인광소광법을 이용한 암세포 내 산소농도 분석연구

Measurement of oxygen concentrations in tumor cells by the phosphorescence quenching method

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1. Introduction

During the last few years, the oxygen-dependent quenching of phosphorescence has been proven as a powerful method for measuring oxygen concentrations in biological samples.[1] This method has a rapid response time and can accurately measure oxygen pressure throughout the physiologically important range (760 torr down to 10^{-2} torr) and can be applied to living cells. Moreover, after addition of phosphors into tumor cells, optical techniques make direct calculation of the oxygen pressure in the observed area as changes occur possible. In this work, we report the simple measurement of oxygen pressure in tumors by using oxygen-dependent phosphorescence quenching of Pd-meso-tetra(4-carboxyphenyl)porphyrin (PdTCPP).

2. Experimental, Results and Discussion

Pt- and Pd-porphyrins are particularly useful for solid state optical sensors to detect low oxygen concentration because intersystem crossing is rapid enough to quantitatively convert the excited single state to the triple state.[2] PdTCPP was synthesized by benzonitrile method. For the phosphorescence imaging and optical measurements, 3 mL of MH134 cells were stained with 10^{-4} M of PdTCPP. The luminescence spectra of PdTCPP-stained MH134 and HeLa cells were very similar to each other in shape, the positions. The maxima of emission bands did not change (ex = 410; em= 713 and 780 nm). This result showed that uptake of PdTCPP was nearly same in the MH134 and Hela cells. In order to evaluate the distribution of probes (and/or oxygen) in the cells, microscopic images were taken by using phase contrast and luminescence modes. The administration of PdTCPP resulted in good localization in the tumor mass, which exhibited an intense emission from all of the illuminated cells. A line profile was evaluated from the phosphorescence image. The profiles were evaluated along a line drawn vertically across the tumor cells. From these line profile results, it was clear that PdTCPP was homogeneously distributed throughout the cells. This result can be interpreted to mean that the oxygen concentrations were nearly constant throughout the cells. In this work, the lifetime method was used to monitor oxygen in living cells instead of luminescence intensity measurements. Lifetime measurements are concerned only with the rate of decrease of the light emission following a flash of excitation light and not dependent on the absolute intensity of

the emitted light. In this context, it is thought that lifetime measurements are superior to intensity ones.

In many biological systems, oxygen is the only quencher present in significant concentrations.[3] In this case, the oxygen concentration in the Stern-Volmer can be replaced with the oxygen pressure [$pO_2 = (1/K_q)\ (1/t - 1/t_0)$]. Most importantly, characteristic of lifetime method is that the calibration is dependent only on the phosphor and its molecular environment. When the environmental parameters are held constant, the calibration is absolute; once the calibration constants are determined they can be generally applied, independent of the operator, within limits. The laser flash photolysis system was used to measure the lifetime of the cells stained with PdTCPP.

3. Conclusions

As shown in Fig. 1, lifetime decay data could be easily fitted by a single decay model, indicating that solely bimolecular quenching between oxygen and probe molecules was occurred in the cell during the quenching process. Interpreted lifetime of MH134 (1 x 10^6 cell/ml) incubated with PdTCPP (1 x 10^{-6} M) was 360 μs. This value corresponds to 5.2 torr based on the calibration data (Fig. 2 ; t_0 = 710 μs and K_q = 260 $torr^{-1}s^{-1}$ at 23℃, pH 7.4), indicating MH134 cells to be severely hypoxic. After all these experiments, no detectable phosphorescence was observed from these supernatants of the samples. These results support that cells were still living and metabolized during the long measurement.

References

1. N. Opitz and D. W. Lubbers, Adv. Exp. Med. Biol. **180**, 261 (1984).
2. S.-K. Lee and I. Okura, Anal. Chim. Acta, **342**, 181 (1997).
3. R. D. Shonat, D. F. Wilson, C. E. Riva, M. Pawlowski, Appl. Optics, **31**, 3771 (1992).

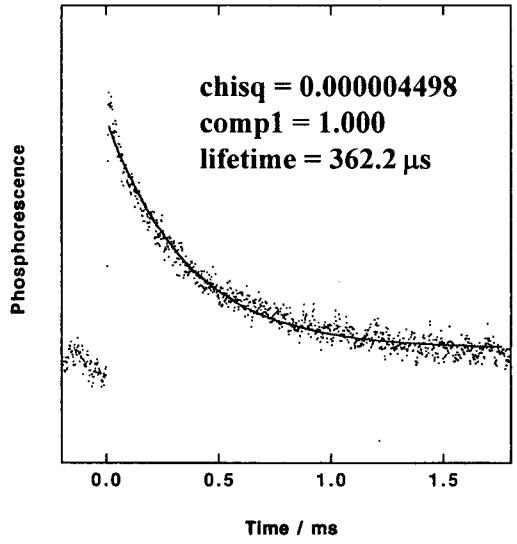

Fig.1 Typical decay of PdTCPP-stained MH134 cells

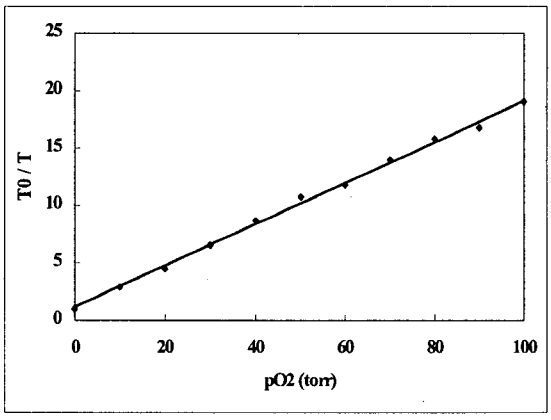

Fig. 2 Calibration curve of PdTCPP -stained MH134 cells