Life Time Measurement of Singlet Excited State of Hematoporphyrin Derivative (HpD) in Tumor Using Pico-second Laser Pulse

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Introduction

We have previously provided evidences that fluorescence spectra originated from native porphyrins are frequently obtained from human colon cancers and transplanted tumors in mice, and the shape of the spectrum is different from that obtained from HpD injected into tumors. However, the kinds of native porphyrin and the dynamic characteristics of HpD in tumor in the presence of native porphyrins still remain unclarified.

The purpose of this study is to measure the life time of the fluorescence from tumor and to clarify the difference of the fluorescent characteristics of tumor with and without HpD.

Materials and Methods

HpD was diluted in PBS with 10% human serum ($8 \times 10^{-5} \text{M}$). HpD solution was treated by laser light (635nm, 100mW/cm^2) for 60 minutes.

Tumor model is a MH-134 transplanted in C3H/He mouse. Mice were treated with either 0 or 5mg/kg of i.p. HpD. Emission life time was measured in vivo and also in resected tumor.

The excitation source was a mode locked Nd:YAG laser (532nm) which delivers 24 psec pulses.

Rise time of fluorescence was measured by streak camera with 10 psec time resolution by the aid of two dimensional signal processing system.

Life time of fluorescence was measured by two methods. One was same as the way of rise time measurement. The other method utilized monochromator, photomultiplier with 250 psec response time and storage oscilloscope, and gave a total temporal resolution of ~ 1.0 nsec. Monochromator was not used in vivo study because fluorescence was very weak. All measurements were carried out by single pulses.

Results and Discussion

The temporal profile of HpD fluorescence showed a fast initial rise time which agreed approximately with the integrated effect of the exciting laser pulse. An anomalous delay or a slow rise time were not observed experimentally (Fig.1).

In the case of untreated HpD solution, the life time measured at 630nm was 12.1 ± 0.7 nsec, at 668nm was 11.5 ± 0.8 nsec and at 690nm was 11.6 ± 0.7 nsec, respectively. On the other hand, in the case of HpD solution treated by laser for 60min, the life time measured at 668nm was significantly reduced to 9.1 ± 0.2 nsec (Table 1-A).

In tumor experiments, the life time of resected tumor was longer than in vivo study. The administration of HpD slightly prolonged the life time of transplanted tumors (Table 1-B).

Conclusion

Main results described in this work can be summarized as follows.
 (i) An anomalous delay or slow rise times of HpD fluorescence in PBS solution were not distinguished within 10psec time resolutions.
 (ii) Life time of singlet excited state whose emission were located at 668nm were found to be shortened after the treatment by laser illumination, (iii) This life time was observed to be lengthened in the case of mouse tumor by the resection.

Origins of these observation should be clarified in the near future.

Fig.1 Rise time of fluorescence of HpD in PBS solution

solid line : fluorescence decay curve
 dash line : temporal profile of laser pulse

A. Solution

	0 min. (n=5)	60min. (n=4)
630nm	12.1 ± 0.7	10.2 ± 3.1 nsec
668nm	11.5 ± 0.8	9.1 ± 0.2 nsec
690nm	11.6 ± 0.7	10.2 ± 1.2 nsec

B. Transplanted Tumor

	no HpD (n=5)		HpD i.p. (n=10)	
Resected	1.7 ± 0.3	11.5 ± 0.5	2.0 ± 0.5	16.6 ± 2.8 nsec
In vivo	1.9 ± 0.3	10.8 ± 2.0	2.2 ± 0.4	11.9 ± 1.7 nsec

Table 1 Life time

A : Solution
 B : Tumor