Phosphorescent oxygen-sensitive materials for biological applications†

Ciara O'Donovan,[a] James Hynes,[a] Dmitri Yashunski[b] and Dmitri B. Papkovsky*[a]

Received 2nd February 2005, Accepted 25th April 2005
First published as an Advance Article on the web 9th May 2005
DOI: 10.1039/b501748c

A number of macromolecular probes employing different carriers and a number of microparticular probes employing different oxygen sensitive dyes were fabricated, giving a panel of oxygen sensitive probes. The photophysical and oxygen sensing properties of these probes were examined comparatively. The probes were used successfully to monitor cellular oxygen uptake and their ability to overcome a number of problems associated with oxygen sensing in biological samples was assessed. Macromolecular probes proved sufficient in a number of cases, particularly where spectral solutions can resolve the interferences. However where physical interactions cause interference the added protection of the polymer in the particle based probes was required.

Introduction

Optical oxygen sensing allows direct quantitation and monitoring of molecular oxygen through the use of fluorescent or phosphorescent oxygen-sensitive materials. The spectral characteristics of fluorescent ruthenium complexes[1,2] and phosphorescent metalloporphyrins[3,4] are well suited for oxygen sensing applications. They allow the use of robust and inexpensive LED and photodiode based instrumentation and facilitate the monitoring of luminescence lifetimes by fluorescence phase measurements.[4]

Oxygen probes may be either solid state or water soluble. Solid-state probes comprise a hydrophobic dye immobilised in or on an oxygen permeable matrix, which prevents sample contamination and protects the dye from potentially interfering compounds. Examples include PtOEPK/polystyrene,[5] $Ru(dpp)_3$/silicone,[6] and $Ru(dpp)_3$/polysulfone.[7] Solid-state sensors are well suited to accurate determination of absolute oxygen concentration and prolonged and/or continuous monitoring resulting in their widespread use.[8–14] An attractive biological application is the measurement of cellular oxygen uptake.[15,16] This allows the assessment of test cell metabolic status and events affecting aerobic pathways such as drug/effector action, pathological processes, *etc.*[12,17] Such applications impose number requirements which diminish the effectiveness of solid-state sensors.

The demands of such biological applications have led to the development of a number of water-soluble oxygen sensitive probes.[12,18,19] Their water soluble nature facilitates greater versatility with respect to measurement format but they display a number of limitations. Their small size and direct exposure to the sample being measured leaves them susceptible to uptake by cells, degradation, and binding to sample components. A number of these limitations have been overcome by the development of macromolecular water-soluble probes,[3,15] where the macromolecular carrier impacts on probe characteristics. Their larger size and hydrophilicity prevent their uptake by cells however they remain susceptible to interference from compounds present in the test sample. This issue has been addressed for intracellular oxygen measurements[14,20] through the development of particle-based oxygen sensors, whereby a dye is encapsulated in a polymer matrix, giving a suspension of uniform particles. A similar yet more simplistic approach may be taken for the measurement of extracellular oxygen in biological samples.

In this paper we describe a number of new oxygen sensitive probes both macromolecular and microparticular in nature. Spectral characterisation and oxygen sensing properties of microparticular probes and macromolecular probes employing different carriers is carried out. Their suitability for monitoring oxygen uptake is assessed along with their ability to overcome a number of problems associated with oxygen sensing in biological samples.

Experimental

Materials

Oxygen-sensitive dyes: monofunctional *p*-isothiocyanatophenyl derivative of platinum(II)-coproporphyrin-I (PtCP-NCS), platinum(II)-octaethylporphine (PtOEP), platinum(II)-octaethylporphine-ketone (PtOEPK) were from Luxcel Biosciences, Ireland. Bis(2,2′-bipyridine)(5-isothiocyanatophenanthroline)rutheniumbis(hexafluorophosphate) (Ru-NCS) was from Fluka, USA. Amino-dextran (MW 40 000) was from Molecular Probes, USA. Mono-amino polyethylene glycol (PEG, MW 20 000 and 5 000) was from Shearwater Corporation, USA. Polystyrene/divinylbenzene microparticles were from Bangs Laboratories, USA. Bovine serum albumin (BSA), dimethylsulfoxide (DMSO), heavy mineral oil, sodium sulfite, caffeine, dantrolene, camptothecein, glucose and glucose oxidase were from Sigma-Aldrich, USA. LB broth, Tryptone peptone and yeast extract were from BD Biosciences, USA. All salts and solvents were of analytical grade; buffers were prepared using Millipore-grade water. PD-10 desalting columns were from Amersham, UK. Tissue culture treated 96-well clear polystyrene plates were from Sarstedt, Ireland.

