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Oxidation of protein tyrosine or methionine residues:  
From the amino acid to the peptide

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Abstract. Methionine and tyrosine are competing targets of oxidizing free radicals in peptides or proteins. The first step is the addition of OH radicals either on the sulphur atom of methionine, followed by OH — elimination, or on the aromatic cycle of tyrosine. The next step can be stabilization of methionine radical cation by a two centre-three electron bond, or intramolecular electron transfer from tyrosine to the methionine radical cation. In this latter case a tyrosine radical is formed, which appears deprotonated. In a first step we have compared the stability of the OH radical adducts on Methionine or on Tyrosine. In agreement with experimental results, the thermodynamical data indicate that the OH adduct on Tyrosine and the radical cation are more stable than those on methionine. In a second step we have investigated the stabilization of the radical cations of Methionine by formation of intramolecular S:X two-center three-electron bond (X=S, N, O). Finally we have compared the spin densities on separated amino acids to that in a radical pentapeptide, methionine enkephalin. One observes a delocalisation of the orbital of the odd electron on the sulfur atom of Met and on the cycle of Tyr. The peptidic chain is also concerned.

1. Introduction
In oxidative stress, among other species the very strong oxidant OH radicals are formed. Their high reactivity renders them extremely deleterious. We are currently investigating the one-electron oxidation of peptides and proteins by experimental and theoretical methods. Experiments often show that among the most important targets of these radicals are the Methionine (Met) and Tyrosine (Tyr) residues. In particular, in neurodegenerative involved peptides and proteins (β amyloid in Alzheimer’s disease, α -synuclein in Parkinson’s disease, Prion protein), the oxidation of Methionine to its sulfoxide is a key event in their conformational change and thus in the development of the diseases. On the other hand, oxidation of Tyr residues in the natural opioid peptides enkephalins, leads to an increase of pain sensation.

The main lines of the mechanisms of the oxidation of these two residues are fairly well known (figure 1). In Methionine the first step would be the addition of OH radical followed by OH — elimination leading to the sulfur centred radical cation. The radical cation could be then stabilized by
forming a two-centre three-electron (2c-3e) bond with any electron-donating atom [1]. As for tyrosine, the first step is also the addition of OH on the cycle, but OH$^{-}$ elimination is slow at neutral pH. With other oxidants it can undergo oxidation giving its radical cation that deprotonates. When Methionine and Tyrosine are simultaneously present in a peptide, oxidation of methionine may be followed by intramolecular reduction by a tyrosine residue ending by the regeneration of Met and the formation of a tyrosinyl deprotonated radical. Depending on the way that is followed, the final compounds are peptide dimer or peptide with DOPA or with oxidized methionine.

Using various radicals as oxidants, this process was characterized in methionine enkephalin [2] (Figure 2), model peptides [3] and ribonuclease A [4]. Very recently this process was reinvestigated in model dipeptides [5] and it was proposed that intramolecular electron transfer needed some proximity of the residues. Preliminary results suggested that a tyrosyl residue (Tyr-125) adjacent to a methionine residue (Met-127) (figure 1) in alpha-synuclein underwent electron transfer. Conversely in thioredoxin [6], calmodulin [7], amyloid peptide [8], the methionyl 2c-3e radical was observed and no long-range intramolecular electron transfer was noticed. In another model system, it was suggested that methionine could serve as a stepping stone between an electron acceptor and Tyr as a donor [9,10]. Clearly, the role of methionine on protein tyrosyl oxidation deserves further investigation.

\[\text{Figure 1. Main steps of the oxidation by OH radicals of a peptide containing Met and Tyr.}\]

