Chapter 8
Measuring Oxygen in Living Tissue: Intravascular, Interstitial, and "Tissue" Oxygen Measurements

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Abstract Oxygen dependent quenching of phosphorescence has been used to measure the oxygen pressure in both the vasculature of the microcirculation and the interstitial spaces of resting muscle tissue. Oxygen sensitive molecules were either dissolved in the blood (intravascular space) or micro-injected into the interstitial space and the distributions, histograms, of the oxygen pressure were measured. The mean oxygen pressures are higher in the blood than in the interstitial space but the oxygen pressures in the lowest 10% of the two spaces were not significantly different, indicating there is minimal (< 1 mm Hg) oxygen gradient between the two spaces in the capillary bed.

8.1 Introduction

Oxygen is bound to hemoglobin when it is transported from the lung to other tissues; but upon reaching those tissues, only diffusion is responsible for the movement of oxygen molecules from the blood plasma through the walls of the micro-vessels into the interstitial (pericellular) space, and finally into the cells to the mitochondria where oxygen is consumed. Quantitative measurements of oxygen pressures within different compartments of tissue are necessary in order to understand how oxygen diffusion affects oxygen delivery and the role of cellular oxygen pressure in tissue biochemistry and physiology.

Oxygen measurement methods, however, are typically either specific for one tissue compartment, such as the intravascular space (e.g., oxy/deoxy-hemoglobin-based

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measurements), or are non-specific, such as for oxygen electrodes inserted into the tissue. One method, oxygen dependent quenching phosphorescence, is unique in that it can be used for measuring oxygen in different tissue compartments.

In this paper, we compare oxygen measurements in the blood plasma of the microcirculation (vascular space) and interstitial space within tissue. The vascular wall is the only physical barrier between the blood plasma and the interstitial space, and oxygen consumption within the interstitial space is very small. The difference in oxygen pressure between the compartments is a function of the diffusion distance and the rate of oxygen consumption by the cells. Oxygen measurements have been made in both awake and anesthetized mice in order to control for the possibility of stress and/or anesthetic induced changes in blood pressure and vascular resistance.

8.2 Materials and Methods

Oxygen dependent quenching of phosphorescence provides a non-invasive optical method for determining oxygen pressures in biological and other samples (1-6,8,9,13-17,21,18,21,25-27). The available oxygen sensitive phosphors, such as Oxyphors R0, R2 and G2, contain Pd-porphyrin cores that are at least partially exposed to the medium, where they readily bind to biological macromolecules, particularly albumin. Albumin plays an important role, both limiting access of oxygen to the porphyrin core, thereby facilitating oxygen measurements in the physiological range (0-120 mm Hg), and providing a relatively homogeneous microenvironment for the porphyrin core.

For measurements of oxygen in the system containing no albumin, we have recently developed a new family of dendritic phosphors (10-12,19,22). The performance of the new probes is illustrated in Figure 1, where the Stern-Volmer plots are shown for Pt-porphyrin-based dendrimers generations 0 (no dendrimer added)-3 (for details, see ref. [12]).

With increase in the dendrimer generation, the oxygen sensitivity decreases. The oxygen sensitivity of the original core (G0) is very high, with a quenching constant of nearly 4000 mm $Hg^{-1}sec^{-1}$, - too high to be useful for measuring oxygen pressures in the physiological range. By generation 2 the quenching constant has decreased to less than 200 mm $Hg^{-1}sec^{-1}$, a value well suited for tissue oxygen measurements. Adding a coat of polyethylene glycol results in an oxygen sensor that is highly soluble in aqueous media and for which the oxygen sensitivity is the same whether it is dissolved in simple phosphate buffer or blood serum. Graph B of the figure shows that the PEG coated sensors are insensitive to the biological agents, including albumin, in blood serum. There was no difference in the Stern-Volmer plot for oxygen quenching whether this particular Oxyphor was dissolved in a simple phosphate buffer or blood serum from a rat.

