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On the Chemical Repair of DNA Radicals by Glutathione: Hydrogen vs Electron Transfer

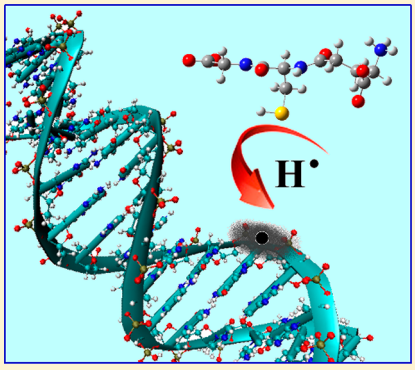
Juan Raúl Alvarez-Idaboy*,† and Annia Galano*,‡

†Departamento de Física y Química Teórica, Facultad de Química, Universidad Nacional Autónoma de México, México DF 04510, México

‡Departamento de Química, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, Col. Vicentina, Iztapalapa, C. P. 09340 México D. F. México

Supporting Information

ABSTRACT: The chemical repair of radical-damaged DNA by glutathione in aqueous solution has been studied using density functional theory. Two main mechanisms were investigated: the single electron transfer (SET) and the hydrogen transfer (HT). Glutathione was found to repair radical damaged DNA by HT from the thiol group with rate constants that are close to the diffusion-limited regime, which means that the process is fast enough for repairing the damage before replication and therefore for preventing permanent DNA damage. The SET mechanism was found to be of minor importance for the activity of glutathione. In addition while SET can be essential for other compounds when repairing radical cation species, repairing the C′-centered guanosyl radicals via SET is not a viable mechanism, due to the very low electron affinity of these species. The importance of considering pH-related physiological conditions and using complex enough models, including the ribose moiety and the H bonding between base pairs, to study this kind of systems is discussed.



INTRODUCTION

Reactive oxygen species (ROS) in general, and free radicals in particular, are naturally produced in healthy organisms at low/moderate concentrations, where they have several beneficial functions. However when their concentrations increase they can cause oxidative damage to a wide variety of biomolecules, including DNA. This situation is known as oxidative stress (OS). If the lesions are not rapidly removed, i.e., before DNA replication, they can lead to permanent damage, which has been associated with the onset of several degenerative diseases such as cancer, cardiovascular disorders, Alzheimer's disease, and Parkinson's disease.[1] Therefore fast DNA repair is crucial for maintaining genomic integrity and a healthy state.

Despite of the vital importance of enzymatic repair in living organisms, it has been reported to have three major drawbacks:[1] (i) The repairing enzymes can also be damaged by ROS, losing their function.[2] (ii) During aging and illness, just when their action is needed the most, their repairing activity decreases.[3] (iii) The enzymatic repairing process can take hours,[4] whereas the half-lives of DNA radicals are in the order of seconds.[5] Accordingly, enzymatic repair might not be enough for protecting against permanent DNA mutations. Fortunately OS-related damages can also be efficiently repaired by nonenzymatic mechanisms,[1] which involve the removal of transient DNA radicals at high reaction rates by natural and synthetic compounds.[1] Some of them are polyphenols,[6−8] singly substituted phenols,[9] dopamine,[10] hydroxycinnamic acid derivatives,[11] uric acid,[10] aniline,[12] indoles,[13] and glutathione,[14,15] which is also able of repairing proteins.[16,17] In the particular case of it, Pellmar et al.[14] proved that glutathione is essential for repairing processes in hippocampal neurons exposed to oxidative damage. In addition, Pujari et al.[15] found that, although glutathione does not act as a radioprotector against DNA damage induced by high dose X-rays, it acts as a modulator of the DNA repair activity.

For DNA-radicals repairing by phenolic compounds it has been proposed that the main chemical mechanisms involved are the single electron transfer (SET)[1−7] and the hydrogen transfer (HT).[6,7] It has also been proposed that SET can be accompanied by a proton transfer (PT).[9,11−13,18] For glutathione, on the other hand, there is no information on the chemical mechanism, or mechanisms, involved in its nonenzymatic DNA repairing activity. In addition glutathione is an excellent antioxidant that exerts such action almost exclusively by HT,[19] essentially from the thiol group. Thus SET is not expected to play a major role in its repairing ability either. However this needs to be demonstrated. Moreover it is important to investigate how fast such repairing takes place, since there are no kinetic data available, and high rates is one of the fundamental aspects regarding chemical repairing. Accordingly it is the main goal of the present work to investigate in detail the kinetics and mechanisms involved in the non-

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enzymatic DNA repair by glutathione. To that purpose we have used complex models including two key features of nucleosides in double stranded DNA: the hydrogen bonding between pairs and the presence of the ribose moiety.

COMPUTATIONAL DETAILS

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional[20] and the 6-31+G(d,p) basis set, in conjunction with the SMD continuum model,[21] using water as solvent. The M05-2X functional has been recommended for thermodynamic and kinetic calculations by their developers,[20] and it has been also successfully used by independent authors to that purposes.[22−26] Local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). In addition, intrinsic reaction coordinate (IRC) calculations have been performed to confirm that the transition state properly connects reactants and products. All of the electronic calculations were performed with the Gaussian 09 package of programs.[27] Thermodynamic corrections at 298.15 K were included in the calculation of relative energies.

The rate constants (k) were calculated using conventional transition state theory (TST)[28] and 1 M standard state as

$$k = \sigma\kappa\frac{k_B T}{h}e^{-(\Delta G^{\ddagger})/RT} \quad (1)$$

where k_B and h are the Boltzmann and Planck constants, $\Delta G^{\ddagger}$ is the Gibbs free energy of activation, σ represents the reaction path degeneracy, accounting for the number of equivalent reaction paths, and κ accounts for tunneling corrections which were calculated using the zero curvature tunneling approach (ZCT).[29] For the electron transfer reactions $\Delta G\ddagger$ was calculated using the Marcus theory[30] as

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

where ΔG is the free energy of reaction and λ is a reorganization term.

Some of the calculated rate constants (k) are close to the diffusion-limit. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work the Collins−Kimball theory is used to that purpose[31]

$$k_{app} = \frac{k_D k_{act}}{k_D + k_{act}} \quad (3)$$

where k_{act} is the thermal rate constant, obtained from TST calculations (equation 1), and k_D is the steady-state Smoluchowski[32] rate constant for an irreversible bimolecular diffusion-controlled reaction

$$k_D = 4\pi R D_{AB} N_A \quad (4)$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of the reactants A (damaged species) and B (repairing species). D_{AB} has been calculated from D_A and D_B according to ref 33, and D_A and D_B have been estimated from the Stokes−Einstein approach[34]

$$D = \frac{k_B T}{6\pi\eta a} \quad (5)$$

where k_B is the Boltzmann constant, T is the temperature, η denotes the viscosity of the solvent, in our case water (η = 8.91 × 10^{-4} Pa s), and a is the radius of the solute.

