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Modeling the activity of glutathione as a hydroxyl radical scavenger considering its neutral non-zwitterionic form

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Abstract Glutathione is an immensely important antioxidant, particularly in the central nervous system. The scavenging mechanism of glutathione towards the OH radical was studied theoretically, considering its neutral, non-zwitterionic form relevant to acidic media. Gibbs free barrier and released energies involved in hydrogen abstraction from the different sites of glutathione by an OH radical were studied at the B3LYP/6-31G(d,p), B3LYP/AUG-cc-pVDZ, M06/AUG-cc-pVDZ, M06-2X/AUG-cc-pVDZ levels of density functional theory. Solvation in bulk aqueous media was also studied at all these levels of theory employing the polarizable continuum model. Our study shows that a hydroxyl radical can abstract a hydrogen atom easily from glutathione. Thus, glutathione is shown to be an efficient scavenger of OH radicals, which is in agreement with the results of previous studies.

Keywords Glutathione · Hydroxyl radical · DFT

Introduction

Glutathione is a tripeptide chain (γ-L-glutamyl-L-cysteinyl-glycine, GSH) made up of glutamic acid, cysteine and glycine [1, 2]. It is an immensely important antioxidant in the central nervous system and plays vital roles in cell metabolism [1–9]. Certain free radicals known as reactive oxygen species (ROS) and reactive nitrogen oxide species (RNOS) cause oxidative and nitrative damage to DNA that leads to mutation and several diseases including cancer [10–12]. Glutathione scavenges ROS and RNOS, including the hydroxyl radical, efficiently from biological media [6–8]. Deficiency of glutathione in cellular environments leads to neurological disorders such as Parkinson's disease, Alzheimer's disease, schizophrenia, autism, etc. [4, 9]. Common anti-oxidants that occur in biological systems or can be obtained from external sources include β-carotene, ascorbic acid (vitamin C), *N*-acetylcysteine (NAC, a glutathione precursor), α-tocopherol (vitamin E), urocanic acid, uric acid, curcumin, etc. [13–17]. These agents protect biological systems from free radicals by transforming them into non-damaging forms while they themselves are modified [13–17]. In such a reaction, two glutathione molecules are converted to glutathione disulfide [18–21] from which glutathione can be regenerated by a nicotinamide adenine dinucleotide dependent reaction [18–26].

The structure of glutathione in aqueous solution and solid state has been studied experimentally [27–31]. At physiological pH, glutathione exists in an anionic form—the glycine moiety being deprotonated while the glutamic acid moiety is in the zwitterionic form [31]. Intramolecular hydrogen transfers involving the sulfur and α-carbon atoms of glutathione have been studied by Zhao et al. [32, 33] and Rauk et al. [34]. Theoretical studies have proved very useful in explaining experimental observations on biomolecules and to establish structure–activity correlations in different contexts [34–40]. Lampela et al. [35] studied the stability of conformers of several charge states of glutathione in aqueous media using classical molecular dynamics simulation. Fiser et al. [36] studied the homolytic bond dissociation energies of hydrogen removal from different parts of glutathione. In this latter study [36], the glutathione molecule was split into three overlapping fragments in both gas phase and aqueous media; further, in gas phase, neutral and non-zwiterionic forms of overlapping fragments of glutathione were taken while in aqueous media, the

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fragment corresponding to glutamic acid was taken to be in zwitterionic form while that corresponding to glycine was taken to be in anionic form. The authors found the OH (COOH) and NH (NH_3^+) bonds of the glutamic acid fragment to be weakest in gas phase and aqueous media, with the corresponding bond dissociation energies calculated at the B3LYP/6-31G(d) level being about 4.2 and 3.5 kcal mol^{-1} less, respectively, than that of the SH bond. Galano and Alvarez-Idaboy [37] studied the scavenging reactions of glutathione towards different ROS, considering its anionic, zwitterionic form in aqueous media. Galano and Alvarez-Idaboy [37] studied abstraction of hydrogen atoms only from carbon and sulfur atoms, and even where there are two hydrogen atoms attached to a carbon atom, they considered abstraction of only one of the two, which appears to be a justified approximation. They found Gibbs barrier energies for hydrogen abstraction by an OH radical from different carbon atoms at the M05-2X/6-311+G(d,p) level not to exceed 5.62 kcal mol^{-1}, while the hydrogen abstraction from the SH group was found to be barrierless.

Glutathione is highly active around neutral pH while its activity usually decreases with decreasing pH [41]. However, there are some significant exceptions to this. For example, it has been reported that a bacterium called *Lactobacillus salivarius* grows at low pH, where glutathione acts as both a nutrient and a protecting agent [42]. Another important situation is that synaptic density is found to be high in certain regions of the brain where acid-sensing ion channel 1 is localized and contributes to the sense of fear [43]. Thus, glutathione does encounter acidic media and it is desirable to study its structure and behavior in such media. In particular, in acidic medium, the structure of glutathione is not expected to be the same as at physiological pH, i.e., having the well known anionic, zwitterionic form investigated earlier as discussed above.

In the present study, we considered the neutral, non-zwitterionic form of glutathione, and studied abstraction of all of its hydrogen atoms by a hydroxyl radical. We also studied the ability of this form of glutathione to act as an anti-oxidant by way of single electron transfer from itself to a hydroxyl radical. The antioxidant nature of the molecule is quite likely to be due to its structure, i.e., a particular combination of the three specific amino acids mentioned above, and we hoped the study would reveal its neutral non-zwitterionic form also. In other words, the structural sensitivity of the molecule to the environment as discussed above may not be fundamental to its antioxidant property. It should be noted that, to the best of our knowledge, reactions relating to the antioxidant nature of the neutral, non-zwitterionic form of glutathione have not been studied previously.

Computational details

A conformational analysis of the normal canonical form of each of the three amino acids (glycine, cysteine and glutamic acid) was performed at the B3LYP/6-31G(d,p) level of theory [44, 45] and thus the most stable conformer of each of the three amino acids in gas phase was obtained. The most stable conformers of two of the amino acids (glycine and cysteine) were joined subsequently by a peptide bond, and various conformers of the dipeptide thus obtained were optimized in gas phase at the B3LYP/6-31G(d,p) level of theory. The most stable conformer of the dipeptide was thus obtained. The third amino acid (glutamic acid) was then joined to the cysteine component of the dipeptide by a peptide bond, and various conformers of the tripeptide so obtained were optimized at the B3LYP/6-31G(d,p) level of theory. Thus the most stable conformer of the neutral non-zwitterionic form of glutathione was obtained. Single point energy calculations for the different conformers of glutathione were also performed at the B3LYP/AUG-cc-pVDZ, M06/AUG-cc-pVDZ and M06-2X/AUG-cc-pVDZ levels [44–47] employing the geometries optimized at the B3LYP/6-31G(d,p) level in both gas phase and aqueous media. We also attempted to obtain transition states in gas phase for hydrogen abstraction by a hydroxyl radical from the various sites of neutral non-zwitterionic form of glutathione employing the M06 and M06-2X functionals along with the 6-31G(d,p) basis set but, in many cases, convergence failure was encountered. Therefore, single point energy calculations were performed using the M06 and M06-2X functionals. The bulk solvent effect of aqueous media was treated by single point energy calculations employing a polarizable continuum model (PCM) [48, 49]. This is a popular model used to treat bulk solvent effects [50]. An alternative approach considering explicit intermolecular interactions and employing the QM/MM-MD scheme has also been employed in certain studies [51, 52]. The most stable conformer of the neutral, non-zwitterionic form of glutathione was found to be the same at the different levels of theory employed here in both gas phase and aqueous media.

