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Theoretical study of the structure of the glutathione-hydrogen peroxide complex

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Summary. Theoretical calculations have been performed in order to investigate the possibility of intermolecular hydrogen bonds between glutathione and hydrogen peroxide. Preliminary investigations of the conformations of GSH in water have been done in the framework of the SIBFA and CHARMm methods. We have proposed some privileged sites on the molecules of GSH for the formation of complexes with H_2O and H_2O_2.

Key words: Glutathione – Water – Hydrogen peroxide – Complexation

1 Introduction

Glutathione (GSH = L-γ-glutamyl-L-cysteinyl-L-glycine) is the major non protein thiol compound present in cells. One of its chief functions is the reduction of hydrogen peroxide H_2O_2 by GSH mediated by glutathione peroxidase [1].

Recently, Abedinzadeh et al. [2], studying the reaction of H_2O_2 with GSH in vitro, in absence of enzyme have put in evidence the initial fast formation of a peroxide (or a chelate) between GSH and H_2O_2:

$$GSH + H_2O_2 \rightleftharpoons [GSH\cdots H_2O_2]$$

This reaction is followed by the disproportionation of $[GSH\cdots H_2O_2]$:

$$2[GSH\cdots H_2O_2] \rightleftharpoons [GSSG\cdots H_2O_2] + 2H_2O$$

From a theoretical point of view, it seemed interesting to test the possibility of such an H-bonded complex formation. Since experimental work has been carried out in aqueous medium buffered at pH 7.4, the negative ion of GSH (see Fig. 1) which represents the state of dissociation of glutathione at this pH value according to both H-NMR [3] and ^{13}C-NMR [4] studies has to be taken into consideration.

Because of the size of the molecule and its flexibility (even when keeping the two peptide links planar with NH and CO in a *trans* position, still eleven degrees of rotational freedom remain [see Fig. 1]), the conformational space could include a great deal of local minima. In preliminary work [5, 6], we had studied the behaviour of a limited number of conformations and found a few local minima

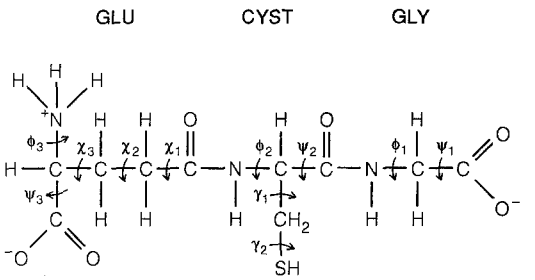

Fig. 1. Negative ion glutathione at pH 7.4; definition of the degrees of rotational freedom: GLU is for the glutamyl, CYST for the cysteinyl and GLY for the glycyl moieties

with respect to intermolecular [GSH···H_2O_2] H-bonded complexes formation. We are conscious that this work only represented a first step towards an answer of this problem; thus we have undertaken the exploration of a more complete [GSH] conformational space. This is the subject of the present paper.

Our work may be divided into two parts:

- A study of the conformation of GSH both in an isolated state and in water.
- A study of intermolecular interactions between H_2O_2 and GSH, taking into account the eventual conformation change of both GSH and H_2O_2. Different structures of [GSH···H_2O_2] have been investigated.

2 Methods

Both intra- and intermolecular energies have been calculated simultaneously in the framework of the SIBFA method (*Sum of Interactions Between Fragments computed Ab-initio*) [7, 8, 9]. SIBFA methodology significantly differs from standard methods by some features that will be briefly described. In order to complete the static picture given by the SIBFA method and because of the great flexibility of GSH, we performed some dynamical calculations with the CHARMm method [14] using standard potentials. A short introduction to both SIBFA and CHARMm methods follows.

2.1 SIBFA

2.1.1 Intermolecular energy [7, 8]. Following an additive procedure, the intermolecular energy is written as a sum of five contributions:

$$E_{INTER} = E_{EL} + E_{POL} + E_{REP} + E_{DISP} + E_{CT} \quad (1)$$

which are calculated from analytical formulae derived from perturbation theory (SAPT = *Symmetry Adapted Perturbation Theory*) [10]. We can point out several characteristic features:

- Use of a multicenter (atom and middle of bonds), multipolar (up to quadrupoles) expansion derived [11] from the *ab-initio* SCF molecular functions for the calculations of *electrostatic* and *polarization* components. In this work, the *ab-initio* wave functions were calculated within an "adapted" minimal basis [12].

• Computation of the *repulsion* term as a sum of 'bond-bond', 'bond-lone pair' and 'lone pair-lone pair' interactions. Such a representation of lone pair accounts for the *radial* and *directional* dependence of repulsion term, the analytical function being of an exponential type.

