

Molecular Orbital Calculations for Firefly Luciferin

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ホタルルシフェリンに対する分子軌道計算

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Received October 30, 1998

Abstract : Semi-empirical molecular orbital calculations are performed for firefly luciferin (Ln). Bioluminescence is caused by the decomposition of Ln and shows high quantum efficiency which is much more than that of other chemical luminescence (more than 10 times). In the process of the decomposition, the intermediate state and the following exited state of Ln cause the electronic excitations in the tetragonal part of Ln. In the calculation, the process of decomposition is simulated in order to study the mechanism of the bioluminescence. The excitation energy of Ln which corresponds to the energy of the bioluminescence is obtained by the calculated HOMO-LUMO levels. The calculated excitation energy shows good agreement with the observation (~ 2.2 eV) for the O-O bond length (~ 3.3 Å) in the tetragon in the intermediate state of Ln, which is reasonable to the stability of the molecule. In the work, the visualization of the molecule is useful to understand the process of the reaction.

Key Words : semi-empirical molecular orbital calculation, firefly luciferin, bioluminescence

抄 録 : ホタルルシフェリンは、非生物系の化合物に比べて10倍以上高い発光効率を示す。そのホタルルシフェリンに対して、半経験的分子軌道計算を行ない、生物発光のメカニズムについて考察した。発光はルシフェリンの分解の際観測されるが、その過程ではルシフェリンの中間状態、更にそれに続く励起状態が生成される。中間状態で存在する四員環の分解過程について計算を行なったところ、四員環内の O-O 結合の距離が3.3 Å程度において、計算された励起エネルギーは発光エネルギーと良い一致を示した。その際、グラフィックソフトの利用は、計算結果が化学的に正しい過程であるかチェックするために有用である。

索引語 : 半経験的分子軌道計算, ホタルルシフェリン, 生物発光

1 Introduction

Firefly luciferin (Ln) shows high quantum efficiency of the bioluminescence under enzyme luciferase. In the biochemical reaction, the intermediate state of Ln and the following excited state are considered to yield the bioluminescence, which are the second and third components in the process in Fig. 1, respectively. The excited state of Ln shows the strong luminescence in the process of decomposition of the tetragonal dioxetane part in the intermediate state of Ln (the second product in Fig. 1). The following removal of CO_2 from the tetragonal part is obtained by the present semi-empirical molecular orbital calculation and the excitation energy of the electrons is compared with the energy of the observed luminescent spectra. The process of the removal of CO_2 is simulated by varying the bond length of C-C and O-O in the tetragon. As the lifetime of the intermediate state is much short, the molecular structure of intermediate Ln has not been determined in experiments. Thus it is meaningful to study by the calculations the process of the bioluminescence, i.e. intermediate state, removal of CO_2 and excitation of oxy-Ln which showing the bioluminescence with high quantum efficiency. It is useful to show the molecular structure by a graphic software in order to conform the calculated results.

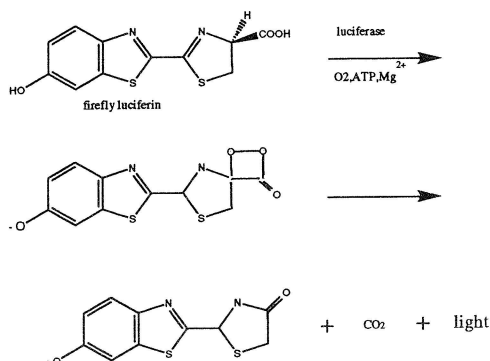


Fig. 1 Firefly luciferin (Ln), intermediate substance and oxy-luciferin with bioluminescence.

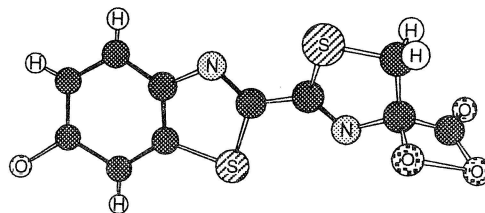


Fig. 2 Model of intermediate substance of Ln in our calculation. O-O bond length was varied in the calculation.

2 Methods of Calculation

We performed semi-empirical molecular orbital (MO) calculation for the intermediate substance of Ln (Fig. 2) by using MOPAC93, which is a wellknown software package in computational chemistry. A dioxetane structure (the tetragonal ring peroxide) in the intermediate of Ln plays an important role to cause the emission. The process of that the tetragonal ring breaks and successively CO_2 is removed was simulated in our calculation. Namely, the bond length O-O in Fig. 2 was varied in some points from the initial length (2.1 Å) to the final

length ($4.0 \text{ \AA}$). The other atomic distance and angle except for the tetragon was fixed at the observed values by X-ray diffraction (Ref. [1]). At these points, the equations were solved and the excitation energies and the MOs are obtained. The Hatree-Fock equations to be solved are

$$FC = C \epsilon_D, \quad (1)$$

$$C' C = I. \quad (2)$$

Here, F is Fock-matrix, C and ϵ_D are the coefficients of MOs and their energies, respectively. Next Eq.(2) is the additional condition as to the normalization about the MOs. In the present calculations, the Fock matrix called MNDO PM3 is adopted, in which 18 kinds of parameters (Coulomb repulsion, size of atomic orbitals, nuclear repulsion for other atoms and so on) exist for an atom. The above secular equations are solved by the self-consistent-field (SCF) approach.

