Photodynamic Action in *Stentor coeruleus*

Yang Ki-chun

나팔벌레(*Stentor coeruleus*)에 있어서의 光力學的 作用(Photodynamic action)

梁 奇 千

Ⅰ. Introduction

Stentor coeruleus, an aneural and unicellular ciliate protozoan, exhibits both step-up photophobic and negative photactic responses to visible light with stentorin as the photoreceptors (Song, 1983). And stentorin contains hypericin as the chromophore linked to apoprotein (Walker et al., 1979) Hypericin has been recognized as an extremely phototoxic sensitizer in nature (Giese, 1980).

Hypericin is known to be a poisonous principle of some plants, like the genus *Hypericum* (Blum, 1941; Johnson, 1982) which are also distributed in the Cheju area (Yang, 1978). Hypericism, a state of skin sensitivity to the visible light in domestic animals, is apparently caused by the ingestion of hypericin-containing food. The photosensitizing effects of skin by hypericin result in severe skin irritation, high body temperature and sometimes death of the domestic animal. The hypericin-sensitized skin sensitization is oxygen dependent, thus hypericism being recognized as a photodynamic action by hypericin (Pace and Mackinney, 1941)

Photosensitization is known to be carried out usually through two diferent photochemical mechanisms(Blum, 1941):

1) Type I mechanism(electron transfer mechanism) in which the light absorbed by the sensitizer molecule is transferred to the substrate via the triplet state of the sensitizer, and ultimately leading to the formatin of superoxide anion radical, a potent oxidizing agent for various substrates.

$^1S \rightarrow {}^3S$

$^3S + A \xrightarrow{O_2} A$ oxidized (Damaged)

2) Type Ⅱ mechanism(energy transfer or singlet oxygen mechanism) in which the interaction of triplet sensitizer with the ground state of oxygen (which is in the triplet state) results in the highly reactive, singlet state of oxigen, $^1O_2$ which can potentially oxidise various substrates.

$^1S \rightarrow {}^3S$

$^3S + 0_2 \rightarrow S + {}^1O_2$

$^1O_2 + A \rightarrow A$ oxidized (Damaged)

農科大學 副教授

Singlet oxygen pathways are very common in photodynamic reactions. *Stentor coeruleus* cells are found to be photodynamically sensitive to the light absorbed by hypericin. The following experiments were conducted in an attempt to understand mechanisms involved in the photodynamic killing of *Stentor* by photoreceptorchromophore hypericin as well as in the photoprotection in *Stentor coeruleus*.

Ⅱ. Materials and Methods

Stentor culture.

Stentor coeruleus named "stella" strain was grown in massive, discontinuous cultures. The growth medium(as described by Walker et al., 1979) consisted of 2.5mM $CaCl_2$, 1.0mM $MgSO_4$, 1.5mM $NaNo_3$, 0.1mM KH_2PO_4 and 1.0mM Trizma-Base buffer; the pH was adjusted to 7.8±0.2 with HCl. 90 boiled wheat grains were mixed with 1.6 liter of growth medium in 26-liter glass bottles. 4000 to 6000 stentors in approximately 250ml of growth medium were inoculated into the fresh medium. The culture was maintained under 12 h-light and 12 h-dark cycle at 20°±1℃. The cells were harvested after 12-14 days by using a pipette from the densely populated wall of the container bottles. The harvested cells were resuspended in fresh growth medium for the experiments.

Irradiation.

Approximately 500 cells were placed in a 1-cm optical cuvette. An Osram 450 W xenon arc lamp was used for the irradiation of the cells. The beam of the light was filtered through a water IR filter combination, bandpass or cutoff filter, and finally a focusing lens which illuminated the entire front side of the cell suspension in the cuvette. The cuvette was air-cooled with an air blower to maintain the solution temperature at 20℃. Control batches of the cells were kept in the dark for the duration of irradiation.

Cell counting

the ratio of dead vs. live *Stentor* cells were determined after each irradiation period with a 450 watt xenon source, using a stereobinocular (Baush & Lomb Stereozoom 5). Since the photodynamically killed cells are immotile and sphere-shaped, they are readily distinguishable from the trumpet-shaped, live cells for visual counting.

Chemicals.