• *Dispersion* is dumped to take into account overestimation of the energy at short distances [13].

• Explicit evaluation of the *charge-transfer* contribution between lone pairs of the electron donor molecule and hydrogen atoms of the electron acceptor molecules.

2.1.2 Intramolecular energy [9]. In the SIBFA method, a large molecule is built out of constitutive molecular fragments separated by single bonds. In fact, one calculates the variation of the conformational energy as a sum of inter-fragments interaction energies:

$$\Delta E_{INTRA} = \sum_{i=1}^{N} \sum_{j=i+1}^{N} E'_{INTER}(i,j) \tag{2}$$

where N is now the number of fragments.

E'_{INTER} is calculated as a sum of the four first contributions given in Eq. (2), plus a term denoted E_{TOR} which is a transferable torsional energy contribution, calibrated for elementary rotations around single bonds (for more details concerning this method see [7–9]).

As an evaluation of the solvent effect, we have only taken into consideration 'hydration water' molecules, i.e. the ones which are very close to the solute and thus interact very strongly with it. In order to estimate the 'hydration energy' (ΔE_{HYDRA}), it may be supposed that each water-solute interaction ($E_{W\text{-}S}$) replaces a water-water interaction ($E_{W\text{-}W}$), N_W being the number of 'hydration water' molecules; we get:

$$\Delta E_{HYDRA} = E_{INTER} - N_W E_{W\text{-}W} \tag{3}$$

We have used the value of $E_{W\text{-}W}$ of 5.4 kcal/mol calculated within the SIBFA method.

We are conscious that ΔE_{HYDRA} only represents part of the total solvation energy in water, but such a study should give an eventual insight into possible intramolecular conformational changes due to these strong water-solute interactions.

2.2 CHARMm

The study of the dynamics of a molecular system requires, first, obtaining a potential energy surface.

The mechanical forces acting on atoms are related to the first derivatives of the potential with respect to the atomic positions. The dynamics of the system are calculated by solving the classical Newton's equations of motion to determine how atomic positions change with time.

The energy functions used in CHARMm are composed of terms representing bonds (b), bond angles (Θ), torsional angles (Φ), Van der Waals interactions and electrostatic interactions.

$$E = \tfrac{1}{2} \sum_{bonds} K_b(b - b_0)^2 + \tfrac{1}{2} \sum_{bond\ angles} K_\Theta(\Theta - \Theta_0)^2 + \tfrac{1}{2} \sum_{torsional\ angles} K_\Phi[1 + \cos(n\Phi - \delta)] + \sum_{i,j\ nb\ pairs} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{C_{ij}}{r_{ij}^{6}} + \frac{q_i q_j}{D r_{ij}} \right) \tag{4}$$

The energy depends on the internal energy parameters K_b, K_Θ, K_Φ, Lennard-Jones parameters A and C, atomic charges q_i, dielectric constant D, and geometrical reference values b_0, Θ_0, n, and δ. The initial positions of atoms of GSH are those obtained within the SIBFA method. In order to account for solvent effects, GSH has been immersed into a sphere containing water molecules (the radius of the sphere is 12 Å). A term denoted E_{DSB} (*Deformable Stochastic Boundary Energy*) has been added to the energy defined in Eq. (5) to keep water molecules inside this sphere, according to the procedure defined by Brooks et al. [15, 16]. We run dynamics trajectories of forty picoseconds. Such periods are long enough to determine the dominant contributions to the atomic fluctuations and water configurations linked to the molecule.

3 Conformation of GSH

In spite of its wide biochemical interest, experimental and theoretical data on the conformation of glutathione are rather scarce:

- A crystal structure determination has been reported for the neutral form GSH [17].
- Among the few NMR studies carried on glutathione, only one [3] was concerned with the geometrical arrangement in water solution at different pH values.
- PCILO calculations [18] have been performed for both GSH and its negative ion in an isolated state.

3.1 Isolated state

We have used bond lengths as determined in the crystal structure [17]. Our results proceed from a simultaneous variation of the eleven torsional angles (defined in Fig. 1) through some optimization process carried out with different initial guesses. In our previous work [5], two initial guesses had been chosen, namely: the '*S*-shaped open' conformation (denoted *S*) observed in the crystal structure and a local energy minimum *S'* obtained from an energy sub-map $E = f(\Psi_2, \Phi_2)$ (all other torsional angles being freezed at the value of [17]). Complete optimization automatic process (involving the eleven dihedral angles) carried out on *S* and *S'* had respectively led to Opt_1 (O_1) (Fig. 2a) and Opt_2 (O_2) (Fig. 2c in [5]) conformations which mainly differ by the value of Ψ_2 angle (22.0 and 126.1 respectively).