RESULTS AND DISCUSSION

General Considerations. The first two pK_a values of glutathione are 3.59 and 8.75,[35] which means that at physiological pH (7.4) the neutral, monoanionic, and dianionic forms represent ∼0%, 96%, and 4%, respectively, of the total population of glutathione (Figure 1S, Supporting Information). In addition it has been previously demonstrated that even when different tautomers of GSH^- might exist, the one presented in Figure 1 accounts for more than 99% of the total GSH^-.[19]

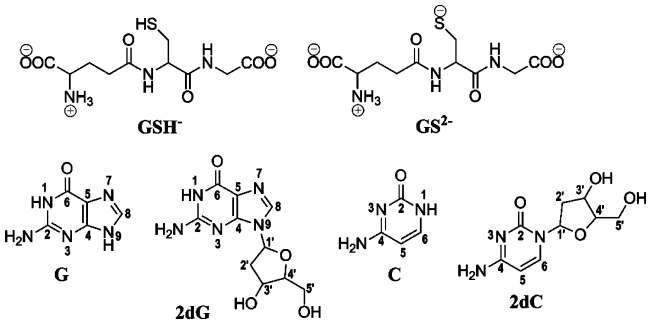

Figure 1. Monoanionic form of glutathione (GSH^-), dianionic form of glutathione (GS^{2-}), guanine (G), 2′-deoxyguanosine (2dG), cytosine (C), and 2′-deoxycytidine (2dC).

Therefore, this is the only tautomer considered in the present work. To investigate the nonenzymatic DNA repair by glutathione we have used the monoanionic (GSH^-, Figure 1) and the dianionic (GS^{2-}, Figure 1) forms of glutathione. Even though GSH^- is the dominant species under physiological conditions, based on their relative populations, if the dianion reacts fast enough its contribution to the overall activity of glutathione could be significant.

The repair of radical damaged DNA by glutathione has been investigated using three different models (Figure 2): (a) the nucleoside 2′-deoxyguanosine (2dG), (b) the pair guanine-cytosine (G-C), and (c) the pair 2′-deoxyguanosine-2′-deoxycytidine (2dG-2dC).

Even though the latter is the most complete one, the other two have been used to assess the relative importance of including the ribose moiety and the hydrogen bonding (HB) between nucleoside pairs on the quality of the results. Due to the large size of these systems, simplified models are commonly used to study them with computational techniques. Therefore it is relevant to know the reliability of results obtained when ignoring both, or one, of these structural features that are actually present in DNA double strands. The optimized geometries of the species involved in models (b) and (c) are provided in Figures 2S and 3S, Supporting Information.

Guanine has been chosen for representing the DNA damage since it is the most easily oxidized of the nucleic acid bases.[36−39] In fact its role as the predominant sink for hole transfer in double-stranded DNA has been justified based on the knowledge that G has the lowest ionization potential among DNA components.[40] This explains why there are numerous chemical agents capable of abstracting one electron from G yielding the corresponding radical cation ($G^{\bullet+}$). Thus this species can be formed by direct SET from G to an oxidant,[41] or

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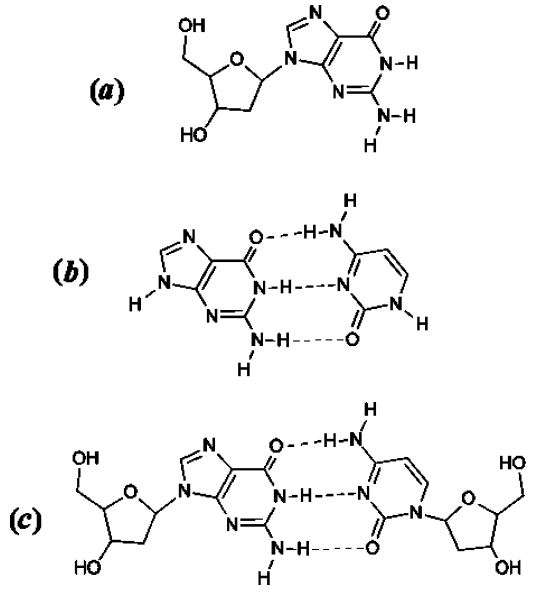

Figure 2. Models used in this work to mimic DNA sites.

also during DNA damage from UV radiation.[42,43] In addition since the *p*Ka value of $G^{\bullet+}$ is 3.9,[36] at physiological pH it rapidly deprotonates yielding a radical. Based on what has been reported in the literature, five main radicals are considered. The first four are those formed at sites 1′, 3′, 4′, and 5′ of the ribose moiety of 2dG (Figure 1).[44−51] The other one is the guanyl radical formed by deprotonation of the purine moiety, which is assumed to take place from site N1[51−56,58] (Figure 1).

Assuming that the repair can take place before or after deprotonation, we have investigated repairing paths involving both kinds of species, the above-mentioned radicals and the radical cation. Two mechanisms of reaction have been considered: the hydrogen transfer (HT) and the single electron transfer (SET). In addition when the DNA damage is caused by reactions with the OH radical, adducts formed by the $^{\bullet}$OH addition to purine sites, mainly at C8, have also been identified.[1,59] Therefore this product has also been considered in this work. Accordingly, the reactions used to investigate the nonenzymatic repairing mechanisms are:

(**a1**) HT from GSH^- to 2dG(-H)C1′$^{\bullet}$
(**a2**) HT from GSH^- to 2dG(-H)C3′$^{\bullet}$
(**a3**) HT from GSH^- to 2dG(-H)C4′$^{\bullet}$
(**a4**) HT from GSH^- to 2dG(-H)C5′$^{\bullet}$
(**a5**) SET from GSH^- to $2dG^{\bullet+}$
(**a6**) SET from GSH^- to 2dG(-H)N1$^{\bullet}$
(**a7**) SET from GSH^- to 2dG(-H)C1′$^{\bullet}$
(**a8**) SET from GSH^- to 2dG(-H)C3′$^{\bullet}$
(**a9**) SET from GSH^- to 2dG(-H)C4′$^{\bullet}$
(**a10**) SET from GSH^- to 2dG(-H)C5′$^{\bullet}$
(**a11**) SET from GSH^- to 2dG8(OH)$^{\bullet}$
(**b5**) SET from GSH^- to $G^{\bullet+}$ −C
(**b6**) SET from GSH^- to G(-H)$^{\bullet}$−C(+H)$^+$
(**c1**) HT from GSH^- to 2dG(-H)C1′$^{\bullet}$−2dC
(**c2**) HT from GSH^- to 2dG(-H)C3′$^{\bullet}$−2dC
(**c3**) HT from GSH^- to 2dG(-H)C4′$^{\bullet}$−2dC
(**c4**) HT from GSH^- to 2dG(-H)C5′$^{\bullet}$−2dC
(**c5**) SET from GSH^- to $2dG^{\bullet+}$ −2dC
(**c6**) SET from GSH^- to 2dG(-H)$^{\bullet}$−2dC(+H)$^+$
(**c7**) SET from GSH^- to 2dG(-H)C1′$^{\bullet}$−2dC
(**c8**) SET from GSH^- to 2dG(-H)C3′$^{\bullet}$−2dC
(**c9**) SET from GSH^- to 2dG(-H)C4′$^{\bullet}$−2dC
(**c10**) SET from GSH^- to 2dG(-H)C5′$^{\bullet}$−2dC
(**a5*α***) SET from GS^{2-} to $2dG^{\bullet+}$
(**a6*α***) SET from GS^{2-} to 2dG(-H)N1$^{\bullet}$
(**a7*α***) SET from GS^{2-} to 2dG(-H)C1′$^{\bullet}$
(**a8*α***) SET from GS^{2-} to 2dG(-H)C3′$^{\bullet}$
(**a9*α***) SET from GS^{2-} to 2dG(-H)C4′$^{\bullet}$
(**a10*α***) SET from GS^{2-} to 2dG(-H)C5′$^{\bullet}$
(**a11*α***) SET from GS^{2-} to 2dG8(OH)$^{\bullet}$
(**b5*α***) SET from GS^{2-} to $G^{\bullet+}$ −C
(**b6*α***) SET from GS^{2-} to G(-H)$^{\bullet}$−C(+H)$^+$
(**c5*α***) SET from GS^{2-} to $2dG^{\bullet+}$−2dC
(**c6*α***) SET from GS^{2-} to 2dG(-H)$^{\bullet}$−2dC(+H)$^+$
(**c7*α***) SET from GS^{2-} to 2dG(-H)C1′$^{\bullet}$−2dC
(**c8*α***) SET from GS^{2-} to 2dG(-H)C3′$^{\bullet}$−2dC
(**c9*α***) SET from GS^{2-} to 2dG(-H)C4′$^{\bullet}$−2dC
(**c10*α***) SET from GS^{2-} to 2dG(-H)C5′$^{\bullet}$−2dC

