The Luminescence Mechanism of Glycitein

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Abstract. Amyloid β-peptide is a well-known therapeutic target for Alzheimer’s disease. Many studies have focused on the design and synthesis of effective fluorescent probes. Glycitein has been reported inhibit amyloid-β peptide aggregation, and solution of glycitein alone can give a maximum fluorescence emission at 465nm with excitation at 350nm. Density functional theory (DFT) and time-dependent density functional theory (TDDFT) have been used to interpret the fluorescence luminescence mechanism of glycitein. Glycitein’s fluorescence luminescence mechanism was the intramolecular charge transfer. The result of this study maybe useful to find the further potent fluorescent probes for alzheimer’s early clinical diagnosis.

1. Introduction
Alzheimer’s disease (AD) is a neurodegenerative disease which is characterized by loss of memory, confusion and speech, which eventually leads to dementia. The pathological features of AD are the presence of neurofibrillary tangles and amyloid plaques[1, 2]. Amyloid-β peptide(Aβ) instinctively aggregate and form plaques. Aβ is accepted as a hallmark of AD[3]. It has become a strong desire to develop fluorescent probes capable of imaging Aβ in vivo.

Flavonoids are a kind of plant secondary metabolites, which are comprised of polyphenolic compounds. Main dietary sources of flavonoids are fruits, vegetables, tea, coffee and red wine. Flavonoids have many physiological functions, such as antioxidant, anti-inflammatory, anti-amyloidogenic[4] and neuroprotective effects[5]. The fluorescence behavior of flavonoids was interesting[6]. Mie Hirohata[7] reported that isoflavones at physiological pH and temperature, especially glycitein and genistein have antigen fibrosis, anti-oligomeric and fibril destabilizing effects on Aβ20 and Aβ42 in vitro. Among them, glycitein exhibited the highest fluorescence enhancement with Aβ25-35. The specific fluorescence of the glycitein solution was the fluorescence emitted at 465nm with an excitation maximum at 350nm. Glycitein maybe useful as fluorescent probes for early diagnosis of AD in its preclinical phase[8]. But the fluorescence mechanism of glycitein was still unknown. So we will use DFT and TDDFT to explain the luminescence mechanism.

2. Computational Method
The density functional theory (DFT) and time-dependent DFT (TDDFT) methods were used to calculate the ground and excited states of all compounds, respectively. All geometric optimizations were performed using the BP-86 functional. The SDD basis set and effective core potential were used for the central I atom and a 6-31G (d, p) basis set was used for the other atoms. In the vibration analysis and calculation, all the ground state and excited state optimized structures have proved to be local minima and no virtual imaginary modes. All theoretical calculations were performed using the Gaussian 09 programs.
3. Result
After optimization, seven conformations were obtained by changing the two hydroxyls, methoxy and the dihedral angle of C1=C2-C1'-C2'. After analyzing the harmonic vibration frequencies of the seven configurations, no virtual frequency was found. The result showed that the conformation 1 exhibited the lowest single point energy and the calculated UV-VIS spectra was 319 nm (Table 2), which was the closest to the experimental value of 350 nm. Therefore, the conformation 1 was selected for the further investigation in this study, as shown in Figure 1.

![Figure 1. Optimized geometry of conformation 1 in the ground state. The gray: C, the red: O, the white: H.](image)

Figure 2 showed the optimized structures of glycitein in different electronic states. Vibration frequency analysis was performed to ensure that they were true minima. Table 1 showed the primary bond lengths and the dihedral angles of glycitein molecule in different electronic state. In the S0 state, the bond length of C2=C3, C3-C4 and C4=O11 were 1.371, 1.485 and 1.247 Å, respectively. The calculated dihedral angle of C2=C3-C1'-C2' was -34.67°.

![Figure 2. Optimized geometries of the glycitein in the S0 (a) and S5 (b) states.](image)
Figure 3. Frontier molecular orbitals of the glycitein.

