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The effect of zinc ion on the absorption and emission spectra of glutathione derivative: Predication by ab initio and DFT methods

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ABSTRACT

Relying on the reaction of *o*-phthalaldehyde (OPA) with glutathione (GSH) to form a highly fluorescence derivative GSH–OPA has been widely used to measure reduced glutathione. In order to better understand spectra property of the GSH–OPA and the effect of zinc ion on it, the ground and the lowest singlet excited state properties, the electronic absorption and emission spectra are predicted by ab initio and DFT methods. The absorption spectra are simulated using time dependent DFT method (TD-DFT) whereas the emission spectra are approximated by optimizing the lowest singlet excited state by HF/CI-Singles and then subsequently using this geometry for the TD-DFT calculations. The solvent effects on transition energies have been described within the conductor-like polarizable continuum model (CPCM). The calculated transition energies (absorption and emission) are in agreement with available experimental information.

1. Introduction

Reduced glutathione (GSH), a tripeptide consisting of glutamic acid, cysteine, and glycine residues (γ-Glu-Cys-Gly), is the most important low molecular weight antioxidant which occurs throughout the animal and plant kingdoms at concentrations varying from units to thousands micromolar [1,2]. Reduced GSH is a critical factor in protecting organisms against toxicity and diseases connected to oxidative stress. Its depletion is linked to a number of chronic diseases such as cancer, neurodegenerative and cardiovascular diseases [3,4]. Therefore, the measurements of the various forms of glutathione concentrations in biological samples are important for the understanding of GSH homeostasis in health and disease.

There are several chemical [5,6] and enzymatic [7] assays available for determination of glutathione. Most methods rely on GSH conjugation with a chromophore or fluorophore and assay the derivatization of GSH using absorbance or fluorescence probes. The fluorophore *o*-phthalaldehyde (OPA) has received a great deal of attention as a derivatizing agent. OPA is nonfluorescent until it reacts as a heterobifunctional reagent with a primary amine in the presence of thiol, cyanide, or sulfite, forming a fluorescent isoindole [8].

Based on the reaction between *o*-phthaldialdehyde (OPA) and amino acids, Hissin and Hilf [9,10] described a fluorometric assay for reduced glutathione. This method uses OPA as the fluorescent reagent, which reacts with both the sulfhydryl and primary amino group of GSH to form a highly fluorescence derivative [11]. The reaction between OPA and GSH might be depicted by Scheme 1, the production is labeled GSH–OPA.

The method, relying on GSH conjugation with OPA, has been widely used to measure GSH. However, to our knowledge, seldom theoretical description of its electronic and vibrancy spectroscopy has been reported. Moreover, recent efforts have focused on developing high efficient spectrophotometry and spectrofluorimetric method for the determination of trace GSH in biological sample [13,14]. Zn^{2+} ion, which has close shell d-orbitals, does not introduce low-energy metal-centered or charge-separated excited states energy and electron transfer processes cannot take place [15]. Therefore, it is expected that a cation like zinc (II) intimately interacting with a fluorophore will cause an observable change in fluorescent intensity [16–20]. Qi [21] had reported that the zinc ion may enhance the fluorescence of GSH–OPA derivative measured at 340 nm excitation and 400 nm emissions.

In order to better understand spectra property of the GSH–OPA and the effect of metal ion on it, the electronic vertical singlet transition energies of the GSH–OPA and the coordination complex of GSH–OPA with Zn (labeled GSH–OPA–Zn) are presented in this work.

From theoretical point of view, the investigation of the properties relating to both ground state and lowest singlet state for

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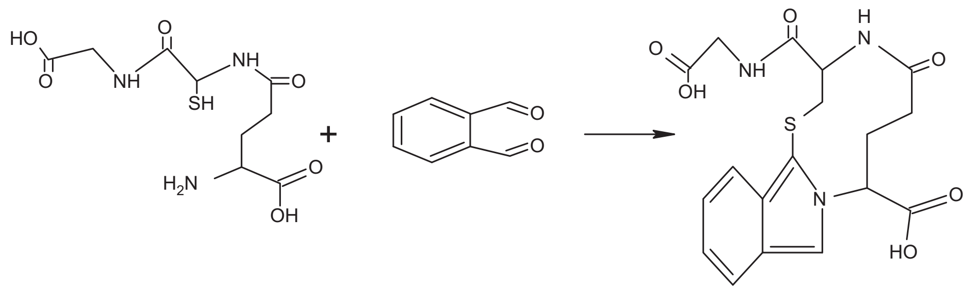

Scheme 1. The reaction of GSH and OPA [12].

GSH–OPA and the complexes (GSH–OPA–Zn) is helpful to well understand their intrinsic relationships among electronic structure and spectroscopic properties, and furthermore to reveal the nature of excitations associated with the charge transfer between ligand and central metal.