where the first letter of the acronyms represents the model used in the calculations, and the ***α*** at the end of the acronyms has been used to differentiate the reaction paths involving GS^{2-} from those involving GSH^-. It should be noticed that for restoring the original structure of 2dG through reactions **a6** to **a10** and **a6*α*** to **a10*α*** the SET step must be followed by protonation of the formed 2dG anion.

Single Electron Transfer (SET) Mechanism from GSH^-. SET reactions from glutathione monoanion were all found to be significantly endergonic (Table 1), with ΔG values larger than any inaccuracy inherent to the calculations. Therefore, they are not expected to contribute to the repair of damaged guanosine. In aqueous solution the energetic cost of removing one electron from GSH^- (aqueous ionization energy, ^{Aq}IE) was estimated to be 6.986 eV. It corresponds to the vertical energy difference calculated as

$$^{Aq}\mathrm{IE} = E_{N-1}(g_N)_{aq} - E_N(g_N)_{aq} \quad (6)$$

where $E_N(g_N)_{aq}$ is the energy of the N-electron system calculated at the geometry g_N, optimized in aqueous solution, and $E_{N-1}(g_N)_{aq}$ is the energy of the $(N-1)$ electron system calculated at the geometry g_N.

The $^{Aq}\mathrm{IE}_{(GSH^-)}$ is relatively high, compared to other chemical compounds, such as phenols. It was found to be about 0.7 eV higher than the ^{Aq}IE value of phenol, calculated in the same way and at the same level of theory ($^{Aq}\mathrm{IE}_{(phenol)}$= 6.300 eV). This explains why GSH^- is a poor electron donor, unlikely to repair radical damaged DNA by the SET mechanism. However there are other compounds, such as polyphenols, that may be able of repairing damaged guanosine by SET.

In addition there is a huge variation on the ΔG values of the SET reactions, depending on the species that is accepting the electron. These values range from 13.9 kcal mol, for reaction **a5**, to 82.9 for reaction **a10**. This indicates that electron accepting capacity of the damaged species drastically varies. To quantify this, aqueous electron affinities (^{Aq}EA) have been calculated as:

$$^{Aq}\mathrm{EA} = E_N(g_N)_{aq} - E_{N+1}(g_N)_{aq} \quad (7)$$

where $E_{N+1}(g_N)_{aq}$ is the energy of the $(N+1)$ electron system calculated at the geometry g_N.

The ^{Aq}EA values (Table 2) explain the considerable variations of ΔG. The radicals formed at sites C1′, C3′, C4′, and C5′ have very low ^{Aq}EA (~2 eV). This value remains very similar when improving the model system from 2dG(-H)Cn′$^{\bullet}$

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Table 1. Gibbs Free Energies of Reaction (ΔG, kcal/mol), Gibbs Free Energies of Activation ($\Delta G^{\ddagger}$, kcal/mol), and Rate Constants (k, M^{-1} s^{-1}), at 298.15 K.

	ΔG	$\Delta G^{\ddagger}$	k_{app}	$k_{app} \times mF_{Gl}$ [b]
a1	−10.35	3.85	1.39×10^9	1.33×10^9
a2	−11.69	4.56	1.02×10^9	9.84×10^8
a3	−10.04	4.25	1.21×10^9	1.16×10^9
a4	−10.17	1.88	1.63×10^9	1.57×10^9
a5	13.91	14.48	1.52×10^2	1.46×10^2
a6	26.75	26.88	negligible[a]	negligible
a7	82.31	103.84	negligible	negligible
a8	78.00	86.63	negligible	negligible
a9	78.99	96.19	negligible	negligible
a10	82.94	102.88	negligible	negligible
a11	66.33	89.09	negligible	negligible
b5	16.40	16.62	4.06E+00	3.89E+00
b6	24.56	24.68	negligible	negligible
c1	−11.78	~3.85	$\sim 7.31 \times 10^8$	$\sim 7.02 \times 10^8$
c2	−11.55	~4.56	$\sim 6.16 \times 10^8$	$\sim 5.92 \times 10^8$
c3	−10.35	~4.25	$\sim 6.78 \times 10^8$	$\sim 6.51 \times 10^8$
c4	−11.20	~1.88	$\sim 7.94 \times 10^8$	$\sim 7.62 \times 10^8$
c5	16.68	16.87	2.65	2.55
c6	24.49	24.61	negligible	negligible
c7	81.42	100.63	negligible	negligible
c8	79.39	89.55	negligible	negligible
c9	80.97	101.70	negligible	negligible
c10	82.51	101.46	negligible	negligible
a5*α*	−31.80	13.18	1.36×10^3	5.43×10
a6*α*	−18.96	0.57	9.60×10^9	3.84×10^8
a7*α*	36.59	39.57	negligible	negligible
a8*α*	32.29	32.31	negligible	negligible
a9*α*	33.27	34.67	negligible	negligible
a10*α*	37.23	39.70	negligible	negligible
a11*α*	20.62	22.22	negligible	negligible
b5*α*	−29.32	7.94	9.38×10^6	3.75×10^5
b6*α*	−21.16	2.06	9.18×10^9	3.67×10^8
c5*α*	−29.03	7.79	1.21×10^7	4.85×10^5
c6*α*	−21.23	2.10	9.15×10^9	3.66×10^8
c7*α*	35.70	37.83	negligible	negligible
c8*α*	33.68	33.82	negligible	negligible
c9*α*	35.26	37.81	negligible	negligible
c10*α*	36.79	38.93	negligible	negligible

[a] $k < 1$ M^{-1} s^{-1}. [b] mF_{Gl}= molar fraction of glutathione at pH = 7.4 ($mF_{GSH^-} = 0.96$, $mF_{GS2^-} = 0.04$).