Our results had shown:

- A drastic instability of *S*-shaped conformation with regards to both O_1 and O_2. The energy difference we have calculated amounts to 50 kcal/mol and is mainly a result from electrostatic component of the intramolecular energy.
- An almost equal stability between both O_1 and O_2: the 2 kcal/mol energy difference we have obtained is not very significant.
- Neither O_1 nor O_2 involves any H-bond between NH_3^+ or COO^- glutamyl group and the peptide backbone.

In the present work, we have chosen as initial guesses, the three most stable conformations calculated within the PCILO method [18]. The first one, P_1, is characterized by intramolecular H-bonds between the NH_3^+ group and the

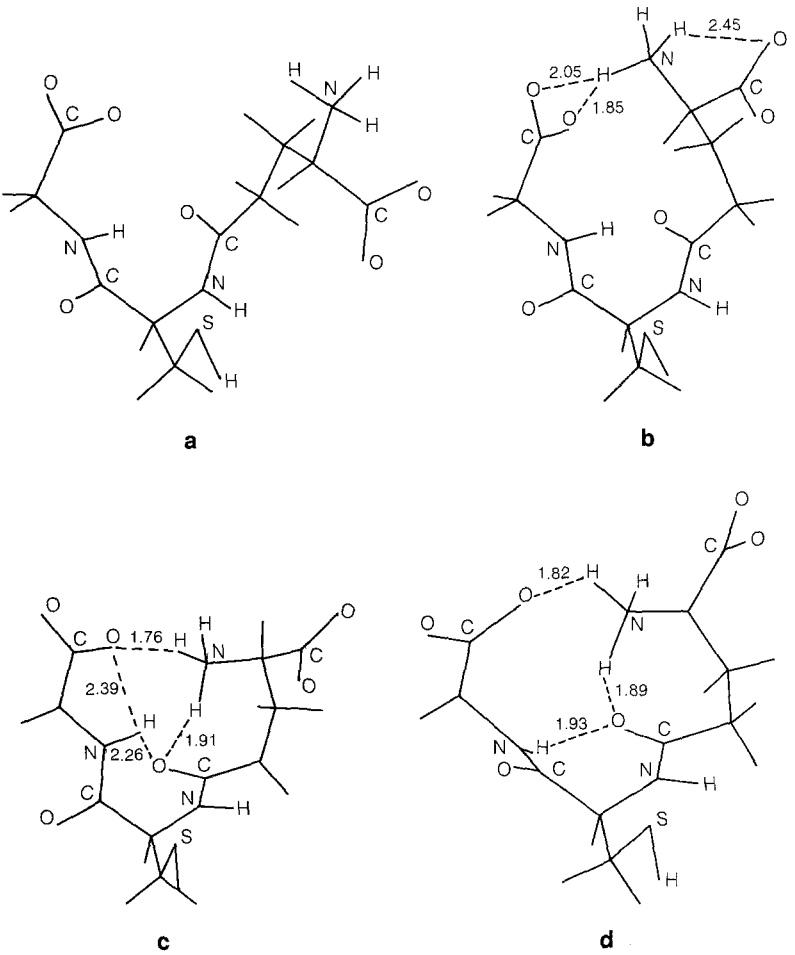

Fig. 2. Most stable conformations of GSH. (a) O_1, (b) F_1, (c) F_2, (d) F_3. The intramolecular H-bonds are represented by *dashed lines*; lengths are in Å

carbonyl of the glu-cyst peptide group, and between the glycyl COO^- group and the NH of the cyst-gly peptide group while the second one, P_2, involves an intramolecular H-bond between the glutamyl COO^- group and the NH of the glu-cyst peptide group. The third one, P_3, is quite similar to P_1.

3.1.1 Optimization of P_1 conformation. As a first step, as a result of a simultaneous variation of the eleven torsional angles, we obtained conformation P'_1, which is stabilized by 4.2 kcal/mol with regards to P_1. P_1 and P'_1 mainly differ by the value of Ψ_1, dihedral angle (in P'_1, Ψ_1 has decreased by about 90° with regards to P_1). In fact, we have noticed in P'_1 (as in O_1) a torsion of the carboxylate oxygen around the C–C bond leading to a weakening of the intramolecular H-bond connecting glycyl NH and COO^- groups ($d_{H\cdots O} = 2.16$ Å and 2.88 Å in P_1 and P'_1 respectively).

Then selecting P'_1 as an initial guess we have produced a sub-map for Φ_2, Ψ_2 rotations, all other torsional angles being freezed at the values calculated for P'_1.

This choice reflects the fact that Ψ_2 and Φ_2 dihedral angles are important with regards to the relative position of the two peptide links and thus for the geometrical arrangement of the central part of the molecule. Then the minimum of $E = f(\Phi_2, \Psi_2)$ was fully optimized taking into account the eleven dihedral angles.

