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Theoretical Study of the Reactivity and Selectivity of Various Free Radicals with Cysteine Residues

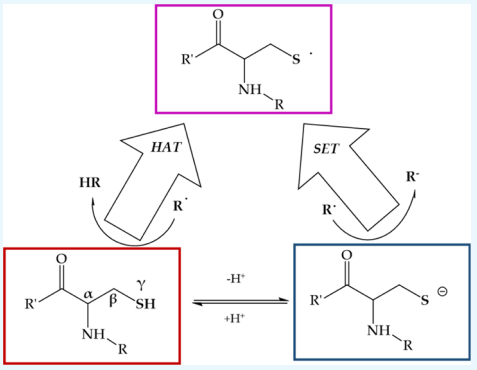
Antonio Romero-Silva,[†] Nelaine Mora-Diez,*[,‡] and J. Raul Alvarez-Idaboy*[,†]

[†]Facultad de Química, Universidad Nacional Autónoma de México, México DF 4510, Mexico

[‡]Department of Chemistry, Thompson Rivers University, Kamloops, British Columbia V2C 0C8, Canada

Supporting Information

ABSTRACT: Radicals in biochemical environments can lead to protein damage. Theoretical studies can help us to understand the observed radical selectivity. In this work, the kinetics and thermodynamics of the hydrogen-transfer (HT) and single-electron transfer (SET) reactions between a cysteine derivative and 17 free radicals of biological significance have been theoretically investigated in aqueous and lipid media. With the exception of the reaction with $^{\bullet}OCCl_3$, all SET reactions in aqueous medium have rate constants in the diffusion-limited regime. The γ site of cysteine was found to be the most reactive for the HT reactions with all the radicals, with rate constants in the diffusion limit for $^{\bullet}OH$, $^{\bullet}OCHCl_2$, and $^{\bullet}OCCl_3$. The HT reactions from the α and γ positions have very similar ΔG° values and even though the β position is the least thermodynamically favored, when the HT from β is exergonic it is a more reactive site than α. The results obtained confirm that the Bell−Evans−Polanyi principle does not apply to the reactions between amino acid residues and free radicals and that reactivity comparisons demand proper kinetic calculations.



1. INTRODUCTION

Oxidative stress (OS) is a recognized contributing factor in the development of numerous diseases such as cancer,[1−4] cardiovascular disorders,[5−8] atherosclerosis,[9−13] fetal growth restriction and preeclampsia,[14−17] and several neurological disorders including Parkinson's and Alzheimer's diseases.[18−21] OS is caused by an imbalance between the production and accumulation of oxidative species, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), which leads to cell and tissue damage.[22] Most ROS are free radicals such as the hydroxyl ($^{\bullet}OH$), alkoxyl ($^{\bullet}OR$), peroxyl ($^{\bullet}OOR$), superoxide radical anion ($O_2^{\bullet-}$), and hydroperoxyl ($^{\bullet}OOH$) radicals. These species are frequently capable of oxidizing essential biological molecules such as fatty acids, proteins, and DNA, causing cell damage.

The reaction of free radicals with cysteine leads to the formation of thiyl radicals ($RS^{\bullet}$) and carbon-centered radicals,[23,24] which could produce hydroperoxides resulting in cell damage.[25] The hydrogen-transfer (HT) reaction between the thiol group (−SH) in free cysteine and the $^{\bullet}OH$ radical has a rate constant of 1.9×10^{10} M^{-1} s^{-1},[26] while the HT reaction between the thiol group in RCH_2SH and the $R-CH_2$ radical has a rate constant of 2×10^{7} M^{-1} s^{-1}.[27] This indicates the high reactivity of the thiol group and suggests that ROS could be reacting mainly with it.

Following the general structure of previously used molecular models to study the damage (and repair) of amino acids by radicals (and antioxidants) in a protein environment,[28−32] we are using *N*-formylcysteinamide (see Figure 1) to mimic

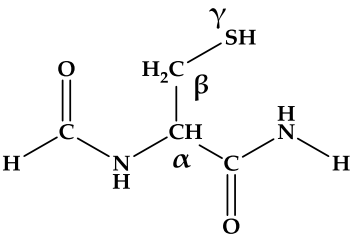


Figure 1. Structure of *N*-formylcysteinamide.

cysteine in a protein environment. Proteins are not the target of usual oxidants, but oxidation can involve residues, located in any protein region. Hence, no specific protein orientation or conformation is necessary for oxidation to take place. Furthermore, the oxidative attack provided by $^{\bullet}OH$ radicals (the most common radical for protein damage in biological systems) is random and nonspecific because of their very high reactivity and low selectivity. Moreover, because of the lack of unsaturation in the protein backbone, electronic effects cannot propagate farther than two sigma bonds.

The reactivity of amino acids in a protein environment could be studied theoretically using reaction barriers. However, an alternative and perhaps more complete approach is that of calculating the rate constants (k) of the possible reactions,