Measurements of the distribution of oxygen (histograms) were carried out as described earlier (20,23,24). The excitation light of a frequency-domain phosphorometer was modulated as the sum of 37 different frequencies ranging from 100 Hz

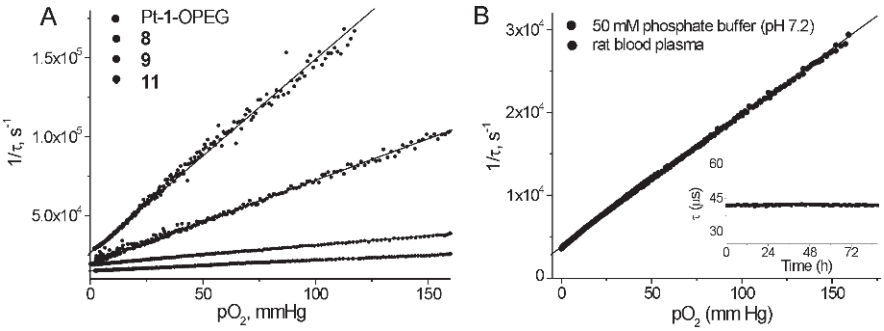

Fig. 8.1 A) Stern-Volmer plots of Pt porphyrin oxygen probes of different dendritic generations. The periphery of the dendrimers is modified with oligoethyleneglycol residues, which makes the probes insensitive to the presence of biological macromolecules, which is illustrated by the experiment shown in graph B), where the dendritic probe was titrated in the aqueous buffer and in the rat blood plasma, rendering identical Stern-Volmer plots. The inset shows signal stability evaluation for the same probe.

to 38,000 Hz. The collected data were used to calculate the distribution of phosphorescence lifetimes and the amount of the phosphor corresponding to each lifetime. The histograms have been normalized by setting the sum (integral) of the Y axis values for each histogram to 1.0. This procedure removes the dependence on the excitation light intensity and phosphorescence collection efficiency. Both the intensities (amplitudes) and lifetimes of phosphorescent signals decrease with increasing oxygen pressures. The decrease in signal strength with increasing oxygen pressure (decreasing the signal to noise) results in the "tail" effect on the high oxygen end of the histogram. This asymmetric broadening is intrinsic to distribution analysis, where the uncertainty in determination of phosphorescence lifetimes increases with decreasing signal-to-noise ratio (S/N).

There is no evidence of toxicity of the Oxyphors, either alone or when illuminated. Injection of the Oxyphor into the blood has no effect on the measured physiological parameters in 2-5 day old pigs or when added to cell culture media had no effect on growth of the cells in culture.

8.3 Results and Discussion

Oxygen pressures in the intravascular and interstitial spaces have been measured using Pd-tetrabenzoporphyrin encapsulated inside generation 2 poly-arylglycine (AG) dendrimer covered with oligoethylene glycol residues (Av. MW 350). This sensor, Oxyphor G3, is biologically inert and can be loaded into the interstitial space by injection into tissue using a 30 guage or smaller needle. Either Oxyphor G3 or Oxyphor G2 can be injected into the blood for measuring the intravascular oxygen pressure. Measurements of the oxygen distribution in the interstitial space have been com-

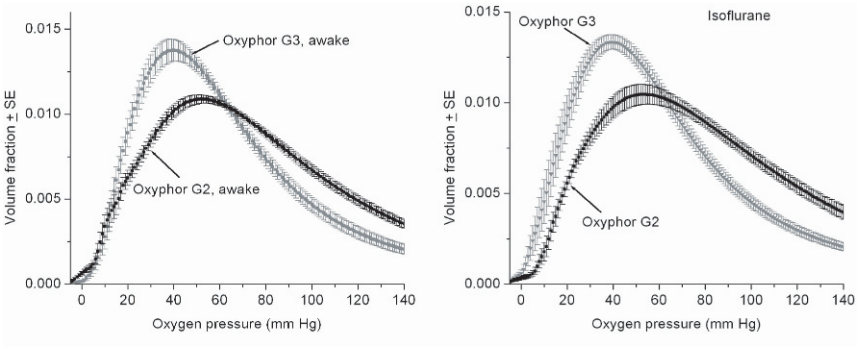

Fig. 8.2 Comparison of the oxygen histograms measured for the interstitial space (Oxyphor G3) and intravascular space (Oxyphor G2) of skeletal muscle in awake (A) and isoflurane anesthetized (B) mice. The histograms are the means for 11 (Oxyphor G3) or 10 (Oxyphor G2) independent experiments with error bars indicating the Standard Error of the Mean (±SE).

pared with those for the intravascular (blood plasma) space in normal and tumor tissue in awake and anesthetized animals. For normal resting muscle in awake mice (Fig. 2), the lowest 10% of oxygen pressures in normal muscle for the interstitial and intravascular spaces were not different (< 1 mm diffusion gradient) whereas in isoflurane anesthetized mice there was a small but significant ($p = 0.01$) difference. In both cases, however, the intravascular oxygen histograms were "skewed" to higher oxygen values compared to the interstitial space, consistent with a population of larger vessels with oxygen pressures significantly higher than in the surrounding interstitial space.