A folded conformation (denoted $F_1(a)$) characterized by a strong interaction between the glutamyl NH_3^+ and glycyl COO^- groups ($d_{H\cdots O} = 1.82$ Å) has been obtained. This folded structure still maintains the intramolecular H-bond between the glutamyl NH_3^+ and the glu-cyst carbonyl groups ($d_{H\cdots O} = 2.03$ Å). Some weak interactions occur between the glutamyl NH_3^+ and the COO^- groups ($d_{H\cdots O} = 2.43$ Å) and between the glycyl COO^- and the cyst-gly NH groups ($d_{H\cdots O} = 2.88$ Å). This folded geometrical arrangement is stabilized with regards to P_1' by 12.1 kcal/mol. Nearly comparable stabilities (to within 3 kcal/mol) are found for O_2 and $F_1(a)$ conformations.

For other degrees of freedom, an $E = f(\Phi_1, \Psi_1)$ energy sub-map has been calculated leading to a minimum which has been refined by an automatic minimization process through the eleven variable dihedral angles simultaneously. The minimum $F_1(b)$, thus obtained, is very similar to $F_1(a)$ from a geometrical point of view, except a shortened distance between glutamyl NH_3^+ and glycyl COO^- groups ($d_{H\cdots O} = 1.71$ Å). $F_1(b)$ lies below $F_1(a)$ by 6.5 kcal/mol.

As a last step, inquiring about the influence of the geometrical arrangement of the glutamyl part of the molecule on the whole molecular conformation, we have explored two series of energy sub-maps $E = f(\chi_1, \chi_2, \chi_3)$ and $E = f(\Phi_3, \Psi_3)$. The two minima, hence obtained, were separately fully optimized following the automatic process defined above. It resulted in a unique minimum denoted F_1 stabilized by 22.2 kcal/mol with regards to $F_1(b)$.

Now F_1 lies 26 kcal/mol below O_2. We have observed that, with regards to $F_1(a)$, the location of this minimum is slightly shifted (by less than 10 kcal/mol) in the $\Pi_2$2-Ψ_2 subspace. As a whole, the stabilization of the F_1 conformation is a result of strong intramolecular bifurcated H-bonds connecting NH_3^+ group with glycyl COO^- group: in fact, one hydrogen of NH_3^+ is connected to the two oxygens of the glycyl COO^- ($d_{H\cdots O} = 1.76$ Å and $d_{H\cdots O} = 2$ Å) (Fig. 2b). Contrary to $F_1(a)$ and $F_1(b)$ conformations, it does not appear that any short contacts between the NH_3^+ and the glu-cyst carbonyl groups occur.

3.1.2 Optimization of P_2 and P_3 conformations. For the P_2 and P_3 conformations, we followed the strategy adopted for the P_1 conformation, performing alternatively full optimization and conformational sub-maps. At least, we have obtained two very similar folded conformations denoted F_2 and F_3: we want to emphasize that full optimization of P_2 directly led to F_2 which remains invariant when the process defined above has been applied. As concerns P_3, as a result of the study of $E = f(\Phi_2, \Psi_2)$ sub-map, an intermediate conformation (stabilized by 10.9 kcal/mol with regards to P_2) has been obtained.

Once again, the folding is a result of strong intramolecular H-bonds between NH_3^+ and the glycyl COO^- groups. But contrary to F_1 conformation the hydrogen of NH_3^+ is H-bonded to only one oxygen of the glycyl COO^- group. Furthermore, in both F_2 and F_3, it has been noticed that a network of intramolecular H-bonds connects the glu-cyst carbonyl group to both glutamyl NH_3^+ and cyst-gly NH groups. In F_2 (and not in F_3 conformation), a weak intramolecular H-bond appears between the glycyl COO^- and cyst-gly NH groups ($d_{O\cdots H} = 2.38$ Å). F_2 and F_3 (Figs. 2c,d) appear to be a little more compact than F_1. These three folded conformations are nearly isoenergetic (Table 1).

In fact, an examination of the values of the dihedral angles (Table 2) shows that:

• The glycyl part of GSH may adopt several geometrical arrangements. A displacement of the glycyl out of the plane (Φ_1) is observed in the F_1 conformation but not in the F_2 or F_3 ones. Furthermore, a torsion of the glycyl carboxylate oxygens around the C–C bond occurs in both F_1 and F_2 conformations.

• The two angles Φ_2 and Ψ_2 also are different in the three folded conformations; consequently, the angle α between the two peptide linkages is modified.

• The three angles χ_1, χ_2, χ_3 (characterizing the geometrical arrangement of the glutamyl skeleton) changes.