Table 2. Aqueous Electron Affinities (^{Aq}EA, in eV) of the Radical Damaged Species

species	^{Aq}EA	species	^{Aq}EA
$2dG^{\bullet+}$	5.788	2dG(-H)C1′•−2dC	2.072
$G^{\bullet+}$−C	5.378	2dG(-H)C3′•−2dC	1.836
$2dG^{\bullet+}$−2dC	5.368	2dG(-H)C4′•−2dC	2.148
2dG(-H)C1′•	2.081	2dG(-H)C5′•−2dC	1.990
2dG(-H)C3′•	1.844	2dG(-H)N1•	4.816
2dG(-H)C4′•	2.172	G(-H)•−C(+H)$^+$	4.996
2dG(-H)C5′•	1.992	2dG(-H)•−2dC(+H)$^+$	5.000
		2dG8(OH)•	3.165

to 2dG(-H)Cn′•−2dC. This is a logical finding since the HB between the bases pair is far enough from the sugar fragment. Therefore its influence on the chemical processes that might involve the ribose moiety is only minor. The low values of ^{Aq}EA for radicals C1′, C3′, C4′, and C5′ explain the very large endergonicity of reactions **a7** to **a10**, and **c7** to **c10** (Table 1). This also means that radicals C1′, C3′, C4′, and C5′ should be repaired by a reaction mechanism other than SET, unless it is coupled with a proton transfer. In the latter case the yielded products would be identical to those formed via HT.

The highest ^{Aq}EA corresponds to the radical cation, as expected based on charge considerations. In this case the differences in the ^{Aq}EA values are larger when different models are used. Our best value, that corresponding to $2dG^{\bullet+}$−2dC is 5.368 eV, which supports the high electrophilicity of this species. Therefore it is expected to be repaired by a wide variety of chemical compounds through SET. The finding that GSH^- is not able to do so, is explained by the high ^{Aq}IE of the latter.

The adduct 2dG8(OH)• and the radical at site N1 have values of ^{Aq}EA between those of the radical cation and the C-centered radicals. However the electron acceptor capability of 2dG8(OH)• is predicted to be significantly lower than that of radical N1. Accordingly the possibility of repairing such species by SET is predicted to be highly dependent on the electron donor capacity of the repairing compound. In addition 2dG8(OH)• is not expected to be easily repaired through this mechanism.

For the particular case of GSH^-, the rate constants of the SET reactions with most of the studied species are negligible. The only exceptions are the reactions involving direct repairing of the radical cation (**a5**, **b5**, and **c5**). However, even these reactions are too slow to represent efficient chemical repairing process (Table 1). Accordingly, it can be concluded that GSH^- is not able of repairing damaged guanosine sites in DNA by electron transfer.

Single Electron Transfer (SET) Mechanism from GS^{2-}. Even though the population of GS^{2-}, at physiological pH, is considerably lower than that of GSH^-, it is expected to be a much better electron donor. In fact the $^{Aq}IE(GS^{2-})$ was estimated to be 4.575 eV. This value is 2.411 eV lower than that of GSH^- and 0.285 eV lower than that of the phenoxide anion (4.860 eV). In this case most of the SET reactions were found to be largely exergonic (Table 1). The only exceptions are those involving radicals C1′, C3′, C4′, and C5′ (**7*α*** to **10*α***) and the radical adduct 2dG8(OH)• (**11*α***). Since GS^{2-} has been found to have an excellent electron donor capability these species are not likely to be chemically repaired via SET by other compounds either.

Despite of the large exergonicity of reactions **a5*α***, **b5*α*** and **c5*α***, their barriers are significantly high (Table 1). This apparently contra-intuitive behavior can be explained based on the fact that these reactions are in the inverted region of the Markus parabola ($\Delta G < -\lambda$),[60] as shown in Figure 3. The most exergonic process (**a5*α***, with $\Delta G = -31.8$ kcal/mol) has the most negative difference ($\Delta G - (-\lambda) = -22.3$ kcal/mol), which causes the largest increase in $\Delta G^{\ddagger}$. For reactions **b5*α*** and **c5*α*** the ΔG values are lower than $-\lambda$ by about 18 kcal/mol, which is also a large difference and lead to relative high values of $\Delta G^{\ddagger}$ (Figure 3). Therefore the most exergonic processes do not correspond to the fastest reactions. In fact reaction **a5*α*** is predicted to be rather slow, with a rate constant in the order 10^1 M^{-1} s^{-1}, considering the molar fraction of GS^{2-}.

Reactions **a6*α***, **b6*α***, and **c6*α*** were found to have the highest rate constants for SET from GS^{2-}. They correspond to the SET repairing of the radical formed at site N1 and are in the order of 10^8 M^{-1} s^{-1}, after considering the relative population of glutathione dianion. However the molar fraction of radical N1

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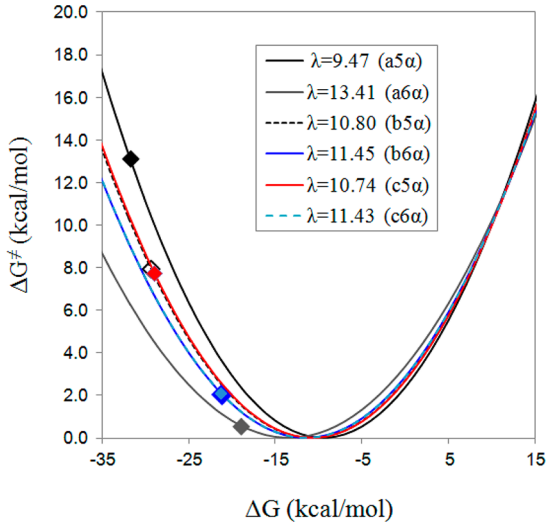

Figure 3. Gibbs free energy of activation, $\Delta G^{\ddagger}$, vs Gibbs free energy of reaction, ΔG. The rhombs correspond to the pair of values for the studied cases.

has not been taken into account at this point. Therefore even though reactions **a6*α***, **b6*α***, and **c6*α*** are fast enough to represent significant chemical repairing, their relative importance cannot be analyzed yet. This aspect will be addressed in the section regarding the overall repairing process.

Hydrogen Transfer (HT) Mechanism. The HT from GSH^- has been modeled with the H being transferred from the thiol group, since it has been previously demonstrated that for the HT reactions of glutathione with free radicals, with the exception of $^{\bullet}OH$, this is the only important path.[19] In addition the HT repairing mechanism is relevant for the radical species, but not for the radical cation or the adduct. This mechanism is only considered for the radicals formed at sites C1′, C3′, C4′, and C5′ for two reasons. The first one is that they are expected to be the most abundant C-centered radicals.[44−51] The second one is that, because of the localization of site N1 in double stranded DNA, a HT reaction involving this site would be highly hindered.

