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DFT studies on the role of glutamate residue in the tyrosine nitration

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ABSTRACT

The influence of the local environment (water, histidine, or glutamate anion) on the properties of tyrosine and the process of 3-nitrotyrosine formation with the presence of glutamate residue have been investigated in detail at the B3LYP/6-31++G(d,p) theory level. The results show that the presence of glutamate anion within a few angstroms from the tyrosine residue (a) may help the tyrosine more likely to ionize and more easily to form radical by acting as a proton acceptor; (b) may stabilize the intermolecular complex **M1** and direct the nitro reagent to the two equivalent *ortho* carbons of the aromatic ring of tyrosine residues mainly by the N···O interaction whose geometry is found to be vertical with the plane of nitro due to the electrostatic interaction between N atom of $^{\bullet}NO_2$ and O atom of glutamate anion; (c) may assist to form the more stable complex **M3** through the concerted double proton transfer, in which the glutamate residue acts as a proton switch to catalyze the proton transfer.

1. Introduction

Tyrosine nitration, which is an *in vivo* posttranslational protein modification, attracts considerable interest in biomedical research, because it can change protein function and associate with the acute and chronic disease states [1]. Proteins including 3-nitrotyrosine have been detected and quantified in several major human diseases including acute lung injury, rheumatoid arthritis, chronic rejection of organ transplantation, atherosclerosis, Parkinson's disease, and Alzheimer's disease [2,3]. A strong correlation between tyrosine nitration and the modification, loss or gain of protein function has been confirmed for Mn superoxide dismutase, prostacyclin synthase, neurofilament-L, tyrosine hydroxylase, protein kinase C, cytochrome P450 2B1, cytochrome C, and nitrated fibrinogen [4–12]. Therefore, the production of 3-nitrotyrosine residues has commonly been used as a marker of pathological disease processes and of oxidative stress.

Interestingly, most mammalian proteins contain on average 3.2–3.9 mol% tyrosine residues with a range of 1–8 mol% [13], however, not all proteins are modified by nitration *in vivo*. Recently, it has been shown that there were only about 40 nitrated proteins out of 1000 during an inflammatory challenge. These included a large number of mitochondrial proteins, for example, Mn superoxide dismutase, tyrosine kinase, and creatine kinase etc., which regulate cellular energy metabolism [14]. Ischiropoulos and co-workers [15,16] have studied the factors determining the selectivity for protein tyrosine nitration, and they proposed that factors included (i) exposure of the aromatic ring of tyrosine to the surface of the protein or to solvent phase; (ii) location of the tyrosine on a loop together with a turn-inducing amino acid such as glycin (Gly) or proline (Pro); and (iii) association with neighboring negatively charged residues such as glutamate (Glu) and aspartate (Asp). The (i) and (ii) mostly concern with the steric restriction of tyrosine nitration. The surface accessibility of the two equivalent carbons CE1 and CE2 in the *ortho* position relative to the hydroxy group of tyrosine residues may be critical for allowing these residues to be modified by nitration. However, analysis of the sites of nitration in glutamine synthase revealed that not all surface exposed tyrosine residues were susceptible to nitration [17]. Moreover, in previous studies on mouse neurofilament-L, and human Mn superoxide dismutase [18–19], the hypothesis that a neighboring glutamate may guide peroxynitrite (PN) to attack a specific tyrosine was proposed. Therefore, Ischiropoulos and co-workers suggested that the (iii), the presence of a nearby negative charge residue within a few angstroms of the tyrosine residue, be critical in determining the site of nitration [15,16] which might help to make the tyrosine more likely to ionize and be more susceptible to radical attack or to remove the hydrogen at the 3-position to facilitate the attack of nitrogen dioxide on the ring of the tyrosine radical to yield 3-nitrotyrosine. Recently, the finding of mutating study of P450 2B1 enzyme [20,21] by PN strongly supported that the hydrogen bond between Tyrosine190 and Glu149 stabilizes the protein for maximal activity and Glu149 may be critical in directing the site of nitration by PN. However, it is not clear how the presence of neighboring negatively charged residue affects the local electrostatic environment of tyrosine residue to form ion or radical and how the carboxylate side chain brings the PN to the tyrosine.

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Although the glutamate and aspartate both have the carboxylate side chain and could enhance nitration, aspartate may be less effective than glutamate because its shorter side chain substantially decreases its flexibility and access to tyrosine [19]. The present study focuses the discussion on the role of glutamate anion for the change of the properties of tyrosine residue and for the mechanism of 3-nitrotyrosine formation. In the first part of this study, the effects of the local environment (water, histidine, and glutamate anion) on the properties of tyrosine are explored at the B3LYP/6-31++G(d,p) theory level. Note that it has been proposed that water and histidine play an important role in the formation of the tyrosyl radical mainly by the hydrogen bond with tyrosine [22–26]. The possible formation mechanisms of tyrosyl radical assisted by the glutamate anion are investigated in the second part. Furthermore, an abundance of evidences have indicated that nitrogen dioxide free radical ($^{\bullet}NO_2$) is capable of nitrating tyrosine residues [27–29] and it is more reactive and toxic than $^{\bullet}NO_2$ *per se* [30]. Then, in the third part, the possible role of glutamate residue to direct $^{\bullet}NO_2$ to the tyrosyl radical and the possible proton-transfer mechanism for the formation of 3-nitrotyrosine are studied. The free radical mechanism, supported by the existence of 3-nitrotyrosine in vivo through combining the tyrosyl radical and $^{\bullet}NO_2$ at diffusion-controlled rates to form 3-nitrotyrosine, is considered (Fig. 1) [1].