† The first and second authors contributed equally to this manuscript.

Phosphorescent oxygen-sensitive probes

Macromolecular oxygen probes were synthesized by conjugation of PtCP-NCS to BSA, amino-PEG and amino-dextran, and Ru-NCS to BSA using a previously described method.[15] Briefly, the macromolecular carrier was incubated with a 5–10 fold molar excess of PtCP-NCS in 0.1 M carbonate buffer, pH 9.5 for 1–6 hours, followed by separation on a PD-10 gel-filtration column in phosphate buffered saline (PBS) (0.3 M NaCl, 0.1 M phosphate, pH 7.4). Conjugates were then desalted, lyophilised and stored dry in the dark at 4 °C.

The microparticle-based oxygen probes were developed in conjunction with Luxcel Biosciences (Ireland). Monodispersed polystyrene/divinylbenzene co-polymer microparticles (D = 3.3 μm) were washed with a gradient of solvents of increasing hydrophobicity before incubation with 4 mM dye in dichloromethane. Following this incubation the particles were washed with solvents of increasing hydrophilicity. After fabrication the probes were lyophilised and stored at 4 °C.

Optical measurements

UV-VIS absorption spectra of the conjugates (range: 250–650 nm) were measured on an HP8453 diode array spectrometer (Agilent, USA) in a 1 cm quartz cell. Excitation and emission spectra were measured on an LS-50B luminescence spectrometer (Perkin Elmer, USA). Prompt fluorescence measurements were performed using a SpectraMax Gemini plate reader, with excitation and emission set at 380 nm and 650 nm respectively and an emission cut-off set at 630 nm. Time-resolved fluorescence (TR-F) measurements were performed on a Victor2 time resolved fluorescence plate reader (Perkin Elmer Life Sciences), using a 340/642 nm set of filters (pre-installed), and a delay and measurement time of 30 μs and 100 μs respectively. Phosphorescence decay curves were measured on the TR-F plate reader ArcDia (ArcDia, Arctic Diagnostics Oy, Finland) using pulsed excitation with 532 nm laser (10 μs square pulses), 650 nm emission filter, and multichannel scaler with a 0.5 μs bin, and lifetimes were calculated using single exponential fit. Depletion of oxygen, for oxygen free measurements, was achieved by addition of sodium sulfite (5 mg ml^{-1}). Quenching constants were calculated from five point calibrations. These calibration measurements were initiated at air saturated oxygen concentrations and continued by bubbling 0, 0.5, 2, 5 and 21% oxygen through a solution of each of the probes, to produce solutions with 0, 2.4, 10, 24 and 100% air saturation. The probes emission was monitored at the appropriate wavelength on an LS-50B luminescence spectrometer (Perkin Elmer, USA).

Monitoring of oxygen uptake

For the monitoring of oxygen uptake by the bacterium *E. coli*, a starter culture of wild type (WT) *E. coli* was prepared by addition of 100 μl of stock WT *E. coli* into 10 ml of LB broth (tryptone peptone, yeast extract and NaCl 10 g l^{-1}, 5 g l^{-1} and 10 g l^{-1} respectively). This was incubated overnight at 37 °C with shaking. 1 ml of this starter culture was added to 100 ml of LB broth and cultured at 37 °C with shaking, until the bacterial OD600 value reached ~0.5 AU. Stock bacterial cells were diluted and counted using a haemocytometer.

To perform respirometric assays, *E. coli* cells were diluted to the desired concentration (10^5 to 10^7 cells ml^{-1}) and oxygen probe was added to give final concentrations of 5 μg ml^{-1} for PtOEP particle based probe or 10^{-7} to 10^{-9} M for PtCP-BSA or PtCP-PEG (depending on the experiment and the instrument used). Samples containing cells and probe were then dispensed in 150 μl aliquots into the wells of clear 96-well plates (Starsted, Ireland). The plates were preheated at 37 °C for 10 min to allow temperature and gas equilibration. The samples were then overlaid with 100 μl of heavy mineral oil to reduce the level of diffusion of atmospheric oxygen into the sample and initiate the respirometric assay. Prompt and time-resolved phosphorescence intensity measurements were carried out on the SpectraMax Gemini fluorescence plate reader and Victor2 TR-F plate reader respectively. The assays were carried out using instrument settings outlined above, with measurements being taken at regular intervals (1–3 min) for approximately 30–120 min.