The HT repairing of free guanosine, reactions **a1** to **a4**, which also represents single stranded DNA, were found to be exergonic by 10.35, 11.69, 10.04, and 10.17 kcal/mol, respectively (Table 1). When this process is studied using the most complex model (**c**), the pair guanosine-cytidine, the Gibbs free energy values remain very similar. This indicates that the results obtained when using model **a** instead of model **c**, would represent a good aproximation to the efficiency of glutathione for repairing radical damaged DNA via HT. This is important since due to the large size of both guanosine and glutathione, calculating the transition state (TS) of the most complete model (**c**) becomes a very expensive computational task, without compromising the accuracy of the calculations by decreasing the level of theory. Therefore we have optimized the TS of reactions **a1** to **a4** but not those of reactions **c1** to **c4**. The Gibbs free energies of activation for the latter have been then assumed to be similar to those of the corresponding reactions **a**. Moreover based on the ΔG values, it is expected that $\Delta G^{\ddagger}$(**c1**) ≈ $\Delta G^{\ddagger}$(**a1**), $\Delta G^{\ddagger}$(**c2**) ≈ $\Delta G^{\ddagger}$(**a2**), $\Delta G^{\ddagger}$(**c3**) ≈ $\Delta G^{\ddagger}$(**a3**), and $\Delta G^{\ddagger}$(**c4**) ≈ $\Delta G\ddagger$(**a4**); thus the rate constant of **a1** should be close to that of **c1**, and the same is expected for the reaction pairs **a2-c2**, **a3-c3**, and **a4-c4**.

The justification of this assumption is that even though the relationship established by Eyring equation (1) is between the rate constant and $\Delta G^{\ddagger}$, the Bell−Evans−Polanyi principle also establishes a linear relationship between the activation energy (E_a) and enthalpy of reaction (ΔH) within a series of closely related chemical processes

$$E_a = A + B\Delta H \tag{8}$$

which is the case for reactions pairs **a1-c1**, **a2-c2**, **a3-c3**, and **a4-c4**. Therefore, the associated entropy change is expected to be similar for each of these pairs, and the Bell−Evans−Polanyi principle applies.

The optimized structure of the transition states for reactions **a1** to **a4** are shown in Figure 4. They all exhibit a HB

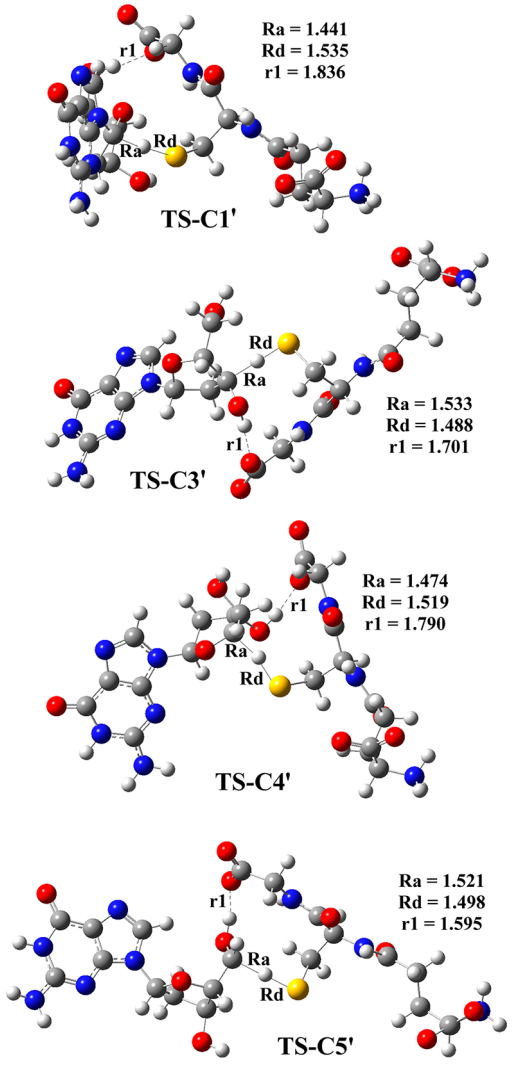

Figure 4. Optimized geometry of the HT transition states.

interaction between the H in the OH group at site 5′ of guanosine and one of the O atoms in the terminal carboxylate moiety of GSH^-. For TS-C5′ the interaction distance was found to be equal to 1.595 Å, which corresponds to an unusually short, therefore strong, HB. In order to characterize this interactions a Bader topological analysis[61] of the M05-2X/6-31+G(d,p) wave functions was performed and a bond critical point (BCP) was found. The values of its electronic charge density, $\rho(r)$, and Laplacian, $\nabla^2\rho(r)$, are 0.0560 and −0.0387,

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respectively, confirming the unusual strength of this HB. Just to put these values in context, for the water dimer in gas phase $\rho(r)$ = 0.0244 and $\nabla^2\rho(r)$ = −0.0213; for the strongest interaction between water and formic acid $\rho(r)$ = 0.0376 and $\nabla^2\rho(r)$ = −0.0271, and for the interaction between hydroxyperoxyl radical and water $\rho(r)$ = 0.0391 and $\nabla^2\rho(r)$ = −0.0286.[62] Therefore, based on the values of the $\rho(r)$ and $\nabla^2\rho(r)$ descriptors, the HB interaction in TS-C5′ is expected to be stronger than those in these three complexes. For transition states TS-C1′, TS-C3′, and TS-C4′, on the other hand, the HB is weaker than for TS-C5′, yet very strong. For TS-C1′ the interaction distance, d(HB), was found to be equal to 1.836 Å, $\rho(r)$ = 0.0316 and $\nabla^2\rho(r)$ = −0.0245, for TS-C3′ d(HB) = 1.701 Å, $\rho(r)$ = 0.0416, and $\nabla^2\rho(r)$ = −0.0333, and in the case of TS-C4′, d(HB) = 1.790 Å, $\rho(r)$ = 0.0345, and $\nabla^2\rho(r)$ = −0.0275. Therefore, among these transition states, TS-C1′ is the one with the least strong HB, and even in this case its strength is comparable to that of the strongest interaction between water and formic acid, and higher than that of the water dimer.

Such strong stabilizing interaction constitutes an additional factor contributing to the low barrier of reactions **a1** to **a4**, and is particularly important for **a4** (TS-C5′) which was found to have $\Delta G^{\ddagger}$ equal to only 1.88 kcal/mol. A similar situation was reported for the reaction of GSH^- with the $^{\bullet}OOH$ radical.[19] This characteristic arrangement might also be important for other related biological systems where such kind of interaction is possible.

