

# Molecular Orbital Calculations for Dioxetane as a Part of the Intermediate of Firefly Luciferin

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## Abstract

Firefly luciferin (Ln) is an absorbing substance showing a high quantum efficiency of bioluminescence. Several proposals about the mechanism of the luminescence in this system have been explained by investigators. A dioxetane structure exists within the intermediate of Ln and plays an important role to occur the emission. In this report, we attempt to calculate the electronic structures of the dioxetane and to correlate the calculated results with the experimental observations as a first step to recognize the mechanism of the bioluminescence of Ln. The obtained results show that the  $2p$  electrons of O and C are important as to the electronic excitations of the luminescence. The calculated excitation energy agrees with the observed value in spite of a simplified system.

## I. INTRODUCTION

Firefly luciferin (Ln) is an attractive chemical material since Ln emits the strong visible ray owing to the much high quantum efficiency of the bioluminescence [1]. The strong bioluminescence occurs by the chemical reaction of Ln and the enzyme luciferase with ATP,  $O_2$  and  $Mg^{2+}$  (Fig. (1)). As the results, the tetragonal ring peroxide (dioxetane structure, the first arrow of Fig. (1)) is generated and successively changes to oxyluciferin with the strong emission [2]. The dioxetane structure is the intermediate in the process of yielding the excited oxyluciferin. Thus, considering the importance of dioxetane structure to the bioluminescence we have tried to perform the semi-empirical molecular orbital (MO) calculation to the tetragonal ring of dioxetane structure Fig. (2). The purpose of this report is to convince the performance of the present MO calculation for the sake of clarify the mechanism of the bioluminescence of Ln in the further step.

## II. METHOD OF CALCULATION

### A. Calculation of the energy of molecular orbitals

We used the program package MOPAC 93 for the MO calculations. By solving the simultaneous equation Eq. (1), the MOs and their energies were obtained.

$$\mathbf{FC} = \mathbf{C}\epsilon_D, \quad (1)$$

$$\mathbf{C}^T \mathbf{C} = \mathbf{I}. \quad (2)$$

Here,  $\mathbf{F}$  is Fock-matrix,  $\mathbf{C}$  and  $\epsilon_D$  are the coefficients of MOs and their energies, respectively. Next

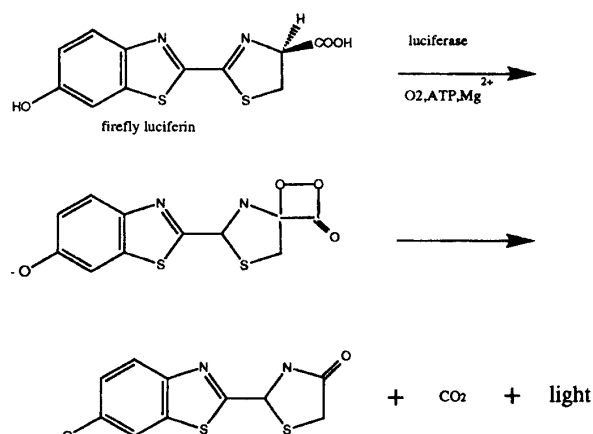


FIG. 1. Firefly luciferin, intermediates and chemiluminescent material. The intermediate includes the dioxetane.

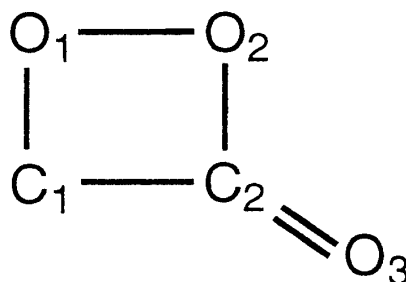


FIG. 2. The schematic molecular structure of the tetragonal ring peroxide (dioxetane structure) with the atomic labels.

(Eq). (2) is the additional condition as to the normalization of 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 and so on) exist for an atom, respectively. The above secular equations are solved by the self-consistent-field (SCF) approach.