For enzymatic oxygen uptake measurements, glucose oxidase (Sigma, USA) was diluted to the desired concentrations in PBS buffer containing 80 mM glucose, mixed with oxygen probe and dispensed in 150 μl aliquots into the wells of a clear 96-well plate (Starstedt, Ireland). Analysis of oxygen consumption was done at room temperature using the respirometric assay set-up outlined above.

Assessment of quenching interferences

For the assessment of quenching interferences a solution of camptothecin, caffeine or dantrolene stock in PBS was added at indicated final concentration to 5 μg ml^{-1} for PtOEP-PS particle probe or 10^{-7} to 10^{-9} M for PtCP-BSA probe in PBS. The samples were then dispensed in 150 μl aliquots into the wells of 96-well plates. Prompt and time-resolved phosphorescence intensity measurements were carried out on the SpectraMax Gemini fluorescence plate reader and the Victor2 TR-F plate reader respectively. The assays were carried out as end point assays using the instrument settings outlined above. Depletion of oxygen for oxygen free measurements was achieved by addition of 5 mg ml^{-1} of sodium sulfite.

Results and discussion

Probe design

Macromolecular oxygen-sensitive probes produced by covalent linkage of the PtCP-NCS dye to BSA have previously been described.[15] These cell impermeable, non-toxic probes display moderate sensitivity to oxygen and satisfactory performance in model cell-respirometric assays. They have convenient spectral characteristics making them compatible with standard fluorescence and time-resolved fluorescence plate readers, spectrometers and imagers. However, these oxygen-sensitive probes, as with antibody conjugates,[4,21,22] display considerable internal quenching of the PtCP label resulting in reduced phosphorescent signals (*i.e.* specific brightness).

In this work we employed a similar method to link the PtCP label to other hydrophilic macromolecular carriers, namely a polysaccharide amino-dextran (MW 40 000) and polyethylene glycols MW 20 000 and 5 000 with a terminal amino-modification (mono-amino-PEG). These macromolecular carriers have different chemical compositions than the previously described polypeptide conjugates, both have low intrinsic charge, linear structure and better defined sites of PtCP attachment. For example mono-functionalised PEG produces 1 : 1 conjugates, with the PtCP label attached to the end of the linear polymeric chain. These oxygen probes are easy to synthesize and standardize, it was also anticipated that by altering the macromolecular carrier one could modulate the resulting probes sensitivity to oxygen and their phosphorescent signal. To broaden the scope of the study, probe performance was also compared to that of a ruthenium based oxygen probe (Ru-NCS linked to BSA). A limitation of such macromolecular probes is that the oxygen sensitive component is directly exposed to the sample being measured. Thus certain chemical and biological compounds present in the sample may interfere with probe function and impair performance in respirometric assays. To circumvent this potential limitation particle probes were fabricated.

Particle-based probes were fabricated by impregnating polymer microparticles with PtOEP and PtOEPK dyes. In solid-state oxygen sensors, polystyrene is a common matrix for these dyes,[4] and monodispersed microparticles of this polymer are readily available, *e.g.* for flow-cytometry and bioaffinity assay applications. Impregnation of the oxygen-sensitive phosphorescent metalloporphyrins into the polystyrene microparticles provides physical protection for the dye. This is particularly important in complex biological samples, as components of the sample may interfere with the dye by quenching or other means. In contrast to the macromolecular conjugates, particle-based probes do not require the use of functionalised dyes. This allows greater flexibility in developing probes with different spectral characteristics.

Photophysical and quenching properties of the probes

The spectral regions 360–400 nm (Soret band) and 525–545 nm (Q-bands) can be used to excite probes based on PtCP and PtOEP dyes. Their phosphorescence is easily detected by steady-state fluorescence measurements using conventional fluorescence spectrometers and plate readers. In addition, the long decay emission of PtOEP and PtCP allows the use of microsecond time-resolved fluorescence (TR-F) detection. TR-F measurements reduce optical interferences and give higher signal to background ratios thereby facilitating detection with significantly higher sensitivity.

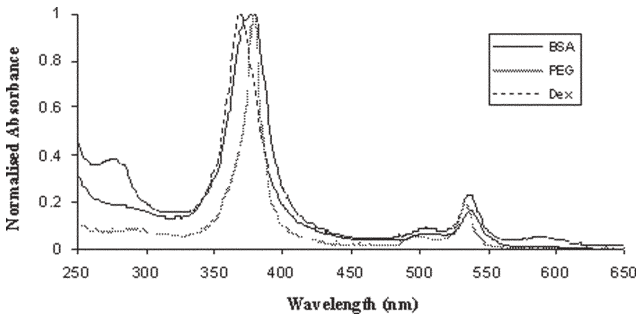

Fig. 1 Normalised absorption spectra of PtCP conjugates with BSA, PEG-20 and dextran.

