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Tryptophan: Antioxidant or Target of Oxidative Stress? A Quantum Chemistry Elucidation

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The Density Functional Theory was used to investigate oxidative stress related reactions of tryptophan in its free zwitterionic form. It was concluded that free tryptophan cannot be considered as antioxidant, and that tryptophan residues in proteins are not especially good targets of oxidative stress. Its previously observed antioxidant abilities can then been attributed to tryptophan metabolites.

Tryptophan is an essential amino acid. Additionally to its function as building block of proteins, it has an important role as a precursor of serotonin and melatonin. In addition to these very well-known and important biomolecules, there are other less known species that are metabolites of tryptophan and that also play important biological roles.

It is assumed that tryptophan possesses antioxidant properties,[1-3] just to mention three examples. However this seems in apparent contradiction with the findings of Christen et al.[4] who proposed that low concentrations of some hydroxylated metabolites of tryptophan, but not tryptophan, scavenge peroxyl radicals with high efficiency. For in vivo studies the answer is straightforward, tryptophan metabolizes before acting as antioxidant. For those studies that use very reactive oxidants the reason could be that almost any organic molecule can be considered "antioxidant" against $^{\bullet}$OH radical. It remains the question for in vitro studies concluding that tryptophan substantially inhibits lipid peroxidation where the oxidant is not specified. Does this action occur via primary antioxidant activity i.e. directly trapping oxidants reducing them?

On the other hand since tryptophan is a building block of proteins, which play crucial role in huge amount of physiological processes, damaging via oxidative stress a tryptophan residue in a protein is an undesirable process. In other words, we do not want for a protein to act as an antioxidant, we need antioxidants to protect proteins from oxidation. This leads to another question, how sensitive to oxidative stress are tryptophan residues in a protein? Are they a particularly easy target for protein damage? Are there any differences between in the oxidative trends of tryptophan when bonded via peptide bond compared to free zwitterionic tryptophan?

It is also important to mention that using an appropriate protocol it is possible to selectively damage tryptophan[5-7] residue in a protein. Therefore this kind of damage, and its repair, has been experimentally studied. A theoretical study on the mechanisms of the damage (and eventually the repair) reactions seems to be an interesting research topic. It is particularly important to study if tryptophan is substantially more sensitive to oxidative stress than other amino acid residues in proteins, as can be inferred from studies in which it is selectively oxidized by the N_3 radical.

In recent years we have developed a quantum mechanics-based test for the overall free radical scavenging activity (QM-ORSA) protocol.[8] This computational protocol has been validated by comparison with experimental results, and its uncertainties have been proven to be no larger than those arising from experiments. More recently we have benchmarked several DFT approaches for radical molecules reaction in solution and we found that LC-ωPBE give results directly comparable with experiments.[9]

We aim in this work to use this powerful tool to study the reactions between two radicals $^{\bullet}$OH as a very reactive oxygen specie[10] and hydroperoxyl radical ($^{\bullet}$OOH) as mild, long lived, reactive oxygen specie very well known for his role in lipid peroxidation which occurs with a rate constant of 1.7 x 10^3 M^{-1} s^{-1}. We will consider tryptophan as an antioxidant if it can react with hydroperoxyl radical faster than lipids and therefore prevent peroxidation. We will perform this task studying all the possible mechanisms and channels

of reaction that could be involved in the oxidation of free tryptophan (TrpZ) and also using a model that mimic the chemical environment of tryptophan in a protein using N–formyl– tryptophanamide (TrpP) (Figure 1).Similar models have been used theoretically (see Ref. 11 and references therein) and experimentally.[6]

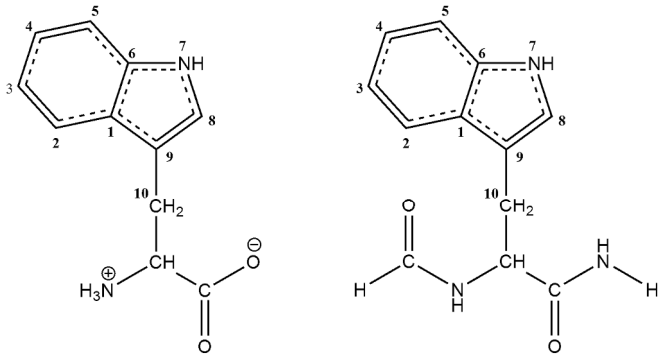

Figure 1. Tryptophan models

Electronic calculations were performed within the framework of the Density Functional Theory[12,13] (DFT). The geometry optimizations and frequency calculations have been carried out using the LC-ωPBE functional[14,15] and the 6-311+G(d,p) basis set, in conjunction with the SMD continuum model[16] using water to mimic aqueous environment. The LC-ωPBE functional has been benchmarked for kinetic calculations and it outperformed the best functionals designed to that purpose. SMD is considered a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known.[16]