The rate constants of reactions **a1** to **a4** ($\sim 10^9$ M^{-1} s^{-1}) and **c1** to **c4** ($\sim 10^8$ M^{-1} s^{-1}) are predicted to be diffusion-controlled. The difference in their values (Table 1) arises from the diffusion rates which are k_D(**a1** to **a4**) = 1.7 × 10^9 M^{-1} s^{-1}, and k_D(**c1** to **c4**) = 8.2 × 10^8 M^{-1} s^{-1}. As the values in Table 1 show, in both cases the apparent rate constant are very close to these limits. The values of the rate constants for reactions **c1** to **c4** are in line with previous reports for the direct reactions between different thiols and various C-centered radicals, which are in the order of 10^7−10^9 $M^{-1}s^{-1}$.[63−65] They are also in agreement with the rate constants reported by Baker et al.[66] for the glutathione repair of a large series of C-centered radicals ($\sim 10^9$ M^{-1} s^{-1}) including ribose (2.1 × 10^9 M^{-1} s^{-1}), deoxyribose (2.5 × 10^9 M^{-1} s^{-1}), and ribose-5-phosphate (1.3 × 10^9 M^{-1} s^{-1}). All of these data indicates that the direct HT reaction between thiol sites and C-centered radicals are actually quite fast. Moreover, according to the results from the present work the HT reactions from GSH^- to radicals C1′, C3′, C4′, and C5′ are fast enough to be predicted as efficient pathways for repairing damaged DNA. Possible competing reactions are discussed in the next section.

Overall Repairing Process. We have chosen our best model (**c**) to analyze the relative importance of the different repairing processes. This is a difficult analysis, as expected based on the complexity of the studied system. There are several competing reactions and most of the reacting species are involved in acid−base, or tautomeric, equilibriums. Accordingly the molar fractions (mF) of all of them should be considered, while for the sake of simplicity in Table 1 only the values of mF for glutathione were included. However, at this point it is imperative to consider also the mF of the radical cation and its deprotonated forms. Otherwise it would be impossible to properly assess the relative importance of the competing reaction paths on the repairing process.

The overall rate coefficient has been calculated as the sum of the rate constants of each path involving the species relevant to model **c**

$$k_{\text{overall}}^{\text{pH7.4}} = \sum_{i=1}^{8} k_{(ci)}^{\text{pH7.4}} + \sum_{j=4}^{8} k_{(cj\alpha)}^{\text{pH7.4}} \tag{9}$$

where

$$k_{(c1)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C1'^{\bullet}-2dC)}k_{\text{app(c1)}} \tag{10}$$

$$k_{(c2)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C3'^{\bullet}-2dC)}k_{\text{app(c2)}} \tag{11}$$

$$k_{(c3)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C4'^{\bullet}-2dC)}k_{\text{app(c3)}} \tag{12}$$

$$k_{(c4)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C5'^{\bullet}-2dC)}k_{\text{app(c4)}} \tag{13}$$

$$k_{(c5)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG^{\bullet+}-2dC)}k_{\text{app(c5)}} \tag{14}$$

$$k_{(c6)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)^{\bullet}-2dC(+H)^{+})}k_{\text{app(c6)}} \tag{15}$$

$$k_{(c7)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C1'^{\bullet}-2dC)}k_{\text{app(c7)}} \tag{16}$$

$$k_{(c8)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C3'^{\bullet}-2dC)}k_{\text{app(c8)}} \tag{17}$$

$$k_{(c9)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C4'^{\bullet}-2dC)}k_{\text{app(c9)}} \tag{18}$$

$$k_{(c10)}^{\text{pH7.4}} = \text{mF}_{(GSH^-)}\text{mF}_{(2dG(-H)C5'^{\bullet}-2dC)}k_{\text{app(c10)}} \tag{19}$$

$$k_{(c5\alpha)}^{\text{pH7.4}} = \text{mF}_{(GS^{2-})}\text{mF}_{(2dG^{\bullet+}-2dC)}k_{\text{app(c5}\alpha\text{)}} \tag{20}$$

$$k_{(c6\alpha)}^{\text{pH7.4}} = \text{mF}_{(GS^{2-})}\text{mF}_{(2dG(-H)^{\bullet}-2dC(+H)^{+})}k_{\text{app(c6}\alpha\text{)}} \tag{21}$$

$$k_{(c7\alpha)}^{\text{pH7.4}} = \text{mF}_{(GS^{2-})}\text{mF}_{(2dG(-H)C1'^{\bullet}-2dC)}k_{\text{app(c7}\alpha\text{)}} \tag{22}$$

$$k_{(c8\alpha)}^{\text{pH7.4}} = \text{mF}_{(GS^{2-})}\text{mF}_{(2dG(-H)C3'^{\bullet}-2dC)}k_{\text{app(c8}\alpha\text{)}} \tag{23}$$

$$k_{(c9\alpha)}^{\text{pH7.4}} = \text{mF}_{(GS^{2-})}\text{mF}_{(2dG(-H)C4'^{\bullet}-2dC)}k_{\text{app(c9}\alpha\text{)}} \tag{24}$$

$$k_{(c10\alpha)}^{\text{pH7.4}} = \text{mF}_{(GS^{2-})}\text{mF}_{(2dG(-H)C5'^{\bullet}-2dC)}k_{\text{app(c10}\alpha\text{)}} \tag{25}$$

The values of the rate constants for each reaction path, as well as the overall rate coefficient, are provided in Table 3. As the values in this table show the rate constants of the reaction paths involving $2dG^{\bullet+}$-2dC and $2dG(-H)^{\bullet}$-$2dC(+H)^+$ become drastically lower once the mF of these species are included in the calculation. On the contrary the decrease of the rate constant for the reactions involving C-centered radicals is relatively small (compared to those in the last column of Table 1) since their mF are higher.

This has major implications for the analysis of the repairing process. Ignoring the mF of the different species to be repaired (Table 1) might lead to miss-conclude that reaction paths **c1** to **c4** are almost equally important than **c6α** (Table 1). However, under the actual conditions at which the DNA repairing would occur, i.e., with the pH buffered at 7.4, paths **c1** to **c4** are the most important ones (Table 3). This is the case when the repair takes place after deprotonation of $2dG^{\bullet+}$-2dC. Therefore glutathione is able of efficiently repairing radical damaged double stranded DNA by HT, at diffusion-limited rates ($\sim 10^7$

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Table 3. Rate Constants for Every Reaction Path and Overall Rate Coefficient (at pH 7.4, in M^{-1} s^{-1}), and Branching Ratios (Γ)

	$k^{pH7.4}$[b]
$k^{pH7.4}_{(c1)}$	4.30×10^7
$k^{pH7.4}_{(c2)}$	5.31×10^7
$k^{pH7.4}_{(c3)}$	4.46×10^8
$k^{pH7.4}_{(c4)}$	1.25×10^8
$k^{pH7.4}_{(c5)}$	negligible[a]
$k^{pH7.4}_{(c6)}$	negligible
$k^{pH7.4}_{(c7)}$	negligible
$k^{pH7.4}_{(c8)}$	negligible
$k^{pH7.4}_{(c9)}$	negligible
$k^{pH7.4}_{(c10)}$	negligible
$k^{pH7.4}_{(c5\alpha)}$	3.66×10
$k^{pH7.4}_{(c6\alpha)}$	1.81×10^3
$k^{pH7.4}_{(c7\alpha)}$	negligible
$k^{pH7.4}_{(c8\alpha)}$	negligible
$k^{pH7.4}_{(c9\alpha)}$	negligible
$k^{pH7.4}_{(c10\alpha)}$	negligible
$k^{pH7.4}_{overall}$	6.24×10^8

