TD-DFT Studies on Hematoporphyrin and Its Dimers
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A theoretical study has been performed on a hematoporphyrin and its dimers which are components of Photofrin, a photosensitizer. Full geometry optimizations have been carried out using the PBEPBE functional and 6-31G(d) basis set. This combination gives better agreement with X-ray crystal data of porphyrin. Among the dimers studied, the C–C linked structure is found to have the highest stability. The predicted change of free energy (ΔG = −13.9 kcal/mol) suggests that the interconversion of ester to ether would be thermodynamically favorable. The time-dependent density functional theory (TDDFT) studies show that Q-band absorption maxima undergo a less intense transition and low oscillator strength, indicating that dimers have activity when treated under higher dosage. [doi:10.2320/matertrans.MB200829]

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1. Introduction
Photodynamic therapy (PDT) is a non-invasive medical technique for the treatment of various types of diseases in oncology and ophthalmology.1–4) The basic principle of PDT for cancer is the combination of a photosensitizing drug capable of absorbing within the body’s therapeutic window (λ = 620–850 nm), a light source (e.g., a laser) of an appropriate wavelength and molecular oxygen. The photosensitizer, which accumulates preferentially in cancer cells and has a low dark toxicity, is injected into human body tissue and then irradiated with visible light. After irradiation, the light-activated molecule undergoes different reactions and can decay from a singlet to a triplet excited state through a radiationless transition (intersystem crossing). The rate of the latter step is enhanced by the presence of an atom with a high atomic number (heavy-atom effect) in the molecule. The key cytotoxic agent is singlet molecular oxygen 1O2, which is generated by an energy-transfer reaction from the photosensitizer triplet state to the ground state molecular oxygen 3O2.5) For an efficient process, the photosensitizer’s triplet-state energy should match the first excitation energy of molecular oxygen (0.98 eV).

The photosensitizers for cancer treatment currently approved for clinical use belong to various groups of photofrin, which is a complex mixture of non-metallic oligomeric porphyrins (Hematoporphyrins).6,7) The mixture is believed to contain two to nine porphyrin units of oligomeric and dehydrated products that are linked by ether, ester or a C–C linkage (Scheme 1).8) The lack of available experimental techniques, to isolate and purify the higher oligomers is a big challenge till to date.7) There are several structure-activity relationship studies on hematoporphyrin and their distribution and photodynamic activity but none of these descriptive studies are quantitative and hence could be used to predict the exact structural information of the compound that are responsible for PDT activity.10)

In recent years, the Time-Dependent Density Functional theory (TDDFT) has proved its efficiency in the evaluation of electronic spectra and could predict the vertical excitation energies and oscillator strength with low computational cost effect.11,12) In this paper, we have undertaken systematic study on the structural, energetic and spectroscopic behaviour for monomer and dimer a component of a classical photosensitizer, photofrin that are used in PDT. The theoretical work presented here is closely tied to experimental work, where to investigate the effect of type of linkage on absorption spectral behaviour of monomer and dimers, as higher oligomer will have very complex spectra that may be hard to understand. In particular, we have determined: (i) the structures and geometric conformation; (ii) the absorption spectra and their electronic origin; (iii) the singlet–triplet energy gap for the monomer and dimers with DFT and TD-DFT methods. Moreover, we have compared them with available experimental data, in order to get a clear insight into the nature of molecule responsible for PDT activity.

2. Computational Methods
All calculations were carried out by using the Gaussian 03 package.13) Full geometrical optimizations, frequency calculations and TD-DFT studies has been carried out employing the PBEPBE functional, based on the generalized gradient functional proposed by Perdew, Burke and Ernzerhof (PBE) with standard 6-31G(d) basis set. Recent results has revealed the choice of 6-31G(d) as basis set, was found to predict the molecular geometry and vertical excitation more precisely.14) No symmetry constraints were imposed during the geometry optimizations. In all cases, frequency calculations were done on optimized structures to confirm the local minima. Restricted formalism was applied for the singlet electronic states and unrestricted formalism for the triplet states. Absorption spectra were computed as vertical electronic excitation energy from the minima of the ground-state structures by using time-dependent density functional response theory.