In this paper, our aim is to make a preliminary thermodynamical analysis of the oxidation of a typical peptide containing Methionine and Tyrosine, Methionine enkephalin (Met enk) (figure 3) by OH radicals. In this peptide, the intramolecular step was not rate-limiting because it was too fast [11], and is noticed only by the appearance of the tyrosinyl deprotonated radical. We chose among the 80 structures in the Protein Data Bank (code 1PLW) a geometry in which Methionine and Tyrosine are close to each other (figure 2). This geometry is similar to that encountered in peptides involved in neurodegenerative diseases (e.g. in alpha synuclein). We have already studied the OH addition to both amino acids [12], thus in this paper we considered the amino acids as residues, i.e. we blocked both the carboxylate and the amine functions (figure 3).
We began the study by considering the first step of the oxidation, i.e. the addition of OH on Met and Tyr separately, and the direct electron transfer reaction between OH radicals and the amino acids (reactions (1) and (2)).
Then we examined some possible fates of Met radical cations. (i) The formation of intramolecular 2c-3e bond that stabilized the entity as it was suggested several times. This was done in some dipeptides. (ii) The fate of the methionine radical if such a stabilization does not occur because of geometrical constraints. Thus we analysed the various conformations of Met enkephalin and selected one in which the sulfur atom is not close to any nitrogen or oxygen atom that could possibly make a 2c-3e bond. This conformation was optimised and the ionisation and the oxidation reactions were studied like for the separated amino acids.

2. Computational methods
The geometries, enthalpies ($\Delta H$) and Gibbs energies ($\Delta G$) of the derivatives of Tyr A and Met B (Figure 3) and products (their adducts and radical cations) were calculated with (U)B3P86/6-31+G(d,p). Enthalpies and Gibbs energies took into account temperature-dependent corrections (zero point energy (ZPE), translational, rotational and vibrational energies) at 298K. All the different ground states were confirmed by vibrational frequency analysis, i.e. no imaginary frequency. The same method was used for the Met Enkephaline and its radical cation.

As for the 2c-3e radical cations the bond lengths and the binding energies are overestimated except with the BH&HLYP functional [13]. We thus privileged the BH&HLYP functional in the spin-unrestricted formalism and used the standard 6-31G(d) basis set. The use of this relatively small basis set is justified by the fact that DFT methods are not very basis-set dependent. All structures were fully optimized using the analytical gradient technique, and the nature of each located stationary point was checked by evaluating harmonic frequencies. When considered, the effect of solvation was taken into account using a continuum model (CPCM). The drawings of Figure 2 and 8 were obtained using the free software Chimera [14].

3. Results

1.1. OH adducts on Tyr and Met
$\Delta H$ (and $\Delta G$) of the addition reaction was $\Delta H_{\text{addition}} = \Delta H (\text{adduct}) - \Delta H (\text{molecule}) - \Delta H (\cdot \text{OH})$. The $\cdot \text{OH}$ addition was thermodynamically favoured on the aromatic ring of Tyr. Depending on the substituent the reaction Gibbs enthalpy ranged from -16 to -10 kcal/mol in vacuum and in water. The most stable stereoisomer of Tyr-OH adduct is shown in figure 4.

![Figure 4. The most stable configuration of the OH adduct on Tyrosine is stabilized by an intramolecular hydrogen bond (dotted line) between the OH group and the amine function.](image-url)
As for methionine, the OH-adduct is less favoured (Gibbs energy of -3.7 kcal/mol in vacuum), which is in agreement with experimental results by pulse radiolysis (i.e. very low (microsecond) and long (millisecond) lifetimes respectively for Met-OH and Tyr-OH adducts respectively [15]). This trend is confirmed in water.

The spin densities are mostly on the cycle in the Tyr adduct and shared between S and O of OH atoms in Methionine (figure 5). The delocalization in Tyr adducts explains the greater stability of these adducts.

![Figure 5](image)

**Figure 5.** Spin densities on the OH adduct (a), on the tyrosinyl radical (b), on methionine OH adduct (c).

1.2. Radical cations of Tyr and Met derivatives

Both radical cations are stable structures. The one formed from B is shown in figure 6. The ionisation potentials (IP) of both molecules are gathered in table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>Met enk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionisation Potential (eV)</td>
<td>8.40</td>
<td>8.75</td>
<td>7.53</td>
</tr>
</tbody>
</table>
Both IP are of the same order of magnitude, as expected, however the one of A is lower than that of B. The spin densities are split on several atoms. In B the spin density is mostly on the sulfur atom (0.62). In A, the spin density is mostly on the cycle (0.70, on the -COH bond and the C in para position).