BSA, dextran and PEG conjugates show subtly different UV-VIS absorption spectra from that of the free PtCP dye. As seen in Fig. 1 BSA and dextran conjugates produce spectra with a broadened Soret band and altered λ_{max}, due to label carrier interaction (restored to that of free dye on addition of surfactant). In contrast PEG conjugates show a sharp peak almost identical to that of free dye with the addition of surfactant having a marginal effect on peak definition. The spectral characteristics of the PtOEP incorporated into PS particles are similar to those of PtCP. Photophysical characteristics of the oxygen probes are summarized in Table 1.

For excitation of Pt-porphyrin phosphorescence, Xe flashlamps, UV lasers and LEDs matching the probe Soret band (maximum ~380 nm) can be used. Furthermore, excitation of PtCP and PtOEP at the Q-band can be achieved with a compact, low cost 532 nm laser[23] which facilitates high sensitivity TR-F detection. PtOEPK based probes have long-wave excitation and emission as well as high photostability.[24] Although not very compatible with PMT-based detectors, their near-infrared emission is easily detectable with silicon photodetectors and CCDs, frequently used in fluorescence imaging applications. Ruthenium metal ligand complexes typically display broad absorption at 480 nm with emission at 650 nm, this allows excitation with standard, inexpensive blue LEDs as well as Xe flashlamps. However these dyes are less bright than Pt-porphyrins and have much shorter emission

Table 1 Spectral characterisation of macromolecular and microparticle probes

Probe	λ_{ex}/nm	λ_{em}/nm	I/IU × 10^{-7} M	I_0/I	τ/μs	τ_0/μs	K_{sv} (% of air saturation)$^{-1}$
Macromolecular							
PtCP-BSA (1 : 1)	380, 535	650	118	3.78	26	67	0.028
PtCP-dextran (1 : 1)	380, 535	650	29	10.2	12	85	0.092
PtCP-dextran (3 : 1)	380, 535	650	29	10.3	12	85	0.093
PtCP-dextran (8 : 1)	380, 535	650	71	10.5	12	85	0.095
PtCP-PEG-5 (1 : 1)	380, 535	650	34	17.9	13	100	0.169
PtCP-PEG-20 (1 : 1)	380, 535	650	29	18.1	13	100	0.171
Ru-BSA	460	640	81	1.4	NM	NM	0.004
Microparticle							
PtOEP-PS	382, 535	650	10[a]	3.7	26	82	NM
PtOEPK-PS	395, 586	760	13[a]	2.1	26	60	NM

[a] IU μg^{-1} ml^{-1}; NM = not measured.

lifetimes (in the range of 1 μs), and are therefore less suitable for TR-F measurements.

Molecular oxygen acts as a dynamic quencher of long-decay photoluminescent dyes such as ruthenium complexes and the metalloporphyrins, leading to their use in oxygen sensitive probes.[25] Quenching by dissolved oxygen was analysed for the various probes by exposing them to air-saturated and deoxygenated buffer solutions (Table 1). Stern–Volmer quenching constants are given in Table 1 and agree with I_0/I data. BSA conjugates display a ~3 fold signal enhancement in phosphorescence intensity, while those of dextran and PEG gave rise to ~10 fold and ~18 fold enhancement respectively, indicating a greater sensitivity to oxygen. The phosphorescence lifetime of PtCP in air saturated buffer was 26 μs for the BSA conjugate, this decreased to 12 μs and 13 μs for the dextran and PEG conjugates respectively. Conversely, in deoxygenated solution, the lifetimes for BSA, dextran and PEG conjugates were 72 μs, 80 μs and 100 μs respectively. Another notable characteristic of the new probes is the variation in signal with level of substitution. While protein conjugates display decreasing levels of phosphorescence of PtCP at higher degrees of substitution,[15] the emission of PtCP-dextran conjugates remained unaffected by this (8 fold increase in substitution gives ~8 fold increase in signal). Also the size of the carrier (see PEG 5 000 and 20 000 Da) does not seem to have a marked effect on the sensitivity of the probe to oxygen. One can conclude that the new soluble probes based on dextran and PEG conjugates provide reduced internal quenching of the PtCP label and greater sensitivity to oxygen. At the same time, in air-saturated solutions they produce lower specific phosphorescence signals and shorter emission lifetimes compared to the protein-based probes.[15] In contrast to the porphyrin based probes, which display marked signal increase between air-saturated and deoxygenated solution, the ruthenium conjugates show a 40% increase in fluorescent signal.

