SPRINGER BRIEFS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

Dmitri B. Papkovsky
Alexander V. Zhdanov
Andreas Fercher
Ruslan I. Dmitriev
James Hynes

Phosphorescent Oxygen-Sensitive Probes

Springer

SpringerBriefs in Biochemistry and Molecular Biology

For further volumes:
http://www.springer.com/series/10196

Dmitri B. Papkovsky · Alexander V. Zhdanov
Andreas Fercher · Ruslan I. Dmitriev
James Hynes

Phosphorescent Oxygen-Sensitive Probes

Springer

Dmitri B. Papkovsky
Department of Biochemistry
University College Cork
Cork
Ireland

Ruslan I. Dmitriev
Department of Biochemistry
University College Cork
Cork
Ireland

Alexander V. Zhdanov
Department of Biochemistry
University College Cork
Cork
Ireland

James Hynes
Luxcel Biosciences Ltd.
BioInnovation Centre, UCC
Cork
Ireland

Andreas Fercher
Department of Biochemistry
University College Cork
Cork
Ireland

ISSN 2211-9353 ISSN 2211-9361 (electronic)
ISBN 978-3-0348-0524-7 ISBN 978-3-0348-0525-4 (eBook)
DOI 10.1007/978-3-0348-0525-4
Springer Basel Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012945485

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Measurement of dissolved oxygen concentration in biological samples by luminescence quenching method has been introduced by the pioneering work of German scientists Dietrich Lubbers and Norbert Opitz who developed in the mid-1970s first solid-state fluorescence-based O_2 sensors called 'optodes', and by the group of David Wilson in the US who introduced the phosphorescence-based probes and O_2 imaging technique in the mid-1980s. High application potential of this technique has been recognised back then, however its use was rather limited, mainly by research groups who had access to or developed themselves dedicated materials and instrumentation for O_2 sensing and possessed special skills.

In the last decade we have witnessed a major change in the uptake of optical O_2 sensing techniques, with many new materials, measurement formats, detection and imaging platforms, analytical methodologies and accessory tools developed and tested. O_2 sensing systems have been adapted for use with standard laboratory equipment such as time-resolved and lifetime-based luminescent readers, live cell imaging systems, liquid handling equipment (microplates, biochips). They have been applied to various measurement tasks and mechanistic studies with complex biological models demonstrating high utility for biomedical research and new insights into cell and tissue function. O_2 sensor technology has now become much more accessible and affordable for ordinary users working in various disciplines of life and biomedical sciences.

On the other hand, the existence of many probes, measurement formats, and detection platforms make it difficult for the user to select optimal combination to address their particular biological problem or measurement task. Also, distinctive features of these probes compared to other fluorescent probes, and general conditions of their use for O_2 monitoring are not always assessed comprehensively by their end-users. This often leads to experimental artifacts, failures, or incorrect interpretation of data. Critical literature describing their practical uses is also in short supply.

This book is aimed to address these aspects and provide a general overview of existing and emerging O_2 sensing probes, detection platforms, and applications in their various modifications, based on authors' long-standing experience in this

area. In the first chapter, the most popular phosphorescent probes based on Pt-porphyrin dyes are described and cross-compared. Subsequently, core biological applications of these probes with different in vitro models (in vivo applications such as imaging of tissue oxygen are outside the scope) are described. For these applications, which are divided into two main groups and chapters—plate reader analysis and O_2 imaging—key technical details are provided on how to set them up, conduct the measurements, extract the analytical and physiological information, interpret the results, and perform troubleshooting. Altogether, this gives potential users a fair representation of merits and limitations, analytical capabilities of the different probes, O_2 sensing and imaging platform(s), and a comprehensive practical guide for their rational selection. The book is expected to facilitate a broader use of the probes and development of new applications.

Dmitri B. Papkovsky

Contents

Chapter 1
O_2-Sensitive Probes Based on Phosphorescent Metalloporphyrins

Ruslan I. Dmitriev and Dmitri B. Papkovsky

Abstract Measurement of molecular O_2 in biological samples represents an important group of analytical methods actively employed in diverse areas of biology (microbes, plants, animals), medicine and toxicology. In this chapter, the significance, classification of main methods and principles of quenched-phosphorescence measurements of O_2 with the help of metalloporphyrin based probes are described. Various measurement platforms are discussed with particular attention to the experimental models.

Keywords Oxygen-sensitive probes · Pt-porphyrins · Phosphorescence quenching · Time-resolved fluorescence · Intracellular probes · Oxygen sensing and imaging · In vitro assays · Cellular oxygen

1.1 Introduction

Measurement of molecular oxygen (O_2) in biological samples containing respiring cells and tissues is of high practical and biomedical importance. O_2 is a small, gaseous, non-polar analyte which has moderate solubility in aqueous media ($\sim$200 μM at air saturation, 37 °C). It is supplied to cells and tissues by passive diffusion and, in higher multicellular organisms, by convectional transport via vasculature, red blood cells and haemoglobin [1, 2]. In mammalian cells O_2 is the key metabolite and the source of energy involved in the production of ATP through the electron transport chain and oxidative phosphorylation [3]. It is a substrate of numerous enzymatic reactions vital for cellular function, involved in cell signalling and genetic adaptation to hypoxia [4, 5]. Therefore, detailed understanding of biological roles of O_2 is of fundamental importance for cell biology, medicine, drug discovery and other disciplines [1, 6].

