

## Excited States of Photoreceptor Molecules (I). Peridinin

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## 광합성 에너지 수용색소 분자의 Excited States (第1報) Peridinin

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**요 약.** 바다 말무리 dinoflagellate의 광합성 에너지 수용 색소인 peridinin의 전자 흡수스펙트럼을 PPP SCF MO 계산결과와 형광 편광 실험결과에 견주어 분석하였다. 청색광 부위의 흡수띠 (470 nm)는  $B \leftarrow A$  천이에 대응하며 분자의 장축과 거의 평행 방향으로 편광되어 있음이 예측된다.

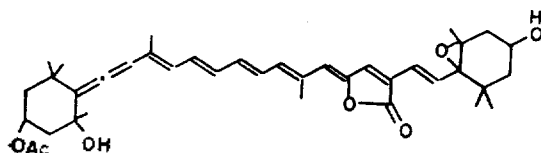
소위 "cis peak" 영역의 근자외선 흡수띠는 두개의 비교적 약한  $\pi \rightarrow \pi^*$ 천이 ( $C \leftarrow A$ 와  $D \leftarrow A$ )에 기인하며 그 편광축은  $B \leftarrow A$  편광축 방향과 근접되어 있음이 MO 계산과 편광 측정에 의해 추정되었다. 그리고 락톤 가르보닐 원자단의 전자구조는  $^1B$  상태에서도  $^1A$  상태 (ground state)에 비해서 과히 변화함이 없으나, allene기는 charge transfer적 성격을 농후하게 띠고 있어서 들뜬상태에서는 allene기가 전자결핍상태가 되는 것이 예측된다.

**ABSTRACT:** The electronic spectrum of marine dinoflagellate antenna pigment, peridinin, has been described in terms of PPP SCF MO CI computations and fluorescence polarization of the peridinin component in photosynthetic pigment complex of *Amphidinium carterae*. The main absorption band at 470 nm,  $^1B \leftarrow A$ , is polarized nearly along the long molecular axis. There appear to be two  $\pi \rightarrow \pi^*$  transitions ( $C$  and  $D \leftarrow A$ ) in the so-called "cis" peak region, and they are polarized roughly parallel to the main  $B \leftarrow A$  absorption. In addition, we have found that the carbonyl group undergoes very little reorganization of the electronic structure in going from the ground to the  $^1B$  excited states of peridinin, while the allenic group shows a strong charge transfer tendency in producing an electron-deficient allente allene group in the excited state.

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## INTRODUCTION

Peridinin is a naturally occurring carotenoid found in several marine dinoflagellates such as *Glenodinium* sp., *Gonyaulax polyedra*, *Amphidinium carterae*, *Amphidinium rhyncocephaleum* and *Gyrodinium dorsum*.<sup>1</sup> Peridinin (1) harvests blue light in the solar spectrum, as its absorption maximum occurs at 460~470 nm, and transfers the energy to chlorophyll *a* to effect photosynthesis. The energy transfer takes place with 100% efficiency.<sup>2,3</sup> Peridinin not only serves as an efficient antenna pigment for photosynthesis in dinoflagellates, but also functions as the photoreceptor for blue light responses such as phototaxis in *Gyrodinium dorsum*.<sup>4</sup>



(1)

In addition to the photosynthetic and phototactic role of peridinin, this carotenoid molecule raises particular intriguing questions as to its unique structure, possessing cross-conjugated lactone ring and allenic group, and its vibrational resolution in the electronic spectrum at 77°K.<sup>3</sup> In an attempt to elucidate the nature of electronic excited states of peridinin, we have undertaken spectroscopic and molecular orbital studies of the carotenoid, which are reported herein.

## EXPERIMENTAL

Peridinin was a generous gift of Professor S. Liaaen-Jensen, University of Trondheim. Spectroquality ethanol from U.S. Industries was used as solvent for the spectroscopic study of peridinin at 77°K, after further purification

via fractional distillation. Absorption, fluorescence and fluorescence polarization of peridinin and the *Amphidinium carterae* pigment complex described below were measured as described elsewhere.<sup>5,6</sup>

The *A. carterae* antenna pigment, peridinin-chlorophyll *a*-protein (PCP), was isolated from the dinoflagellate, as described previously.<sup>2,3</sup> The fluorescence polarization of chlorophyll *a* in the PCP complex was measured with respect to the peridinin absorption bands according to our previously published work.<sup>2,3</sup>

Semiempirical integrals used for the Pariser-Parr-Pople (PPP) SCF MO CI computation were identical with those employed for cross-conjugated carotenoids elsewhere.<sup>7</sup> In addition to nearest neighbor core resonance integrals, the next-to-the nearest neighbor integrals were also included in the computation in order to compensate for the PPP's tendency to yield a 0.1~0.2 eV blue shift for bond alternating systems. Methyl groups were treated by an inductive model.<sup>8</sup>