[a] $k < 1$ M^{-1} s^{-1}. [b] $mF_{2dG(-H)C1'\bullet\text{-}2dC} = 0.0613$, $mF_{2dG(-H)C3'\bullet\text{-}2dC} = 0.0898$, $mF_{2dG(-H)C4'\bullet\text{-}2dC} = 0.6849$, $mF_{2dG(-H)C5'\bullet\text{-}2dC} = 0.1638$, $mF_{2dG\bullet+\text{-}2dC} = 0.0001$, and $mF_{2dG(-H)\bullet\text{-}2dC(+H)+} = 0.0001$.

to 10^8 M^{-1} s^{-1}, Table 3). On the other hand, if the repair takes place before deprotonation, i.e., when only $2dG^{\bullet+}$-2dC is present, the repairing ability of glutathione is not significant ($\sim 4 \times 10^1$ M^{-1} s^{-1}). This would correspond to SET from GS^{2-} to $2dG^{\bullet+}$-2dC (reaction **c5α**). In addition the large difference between the rate constants of reactions **c1** to **c4** and **c5α** indicates that glutathione would repair damaged DNA in a particularly efficient way when the C-centered radicals are formed. This is proposed to be a key chemical repairing process since these radicals represent the most important type of DNA damage (strand breaks).[67−70]

Repairing of $2dG(-H)^{\bullet}$-$2dC(+H)^{+}$ by glutathione seems to be irrelevant to the overall repairing process of DNA because of its very low population. Even when reaction **c6α** is intrinsically very fast (Table 1) when the mF of $2dG(-H)^{\bullet}$-$2dC(+H)^{+}$ is taken into account it becomes 4 orders of magnitude lower (Table 3). This means that under physiological conditions the contributions of path **c6α** to the overall repairing process would be negligible. Analyzing the gathered data altogether it can be concluded that the main reaction path involved in the fast nonenzymatic repair of radical damaged DNA by glutathione is the HT from GSH^{-} (paths **c1** to **c4**).

Moreover, since their rate constants actually correspond to the diffusion-limited regime, if there is a molecule of glutathione in the vicinity of radical damaged DNA when the oxidative damage occurs, it would be able of repairing the damage before replication, and before possible further reaction with O_2. This is actually a likely event because the intracellular concentration of glutathione in humans is relatively high (~0.1−10 mM).[71] Therefore glutathione is predicted to be very efficient for preventing permanent DNA damage and the formation of peroxyl radical sites via HT.

At this point it seems worthwhile to consider in more detail competing reactions that may affect the efficiency of GSH^{-} for repairing radical damaged DNA. It has been reported that while the extent of repair by glutathione is ~100% for alcohol-derived C-centered radicals, it is only 30% to 60% for carbohydrates.[66] This decreasing in the repair efficiency of glutathione has been interpreted as an evidence of the occurrence of competing reactions. As mentioned before, reactions with O_2 are possible competing process. However, it has been reported that the rate constants for the reactions of O_2 with the target radical can be influenced by the viscosity of the environment, as well as by the O_2 solubility. Thus, while in aqueous solution it is $\sim 2 \times 10^9$ M^{-1} s^{-1} in biological systems (experiments with bacteria) it is reduced to $\sim 5 \times 10^8$ $M^{-1}s^{-1}$.[72] Since O_2 fixation and chemical repair are competing processes, a faster repair would also result in a shorter lifetime of the O_2-dependent damage. von Sonntag[72] has analyzed the first half-lives ($t_{1/2}$) of the decay of the O_2-dependent damage to various strains of *E. coli* K 40, and found that compared to the wild-type, other strains containing less GSH present higher values of $t_{1/2}$. Therefore this comparison supports the protective effects of glutathione. However it should be kept in mind that protection in this context does not mean "full" protection, i.e. complete elimination of O_2 fixation, but a reduction of it. Moreover, the relative importance of these two competing processes would also be influenced by the local concentration of both, GSH and O_2, in the vicinity of the reaction site.

Another possible competing process is the reverse reaction of the HT repair by thiols, i.e., the H abstraction from a CH site by a thiyl radical. The rate constant of the reverse process has been reported to be $\sim 10^3$−10^4 M^{-1} s^{-1},[63,73] i.e., the reverse reaction is four to five orders of magnitude slower than the repair via HT by the thiol. This means that the net transformation corresponds to the repair of C-centered radicals by thiols. In addition to an equilibrium constant in the order of 10^4−10^5 for the HT reaction between glutathione and radical DNA, the products yield by such reaction are susceptible to be rapidly consumed by further reactions with other species in the environment. For example it has been proposed that thiyl radicals can be deactivated by ascorbate,[72] or even by oxygen.[63] Thus thiyl-related DNA damage are expected to be much less important than the thiol-related repair.

The reversibility of the reaction S-H + C-centered radical depends on the relative strength of the two bonds S-H and C-H. While the bond dissociation energy (BDE) of S-H is almost the same in any aliphatic thiol, ~87.5 kcal/mol,[74] the situation is very different for C-H bonds, which have BDE values that can vary from 87.7 kcal/mol for allyl C-H to 101.1 kcal/mol for primary C-H. The first case corresponds to an almost isothermic reaction, and the second one to an exothermic reaction with $\Delta H = -13.6$ kcal/mol, i.e., with negligible reversibility at room temperature. Because of the nature of C-H bonds in carbohydrates, and specifically in deoxyribose, we should expect BDE about 93.9 kcal/mol (similar to that of 2-propanol) that corresponds to $\Delta H = -6.5$ kcal/mol. This value is in line with a direct rate constant five orders larger than the reverse one, i.e., the equilibrium constant for the direct process would be $\sim 10^5$ which means that the reversible reaction is of minor, or even negligible, importance. This is in perfect agreement with the proposals by von Sonntag that "the rate of H-donation by the thiol is four to five orders of magnitude faster than the rate of H-abstraction by the thiol, that is, these data are not in contradiction with the general conclusion that thiols do reduce C-centered free-radicals and that the reverse reaction is usually not observed".[75]

Comparing our data with experimental kinetic and thermodynamic data available we can safely conclude that the error in our forward rate constants is negligible, but the

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calculated ΔG can be overestimated by up to 2.8 kcal/mol, which means that the reverse rate constant would be underestimated. However, even for ΔG values 2.8 kcal/mol higher the studied processes would remain practically irreversible and our conclusions still valid.