Unrestricted calculations were used for open shell systems and local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). In the case of the transition states they were verified that the imaginary frequency corresponds to the expected motion along the reaction coordinate, by Intrinsic Coordinate calculations (IRC). All the electronic calculations were performed with Gaussian 09 package of programs.[17] Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. In addition, the solvent cage effects have been included according to the corrections proposed by Okuno,[18] taking into account the free volume theory of Benson.[19] The rate constants (k) were calculated using the Conventional Transition State Theory (TST)[20,-22] and 1M standard state. In case of rate constants limited by diffusion Collins-Kimball[23] theory was used. This is used in combination with the Smoluchowski[24] approximation for calculation of diffusion constants for an irreversible bimolecular diffusion-controlled reaction and Stokes-Einstein[25] to calculate the diffusion coefficients of the reacting species. As implemented in the QM-ORSA protocol.[8]

Three different reaction mechanism were investigated. They are the single electron transfer (SET), the hydrogen transfer (HT), and the radical adduct formation (RAF). The optimized geometries of the transition states corresponding to HT and RAF mechanisms for the reactions between both models of tryptophan and •OH and •OOH radicals have been shown from Figure 3S to 6S (supplementary information). The calculated Gibbs free energies of reaction, Gibbs free energies of activation, reaction rate constants and branching ratios of tryptophan with •OH radical using as solvent water are shown in Table 1.

Table 1. Gibbs free energies of reaction (ΔG°, kcal/mol), Gibbs free energies of activation ($\Delta G^{\neq}$, kcal/mol), rate coefficients (k_{app}, $M^{-1}s^{-1}$), and branching ratios (Γ, %) for tryptophan in zwitterionic form (TrpZ) and in a protein model (TrpP), with the •OH radical in aqueous solution, at 298.15 K.

·OH	ΔG°		$\Delta G^{\neq}$		k_{app}		Γ	
	TrpP	**TrpZ**	**TrpP**	**TrpZ**	**TrpP**	**TrpZ**	**TrpP**	**TrpZ**
SET	-15.69	-13.31	2.12	1.09	8.15E+09	8.34E+09	29.29	30.72
HT								
c10	-33.93	-34.47	3.40	0.00	2.77E+09	3.02E+09	9.96	11.12
n7	-29.88	-32.64	(a)	(a)	~2.98E+09	~3.02E+09	10.70	11.13
RAF								
c2	-19.88	-19.81	3.38	3.30	2.78E+09	2.84E+09	9.98	10.45
c3	-14.15	-13.53	3.18	3.93	2.83E+09	2.55E+09	10.18	9.39
c4	-16.55	-15.77	3.95	4.93	2.51E+09	1.51E+09	9.00	5.55
c5	-19.52	-19.70	3.14	3.25	2.84E+09	2.85E+09	10.20	10.51
c8	-27.89	-26.85	0.00	0.00	2.98E+09	3.02E+09	10.70	11.13
k_{total}					**2.78E+10**	**2.72E+10**	100.00	100.00

(a) Transition states couldn't be located, due to the huge exergonicity diffusion control was assumed.

As can be seen from the table all the reaction channels are very exergonic, with the largest exergonicity corresponding to HT from site C10 (~ -34 kcal/mol) independently of the used model, zwitterionic or protein-like. Based on the Bell–Evans–Polanyi principle, highly exergonic reactions are expected to be very fast. For channel c10, the corresponding barrier is 0 kcal/mol because even it was possible to locate transition state it was found below reactants in terms of Gibbs free energy. The main conclusion nonetheless from table 1 is that the reaction with •OH radical is very fast, nonspecific and nonselective. Almost all channels and mechanisms are very favoured from thermodynamic and kinetic points of view. The reaction is diffusion controlled and all products are possible, being the single electron transfer (SET) channels the most probable.

This behaviour could be interpreted that both free and protein bonded tryptophan are an antioxidant and/or easy victims of oxidative stress. The problem with this interpretation, which is not

missing from current literature is that it is not necessarily correct since •OH radical is so reactive that it reacts at close to diffusion limit reaction rates with almost any organic compound.

To perform a more precise evaluation of the primary antioxidant activity of tryptophan it is necessary to study the reaction with another, less reactive and more selective, oxidant. The hydroperoxyl radical, which is the conjugated acid of the ubiquitous superoxide radical anion, satisfy this requirement. The thermodynamic and kinetic parameters for the reaction channels of the •OOH reactions with both tryptophan models are reported in Table 2.

