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The One-Electron Reduction Potential of Methionine-Containing Peptides Depends on the Sequence

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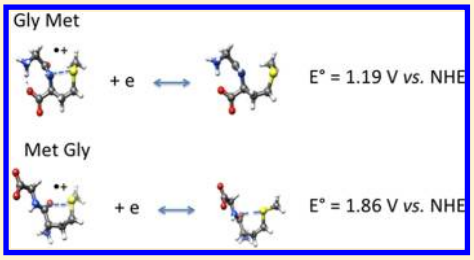
Supporting Information

ABSTRACT: The protein residue methionine (Met) is one of the main targets of oxidizing free radicals produced in oxidative stress. Despite its biological importance, the mechanism of the oxidation of this residue is still partly unknown. In particular the one-electron redox potentials of the couple $Met^{\bullet+}$/Met have not been measured. In this work, two approaches, experimental as well as theoretical, were applied for three dipeptides L-Met L-Gly, L-Gly L-Met and L-Met L-Met. Measurements by electrochemistry indicated differences in the ease of oxidation. Two DFT methods (BH&HLYP and PBE0) with two basis sets (6-31G(d) and 6-311+G(2d,2p)) were used to determine the redox potentials of Met in these peptides present in different conformations. In agreement with experimental results, we show that they vary with the sequence and the spatial structure of the peptide, most of the values being higher than 1 V (up to 2 V) vs NHE.



INTRODUCTION

Methionine (**Met**) is an essential amino acid in human nutrition and a key metabolite in several processes. Met deficiency can be a limiting factor in protein synthesis. Its oxidation leads to numerous diseases mostly related to protein misfolding like neurodegenerative conditions. The Met thioether function is one of the main targets of oxidizing free radicals and of hydrogen peroxide, belonging to the so-called "reactive oxygen species". However, despite its biological importance, the mechanism of the oxidation of this residue is still partly unknown. Several studies have revealed that all protein Met residues could not be oxidized.[1−3] The extent to which they are oxidized is strongly dependent on the structure of the protein or the peptide. Moreover, the product of the oxidation is still debated.[4] In peptides and proteins the only product that has been characterized is methionine sulfoxide.[5−9] However, depending on the position of the residue in the polypeptidic chain (on the surface or inside the protein matrix, neighboring residues etc.), other compounds might be formed.

In one-electron oxidations of proteins, pulse radiolysis data indicate that Met is most readily oxidized.[10] The transients formed in the oxidation of Met by $^{\bullet}OH$ radicals have been characterized in peptides and proteins;[11−13] the first steps lead to Met radical cation $Met^{\bullet+}$, which is centered on the sulfur atom. It can subsequently complex with oxygen, nitrogen or other sulfur containing groups depending on the local arrangement. These groups act as Lewis bases by providing electron lone pairs leading to two-center three-electron (2c−3e) bonds,[14] designated as S∴X, with X = N, O or S. This complexation would increase the lifetime of the free radical and might determine its final fate.

Up to now, there are no data about the one-electron reduction potentials of methionine despite the crucial importance of this parameter. The reduction potential of dimethyl sulfide was determined by pulse radiolysis to be 1.66 ± 0.03 and 1.40 ± 0.02 V vs the normal hydrogen electrode (NHE), respectively, for the $(DMS^{\bullet+}/DMS)$ and $(DMS)_2^{+}$/2DMS (disulfide radical cation) couples [15]. For Se-methionine (SeM) the reduction potential of the $SeM^{\bullet+}$/SeM couple was found equal to 1.2 ± 0.05 V vs NHE at pH = 7.[16] As for methionine in proteins, experimental results showed that it could sometimes be oxidized by weak oxidants.[3,17] In addition, methionine is sometimes able to reduce Cu^{II} linked to proteins into Cu^{I}.[18] The Cu^{II}/Cu^{I} reduction potentials of protein copper centers vary considerably from 184 mV to above 1 V (in human ceruloplasmin), which indicates that the one of the methionines could also vary importantly and reach much lower values than those that were expected. Finally, the oxidation of some methionine residues in proteins can proceed by intramolecular electron transfer, in which Met is either the electron donor[19] or a hopping site.[20] Again the authors claimed that the redox potential could be lowered by 0.5 V through neighboring effects. A thorough investigation of the variations of the reduction potential of this residue in peptides appears thus necessary.

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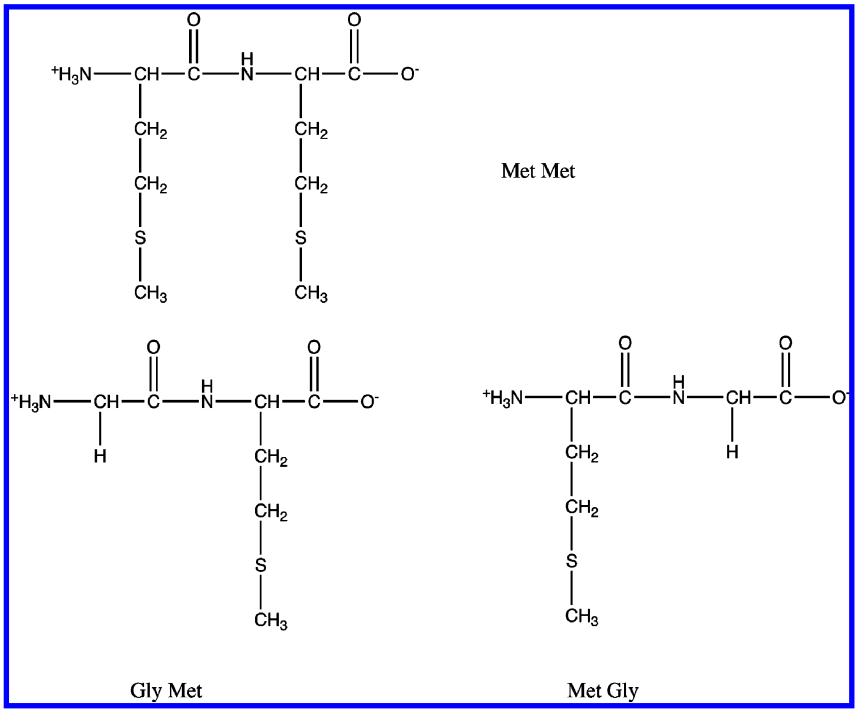
Chart 1. Developed Formulas of the Dipeptides Considered in This Study[a]



[a]In the methylated ones, the $-NH_3^+$ and the $-COO^-$ terminals were replaced by $COCH_3$ and $NHCH_3$ respectively.

