively calculated the activation energy for the transition between the π-complex of HS• and toluene and the C_3-cyclohexadienyl radical. This activation energy is rather low, 0.9 kcal/mol, and associated with an S−C_3 bond length of 2.182 Å.

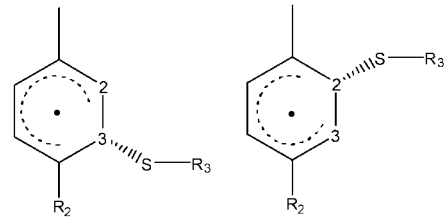
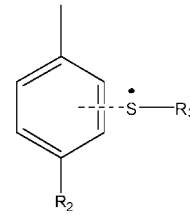
Discussion

The calculations presented in this paper suggest that peptide/protein Cys thiyl radicals can associate with aromatic residues. Our time-resolved experimental data obtained with PheCysS•[18] ($R_1 = O^-$, $R_2 = H$ for the structures shown in Table 1) were initially rationalized by the formation of a cyclohexadienyl-type radical (σ-like complex[31]) based on the similarity of the transient spectra to analogous spectra of known cyclohexadienyl radicals. The present calculations show that the C_3-cyclohexadienyl radical **5** of PheCysS• (Table 1; $R_1 = O^-$, $R_2 = H$) is isoelectronic with the open conformation **3**, suggesting that an equilibrium between **3** and **5** may be experimentally observable. However, the π-complex **4** of PheCysS• (Table 1; $R_1 = O^-$, $R_2 = H$) is energetically significantly more favorable, being 7.7−9,1 kcal/mol more stable than **3** and **5**, depending on the level of theory. Qualitatively, these results agree with model calculations on *inter*molecular reactions of small thiyl radicals, HS•/CH_3S•, with toluene and *p*-hydroxytoluene (displayed in Table 2). These calculations also demonstrate that the activation energy for interconversion between a C_3-cyclohexadienyl radical and a π-complex (of HS• and toluene) is very low, 0.9 kcal/mol.

In contrast to PheCysS• with $R_1 = O^-$ and $R_2 = H$, the C_3-cyclohexadienyl radicals **5** of all other PheCysS• and TyrCysS• derivatives are 1.4−7.7 kcal/mol higher in energy compared to the open conformations **3**. All PheCysS• and TyrCysS• derivatives show π-complexes **4**, which are more stable than the open conformations **3** and the cyclohexadienyl radicals **5**.

In all π-complexes, the shortest S−C distance is that between S and C_2 compared to the S−C_1 and S−C_3 distances. On the basis of the inequality of the distances for S−C_1 and S−C_3 for four out of six combinations in Table 1, the π-complexes **4** may be well described as bridged $\eta_2-\pi$ complexes, where the thiyl radical preferentially associates with two carbon atoms of the aromatic ring. Comparable structures have been described for the addition of Br• and Cl• to ethylene (though it was debated whether to describe them as symmetrically bridged or rapidly interconverting asymmetric β-halogen-substituted alkyl radicals[32,33]), and for the addition of Cl• radicals to benzene.[34] Interestingly, the latter reaction seems far from being completely understood with recent DFT calculations favoring the formation

TABLE 2: Reaction Enthalpies ΔH (kcal mol^{-1}) for the Formation of C_2/C_3-Cyclohexadienyl Radical and π-Complex during the Reaction of HS$^{\bullet}$/$CH_3S^{\bullet}$ with the Aromatic Ring (Displayed in Figure 5) As a Function of Substituents R_1 and R_2, Calculated in Vacuum and Water (BH&HLYP/6-311+G/PBF, Jaguar 7.0 program), and the Shortest Bond *L* between Sulfur and the C Atom of the Aromatic Ring (Å)**

	 cyclohexadienyl radical					 π-complex			
R_2	R_2=H	R_2=H	R_2=OH	R_2=OH		R_2=H	R_2=H	R_2=OH	R_2=OH
R_3	R_3=H	R_3=CH_3	R_3=H	R_3=CH_3		R_3=H	R_3=CH_3	R_3=H	R_3=CH_3
ΔH(C3) vacuum	0.4	2.9	-2.7	1.6		-5.2	-4.4	-2.9	-8.6
ΔH(C_3) water	0.3	0.4	-3.9	2.6		-4.9	-5.7	-3.7	-5.5
ΔH(C_2) water	-2.1	-0.5	0.9	3.9					
L(S-C_3)	1.876	1.868	1.891	1.880		3.018	3.224	3.048	3.074
L(S-C_2)	1.884	1.880	1.888	1.878					

of an $\eta_1-\pi$ complex.[31] Experimental results for charge-transfer complexes between Cl$^{\bullet}$ and aromatic substrates[35,36] have also been rationalized by formation of a $\eta_6-\pi$ complex[36] and further consideration has been given to such intermediates as lying somewhere inbetween the two extremes, centrosymmetric charge transfer complex and cyclohexadienyl radical (σ-like complex).[37]

