

Clarification of degradation mechanism on retinal prosthesis using photoelectric dyes coupled to polyethylene film by mass spectrometry

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Abstract

Photoelectric dyes have stimulated retinal neurons by absorbing light and generating an electric potential. Therefore, a photoelectric dye was used to develop a retinal prosthesis to restore vision loss due to retinitis pigmentosa. The retinal prosthesis, referred to as a dye-coupled film, was prepared by chemically coupling the dyes to a polyethylene film surface through amide bonds. However, the coupled dyes decreased during an implantation test in a monkey eye. This study clarifies the degradation mechanism of the dye-coupled film. Since the dyes were selectively coupled to the film surface, it is difficult to detect the eliminated dyes in a solution. Therefore, a model compound that mimicked the chemical structure of the dye-coupled film by converting the carboxylic acid of dye to the amide bond is used. It was found that the elimination of the side chain, including the amide bond, occurred before the degradation of the conjugated structure according to mass spectrometry of the model compound. The

degradation mechanism of the dye-coupled film was predicted based on that of the model compound. It was concluded that chemical bonds between the dye and the film surface were preferentially decomposed, leading to the detachment of the dyes from the film surface.

Keywords. Degradation, Photoelectric dye, Benzothiazole, Mass spectrometry, Retinal prosthesis

1. Introduction

In the mammalian retina, photoreceptor cells detect light and transduce it into a neuroelectric signal. Retinitis pigmentosa is a hereditary disease in which the degeneration of peripheral photoreceptors progresses slowly and eventually leads to blindness due to macular involvement. Therefore, we developed a photoelectric dye-based retinal prosthesis (**Fig. 1(a)**) that provides electrical stimulation in response to light to treat retinitis pigmentosa. We used a thin polyethylene (PE) film as a substrate because photoelectric dyes cannot be implanted directly in the retina due to their powdery nature. By etching with fuming nitric acid, Carboxyl groups were selectively introduced onto the PE film surface. Finally, the photoelectric dye was coupled to the Carboxyl group on the PE film surface through 2 amide bonds. We optimized the synthesis process, leading to the development of the Okayama University-type retinal prosthesis (OUReP), i.e., a dye-coupled film (**Fig. 1(b)**) [1,2].

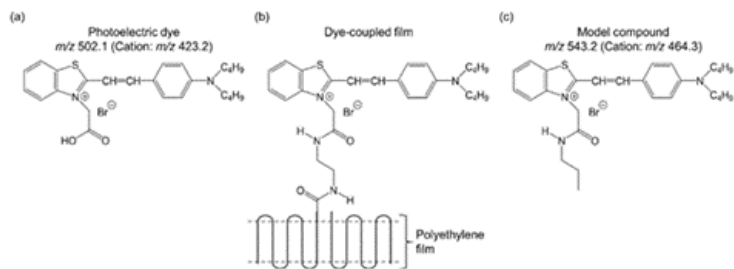


Fig. 1

Various medical devices safety and effectiveness testing have now been completed [3-5], and it has been demonstrated that the dye-coupled film exhibits no toxicity. In an animal model study, the vision of monkeys with macular degeneration was restored after implanting the dye-coupled film into the monkey eye [4]. However, the amount of dye coupled to the film surface gradually decreased in the monkey's eye. Elucidating the degradation mechanism is necessary for improving the long-term durability of the film. As the dye-coupled film is newly reported here, no previous studies are available for comparison. In addition, as the dyes were selectively coupled to the PE film surface, detecting and analyzing the coupled and eliminated dyes was a difficult task. To clarify the degradation mechanism of the dye-coupled film, we used a model compound that mimicked the chemical structure of the dye-coupled film by converting the carboxylic acid group of the dye to an amide bond (**Fig. 1(c)**). We attempted to identify an easily decomposable chemical bond in the model compound using electrospray ionization (ESI)-ion trap (IT)-mass spectrometry (MS) [6]. First, fragment ions of the model compound were generated by collision-induced dissociation (CID) [7] and the fragmentation was analyzed via tandem mass spectrometry (MS/MS) [8]. Subsequently, the model compound was decomposed via ultraviolet (UV) light and heat, and the change in molecular weight was analyzed using MS. The progress of degradation process was quantitatively evaluated

by measuring the absorbance of the sample. The degradation mechanism of the dye-coupled film was predicted based on that of the model compound.

2. Material and methods

2.1. Preparation of model compound

Photoelectric dye (1.0 equiv.), n-propylamine (2.0 equiv.), and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, 2.0 equiv.) were added to acetonitrile. The dye solution was allowed to stand at room temperature for 3 days. Then, the dye powder was obtained by filtering the dye solution.