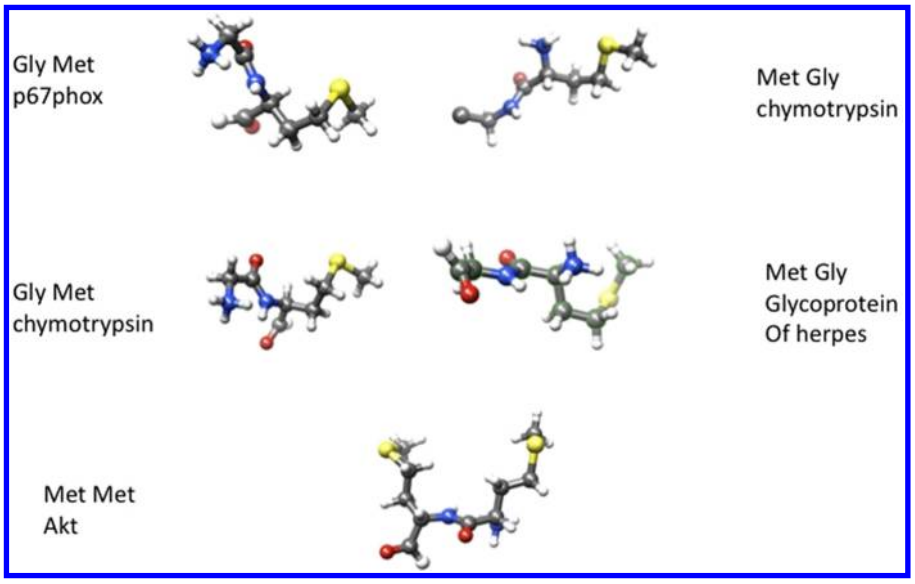


Figure 1. Some sequences found in the PDB ($p67^{phox}$ PDB 1E96; glycoprotein of herpes 3NW8; Akt 3O96; chymotrypsin 2YT6). Such geometries were considered as benchmarks for the optimizations. The sulfur atom of Met could be closer to a heteroatom (N or O) when oxidized.

In this work we report on new attempts, by electrochemical methods and quantum-mechanical calculations, to determine the one-electron reduction potentials for the following dipeptides: L-Gly L-Met (GlyMet), L-Met L-Gly (MetGly) and L-Met L-Met (MetMet) (Chart 1). For computations, we considered various initial conformations and their subsequent radical cations stabilized or not by the formation of 2c−3e bonds. We took advantage of related sequences in the Protein Data Bank (PDB) that provided us some starting points for optimizations (p53 PDB 1YCS, thioredoxin PDB 1EP7, prion PDB 1HLP etc.), (See Figure 1 for some examples.)

We show that the one-electron reduction potential is in most cases higher than +1 V vs NHE, however it can vary from ca. 1 V to ca. 2 V with the sequence and the various conformations available for a dipeptide and its cation. The reduction potential might thus depend on the constraints that exist in a protein limiting the possible rearrangements of the cation. This might explain why the oxidation of methionine residues in proteins or peptides appears so different according to the protein.

EXPERIMENTAL SECTION

Cyclic voltammetry was carried out with a three-electrode system connected to a EG&G PAR 273A potentiostat which

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was computer-controlled via the M270 software. The system consisted of a Teflon electrochemical cell containing a boron-doped diamond working electrode, a platinum gauze counter electrode and a saturated Calomel reference electrode (SCE). A sodium perchlorate (VWR, France) 0.1 M, pH = 2.0 buffer was prepared with pure water obtained from a Milli-RiOs 8 unit followed by a Milli-Q academic purification set (water resistivity: 18.2 MΩ·cm). Solutions were made with the dipeptides GlyMet, MetGly and MetMet from Bachem (Switzerland), and with the amino acids methionine (Met) and glycine (Gly) from Sigma (France), used as received from the suppliers. All solutions had a concentration of 1 mM in their solutes and were thoroughly deoxygenated with argon prior to the cyclic voltammetry experiments.

COMPUTATIONAL METHODOLOGY

DFT Methods and Basis Set. To the best of our knowledge no DFT method has been specifically adapted to the calculations of redox potentials. In addition the ones that should be applied to describe 2c−3e bonds are still debated. B3LYP, the most common density functional, is known to be inadequate. In this work, we have chosen two DFT hybrid methods (BH&HLYP and PBE0) because of their different properties concerning free radicals. Braïda et al.[21] have demonstrated that the BH&HLYP functional, which includes 50% Hartree−Fock exchange, 50% Slater exchange and the additional correlation effects of the LYP functional[22] were in its spin-unrestricted formalism, the most accurate method to describe 2c−3e bonds. In addition the authors[23] have obtained, on a set of small radicals, similar results with BH&HLYP as with the CCSD(T) approach. As for the PBE0 model,[24] which is based on the Perdew−Burke−Erzenrhof exchange-correlation functional,[25] it is accurate for the ground-state properties of organic free radicals; however it has not yet been tested for those containing 2c−3e bonds.

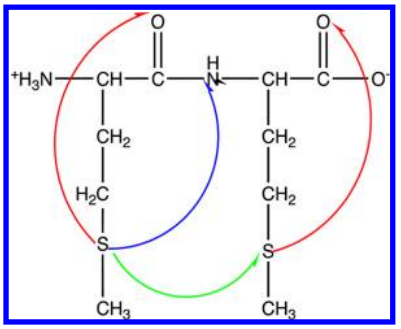
We used the relatively small basis set 6-31G(d) (SB) that usually gives satisfactory results for the optimizations of such radicals. In addition, considering the experimental and the theoretical difficulties linked to measurements of redox potentials, we also performed some calculations with the greater basis set 6-311+G(2d,2p) (GB).

Solvation. Solvation effects were accounted for with the COSMO option for the polarized continuum model CPCM considering an aqueous environment. Some calculations were also performed with IEFPCM, which gave identical results.

We chose the set of initial conformations so that various pseudocycles from 5- to 9-membered cycles with a SN or SO bond could be formed in the three dipeptide radical cations (Chart 2). The intramolecular SS bond in MetMet was also considered. We added some conformations in which there was no proximity of S to O or N, e.g., GlyMet in chymotrypsin or in P67^{phox} (depicted in Figure 1). In order to take into account different situations of these small sequences in polypeptides or proteins, we considered both the zwitterionic forms (ZW) as models of C- or N-terminals and the 2-methylated species as models of protein sequences.

All structures were fully optimized in their molecular and in their radical cation states, to obtain adiabatic and not vertical redox potentials. Each located stationary point was checked by evaluating harmonic frequencies. We used the geometries obtained after these optimizations with BH&HLYP as benchmarks for the PBE0calculations.

Chart 2. The Various Possibilities of 2c−3e Bonds within MetMet[a]



[a]Blue: SN bonds. Red: SO bonds. Green: SS bond.