2. Computational methods

All calculations were performed using the Gaussian 03 program package [22]. The ground state (S_0) geometry optimizations both in vacuo and in solvent were carried out using Hartree–Fock method [23] and density functional theory (DFT) known as B3LYP which includes Becke's [24,25] three parameter exchange functionals along with Lee, Yang, and Parr type non-local correlation functionals [26]. The 6-31G (d,p) basis set was used for all atoms except the Zn^{2+} ion for which the model core potential LANL2DZ pseudopotential [27] had been considered. The configuration interaction singles (CIS) [28] calculations had been carried out for the optimized the lowest singlet electronic excited state of GSH–OPA and GSH–OPA–Zn both in vacuo and in solvent. The stability of the optimized geometries had been verified by performing calculations on vibrational frequencies. Time-dependent density function theory (TD-DFT) [29] B3LYP was also used for subsequent investigation on the spectral features of vertical S_0–S_1 transitions of GSH–OPA and GSH–OPA–Zn complex. Solute–solvent interaction was evaluated by use of the conductor-like polarizable continuum (CPCM) [30–32] model, in which the cavity was built up using the united atom topological model applied on UFF radii [33]. Water and methanol were modeled with dielectric constant of $e = 79.39$ and $e = 32.63$, respectively.

3. Results and discussion

3.1. Ground and excited-state geometries of GSH–OPA and GSH–OPA–Zn

All possible modes of complexation had been calculated, the interaction of the GSH–OPA with Zn^{2+} ion gave rise to eight complexes (structures can be found in Supplementary Material). Between them, only three structures are significant from an energetic point of view. They are global minimum GSH–Y–Zn-1 followed by GSH–Y–Zn-2 at 16.52 kcal/mol, and GSH–Y–Zn-3 at 14.86 kcal/mol. All other systems are found beyond 40 kcal/mol above the most stable adduct.

In the complex GSH–Y–Zn-1, the Zn^{2+} ion has tridentate complexation to three oxygen atoms, one arising from the carboxylic group of the Gly, the another two from carbonyl of Gly and Cys residue of GSH, respectively. In the GSH–Y–Zn-2 complex, the atoms directly involved in the interaction with the Zn^{2+} ion belong to oxygen of Gly residue, nitrogen of Gly and sulfur of Cys residue. In the GSH–Y–Zn-3, the Zn^{2+} ion has bidentate complexation to two oxygen atoms arising from the carboxylic group and carbonyl of Gly residue, respectively.

TD-DFT had been used to determine the excitation energies and Oscillator strengths of the three stable structure of Zn^{2+} complexes, only the GSH–Y–Zn-2 complex (Zn2+ ion binding to the nitrogen, oxygen and sulfur atom of GSH–OPA) was chose in this article to investigate the spectra property because the excitation energies of this structure were close to the main characteristics of the experimental spectra (in reference [21]).

The binding energy (ΔE) of the Zn^{2+} with GSH–OPA of the GSH–Y–Zn-2 complex in vacuum was calculated as:

$$\Delta E = E_{AB} - (E_A + E_B)$$

Here, E_{AB} was the energy of the complexes, E_A the energy of GSH–OPA and E_B the energy of Zn^{2+} ion. The binding energy was corrected with BSSE correction [34] and ZPE correction. The binding energy of the complex in vacuum is about −242.84 kcal/mol. The amount of charge transfer between GSH–OPA and Zn^{2+} ion is easily determined as the difference between the charge of the isolated ion and the net atomic charge of the metal in complex. From an analysis of the atomic charges evaluated by the NBO method [35], it is found that the charge of Zn^{2+} ion changes from 2 to 0.881 in the complex. The charge transfer was related to the charge redistribution between donor (GSH–OPA) and acceptor (Zn^{2+} ion) when the coordination reaction occurred. The calculations suggest that strong bonding interaction is form between GSH–OPA and Zn^{2+} ion, and the strong bonding interaction might improve the rigid of GSH–OPA. It was also point out by Wang [36] that the binding of Zn^{2+} makes the rigidity of ligand increase, while the fluorescence is enhanced.

GSH–OPA and GSH–OPA–Zn optimized by Hartree–Fock for ground state and CIS method for the lowest singlet electronic excited state. The key geometrical parameters of all stationary points are shown in Fig. 1. The calculated results shows that in ground state, the pyrrole and benzene ring in molecular appear coplanar. In excited state, the two rings are slightly changed compared to ground state but still nearly coplanar (the change of dihedral is 0.15–0.3°). From ground to excited state, the C—C bonds and C—N bond in the pyrrole ring are lengthened for GSH–OPA and GSH–OPA–Zn complex. For GSH–OPA, the bonds connecting two rings are slightly lengthened by 0.02 and 0.04 Å; the C—C bonds in benzene ring are with alternate variation, one is shorted and the next one is lengthened.

In GSH–OPA–Zn complex, the variations in geometries from ground to excited state are obvious. The bonds of Zn—O, Zn—N and Zn—S (where the Zn and GSH–OPA coordinated) are lengthened by 0.16, 0.27 and 0.43 Å, respectively. During the excitation, the main changes of dihedral in GSH–OPA are shown in Fig. 1(A) (red for excited state and black for ground state). The $C_7N_{38}C_{32}C_{34}$ torsion angle is obviously increased from 47.65° to 62.33°; the $C_{29}C_{32}C_{34}O_{35}$ from 13.26° to 98.78°; the $N_{39}C_{41}C_{44}O_{46}$ from 0.34° to 11.67°; the $N_{38}C_{32}C_{34}C_{35}$ from −112.16° to −27.98°. As shown