The microparticle based probes displayed sufficient specific signals and moderate sensitivity to oxygen, with the phosphorescence enhancement factors of 3.7 and 2.1 for PtOEP-PS and PtOEPK-PS respectively (Table 1). Their phosphorescence lifetimes in air-saturated solution were 26 μs for both PtOEP-PS and PtOEPK-PS and 82 μs and 60 μs in deoxygenated solution for PtOEP-PS and PtOEPK-PS respectively (Table 1). These values are very close to those of the solid-state sensors based on PtOEP-PS and PtOEPK-PS film coatings.[4,26] The photophysical properties of the microparticle-based probes appear appropriate for their use in oxygen uptake measurements.

Following this investigation, three of the oxygen probes were selected for further studies: the PtCP-BSA probe (standard), the PtCP-PEG-20 000 probe and the PtOEP-PS particle based probe.

Application of probes to the analysis of oxygen uptake

Analysis of oxygen uptake has been achieved previously using both solid state and water soluble Pt-porphyrin based oxygen probes.[15,27] Described above are water soluble probes with higher sensitivity to oxygen and a dispensable solid state probe. Here we evaluate the ability of these new probes to analyse oxygen uptake. Both PtCP-PEG and PtOEP-PS microparticles are used to analyse oxygen uptake by *E. coli* cells, and their performance is compared to that of PtCP-BSA.

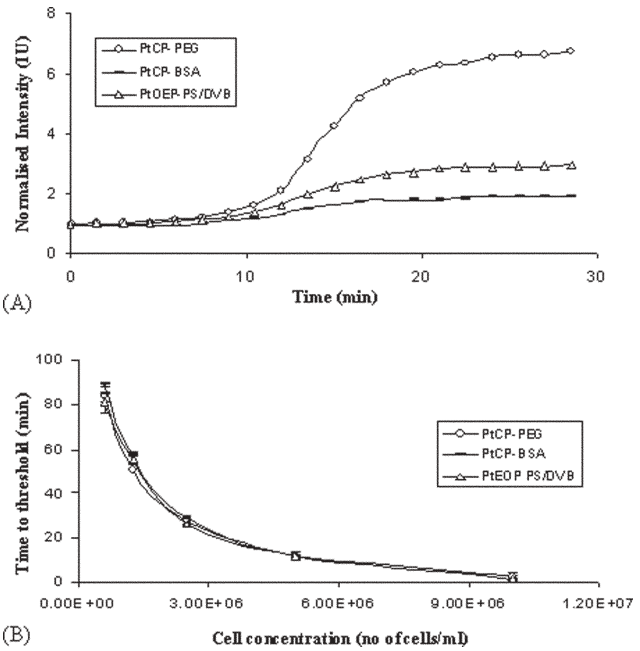

Fig. 2 (A) Respiration profiles of samples containing *E. coli* cells measured with PtCP-BSA, PtCP-PEG and PtOEP-PS probes. (B) Dependence of initial slope of PtCP-BSA, PtCP-PEG and PtOEP-PS probe phosphorescence signal on *E. coli* cell concentration.

E. coli is a bacterium characterized by high levels of aerobic respiration and rapid proliferation with doubling times of ~20 min. Fig. 2A illustrates the effect of cellular respiration on probe emission intensity reflecting oxygen depletion. When oxygen uptake rates exceed back diffusion, probe emission increases. Rapidly respiring cell types such as *E. coli* eventually consume almost all dissolved oxygen within the sample, producing characteristic sigmoidal curves and distinct signal changes. Both PtCP-PEG and PtOEP-PS can be successfully applied to such analysis. It should be noted that despite the significantly larger intensity change of PtCP-PEG in response to oxygen uptake, there is no evident difference in its sensitivity with respect to initial cell concentrations (Fig. 2B). Determining sensitivity is difficult in this system as, due to cell division, oxygen uptake rates increase rapidly during the course of the assay.

