Measurement of Oxygen Concentrations in Tumor Cells by the Phosphorescence Quenching Method

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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) in order to useful information concerning cancer treatments such as photodynamic therapy. 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] As a result, these compounds show little or no fluorescence and their resulting phosphorescence quantum efficiency is high. Thus the phosphorescence can be readily measured not only by using conventional photomultipliers but also by using intensified video cameras. Measured phosphorescence can be quantitatively converted by the Stern-Volmer relationship:

$$I_0 / I = \tau_0 / \tau = 1 + K_q \tau_0 [O_2] \quad (1)$$

where I_0 and I are phosphorescence intensities in the absence and in the presence of oxygen concentration, $[O_2]$, respectively; τ_0 and τ are lifetimes in the absence and in the presence of oxygen, respectively; K_q is the bimolecular quenching constant.

PdTCPP was synthesized by benzonitrile method. MH134 (mouse liver carcinoma) and HeLa (human cervical carcinoma) cells were used for mainly photometric measurements and phosphorescence imaging, respectively. MH134 cells were grown as follows. First, C3H/HeJ mice were given an injection i.p. with approximately 10^7 cells from a culture of MH134 cells and the cells were allowed to grow for a period of about 10 days. HeLa cells were grown in MEM (minimum essential medium) supplemented with 10 % FBS (fetal bovine serum) and antibiotics. Cells were first sown (6×10^5) onto 6 cm tissue culture dishes with 4ml of 10% FBS containing MEM and incubated overnight at 37 ºC. Next they were washed with PBS (phosphate buffered solution) and placed with MEM, and further measurements were performed immediately. 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. As shown in Figure 1, 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 reported in Figure 1(b). 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. Although intensity measurements are simple, the most common difficulties with intensity measurements are that (a) they are influenced by other chromophores in the system which absorb either the excitation or emitted light and (b) the intensity of phosphorescence cannot be distinguished from fluorescence emitted in the detected wavelength range (In Fig. 1b, we can see autofluorescence of the cells depicted by green and blue lines). Neither of these difficulties affect phosphorescence lifetime 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.

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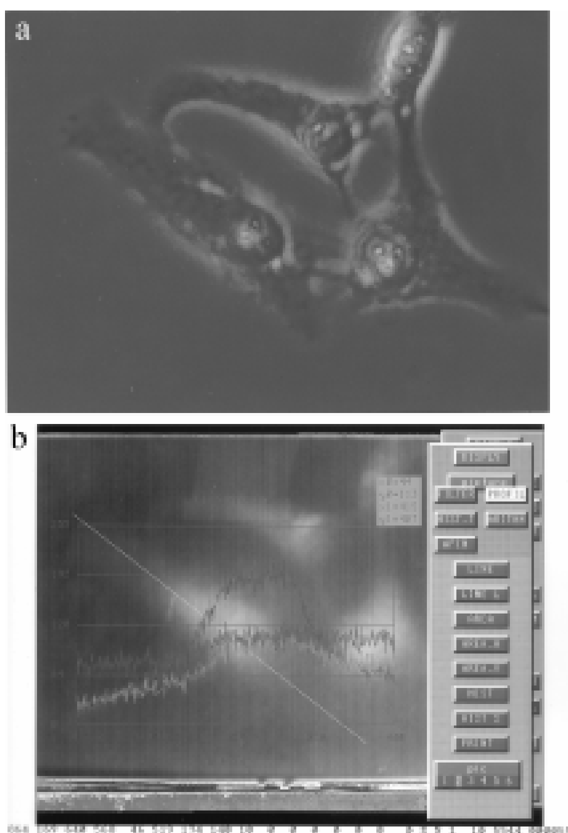

Figure 1. Images of PdTCPP-stained HeLa cells taken by using (a) phase contrast and (b) phosphorescence modes. Phosphorescence images were line-profiled to investigate oxygen distribution in the cells.

In many biological systems, oxygen is the only quencher present in significant concentrations.[3] In this case, the oxygen concentration in Eq. (1) can be replaced with the oxygen pressure. Equation (1) then becomes

$$pO_2 = (1/K_q)\,(1/\tau - 1/\tau_0) \quad (2)$$

Most importantly, characteristic of lifetime method is that the calibration is dependent only on the phosphor and its molecular environment.[4] 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.[5] The measurement setup and data analysis were carried out according to the previous report.[6] Removal of oxygen was achieved through Strambinis method.[7] The gas mixtures containing well-defined oxygen pressures are prepared by controlling the relative flow rates of nitrogen and oxygen. The total pressure was maintained at 760 Torr. As shown in Figure 2, 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.[6] Interpreted lifetime of MH134 (1×10^6 cell/mL) incubated with PdTCPP (1×10^6 M) was 360 μs. This value corresponds to 5.2 torr based on the calibration data (Fig. 2 inset; τ_0 = 710 μs and K_q = 260 $torr^{-1}s^{-1}$ at 23 °C, 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.

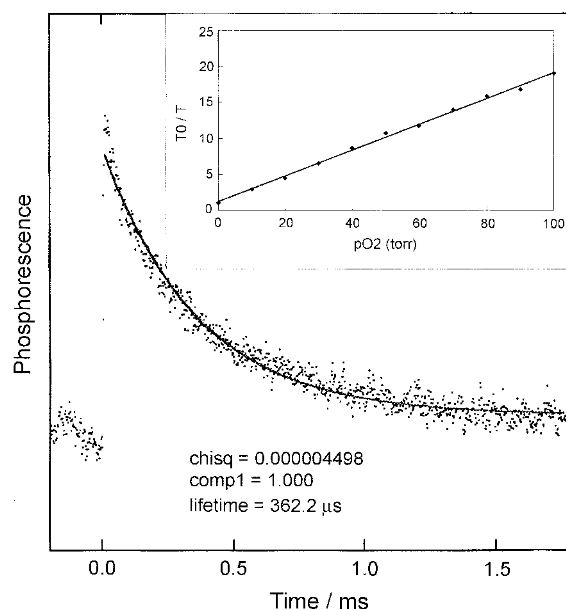

Figure 2. Typical decay of PdTCPP-stained MH134 cells after laser flash photolysis and the calibration curve (inset).

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