Redox Potentials. The one-electron reduction potentials were calculated as described in ref 26. Briefly the standard reduction potential of a dipeptide $RS^{\bullet+}/RS$ couple, relative to the normal hydrogen electrode (NHE), $E°(RS^{\bullet+}/RS)$, is defined by eq 1:

$$E°(RS^{\bullet+}/RS) = -(\Delta G_{(aq)S} - \Delta G_{(aq)NHE})/F \quad (1)$$

where F is the Faraday constant, F = 96,485 C mol^{-1}, $\Delta G_{(aq)NHE}$ is the free energy change for the standard hydrogen cell half-reaction,

$$H^{+}_{(aq)} + e^{-} \rightarrow (1/2)H_{2(g)}, \quad \Delta G_{(aq)NHE} = -418\ \text{kJ mol}^{-1}$$

(ignoring the electron).[27] $\Delta G_{(aq)S}$ is the calculated free energy change for reaction 2, again ignoring the electron.

$$RS^{\bullet+}_{(aq)} + e^{-} \rightarrow RS_{(aq)} \quad (2)$$

For each dipeptide, the Gibbs energies ΔG_1 and ΔG_2 (in kJ·mol^{-1}) of molecule and cation species, respectively, relative to the molecule of lowest energy (taken as reference, 0 kJ·mol^{-1}) were calculated. $\Delta G_{(aq)S}$ is thus equal to $\Delta G_1 - \Delta G_2$.

Calculations were performed with the Gaussian09 package.[28] Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081).[29]

EXPERIMENTAL RESULTS

Met is irreversibly oxidized on a boron-doped diamond electrode, the corresponding wave onset (value at which the current starts increasing) and anodic peak (value at which the maximum current is reached) potentials being E_{onset} = 1.270 V and E_{pa} = 1.520 V, respectively (Figure 2). The MetMet E_{onset} coincides with that of Met, hinting that the same chemical group (probably −SCH$_3$) undergoes oxidation in these two species and peaks at E_{pa} = 1.650 V. Indeed there is no electrochemical response with Gly alone (not shown). The current magnitude for the MetMet wave is roughly 2-fold that of Met, as one would expect for a molecule having two −SCH$_3$ groups. GlyMet, like the previous two compounds, starts being oxidized at 1.270 V (Table 1), and its less intense wave when compared to that of Met may be explained by a smaller diffusion coefficient. MetGly is more difficult to oxidize that GlyMet, which is confirmed by both the wave onset potentials (E_{onset} = 1.370 V vs E_{onset} = 1.270 V) and the anodic peak potentials (E_{pa} = 1.750 V vs E_{pa} = 1.570 V, Table 1). Surprisingly, the anodic peak current, I_{pa}, is higher (≈50%) for

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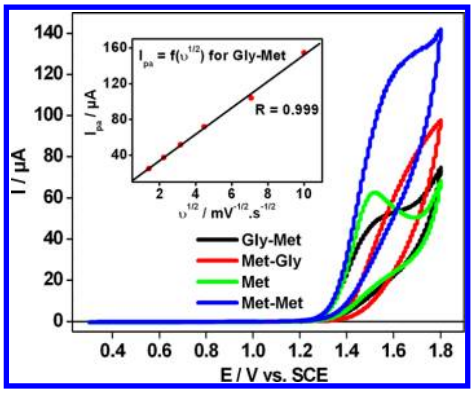

Figure 2. Cyclic voltammograms of 1 mM solutions of Met, GlyMet, MetGly, MetMet and Met in sodium perchlorate 0.1 M, pH = 2.0, obtained on a boron-doped diamond working electrode at 10 mV s^{-1}. The inset shows the dependence of I_{pa} on $v^{1/2}$ for GlyMet.

Table 1. Compilation of E_{onset} and E_{pa} for All Studied Compounds

compound	E_{onset} (V vs SCE)	E_{pa} (V vs SCE)
Met	1.27	1.52
MetMet	1.27	1.65
GlyMet	1.27	1.57
MetGly	1.37	1.75

MetGly than for GlyMet, a result which is not easy to rationalize.

The inset in Figure 2 shows the dependence of the anodic peak current, I_{pa}, on the square root of the scan rate, $v^{1/2}$, for the dipeptide GlyMet. The linear dependence indicates that diffusion controls the mass transport to the electrode surface, and a similar behavior was observed for all the other species studied. It was not possible to determine the redox potentials of these peptides because none of their voltammograms were reversible. The difference in the anodic peak potentials would indicate that the redox potential of MetGly would be ca. 0.2 V higher than that of GlyMet.

THEORETICAL RESULTS

"Redox couples" made of a dipeptide and its radical cation were constructed by optimizing first one entity of the moiety (either the molecule or the radical) and then the second one starting from the optimized structure of the first partner of the couple. In a preceding work, we had identified several stable configurations of the cyclic radical cations coming from the same three dipeptides containing methionine.[30] In this work we have also considered extended peptide conformations for radicals and parent molecules, i.e., conformers in which the shortest SX distance was larger than 4 Å. Zwitterionic and methylated (at the N and C terminal atoms) structures were all considered in water except for tests of the influence of dielectric constant. It should be remembered that the dielectric constant of protein interiors is not homogeneous. It varies according to several factors like the presence of charged side chains etc. According to several authors, its value can be either as low as 2 or much higher than that of water.[31,32]

We have adopted a nomenclature for the various cyclizations based on the geometries of the radical cations. Whenever they involved pseudocycles of x = 5 to 9 atoms resulting from the bonding of S−N of the peptidic link, they were denoted SNxp. SOxp means an existing S−O bond with the peptidic oxygen, SOxc, with oxygen of the carboxylate group (Chart 2). Whenever there was no 2c−3e bond, the structures were considered as extended and noted "Ext".

The absolute Gibbs energies obtained at the BH&HLYP or PBE0/6-31G(d) level are in Table S2 in the Supporting Information. In Table S3 in the Supporting Information the values obtained with both methods and the 6-311+G(2d,2p) basis set are gathered.

Table 2. Gibbs Energies (in kJ·mol^{-1}) of Zwitterionic Molecules and Cations Computed with BH&HLYP or PBE0 Methods[a]