To further investigate the influence of probe I_0/I values (Table 1) on its sensitivity to detect low levels of oxygen consumption in a sample, a more simple enzymatic system was employed. Glucose oxidase is a well defined oxygen consuming enzyme, which in the presence of excess glucose consumes oxygen at a rate proportional to enzyme concentration. This provides constant rates of oxygen uptake and is convenient for probe comparison. Fig. 3 illustrates that although PtCP-PEG produces larger initial slopes of phosphorescence signal for a given concentration of glucose oxidase, there is a marginal difference in its sensitivity compared to the other probes with lower I_0/I. The limits of detection for glucose oxidase are

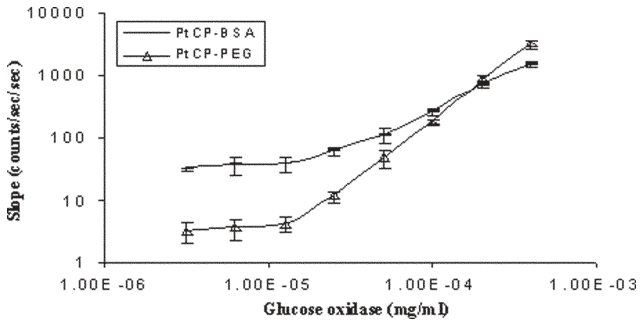

Fig. 3 Dependence of the initial slope of probe phosphorescent signal on the concentration of glucose oxidase enzyme.

2.3×10^{-5} mg ml^{-1} and 2.5×10^{-5} mg ml^{-1} for PtCP-PEG and PtCP-BSA respectively.

Examination of probe interferences

In our previous experience macromolecular oxygen probes work reliably in various cellular oxygen uptake assays, allowing the analysis of various cellular effectors and chemical compounds. However a number of cases have arisen where the probes are influenced by sample components. As outlined above, the macromolecular probes may be susceptible to interferences. For this reason, the macromolecular and microparticle based probes were compared in the presence of a number of interfering compounds.

Camptothecin, a selective inhibitor of DNA topoisomerase, is used as an inducer of apoptosis.[28] It is a fluorescent compound which absorbs at ~360 nm and emits at ~440 nm. Addition of high concentrations of camptothecin to samples containing a macromolecular probe caused significant increases in intensity signals, when exciting at 340 ± 50 nm and measuring at 642 ± 6.5 nm (Fig. 4A). The emission lifetime of camptothecin is 4 ns,[29] however the inclusion of a 20 μs delay did not eliminate camptothecin emission, due to the limited time resolution associated with Xe flashlamp based instruments (lamp afterglow). This interference can be avoided by exciting the oxygen probe at 532 nm (data not shown). The use of a microparticle based oxygen probe also eliminates the enhancement effect of camptothecin, suggesting that close contact between this compound and the phosphorescent dye is necessary for enhancement to occur.

Caffeine was also seen to interfere with the macromolecular PtCP based probes causing an increase in probe emission. A similar effect has been reported for fluorescent calcium indicator dyes such as mag-fura-2 and fura-2.[30] The enhancement effect of caffeine was shown to be due to direct interaction with the PtCP label, resulting in no alteration in measured I_0/I values (Fig. 4B). While the effect can be removed by normalising for initial intensity, the insusceptibility of the particle probes to interferences by this compound provides a more convenient solution.

Dantrolene, a skeletal muscle relaxant used in malignant hyperthermia studies, absorbs light at 350–450 nm and therefore has the potential to interfere with measurements carried out with the probes due to an "inner filter effect". In our study we found that high concentrations of dantrolene

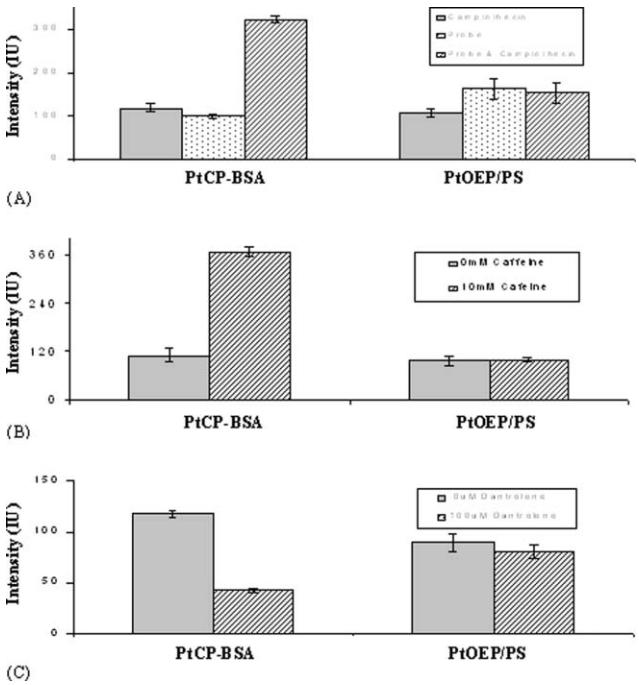

Fig. 4 Effects of different compounds on the phosphorescence intensity of PtCP-BSA and PtOEP-PS probes: A camptothecin; B caffeine; C dantrolene. Measurements were carried out on the SpectraMax Gemini fluorescent plate reader, with probe concentrations of 10^{-7} to 10^{-9} M and 5 μg ml^{-1} for PtPC-BSA and PtOEP-PS respectively.