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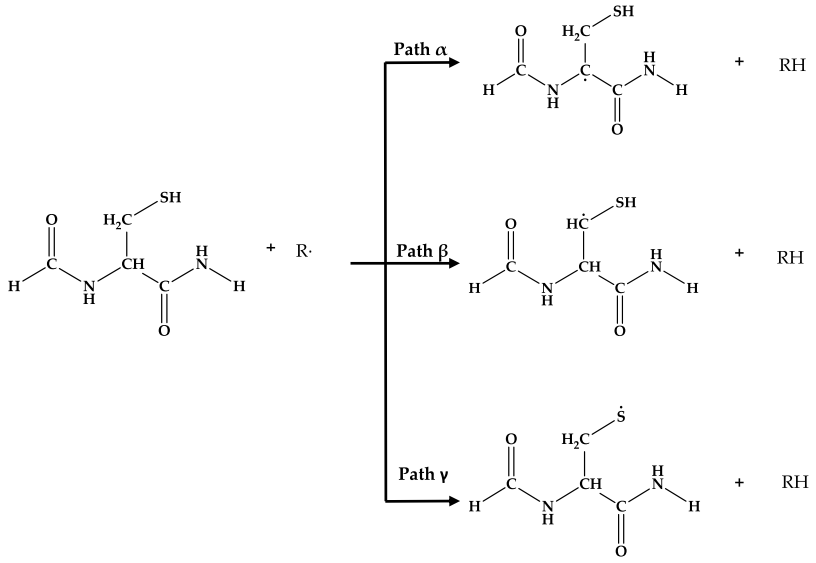

Figure 2. Reaction channels for the HT between free radicals and cysteine.

which has the additional advantage of being directly comparable with experimental data. Unfortunately, kinetic data available for the reactions of free radicals with amino acid residues in peptide environments are very scarce.

The main goal of the present work is to provide theoretical kinetic data for the reactions of cysteine residues with various biologically relevant free radicals in both aqueous and lipid environments. Cysteine, one of the most easily oxidized residues in proteins, has been chosen because of the lack of experimental and computational studies about its site reactivity. The ROS and RNS chosen for our systematical investigation due to their different intrinsic reactivity are $^{\bullet}N_3$, $^{\bullet}NO_2$, $^{\bullet}OR$, and $^{\bullet}OOR$, with R = H, CH_3, CH_2Cl, $CHCl_2$, CCl_3, and $CHCH_2$.[33] Furthermore, the radicals $^{\bullet}$DPPH (2,2-diphenyl-1-picrylhydrazyl), $^{\bullet}ClO_2$, and $^{\bullet}BrO_2$ were also considered, for a total of 17 radicals.

Because the pK_a values for cysteine residues vary in enzymes,[34] it is important to take into account the population of the thiol and the thiolate anion at physiological pH, in order to adequately model the kinetics of the reaction between cysteine and free radicals. Hence, we have studied two different reaction mechanisms, the HT from neutral cysteine (which involves three reaction channels from positions α, β, and γ, see Figure 1) and the single-electron transfer (SET) from the anionic (thiolate) form of cysteine to the chosen radical.

2. COMPUTATIONAL METHODOLOGY

Electronic calculations were performed using the Gaussian 09 software package.[35] Geometry optimization and frequency calculations were carried out using the M06-2X functional[36] and the 6-311+G(2d,2p) basis set, in conjunction with the SMD continuum solvation model,[37] using water and pentyl ethanoate (PE) as solvents. Because fats are esters of glycerin with fatty acids and PE is the largest ester for which solvent parameters are available in Gaussian 09, it has been chosen to mimic a lipid environment. PE has been used in this role in several computational biochemical studies.[40,42,56−58]

The M06-2X functional has been recommended for thermodynamic and kinetic calculations by its developers,[38] and it has been successfully used (other than by the developers) for that purpose.[39−42] It is also one of the best performing functionals for modeling reaction energies involving free radicals.[43] However, it could fail if the transition state (TS) presents multireference character, which is not to be expected in this work. SMD is considered a universal and reliable solvation model because of its applicability to any charged or uncharged solute in any solvent or liquid medium,[37] and it can be successfully used for optimization and frequency calculations in solution.[44] However, other continuum solvation methods, such as COSMO or IEF-PCM, could lead to artificial inconsistencies and their use for thermodynamic corrections in solution has thus been discouraged.[45]

Unrestricted calculations were used for open shell systems. TSs and local minima were identified by the number of imaginary frequencies (1 and 0, respectively). Relative energies were calculated with respect to the isolated reactants. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies, which correspond to the 1 M standard state. Furthermore, the solvent cage effects have been included according to the corrections proposed by Okuno,[46a] taking into account the free volume theory.[46b] Because of the cage effect of the solvent, there is an entropy loss associated with any chemical reaction with molecularity equal or higher than two. Reactant complexes were not included in this study because they rarely have an effect on the rate constant of a reaction.

Rate constants were calculated using conventional TS theory (TST).[47−49] Reaction path degeneracies were assigned the value of 2 for attacks to the β position of the cysteine residue and 1 for attacks to the α and γ positions (see Figure 1). Tunneling was calculated assuming an unsymmetrical, one-dimensional Eckart function barrier[50] using Brown's[51] numerical integration program, which requires the reaction and activation energies at 0 K, the imaginary frequency of the corresponding TS and the absolute temperature.

When the calculated TST rate constants were in the diffusion-limited regime ($k > 1.0 \times 10^8$), apparent rate constants (k_{app}) were calculated using the rate constant of the diffusion-controlled reaction (k_D) applying the Collins−Kimball theory[52] in conjunction with the steady-state von Smoluchowski[53] and the Stokes−Einstein approaches.[54,55] For