	BH&HLYP			PBE0		
	molecule	cation		molecule	cation	
	ΔG_1	ΔG_2	$\Delta\Delta G_2$	ΔG_1	ΔG_2	$\Delta\Delta G_2$
GlyMet SN5p1	0.00	532.69	0.00	11.46	552.71	41.86
GlyMet SO7p1	25.81	599.00	66.31	0.00	(−CO_2)	
GlyMet SO7p2	27.51	599.27	66.58	16.00	575.61	64.76
GlyMet SN5p2	32.47	552.92	20.23	10.94	(−CO_2)	
GlyMet SO6c1	34.05	542.16	9.47	17.21	510.85	0.00
GlyMet SO6c2	65.70	570.78	38.09	49.94	540.59	29.74
MetGly Ext	0.00	621.67	13.57	0.00	629.43	30.95
MetGly SO6p1	10.28	608.11	0.0	11.02	598.48	0.00
MetGly SO6p2	23.82	623.69	15.59	22.11	621.59	23.11
MetGly SO9c	36.85	619.81	11.70	32.37	605.32	6.84
MetGly SN6p1	45.27	639.50	31.40	47.58	624.39	25.91
MetGly SN6p2	65.00	679.92	71.82	62.24	663.16	64.68
MetMet SN5p1	0.00	551.76	0.00	39.45	(−CO_2)	
MetMet SS	35.99	565.98	14.22	18.10	583.79	17.65
MetMet SN5p2	45.82	591.24	39.48	0.00	566.14	0.00
MetMet SO6p	57.08	623.34	71.58	43.88	606.32	40.18
MetMet SO9c	63.90	551.89	0.13	48.52	588.17	22.03
MetMet Ext	84.75	693.85	142.09	74.75	(−CO_2)	

[a] ΔG_1 and ΔG_2 are relative to the molecule of lowest energy (taken as reference). $\Delta\Delta G_2$ are relative to the cation of lowest energy. (−CO_2) means loss of CO_2 (see the text).

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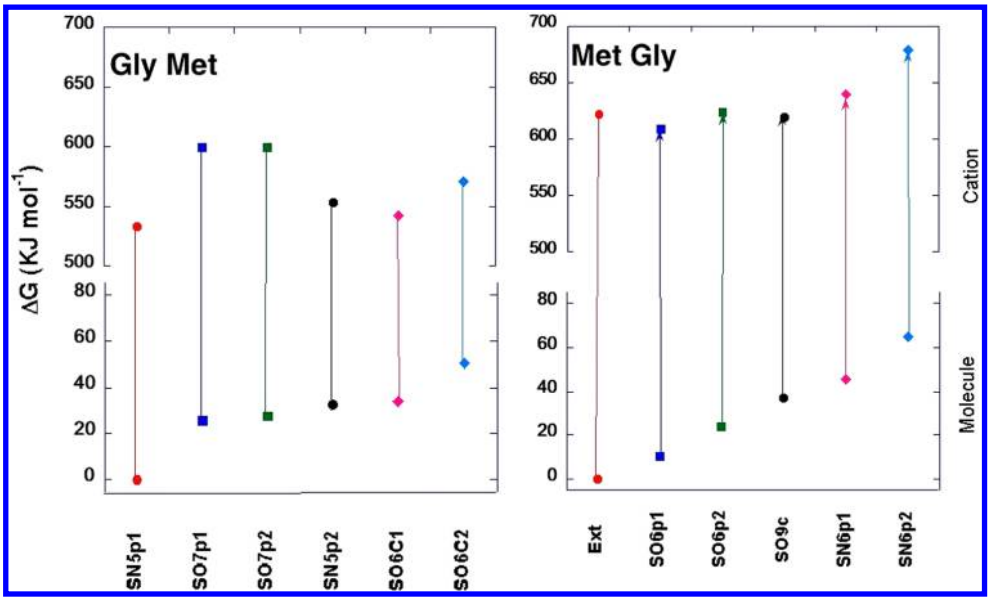

Figure 3. Relative Gibbs energies of the optimized molecules and radical cations coming from the dipeptide GlyMet (left) and MetGly (right) calculated at the BH&HLYP/6-31G(d) level. Lower scale: molecule. Upper scale: cation.

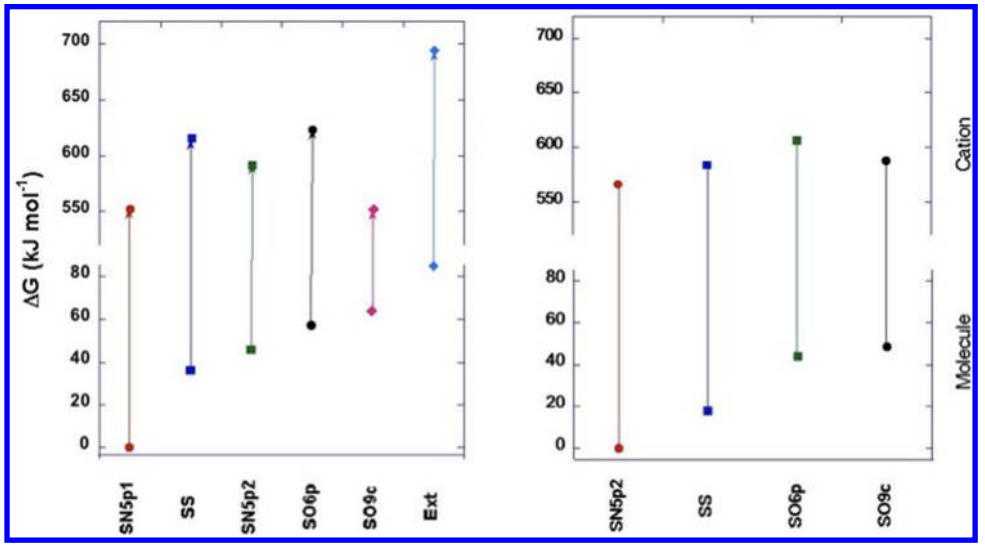

Figure 4. Relative Gibbs energies of the optimized molecules and radical cations coming from the dipeptide MetMet. Lower scale: molecule. Upper scale: radical cation. Left: BH&HLYP. Right: PBE0. Basis set 6-31G(d).

Small Models. In order to validate our calculations, we have first computed the redox potential of the dimethyl sulfide $DMS^{\bullet+}$/DMS couple, for which experimental values have been measured. The absolute energies are reported in Table S1 (Supporting Information). We found, respectively with SB and GB, 1.70−1.73 V (BH&HLYP) and 1.76−1.79 V (PBE0) vs NHE, which compares adequately with the experimental value (1.66 V[12]). As for methionine amino acid, computation gave 1.51−1.65 V (BH&HLYP) and 1.58−1.88 V (PBE0) vs NHE, which are also in the range expected.

Zwitterionic Dipeptides. SB. *Structures and Gibbs Energies.* The Gibbs energies ΔG_1 and ΔG_2 (in kJ·mol^{-1}) of all species relative to the molecule of lowest energy (taken as reference, 0 kJ·mol^{-1}) obtained with both BH&HLYP and PBE0 methods and the 6-31G(d) basis set are gathered in Table 2. Moreover the Gibbs energies of the cations $\Delta\Delta G_2$ relative to the cation of lowest energy were added. For the three dipeptides the Gibbs energies of the various couples (parent molecules − radical cations) are compared in Figures 3 and 4. The optimized geometries of some couples together with the SX bond lengths and the redox potentials are displayed in Tables 3−5.