3.2 Influence of solvent

1. Hydration energy calculated within SIBFA method

As emphasized in [5], it has been noticed that hydration of the O_2 conformation is satisfied (in the sense of hydration waters) by ten water molecules. Thus the hydration process has been carried out with O_1 and folded F_1, F_2 and F_3 conformations. Nineteen water molecules participate in the hydration shell of each conformer. As a result of simultaneous intermolecular and intramolecular energy optimizations notice that:

• The intramolecular geometrical arrangements are practically similar to the one obtained in an isolated state, but a loss of intramolecular energy is observed. The eleven dihedral angles do not differ by more than 20°. The hydrated structures practically maintain the intramolecular H-bonds which occur in water free GSH.

• This loss of intramolecular energy is more important in O'_1 (7.9 kcal/mol), F'_2 (6 kcal/mol), F'_3 (6.3 kcal/mol) than in F'_1; but it is balanced by an hydration energy gain (which is larger for O'_1: −170.1 kcal/mol than F'_1: −137.5 kcal/mol) (see Tables 1 and 3).

• Overall when considering the total energy of the different hydrated complexes, our results show that O'_1, F'_1 and F'_3 have comparable stabilities within 2.5 kcal/mol.

Table 1. Intramolecular energy difference between some calculated conformations and crystal structure. (All values in kcal/mol)

Conformations	O_1	O_2	F_1	F_2	F_3
ΔE_{INTRA}	−48.4	−50.0	−75.5	−74.3	−75.0

Table 2. Values of dihedral angles defining glutathione conformations. (All values in degrees)

	Φ_1	Ψ_1	Φ_2	Ψ_2	Φ_3	Ψ_3	γ_1	γ_2	χ_1	χ_2	χ_3
F_1	118.2	116.6	41.5	37.6	316.5	72.8	70.8	193.8	120.7	61.0	196.5
F_2	199.5	188.4	302.7	1.9	202.1	75.0	176.1	177.7	276.7	68.4	43.8
F_3	157.2	109.1	307.1	200.7	270.1	57.1	157.6	173.1	200.7	57.0	261.9
O_1	172.2	119.1	197.8	22.0	30.1	31.8	56.1	211.2	91.4	305.7	297.6

Table 3. Variation of the total energy of the hydrated structures. $\Delta E_{TOT} = \Delta E_{INTRA} + \Delta E_{HYDRA}$; ΔE_{INTRA} has the same meaning as in Table 1 and ΔE_{HYDRA} is defined by Eq. (8). (All values in kcal/mol)

	O'_1	F_1	F'_2	F'_3
ΔE_{INTRA}	−40.5	−75.5	−68.3	−68.7
ΔE_{HYDRA}	−170.1	−137.5	−133.0	−142.3
ΔE_{TOT}	−210.6	−213.0	−201.3	−211.0

Figure 3 gives an illustration of the three more stable hydrated complexes (namely O'_1, F'_1 and F'_3) we have studied. It may be noticed that besides the usual 'hydration' water linked to C=O, NH or SH group, it appears that some water molecules form H-bonds between two interaction sites of GSH molecule, and are thus interacting very strongly. In order to gain insight of these two interaction

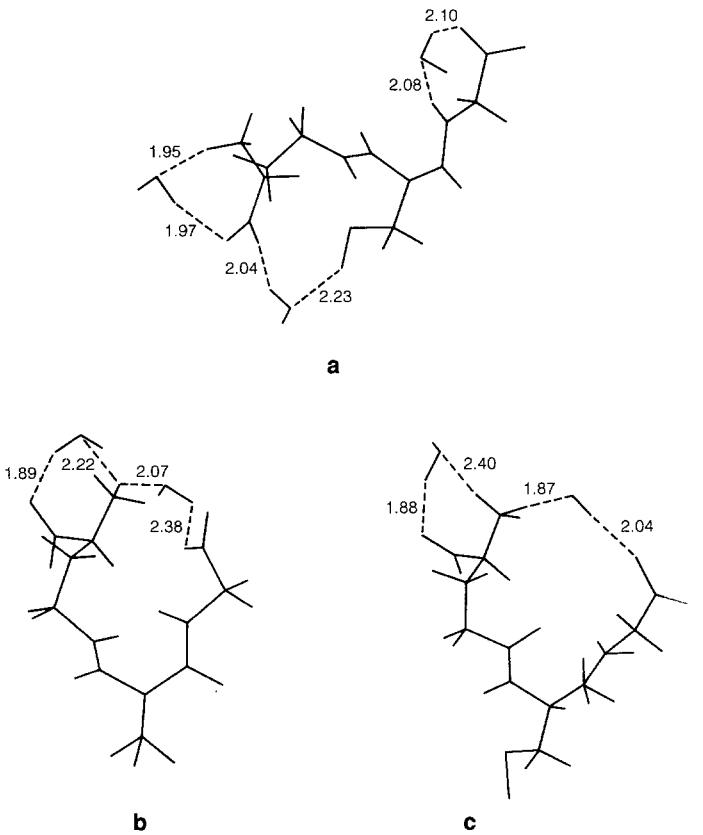

Fig. 3. Hydrated structures of glutathione. (a) O'_1, (b) F'_1, (c) F'_3. Intermolecular H-bonds are represented by *dashed lines*; lengths are in Å