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Table 1. Gibbs Energies ($\Delta G°$, kcal mol^{-1}) in Aqueous and Lipid Media for the HT Reactions Studied at 298.15 K

	aqueous media			lipid media		
radical	path α	path β	path γ	path α	path β	path γ
$^{\bullet}$DPPH	9.46	17.91	8.68	6.12	15.30	5.73
$^{\bullet}N_3$	−7.49	0.96	−8.27	−9.94	−0.76	−10.33
$^{\bullet}NO_2$	0.75	9.19	−0.04	2.33	11.51	1.94
$^{\bullet}ClO_2$	3.18	11.63	2.40	4.11	13.30	3.72
$^{\bullet}BrO_2$	−4.54	3.91	−5.33	−4.38	4.80	−4.77
$^{\bullet}OH$	−33.13	−24.68	−33.92	−33.71	−24.53	−34.10
$^{\bullet}OCH_3$	−18.15	−9.70	−18.93	−18.48	−9.30	−18.87
$^{\bullet}OCH_2Cl$	−18.87	−10.43	−19.66	−18.85	−9.67	−19.24
$^{\bullet}OCHCl_2$	−24.53	−16.09	−25.32	−24.77	−15.58	−25.15
$^{\bullet}OCCl_3$	−26.63	−18.19	−27.42	−26.74	−17.56	−27.13
$^{\bullet}OCHCH_2$	2.01	10.46	1.23	0.94	10.12	0.55
$^{\bullet}OOH$	−0.57	7.87	−1.36	−0.69	8.49	−1.08
$^{\bullet}OOCH_3$	0.76	9.21	−0.02	−0.46	8.72	−0.85
$^{\bullet}OOCH_2Cl$	−4.36	4.08	−5.15	−3.62	5.57	−4.01
$^{\bullet}OOCHCl_2$	−7.29	1.16	−8.08	−10.56	−1.38	−10.95
$^{\bullet}OOCCl_3$	−8.24	0.20	−9.03	−7.69	1.49	−8.08
$^{\bullet}OOCHCH_2$	−0.65	7.79	−1.44	−0.38	8.81	−0.77

details on the expressions used, please refer to our previous publications on this subject.[29−32,56−58]

Branching ratios (Γ_i) for each HT channel (i) considered were calculated using eq 1

$$\Gamma_i = \frac{k_i}{k_{total}} \times 100 \quad (1)$$

Marcus Theory[59,60] was used to estimate the reaction barriers of SET reactions, where the SET activation barrier ($\Delta G^{\ddagger}_{SET}$) was calculated using eq 2 in terms of two dynamic parameters, the Gibbs energy of reaction (ΔG^{0}_{ET}), and the nuclear reorganization energy (λ):

$$\Delta G^{\ddagger}_{SET} = \frac{\lambda}{4}\left(1 + \frac{\Delta G^{0}_{ET}}{\lambda}\right)^2 \quad (2)$$

The value of λ could be calculated as indicated by eq 3, where ΔE_{ET} is the nonadiabatic energy difference between reactants and vertical products. This approach is similar to that previously used by Nelsen and co-workers for a large set of reactions.[61,62]

$$\lambda \approx \Delta E_{ET} - \Delta G^{0}_{ET} \quad (3)$$

The methodology used in this work has been previously shown to accurately reproduce experimental rate constants in solution[56] and has been applied in a number of theoretical kinetic studies.[29−32]

3. RESULTS AND DISCUSSION

3.1. HT Reactions. *3.1.1. Thermodynamic Study.* The HT reactions considered in this study are shown in Figure 2, and the corresponding $\Delta G°$ values in aqueous and lipid media are displayed in Table 1. In general, it was found that as the electrophilicity of the radical increases, so does the exergonicity of the reactions studied.[63]

The three HT reactions of 5 of the 17 radicals studied ($^{\bullet}$OH > $^{\bullet}OCCl_3$ > $^{\bullet}OCHCl_2$ > $^{\bullet}OCH_2Cl$ > $^{\bullet}OCH_3$) are exergonic, with similar $\Delta G°$ values for the α and γ reactions in both media. Among these radicals, $^{\bullet}$OH produces the most exergonic reactions (followed by $^{\bullet}OCCl_3$ and $^{\bullet}OCHCl_2$). In general, it was found that the HT reactions from the β site are the least exergonic when compared to those from the α and γ sites, which tend to show similar $\Delta G°$ values, in all cases (although slightly more exergonic for the HT reactions from γ). These observations, based on thermodynamic considerations, might (wrongly) lead us to conclude that the α and γ sites of cysteine residues have similar reactivity toward HT reactions or that the reactivity toward the α position is much greater than that of β. This point will be further addressed below.

The β reactions with $^{\bullet}$DPPH, $^{\bullet}NO_2$, $^{\bullet}ClO_2$, $^{\bullet}BrO_2$, $^{\bullet}OCHCH_2$, $^{\bullet}OOH$, $^{\bullet}OOCH_3$, $^{\bullet}OOCH_2Cl$, $^{\bullet}OOCHCH_2$ are endergonic in both media. Thus, these radicals are not capable of directly damaging cysteine at the β site. The aqueous reactions involving $^{\bullet}N_3$, $^{\bullet}OOCHCl_2$, and $^{\bullet}OOCCl_3$ (also in lipid medium) at the β site are slightly endergonic with $0 < \Delta G° < 2.0$ kcal/mol. This is also the case of the α and/or γ reactions involving $^{\bullet}NO_2$, $^{\bullet}OCHCH_2$, and $^{\bullet}OOCH_3$ in one or both environments. From a thermodynamic point of view, the reactions with $^{\bullet}$DPPH and $^{\bullet}ClO_2$ are the least viable in both environments, as they are the most endergonic.

3.1.2. Kinetic Study. Kinetic results in aqueous and lipid media are reported in Tables 2 and 3, respectively. The endergonic reactions were not included in the kinetic study of the different HT reaction paths, because if $\Delta G°$ is significantly positive, the equilibrium constant ($\Delta G° = -RT \ln K$) is significantly smaller than 1 and in such cases product formation would not be observed. However, slightly endergonic reactions in which $0 < \Delta G° < 2.0$ kcal/mol should be considered for SET reactions. The Cartesian coordinates of the optimized geometries of the TSs calculated in water and PE are reported in the Supporting Information section together with their thermodynamic data at 298.15 K. The structures of the TSs in water are also reported in the Supporting Information.