For the interpretation of our experimental results with PheCysS$^{\bullet}$ (R_1 = O^-, R_2 = H), our calculations have the following implications: the C_3-cyclohexadienyl radical **5** (R_1 = O^-, R_2 = H) is energetically possible relative to the open conformation **3**. Our transient spectra[18] suggest that cyclohexadienyl radical **5** (R_1 = O^-, R_2 = H) forms in water to an extent of ca. 20%. The remainder may exist in the open conformation **3** or the π-complex **4**. The latter cannot have a strong absorbance between 350 and 500 nm as our time-resolved absorbance data[18] do not indicate any significant absorbance in this spectral region. This fact could argue against significant experimental yields of such π-complexes, as experimental data on π-complexes between toluene and Cl$^{\bullet}$, Br$^{\bullet}$, and I$^{\bullet}$ show broad charge-transfer bands with absorption maxima in the visible UV region between 479 and 535 nm.[36] On the other hand, we do not know the absorption characteristics of analogous charge-transfer bands of thiyl radical π-complexes. To this end, we do not discount the experimental formation of **4** (which is energetically most favorable by our applied levels of theory), but simply note that our time-resolved experiments[18] seem not to indicate significant experimental yields of π-complexes. Therefore, it is probably best to assume that PheCysS$^{\bullet}$ can exist in a rapid equilibrium between all three species, **3**, **4**, and **5** (supported by the low activation energy for interconversion between cyclohexadienyl radical and π-complex). The important aspect is that the association of the thiyl radical with the aromatic residue, no matter whether cyclohexadienyl radical or π-complex, may have significant biological consequences.

Our calculations suggest that π-complex formation with Phe is more efficient compared to that with Tyr, though the values for ΔE and ΔG suggest that also the *intra*molecular addition of thiyl radicals to Tyr may be experimentally observable (of course, there is the possibility of competitive *intra*molecular hydrogen abstraction from the Tyr phenol group, but recent data with Cys- and Tyr-containing model peptides indicate that the reverse reaction, *intra*molecular hydrogen/electron transfer from Cys to tyrosyl radicals, appears significantly more efficient[38]). The latter would be of interest, as thiyl radical addition to Tyr, followed by oxidation and rearomatization, could theoretically represent the first step in an alternative mechanism for the formation of cysteinyltyrosine cross-links, such as observed in the enzyme galactose oxidase.[22] Hence, thiyl radical addition to aromatic rings could represent a more general mechanism for the irreversible formation of cross-links in proteins under biologic conditions of oxidative stress. In this respect, we recently detected cysteinyltyrosine cross-links during the photolysis of insulin.[39]

Another example for the chemically important weak interaction of a radical with an aromatic ring was recently presented for the *inter*molecular addition of peroxyl radicals to benzene en route to the formation of benzene oxide.[40] Here, cyclohexadienyl radicals were theoretically and experimentally supported (through products) even though associated with a positive ΔG = 20.6 kcal/mol.

Theoretically, the π-complex between CysS$^{\bullet}$ and Phe could undergo *intra*molecular electron transfer to yield $CysS^-$ and $Phe^{+\bullet}$, followed by rapid deprotonation of $Phe^{+\bullet}$ to benzyl radical and protonation of $CysS^-$ to CysSH. Subsequently, benzyl radicals will abstract a hydrogen atom from CysSH[41] so that they may not accumulate to a significant extent, consistent with our time-resolved experimental data.[18] However, kinetic NMR experiments with cysteamine thiyl radicals and either Phe or

$PheNH_2$ in D_2O indicate covalent exchange of $^{\beta}C-H$ to $^{\beta}C-D$ in the amino acid.[15] This is consistent with the formation of benzyl radicals and thiol, where thiol H/D-exchange in D_2O (RSH → RSD) precedes deuterium transfer from RSD to the benzyl radical.

Acknowledgment. Financial support by the NIH (PO1AG12993) is gratefully acknowledged.