Table 4. Intermolecular interaction energy between GSH (within O_1, F_1 and F_3 conformations) with H_2O (and H_2O_2) for different $C_{X\text{-}Y}$ (with X and Y for glu, gly, cyst) complexes. In each column the first value concerns GSH···H_2O complex, while the second (between parentheses) stands for GSH···H_2O_2 complexes. All values in kcal/mol

	$C_{gly\text{-}gly}$	$C_{glu\text{-}cyst}$	$C_{glu\text{-}glu}$	$C_{glu\text{-}gly}$
O'_1	−15.3 (−12.9)	−20.8 (−24.8)	−21.2 (−22.3)	
F'_1			−10.8 (−15.4)	−13.0 (−23.8)
F'_3			−12.6 (−19.5)	−15.2 (−21.5)

sites of GSH, we have adopted the following convention for denoting the different GSH···H_2O complexes:

- $C_{glu\text{-}glu}$ (glutamyl COO^- and NH_3^+ groups).
- $C_{gly\text{-}gly}$ (glycyl COO^- and cyst-gly NH groups).
- $C_{glu\text{-}gly}$ (glutamyl NH_3^+ and glycyl COO^- groups).
- $C_{glu\text{-}cyst}$ (glutamyl COO^- and cysteinyl SH groups).

Several remarks could be done from the examination of the different complexes:

(a) $C_{glu\text{-}glu}$ complex has been obtained within the three GSH structures we have studied. It may be noticed that a similar situation has been observed with *ab-initio* calculations [19] (within 6-31G** and Dunning basis sets) of glycine zwitterion which has the same topology as the one existing in $COO^-CH_2NH_3^+$ group of glutamyl part. In $C_{glu\text{-}glu}$ complexes, a water molecule interacts more strongly with O'_1 (−21.2 kcal/mol) than with F'_1 or F'_3 (−10.8 kcal/mol and −12.6 kcal/mol) (see Table 4).

(b) In O'_1 both $C_{gly\text{-}cys}$ and $C_{gly\text{-}gly}$ complexes can be formed ($\Delta E_{INTER} = -20.8$ kcal/mol and −15.3 kcal/mol respectively).

(c) In F'_1 and F'_3 conformations we have obtained $C_{glu\text{-}gly}$ ($\Delta E_{INTER} = -13.0$ kcal/mol and −15.2 kcal/mol respectively). In $C_{glu\text{-}gly}$ complexes, it may be noticed (see Fig. 3) that the particular location of the water molecule contributes to maintain the compactness of the GSH folded structures.

We may sound ourselves about the reality of the hydration water molecules we have obtained without taking into account the whole solvent. So for the sake of information a study of dynamical properties of GSH surrounded by solvent has been considered.

2. Dynamic simulations within CHARMm method

Calculations have been performed only with O_1 and F_1 conformations of GSH. GSH has been immersed into a sphere containing 218 water molecules (nearly five shells of water molecules). Such calculations lead to two very important conclusions:

(a) The two conformations of GSH remain stable during some forty picoseconds. CHARMm dynamical calculations confirm SIBFA results as concerns the isostability of both O_1 and F_1 conformations of GSH in water.

(b) An analysis of location of water molecules has shown the presence of both:

- $C_{glu\text{-}glu}$ and $C_{gly\text{-}gly}$ complexes in O_1 conformation.
- $C_{glu\text{-}glu}$ and $C_{glu\text{-}gly}$ complexes in F_1 conformation.

4 GSH···H_2O_2 Complexes

As in our preliminary work, our calculations have been performed with the experimental skew geometry of H_2O_2 ($\tau = 120°$). No optimization has been performed on the isolated state of H_2O_2, since it is well known [20] that available results on H_2O_2 are only obtained in the framework of *ab-initio* calculations fulfilling at least two criteria:

- the basis set employed has to be augmented;
- all geometrical parameters have to be optimized for all values of τ to be considered.