Even though the exergonicities of the HT reactions from the α and γ sites are very similar (with those from γ being only 0.79 kcal/mol more exergonic), in all cases, the most reactive site for HT is γ (the thiol hydrogen atom). Our results suggest that the α hydrogen atom might be deactivated with respect to

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Table 2. Gibbs Energies ($\Delta^\circ G^\ddagger$, kcal mol^{-1}) and Enthalpies of Activation ($\Delta^\circ H^\ddagger$, kcal mol^{-1}), Thermal Rate Constants (k, M^{-1} s^{-1}), Tunneling Corrections (κ), Diffusion Rate Constants (k_D, M^{-1} s^{-1}), Apparent Rate Constants (k_{app}, M^{-1} s^{-1}), and Branching Ratios (Γ, %) for the HT Reactions in Aqueous media at 298.15 K[a]

path	$\Delta^\circ G^\ddagger$	$\Delta^\circ H^\ddagger$	k	κ	k_D	k_{app}	Γ
				$^\bullet N_3$			
α	15.72	9.15	203	10.92			0.01
γ	10.45	5.37	1.39×10^6	10.26			99.99
total			1.39×10^6				
				$^\bullet NO_2$			
γ	25.13	17.88	1.07	[b]			
total			1.07				
				$^\bullet BrO_2$			
α	21.93	12.69	2.12×10^{-2}	40.62			0.00
γ	16.27	9.32	1.66×10^3	225.41			100.00
total			1.66×10^3				
				$^\bullet OH$			
α	7.09	1.23	8.16×10^7	2.09		8.16×10^7	3.59
β	6.49	0.93	2.27×10^8	2.08	3.13×10^9	2.12×10^8	9.31
γ	4.24	−0.47	4.79×10^9	1.00	3.38×10^9	1.98×10^9	87.11
total						2.27×10^9	
				$^\bullet OCH_3$			
α	12.31	4.66	3.25×10^4	5.51			0.38
β	12.32	4.76	6.86×10^4	6.01			0.80
γ	8.43	0.68	8.48×10^6	2.07			98.82
total			8.58×10^6				
				$^\bullet OCH_2Cl$			
α	11.76	3.25	3.53×10^4	2.37			3.92
β	17.63	9.13	2.76	1.86			0.00
γ	9.84	1.37	8.65×10^5	2.27			96.08
total			9.00×10^5				
				$^\bullet OCHCl_2$			
α	10.54	1.84	1.65×10^5	1.41		1.65×10^5	0.01
β	8.43	0.53	9.83×10^6	1.20		9.83×10^6	0.34
γ	0.24	−7.48	4.14×10^{12}	1.00	2.84×10^9	2.84×10^9	99.65
total						2.85×10^9	
				$^\bullet OCCl_3$			
α	7.61	−1.79	1.63×10^7	1.00		1.63×10^7	0.30
β	0.00	−10.58	6.21×10^{12}	1.00	2.59×10^9	2.59×10^9	46.64
γ	[c]	[c]	[c]	1.00	2.83×10^9	2.83×10^9	52.06
total						5.44×10^9	
				$^\bullet OOH$			
α	24.4	15.83	2.56×10^{-3}	316.29			0.00
γ	16.38	8.92	4.01×10^2	65.56			100.00
total			4.01×10^2				
				$^\bullet OOCH_3$			
γ	15.95	8.19	0.962	54.02			
total			0.962				
				$^\bullet OOCH_2Cl$			
α	21.62	12.21	4.47×10^{-2}	50.73			0.00
γ	14.72	6.99	3.76×10^3	37.26			100.00
total			3.76×10^3				
				$^\bullet OOCHCl_2$			
α	17.83	9.01	26.8	50.65			0.67
γ	14.54	6.20	3.98×10^3	29.08			99.32
total			4.01×10^3				
				$^\bullet OOCCl_3$			
α	17.75	8.18	7.73	12.76			0.04
γ	13.45	5.16	1.83×10^4	21.32			99.95
total			1.83×10^4				
				$^\bullet OOCHCH_2$			
α	21.67	12.08	0.132	163.12			0.00
γ	14.60	6.71	4.23×10^3	34.41			100.00
total			4.23×10^3				

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Table 2. continued

[a] k_D and k_{app} values are only reported when k is greater than 1.00×10^8. [b] $\kappa > 10^5$; k (neglecting tunneling) $< 1.07 \times 10^{-5}$. This is not an important reaction. [c] There is no TS; this reaction is purely diffusion-controlled, as in the case of recombination reactions.

the most exposed site. That is also the case relative to the HT from the β site in most cases when the β reaction is exergonic. This situation contributes to the protection of the integrity of the peptide backbone because of the deactivating polar effect previously proposed.[28,64] There do not seem to be a consistent pattern when comparing rate constants in hydrophobic and hydrophilic environments.

As expected, there is an inverse relationship between reactivity and selectivity. The HT reactions with the three most reactive radicals in both solvents ($^\bullet$OH, $^\bullet OCCl_3$, and $^\bullet OCHCl_2$) have contributions from all sites, with β and γ being the most reactive. These are the radicals that produce the most exergonic reactions. The HT reactions from positions in β and γ to $^\bullet$OH and $^\bullet OCCl_3$ are diffusion-controlled in a polar environment, while in a nonpolar one, all the positions lead to diffusion-controlled reactions. The third most reactive radical in both environments, $^\bullet OCHCl_2$, has diffusion-controlled rate constants for the γ attack.