Synthetic hypericin was purchased from ICM Pharmaceuticals (New York). Beta-Carotene was a gift from Hoffman-La Roche, Nutley, NJ, and a water-soluble carotenoid crocetion was obtained from Sigma Chemical Co.. Deuterium oxide (D_2O), gold-label grade, 99 atom %, was found to be toxic to *Stentor*, instantly killing the cells in D_2O. However, the toxicity of D_2O was quenched by redistilling the heavy water. Thus, water-D_2O (1 : 1, v/v) mixture was used after the redistillation of D_2O. All other chemicals were from Sigma Chemical Co. and were used without further purification.

Ⅲ. Results

After exposure to increasing intensities of visible light from a 450 watt xenon arc source through a cutoff filter, *Stentor coeruleus* began to lose its motility and the normal cell morphology (shape). The cells were thus photodynamically killed and did not recover from the photoinactivation, even after a prolonged incubation in the dark.

According to the different wavelengths of cutoff filters (as can be seen in Fig. 1), each irradiation time was prolonged gradually in longer wavelengths than 430nm cutoff for the photokill-

ing of the cells(Fig. 2). A crude action spectrum obtained from the previous data showed that the long wavelength light did not seem to be very effective for the photodynamic killing of *Stentor coeruleus* (Fig. 3).

Fig. 4 and 5 indicate that β-carotene and crocetin increase the extent of survivability of *Stentor* when exposed to defferent light intensities. Both β-carotene and crocetin at the concentrations of 50uM showed 50-60% increase in the tolerance of the cells towards the higher light intensities.

When stentors were subjected to exogenous hypericin, the cells became more susceptible to the irradiation as compared to that of the untreated ones (Fig. 6). Interestingly, 1 uM of benzoquinone rendered significant protection against the hypericin-mediated photodynamic killing of stentors.

The photodynamic action sensitized by endogenous stentorin pigments and exogenous hypericin added is considerably enhanced in solvent containing D_2O, as shown in Fig. 7. The photodynamic killing sensitized by stentorin is also suppressed by 1% ethanol, when compared with the control, i.e. sensitization by endogenous stentorin in the absence of ethanol (Fig. 6). Again, the exogenously added sensitizer markedly promoted the photodynamic killing of *Stentor*. Both endogenous and exogenous sensitizer-induced killing of the cells are significantly inhibited by β-carotene (Fig. 7).

Ⅳ. Discussion

The photodynamic action of visible light on the ciliate *Stentor coeruleus* involves a readily discerni-

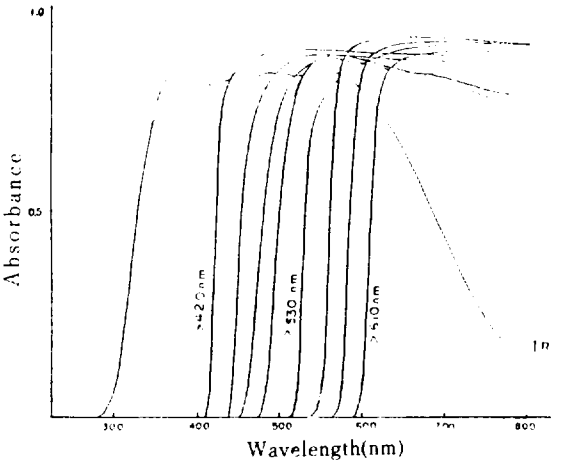

Fig. 1. The absorption spectra of each cutoff filter —IR filter combination.

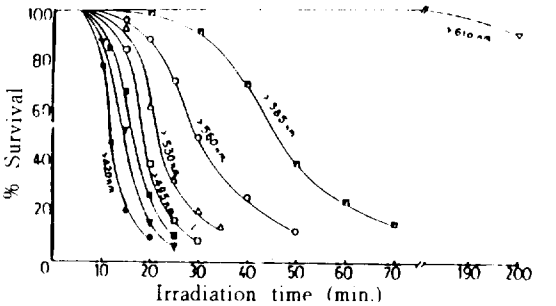

Fig. 2. Using a set of cutoff and IR—absorbing filters, light-induced killing of *Stentor* measured as a function of irradiation time.