Our first strategy in [5] consisted in studying the three (GSH···H_2O_2) complexes suggested by Abedinzadeh et al. [2] namely the ones involving interactions between H_2O_2 and (1) both CO groups, (2) both NH groups belonging to the peptide links and (3) cyst-gly NH and glu-cyst CO groups. It has appeared from our results that such complexes are rather weak, in fact our optimization process brings H_2O_2 closer to the glycyl COO^- group ($d_{H\cdots O} = 1.94$ Å) leading to an interaction energy of -12.9 kcal/mol. It has appeared that H_2O_2 has replaced one water molecule which occupies the same site in the hydrated O_1 structure.

As a second strategy, we decided, in this work, to replace by H_2O_2, the H_2O molecule forming H-bond bridges between two interaction sites of GSH and leading to different $C_{X\text{-}Y}$ (with X or Y as glu, gly or cyst). So for GSH···H_2O_2 complexes we used the notation adopted for GSH···H_2O complexes. An illustration of different GSH···H_2O_2 complexes is given by Fig. 4.

Table 4 gives an insight to the stability of these complexes. It appears that:

- For O_1 conformation, $C_{glu\text{-}cyst}$ and $C_{glu\text{-}glu}$ are the most stable ones. $C_{gly\text{-}gly}$ lies $\simeq 10$ kcal/mol above them.
- For F_1 folded conformations $C_{glu\text{-}gly}$ is favored by 8.4 kcal/mol with regards to $C_{glu\text{-}glu}$ complex.
- For F_3 folded conformation $C_{glu\text{-}glu}$ and $C_{glu\text{-}gly}$ have almost the same stability, within 2 kcal/mol.

We may notice that when GSH adopts:

- *The conformation* O_1

The interaction with a water molecule is preferred by 2.4 kcal/mol to the one involving H_2O_2 in $C_{gly\text{-}gly}$ complexes. The situation is reversed with $C_{glu\text{-}cyst}$ and $C_{glu\text{-}glu}$ complexes which are a little more stable (by 4 kcal/mol and 1 kcal/mol respectively) when H_2O_2 (rather than H_2O) is involved in the interaction process.

- *The two folded conformations denoted* F_1 *or* F_3

In the two complexes we have obtained (namely $C_{glu\text{-}glu}$ and $C_{glu\text{-}gly}$), GSH···H_2O_2 is preferred to GSH···H_2O interactions. The energy difference between these two kinds of interaction may be important, until -10.6 kcal/mol for $C_{glu\text{-}gly}$ within F_1 conformation.

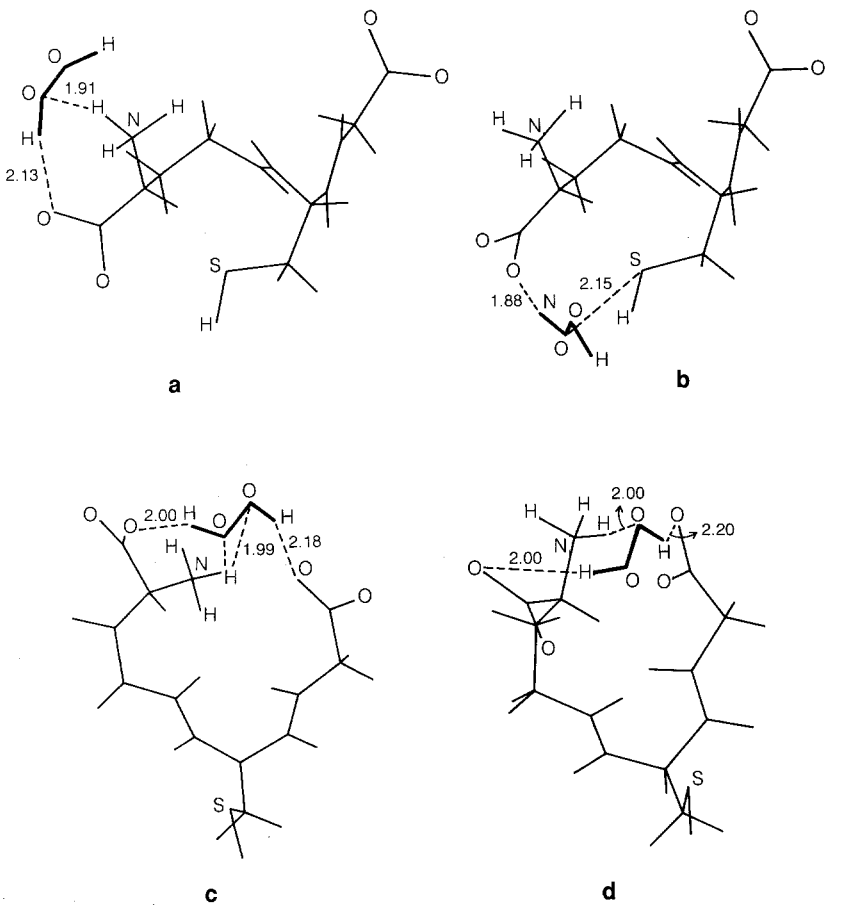

Fig. 4. GSH···H_2O complexes. (a) $C_{glu\text{-}cyst}$, (b) $C_{glu\text{-}glu}$ (with O_1), (c) $C_{glu\text{-}gly}$ (with F_1), (d) $C_{glu\text{-}gly}$ (with F_3). The intermolecular H-bonds are represented by *dashed lines*; lengths are in Å