The alkoxyl radicals are always more reactive than their equivalent peroxyl radicals (with the exception of $^\bullet OCHCH_2$ and $^\bullet OOCHCH_2$) in both solvents. For the halogenated alkoxyl and peroxyl series of radicals, the reactivity of each of the three reaction sites increases (greater rate constants are obtained) with the degree of halogenation of the radical in both solvents ($^\bullet OCH_2Cl < {}^\bullet OCHCl_2 < {}^\bullet OCCl_3$ and $^\bullet OOCH_2Cl < {}^\bullet OOCHCl_2 < {}^\bullet OOCCl_3$); lower Gibbs energies of activation ($\Delta^\circ G^\ddagger$) are obtained for each position in both solvents. Regarding $^\bullet OCHCl_2$ and $^\bullet OCCl_3$ in both solvents, in addition to what was previously noted, it can be observed that the $\Delta^\circ G^\ddagger$ values decrease in the series $\alpha > \beta > \gamma$ to the point that it is impossible to find a TS for the HT reaction from γ to $^\bullet OCCl_3$. The γ HT reactions with the $OCCl_3$ radical are barrierless and limited only by the diffusion rates because every collision is effective, as it occurs in recombination reactions. We arrived at this conclusion after having searched for the corresponding prereactive complexes. Every attempt to find them led to the reaction products. Moreover, the relaxed scan of the S−H distance in the presence of the radical confirms this because the energy decreases as the S−H distance increases. The structures of the TSs for the α and β attacks with $^\bullet OCCl_3$ in polar environment are displayed in Figure 3.

The radicals $^\bullet OCCl_3$, $^\bullet OCHCl_2$, and $^\bullet$OH are the most damaging for cysteine residues in proteins via HT, particularly for the γ (thiol) site in either hydrophilic or hydrophobic environments. The radicals $^\bullet OCH_3$, $^\bullet N_3$, $^\bullet OCH_2Cl$, $^\bullet OOCCl_3$, $^\bullet OOCHCl_2$, $^\bullet OOCH_2Cl$, $^\bullet OOCHCH_2$, $^\bullet BrO_2$, $^\bullet OOCH_3$, and $^\bullet$OOH are also predicted to threaten the integrity of proteins in hydrophilic and/or hydrophobic environments, but to a lesser extent (at least via HT; with rate constants between 10^7 and 10^2 M^{-1} s^{-1}). In contrast, the HT reactions with the radicals $^\bullet NO_2$, $^\bullet$DPPH, $^\bullet ClO_2$, and $^\bullet OOCHCH_2$ are slow enough (with rate constants smaller than 100 M^{-1} s^{-1}) not to represent a risk for cysteine residues.

One very important observation from our work is that the exergonicity of the three possible HT reactions from the cysteine residue is not always directly linked to the degree of reactivity and selectivity of the different radicals studied. The α and γ reactions have very similar exergonicities, yet the difference in reactivity is remarkable. Furthermore, in cases in which the β reactions were exergonic and studied from a kinetic point of view, even though they were much less exergonic than the α reactions, they were in 8 of 11 instances a more reactive site. In other words, the Bell−Evans−Polanyi principle does not apply, as was previously demonstrated for amino acids with nonpolar sidechains.[28,64]

Hence, reactivity comparisons demand proper kinetic calculations, not a thermodynamic- or thermochemical-based analysis making use of ΔG° values, bond dissociation, or reaction energies. The crucial role of performing kinetic studies for reactions between amino acids and free radicals has been previously pointed out by Chan et al.,[28] who asserted that regioselectivity depends on structural factors, polar effects, and solvent effects. Our results, with a different amino acid not previously studied, are in agreement with the data reported by Chan et al.[28] and confirm their proposal. As previously stated,[28] calculated rate constants and Gibbs energy reaction profiles can clearly explain the "abnormal" reactivity of the α position without needing to use the "kinetic trap" hypothesis of Scheiner and Kar, which lacks solid foundations.[65]

On the basis of the calculated kinetic data, the reactivity toward cysteine residues in hydrophilic environments of the radicals studied is proposed to be $^\bullet OCCl_3$ ($k_{TOTAL} = 5.44 \times 10^9$ M^{-1} s^{-1}) > $^\bullet OCHCl_2$ ($k_{TOTAL} = 2.85 \times 10^9$ M^{-1} s^{-1}) > $^\bullet$OH ($k_{TOTAL} = 2.27 \times 10^9$ M^{-1} s^{-1}) > $^\bullet OCH_3$ ($k_{TOTAL} = 8.58 \times 10^6$ M^{-1} s^{-1}) > $^\bullet N_3$ ($k_{TOTAL} = 1.39 \times 10^6$ M^{-1} s^{-1}) > $^\bullet OCH_2Cl$ ($k_{TOTAL} = 9.00 \times 10^5$ M^{-1} s^{-1}) > $^\bullet OOCCl_3$ ($k_{TOTAL} = 1.83 \times 10^4$ M^{-1} s^{-1}) > $^\bullet OOCHCH_2$ ($k_{TOTAL} = 4.23 \times 10^3$ M^{-1} s^{-1}) ≈ $^\bullet OOCHCl_2$ ($k_{TOTAL} = 4.01 \times 10^3$ M^{-1} s^{-1}) ≈ $^\bullet OOCH_2Cl$ ($k_{TOTAL} = 3.76 \times 10^3$ M^{-1} s^{-1}) > $^\bullet BrO_2$ ($k_{TOTAL} = 1.66 \times 10^3$ M^{-1} s^{-1}) > $^\bullet$OOH ($k_{TOTAL} = 4.01 \times 10^2$ M^{-1} s^{-1}) > $^\bullet NO_2$ ($k_{TOTAL} = 1.07$ M^{-1} s^{-1}) > $^\bullet OOCH_3$ ($k_{TOTAL} = 0.962$ M^{-1} s^{-1}) > $^\bullet$DPPH, $^\bullet ClO_2$, $^\bullet OCHCH_2$.

