THE OPTICAL PROPERTIES OF PHEOPHORBIDE A

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Photochemical therapy has become a new area in treating malignant tumour with photosensitive medicines. In recent years, a new photosensitive medicine-pheophorbide A (PhA) is developed, with curative effect ten times as high as that of Hematoporphyrin[1]. In this note, the method of preparing PhA from pteridophyte is reported, the electronic spectra of PhA is qualitatively analyzed by HMO figure theory and the mechanism of photochemically treating cancer is discussed.

I. Material and Experiment

1. *Preparation of PhA*

Chlorophyl was extracted from fern with aceton as extractant, then chlorophyl A separated out by a cation chromatographic column. Mg ions were removed with diluted hydrochloric acid. Concentrated sulphuric acid was used to remove phytoalcohol from ChlA to obtain PhA.

2. *Measured Spectra of PhA*

Absorption spectra were measured with a UV-200 spectrophotometer. An F-4000 fluorescence spectrophotometer and a GDM-1000 monochromator were used respectively to measure the emission spectrum and determine the photosensitive properties.

3. *Sensitization of $^1O_2$ Fluorescence by PhA Aqueous Solution*

Sensitization of $^1O_2$ fluorescence by PhA aqueous solution with concentration of 5×10^{-4} mol/L and 5% ethanol was put in transparent cells A, B and C, respectively. N_2 was introduced in cell A for 5 min to remove oxygen and O_2 in cells B and C for 5 min and 10 min respectively, with a speed of 0.5 L/min. Then the cells were sealed, and their emission spectra measured.

II. Results

The excitation and emission spectra of PhA are shown in Fig. 1.

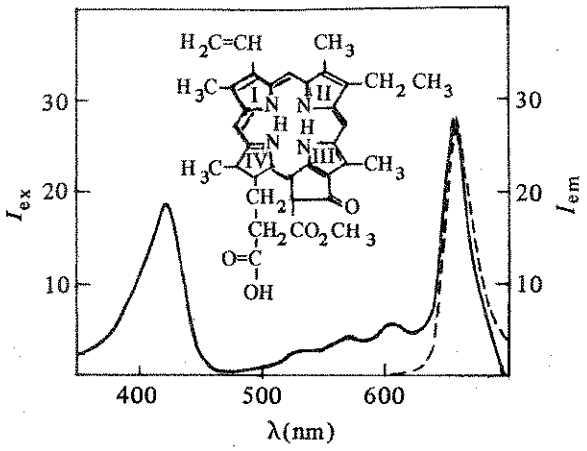

Fig. 1. Electronic spectra and molecular structure of PhA. ———, Excitation spectra; ------, fluorescence spectra.

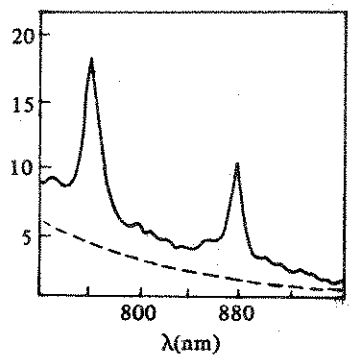

Fig. 2. Luminescence of $^1O_2$.

PhA has two excitative bands with peaks at 418 nm and 669 nm, while only a 672 nm band appears in emission spectra and is in mirror symmetry with the 669 nm excitation band. The sensitization of $^1O_2$ fluorescence is shown in Fig. 2. The solid is the emission spectrum of PhA aqueous solution with oxygen solved, and the dashed line is the spectrum of the sample without oxygen. Both of them are excited by 488 nm from an argon laser, the peaks at 760 nm and 870 nm are only found in the spectra of PhA solution with oxygen. They are the luminescence of singlet oxygen, quenching of PhA luminescence increases with increasing solved oxygen.

III. Discussion

1. *The Electronic Spectra of PhA*

The molecular structure of PhA is shown in Fig. 1. It is well konwn that the absorption of biomolecules arises from electronic transition of conjugate π-electron. In order to understand the main feature of electronic spectra of PhA, the πMO energy of PhA is evaluated by HMO figure theory.

From the molecular structure of PhA, we can see that the N atom at site IV participates in the conjugate system. Considering only the π-electrons on the porphyrin frame, the secular determinant of the heteroatomic conjugate system may be written starting from

N atom at IV as

$$D_{19}=\begin{vmatrix} y & -\eta & & & -\eta \\ -\eta & x & & 0 & \\ & & \ddots & & \\ & & & & -\eta \\ & 0 & & & \\ -\eta & & -\eta & & x \end{vmatrix}=0. \tag{1}$$

The elements of the determinant are determined by the interaction between two closest atoms, especially,

$$y=\frac{E-(\alpha+h\beta)}{\beta}=\frac{E-\alpha}{\beta}-h=x-h, \tag{2}$$

$$\eta=-\frac{\beta_{cN}}{\beta}=-k, \tag{3}$$

where α and β are Coulomb integral and bond integral, h and k integral parameters of the N atom, respectively. Expanding (1) by the first row, we have

$$D_{19}=yg_{13}(x)-2\eta^2 g_{17}(x)-2\eta^2, \tag{4}$$

$$g_n(x)=\sum_r(-1)^r\frac{(n-r)!}{r!(n-2r)!}x^{n-2r}\quad\left(r=0,\ 1\cdots\frac{n}{2}\ or\ \frac{n-1}{2}\right). \tag{5}$$

Substituting $h=2$, $\eta=1$ into (4), we get

$$\begin{aligned} D_{19}&=(x-2)g_{18}(x)-2g_{17}(x)-2\\ &=x^{19}-2x^{18}-19x^{17}+34x^{16}+152x^{15}-240x^{14}-665x^{13}+910x^{12}\\ &\quad+1729x^{11}-2002x^{10}-2717x^9+2574x^8+2508x^7-1848x^6\\ &\quad-1254x^5+660x^4+285x^3-90x^2-19x=0. \end{aligned} \tag{6}$$

Substituting its solution into (2), the πMO energies of PhA can be derived as listed in Table 1.