As for the reaction of oxidation of each residue by OH radicals (reaction (2)) the energy variations are 141.9 and 150.1 kcal/mol respectively for A and B in vacuum. They follow the order of ionisation potentials. Including solvation strongly decreases these values as expected but the order remains the same. Globally it appears that it the reaction of OH is easier with the Tyr derivative than with the Met one, for the electron transfer as well as for the addition reaction. In Tyrosine, experimental results indicate that H atom abstraction from the OH group of Tyr by OH radical is not favoured. Conversely the radical cation undergoes very fast deprotonation of the phenol giving the tyrosinyl radical. With other oxidants, H atom transfer either direct or indirect (i.e., via (i) coupled electron-proton transfers or (ii) adduct formation followed by H2O release) leads to the same tyrosinyl radical. The O-H bond dissociation enthalpy (BDE) of Tyr is around 90 kcal/mol. The radical is stabilized by the spin density delocalization over the entire aromatic ring (figure 5). The spin density on the O-atom from where the H-atom was removed is relatively low (around 0.37).

1.3. Stabilization of the Met radical cations in peptides

The radical cations of Met could be stabilized by 2c 3e bonds with N or O atoms of the vicinal peptidic bond. The most stable are 5 membered cycles involving nitrogen of the peptidic bond or 6 membered rings of the peptidic bond (figure 7) [16]. The SN bonded entity is more stable than the SO one by 16 kcal/mol. It means that even with this stabilisation, the oxidation of Tyr is more favourable energetically.

![Figure 6. Radical cation from methionine derivative B.](image)

![Figure 7. Stabilisation of the sulfur-centred radical cation by a 2c-3e bond making a pseudo cycle of 5 atoms.](image)
1.4. **Insight into the one-electron oxidation of Met enkephalin**

There are 80 experimental conformations obtained by NMR in micelles (PDB code 1PLW). A conformational analysis of the enkephalins was performed [17,18]. The peptide backbone exists as a mixture of folded and unfolded forms (approximately 50% each). In the folded ones the Tyr and Met residues are close to each other (distance S…OH around 3.5 Å). We extracted some typical conformations that we have optimized and we show here the results obtained with a folded one (structure 50, figure 2), distance S…OH = 3.3 Å). The radical cation was optimised (figure 8). In this cation the folding has increased, but the distance S…O(phenol) remained of the same order of magnitude (3.8Å). This relatively large distance indicates that no 2c-3e bond was created between these two atoms.

The ionisation potential is much lower than that of the separated amino acids. Similarly the energy variation of the reaction of oxidation corresponding to Reaction (2) is lower than for the amino acids (121kcal/mol, Table 1). The spin density is remarkably delocalised. The S atom bears only 16% of the total spin density, 15 % is found on the cycle of Tyrosine and 30 % on the N-terminal amine, the rest being dispersed in the molecule.

![Optimised structure of Methionine enkephalin radical cation (starting point: structure 50 of PDB 1PLW, see figure 2).](image)

**Figure 8.** Optimised structure of Methionine enkephalin radical cation (starting point: structure 50 of PDB 1PLW, see figure 2).

4. **Conclusion**

In this work we compared the one-electron oxidation of two protein residues, tyrosine and methionine by OH radicals in derivatives and in a pentapeptide, Methionine enkephalin. In general, from a thermodynamical point of view, 'OH radicals may add more favourably on the aromatic rings of Tyr than on Met in agreement with results by pulse radiolysis. The radical cations are stable structures. However their spin densities are somewhat delocalised. Especially in the pentapeptide that we studied, the major part of the spin density is neither on the sulfur nor on the tyrosine residue. More investigations are necessary to understand the structure of the radicals coming from one electron abstraction on peptides.

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References