The mechanisms of hydrogen abstraction by a hydroxyl radical from the different sites of neutral, non-zwitterionic form of glutathione were studied considering its most stable conformer. All the molecular geometries of reactant complexes (RCs), transition states (TSs) and product complexes (PCs) were optimized at the B3LYP/6-31G(d,p) level of theory in gas phase. Single point energy calculations were performed in both gas phase and aqueous media at the three levels of theory mentioned above, employing the B3LYP/6-31G(d,p) level gas phase optimized geometries and treating solvation in bulk aqueous media employing the PCM [48, 49]. Gibbs barrier (ΔG^b) and released energies (ΔG^r) were calculated at all mentioned levels of theory in both gas phase and aqueous media. The thermal energy corrections giving the

Gibbs free energies in gas phase at the B3LYP/6-31G(d,p) level were considered to be valid at all levels of theory employed here in both gas phase and aqueous media.

Values of <S**^{2}> were found consistently to be close to 0.75 during the entire process of geometry optimization in each case, which shows that there was no spin contamination on the surface with doublet spin multiplicity. Vibrational frequency analysis was carried out for all the optimized molecular geometries of RCs, TSs and PCs in gas phase. These analyses revealed that all the RCs and PCs were characterized by all real frequencies whereas all TSs had one imaginary frequency each. At all TSs, corresponding to the imaginary frequencies, the vibrational motions connected the RCs and PCs quite clearly. Therefore, intrinsic reaction coordinate (IRC) analysis was not performed. All the molecular geometries were optimized using the Windows version of the Gaussian 09 (G09W) suite of program [53]. Molecular structures and vibrational motions were visualized using the Windows version of the GaussView software (version 5.0) [54].

Results and discussion

Stability of conformers of glutathione

The most stable conformers of the three amino acids are presented in Fig. 1, which also shows the values of some important geometrical parameters. The orders of total energies of the various conformers of glycine and cysteine obtained in the present study (not given) matched well with those obtained in previous studies [17, 55]. The structures of the six most stable conformers of glutathione optimized as mentioned in Computational details, denoted as GSH I, GSH II, GSH III, GSH IV, GSH V and GSH VI with relative total energies in increasing order, are presented in Fig. 2 along with their relative Gibbs free energies obtained at the B3LYP/AUG-cc-pVDZ level of theory in aqueous media. These conformers were optimized at the B3LYP/6-31G(d,p) level in the absence of any water molecule. The atomic numbering scheme for glutathione employed here is also shown in Fig. 2a. In this figure, each type of atom, i.e., carbon, nitrogen, oxygen, sulfur and hydrogen, is numbered independently. Single point energies of the six conformers obtained in both gas phase and aqueous media at the B3LYP/AUG-cc-pVDZ, M06/AUG-cc-pVDZ, and M06-2X/AUG-cc-pVDZ levels are given in Table 1. The relative Gibbs free energies of the six conformers of glutathione obtained at the B3LYP/6-31G(d,p) level of theory in gas phase were found to lie in the range 0–8.8 kcal mol^{-1} while the corresponding energies in aqueous media lie within the range 0–5.7 kcal mol^{-1}. The conformer GSH I is most stable in both gas phase and aqueous media at all levels of theory employed here.

The most stable conformer of glutathione (GSH I) is stabilized by two intramolecular hydrogen bonds, one between the O1 and H17 and the other between O3 and H14. The latter hydrogen bond is also present in the conformers GSH I, GSH II, GSH III and GSH IV. Further, on the basis of interatomic distances, it appears that the latter hydrogen bond is slightly weaker in GSH I than those in the other three conformers. No intramolecular hydrogen bond exists in the conformers GSH V and GSH VI. The orientations of the amino group and carboxylic acid group attached to the C2 site of glutathione differ significantly in the different conformers of glutathione. In GSH I, the dihedral angles C3–C2–N1–H3 and C3–C2–C1–O2 were found to be 178.7° and −157.7° while in GSH II, these dihedral angles are found to be 175.2° and −81.7°, respectively. In GSH III, the corresponding dihedral angles are 179.7° and 99.47° respectively. In GSH IV, the dihedral angles C3–C2–N1–H3 and C3–C2–C1–O2 are found to be −62.4° and 89.4° while in GSH V, these dihedral angles are 175.9° and −82.9°, respectively. In the conformer GSH VI which has the highest Gibbs free energy among the six conformers, the corresponding dihedral angles are found to be 177.1° and 82.0°, respectively. The above discussion shows that the various conformers of glutathione differ mainly with respect to the dihedral angle C3–C2–C1–O2. Since the conformer GSH I is most stable among the six conformers of glutathione in both gas phase and aqueous media (Fig. 2), it was considered in the study of reactions related to single electron transfer and scavenging of a hydroxyl radical. Corrections for basis set superposition error (BSSE) obtained at the B3LYP/AUG-cc-pVDZ level in gas phase were applied to the Gibbs free energies of the first three most stable conformers of glutathione (GSH I–III). The corrected relative Gibbs free energies of these three conformers were found to be 0.0, 0.2 and 0.5 kcal mol^{-1} while those without correction were 0.0, 1.8 and 2.3 kcal mol^{-1}, respectively (Table 1). Thus GSH I is the most stable conformer of glutathione even after BSSE correction though the Gibbs free energy difference between GSH I and GSH II has become quite small after the correction.

Ko et al. [56] studied the structures of neutral and anionic glutathione (GSH) using negative ion photoelectron spectroscopy and quantum chemical calculations. Three important intramolecular hydrogen bonding distances obtained by Ko et al. [56] in the neutral form of GSH at the B3LYP/6-31+G(d) level of theory are as follows (the amino acid Cys, Glu or Gly to which a specific atom belongs is indicated): H13(Cys)O4 (Cys)=2.48, O3(Glu)H14(Gly)=2.01 and H1(Glu)O6(Gly)= 1.76 Å (Fig. 2a). The corresponding intramolecular hydrogen bonding distances obtained in the present work are 2.25, 2.00 and 1.90 Å. The small differences between the two sets of corresponding intramolecular hydrogen bonding distances arise due to the difference between the basis sets and show that similar relative orientations of the three amino acid

