Quantitative Analysis of Molecular Oxygen Using Palladium Porphyrins

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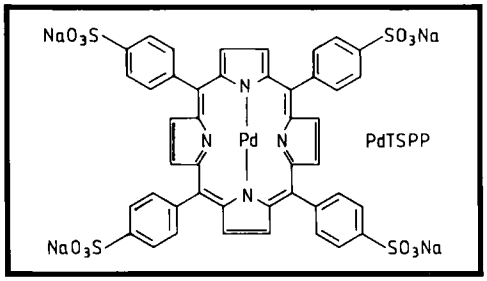
A simple optical method for the quantitative determination of dissolved oxygen under physiological conditions is described. The technique involves measurement of room temperature phosphorescence from palladium(II) porphyrins. Such emission is rare for large organic molecules, and its long lifetime, together with the favourable absorption characteristics of the porphyrin receptor, ensure that the phosphorescence can be resolved easily, even in heterogeneous media. Phosphorescence yields and lifetimes are shown to be highly sensitive to the concentration of dissolved oxygen, but do not depend upon the nature of the environment, and can be used separately or together to determine oxygen levels within a biological substrate. This technique should be applicable to all areas of clinical, medicinal and biomedical chemistry.

In order to facilitate development of novel anti-cancer agents it is necessary to be able to monitor their in-situ reactivity with molecular O_2, since activated forms of O_2 (for example superoxide ions, hydroxyl and peroxyl radicals, singlet molecular O_2) are believed to be responsible for the initiation of the chemotherapeutic process. This requires analytical determination of dissolved O_2 concentrations under physiological conditions. Several experimental methods have been devised to obtain such information, including membrane polarographic detectors (1), oxygen-dependent fluorescence quenching (2), ESR active spin-labels (3), chemiluminescence (4), and phosphorescence quenching (5). Each of these approaches has its merits and disadvantages but, in terms of universal application and simplicity of operation, the luminescence quenching method is the most attractive. Because a long-lived triplet excited state will always be very much more sensitive towards O_2-quenching than the corresponding short-lived singlet excited state, the phosphorescence quenching technique is to be preferred over the fluorescence approach. However, very few dyes are known that are biocompatible, stable and easily derivatised and which exhibit room temperature phosphorescence. Recent work has shown that palladium(II) porphyrins exhibit moderately intense phosphorescence in solution at room temperature, and this is quenched by O_2(5). We have since found that the phosphorescence lifetime of the porphyrin can be used to give a direct measurement of the concentration of dissolved O_2 in the solution. Palladium(II) porphyrins, therefore, could be used as molecular probes for determination of dissolved O_2 concentrations in a wide range of environments, including biomaterials.

Discussion and Results

Photophysical Studies in Water

As an illustration of the proposed technique, we present recently obtained results describing the luminescence properties of a water-soluble palladium(II) porphyrin (PdP) in dilute aqueous solution. The porphyrin used for this study was palladium(II) tetrakis(4-sulphonatophenyl)porphyrin (PdTSPP), the structure of which is given overleaf. This compound shows prominent absorption bands in the visible region, as shown, see Figure 1. Excitation of PdTSPP in N_2-saturated



aqueous solution gives rise to the emission spectrum given in Figure 2. Both fluorescence and phosphorescence emission can be observed under such conditions. Time-resolved luminescence studies allow resolution of the two emissions since fluorescence (τ_f = 0.1 ns) is very much shorter lived than phosphorescence (τ_p = 1 ms). The two spectra are well separated, with minimal overlap, and the relative ratio of their maximum intensities can be measured easily. Under these conditions, the yield of total luminescence is reasonably high and can be measured with any commercial spectrofluorimeter.

Fluorescence is unaffected by the presence of molecular oxygen, whereas both phosphorescence intensity and lifetime are quenched by added O_2. As shown in Figure 3, there is a linear correlation between the rate constant for decay of triplet PdTSPP, measured by laser flash photolysis methods, and the concentration of dissolved O_2. For the same solutions, there is a corresponding correlation between the ratio of phosphorescence-to-fluorescence maximum intensities and the concentration of dissolved O_2, Figure 4. These latter two plots can be used to determine the concentration of dissolved O_2 in an unknown solution by comparing values measured for the unknown solution with the calibration plots.