BH&HLYP. The global shapes of each molecular structure were generally conserved upon one-electron withdrawal (Tables 3−5). As expected, the SN or SO bonds were always shorter in the cations in which the formation of a 2c−3e bond took place. In some cases the sulfur atom was as close to a nitrogen atom as to an oxygen in the molecular and in the radical compounds. Generally, whatever the dipeptide, the most stable molecule conformers did not lead to the most stable cation conformers (Figures 3 and 4).

GlyMet. The structure of lowest energy of the dipeptide molecular conformers is that involving a pseudocycle of 5 atoms and a hydrogen bond between the carboxylic and the amine groups (SN5p1, Table 3), which stabilizes this structure (SN5p2 being around 30 kJ·mol^{-1} above it). The extended

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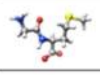
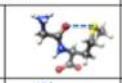
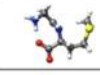
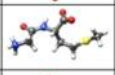
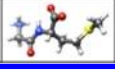
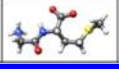
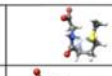
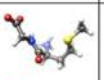
Table 3. Optimized Geometries of Some GlyMet Redox Couples Obtained with BH&HLYP/6-31G(d), Together with the SX Bond Lengths of the 2c−3e Bonds in the Cations When Present and the One-Electron Reduction Potentials Computed with BH&HLYP/6-31G(d) or PBE0/6-31G(d)[a]

	Molecule	Cation	d(SX) (Å)		E°vs NHE (V)	
	BH&HLYP		BH&HLYP	PBE0	BH&HLYP	PBE0
SO7p1			2.56	-CO_2	1.61	
SO7p2			2.53	2.47	1.59	1.47
SN5p1			2.65	2.66	1.19	1.28
SN5p2			2.44	-CO_2	1.06	
SO6c1			2.32	2.35	0.80	0.78
SO6c2			2.31	2.35	0.90	0.75

[a]The geometries obtained with PBE0 were very similar. −CO_2: the radical cation was not stable and underwent decarboxylation.

Table 4. Optimized Geometries of Some MetGly Redox Couples Obtained with BH&HLYP/6-31G(d), Together with the SX Bond Lengths of the 2c−3e Bonds in the Cations When Present and the One-Electron Reduction Potentials Computed with BH&HLYP/6-31G(d) or PBE0/6-31G(d)[a]

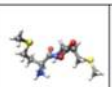
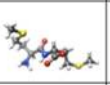
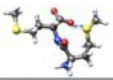
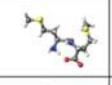
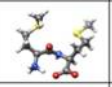
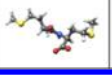
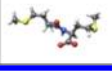
	Molecule	Cation	d(SX) (Å)		E° (V *vs.* NHE)	
	BH&HLYP		BH&HLYP	PBE0	BH&HLYP	PBE0
SN6p2			2.60	2.64	2.04	1.90
SO6p1			2.53	2.45	1.86	1.88
SO9c			2.37	2.41	1.71	1.61
Ext.			-	-	2.11	2.19

[a]The geometries obtained with PBE0 were very similar.

structures of molecules, which lead to the SO7p cations, have ΔG_1 of 26−27 kJ·mol^{-1} above the minimum whereas the SO6c rings are in a range of ΔG_1 of 34−66 kJ·mol^{-1}.

As for the cations, the most stable conformer has still a SN5p1 structure with a SN bond relatively long (2.65 Å) and keeps an intramolecular hydrogen bond between the carboxylate and the amine groups. Conformation SN5p1 is followed by SO6c1 with a $\Delta\Delta G_2$ value higher by 9.47 kJ·mol^{-1} relative to the minimum. GlyMet SN5p2 is 20 kJ·mol^{-1} higher than GlyMet SN5p1. It has no intramolecular hydrogen bond but a shorter SN bond length (2.44 Å). The other SO cations have higher relative Gibbs energies (SO6c2, 38 kJ·mol^{-1}; SO7p structures, 66 kJ·mol^{-1}). The SO bond lengths are around 2.50 Å in the SO7 structures and are equal to 2.31 Å in the SO6c ones. It is noteworthy that the S∴O$^+$ compounds are more

Table 5. Optimized Geometries of Some MetMet Redox Couples Obtained with BH&HLYP/6-31G(d), Together with the SX Bond Lengths of the 2c−3e Bonds in the Cations When Present and the One-Electron Reduction Potentials Computed with BH&HLYP/6-31G(d) or PBE0/6-31G(d)[a]

	Molecule	Cation	d(SX) (Å)		E° vs. NHE (V)	
	BH&HLYP		BH&HLYP	PBE0	BH&HLYP	PBE0
SO6p			2.47	2.42	**1.54**	**1.50**
SO9c			2.35	2.41	**0.73**	**1.26**
SN5p1			2.65	2.66	**1.32**	**1.54**
SS			3.04	3.04	**1.68**	**1.53**
Ext			—	-CO_2	**1.98**	

[a]The geometries obtained with PBE0 were very similar. −CO_2: the radical cation was not stable and underwent decarboxylation.

stable when the positively charged sulfur is linked to the carboxylate group O, which is negatively charged, instead of the peptidic one.[12,23] Interestingly, the selected extended conformations all led to SO7p structures.

MetGly. The geometries of some couples are shown in Table 4. In the molecular dipeptides, the "extended" conformations were the most stable ones, especially when they exhibited an intramolecular H-bond between the NH_3^+ and CO_2^- terminal groups. Again, starting from the molecular compounds going to the 2c−3e cations, the SO and SN distances decreased (from 3.6 to 3.8 to 2.6 Å) keeping similar pseudocyclic structures.

As for the MetGly cations, the SO6p1 one with bond length of 2.53 Å is the most stable one, followed within 16 kJ·mol^{-1} by SO9c (2.37 Å), the Ext conformation and SO6p2. The SN cycles exhibit much higher $\Delta\Delta G_2$, up to 72 kJ·mol^{-1} (Figure 3).

Comparing GlyMet to MetGly cations, one can see that the most stable structures are GlyMet SN5p1 and MetGly SO6p1. It should be noted that there is a large difference in the energies of the cations. Generally, those coming from one-electron withdrawal from GlyMet exhibit lower energies than those coming from MetGly (Figure 3).

Met Met. As in GlyMet the most stable molecule remains the SN5p1 followed by the SS and the SN5p2 ones (with ΔG_1 = 36 and 46 kJ·mol^{-1}). The SO structures are less stable, SO6p and SO9c being respectively 57 and 64 kJ·mol^{-1} above the minimum energy. The extended structure is the less stable one (ΔG_1 = 85 kJ·mol^{-1}).

