Chapter 7
Oxygen Pressures in the Interstitial Space of Skeletal Muscle and Tumors in vivo

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Abstract A new Oxyphor (Oxyphor G3) has been used to selectively determine the oxygen pressure in interstitial (pericellular) spaces. Oxyphor G3 is a Pd-tetrabenzoporphyrin, encapsulated inside generation 2 poly-arylglycine (AG) dendrimer, and therefore is a true near infrared oxygen sensor, having a strong absorption band at 636nm and emission near 800nm. The periphery of the dendrimer is modified with oligoethylene glycol residues (Av. MW 350) to make the probe water soluble and biologically inert. Oxyphor G3 was injected along “tracks” in the tissue using a small needle (30gage or less) and remained in the pericellular space, allowing oxygen measurements for several hours with a single injection. The oxygen pressure distributions (histograms) were compared with those for Oxyphor G2 in the intravascular (blood plasma) space. In normal muscle, in the lower oxygen pressure region of the histograms (capillary bed) the oxygen pressure difference was small. At higher oxygen pressures in the histograms there were differences consistent with the presence of high flow vessels with oxygen pressures substantially above those of the surrounding interstitial space. In tumors, the oxygen pressures in the two spaces were similar but with large differences among tumors.

In mice, anesthesia with ketamine plus xylazine markedly decreased oxygen pressures in the interstitial and intravascular spaces compared to awake or isoflurane anesthetized mice.

7.1 Introduction

Oxygen transported to tissue, after reaching the tissue microcirculation, diffuses from the blood plasma through the walls of the micro-vessels into the interstitial (pericellular) space and then from interstitial space into the cells and

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finally to the mitochondria. As it diffuses, from the source (blood plasma) to a sink (mitochondria), an oxygen pressure gradient is formed in which the pressure is lower at the sink than at the source. The difference in oxygen pressure between the blood plasma and the mitochondria increases with increase in the rate of oxygen consumption by the mitochondria and the distance from the vessel to the mitochondria. The distance over which oxygen can be supplied to the mitochondria is, therefore, determined by a) the rate of oxygen consumption by the mitochondria, b) the distance from the blood plasma (the oxygen source) to the mitochondria and c) the oxygen pressure in the blood plasma.

Oxygen dependent quenching of phosphorescence is a minimally non-invasive optical method that can quantitate oxygen pressures in biological and other samples [1–4]. Although it has been widely used [1–17] for measurements in vivo, focus has been on the intravascular space. The available oxygen sensitive phosphors, such as Oxyphors R0, R2 and G2 (Oxygen Enterprises, Ltd, Philadelphia, PA), contained Pd-porphyrin cores that are at least partially exposed to the medium. As a result, the oxygen sensitivity is dependent on the microenvironment of the porphyrin and therefore on the macromolecule to which it is bound, and on the fraction of the Oxyphor bound to that macromolecule. In blood plasma, Oxyphors R0, R2 and G2 are essentially quantitatively bound to albumin. Albumin plays an important role, helping both to limit access of oxygen to the porphyrin core, facilitating oxygen measurements in the physiological range (0–120 Torr), and to provide a relatively homogeneous microenvironment for the phosphor.

A new family of Oxyphors has been synthesized that can be used in a much wider range of media, particularly in highly heterogeneous environments such as the interstitial space. The porphyrin core is first coated with dendrons and then the external surface of the dendrimer modified with oligoethylene glycol fragments [18–20]. Oxyphor G3 is a member of this oxygen sensor family. Not only are its oxygen quenching properties unaffected by biological macromolecules such as albumin, but also its oxygen quenching constant and phosphorescent lifetimes are well suited for measuring oxygen in vivo and in vitro.

7.2 Materials and Methods

7.2.1 Measurement of Oxygen Pressure Histograms

Phosphorescence lifetime measurements were performed using a PMOD-5000 phosphorometer (Oxygen Enterprises, Ltd., Philadelphia, PA, USA) [4], a frequency domain instrument with a range of 100–100,000 Hz. Phosphorescence lifetimes are independent of local phosphor concentration and insensitive to endogenous tissue fluorophores and chromophores. The PMOD-5000 was used in multifrequency mode [4] in order to determine distributions of phosphorescence lifetimes. The lifetime distributions were used to calculate