According to the above discussion it becomes evident that the study of radical DNA chemical repair by glutathione, or any other free radical scavenger, in biological systems is a very complex phenomenon. Based on the previously gathered, and above-discussed, data as well as on the results from this work, we are convinced that glutathione has the potential of effectively perform such repair via HT from its thiol moiety. However we are not ruling out the possibility that competing process might lead to reduced values of the apparent rate constants ($\sim 10^6$ M^{-1} s^{-1}).[72] Moreover it would be in line with the extent of repair (30%−60%) for carbohydrates reported by Baker et al.[66]

Assessing the Accuracy of the Used Methodology. In order to evaluate the reliability of the data provided here for the first time, different tests have been conducted including comparisons with experimental data and with higher levels of theory. To that purpose two additional reactions have been considered. The first one is that between 2′-deoxyuridin-1′-yl radical (Figure 5, RM1) and 2-mercaptoethanol (2ME). It has been chosen because there are experimental kinetic data available for it. Emanuel et al.[76] and Chatgilialoglu et al.[77] reported rate constant values equal to $(2.3 \pm 0.5) \times 10^6$ M^{-1} s^{-1} and $(2.9 \pm 0.4) \times 10^6$ M^{-1} s^{-1}, respectively. The second one is the reaction between methanethiol (MT) and a smaller radical that retains the main chemical features of the radical site in RM1 (Figure 5, RM2). This system has been chosen because its reduced size allows performing high level calculations at a reasonable computational cost.

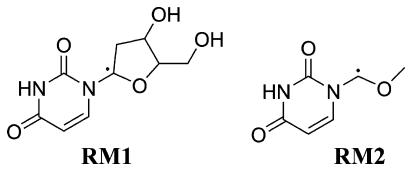

Figure 5. Structures of radicals RM1 and RM2.

We have analyzed the effect of increasing the basis set from 6 to 31+G(d,p) to 6−311++G(d,p) in both cases. We have also performed CCSD(T)/ 6-311++G(d,p), and CBS-QB3 calculations at M05−2X geometries for the smaller system. The effect of increasing the basis set was found to be minimal. The maximum differences in ΔG and $\Delta G^{\ddagger}$ were found to be 0.13 and 0.17 kcal/mol respectively (Table 1S). Regarding the rate constants, it becomes 1.03 times higher for the RM1 + 2ME reaction and 1.01 times lower for RM2 + MT reaction when the basis set is increased (Table 4). The calculated rate constants were found to be in excellent agreement with the experimental values. In fact the value calculated at M05-2X/6-31+G(d,p) level is within the error range reported for the experimental measurements, which supports the reliability of the data reported in this work.

It should be noted that the HT reaction from 2ME to RM1 was found to be significantly slower than that from GSH^- to 2dG(-H)C1′$^{\bullet}$ (1.33×10^9 M^{-1} s^{-1}, Table 1). This indicates that the radical formed at C1′ site in 2dG is more reactive than 2′-deoxyuridin toward thiols, i.e., it is easier to repair by this kind of compounds via HT. This difference in reactivity is reflected in the structures of the transition states (Figures 4 and 4S). The rate constants decrease for the three similar reactions in the following order RM2+MT > 2dG(-H)C1′$^{\bullet}$ + GSH^- > RM1 + 2ME, and the earliness of the transition states decreases in the same order. To quantify how early the transition states are the L parameter was calculated according to

$$L = \frac{\delta r(SH)}{\delta r(CH)} \tag{26}$$

where δr(SH) represents the difference in the breaking bond distance (transition state−reactant), and δr(CH) represents the difference in the forming bond distance (transition state − product). The values of the L parameters were found to be 0.47, 0.51, and 0.55 for the reactions RM2 + MT, 2dG(-H)C1′$^{\bullet}$ + GSH^-, and RM1 + 2ME, respectively. Thus, these values support the reactivity trend.

Table 4. Rate Constants (M^{-1} s^{-1}), at 298.15 K, for Reactions RM1 + 2ME and RM2 + MT.

	RM1 + 2ME	RM2 + MT
M05-2X/6-31+G(d,p)	2.65×10^6	1.55×10^9
M05-2X/6-311++G(d,p)	2.74×10^6	1.41×10^9
CCSD(T)/6-311++G(d,p)		2.09×10^9
CBS-QB3		2.09×10^9
exp.[76]	$(2.3 \pm 0.5) \times 10^6$	
exp.[77]	$(2.9 \pm 0.4) \times 10^6$	

Comparing the M05-2X/6-31+G(d,p) data for the reaction RM2 + MT with those obtained when using CCSD(T) and CBS-QB3 it was found that the ΔG value is more negative by 0.38 and 2.00 kcal/mol, respectively, while the $\Delta G\ddagger$ is about 1.2 kcal/mol higher than those obtained with both CCSD(T) and CBS-QB3. The rate constants obtained with them are identical, and equal to 2.09×10^9 M^{-1} s^{-1}, because both are within the diffusion-limited regime. The value obtained with the methodology used in this work is only 1.35 times lower than that. All of these comparisons indicate that our results are reliable for both thermochemical and kinetic data, especially for the later. Moreover, since the rate constant obtained using M05-2X/6-31+G(d,p) is slightly lower than those arising from using CCSD(T) and CBS-QB3 it seems that any error inherent to the used methodology would represent a slight underestimation of the rate constant. Therefore the highly efficient repairing process of damaged DNA by glutathione, via HT, is confirmed. It should be noted, however, that the data reported in this work correspond to individual reactions, and do not include the possible effects of the competing processes described in the previous section. Thus, the results presented here should be interpreted accordingly. It seems important to call attention to the fact that this is one of the strengths of theoretical calculation, i.e., the possibility of independently studying chemical process that takes place concurrently in real systems. This would, hopefully, allow detailed comparisons and further interpretations of experimental evidence.

CONCLUSIONS

Different mechanisms for the chemical repair of radical damaged DNA by glutathione in aqueous solution, at physiological pH, have been modeled. The results support the efficiency of glutathione for nonenzymatic repair of radical damaged DNA. For this particular compound SET processes are not expected to contribute to such important activity, which

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was identified to take place almost exclusively by H transfer from the thiol group.

This is the first theoretical study establishing that HT is the prevalent mechanism involved in the reaction of GSH^- with radical damaged DNA based on the rate constants of the H-atom and electron transfer processes. This subject certainly deserves further attention since while potential repairing species are not always good electron donors, they can still be good H donors. The HT reaction between GSH^- and damaged guanosine sites is proposed to be very fast, with rate constants $\sim 10^7-10^8$ M^{-1} s^{-1}. In fact they are close to the diffusion-limited regime, which indicates the great capacity of glutathione for preventing permanent DNA modification and thus for ameliorating the effects of oxidative stress. However competing reactions may reduce such values to some extent. This point also deserves further investigation.

Including the effects of physiological pH and models complex enough, including the ribose moiety and the H bonding between base pairs, is highly recommended to obtain results that are relevant to the actual conditions where the repairing processes would take place.

■ ASSOCIATED CONTENT

Supporting Information

Distribution diagram of glutathione in aqueous solution. Fully optimized geometries of G−C, $G^{\bullet+}$−C, $G(-H)^{\bullet}$−$C(+H)^+$, 2dG−2dC, $2dG^{\bullet+}$−2dC, and $2dG(-H)^{\bullet}$−$2dC(+H)^+$. Gibbs free energies of reaction (ΔG), Gibbs free energies of activation ($\Delta G^{\ddagger}$) for reactions RM1 + 2ME and RM2 + MT. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jidaboy@unam.mx; agal@xanum.uam.mx.

Notes

The authors declare no competing financial interest.

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