(100 mM) reduced the emission intensity of PtCP-BSA. This quenching is greater in deoxygenated solutions than in air-saturated (Fig. 4C). The interference on the macromolecular probe observed on excitation at 380 nm was also seen on excitation at 535 nm; together with the absence of an effect on the particle based probes these data suggest that interference by dantrolene may be due to its dynamic quenching effect on the oxygen probe rather than an "inner-filter effect".

The above examples illustrate that interferences by chemical compounds with the phosphorescent oxygen probes can be resolved by both instrumental and chemical means. Thus, the use of longwave excitation (532 nm as opposed to 340–380 nm) and TR-F detection can eliminate the effects of sample/compound autofluorescence. A more convenient alternative is the use of microparticle based oxygen probes which are less susceptible to interferences involving dynamic or static quenching of the oxygen-sensitive dye or solution RET (resonance energy transfer). The physical protection given to these probes by the polymer carrier allows them to be used in less forgiving environments than the macromolecular probes.

Conclusions

A panel of macromolecular and microparticle-based oxygen probes were fabricated. The macromolecular probes were created using a number of different macromolecular carriers conjugated to PtCP. The probes were lyophilised directly after fabrication and subsequently used over a period of several months. Altering the carrier produced probes with slightly

different spectral characteristics and had a dramatic effect on sensitivity to oxygen, with I_0/I values ranging from 3 to 18. The particle based probes showed spectral characteristics similar to those reported for solid state sensors based on the same oxygen sensitive dyes, however the fact that they can be dispensed simply allows for more convenient use. In addition the redundancy of functionalised derivatives for such probes allows development of probes with wide ranging spectral characteristics.

Macromolecular oxygen sensitive probes have previously been shown to be applicable to the monitoring of oxygen uptake by biological samples. In this paper we examined the applicability of newly developed probes to such analysis. This was particularity important for particle based probes, as they are markedly different from previously used probes. These particle based probes proved appropriate for the monitoring of oxygen uptake by *E. coli* cells, showing sensitivities similar to those of BSA and PEG macromolecular based probes. A well defined oxygen consuming enzymatic system was used to assess the sensitivity of macromolecular probes to oxygen consumption in air-saturated samples. It is apparent from these probes' I_0/I values that they have markedly different responses to actual concentrations of dissolved oxygen. However parallel analysis of glucose oxidase catalysed oxygen consumption using each of these probes demonstrated marginal differences in sensitivity to oxygen uptake.

Analysis in complex biological samples can result in probes being exposed to a number of interfering compounds. The long lived emission of these probes facilitates the use of TR-F measurements, allowing the exclusion of short lived fluorescence interferences present in the sample. The use of Q-band excitation has also been shown as a potential method of avoiding interferences. While these approaches provide solutions in a number of cases, they are not always sufficient and in such instances the physical protection offered by the particle based probes outlined above can provide a convenient alternative.

Acknowledgements

Financial support of this work by the Irish Health Research Board, grant RP/106/2002 and by the Irish Higher Educational Authority and National Development Plan is gratefully acknowledged.

Ciara O'Donovan,[a] James Hynes,[a] Dmitri Yashunski[b] and Dmitri B. Papkovsky*[a]
[a]*Biochemistry Department, University College Cork, Lee Maltings, Prospect Row, Cork, Ireland*
[b]*Institute of Biomedical Chemistry, Pogodinskaia St. 10, Moscow, 119992, Russian Federation*