As for the cations, several different cyclic structures have very close Gibbs energies: the SN5p1 and SO9c are quasi isoenergetic, followed by SS 14 kJ·mol^{-1} above the SN5p1 cation. As for the molecule, the cation of MetMet Ext is the less stable ($\Delta\Delta G_2$ = 142 kJ·mol^{-1}). As for the SX distances, the same trend as for the other dipeptides is observed: they decrease upon electron withdrawal from ca. > 3 Å to ca. 2.6 Å (Table 5). However, for a few couples (SN6 and SO9c) the

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Table 6. Gibbs Energies (in kJ·mol^{-1}) of the Three Dimethylated Molecules and Cations and Redox Potentials in V Computed at the BH&HLYP or PBE0/6-31G(d) Levels[a]

	BH&HLYP				PBE0			
	molecule ΔG_1	cation ΔG_2	cation $\Delta\Delta G_2$	$E°$	molecule ΔG_1	cation ΔG_2	cation $\Delta\Delta G_2$	$E°$
GlyMet Ext	0.0	588.30	6.48	1.76	0.0	539.51	0.0	1.26
GlyMet SO7p	4.54	581.82	0.0	1.65	4.72	574.95	35.44	1.58
GlyMet SO6c	11.17	582.22	0.40	1.59	11.96	580.14	40.63	1.56
GlyMet SN5p1	20.07	586.57	4.75	1.51	19.49	575.48	35.97	1.43
MetGly SN6p1	0.0	585.86	0.0	1.49	0.0	581.98	0.0	1.42
MetGly SO9c	3.91	586.99	1.13	1.71	4.46	592.63	10.65	1.76
MetGly SN6p2	28.14	624.05	38.19	1.84	28.04	613.38	31.40	1.73
MetGly Ext	51.54	644.62	6.48	1.81	53.64	605.85	23.87	1.39
MetGly SO6p	90.52	651.88	66.02	1.49	91.99	646.50	64.52	1.41
MetMetSO9c	0.0	587.28	23.35	1.75	0.0	589.59	36.86	1.78
MetMet SN5p2	2.36	589.46	25.23	1.75	7.34	580.98	28.25	1.61
MetMet Ext	14.71	588.77	24.84	1.62	16.31	552.73	0.0	1.23
MetMet SN5p1	19.72	583.34	19.41	1.51	21.17	576.87	24.14	1.43
MetMet SO6p	30.61	563.93	**0.0**	1.20	34.05	559.32	6.59	1.11
MetMet SS	34.39	586.91	22.98	1.39	34.89	571.70	18.97	1.23

[a] ΔG_1 and ΔG_2 are relative to the molecule of lowest energy (taken as reference). $\Delta\Delta G_2$ are relative to the cation of lowest energy.

global geometry is modified going from the peptide to the cation.

Redox Potentials. Their values are summarized in Tables 3−5. The values obtained for MetGly fall in a relatively narrow range (1.7−2.1 V) (Table 4). However, for GlyMet and MetMet they exhibit strong variations and some values are far away from this range. For instance, redox potential of the MetMet SO9c couple was much smaller (0.73 V, Table 5). Similarly, that of GlyMet SO6c was equal to 0.80 V (Table 3). Furthermore, one can establish a rough scale for the different dipeptides based on the increasing $E°$ values: GlyMet < MetMet < MetGly. This perfectly matches the trend observed in cyclic voltammetry for E_{pa}.

PBE0. Generally the results are in good agreement with most of those obtained with the BH&HLYP functional (Table 2 and Table S2 in the Supporting Information).

GlyMet. There is a striking difference in some cations for which there is no stabilization but a loss of CO_2. The redox potentials $E°$ for SN5p1, SO6c and SO7p2 are in a relatively large range, from 0.8 V (SO6c1) to 1.5 V (SO7p2) as with BH&HLYP, the most important differences between both functional results being of 0.1 V (Table 3).

MetGly. Results are similar to those of BH&HLYP. All cations are as stable as they were with BH&HLYP; there is no decarboxylation. As shown in Table 4, the redox potentials are higher than those of GlyMet (from 1.6 to 2.2 V). There is a gap <0.1 V except for SN6p1 (0.2 V).

MetMet. As in GlyMet, some radicals dissociate by losing a CO_2 (SN5p1 and Ext). The $E°$ values are closer to each other (from 1.3 to 1.5 V, Table 5) than those of GlyMet (0.8−1.5 V, Table 3). These results are markedly different from those calculated with BH&HLYP, which are more dispersed (from 0.7 to 1.7 V). In addition, for MetMet SN5p1 $E°$ is higher with PBE0 (1.5 V) than with BH&HLYP (1.3 V), whereas it is the opposite for MetMet SS (1.5 vs 1.7 V, respectively, Table 5).

Methylated Dipeptides. SB. The Gibbs energies (in kJ·mol^{-1}) and redox potentials are summarized in Table 6. Again, for each dipeptide the most stable molecular structures are taken as references. Their absolute energies are in Table S4 in the Supporting Information with the SX distances.

Structures and Gibbs Energies. As in zwitterions, whenever possible the sulfur atom was closer to a heteroatom in the radical cation than in the molecule. Nevertheless the radical cations SO6c of GlyMet and SO9c of MetGly and MetMet species have longer SO distances than for zwitterions, because the SO bond is no more neutral, it bears a positive charge. Furthermore, there are large changes in the energies and in the optimized geometries. For example the SN5p2 structure of the GlyMet cation does not exist anymore; after optimization it becomes SO7p (BH&HLYP) or an extended one (PBE0). Some structures of GlyMet are compared to the zwitterionic ones in the Supporting Information (Figure S1).

BH&HLYP. GlyMet. The Gibbs energy differences are less important than for zwitterions, the largest ΔG_1 for the molecules being 20 kJ·mol^{-1} for SN5p1. It is the same for the cations ($\Delta\Delta G_2 \leq 6.5$ kJ·mol^{-1}). The most stable molecular conformation is Ext followed by SO7p (4.54 kJ·mol^{-1} above Ext) (Table 6). The intramolecular H bond between the carbonyl and the amine groups remained in SN5p1, but it did not bring stabilization as in the ZW species.

MetGly. The energies are much more dispersed than for GlyMet, up to 90 kJ·mol^{-1} for the molecules and up to 66 kJ·mol^{-1} for the cations (Table 6). For the cations as well as for the molecules, the order of stabilities is roughly the same: the SN6p1 structure is the most stable, followed closely by the SO9c (3.91/1.13 kJ·mol^{-1} above SN6p1). They are followed by SN6p2 for the molecule (28 kJ·mol^{-1}) and the extended conformation, Ext, for the cation (6.5 kJ·mol^{-1}), the highest energy conformer being the SO6p.

MetMet. The results are strongly different for this dipeptide, with respect to the other ones: the energy range is lower than 35 kJ·mol^{-1} for the molecules. The order of Gibbs energies is fully changed after oxidation: the SO9c is the most stable molecule whereas the SO6p cycle is the most stable cation. Surprisingly, all the cation energies are very close to each other, around 23 kJ·mol^{-1}, except for the most stable SO6p.