Before ending this section, we want to emphasize that the intramolecular energy of GSH remains almost unchanged upon complexation between GSH (within O_1, F_1 and F_3 conformations) and H_2O_2. The variations of ΔE_{INTER} does not exceed 2.0 kcal/mol. In fact dihedral angles defining the different geometrical arrangements do not vary by more than 20°. In the same way, the value of the angle τ defining the conformation of H_2O_2 does not deviate significantly from 120°, the value obtained for the minimal conformation.

We are conscious that in this work, we have not taken in account the solvent effect on GSH···H_2O_2 complexes, so we cannot decide that one of the complexes we have studied is the most stable one; we may only conclude that effectively intermolecular complexes between H_2O_2 and GSH seem possible: one GSH···H_2O interaction may be replaced by one GSH···H_2O_2 interaction. Furthermore, in light of our results, CO and the charged glutamyl or glycyl COO^- groups and the glutamyl NH_3^+ group. In some cases the cysteinyl SH group may be involved in the complexation process.

5 Conclusions

In light of the results of the calculations reported above, four main conclusions may be drawn up:

1. In the conformational space, besides closed (gauche) conformation involving intramolecular H-bonds between terminal glutamyl ionized groups and atoms belonging to the peptide links (like the III-1 rotamer found in PCILO calculations) some closed conformations exist in which neither CO nor NH peptide groups are involved in internal H-bonds (O_1, O_2 conformations) and some folded conformation with interaction between glutamyl NH_3^+ group and glycyl COO^- as well as between glutamyl NH_3^+ and CO groups.

2. The solvent does not strongly modify the conformations of GSH.

3. Some water molecules strongly interact with GSH. Some H-bond bridges between H_2O and two interaction sites of GSH have been observed: this result has been obtained by both SIBFA and CHARMm methods.

It has been interesting to notice that dynamical simulations performed with two different conformations (namely O_1 and F_1) of GSH immersed into a sphere containing 218 water molecules confirm our SIBFA results as concerns the isostability of these two conformations in water. We may reasonably think that there are probably many accessible conformations in water for GSH and we are conscious that the present work is only a preliminary study. We intend to explore a more complete conformational space, taking into account the whole solvent (using a discrete-continuum model for instance), so we will be able to discuss the entropy effects in water.

4. Intermolecular complexes between GSH and H_2O_2 are possible. This result is not without interest from a biological point of view, since it may be conceived that even in vivo in absence of glutathione peroxydase, GSH may mask H_2O_2 by complexing it.

Before ending this paper, we want to mention and discuss the recent results obtained from a kinetic study on the oxydation of N-acetyl cysteine (Fig. 5) by H_2O_2.

It has been shown that such a reaction presents some analogies with the one involving glutathione, particularly the incidence of the initial fast formation of a complex with H_2O_2. The equilibrium constant thus calculated (1500 ± 200) [21] is smaller than the one evaluated for glutathione (1950 ± 50).

H_2O_2 may interact with both the COO^- and NH groups of N-acetyl cysteine leading to a H-bonded bridged complex similar to $C_{gly\text{-}gly}$ one in GSH. In fact, this interaction site is the only one possible in acetyl cysteine; but when considering the results of Table 4, it immediately appears that $C_{gly\text{-}gly}$ complex is the weakest one, so we may reasonably think that it is unlikely to be formed in GSH.

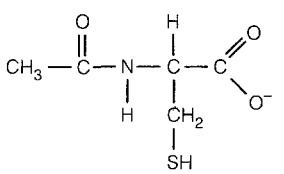

Fig. 5. N-acetyl cysteine

This could explain the difference between the association constants evaluated in N-acetyl cysteine and in GSH. In fact, further experimental works, particularly on the reaction of cysteine itself with H_2O_2 could provide us with some useful results concerning $C_{glu\text{-}glu}$-like complexes. The value of the association constant of such a complex would be of a great interest for us. Concerning the occurrence of $C_{glu\text{-}gly}$, let us recall that GSH is the smallest polypeptide (3 peptide units) in which a folded conformation may be found and in fact complex $C_{glu\text{-}gly}$ is inherent in this particular geometrical arrangement. What would happen with a polypeptide with four or more peptide units?

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