Table 1 The bond lengths (Å) and dihedral angle (°) of glycatein were calculated in the ground and excited states.

| Bond         | S₀   | S₅   |
|--------------|------|------|
| C₂=C₃        | 1.371| 1.425|
| C₃-C₄        | 1.485| 1.477|
| C₄=O₁₁       | 1.247| 1.257|
| C₂=C₃-C₁’-C₂’| -34.7| -12.4|

Table 2 Energies (E), wavelengths (λ) and oscillator strengths (OS) of relevant excited states and the contribution of the electronic transitions between molecular orbitals for glycitein.

| Transition | E/eV  | λ/nm  | OS    | MOs               |
|------------|-------|-------|-------|-------------------|
| S₀-S₁      | 3.0489| 406.65| 0.0069| HOMO → LUMO       |
| S₀-S₂      | 3.2609| 380.22| 0.0003| HOMO-1 → LUMO     |
| S₀-S₃      | 3.6875| 336.23| 0.0842| HOMO-2 → LUMO     |
| S₀-S₄      | 3.8338| 323.40| 0.0283| HOMO-1 → LUMO+1   |
| S₀-S₅      | 3.8865| 319.01| 0.3225| HOMO → LUMO+1     |

To study the charge distribution and charge transfer in the excited state, the frontier molecular orbital analysis (FMOs) and electronic transition energy as well as the corresponding oscillator strengths were calculated by the TDDFT method. The result are listed in Table 2. The S₅ state had a large oscillator strength of 0.3225 so that after light absorption, glycitein was predominantly excited to the S₅ state. The electronic transition energy of the S₁ state was calculated to be 3.0489 eV (406 nm) and the electronic transition energy of the S₅ state was 3.8865 eV (319 nm). As shown in Table 2, the S₁ state was contributed by the electronic transition from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) while the S₅ state was contributed by the HOMO to the LUMO+1. In Figure 3, we can see the electron density is distributed in different parts. Upon electronic excitation, the electronic density transited from B ring to A, C rings via a π-π* transition as shown in Figure 3.

The geometric optimization of glycitein in different electronic states was calculated using DFT and TDDFT. Based on the geometric optimization, the IR spectra were calculated to monitor the vibrational stretching modes under different electronic states. The calculated IR spectra was shown in Figure 4. In the S₀ state the stretching vibrational frequency of C₄=O₁₁ appeared at 1652 cm⁻¹. Upon excitation, It shifted to 1619 cm⁻¹ in the S₅ state. The redshift suggested that the C₄=O₁₁ bond lengthen was caused by the electronic excitation. The stretching vibrational frequency of two hydroxyl groups were slightly changed.
In the $S_5$ state the optimized geometric structure and corresponding geometric parameters were shown in Figure 2 and Table 1. In the $S_5$ state, the bond lengths of $C_2=C_3$, $C_3-C_4$ and $C_4=O_{11}$ were 1.425, 1.477 and 1.257 Å, respectively, which were longer than those in the $S_0$ state. The change of bond length of $C_4=O_{11}$ is consistent with the indication of infrared spectrum. Compared with the $S_0$ state, in the $S_5$ state, the dihedral angle of $C_2=C_3-C_1'-C_2'$ increased from $-34.7^\circ$ to $-12.4^\circ$. It suggested that the benzopyrone and phenyl rings tend to be coplanar. The planar structure might be favorable to intramolecular charge transfer.

4. Conclusion
In this study, the fluorescence luminescence mechanism of glycitein was theoretically studied by using DFT and TDDFT methods. The analysis of bond lengths, dihedral angle and IR vibrational spectra showed that the carbonyl bond was weakened in the excited state. The dihedral angle tends to $0^\circ$ between the benzopyran ring and phenolic B ring indicated that the plane structure would be favorable to the charge transfer. The frontier molecular orbital analysis and electron density distribution demonstrated that it via a $\pi-\pi^*$ transition. The fluorescence luminescence mechanism was the intramolecular charge transfer. The fluorescence luminescence mechanism of glycitein could be useful for the future imaging agent design and synthesis.

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