### B. Estimation of the excitation energy

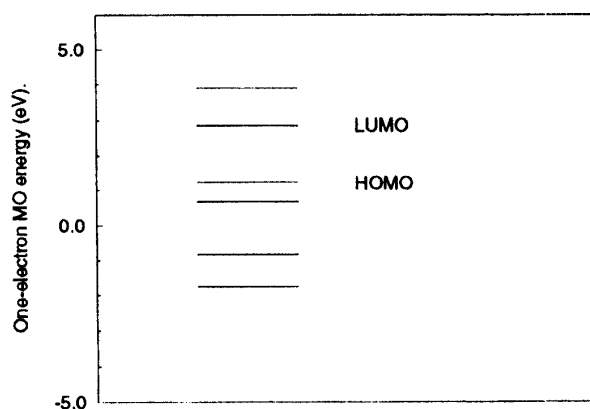
By using the obtained one-electron energy levels and total energies, we estimate the excitation energy of dioxetane structure. Since oxyluciferin are yielded following to the excitation of dioxetane, the excitation energy of dioxetane structure is meaningful to compared with the energy of the luminescence. We adopted the two formalisms to calculate the excitation energy of the system. One is to calculate the difference of the one-electron MO energies between the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO). In the ground state of dioxetane structure, each of MOs from in the bottom level up to in the  $i$ -th level (HOMO) are occupied by the two electrons. When one of the electrons in the  $i$ -th level moves to the upper  $i+1$ -th level (LUMO), the excitation of the system occurs. Then we calculate,

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

where  $\epsilon_{i,gr}$  is the energy of the  $i$ -MO in the ground state. The other procedure is to compare the total

**TABLE I. Atomic structure of dioxetane structure (in Å for bond lengths and degrees for bond angles.)**

Bond lengths		Bond angles	
C <sub>1</sub> —C <sub>2</sub>	1.52	C <sub>1</sub> —C <sub>2</sub> —O <sub>2</sub>	90.0
C <sub>2</sub> —O <sub>3</sub>	1.16	C <sub>1</sub> —C <sub>2</sub> —C <sub>3</sub>	135.0
C <sub>2</sub> —O <sub>2</sub>	1.36	O <sub>1</sub> —O <sub>2</sub> —C <sub>2</sub>	87.26
O <sub>1</sub> —O <sub>2</sub>	1.48		

**FIG. 3. One-electron energy of the dioxetane nearby HOMO and LUMO in the ground state. The total energy is  $-1111.04029$  (eV).**

energies both in the ground and in the excited state.

$$\Delta E_{tot} = E_{tot,exc} - E_{tot,gr}, \quad (4)$$

which is called  $\Delta$ SCF method. We estimated the excitation energies by these procedures.

### III. RESULTS

The bondlengths and the bond angles of the dioxetane structure is listed in Table I. With the use of this structure, the one-elctron MO energies were calculated and specified in Fig. 3 nearby HOMO and LUMO. In the semi-empirical Hartree-Fock calculation, only the valence orbitals of atoms are explicitly solved. As is mentioned above, since the emission is caused by the electronic excitations in the HOMO and LUMO, the energy levels of the two MOs should be considered to be important about the emission. In the ground state, each of the MOs below the HOMO are doubly occupied and the LUMO is empty. The excited state was calculated by the configuration of singly occupied HOMO and LUMO with the spin singlet state. The obtained one-electron energies are 1.235 (eV) for HOMO and 2.852 (eV) for LUMO in the ground state. Then, we obtained the excitation energy by Eq. (3) as 1.617 (eV). The total evergies are  $-1111.04029$  (eV) for the ground state and  $-1108.90906$  (eV) for the singlet excited state, then, we obtained the excitation energy by Eq. (4) as 2.13123 (eV). In Table II, the calculated energies and the experimental observation are listed. It seems that our results gave close energies to the experiment. The calculated energies are in the region of the visible ray. This agrees with the bioluminescence of firefly. Furthermore, in order to investigate the relationship between the electronic structure and

**TABLE II. Excitation energies of the dioxetane structure.**

	(eV)	( $10^3 \text{ cm}^{-1}$ )
By Eq. (3)	1.62	13.05
By Eq. (4)	2.13	17.18
Exp. [1]	2.73	22.01

**TABLE III. Atomic orbitals in HOMO and LUMO in the ground state.**

Atom AO	C <sub>1</sub>		C <sub>2</sub>		O <sub>1</sub>		O <sub>2</sub>		O <sub>3</sub>	
	2s	2p	2s	2p	2s	2p	2s	2p	2s	2p
LUMO	0.069	0.302	0.001	0.297	0.012	0.094	0.000	0.064	0.032	0.149
HOMO	0.023	0.330	0.320	0.033	0.020	0.164	0.008	0.074	0.000	0.029

the bioluminescence, the atomic character of MOs is shown in Table III, The dominant atomic characters are C-2p and O-2p $\sigma$ -orbitals. It is noted that the HOMO and LUMO are antibonding orbitals. This suggests that the HOMO of dioxetane is unstable and the possibility of the decomposition of this dioxetane structure into several materials, which agrees with the case of the chemical reaction Ln to oxy-Ln.

#### IV. CONCLUSION

We performed semi-empirical Hatree-Fork calculations for the dioxetane which is a part of firefly luciferin. HOMO-LUMO gap and  $\Delta\text{SCF}$  estimations showed the close value to the bioluminescence in the visible region.

#### ACKNOWLEDGMENTS

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