The oxygen pressures in K1735 tumor tissue were very low in both the intravascular and interstitial spaces when measured in awake mice but these increased dramatically when the mice were anesthetized with isoflurane. This was in contrast to resting muscle where the histograms for awake and anesthetized mice were not significantly different. The increase in oxygen pressure in the tumor is consistent with the isoflurane lowering the resistance of the tumor vessels relative to normal muscle, increasing blood flow to the tumor tissue. Tumor tissue also differs from muscle in that the lowest 10% of the oxygen distribution in the interstitial space was significantly lower than for the intravascular space. The presence of an increased oxygen gradient is consistent with tumor tissue having regions with significantly greater distances from the vessels to the cells than does muscle.

8.4 Conclusions

1. In normal resting muscle the oxygen pressure difference from the vascular space of the capillaries to the interstitial space is less than 1 mm Hg. Thus, the vascular

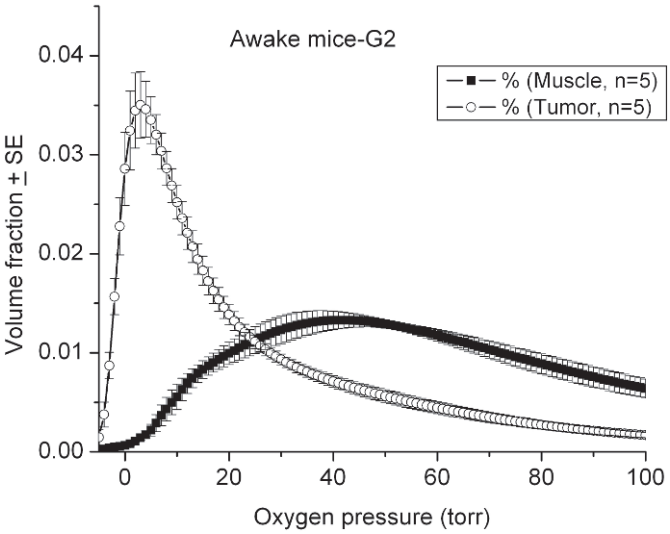

Fig. 8.3 Comparison of the oxygen histograms for the blood in the microcirculation (Oxyphor G2) of K1735 tumors (open squares) and normal muscle (solid squares) in awake mice.

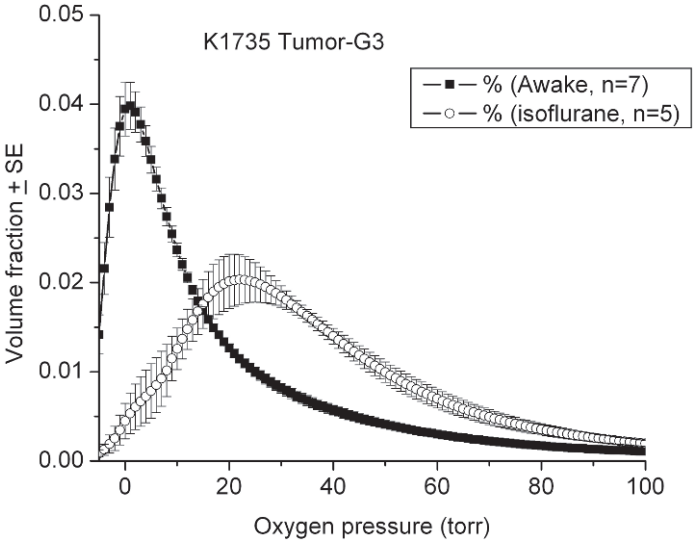

Fig. 8.4 Histograms of the oxygen distributions in the interstitial space (Oxyphor G3) of K1735 tumors in awake (solid squares) and isoflurane anesthetized (open squares) mice.

wall does not cause a significant diffusion/metabolism induced decrease in oxygen pressure between the blood plasma and the pericellular space.

2. In K1735 tumors of awake animals, the oxygen pressures in both the vascular and interstitial spaces of the tumor are much lower than in normal muscle. There

is also a substantial intravascular/interstitial space oxygen difference, consistent with substantially increased distances from the capillary walls to the cells.
3. The oxygen levels in K1735 tumors greatly increase when mice are given isofluorane anesthesia, rising nearly to those of normal tissue. This suggests an anesthetic induced decrease in vascular resistance in the tumors relative to the surrounding tissue.

Acknowledgments

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