PBE0. As it could be expected, when the C terminal is methylated there is no more decarboxylation (Table 6). It is important to note that the order of stability for molecules is the same as for BH&HLYP. It is not the case for the cations except

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Table 7. Energies (a. u.), SX Bond Lengths (Å) and Redox Potentials (V) of the GlyMet SN5pep1 molecule and of Its Radical Cation with 2Me in Different Solvents

	BH&HLYP			PBE0		
	molecule	cation	$E°$ (V)	molecule	cation	$E°$ (V)
in water (ε = 78.4)	−1086.51997	−1086.30399	1.54	−1086.01507	−1085.80310	1.43
	SN 3.42	SN 2.65		SN 3.39	SN 2.62	
ε = 7.4	−1086.51738	−1086.29339	1.76	−1086.01273	−1085.79289	1.64
	SN 3.42	SN 2.64		SN 3.39	SN 2.61	
ε = 2.2	−1086.51205	−1086.26717	2.32	−1086.00740	−1085.77828	1.90
	SN 3.42	SN 2.62		SN 3.42	SN 3.18	
in vacuum (ε = 1.0)	−1086.50604	−1086.22474	3.31	−1086.00237	−1085.73241	3.01
	SN 3.44	SN 2.61		SN 3.41	SN 3.13	
	SO 3.53			SO 3.54		

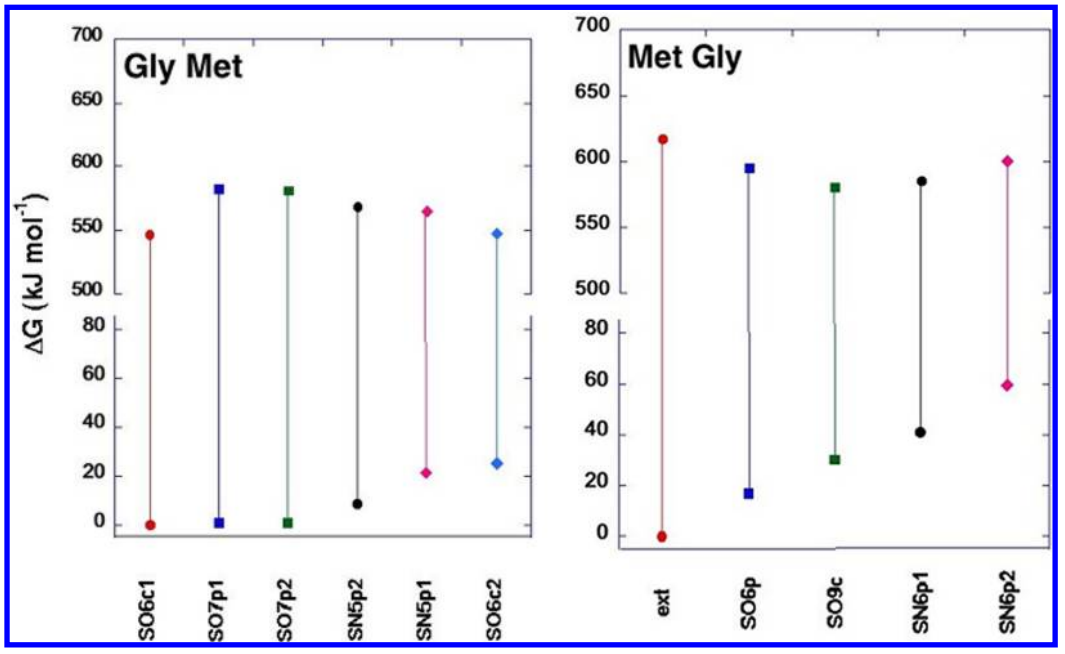

Figure 5. Relative Gibbs energies of the optimized molecules and radical cations coming from the zwitterionic dipeptides GlyMet (left) and MetGly (right). Lower scale: molecule. Upper scale: cation. BH&HLYP/6-311+G(2d,2p).

for those of MetGly. For GlyMet, the energetic gap between the most stable conformer and the other ones is six times larger.

Redox Potentials. Though the ΔGs are very different from one dipeptide to another, the $E°$ values are globally less disperse for the various conformations. They are in a range of 1.5−1.8 V within the BH&HLYP method (except for MetMet SS and MetMet SO6p, respectively 1.4 and 1.2 V). The range is similar with PBE0 (1.4−1.8 V). Some conformations exhibit lower values: MetMet SO6p (1.1 V), GlyMet Ext, MetMet Ext and MetMet SS (around 1.2 V).

Influence of the Dielectric Constant. The methylated entities being considered as models of protein sequences, they could be inside a protein matrix. The dielectric constant of a protein is still a matter of debate, but it is generally admitted that it should be much lower than that of water. Thus, we have reoptimized the dipeptide GlyMet in its SN5pep1 conformation using three values of the dielectric constant (7.4, 2.2 and 1) (Table 7). As expected, the hydrogen bond between the −NH and the −CO groups (well preserved in ZW as well as in methylated compounds in water) is no more present in all the other environments.

Their redox potentials increase only by 0.2 V upon decreasing the dielectric constants from 78 to 7. However they become extremely high for very low ε values, i.e., in very apolar media.

Zwitterionic and Methylated Species. GB. The results are summarized in Tables S4 and S5 in the Supporting Information. The calculation of frequencies became very time-consuming with the basis considered (6-311+G(2d,2p)), especially for the MetMet methylated species. Thus, this basis is probably the highest level that we can reach for the redox potential calculations.

The most important effects occurred for the dipeptides in their ZW form (Table S3 in the Supporting Information and Figure 5). No decarboxylation of cations was observed. The energies of molecules are closer to each other for GlyMet and MetGly. Similarly, the energy range for cations is also reduced compared to SB. As a consequence, the $E°$ values are less disperse. In addition, they are comparable with both BH&HLYP and PBE0 methods (for GlyMet, 1.3−1.7 V vs 1.2−1.7 V respectively; for MetGly, 1.7−2 V vs 1.6−1.9 V). The redox potentials are more sensitive to the method for MetMet: 1.4−2 V vs 1.4−1.7 V. The rough scale for the different dipeptides based on the increasing $E°$ values is the same as with SB: GlyMet < MetMet < MetGly.

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As for methylation, the increase of the basis set had no noticeable effects; for instance the E° values for all the dipeptides are globally in a short range except MetGly SN6p2 (E° is lowered by ca. 0.6 V), MetGly SN6p1 and MetGly SO6p1 (E° increases by 0.2 V) (Table 6 and Table S5 in the Supporting Information). The influence of the dielectric constant is also very weak.