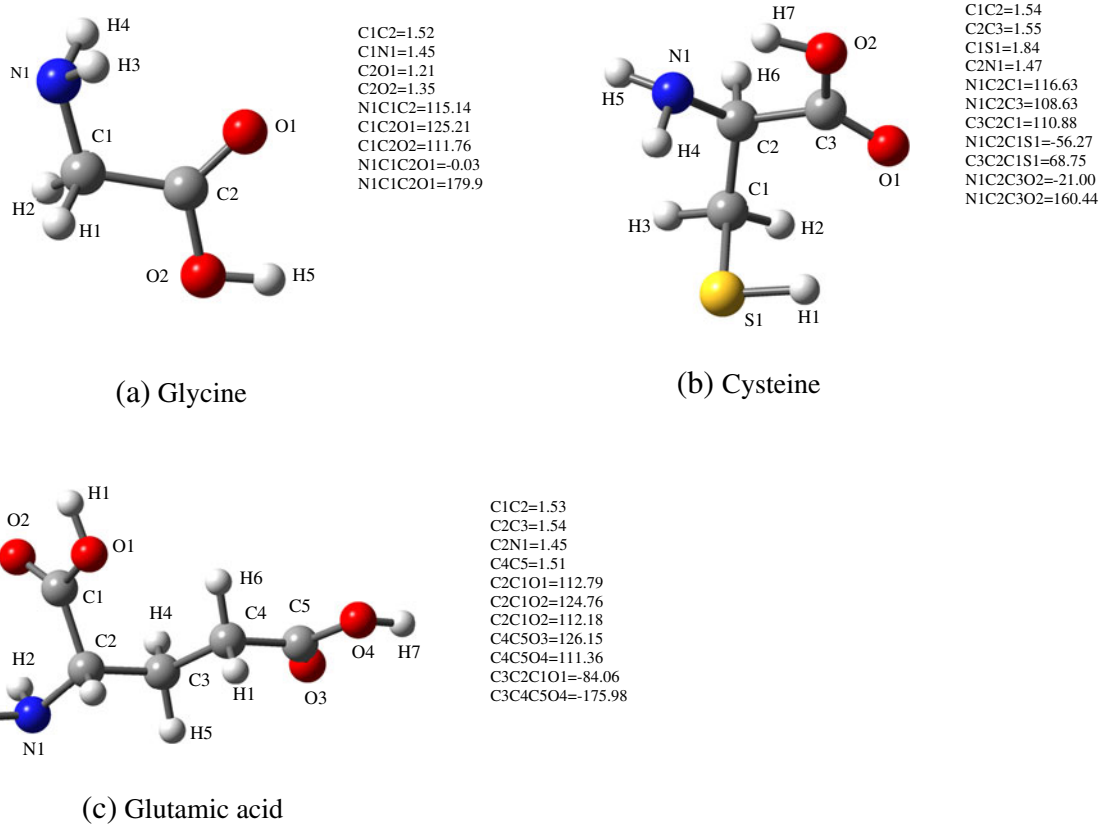

Fig. 1 Geometries of the most stable conformers of (**a**) glycine, (**b**) cysteine and (**c**) glutamic acid optimized at the B3LYP/6-31G(d,p) level in gas phase. Some important interatomic distances, angles and dihedral angles (Å, degrees) are also given

components are obtained in the two studies. Tehrani and Fattahi [57] have carried out conformational analysis of neutral, zwitterionic, cationic and anionic forms of glutathione. In this study [57], minima were initially searched on the potential energy surfaces employing molecular mechanics and, subsequently, the most stable structures were optimized at the B3LYP/6-31+G (d,p) level. Of the three hydrogen bonding distances given above, one, i.e., O3(Glu)H14(Gly), was found to be 1.971 Å by Tehrani and Fattahi [57], which is close to the values 2.00 and 2.01 Å obtained in the present study and that of Ko et al. [56], respectively, while the other two distances are not included in the list of hydrogen bonding distances by Tehrani and Fattahi [57].

Single electron transfer reaction

Single electron transfer from glutathione (GSH I) to an OH radical can be represented as follows.

$$GSH + OH^{\bullet} \rightarrow GSH^{\bullet+} + OH^{-} \quad (1)$$

In this reaction, GSH stands for GSH I and acts as an electron donor while the OH radical acts as an electron acceptor. The geometries of all four species involved in this reaction were optimized at the B3LYP/6-31G(d,p) level of theory. The Gibbs free energy change (ΔG) involved in this process would equal the adiabatic ionization potential (AIP). The AIP values calculated for this reaction at the B3LYP/6-31G(d,p), B3LYP/AUG-cc-pVDZ, M06/AUG-cc-pVDZ, and M06-2X/AUG-cc-pVDZ levels of density functional theory in both gas phase and aqueous media are presented in Table 2. AIP values were found to be much smaller in aqueous media than in gas phase at all the above mentioned levels of theory (Table 2), showing that glutathione can be oxidized and the hydroxyl radical reduced by single electron transfer much more easily in aqueous media than in gas phase.

Hydrogen abstraction reaction

Abstraction of hydrogen atoms by a hydroxyl radical from the different sites of glutathione (GSH I) can be represented as follows

$$GSH - H_n + OH^{\bullet} \xrightarrow{\Delta G_n^b} [TS]_n \xrightarrow{\Delta G_n^r} GSH^{\bullet} + H_nOH \quad (2)$$

where GSH-H_n stands for nth hydrogen atom of GSH I (n=1–17) (Fig. 2a) and H_nOH stands for a water molecule. ΔG_n^b is the Gibbs barrier energy while ΔG_n^r is the Gibbs released energy consequent to abstraction of H_n by an OH radical. The values of ΔG_n^b and ΔG_n^r obtained by geometry optimization at