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3. Results and Discussion

3.1 Molecular geometries

Hematoporphyrin monomer can undergo oligomerization under various pH conditions to form three different possible linkages. Experimentally, known dimers are ether(-C-O-C-) linked, ester(-CO-O-C-) linked and -C-C- linked hematoporphyrins [Fig. 1]. Since hematoporphyrin derivatives are difficult to isolate in its pure state, no single crystal data are available and hence comparison has been made with the analogues porphyrin system. Moreover, porphyrin and hematoporphyrin have similar electronic-structure based properties. Monomer of hematoporphyrin has been optimized at the different theory levels such as HF, B3LYP, B3P86 and PBEPBE with basis set of 6-31G(d) in the gas phase. Previous results has show the choice of 6-31G(d) as basis set, was able to predict the molecular geometry and vertical excitation more precisely on porphyrin system. Table 1 shows the selected bond lengths obtained under various theory levels. Among the methods tested, PBEPBE has better agreement with experimental values of the porphyrin system. Therefore, we have used PBEPBE method with 6-31G(d) basis set for optimizing the dimer structures.

To identify the most stable conformer for the dimers, optimizations were carried out with different initial geometries such as, the one in which two porphyrin units are in-plane and the other in which they are in perpendicular to each other. The fully optimized conformers with the lowest energy are shown in Fig. 2. The structures shows that the C–C linked isomer have a partial linear structure, while the other two isomers have a folded structure. In addition to the folding observed between the two porphyrin rings effective/C25\-C25/C interactions between the two porphyrin rings were observed in the case of ether. Among the dimers studied, the C–C linked structure has been found have the highest stabilization energy and between the ether and ester isomers, ether was found to be 13.3 kcal/mol more stable than ester isomer. The predicted free energy change (\(\Delta G = -13.9\) kcal/mol) suggests that the interconversion of ether to ester would be thermodynamically favorable at room temperature, which has already been observed experimentally in aqueous alkaline media. Moreover, the frequency calculations have been performed for most stable configurations of three types linked isomers. These calculations reveal that all of Eigen values of Hessian matrix are positive, and hence, the corresponding frequencies are real. This means that these structures are indeed (at least local) minima.

3.2 Electronic spectra of monomer and dimer

Absorption spectra of monomer and dimers of hematoporphyrin were computed using TDDFT studies and results are provided in Table 2. The computed \(\lambda_{\text{max}}\) value for the monomer agrees well with experimental data. As their exist highly conjugated ring, these porphyrin-like systems show intense absorption bands around 400 nm, which is called as B-band region, followed by weaker satellites peaks between 500 to 800 nm, known as the Q-band region. We have computed spin-allowed singlet transitions for the compounds in vacuum and their relative results are reported in Table 2.
The oscillator strengths and the transition character along with the percentage contribution of orbitals to the transition in parenthesis for peak around 620 nm are provided in Table 3.

The lowest excitation energy in the wavelength region of 620 nm in dimers gives rise to the weaker transition, with oscillator strength varying from 0.0072 to 0.00001. This transition stems mainly from the HOMO-2–LUMO+3 excitation and HOMO-2 to LUMO for the ester linked dimer. While three transitions HOMO-2–LUMO, HOMO-3–LUMO+3 and HOMO-2 to the LUMO+2 are responsible for the transition in the case of the ether linked dimer. In the case of C–C linked dimer there is a contribution from the HOMO-1–LUMO+2 and HOMO-1–LUMO+4. The oscillator strength of C–C linked dimer is 100 times higher than other type of linkages shows that C–C linked type can have better PDT. This lowest excitation energy varying between 2.02–2.07 eV corresponds to the band, which plays a basic role in PDT applications. There is no extensive π-conjugation between the two hematoporphyrin units in all dimers and hence no significant shift in the excitation energy has been observed. Moreover, as there is no change in the number of π-electrons in the porphyrin core, which contributes to the increase and shift in absorbance maxima in the case of porphyrin, chlorin, and bacteriochlorin.18)