## RESULTS AND DISCUSSION

Fig. 1 compares the experimental and theoretical spectra for  $\pi \rightarrow \pi^*$  transitions in peridinin. The long wavelength band can be readily assigned to the  $B \leftarrow A$  ( $B_u \leftarrow A_g^-$  in  $C_{2h}$  notation<sup>9</sup>) transition, on the basis of a good agreement between the observed and experimental spectral quantities ( $\lambda_{\max}$  and intensity). The O-O band of  $B_u \leftarrow A_g^-$  is at ca. 506nm. This represents a red shift of 8 nm from the O-O band of 15, 15'-*cis*- $\beta$ -carotene.<sup>10,11</sup> Unlike the latter, the  $B_u$  envelope of the former is not sharply structured, apparently due to a strong charge transfer effect of the allenic group (*vide infra*), which broadens the absorption band.

Peridinin in ethanol at room temperature exhibits three broad bands at ca. 320, 295 and

240 nm.<sup>3,9</sup> The first two bands develop vibrational structure at 77 °K, as shown in Fig. 1. Calculations by the PPP method predict at least two  $\pi \rightarrow \pi^*$  transitions in this spectral region, *i. e.*, 352 nm and 330 nm, with comparable oscillator strengths. In addition, a  $\pi \rightarrow \pi^*$  transition is predicted at 287 nm with  $f=0.206$ . Thus, it is apparent that, for peridinin, the so-called "cis" peak of carotenoids appears as allowed

bands of more than one  $\pi \rightarrow \pi^*$  origin in the 280 ~360 nm region. Following the Platt notation, we assign these bands C, D and E in order of increasing energy.

Fig. 2a predicts polarization directions for the visible and UV bands of the peridinin absorption spectrum. The main  $B \leftarrow A (S_1 \leftarrow S_0)$  transition is polarized approximately along the long molecular axis, consistent with the linear dichroic spectrum of  $\beta$ -carotene.<sup>10</sup>

Except for the  $C \leftarrow A (S_2 \leftarrow S_0)$  transition, the  $D \leftarrow A (S_3 \leftarrow S_0)$  and  $E \leftarrow A (S_4 \leftarrow S_0)$  transitions are polarized nearly parallel to the  $B \leftarrow A$  polarization axis. It is likely that the lactone carbonyl exerts a strong influence on the polarization directions of these near UV-transitions, while the  $B \leftarrow A$  transition is not significantly affected by the carbonyl, *vide infra*. This may account for the resolution of vibrational fine structure over the "cis" peak region (Fig. 1), as found in other conjugated carbonyl-containing carotenoid—which exhibit  $C=O$  vibrational progression.<sup>7,12</sup>

Since peridinin is non-fluorescent under various conditions employed,<sup>3</sup> it is not possible to use photoselective fluorescence anisotropy methods to deduce relative polarization directions of different electronic transitions in peridinin. However, it is possible to measure the fluorescence polarization of peridinin