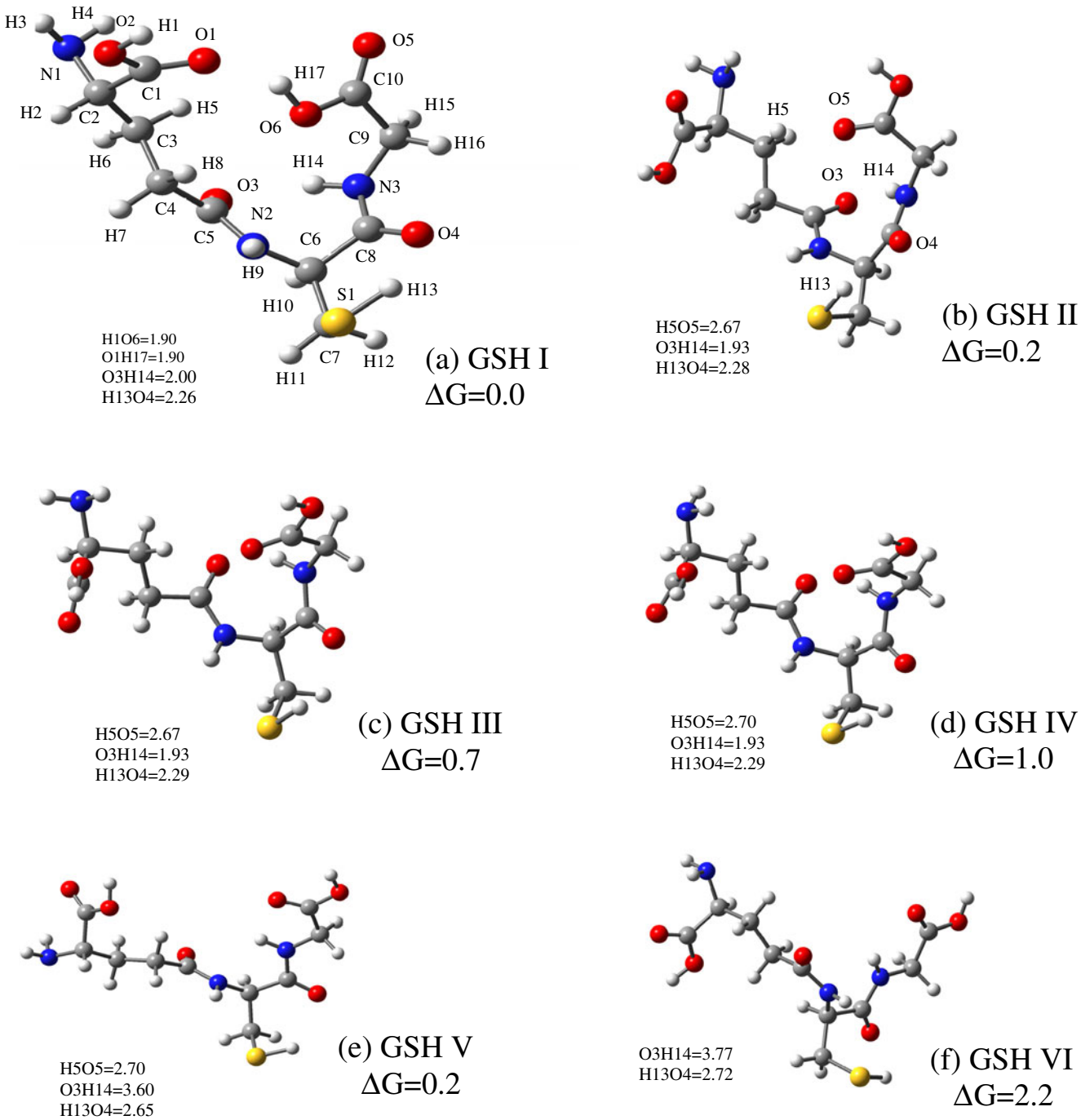

Fig. 2 Structures of the six lowest-energy conformers of glutathione (**a** GSH I, **b** GSH II, **c** GSH III, **d** GSH IV, **e** GSH V, **f** GSH VI) optimized at the B3LYP/6-31G(d,p) level of theory in gas phase. Relative Gibbs free energies (kcal mol^{-1}) of the conformers obtained at the B3LYP/AUG-cc-pVDZ level of theory in aqueous media are given relative to that of GSH I

the B3LYP/6-31G(d,p) level of theory and single point energy calculations at the B3LYP/AUG-cc-pVDZ, M06/AUG-cc-pVDZ, and M06-2X/AUG-cc-pVDZ levels in both gas phase and aqueous media are presented in Table 3. Note that we could locate separate RCs for abstraction of the various hydrogen atoms, except for H8. A search for RC8 always led to RC9, implying that the energy minimum resulting from hydrogen bonding between the oxygen atom of the hydroxyl group (O38) and H8 is shallow while that corresponding to hydrogen bonding between O38 and H9 is comparatively deeper. Therefore, the Gibbs barrier energy for abstraction of H8 was obtained as an approximation with respect to the Gibbs free energy minimum corresponding to RC7, since both H7 and H8 are bonded to the same carbon

Table 1 Relative Gibbs free energies at 298.15 K (kcalmol^{-1}) of the six lowest-energy conformers of glutathione (GSH I–GSH VI) obtained at different levels of theory in the gas phase and in aqueous media[a] with respect to those of the most stable conformer of glutathione (GSH I)

Conformer	B3LYP/6-31G(d,p)	B3LYP/AUG-cc-pVDZ	M06/AUG-cc-pVDZ	M06-2X/AUG-cc-pVDZ
GSH I	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
GSH II	1.8 (1.4)	0.4 (0.2)	2.8 (2.1)	2.5 (2.2)
GSH III	2.3 (2.0)	0.7 (0.7)	3.0 (2.8)	2.9 (2.7)
GSH IV	3.2 (2.6)	1.3 (1.0)	3.4 (3.0)	3.4 (2.9)
GSH V	4.5 (2.5)	2.0 (0.2)	5.7 (3.5)	5.4 (3.3)
GSH VI	8.8 (5.7)	6.0 (2.2)	8.8 (5.2)	8.8 (5.3)

[a] Quantities given in parentheses correspond to aqueous media

atom, i.e., C4 and their abstraction energies are expected to be similar.

The hydrogen abstraction reactions from the different sites of GSH. I including the values ΔG_n^b and ΔG_n^r obtained at the B3LYP/AUG-cc-pVDZ level of theory in aqueous media are presented in Figs. 3, 4 and 5. In these figures, reactant complexes RCn, transition states TSn and product complexes PCn (n=1–17) correspond to abstraction of the hydrogen atom Hn. Five hydrogen abstraction reactions involving the H2, H3, H4, H11 and H12 atoms of GSH by an OH radical, which correspond to the lowest five Gibbs barrier energies, are presented in Fig. 3. The abstraction reactions of the remaining hydrogen atoms are presented as supporting information (Fig. S1 for H1, H5, H6 and H7, Fig. S2 for H8, H9, H10 and H13 and Fig. S3 for H14, H15, H16 and H17).

If we compare the different Gibbs barrier energies obtained using the AUG-cc-pVDZ basis set along with three different functionals, we find that the following order is usually followed: B3LYP/AUG-pVDZ <M06/AUG-cc-pVDZ <M06-2X/AUG-cc-pVDZ (Table 3). In the following analysis, we consider the Gibbs barrier energies obtained at the B3LYP/AUG-cc-pVDZ and M06/AUG-cc-pVDZ levels of theory in aqueous media, with the values obtained at the latter level given in parenthesis. The Gibbs barrier energies for abstraction of H2, H3, H4, H11 and H12 atoms from glutathione by a hydroxyl radical as obtained at these two levels of theory are −2.8 (−0.2), −0.8 (−0.03), −2.9 (−0.8), −1.6 (0.4) and −0.9 (−1.0) kcalmol^{-1} (Fig. 3), while those for abstraction of H1, H5, H6 and H7 atoms by the same radical at the same levels of theory are 0.1 (3.6), 4.0 (6.2), 0.7 (2.6), 0.3 (2.4) kcal mol^{-1} (Fig. S1). The Gibbs barrier energies for abstraction of H8, H9, H10, H13 atoms by the same radical at the same levels of theory are 0.2 (1.5), 15.2 (11.6), 0.7 (1.7), 4.8 (4.7) kcalmol^{-1} (Fig. S2), while for abstraction of H14, H15, H16 and H17 atoms, the Gibbs barrier energies are 4.6 (5.9), −0.5 (2.0), 0.1 (2.4) and 0.9 (2.9) kcalmol^{-1} (Fig. S3), respectively (Table 3).

Table 2 Gibbs free energy changes (ΔG) corresponding to adiabatic ionization potential (AIP; kcalmol^{-1}) involved in single electron transfer from glutathione (GSH I) to an OH radical obtained at different levels of theory in gas phase and aqueous media

Medium	B3LYP/6-31G(d,p)	B3LYP/AUG-cc-pVDZ	M06/AUG-cc-pVDZ	M06-2X/AUG-cc-pVDZ
Gas phase	183.8	144.6	152.4	155.3
Aqueous media	61.5	26.9	33.8	22.0

We find that the Gibbs barrier energies obtained at the B3LYP/AUG-cc-pVDZ and M06/AUG-cc-pVDZ levels of theory are usually qualitatively similar and, where their signs are different, the magnitudes are small. Largest Gibbs free barrier energies are obtained for abstraction of H9 bonded to N2, lying in the range 11.6–15.5 kcalmol^{-1} at the different levels of theory in aqueous media (Table 3). In the study of bond dissociation energy by Fiser et al [36] also, the largest dissociation energy was obtained for the N2H9 bond. Moderate Gibbs barrier energies lying in the range 4.6–6.8 kcalmol^{-1} corresponding to abstraction of H14 bonded to N3 in aqueous media were obtained at various levels of theory (Table 3).