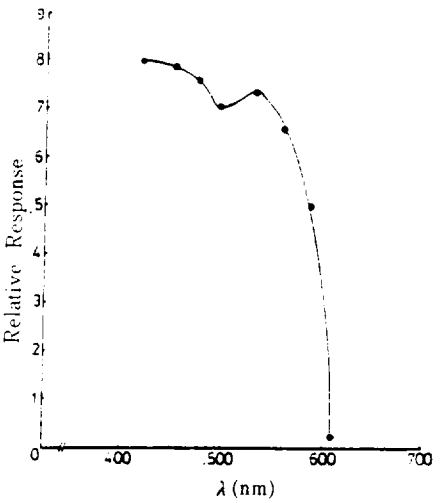

Fig. 3. A crude action spectrum was obtained from the previous data.

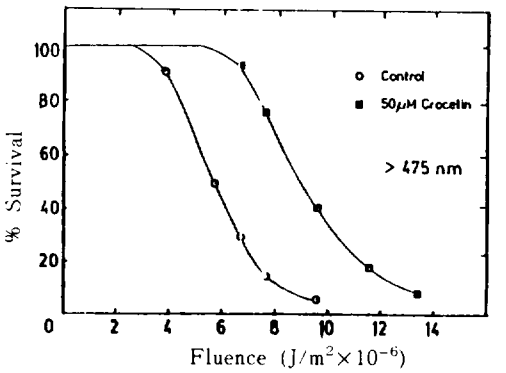

Fig. 4. Fluence-rate survival curves of *Stentor coeruleus*. 50 μM of crocetin gives significant protection to the cell against photodynamic killing.

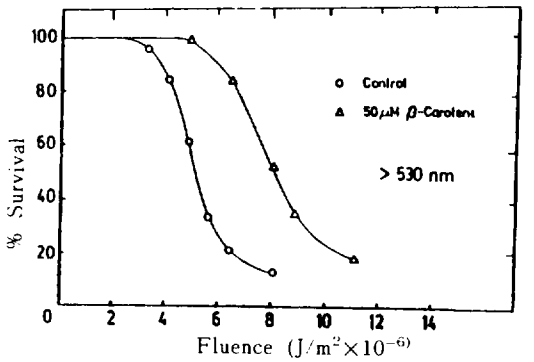

Fig. 5. Fluence-rate survival curves of *Stentor*. 50 μM β—carotene gives significant protection to the cell against photodynamic killing.

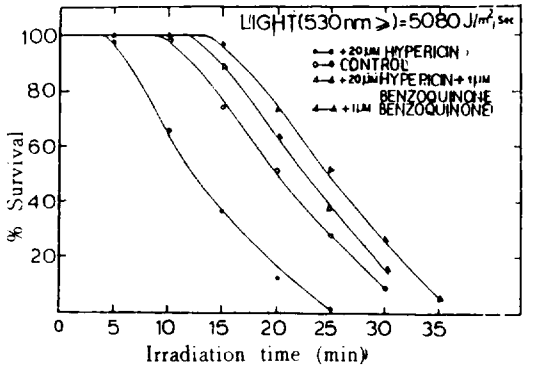

Fig. 6. Fluence (time-course) survival curves of *Stentor coeruleus* at the light intensity of 5080 J/m²/sec (530nm>).

ble morphological change, transforming the trumpet-shaped cell to the spherical appearance and resulting in the death of the cell which cannot be revived after incubation in the dark. The endogenous sensitizer is likely to be stentorin or its analog in the cell, with hypericin or hypericin-like derivative as the chromophore. Because of the known photosensitizing toxicity of hypericin and its derivatives in nature (Giese, 1980), it is tempting to speculate that the organism developed as a self-defense mechanism, utilizing stentorin as the photoreceptor.

The photosensitization mechanism for the photodynamic killing of *Stentor coeruleus* cannot be identified in terms of either Type I or II or both photosensitizations on the basis of results presented in this paper. The fact that carotenoids effectively protect the ciliate *Stentor* from photodynamic damage (Figs. 4, 5 and 6) is suggestive of the involvement of singlet oxygen, as carotenoids are known to be efficient quenchers of singlet oxygen (Foot, 1968; Krinsky, 1968; Koka and Song, 1978).