In HMO method β is an original constant which is related to the properties of one or a pair of atoms, therefore, its value may be got from fitting experimental data. In this note $\beta/hc=-20000\ cm^{-1}$ fitted from benzene spectra is used[2]. The excitation wavelengths of electronic transitions from E_{10} to E_{12}, E_{13} and E_{14} are at 670 nm, 612 nm and 413 nm, respectively corresponding to the three excitation peaks in Fig. 1. From the results, we found

Table 1

πMO Energy of PhA

BMO		ABMO	
E_1	$\alpha + 2.828\beta$	E_{11}	$\alpha - 0.165\beta$
E_2	$\alpha + 1.964\beta$	E_{12}	$\alpha - 0.744\beta$
E_3	$\alpha + 1.896\beta$	E_{13}	$\alpha - 0.804\beta$
E_4	$\alpha + 1.703\beta$	E_{14}	$\alpha - 1.21\beta$
E_5	$\alpha + 1.582\beta$	E_{15}	$\alpha - 1.354\beta$
E_6	$\alpha + 1.251\beta$	E_{16}	$\alpha - 1.644\beta$
E_7	$\alpha + 1.091\beta$	E_{17}	$\alpha - 1.757\beta$
E_8	$\alpha + 0.658\beta$	E_{18}	$\alpha - 1.912\beta$
E_9	$\alpha + 0.489\beta$	E_{19}	$\alpha - 1.972\beta$
E_{10}	$\alpha + 0.002\beta$		

that HMO method is rather simple and straightforward, may be more successful than accurate calculation in predicting some properties of organic molecules, though more approximations should be applied there.

2. *Energy Transfer Between PhA and* O_2

It is shown in Fig. 3 that the excited states of O_2 are lower than those of PhA.

The excited states of PhA get lower and the space of energy level between T_1 of PhA and $^1\sum_g^+$ of O_2 gets smaller, thus the energy transfers more efficiently. Since the N atom takes part in the conjugate system of PhA, the degeneracy of πMO of the porphyrin cycle is removed, the excitation energy is transferred from PhA to O_2 and the sensitized fluorescence would be produced. Here PhA is the sensitizer, while $^1O_2$ is the activator, the energy transfer process is

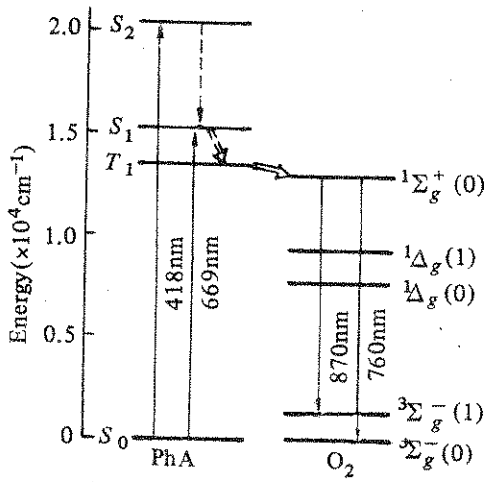

Fig. 3. Energy transfer between PhA and O_2.

$$\begin{aligned}
&\text{PhA}\ (S_0) + h\nu_a \rightarrow \text{PhA}\ (S^*),\\
&\text{PhA}\ (S^*) \xrightarrow[\text{singlet and triplet}]{\text{cross relaxation between}} \text{PhA}\ (T_1^*),\\
&\text{PhA}\ (T_1^*) + \text{O}_2\ (T) \xrightarrow[\text{transfer}]{\text{resonance energy}} \text{PhA}\ (S_0) + {}^1\text{O}_2\ (S^*),\\
&{}^1\text{O}_2\ (S^*) \rightarrow \text{O}_2\ (T) + h\nu_f,
\end{aligned} \tag{7}$$

and is schematically shown in Fig. 3, where S, T and $*$ denote singlets, triplets and excited states respectively. By measuring quenching of PhA fluorescence, the transfer efficiency between PhA and O_2 can be estimated by the following formula[3]

$$T = 1 - \frac{f_{\text{P·O}}}{f_{\text{P}}}, \tag{8}$$

where $f_{\text{P·O}}$ and f_{P} are the emission intensities of PhA in the solution without and with O_2 under 488 nm excitation respectively, and T is transfer efficiency between PhA and O_2. Substituting the experimental value into (8), we get $T = 45\%$. This indicates that the intermediate state ${}^1O_2$ is present in the process of energy transfer between PhA and O_2. Since ${}^1O_2$ is a strong oxydizer, cancer cells might be killed by oxydization of ${}^1O_2$, as they are exposed under laser light after PhA is injected into vein.

References

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