In view of the results discussed above, for further analysis, we consider only the Gibbs barrier energies obtained at the B3LYP/AUG-cc-pVDZ level of theory. The Gibbs barrier energies obtained at this level of theory for hydrogen abstraction corresponding to the different sites can be divided into four categories: (1) Gibbs barrier energies on the larger side (8 kcalmol^{-1} or more), (2) Gibbs barrier energies in the middle region (4–8 kcalmol^{-1}), (3) Negligible or small positive Gibbs barrier energies (0–4 kcalmol^{-1}) and (4) negative Gibbs barrier energies. The Gibbs barrier energies corresponding to abstraction of the various hydrogen atoms obtained at the B3LYP/AUG-cc-pVDZ level of theory lie in these categories as follows (Gibbs barrier energies in kcalmol^{-1} are given in parentheses): Category (1): H9 (15.2), (2) H13 (4.8), H14 (4.6) and H5 (4.0), (3) H17 (0.9), H10 (0.7), H6 (0.7), H7 (0.3), H8 (0.2), H16 (0.1) and H1 (0.1), and (4) H15 (−0.5), H3 (−0.8), H12 (−0.9), H11 (−1.6), H2 (−2.9) and H4 (−2.9). It is obvious that abstraction of only H9 would encounter a significant barrier. Further, abstraction of three hydrogen atoms lying in category (2) would involve moderate Gibbs barriers lying between 4 and 4.8 kcalmol^{-1}. The Gibbs barriers of abstraction of seven hydrogen atoms lying in category (3) are small, positive, being in the range 0.1–0.9 kcalmol^{-1}. Six hydrogen atoms belonging to category (4) would be abstracted by an OH radical without barrier as the calculated Gibbs barriers in these cases are negative. This list includes the hydrogen atoms of the two carboxylic acid groups and the NH_2 group of glutathione.

Table 3 Gibbs barrier (ΔG_i^b) and released (ΔG_i^r) (i=1–17) energies (kcal mol^{-1}) involved in hydrogen abstraction reactions from the different sites of glutathione by a hydroxyl radical at different level of theory in both gas phase and aqueous media[a]

Reaction site	Gibbs free barrier and released energy	B3LYP/ 6-31G(d,p)	B3LYP/ AUG-cc-pVDZ	M06/ AUG-cc-pVDZ	M06-2X/ AUG-cc-pVDZ
O2(H1)	ΔG_1^b	−0.3(−0.5)	0.4(0.1)	2.8(3.6)	5.2(5.1)
	ΔG_1^r	−25.2(−25.9)	−25.5(−26.0)	−31.0(−31.4)	−30.0(−30.6)
C2(H2)	ΔG_2^b	1.8(1.9)	−1.7(−2.8)	0.9(−0.2)	3.8(4.1)
	ΔG_2^r	−47.5(−47.5)	−48.0(−48.1)	−50.1(−67.3)	−52.6(−53.7)
N1(H3)	ΔG_3^b	−4.9(−2.5)	−3.2(−0.8)	−2.2(−0.03)	−3.6(−1.6)
	ΔG_3^r	−9.3(−11.9)	−12.1(−14.1)	−15.8(−18.1)	−16.4(−18.8)
N1(H4)	ΔG_4^b	−1.3(−4.2)	−2.0(−2.9)	0.4(−0.8)	−0.2(−1.7)
	ΔG_4^r	−18.3(−19.5)	−19.6(−20.3)	−21.0(−36.1)	−27.7(−36.6)
C3(H5)	ΔG_5^b	4.1(0.4)	2.4(4.0)	4.9(6.2)	7.3(8.4)
	ΔG_5^r	−23.5(−23.1)	−25.2(−26.2)	−27.5(−28.5)	−28.5(−29.4)
C3(H6)	ΔG_6^b	3.6(2.4)	1.9(0.7)	3.4(2.6)	4.4(3.7)
	ΔG_6^r	−20.7(−21.2)	−21.3(−21.8)	−23.9(−24.8)	−23.4(−49.3)
C4(H7)	ΔG_7^b	1.4(1.6)	0.1(0.3)	−0.01(2.4)	3.0(5.1)
	ΔG_7^r	−28.1(−26.5)	−26.3(−24.7)	−30.7(−29.2)	−31.8(−30.6)
C4(H8)	ΔG_8^b	0.7(0.3)	0.3(0.2)	−0.4(1.5)	2.8(4.2)
	ΔG_8^r	−34.3(−31.0)	−40.0(−27.9)	−34.0(−30.5)	−35.1(−31.6)
N2(H9)	ΔG_9^b	6.0(15.2)	12.2(15.2)	9.3(11.6)	10.7(11.9)
	ΔG_9^r	−13.7(−14.6)	−15.9(−16.0)	−15.9(−15.5)	−17.2(−15.8)
C6(H10)	ΔG_{10}^b	2.9(2.5)	0.1(0.7)	0.4(1.7)	4.2(1.7)
	ΔG_{10}^r	−24.4(−25.1)	−26.2(−27.3)	−29.1(−30.7)	−28.4(−30.3)
C7(H11)	ΔG_{11}^b	1.4(0.2)	−1.1(−1.6)	0.9(0.4)	3.1(3.8)
	ΔG_{11}^r	−21.1(−5.8)	−22.8(−22.6)	−25.3(−25.2)	−25.1(−25.2)
C7(H12)	ΔG_{12}^b	2.3(−1.8)	0.2(−0.9)	1.8(−1.0)	1.9(−0.7)
	ΔG_{12}^r	−18.5(−20.4)	−20.4(−20.3)	−24.1(−24.5)	−23.0(−25.5)
S1(H13)	ΔG_{13}^b	3.3(3.1)	4.6(4.8)	4.7(4.7)	4.9(5.0)
	ΔG_{13}^r	−34.1(−33.1)	−32.9(−31.5)	−35.5(−34.2)	−38.9(−38.8)
N3(H14)	ΔG_{14}^b	5.6(5.4)	3.5(4.6)	3.8(5.9)	9.4(6.8)
	ΔG_{14}^r	−10.4(−11.1)	−11.1(−12.6)	−14.3(−14.7)	−15.2(−15.9)
C9(H15)	ΔG_{15}^b	2.6(0.6)	0.3(−0.5)	2.3(2.0)	6.0(5.6)
	ΔG_{15}^r	−35.5(−35.3)	−35.3(−34.9)	−37.4(−37.6)	−40.1(−40.2)
C9(H16)	ΔG_{16}^b	3.5(1.1)	1.3(0.1)	3.2(2.4)	9.1(5.3)
	ΔG_{16}^r	−38.4(−40.3)	−42.1(−43.3)	−41.3(−43.0)	−43.0(−44.7)
O6(H17)	ΔG_{17}^b	0.1(0.2)	−0.2(0.9)	2.4(2.9)	3.7(4.2)
	ΔG_{17}^r	−6.0(−6.1)	−6.1(−7.2)	−8.2(−9.1)	−7.0(−7.9)

For atomic numbering scheme, see Fig. 2a.