The reactivity order in hydrophobic environments is proposed to be $^\bullet$OH ($k_{TOTAL} = 3.51 \times 10^9$ M^{-1} s^{-1}) ≈ $^\bullet OCCl_3$ ($k_{TOTAL} = 3.50 \times 10^9$ M^{-1} s^{-1}) > $^\bullet OCHCl_2$ ($k_{TOTAL} = 1.51 \times 10^9$ M^{-1} s^{-1}) > $^\bullet OCH_3$ ($k_{TOTAL} = 1.38 \times 10^7$ M^{-1} s^{-1}) > $^\bullet N_3$ ($k_{TOTAL} = 7.52 \times 10^6$ M^{-1} s^{-1}) > $^\bullet OCH_2Cl$ ($k_{TOTAL} = 2.50 \times 10^6$ M^{-1} s^{-1}) > $^\bullet OOCHCl_2$ ($k_{TOTAL} = 9.73 \times 10^5$ M^{-1} s^{-1}) > $^\bullet OOCCl_3$ ($k_{TOTAL} = 9.43 \times 10^3$ M^{-1} s^{-1}) > $^\bullet OOCH_3$ ($k_{TOTAL} = 6.57 \times 10^3$ M^{-1} s^{-1}) > $^\bullet OOCHCH_2$ ($k_{TOTAL} = 1.44 \times 10^3$ M^{-1} s^{-1}) > $^\bullet BrO_2$ ($k_{TOTAL} = 7.25 \times 10^2$ M^{-1} s^{-1}) > $^\bullet$OOH ($k_{TOTAL} = 5.55 \times 10^2$ M^{-1} s^{-1}) > $^\bullet OOCH_2Cl$ ($k_{TOTAL} = 4.17 \times 10^2$ M^{-1} s^{-1}) > $^\bullet$DPPH, $^\bullet ClO_2$, $^\bullet OCHCH_2$, $^\bullet NO_2$.

3.2. SET Reactions. Cysteine residues in proteins are involved in an acid−base equilibrium as shown in Figure 4. The pK_a value for cysteine residues in enzymes varies greatly with some values in the 7.4−9.1 range.[66] A value of 8.22 ± 0.16 has been reported for the aqueous pK_a of free cysteine,[67] while a value of 8.3 ± 0.2 has been reported for a cysteine residue in a particular enzyme.[68] The pK_a of the cysteine residue in glutathione (L-γ-glutamyl-L-cysteinylglycine) has been reported to be 9.2 ± 0.15[69] and 9.42 ± 0.17.[67]

Because at physiological pH the concentration of the thiolate form of cysteine (Cys-S$^-$) is significant, SET reactions

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Table 3. Gibbs Energies ($\Delta^\circ G^\ddagger$, kcal mol^{-1}) and Enthalpies of Activation ($\Delta^\circ H^\ddagger$, kcal mol^{-1}), Thermal Rate Constants (k, M^{-1} s^{-1}), Tunneling Corrections (κ), Diffusion Rate Constants (k_D, M^{-1} s^{-1}), Apparent Rate Constants (k_{app}, M^{-1} s^{-1}), and Branching Ratios (Γ, %) for the HT Reactions in Lipid Media at 298.15 K[a]

path	$\Delta^\circ G^\ddagger$	$\Delta^\circ H^\ddagger$	k	κ	k_D	k_{app}	Γ
				$^\bullet N_3$			
α	11.64	5.45	1.05×10^5	5.79			1.40
β	12.2	6.89	1.29×10^5	9.12			1.71
γ	9.28	3.81	7.29×10^6	7.48			96.89
total			7.52×10^6				
				$^\bullet BrO_2$			
α	19.46	11.15	0.853	25.23			0.12
γ	16.59	9.45	7.24×10^2	168.69			99.88
total			7.25×10^2				
				$^\bullet OH$			
α	4.65	0.13	3.18×10^9	1.31	1.71×10^9	1.11×10^9	31.62
β	5.13	1.43	2.04×10^9	1.89	1.69×10^9	1.20×10^9	34.19
γ	4.29	−0.57	4.45×10^9	1.00	1.65×10^9	1.20×10^9	34.19
total						3.51×10^9	
				$^\bullet OCH_3$			
α	9.86	2.94	1.34×10^6	3.64			9.71
β	12.63	5.05	6.23×10^4	9.08			0.45
γ	8.19	0.73	1.24×10^7	2.02			89.84
total			1.38×10^7				
				$^\bullet OCH_2Cl$			
α	10.27	2.20	3.16×10^5	1.72			12.64
β	10.70	2.89	3.90×10^5	2.19			15.60
γ	9.46	2.18	1.79×10^6	2.90			71.60
total			2.50×10^6				
				$^\bullet OCHCl_2$			
α	8.43	0.38	4.34×10^6	1.06		4.34×10^6	0.29
β	7.72	1.41	3.19×10^7	1.20		3.19×10^7	2.11
γ	0.28	−7.23	3.86×10^{12}	1.00	1.47×10^9	1.47×10^9	97.35
total						1.51×10^9	
				$^\bullet OCCl_3$			
α	4.98	−3.21	1.39×10^9	1.00	1.46×10^9	7.11×10^8	20.31
β	1.92	−2.47	2.43×10^{11}	1.00	1.45×10^9	1.44×10^9	41.14
γ	[b]	[b]	[b]	1.00	1.35×10^9	1.35×10^9	38.57
total						3.50×10^9	
				$^\bullet OOH$			
α	22.87	14.63	3.98×10^{-2}	372.22			0.01
γ	16.06	9.02	5.55×10^2	52.84			99.99
total			5.55×10^2				
				$^\bullet OOCH_3$			
α	21.01	12.66	0.834	337.65			0.01
γ	14.21	6.40	6.57×10^3	27.61			99.99
total			6.57×10^3				
				$^\bullet OOCH_2Cl$			
α	20.35	13.06	0.243	32.22			0.06
γ	15.04	7.32	4.17×10^2	27.81			99.94
total			4.17×10^2				
				$^\bullet OOCHCl_2$			
α	12.9	4.99	2.82×10^4	12.95			2.90
γ	10.57	3.32	9.45×10^5	8.52			97.12
total			9.73×10^5				
				$^\bullet OOCCl_3$			
α	16.61	7.57	31.9	7.69			0.34
γ	14.87	6.53	9.40×10^3	120.25			99.65
total			9.43×10^3				
				$^\bullet OOCHCH_2$			
α	19.64	11.4	2.60	104.53			0.18
γ	15.2	7.84	1.44×10^3	32.12			99.82
total			1.44×10^3				