Supporting Information Available: Atomic data for optimized cyclic structures in water at the B3LYP/6-311g**+/Pbf (Version 4.00, Jaguar 8.0) level of theory. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Wardman, P.; von Sonnta, C. *Methods Enzymol.* **1995**, *251*, 31–45.
(2) Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705–762.
(3) Himo, F.; Siegbahn, P. E. M. *Chem. Rev.* **2003**, *103*, 2421–2456.
(4) Stubbe, J. *Chem. Commun.* **2003**, 2511−2513.
(5) Reddy, S. G.; Wong, K. K.; Parast, C. V.; Peisach, J.; Magliozzo, R. S.; Kozarich, J. W. *Biochemistry* **1998**, *37*, 558–563.
(6) Becker, A.; Fritz-Wolf, K.; Kabsch, W.; Knappe, J.; Schultz, S.; Wagner, A. F. V. *Nat. Struct. Biol.* **1999**, *6*, 969–975.
(7) Gauld, J. W.; Eriksson, L. A. *J. Am. Chem. Soc.* **2000**, *122*, 2035–2040.
(8) Becker, A.; Kabsch, W. *J. Biol. Chem.* **2002**, *277*, 40036–40042.
(9) Krieger, C. J.; Roseboom, W.; Albracht, S. P. J.; Spormann, A. M. *J. Biol. Chem.* **2001**, *276*, 12924–12927.
(10) Himo, F. *J. Phys. Chem. B* **2002**, *106*, 7688–7692.
(11) Jourd'heuil, D.; Jour'heuil, F. L.; Feelisch, M. *J. Biol. Chem.* **2003**, *278*, 15720–15726.
(12) Kolano, C.; Helbong, J.; Bucher, G.; Sander, W.; Hamm, P. *J. Phys. Chem. B* **2007**, *111*, 11297–11302.
(13) Schöneich, Ch. *Chem. Res. Toxicol.* **2008**, *21*, 1175–1179.
(14) Nauser, T.; Schöneich, Ch. *J. Am. Chem. Soc.* **2003**, *125*, 2042–2043.
(15) Nauser, T.; Pelling, J.; Schöneich, Ch. *Chem. Res. Toxicol.* **2004**, *17*, 1323–1328.
(16) Mozziconacci, O.; Sharov, V. S.; Williams, T. D.; Kerwin, B. A.; Schöneich, Ch. *J. Phys. Chem. B* **2008**, *112*, 9250–9257.
(17) Nauser, T.; Casi, G.; Koppenol, W. H.; Schöneich, Ch. *J. Phys. Chem. B* **2008**, *112*, 15034–15044.
(18) Nauser, T.; Casi, G.; Koppenol, W. H.; Schöneich, Ch. *Chem. Commun.* **2005**, 3400−3402.
(19) Griller, D.; Marriott, P. R.; Nonhebel, D. C.; Perkins, M. J.; Wong, P. C. *J. Am. Chem. Soc.* **1981**, *103*, 7761–7763.
(20) Ingold, K. U.; Lusztyk, J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 343–348.
(21) Alberti, A.; Pedulli, G. F. *Gazz. Chim. Ital.* **1987**, *117*, 381–384.
(22) Firbank, S. J.; Rogers, M. S.; Wilmot, C. M.; Dooley, D. M.; Halcrow, M. A.; Knowles, P. F.; McPherson, M. J.; Phillips, S. E. V. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12932–12937.
(23) Heo, J.; Prutzman, K. C.; Mocanu, V.; Campbell, S. L. *J. Mol. Biol.* **2005**, *346*, 1423–1440.
(24) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
(25) Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–89.
(26) (a) Becke, A. D. *J. Chem. Phys.* **1996**, *104*, 1040–1046. (b) Bally, T.; Borden, W. T. *Rev. Comput. Chem* **1999**, *13*, 1–97.
(27) Lynch, B. J.; Fast, P. L.; Harris, M.; Truhlar, D. G. *J. Phys. Chem. A* **2000**, *104*, 4811–4815.
(28) Xu, X.; William, A.; Goddard, I. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2673–2677.
(29) *Jaguar 7.0*; Schrödinger LLC: New York, 2008.
(30) Tannor, D.J. M.; Murphy, B.; Friesner, R.; Sitkoff, R. A.; Nicholls, D.; Ringnalda, A.; Goddard, W. A. M.; Honig, B. *J. Am. Chem. Soc.* **1994**, *116*, 11875–11882.
(31) Croft, A. K.; Howard-Jones, H. M. *Phys. Chem. Chem. Phys.* **2007**, *9*, 5649–5655.
(32) Skell, P. S.; Traynham, J. G. *Acc. Chem. Res.* **1984**, *17*, 160–166.
(33) Skell, P. S.; Pavlis, R. R.; Lewis, D. C.; Shea, K. J. *J. Am. Chem. Soc.* **1973**, *95*, 6735–6745.
(34) Jarezba, W.; Thakur, K.; Hörman, A.; Barbara, P. F. *J. Phys. Chem.* **1995**, *99*, 2016–2023.
(35) Bühler, R. E. *Helv. Chim. Acta* **1968**, *51*, 1558–1571.
(36) Raner, K. D.; Lusztyk, J.; Ingold, K. U. *J. Phys. Chem.* **1989**, *93*, 564–570.
(37) Walling, C. *J. Org. Chem.* **1988**, *53*, 305–308.
(38) Zhang, H.; Xu, Y.; Joseph, J.; Kalyanaraman, B. *J. Biol. Chem.* **2005**, *280*, 40684–40698.
(39) Mozziconacci, O.; Williams, T. D.; Kerwin, B. A. Schöneich, Ch. *J. Phys. Chem. B* **2008**, *112*, 15921–15932.
(40) Broyles, D. A.; Carpenter, B. K. *J. Org. Chem.* **2005**, *70*, 8642–8644.
(41) Notre Dame Radiation Laboratory - Radiation Chemistry Data Center (2003) RCDC Kinetics Data Base, www.rcdc.nd.edu.

JP8076358