2. Computational details

The geometries of all complexes have been fully optimized by the analytic gradient techniques using the density functional theory with Becke's three parameter (B3) exchange functional along with the Lee-Yang-Parr (LYP) nonlocal correlation functional (B3LYP) [31–33]. The standard Pople's basis set, 6-31++G(d,p), was used in conjunction with the B3LYP method. Previous theoretical calculations have shown that the B3LYP approach is a cost-effective method to provide accurate electronic structures and properties of various molecules, radicals, and molecular clusters [34–36]. No symmetries constrained in optimizations. Harmonic vibrational frequency analyses were also performed at the B3LYP/6-31++G(d,p) theory level to identify minima and saddle points. Interaction energies were computed, using the optimized structures, as the energy difference between the dimer and the interacting monomers:

$$E_{\mathrm{int,pair}}(\mathrm{XY}) = E_{\mathrm{XY}} - [E_{\mathrm{X}} + E_{\mathrm{Y}}]$$

The interaction energy for the complexes involving the three subsystems, $E_{\mathrm{int,complex}}$ (XYZ), can be expressed as the energy differences between the total energy of the complex and the sum of the total energies of all monomers,

$$E_{\mathrm{int,complex}}(\mathrm{XYZ}) = E_{\mathrm{XYZ}} - [E_{\mathrm{X}} + E_{\mathrm{Y}} + E_{\mathrm{Z}}]$$

The basis set superposition error (BSSE) was corrected using the Boys-Bernardi counterpoise technique [37].

In order to consider the solvent effect, for the reaction of the 3-nitrotyrosine formation, the solvation energies for reactants, transition states, intermediates, and products were computed using a polarizable continuum model (CPCM) with a permittivity of 78.39, the value for water, the solvent in the protein environment.

The bonding characteristics of the different complexes were analyzed by using the "atoms in molecules" (AIM) theory of Bader [38], which is based on a topological analysis of the electron charge density and its Laplacian. The AIM theory has proved itself a valuable tool to conceptually define what an atom is, and above all what a bond in a quantum calculation of a molecular structure is. The charges on selected atoms were calculated using the natural bond orbital (NBO) theory method [39]. The B3LYP/6-31++G(d,p) electron density was used throughout the paper. All computations were carried out using the Gaussian 03 suite of programs running on several Linux PC clusters [40].

3. Results and discussion

3.1. Effects of glutamate anion on the structure and property of Tyrosine

To study the formation of tyrosyl radical and proton transfer mechanisms between tyrosine and glutamate anion, it is necessary first to investigate the structural changes of tyrosine with the presence of the glutamate anion. Fig. 2 shows the effects of glutamate anion on the geometric structure of tyrosine. In the Yz···Glu(anion) complex, the aromatic ring of tyrosine and the carboxyl of glutamate anion is nearly planar ($\angle C_1O_8O_{13}C_{14} = -0.14°$), and the interaction energy between tyrosine and glutamate anion (ΔE_{TG}) is −30.3 kcal/mol. Two hydrogen bonds are formed in this complex, which is confirmed by the two bond paths linking $(O_8)H_9$ and $(C_2)H_{10}$ of tyrosine and O_{13} and O_{15} of Glutamate anion, respectively: the $(O_8)H_9\cdots O_{13}$ hydrogen bond is a classical hydrogen bond and the optimized $(O_8)H_9\cdots O_{13}$ distance (2.545 Å) is comparable to the experimental value [41–44]; the $(C_2)H_{10}\cdots O_{15}$ weak contact ($(C_2)H_{10}\cdots O_{15}$ = 3.32 Å) is of the typical C–H···O hydrogen bond which has bond energies around 5 kJ/mol and plays a crucial role in many chemical and biological systems [45,46]. Obviously, the formation of hydrogen bond in the Yz···Glu(anion) complex leads to the structural change of tyrosine residue. The C_1—O_8 bond length is shorted from 1.374 to 1.340 Å; O_8—H_9 is elongated from 0.966 to 1.032 Å; the C_2–H_{10} is almost unaffected. The structural change is associated with the changes of electron density distributions (in Table 1). In neutral tyrosine, most of the negative charge is

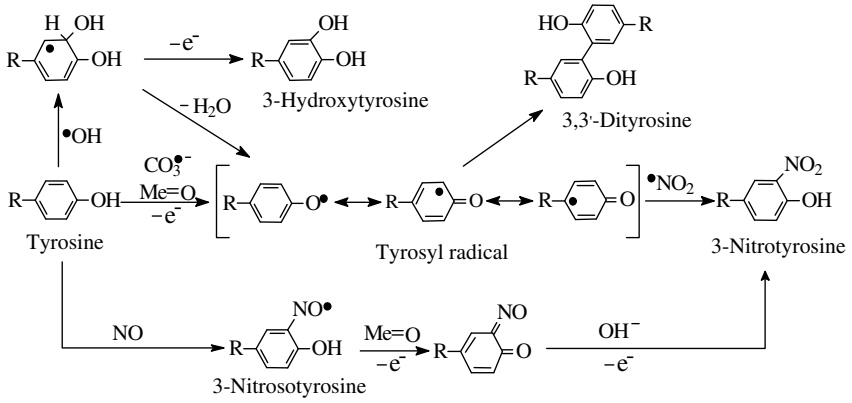

Fig. 1. The free radical mechanism of tyrosine nitration.