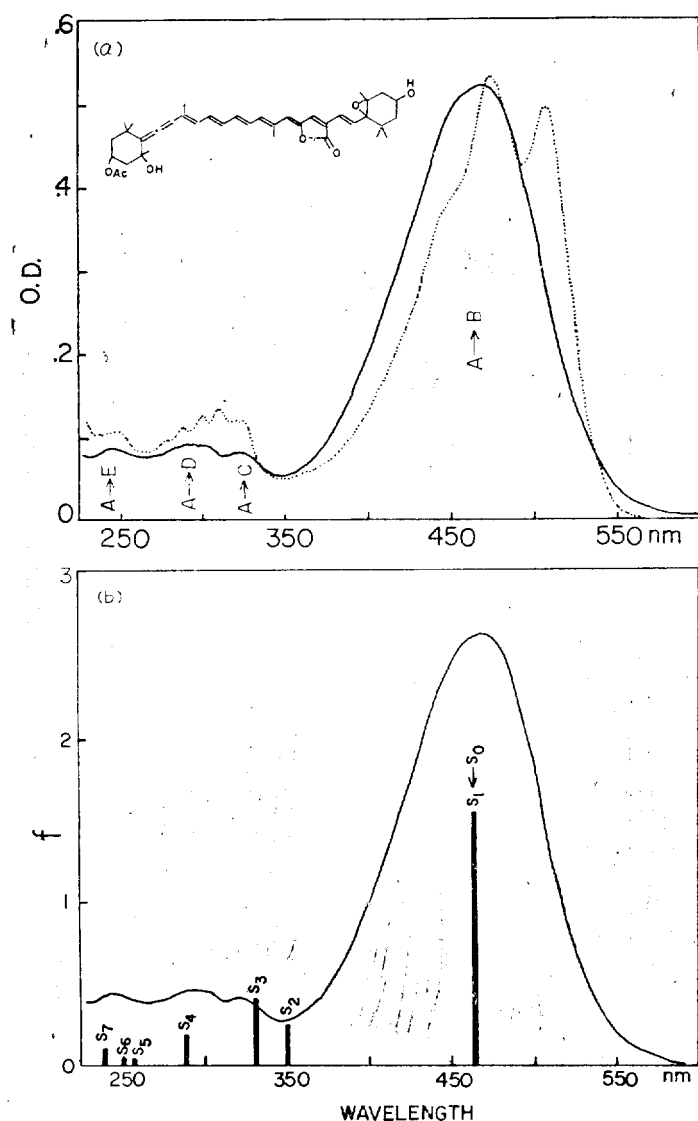


Fig. 1 (a) The absorption spectra of peridinin in ethanol at room temperature (—) and 77°K (···). (b) Calculated spectrum of peridinin.  $f$ ; oscillator strength. The room temperature spectrum is also shown for comparison.

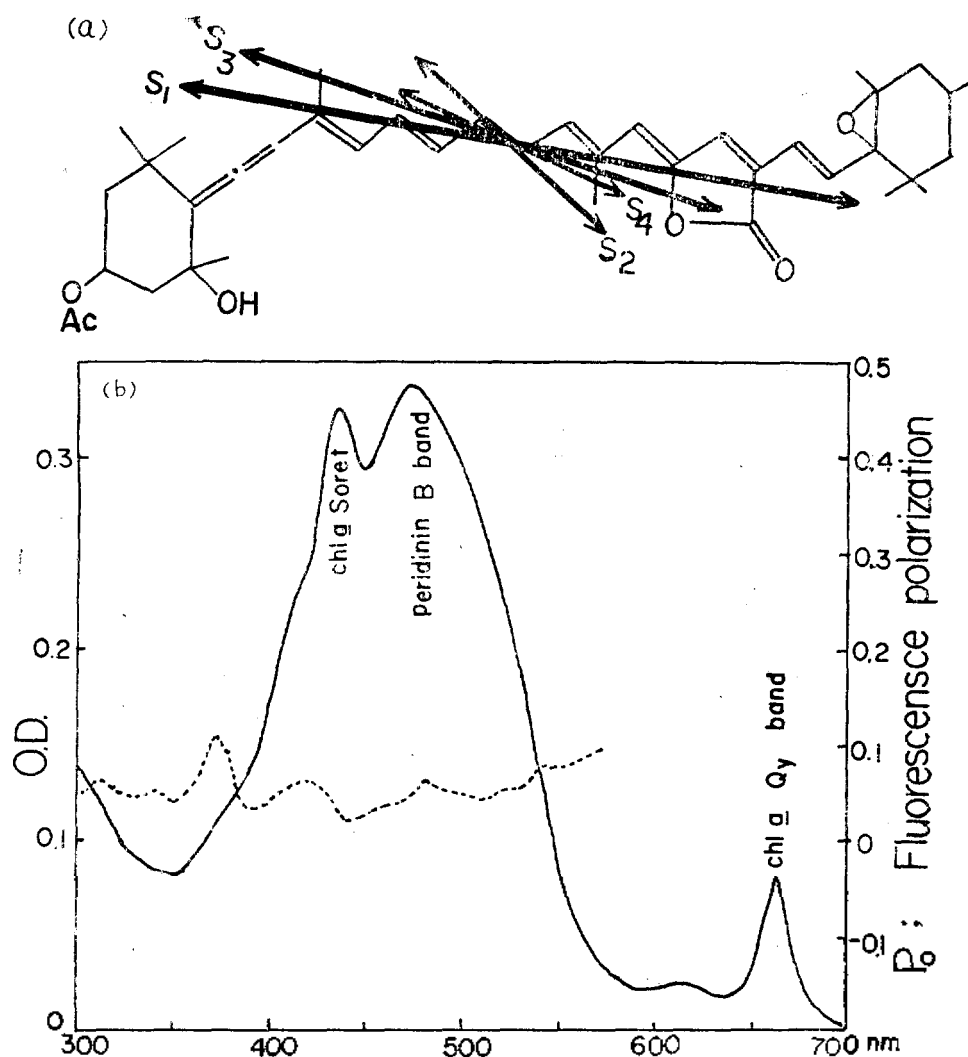


Fig. 2. (a) Polarization directions of low energy  $\pi \rightarrow \pi^*$  transitions calculated by the PPP SCF MO CI method. The figure is not quantitative. See the calculated oscillator strengths in Fig. 1b for a quantitative picture of the relative lengths of transition dipoles. (b) Polarized fluorescence excitation spectrum (---) of peridinin-chlorophyll a-protein complex with respect to the fluorescence emission from chlorophyll a as the result of energy transfer from peridinin to chlorophyll a. The protein complex was dissolved in Tris-glycerol (1:4, v/v), 200°K. The absorption spectrum of the pigment complex is shown in solid line.

by monitoring the fluorescence of an acceptor to which the former can transfer the excitation energy. Thus, we have been able to determine the fluorescence excitation polarization of peridinin relative to the sensitized fluorescence of chlorophyll a in the photosynthetic antenna pigment protein complex consisting of peri-

dinin : chlorophyll a : protein (4:1:1)<sup>3</sup> of *Amphidinium carterae* (Fig. 2b).