3.3 Singlet–triplet energy difference

One of the basic requisites of a photosensitizer for achieving an optimal performance in PDT is represented by its singlet–triplet energy gap (greater than or equal to 0.98 eV).19) The singlet–triplet energy gaps (ΔE) for the studied dimers are reported in Table 4. The series has ΔE values ranging from 1.52 eV to 1.69 eV, where values higher than 0.98 eV are found. Thus the series should induce the triplet–singlet molecular-oxygen transitions. However, it is worth noting that, owing to the small difference in ΔE values, this has to be approached with caution.

4. Summary and Conclusion

In conclusion we studied the ground-state geometries and electronic absorption spectra of hematoporphyrin and its dimers using TD-DFT approach. The PBEPBE functional along with the all electron 6-31G(d) basis set was found to give better results, indicating that the possibility of employing the same functional with basis set for higher oligomers. Dimers of different possible linkages have been optimized and they shows that the C–C linked isomer have a partial linear structure, while the other two isomers have a folded structure. The inter-conversion of ester- to ether-linked dimer is thermodynamically favoured process, which agrees well the experimental observation. Owing to their use in photo-

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Table 2 Comparison of UV-Vis data and TD-DFT values.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>B-band (nm)</th>
<th>Q-bands (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer</td>
<td>397.0</td>
<td>505.0</td>
</tr>
<tr>
<td></td>
<td>(396.0)*</td>
<td>(505.0)</td>
</tr>
<tr>
<td>Ester linked dimer</td>
<td>398.4</td>
<td>507.2</td>
</tr>
<tr>
<td>Ether linked dimer</td>
<td>399.2</td>
<td>505.6</td>
</tr>
<tr>
<td>C-C linked dimer</td>
<td>399.2</td>
<td>505.6</td>
</tr>
</tbody>
</table>

*Experimental values of wavelength in nm are given in parenthesis.

Table 3 Excitation energies (ΔE) in eV for peak around 620 nm of Q band with orbitals contributions.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Symmetry</th>
<th>Excitation energy ΔE [eV]</th>
<th>Oscillator strength</th>
<th>Transition character*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether linked</td>
<td>A₁u</td>
<td>2.04</td>
<td>0.0001</td>
<td>HOMO-2 → LUMO (63.6%)</td>
</tr>
<tr>
<td>C-C linked</td>
<td>A₁u</td>
<td>2.02</td>
<td>0.0072</td>
<td>HOMO-1 → LUMO+2 (34.6%)</td>
</tr>
<tr>
<td>Ester linked</td>
<td>A₁u</td>
<td>2.07</td>
<td>0.0002</td>
<td>HOMO-2 → LUMO+2 (19.4%)</td>
</tr>
</tbody>
</table>

*The percentage contribution of orbitals to the transition are given in parenthesis.

Table 4 Total energies and singlet–triplet energy gaps (ΔE) for the studied dimers.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Electronic states</th>
<th>Total energy (Hartrees)</th>
<th>ΔE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether linked</td>
<td>¹A</td>
<td>-3896.74138971</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>³A</td>
<td>-3896.68414099</td>
<td>1.56</td>
</tr>
<tr>
<td>Ether linked</td>
<td>¹A</td>
<td>-3896.7171993</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>³A</td>
<td>-3896.6562231</td>
<td>1.69</td>
</tr>
<tr>
<td>C-C linked</td>
<td>³A</td>
<td>-3824.60693816</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>³³A</td>
<td>-3824.55089916</td>
<td>1.52</td>
</tr>
</tbody>
</table>
dynamic therapy, particular attention was devoted to two spectral aspects that are important for an ideal photosensitizer: (1) determination of the energetic gap between singlet and triplet excited states, shows that the values are greater than 0.98 eV and (2) computation of the Q-band absorption maximum shows that they have weaker transitions with low oscillator strength. Among the dimers C–C linked dimer has higher oscillator strength. Thus dimers may involve in the PDT activity, when used at higher dosage.

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