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Table 3. continued

[a]k_D and k_{app} values are only reported when k is greater than 1.00×10^8. [b]There is no TS; this reaction is purely diffusion-controlled, as in the case of recombination reactions.

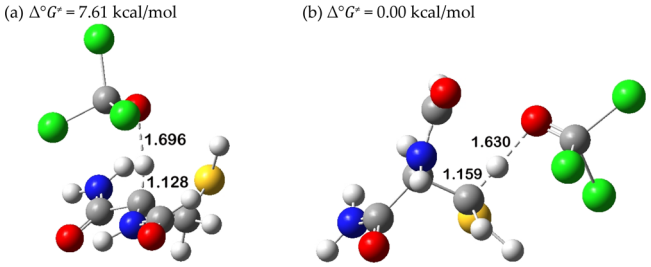

Figure 3. Structures of the TSs for (a) α and (b) β attacks with $^{\bullet}OCCl_3$ in polar environment.

could take place and have been taken into account using the reactions indicated by Schemes 1 and 2 for aqueous and lipid media, respectively. The kinetic results obtained are reported in Table 4.

Very high $\Delta^{\circ}G^{\ddagger}_{SET}$ values were calculated for the SET reactions in nonpolar environments, which suggests that this is not an important pathway in this medium. This was an expected result because SET reactions in general are more common in polar than in lipid environments. The fastest SET process in lipid medium involves $^{\bullet}OCHCl_2$ with a rate constant of 2.81 M^{-1} s^{-1}, which is too small for this reaction to be important.

With the exception of the reaction with $^{\bullet}OCCl_3$, all SET reactions in aqueous medium have rate constants in the diffusion-limited regime. These results suggest that the thiolate anion of cysteine is very reactive toward SET reactions with free radicals in polar environments, leading to the formation of the anion derived from the free radical and the thiyl radical (Cys-S$^{\bullet}$). Thus, the SET mechanism should be considered to properly investigate the reactivity of cysteine with free radicals in polar environments.

Finding that the most reactive radical via HT in aqueous medium, $^{\bullet}OCCl_3$, is the least reactive via SET seems an apparent contradiction. However, this result is a consequence of the very large negative ΔG^0_{ET} of the SET reaction with $^{\bullet}OCCl_3$, which places it in the Marcus inverted region. This is one of the most surprising results of Marcus's theory.

To adequately model the kinetics of the SET reaction between cysteine residues and free radicals in hydrophilic environments, it is important to take into account the population of the thiolate anion (Cys-S^-) at physiological pH. Its mole fraction (χ) depends on the K_a value of the cysteine residue and $[H^+]$, as shown in eq 4. A correct rate constant for this process must be multiplied by the corresponding χ (Cys-S^-).

Scheme 1

$$\text{Cys-S}^{-} + {}^{\cdot}\text{R} \rightarrow \text{Cys-S}^{\cdot} + \text{R}^{-}$$

Scheme 2

$$\text{Cys-SH} + {}^{\cdot}\text{R} \rightarrow [\text{Cys-SH}]^{\cdot+} + \text{R}^{-}$$

$$\chi(\text{Cys-S}^-) = \frac{k_a}{[H^+] + k_a} \quad (4)$$

The variability of pK_a values for cysteine residues in proteins depending on environment makes the SET kinetic results inconclusive. Using the pK_a value of 8.3 ($K_a = 5.01 \times 10^{-9}$) previously reported for free cysteine,[67] at physiological pH (7.40), $[H^+] = 3.98 \times 10^{-8}$ M, and χ(Cys-S^-) is calculated to be 0.11. Using this value, the previously calculated range of rate constants for the SET reactions (listed in Table 4), which goes from 8.00×10^9 M^{-1} s^{-1} (with $^{\bullet}OH$) to 1.37×10^8 M^{-1} s^{-1} (with $^{\bullet}OCHCl_2$), becomes 8.80×10^8 to 1.51×10^7 M^{-1} s^{-1}. These results indicate that, in addition to the neutral pathways via HT, the ionized (SET) pathway plays an important role in hydrophilic environments.

4. CONCLUSIONS

The kinetic study of the HT and SET reactions between *N*-formylcysteinamide (the model used to mimic cysteine in a protein environment) and 17 free radicals in aqueous and lipid environments was carried out using density functional theory.