[a]Quantities given in parentheses correspond to aqueous media

We also optimized the geometry of glutathione considering the zwitterionic form of its glutamic acid part at the B3LYP/6-31 G(d,p) level of theory in aqueous media employing the PCM model for solvation [45, 46]. Subsequently, we obtained Gibbs barrier energies for hydrogen abstraction from the NH_3^+ group at the B3LYP/6-31 G(d,p) and B3LYP/AUG-cc-pVDZ levels of theory in aqueous media. These Gibbs barrier energies were found at the two levels of theory in aqueous media to be −1.4 and −0.5 kcal mol^{-1}, respectively, implying that the hydrogen abstraction reaction under consideration is barrierless. These results and those discussed above for abstraction of the NH_2 group hydrogen atoms show that the result remains invariant (barrierless) whether the NH_3^+ or NH_2 form of the amino group of the glutamic acid part of glutathione is considered.

The Gibbs barrier energies corresponding to abstraction of H13 bonded to the sulfur atom in aqueous media obtained at the different levels of theory lie in the range 3.1–5.0 kcal mol^{-1} (Table 3). Further, our results show that, in going from gas phase to aqueous media, the Gibbs barrier energy for hydrogen abstraction from the SH bond is changed to a small extent (0–6 %) at the different levels of theory employed here (Table 3, Fig. 4). Fiser et al. [36] found the SH bond dissociation energy of the component corresponding to cysteine to increase in going from gas phase to aqueous media to a small extent (~4 %) at various levels of density functional theory. Our results and those of Fiser et al. [36] suggest that the SH bond of glutathione is not very sensitive to change of environment from gaseous to aqueous. As mentioned earlier, hydrogen abstraction from the SH bond of glutathione by a hydroxyl radical was found by Galano and Alvarez-Idaboy

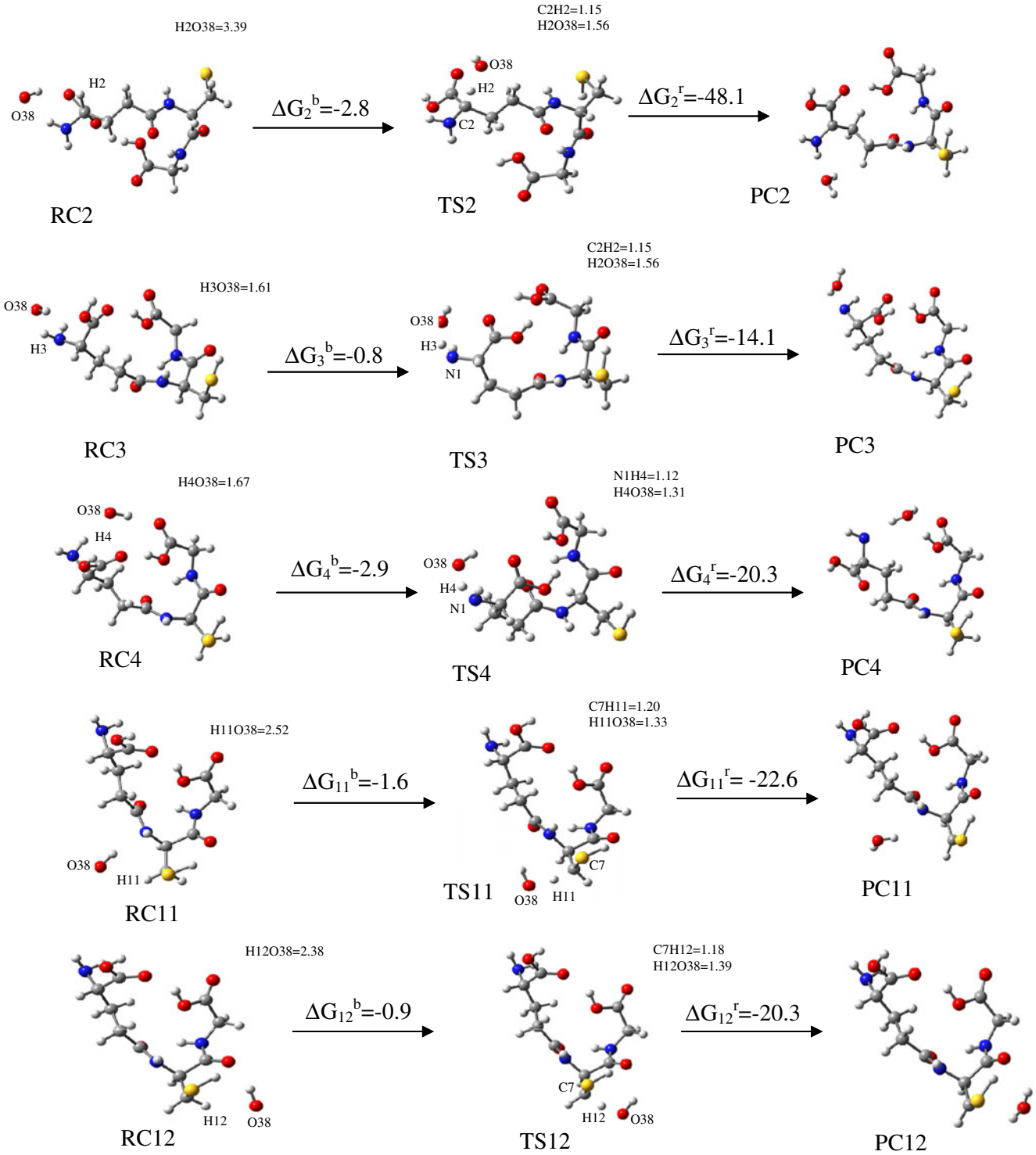

Fig. 3 Reactant complexes (RCs), transition states (TSs) and product complexes (PCs) corresponding to hydrogen abstraction (H2, H3, H4, H11 and H12) from the most stable conformer of glutathione (GSH I) by a hydroxyl radical obtained by geometry optimization at the B3LYP/6-31G(d,p) level in gas phase. The Gibbs barrier and released energies obtained at the B3LYP/AUG-cc-pVDZ level in aqueous media are given. Some important interatomic distances (Å) are also given. RCn, TSn and PCn correspond to abstraction of hydrogen atom Hn

[37] by geometry optimization at the M05-2X/6-311+G(d,p) level in aqueous media to be barrierless. Since these authors [37] have not reported the corresponding gas phase Gibbs barrier energy, the change in going from gas phase to aqueous media in this case cannot be obtained. The SH group is common to N-acetylcysteine (NAC) and glutathione.