In conclusion, we have used the method described in Computational Methodology to evaluate the redox potentials of the three dipeptides. As explained earlier, there is a methodological problem with radicals involving 2c−3e bonds. Therefore, we chose BH&HLYP and in addition we tested PBE0. We used the basis set of moderate size 6-31G(d), which gives accurate results for conformational analysis. Since no method was established for redox potentials, we compared the results obtained with this relatively small basis to those obtained with a more sophisticated basis 6-311+G(2d,2p). For such a large dipeptide as MetMet it is the upper limit.

Turning to dipeptides, we have built redox couples, computed the thermodynamical values related to each partner of the couple and deduced the redox potentials. Using SB, a striking difference between both methods is that with BH&HLYP all radical cations were stable, whereas with PBE0 some GlyMet and MetMet radicals underwent decarboxylation. However, this was not confirmed with the larger basis. Thus both functionals coupled with both basis sets gave different information and none of them can be excluded for such problems.

Adding methyl groups to both ends did not change much the geometries of molecules and radicals (see for examples Supporting Information, Figure S1). The optimized structures of some molecular and cationic species are similar to some encountered in the PDB (Figure 1). For instance, GlyMet from chymotrypsin is close to GlyMet SO7p2 while MetGly of the glycoprotein of herpes has similarities with SN6 structures and MetMet from Akt could make a SS bond if oxidized (Tables 2−4).

It was interesting to note that the most stable conformations of the peptides did not always lead to the most stable radical cations (Figures 3 and 4). The hydrogen bond between the amine and the carbonyl terminal groups was an important but not essential factor in the stabilization of all dipeptide molecules. This comment is also valid for zwitterionic cations (Tables 3−5). Indeed, apart from the distance changes, the global geometries of the dipeptides are roughly conserved upon electron withdrawal.

DISCUSSION AND CONCLUSION

The one-electron oxidation of methionine residue in peptides and proteins has been studied for more than 30 years, and still there are many uncertain aspects in the mechanism. As for the first step, the formation of inter- or intramolecular 2c−3e bonds involving the sulfur radical cation has been shown decades ago (for review see ref 33 and references therein). Several experimental studies based on oxidation by pulse radiolysis or flash photolysis were devoted to the characterization of the resulting S.∴X bonds in peptides or in small model compounds. These results prompted theoretical calculations to ascertain the identification of the free radicals.

The aim of this work was to evaluate the one-electron redox potential of methionine in simple dipeptides (L-Met L-Gly, L-Met L-Met). Moreover, through the conformational variations, it led also to appreciate the reorganization linked to electron transfer.

Attempts to measure the redox potentials by electrochemistry led to peak potentials in model compounds that proved to vary considerably with the surrounding of the thioether function.[34,35] However, they are not fully conclusive as far as one-electron redox processes are concerned, even if in our experimental conditions it is clear that the oxidation of GlyMet and MetMet is easier than that of MetGly. Actually the irreversibility of the electrochemical waves can be explained by some experimental results. It has been shown that the sulfur-centered radical cation can be delocalized on the vicinal carbon atoms. Moreover loss of CO_2 induced by oxidation has been observed, which might indicate a very fast electron or hole transfer to the carboxylate function followed by a C−C bond breaking. The occurrence of this fast process is in full agreement with the irreversibility of electrochemical waves. Interestingly, optimization of some radical cations with PBE0 and SB led to results in agreement with this process. However all experimental results by pulse radiolysis (see for example refs 12, 33, and 36 and references therein) and the main final compound, methionine sulfoxide,[5] are only compatible with a sulfur-centered radical, which should be the major mesomeric form of the radical cation. This is in agreement with all calculations performed up to now (see for example ref 12 and references therein).

One might try to predict the ease of oxidation by looking at the atoms in the neighborhood of the sulfur atom. Our results show that it is not so simple and that results might vary with the level of theory. Nitrogen as well as oxygen vicinity can lead to the most stable radical. The nature of the atom involved in the 2c−3e bond does not help to rationalize the scale of redox potential. Of course, in proteins, one should take into account the other residues in the vicinity of the thioether function that may provide other partners for 2c−3e bonds and constraints in mobility inherent to a polymer.

Some energy differences between molecule conformations are extremely high (up to 90 kJ·mol^{-1}), which might indicate that these structures cannot be encountered at room temperature. However, in proteins this difference can be compensated by other interactions.

The redox potentials of the dipeptides were in the range expected around that of methionine amino acid. In some conformations it reached lower (1.3 V) or much higher values (2 V). Experimental results indicated such possible variations.[3,17−20] However, in proteins other phenomena take place, like intramolecular electron transfers that could complicate the interpretation of results. Electrochemistry showed that oxidation became more difficult according to the sequence GlyMet < MetMet < MetGly, and it is expected that the redox potentials follow this trend. This is in good agreement with the calculations, confirming the influence of the sequence on the redox properties of this residue.

These calculations also provide kinetic information. In many cases there were only slight geometry differences between the molecule and the radical even without the constraint of a protein polypeptidic chain surrounding the dipeptide (all GlyMet models, MetGly Ext, MetGly SO6p1, MetMetSS, MetMet Ext, MetMet SN5 etc.) (Tables 3−5). It would mean that oxidation would not perturb the global geometry of the peptide and that the geometrical changes induced by electron withdrawal might involve relatively low reorganization energy in the protein. Conversely, in other cases, there are greater

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changes in the global geometry linked to electron withdrawal (MetGly SO9c, MetGly SN6p, MetMet SO6p, MetMet SO9c) (Tables 3−5), which, in proteins, might increase the reorganization energy and hence slow down the oxidation reaction.

There is an uncertainty about the dielectric constant of a protein matrix. Recent results would indicate that in protein interiors it is not homogeneous. According to some authors, its value can be either as low as 2 or much higher than that of water,[31,32] average values being around 2−6. We thus investigated this range (1−8). Decreasing the dielectric constant from the most polar (water, 78.4) to vacuum (1) led to a redox potential increase up to ca. 3 V, rendering the residue much more difficult to oxidize. We have not considered dielectric constants higher than that of water. However it means that residues close to the surface can be more easily oxidized than those inside the protein matrix not only because of accessibility but also because of the polar surrounding.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1: comparison between optimized geometries of zwitterions vs methylated dipeptides for two couples. Table S1: absolute Gibbs energies and redox potentials of molecules and radical cations of DMS, $(DMS)_2$, Met and Met_2Me (basis 6-311+G(2d, 2p)). Table S2: absolute Gibbs energies and SX distances of molecules and radical cations of the three dipeptides in their zwitterionic states (basis 6-31G(d)). Table S3: absolute Gibbs energies and SX distances of molecules and radical cations of the three dipeptides in their zwitterionic states (basis 6-311+G(2d, 2p)). Table S4: absolute Gibbs energies and SX distances of molecules and radical cations of the three methylated dipeptides (basis 6-31G(d)). Table S5: absolute Gibbs energies and SX distances of molecules and radical cations of the three methylated dipeptides (basis 6-311+G(2d, 2p)). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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