The energy levels of the i -th highest occupied molecular orbital (HOMO) and the $i+1$ -th lowest unoccupied molecular orbital (LUMO) were obtained for the various O-O bond lengths in the tetragon (Fig. 2). The HOMO-LUMO gap energy $\Delta \epsilon$ is defined as the excitation energy and compared with the experimental bioluminescence.

$$\Delta \epsilon = \epsilon_{D, i+1} - \epsilon_{D, i}. \quad (3)$$

3 Results and Discussion

The molecular structure for the expanded O-O bond is shown in Fig. 3. Successively C-C bond also breaks after that. It is considered that the bioluminescence is caused when O-O and C-C bonds breaks and oxy-Ln is generated. The energy scheme of these process is in Fig. 4. The transition state (the second in Fig. 1) moves to the excited state, immediately. In this scheme, the gap energy between the ground state and the excited state (HOMO-LUMO gap

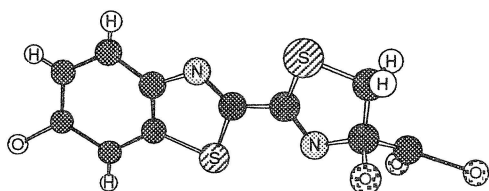


Fig. 3 Removal of CO_2 (O-O is $3.5 \text{ \AA}$).

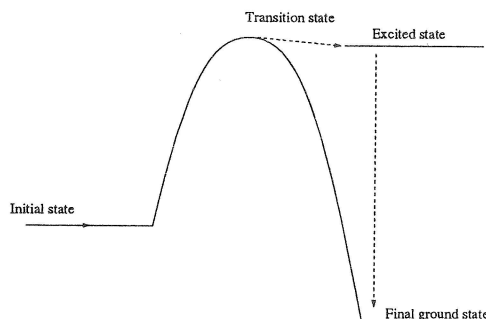


Fig. 4 Energy scheme of intermediate state, excited state and final ground state with luminescence.

energy) corresponds to the luminescence energy.

HOMO-LUMO gap energy versus O-O bond length is shown in Fig. 5. Wavelength for a firefly "*Luciola Cruciata*" 562 nm (~ 2.2 (eV)) was observed in the experiments. Considering the reported 562 nm, the excitation energy is reasonable for O-O bond length between 2.1 Å and 3.3 Å. In Fig. 5, the excitation energy more than 3.7 Å is much overestimated and the results shows that the bioluminescence is caused at 3.3 Å. In spite of that the bond length of O-O has not been measured in experiments, it could be considered that the excitation of oxy-Ln is caused near at the length by the estimation of the excitation energy. Thus, in the present calculation, it seems that the electronic excitation in the tetragonal part in Fig. 2 is caused at the moment of breaking out of O-O bond and that agrees with the theoretical expectation.

The present calculation has been performed by UNIX workstation. The total CPU time for the calculations for the points of O-O distance in Fig. 5 is about five hours on average. Recently, semi-empirical calculations can be performed by a personal computer, in which the developed graphic user interface has been installed and the transformation of the molecular structure by molecular dynamics can be obtained successively at sight. It is useful to use such a friendly software for understanding complex chemical process, furthermore, in respect of computer science, also. Making graphic software for those calculations is the important works of information science. As the ability of computations make progress, it may be more needed to study the problem about human interface.

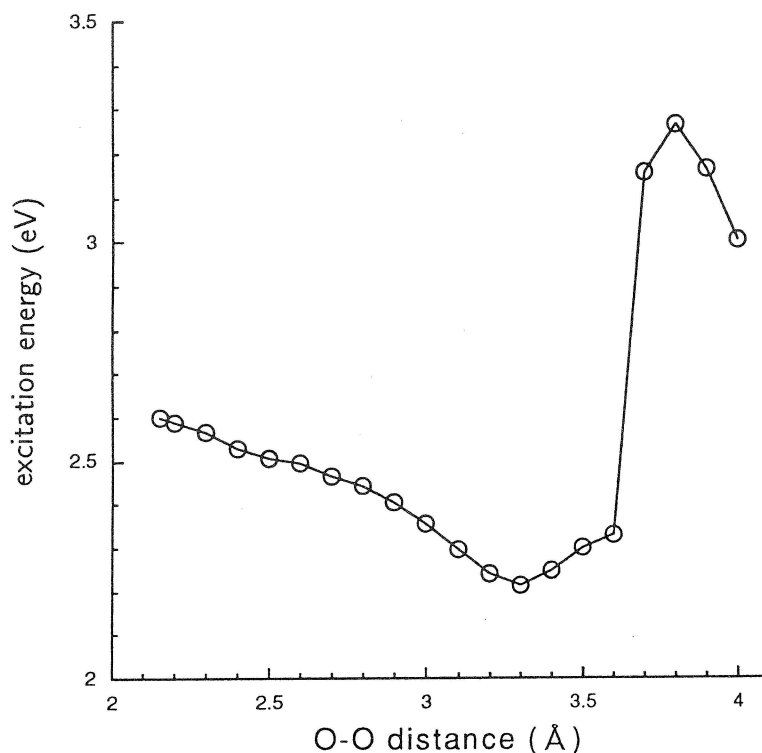


Fig.5 Calculated excitation energy versus O-O bond length of the intermediate Ln.

References

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