D. B. Papkovsky et al., *Phosphorescent Oxygen-Sensitive Probes*,
SpringerBriefs in Biochemistry and Molecular Biology,
DOI: 10.1007/978-3-0348-0525-4_1, © The Author(s) 2012

The main analytical tasks in O_2 measurement are: (1) assessment of bulk oxygenation of samples containing cells, tissues, organs and whole organisms; (2) measurement of O_2 consumption rates (OCR); (3) analysis of O_2 distribution, localised gradients and O_2 maps in heterogeneous samples; (4) analysis of subcellular O_2 gradients, and (5) monitoring of dynamics of parameters 1–4 in response to changes in cellular function, for example, in normal/resting and diseased/stimulated cells and tissues.

Analytical task 1 probably has the highest importance: under normal physiological conditions, O_2 levels in different tissues are maintained within the defined limits, which are tissue-specific [7, 8]. Significant alterations in oxygenation from the norm are observed in diseased tissues and under pathological conditions, e.g. in solid tumours, under ischaemia/stroke, anaemia, neurodegeneration, hypertension, metabolic disorders. Short-term and sustained hypoxia can lead to cell death or protection and adaptive responses via rearrangement of cell metabolism. The latter includes Warburg effect, hypoxia-induced expression of regulatory genes and proteins such as HIF-1α, PGC-1α [2, 5, 9–11] and their downstream products. On the other hand, significant spatial and temporal fluctuations in O_2 occur in exercised skeletal and cardiac muscles, excited regions of the brain, kidney during their normal function [2, 12–14].

OCR reflects respiratory activity of a sample and, together with other biomarkers such as ATP content, mitochondrial membrane potential, metabolite concentrations and ion fluxes, provides important information on the metabolic activity and bioenergetic status. Significant alterations in cellular OCR reflect perturbed metabolism, mitochondrial dysfunction, disease state or drug toxicity [3]. Analytical tasks 3–5 are best addressed by means of O_2 imaging techniques, which allow mapping of O_2 concentration within biological samples in 2D, 3D and 4D (time lapse experiments), and with sub-micrometer spatial resolution.

Due to the high importance of O_2 measurement, the diversity of analytical tasks and biological objects to be analysed, different measurement methodologies have been developed for O_2 sensing. The particular sample, measurement location, concentration range, sampling frequency and resolution of O_2 to be measured determine the choice of experimental technique, measurement format, detection modality, the particular probe, instrumentation and other tools.

Among these the following main groups can be defined:

1. Electrochemical methods utilising electrodes, such as the Clark electrode system.
2. Physical methods utilising paramagnetic properties of O_2, such as EPR spectrometry.
3. Optical methods.

The Clark-type electrode [15] has a relatively simple set-up and low cost. In this system, sample O_2 diffuses through a Teflon membrane to a Pt electrode polarised at about +0.7 V against the Ag/AgCl electrode, where it gets reduced generating current proportional to O_2 concentration. Its main applications are point measurement of dissolved O_2 and absolute OCR of macroscopic biological

samples containing suspension cells or isolated mitochondria in a sealed, stirred and temperature controlled cuvette, as well as measurement of local O_2 levels in cells and tissues with microelectrodes [16, 17]. Their principal drawbacks are O_2 consumption by the electrode itself, the need for stirring and regular maintenance, baseline drift, poisoning and fouling of the electrode, poor compatibility with adherent cells and effector treatments, limited throughput. In the past years, much progress has been made in addressing these limitations and adapting the technology for use with adherent cell lines in multiparametric biochips [16] or customised systems for cerebellar granule neurons [17, 18].

Electron paramagnetic resonance (EPR) is a physical method for detecting molecules with unpaired electrons. Since O_2 is a paramagnetic molecule, EPR can be used for its quantification, directly or indirectly using dedicated probes. Extracellular EPR probes such as India Ink [19] were developed for clinical use and assessment of cell populations. Nitroxyl and esterified trityl radicals (e.g. triarylmethyl) represent promising probes for EPR imaging of intracellular O_2 [20, 21]. In vivo EPR imaging with micron resolution ($30 \times 30 \times 100$ μm) has been demonstrated [22] which complements the other O_2 sensing techniques well. EPR spectra and intensity signals can be used for quantification.

Optical methods rely on endogenous or exogenous probes which alter their properties in response to fluctuations in O_2 concentration. The absorption-based methods (e.g. myoglobin in muscle tissue [23]) have been complemented by the luminescence-based techniques which include the measurement of fluorescence of redox indicators (e.g. NADH and FAD) [24], delayed fluorescence of endogenous protoporphyrin IX [25, 26], photoacoustic tissue imaging [27], GFP-based biosensor constructs [28–30] and quenched-luminescence O_2 sensing [31–33].

Luminescence quenching represents one of the most powerful and versatile techniques which allows direct, minimally or non-invasive, real-time monitoring and imaging of O_2 in biological samples with good selectivity and tunable sensitivity [32, 34]. This technology provides reliable and accurate detection of O_2 in different formats including single point macroscopic sensors and microsensors, in vitro bioassays based on O_2 detection, screening platforms (cell, enzyme and animal based) operating on commercial fluorescent readers, sophisticated live cell and in vivo imaging systems, multi-parametric systems in which O_2 detection is coupled with the other probes and biomarkers. A number of such systems and applications have already gained wide use and are produced commercially [35, 36].

The key components of the optical O_2 sensing technique are dedicated luminescent materials that enable O_2 to be probed in complex biological objects, particularly those containing respiring cells. The main O_2 pools that require quantification and monitoring are: (i) dissolved extracellular O_2 in growth medium or vasculature; (ii) pericellular O_2 in the interstitial space, at cell surface; (iii) intracellular O_2 in the cytosol, mitochondria or other compartments; (iv) in vivo measurement of O_2 distribution in live tissues, organs and whole organisms.

Pt(II)- and Pd(II)-porphyrins and some related structures possessing strong phosphorescence at room temperature, moderate quenchability by O_2 and high chemical stability are among the common indicator dyes used in O_2-sensing