Table 2. Gibbs free energies of reaction (ΔG°, kcal/mol), Gibbs free energies of activation ($\Delta G^{\neq}$, kcal/mol) and rate coefficients (k_{app}, $M^{-1}s^{-1}$) for tryptophan zwitterionic form (TrpZ) and protein model (TrpP), with the •OOH radical in aqueous solution, at 298.15 K.

•OOH	ΔG°		$\Delta G^{\neq}$		*k* app	
	TrpP	TrpZ	TrpP	TrpZ	TrpP	TrpZ
SET	15.89	18.27	-	-	-	-
HT						
c10	0.79	0.24	23.37	23.99	3.93E-02	7.05E-02
n7	5.17	2.07	-	-	-	-
RAF						
c2	8.77	9.59	-	-	-	-
c3	14.97	14.85	-	-	-	-
c4	12.91	11.93	-	-	-	-
c5	10.00	9.07	-	-	-	-
c8	1.51	2.32	-	-	-	-

These values are counterparts those reported in Table 1 for •OH radical, however they are very different qualitatively and quantitatively. In the case of the •OOH reactions, Gibbs free energies are endergonic regardless of the reaction site. This means that even if these reaction are fast enough the reverse rate constant will be even faster. Moreover, in the case of addition channel under real physiological conditions with low concentrations of •OOH, or any other radical, the conditional equilibrium constant will largely favour the reverse unimolecular reaction. For this reason we have calculated only the rate constant for HT from the C10 site, the only almost isoergonic channel. The reaction using the model of tryptophan linked to a protein present a rate constant equal to 3.93×10^{-2} M^{-1} s^{-1}. That is five orders slower than the rate of the polyunsaturated fatty acids peroxidation, the inhibition of which is considered as a reference reaction for antioxidant capacity. This means that even for the less disfavoured channel the rate of reaction is so slow that it can be considered negligible taking into account the complexity of the biological systems and the presence of many real antioxidants including endogenous ones like glutathione, which is present at high concentrations and reacts with •OOH with rate constant several orders higher.[26]

Comparing the results of Table 1 and Table 2 for both radicals important information can be gathered. All the reaction channels are exergonic for the reaction with the •OH radical, while all of them are endergonic with the •OOH radical. Using a radical of intermediate reactivity would allow to selectively obtain the most stable C10 carbon "centred" radical, which in fact is a very stable delocalized radical. The radical with this intermediate reactivity might be the •N3 radical used in experiments to selectively oxidize tryptophan, which mainly react via SET. In such a case, deprotonation from radical cation produced via SET, would follow. The most likely deprotonation site being the most acid H-N centre, which would lead to the corresponding N centred radical as the reaction product.

According to our results, it can be concluded that tryptophan in its both forms, free zwitterionic and as a protein residue, are equally sensitive to oxidative stress. It can be damaged by very reactive radicals such as •OH in both chemical environments.

For the reactions with the •OH radical all mechanisms are possible i.e. RAF, HT and SET. However, the reactions with hydroperoxyl radical with free and peptidic tryptophan are both very slow. Moreover the reaction via SET is largely endergonic, while the HT reaction is almost isoergonic, but has very high activation energy. The rate constant of the reaction is 5 orders of magnitude slower than the rate constant of lipid peroxidation. Accordingly, it can be safely concluded that free tryptophan cannot be considered as antioxidant, and that tryptophan residues in proteins are not especially good targets of oxidative stress. Peroxyl radicals would not significantly oxidize tryptophan in any of these chemical environments. The previously observed antioxidant abilities of tryptophan can then been attributed to its metabolites. N_3 is predicted to selectively react with tryptophan, albeit this reaction lacks of biological importance because to our best knowledge there is no N_3 radicals in biological systems. More research is necessary to elucidate if free radicals present in biological system, other than •OH, are actually capable to oxidize tryptophan. So far it is certain that this amino acid is not a good antioxidant, and probably not a good target for oxidative stress when it is part of a protein. An indirect evidence of such proposal is the fact that usually enzymes does not repair tryptophan damaged residues, but they are designed to repair cysteine and methionine instead. However, free tryptophan can be metabolized in vivo and play important roles as antioxidant, which solves the paradox of being relatively inert to damage and good antioxidant.

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† Electronic Supplementary Information (ESI) available: Molecular graphics whit main geometrical parameters and Cartesian coordinates of the compounds under study. Gibbs See DOI: 10.1039/c000000x/

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