In this complex, the blue light absorbed by peridinin is transferred to chlorophyll a, resulting in fluorescence emission from the latter. The near UV absorption of this complex at 300 ~ 400 nm is predominantly contributed by peri-

dinin. Since the energy transfer from peridinin to chlorophyll *a* occurs with 100 % efficiency,<sup>2,3</sup> it is then possible to assign the observed polarization degrees (Fig. 2b) in the 300~400 nm region to the peridinin absorption bands. The polarization degree for the 300~350 nm region is constant, with an average value of 0.05. A higher value of polarization is recorded at 370 nm (Fig. 2b).

The *B*←*A* transition moments of the four peridinin molecules within the peridinin-chlorophyll *a*-protein complex are oriented about 45° with respect to *Q<sub>y</sub>* axis of chlorophyll *a*, as has been assigned on the basis of polarized fluorescence excitation spectrum.<sup>2,3</sup> Since the 300~350 nm band(s) show essentially identical degrees of fluorescence polarization as the main peridinin bands, it is most likely that the "*cis*" peaks are polarized nearly parallel to the *B*←*A* transition dipole. Calculated polarization directions shown in Fig. 2a are in good agreement with the above conclusion. However, an angle of 32° is predicted between the *B*←*A* and *C*←*A* transition axes. The higher polarization at 370 nm may be due to contribution of a strongly positive polarized band of chlorophyll *a*.<sup>3</sup>

Fig. 3 shows changes in the electronic struc-

ture of allenic and lactone groups upon excitation. The *S*<sub>1</sub>←*S*<sub>0</sub> excitation causes no changes in the  $\pi$ -electronic charge and bond distance at the lactone carbonyl. However, other near UV excitations underscore some alteration in the electronic structure of the carbonyl group, as can be predicted from the calculated polarization directions of these transitions, *vide supra*. In contrast, the allenic group undergoes more significant redistribution of  $\pi$ -electron density upon excitation to the *B* state (Fig. 3), showing a strong charge transfer from the allenic group to the rest of heptaene chain. Thus, the allenic group tends to be electron deficient in the *B* excited state.

## CONCLUSION

The main absorption band of peridinin is not strongly perturbed by the cross-conjugated intrachain lactone group. However, the allenic group exerts charge transfer to the heptaene moiety, which accounts for the red shift of the main band in peridinin (8 conjugated C=C and allenic double bonds) relative to 11 double bonded carotenoids such as  $\beta$ -carotene. The *cis* peak region of peridinin absorption spectrum is

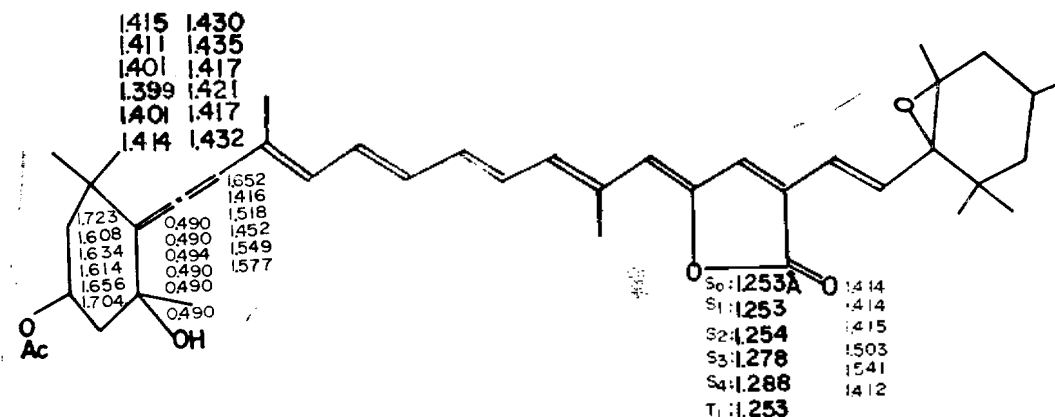


Fig. 3.  $\pi$ -Electron density distribution and bond distances for different electronic states of peridinin calculated by the PPP SCF MO CI method.

composed of at least two separate  $\pi \rightarrow \pi^*$  transitions, and their polarization directions are not far from being parallel to the  $B \leftarrow A$  polarization axis, which coincides with the long molecular axis.

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