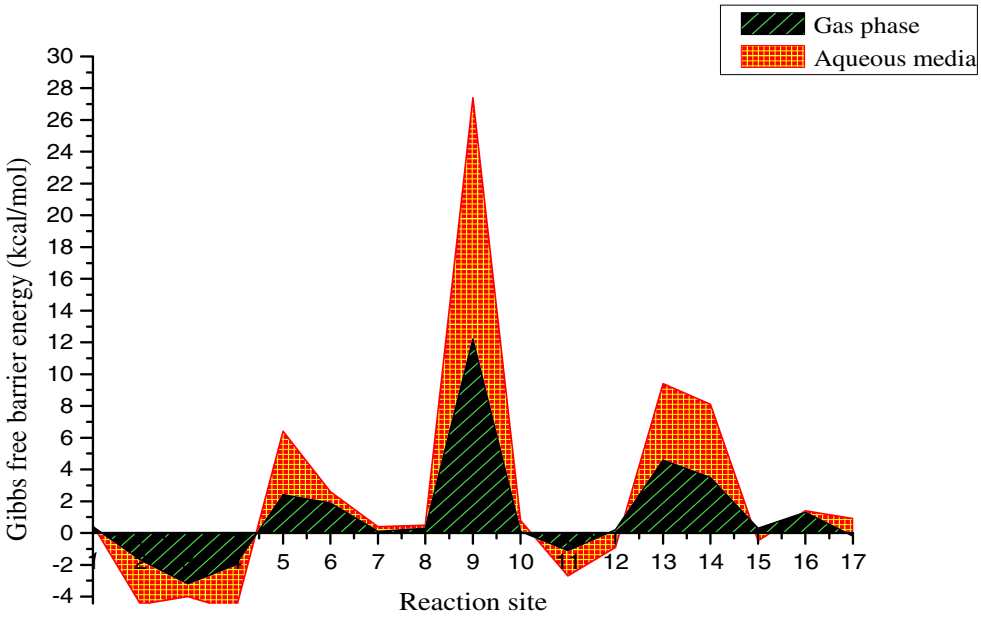

Fig. 4 Diagram showing Gibbs barrier energies for hydrogen abstraction from the different sites of glutathione obtained at the B3LYP/AUG-cc-pVDZ level of theory in gas phase and aqueous media

Therefore, the Gibbs barrier energies for hydrogen abstraction from the SH groups of these two anti-oxidants obtained at the same level (B3LYP/Aug-cc-pVDZ) may be compared. At this level of theory, the Gibbs barrier energies for hydrogen abstraction from the SH group of NAC in gas phase and aqueous media were found to be 3.2 and 3.1 kcalmol^{-1}, respectively [16], while the corresponding barrier energies for hydrogen abstraction from the SH group of glutathione were found to be 4.6 and 4.8 kcalmol^{-1}, respectively. Thus the barrier energy for hydrogen abstraction from the SH group of glutathione is larger by about 30 % in comparison to that in NAC.

A diagram showing Gibbs barrier energies for abstraction of hydrogen atoms by a hydroxyl radical from the different sites of glutathione found at the B3LYP/AUG-cc-pVDZ level of theory in both gas phase and aqueous media is presented in Fig. 4. This figure reveals that the Gibbs barrier energies vary qualitatively in a similar way in going from one site to another in going from gas phase to aqueous

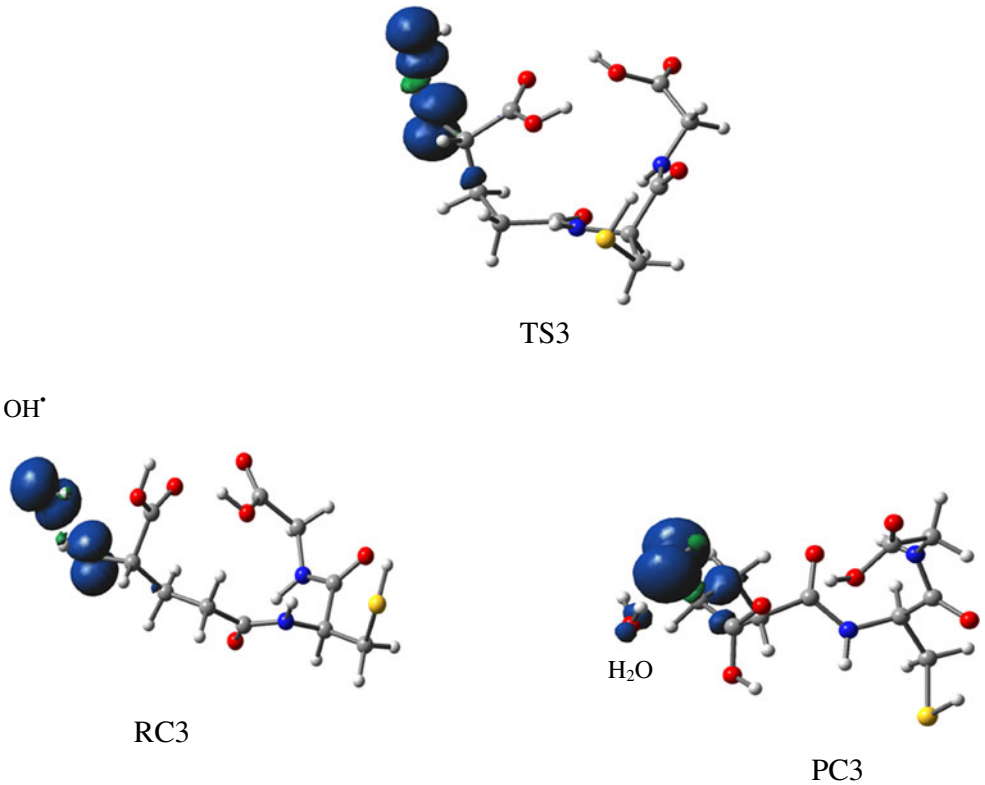

Fig. 5 Iso-spin density maps for the spin density value of 0.004 electron/bohr^3 for RC3, TS3 and PC3 of reaction three at B3LYP/AUG-cc-pVDZ level of theory in aqueous media. *Blue* Positive spin density, *green* negative spin density, the magnitude being the same for both colors

media (Fig. 4). It is also found that the magnitudes of barrier energies (positive or negative) are usually enhanced in going from gas phase to aqueous media. As several Gibbs barrier energies for hydrogen abstraction by a hydroxyl radical are small, it is obvious that glutathione would act as a very efficient hydroxyl radical scavenger.

Spin density distribution

Iso-spin density distributions were studied in the various RCs, TSs and PCs involved in the hydrogen abstraction reactions studied here. The iso-spin density distributions for the value 0.004 electrons/Bohr^3 for the reaction that corresponds to the lowest barrier energy in gas phase, i.e., in RC3, TS3 and PC3 are presented in Fig. 5. The spin density is mostly positive (blue color) but in one case it is also negative (green color). Positive and negative spin densities behave differently in an applied magnetic field [58]. We find that, in RC3, an appreciable amount of positive spin density is localized not only at the OH group but also at the NH_2 group. In TS3, the positive spin density distribution is similar to that in RC3, but in this case, a noticeable negative spin density is also localized at the hydrogen atom that is in the process of being extracted by the OH group. In PC3, spin density distribution is a bit more extended. Thus, although a major amount of positive spin density is localized at the NH group of PC3, small amounts of it are also localized at the oxygen atom of the water molecule and two carbon atoms (C1, C3) of the glutamic acid moiety. Further, small amounts of negative spin density are localized at the hydrogen atom of the NH group and a carbon atom (C2) of the glutamic acid moiety.