The extent of singlet oxygen in the photodynamic killing of the ciliate *Stentor* cannot be quanti-

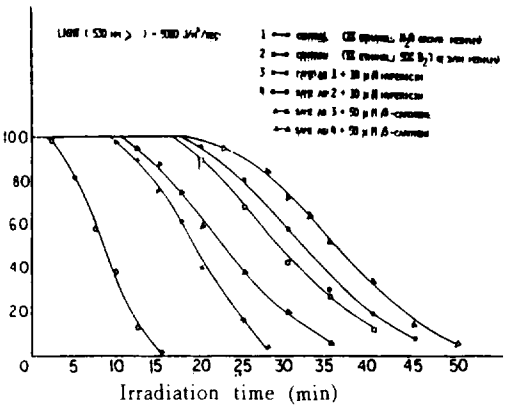

Fig. 7. Fluence (time-course) survival curves of *Stentor coeruleus* and the effect of D_2O, hypericin and β—carotene on the photodynamic killing of the cells.

tated on the basis of the present study. However, the fact that the photodynamic killing sensitized by the endogenous pigment and the exogenous hypericin is significantly amplified in D_2O containing media (Fig. 7) is consistent with the involvement of singlet oxygen, as the lifetime of the reactive oxygen species is considerably lenthened by the isotopic solvent (Merkel and Kearns, 1972 cited by Song). The solvent isotope effect is also quenched by carotenoids in support of the Type II photosensitization mechanism.

In addition to the Type II mechanism of photosensitization in *Stentor coeruleus*, a free radical mechanism of Type I photosensitization appears to play a supplementary role in the photodynamic action. p-Benzoquinone is known for its protective effect from the photodynamic decomposition of proteins(e.g., Koka and Song, 1978). It is also a singlet oxygen quencher, although its efficiency is relatively low(Krinsky, 1968; Koka and Song, 1978).

In conclusion, the present study establishes stentorin with hypericin or hypericin-like chromophore as the sensitizing pigment in the photodynamic killing of *Stentor coeruleus*. The photodynamic killing of *Stentor* can be additionally sensitized by exogenously added hypericin, suggesting that the endogenous pigment is somewhat "detoxicated", possibly due to its role as an efficient photoreceptor for photomovement under low fluence conditions and its particular topographic orientation within the cell. The photosensitization mechanism of the photodynamic killing of the organism appears to be of Type II. However, Type I mechanism also contributes to the photodynamic killing.

Acknowledgement

The author greatly appreciate the considerate advice and thechnical assistance of Dr. P. S. Song, and Mr. R. K. Prusti at Dept. of Chemistry, Texas Tech University, Texas, during my visit, 1982–1983.

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국 문 초 록

나팔벌레(*Stentor coeruleus*)에 있어서의 光力學的 作用(Photodynamic action)

나팔벌레에 있어서 光線으로부터 回避하려는 嫌光性反應은 나팔벌레의 色素顆粒內의 Stentorin이라는 物質이 光受容體로 作用하게 되며, 이 Stentorin은 그와 結合되어 있는 發色素인 hypericin이라는 物質을 含有하고 있다는 것은 잘 알려진 事實이다. 그러나 이 hypericin은 고추나물과 같은 植物에 含有되어 있어 草食家畜의 光過民症(Photosensitization)을 誘發하기도하는 有毒한 成分으로서 强力한 光力學的 作用 因子이기 때문에, 나팔벌레가 그가 갖고 있는 hypericin에 依해서 吸收된 光線에 光力學的으로 敏感할 것인가 하는 것과, 그렇다면 그 光力學的 轉機는 무엇인가 하는 것을 確認하는 것은 家畜의 hypericism의 光化學的 轉機를 究明할 수 있는 興味있는 일일 것이다.

本 實驗에 依하면 可視光線의 線量을 增加시켰을때 나팔벌레가 甚하면 cell lysis를 일으키면서 죽는 것을 確認하였다. 그러나 나팔벌레를 죽이는 이러한 光力學的 作用은 β-carotene, crocetin 및 benzoquinone에 依해서 抑制되는 것을 發見하였다.

β-carotene과 crocetin 같은 carotenoid 및 benzoquinone은 光力學的 作用轉機의 하나인 singlet oxygen ($^1O_2$)의 消去劑(quencher)로 알려져 있기 때문에 나팔벌레를 죽이는 光力學的 作用은 Singlet Oxygen에 의해서 惹起되는 이른바 第二型의 機轉(Type II mechanism)이 主가 된다는 것을 强力히 提示해 주었다.