In all cases, the preferred HT reaction site is γ with rate constants in the diffusion limit for $^{\bullet}OH$, $^{\bullet}OCHCl_2$, and $^{\bullet}OCCl_3$, which, of the radicals studied, are the most damaging for cysteine residues in proteins. The radicals $^{\bullet}OCH_3$, $^{\bullet}N_3$, $^{\bullet}OCH_2Cl$, $^{\bullet}OOCCl_3$, $^{\bullet}OOCHCl_2$, $^{\bullet}OOCH_2Cl$, $^{\bullet}OOCHCH_2$, $^{\bullet}BrO_2$, $^{\bullet}OOCH_3$, and $^{\bullet}OOH$ are also predicted to threaten the integrity of cysteine residues in hydrophilic and/or hydrophobic environments. The HT reactions from the α and γ positions have very similar ΔG° values, and even though the β position is the least thermodynamically favored, when the HT from β is exergonic it is a more reactive site than α. The results obtained confirm that the Bell–Evans–Polanyi principle does not apply to the reactions between amino acid residues and free radicals and that reactivity comparisons demand proper kinetic calculations, not a thermodynamic- or thermochemical-based analysis making use of ΔG° values, bond dissociation, or reaction energies.

It has been shown that SET reactions are an important damaging mechanism for cysteine residues in hydrophilic environments with most of the rate constants in the diffusion-

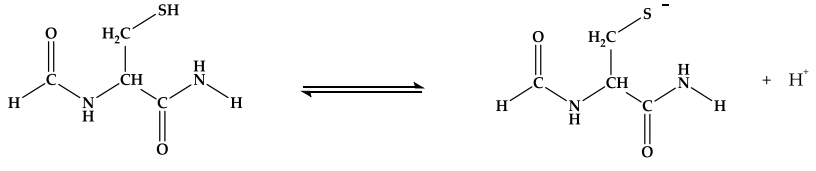

Figure 4. Acid dissociation of the cysteine residue in aqueous solution.

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Table 4. Gibbs Energies of Activation ($\Delta^{\circ}G^{\ddagger}_{SET}$, kcal mol^{-1}, in Lipid and Aqueous Media), Thermal Rate Constants (k, M^{-1} s^{-1}), Diffusion Rate Constants (k_D, M^{-1} s^{-1}), and Apparent Rate Constants (k_{app}, M^{-1} s^{-1}) in Aqueous Media for the SET reaction at 298.15 K[a]

N	$\Delta^{\circ}G^{\ddagger}_{SET}$ (lipid)	$\Delta^{\circ}G^{\ddagger}_{SET}$ (aqueous)	k	k_D	k_{app}
•DPPH	59.66	1.97	2.23×10^{11}	7.65×10^{9}	7.64×10^{9}
$^{\bullet}N_3$	54.20	4.66	2.38×10^{9}	7.61×10^{9}	7.60×10^{9}
$^{\bullet}NO_2$	64.36	1.18	8.48×10^{11}	7.68×10^{9}	7.61×10^{9}
$^{\bullet}ClO_2$	64.36	0.05	5.71×10^{12}	7.85×10^{9}	7.84×10^{9}
$^{\bullet}BrO_2$	57.89	0.00	6.21×10^{12}	7.55×10^{9}	7.30×10^{9}
•OH	92.76	0.78	1.67×10^{12}	8.03×10^{9}	8.00×10^{9}
$^{\bullet}OCH_3$	105.65	0.00	6.21×10^{12}	7.71×10^{9}	7.70×10^{9}
$^{\bullet}OCH_2Cl$	28.16	1.36	6.12×10^{11}	7.58×10^{9}	7.49×10^{9}
$^{\bullet}OCHCl_2$	16.84	6.34	1.40×10^{8}	7.48×10^{9}	1.37×10^{8}
$^{\bullet}OCCl_3$	56.49	13.86	4.30×10^{2}		
$^{\bullet}OCHCH_2$	96.43	2.28	1.32×10^{11}	7.62×10^{9}	7.20×10^{9}
•OOH	110.29	5.73	3.92×10^{8}	7.83×10^{9}	3.73×10^{8}
$^{\bullet}OOCH_3$	111.67	3.77	1.07×10^{10}	7.59×10^{9}	4.44×10^{9}
$^{\bullet}OOCH_2Cl$	74.70	0.92	1.31×10^{12}	7.51×10^{9}	7.47×10^{9}
$^{\bullet}OOCHCl_2$	64.79	0.27	3.94×10^{12}	7.47×10^{9}	7.46×10^{9}
$^{\bullet}OOCCl_3$	55.21	0.00	6.21×10^{12}	7.45×10^{9}	7.44×10^{9}
$^{\bullet}OOCHCH_2$	89.20	2.22	1.47×10^{11}	7.54×10^{9}	7.17×10^{9}

[a] k_D and k_{app} values are only reported when k is greater than 1.00×10^{8}.

limited regime, or very close to it. However, SET reactions are blocked in lipid media because of the lack of appropriate solvation for the anions, which are formed in the transfer of electrons. SET reactions produce exclusively S-centered radicals, so they are very selective despite the reactivity of the attacking radical.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02964.

M06-2X-SMD/6-311+G(2d,2p) Cartesian coordinates, structures (with important bond distances shown in Å), and thermodynamic data (in hartrees at 298.15 K) of the optimized geometries of the TSs calculated in water; M06-2X-SMD/6-311+G(2d,2p) Cartesian coordinates and thermodynamic data (in hartrees at 298.15 K) of the optimized geometries of the TSs calculated in PE (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: nmora@tru.ca (N.M.-D.).
*E-mail: jidaboy@unam.mx (J.R.A.-I.).

ORCID

J. Raul Alvarez-Idaboy: 0000-0002-2901-5412

Notes

The authors declare no competing financial interest.

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