Conclusions

We arrive at the following conclusions from the present study of the hydroxyl radical scavenging ability of the reduced neutral non-zwitterionic form of glutathione:

1. Abstraction of the hydrogen atom H9 attached to N2 by a hydroxyl radical would involve the highest Gibbs barrier energy. Gibbs barrier energies for abstraction of certain other hydrogen atoms, e.g., H13 bonded to the sulfur atom and H14 bonded to N3, are moderate. The aqueous medium does not have a significant effect on the Gibbs barrier energy of abstraction of the hydrogen atom of the SH group.
2. The Gibbs barrier energies of abstraction of certain hydrogen atoms attached to carbon atoms are small positive or negative.
3. The Gibbs barrier energies for abstraction of hydrogen atoms of the carboxylic acid groups in aqueous media are usually small positive while those for abstraction of hydrogen atoms of the NH_2 group in aqueous media are negative. Gibbs barrier energies for hydrogen abstraction from the NH_3^+ group in aqueous media were also found to be negative. Therefore, as far as hydrogen abstraction by a hydroxyl radical is concerned, there appears to be no need to consider the glutamic acid part of glutathione to be in the zwitterionic form.
4. Spin density is localized mostly near the site from where a hydrogen atom is abstracted by an OH radical.
5. The Gibbs barrier energies discussed above show that the neutral non-zwitterionic form of glutathione would act as a very efficient hydroxyl radical scavenger.

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References

1. Chatterjee S, Noack H, Possel H, Keilhoff G, Wolf G (1999) Glia 27:152–161
2. Dringen R, Kussmaul L, Gutterer JM, Hirrlinger J, Hamprecht B (1999) J Neurochem 72:2523–2530
3. Moran LA, Scrimgeour KG, Horton HR, Ochs RS, Rawn JD (1994) Biochemistry, 2nd edn. Prentice Hall, Englewood Cliffs
4. Bains JS, Shaw CA (1997) Brain Res Rev 25:335–358
5. Axelsson K, Mannervik B (1983) FEBS Lett 152:114–118
6. Wardman P (1988) In: Sies H, Ketterer B (eds) Glutathione conjugation: mechanism and biological significance. Academic, London, pp 43–72
7. Saez GT, Bannister WH, Bannister JV (1990) CRC, Boca Raton, pp 237–254
8. Buxton GV, Greenstock CL, Helman WP, Ross AB (1988) J Phys Chem 17:513–531
9. Do KQ, Bovet P, Cuenod M (2004) Schweiz Arch Neurol Psychiatr 155:375–385
10. Jena NR, Mishra PC (2005) J Phys Chem B 109:14205–14218
11. Shukla PK, Mishra PC (2007) J Phys Chem B 111:4603–4615
12. Yadav A, Mishra PC (2012) Int J Quantum Chem 112:2000–2008
13. Tanaka T, Makita H, Ohnishi M, Mori H, Satoh K, Hara A, Sumida T, Fukutani K, Ogawa H (1997) Cancer Res 57:246–252
14. Vaya J, Aviram M (2001) Curr Med Chem Immunol Endocr Metab Agents 1:99–117
15. Shukla MK, Mishra PC (1996) J Mol Struct 377:247–259
16. Agnihotri N, Mishra PC (2009) J Phys Chem B 113:12096–12104
17. Agnihotri N, Mishra PC (2011) J Phys Chem A 115:14221–14232
18. Pastore A, Federici G, Bertini E, Piemonte F (2003) Clin Chim Acta 333:19–39
19. Perricone C, De Carolis C, Perricone R (2009) Autoimmun Rev 8:697–701
20. Estrela J, Ortega A, Obrador E (2006) Crit Rev Clin Lab Sci 43:143–181
21. Giustarini D, Dalle-Donne I, Tsikas D, Rossi R (2009) Crit Rev Clin Lab Sci 46:241–281
22. Viguie CA, Frei B, Shigenaga MK, Ames BN, Packer L, Brooks GA (1993) J Appl Physiol 75:566–572
23. Laires MJ, Madeira F, Sergio J (1993) Magnes Res 6:233–238
24. Liu H, Wang H, Shenvi S, Hagen TM, Liu RM (2004) Ann NY Acad Sci 1019:346–349

25. Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL (2009) Biol Chem 390:191–214
26. Schulz JB, Lindenau J, Seyfried J, Dichgans J (2001) Eur J Biochem 267:4904–4911
27. Fujiwara S, Formicka-Kozlowska G, Kozlowski H (1977) Bull Chem Soc Jpn 50:3131–3135
28. Rabenstein DL (1973) J Am Chem Soc 95:2797–2803
29. York MJ, Beilharz GR, Kuchel PW (1987) Int J Pept Protein Res 29:638–646
30. Gorbitz CH (1987) Acta Crystallogr 41:362–366
31. Han Y, HaoMiao Z, Jian S (2007) Sci China Ser B 50:660–664
32. Zhao R, Lind J, Merenyi G, Eriksen TE (1994) J Am Chem Soc 116:12010–12015
33. Zhao R, Lind J, Merenyi G, Eriksen TE (1997) J Chem Soc Perkin Trans 2:569–574
34. Rauk A, Armstrong DA, Berges J (2001) Can J Chem 79:405–417
35. Lampela O, Juffer AH, Rauk A (2003) J Phys Chem A 107:9208–9220
36. Fiser B, Szori M, Jojart B, Izsak R, Csizmadia IG, Viskolcz B (2011) J Phys Chem B 115:11269–11277
37. Galano A, Alvarez-Idaboy JR (2011) R Soc Chem 1:1763–1771
38. Mohan CG, Kumar A, Mishra PC (1996) Int J Quantum Chem 60:699–708
39. Kumar A, Bhattacharjee AK, Mishra PC (1992) Int J Quantum Chem 43:579–589
40. Jena NR, Mishra PC, Suhai S (2009) J Phys Chem B113:5633–5644
41. Ikebuchi M, Kashiwagi A, Asahina T, Tanaka Y, Takagi Y, Nishio Y, Hidaka H, Kikkawa R, Shigeta Y (1993) Metabolism 42:1121–1126
42. Lee K, Pi K, Kim EB, Rho BS, Kang SK, Lee HG, Choi YJ (2010) Biotechnol Lett 32:969–972
43. Wemmie JA, Askwith CC, Lamani E, Cassell MD, Freeman JH, Welsh MJ (2003) J Neurosci 23:5496–5502
44. Becke AD (1993) J Chem Phys 98:5648–5652
45. Lee C, Yang W, Perr RG (1988) Phys Rev B 37:785–789
46. Zhao Y, Truhlar DG (2008) Theor Chem Acc 120:215–241
47. Zhao Y, Truhlar DG (2006) J Phys Chem 110:5121–5129
48. Miertus S, Tomasi J (1982) Chem Phys 65:239–245
49. Miertus S, Scrocco E, Tomasi J (1981) Chem Phys 55:117–129
50. Tomasi J, Mennucci B, Cammi R (2005) Chem Rev 105:2999–3093
51. Schwenk CF, Hofer TS, Randolf BR, Rode BM (2005) Phys Chem Chem Phys 7:1669–1673
52. Park K, Götz AW, Walker RC, Paesani F (2012) J Chem Theory Comput. doi:10.1021/ct300331f
53. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA Jr, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ (2009) Gaussian 09, Revision A.1. Gaussian, Wallingford
54. Dennington R, Keith T, Millam J (2009) GaussView, Version 5. Semichem, Shawnee Mission
55. Tiwari S, Mishra PC, Suhai S (2008) Int J Quantum Chem 108:1004–1016
56. Ko YJ, Wang H, Li X, Bowen KH, Lecomte F, Desfrancois C, Poully JC, Gregoire G, Schermann JP (2009) Chem Phys Chem 10:3097–3100
57. Tehrani ZA, Fattahi A (2012) Struct Chem. doi:10.1007/s11224-012-0023-1
58. Santos EJG, Sánchez-Portal D, Ayuela A (2010) Phys Rev B 81:125433-1-6