2.2. Degradation of a model compound with UV light and heat

Dye solution (3 $\mu\text{g/ml}$) was prepared by dissolving the dye powder in acetonitrile. The dye solution was degraded by irradiating UV light (wavelength: 254 nm, intensity: 1095 $\mu\text{W/cm}^2$ where the distance from the light source is 50 mm) at room temperature for 4 weeks. The dye solution was degraded under high temperature at 60 $^{\circ}\text{C}$ in a dark environment for 4 weeks.

2.3. Chemical property evaluation

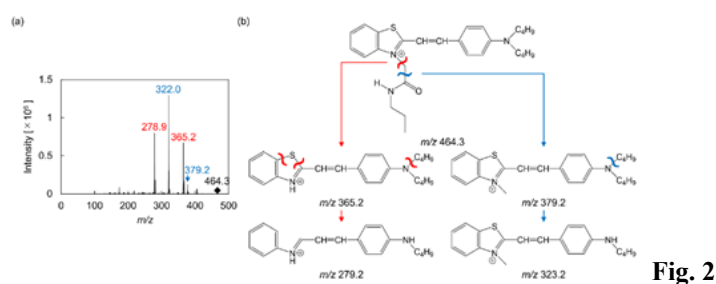
The absorbance spectra of the dye solution were recorded using an ultraviolet-visible (UV-vis) spectrophotometer with an integrating sphere unit (V-750 and PIV-756, JASCO Corp., Japan). The maximum absorbance value was used to compare the dye amount in the solution quantitatively. The baseline spectrum was obtained using acetonitrile. MS analysis was performed using an HCT-Plus ion trap mass spectrometer with ESI (Bruker Daltonics, Germany). The dye solution was injected into the ESI

source by direct infusion. ESI was conducted at 150 °C. Positive ionization mode was selected. MS/MS analysis of cation at m/z 464.3, the molecular weight of the model compound, was performed. Absorbance measurement and MS analysis of the dye solution was conducted at 0th week (before degradation) and 4th week (after degradation).

3. Results and discussion

Fig. 2(a) shows MS/MS spectrum of the cation at m/z 464.3. Rhombus in **Fig. 2(a)** represents the peak position of the model compound. **Fig. 2(b)** shows chemical structures corresponding to fragment ions.

Peaks at m/z 379.2, 365.2, 322.0, and 278.9 were observed. First, a methyl group was formed by decomposing the chemical bond next to the carbonyl group in the amide bond (m/z 379.2). Subsequently, the butyl group was eliminated (m/z 322.0). Furthermore, the chemical bond next to the N atom of benzothiazole was decomposed, and the entire side chain, including the amide bond, was eliminated (m/z 365.2). Subsequently, the butyl group and the S atom were eliminated (m/z 278.9). Therefore, it was found that the side chain, including the amide bond, was decomposed, followed by the elimination of the butyl group. In other words, it was found that the conjugated structure was relatively stable.



Absorbance spectra of the dye solution are shown in **Fig. 3(a)**. The absorbance value of the dye

solution before degradation was 0.016 [-]. By degrading the dye solution with UV light and heat at 60 °C for 4 weeks, absorbance values were decreased to 0.007 [-] and 0.003 [-], respectively. The relationship between the dye concentration and absorbance follows the Lambert-beer law. Therefore, it was found that the degradation of the dye solution proceeded by heat at 60 °C than UV light. MS spectra of the dye solution before degradation, after degradation with UV light for 4 weeks, and after degradation with heat at 60 °C for 4 weeks show **Fig. 3(b), (c), (d)**, respectively. The peaks at m/z 464.3 derived from the model compound and at m/z 423.2 derived from the unreacted dye were observed in the dye solution before degradation. By degrading the dye solution with UV light, the peak at m/z 464.3 decreased, and the peak at m/z 423.2 disappeared. By degrading the dye solution with heat at 60 °C, both m/z 464.3 and 423.2 disappeared. The peak intensity at m/z 464.3 decreased depending on the progress of the degradation of the dye solution; the degradation of the model compound proceeded by heat at 60 °C than UV light. The peak at m/z 423.2 was disappeared due to decarboxylation. However, the peak intensity at m/z 379.2 increased due to the degradation of the model compound and the unreacted dye. Additionally, it was found that the chemical structure corresponding to m/z 379.2 is the most stable because only the peak at m/z 379.2 remained after degradation with heat at 60 °C. Therefore, the model compound degraded in the following order: elimination of the side chain, including the amide bond, and degradation of the conjugated structure. This degradation process was the same as the MS/MS analysis.