References

1 O. S. Wolfbeis, *Fibre Optic Chemical Sensors and Biosenors*, CRC Press, Boca Raton, FL, 1991.
2 J. R. Bacon and J. N. Demas, *Anal. Chem.*, 1987, **59**, 2780–2785.
3 J. M. Vanderkooi, G. Maniara, T. J. Green and D. F. Wilson, *J. Biol. Chem.*, 1987, **262**, 5476–5482.
4 D. B. Papkovsky, G. V. Ponomarev, W. Trettnak and P. O'Leary, *Anal. Chem.*, 1995, **67**, 4112–4117.
5 D. B. Papkovsky, *Sens. Actuators B: Chem.*, 1995, 213–218.
6 C. Kolle, W. Gruber, W. Trettnak, K. Biebernik, C. Dolezal, F. Reininger and P. OLeary, *Sens. Actuators B: Chem.*, 1997, **38**, 141–149.
7 H. S. Voraberger, H. Kreimaier, K. Biebernik and W. Kern, *Sens. Actuators B: Chem.*, 2001, **74**, 179–185.
8 D. B. Papkovsky, N. Papkovskaia, A. Smyth, J. Kerry and V. I. Ogurtsov, *Anal. Lett.*, 2000, **33**, 1755–1777.
9 W. K. Barnikol, O. Burkhard, H. Trubel, F. Petzke, N. Weiler and T. Gaertner, *Biomed. Technol. (Berlin)*, 1996, **41**, 170–177.
10 R. Fraatz, C. Sachs, J. F. Mollard, P. Finetti and J. Tusa, *Clin. Chem.*, 1996, **42**, 768–768.
11 B. H. Weigl, A. Holobar, W. Trettnak, I. Klimant, H. Kraus, P. O'Leary and O. S. Wolfbeis, *J. Biotechnol.*, 1994, **32**, 127–138.
12 S. A. Vinogradov and D. F. Wilson, *Adv. Exp. Med. Biol.*, 1997, **411**, 597–603.
13 E. Puklin, B. Carlson, S. Gouin, C. Costin, E. Green, S. Ponomarev, H. Tanji and M. Gouterman, *J. Appl. Polym. Sci.*, 2000, **77**, 2795–2804.
14 H. A. Clark, R. Kopelman, R. Tjalkens and M. A. Philbert, *Anal. Chem.*, 1999, **71**, 4837–4843.
15 J. Hynes, S. Floyd, A. E. Soini, R. O'Connor and D. B. Papkovsky, *J. Biomol. Screen.*, 2003, **8**, 264–272.
16 M. Wodnicka, R. D. Guarino, J. J. Hemperly, M. R. Timmins, D. Stitt and J. B. Pitner, *J. Biomol. Screen.*, 2000, **5**, 141–152.
17 F. C. O'Mahony, C. O'Donovan, J. Hynes, T. Moore, J. Davenport and D. B. E. S. T. Papkovsky, *Environ. Sci. Technol.*, 2005, in press.
18 D. Papkovsky', M. A. Uskova, G. V. Ponomarev, T. Korpela, S. Kulmala and G. G. Guilbault, *Anal. Chim. Acta*, 1998, **374**, 1–9.
19 F. N. Castellano and J. R. Lakowicz, *Photochem. Photobiol.*, 1998, **67**, 179–183.
20 Y. E. Koo, Y. Cao, R. Kopelman, S. M. Koo, M. Brasuel and M. A. Philbert, *Anal. Chem.*, 2004, **76**, 2498–2505.
21 T. C. O'Riordan, A. E. Soini and D. B. Papkovsky, *Anal. Biochem.*, 2001, **290**, 366–375.
22 J. T. Soini, M. E. Waris and P. E. Hanninen, *J. Pharm. Biomed. Anal.*, 2004, **34**, 753–760.
23 T. C. O'Riordan, A. E. Soini, J. T. Soini and D. B. Papkovsky, *Anal. Chem.*, 2002, **74**, 5845–5850.
24 D. B. Papkovsky, G. V. Ponomarev and O. S. Wolfbeis, *Spectrochim. Acta Part A*, 1996, 1629–1638.
25 D. B. Papkovsky, *Methods Enzymol.*, 2004, **381**, 715–735.
26 D. Papkovsky, J. Olah, I. Troyanovsky, N. Sadovsky, V. Rumyantseca, A. Mironov, A. Yaropolov and A. Savitsky, *Biosens. Bioelectron.*, 1991, **7**, 199–206.
27 T. C. O'Riordan, D. Buckley, V. Ogurtsov, R. O'Connor and D. B. Papkovsky, *Anal. Biochem.*, 2000, **278**, 221–227.
28 S. D. Desai, L. F. Liu, D. Vazquez-Abad and P. D'Arpa, *J. Biol. Chem.*, 1997, **272**, 24159–24164.
29 J. Dey and I. M. Warner, *J. Lumin.*, 1997, **71**, 105–114.
30 M. Muschol, B. R. Dasgupta and B. M. Salzberg, *Biophys. J.*, 1999, **77**, 577–586.