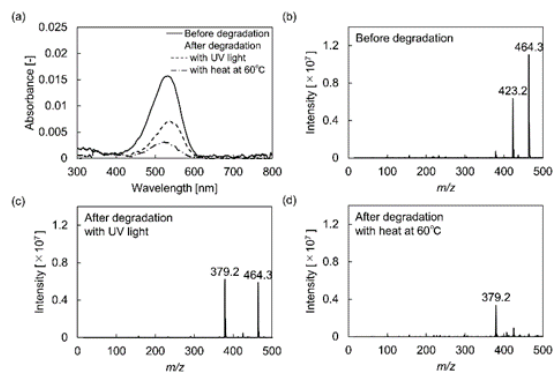


Fig. 3

The degradation mechanism of the dye-coupled film was predicted based on that of the model compound. It was concluded that the chemical bond next to the carbonyl group in the amide bond was preferentially decomposed. Furthermore, the chemical bond between N atoms of the benzothiazole would be decomposed. The degradation of chemical bonds between the dye and the PE film surface led to the elimination of the dyes from the film surface.

4. Conclusion

In this study, we worked on elucidating the degradation mechanism of the dye-coupled film. The MS analysis of the model compound revealed that the chemical bonds next to the carbonyl group in the amide bond and the N atom of benzothiazole were decomposed and the detachment of the side chain, including the amide bond, occurred before the degradation of the conjugated structure. The degradation mechanism of the dye-coupled film was predicted based on that of the model compound. It was concluded that chemical bonds between the dye and the PE film were preferentially decomposed, leading to the detachment of the dyes from the film surface. Using the methods provided in this study, the degradation mechanism of a model compound was elucidated and chemical structure of the degraded material was

identified. Additionally, this approach is applicable for clarifying the degradation mechanism of photosensitive dyes incorporated in a solar cell.

Acknowledgments

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References

- [1] T. Matsuo, A simple method for screening photoelectric dyes towards their use for retinal prostheses, *Acta Med. Okayama*. 57 (2003) 257–260. <http://doi.org/10.18926/AMO/32824>.
- [2] T. Uchida, M. Nitta, S. Kanashima, Synthesis and light-induced surface potential observation of retinal prosthesis using polyethylene thin films immobilized with photoelectric dyes, *J. Photopolym. Sci. Technol.* 28 (2015) 261–267. <https://doi.org/10.2494/photopolymer.28.261>.
- [3] Alamusi, T. Matsuo, O. Hosoya, K.M. Tsutsui, T. Uchida, Behavior tests and immunohistochemical retinal response analyses in RCS rats with subretinal implantation of Okayama-University-type retinal prosthesis, *J. Artif. Organs*. 16 (2013) 343–351. <https://doi.org/10.1007/s10047-013-0697-1>.
- [4] T. Matsuo, T. Uchida, J. Sakurai, K. Yamashita, C. Matsuo, T. Araki, Y. Yamashita, K. Kamikawa, Visual evoked potential recovery by subretinal implantation of photoelectric dye-coupled thin film retinal prosthesis in monkey eyes with macular degeneration, *Artif. Organs*. 42 (2018) E186–E203. <https://doi.org/10.1111/aor.13120>.
- [5] T. Matsuo, C. Matsuo, T. Uchida, K. Yamashita, T. Tanaka, Y. Kawakami, T. Hitomi, K. Taga, T. Sanada, Y. Yamashita, Curved-tip disposable injector (OUReP injector) to insert photoelectric dye-coupled polyethylene film (OUReP) as retinal prosthesis into subretinal space of rabbit eyes, *J. Surg. Tech. Proced.* 4 (2020) 1040–1045.

- [6] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Electrospray ionization for mass spectrometry of large biomolecules, *Science*. 246 (1989) 64–71.
<http://doi.org/10.1126/science.2675315>.
- [7] S.A. McLuckey, Principles of collisional activation in analytical mass spectrometry, *J. Am. Soc. Mass Spectrom.* 3 (1992) 599–614. [https://doi.org/10.1016/1044-0305\(92\)85001-Z](https://doi.org/10.1016/1044-0305(92)85001-Z).
- [8] K.L. Busch, G.L. Glish, S.A. McLuckey, *Mass spectrometry/mass spectrometry: techniques and applications of Tandem mass spectrometry*, VCH Publishers, New York, 1988.

Figure captions

Fig. 1 Chemical structures of (a) the photoelectric dye, (b) the dye-coupled film, and (c) the model compound.

Fig. 2 (a) MS/MS spectrum of cation at m/z 464.3, which is the molecular weight of the model compound. (b) Chemical structures are corresponding to the fragment ions.

Fig. 3 (a) Absorbance spectra of the dye solution. MS spectra of the dye solution (b) before degradation, (c) after degradation with UV light for 4 weeks, and (d) after degradation with heat at 60 °C for 4 weeks.