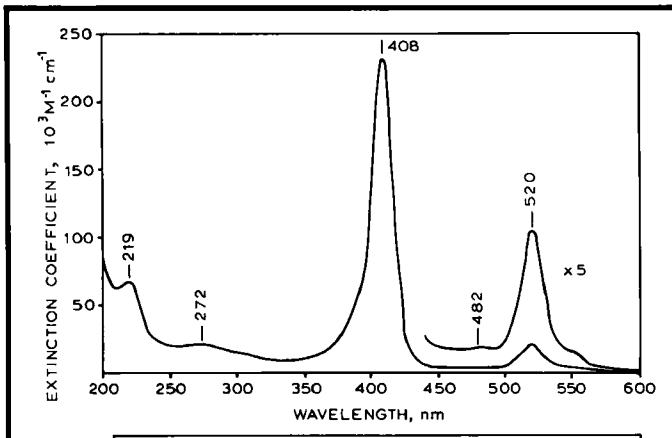


Fig. 1 The absorption spectrum of palladium(II) tetrakis (4-sulphonatophenyl)porphyrin in water shows the prominent absorption in the visible region

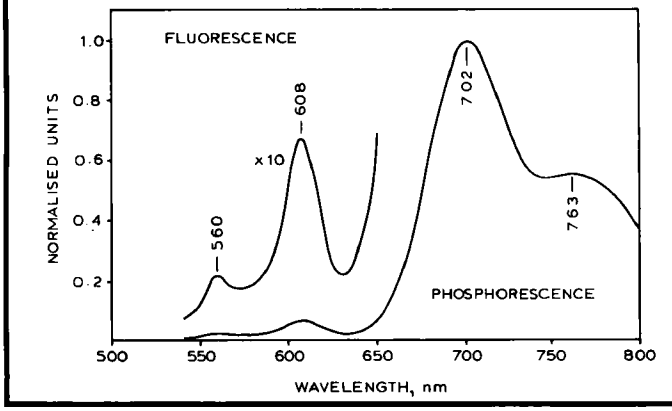


Fig. 2 The total emission spectrum recorded for PdTSPP in nitrogen-saturated water at room temperature shows both the fluorescence and the phosphorescence spectra

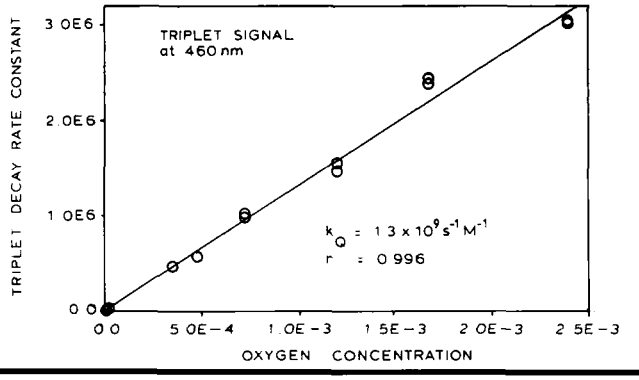


Fig. 3 The plot of the observed rate constant for the decay of the PdTSPP triplet excited state versus the concentration of dissolved oxygen in water at room temperature shows a linear correlation; k_Q is the quenching rate constant, and r is the correlation coefficient which indicates how close the plot is to a linear display (for a perfect straight line r = 1)

Identical effects are observed with other water-soluble PdP derivatives and with water-insoluble PdP compounds dissolved in organic solvents. In all cases, the phosphorescence lifetime and total emission spectrum can be used to give a quantitative determination of the concentration of dissolved O_2 in the system.

Analytical Considerations

The studies outlined above have shown that dilute solutions of palladium(II) porphyrins (PdP) exhibit both fluorescence and phosphorescence at room temperature. The fluorescence yield remains independent of the concentration of dissolved O_2, since the fluorescence lifetime is too short for diffusional quenching, but the phosphorescence yield (ϕ_p) decreases progressively as the concentration of dissolved O_2 increases. Under the same conditions, there is a corresponding decrease in the phosphorescence lifetime (τ_p); in the absence of O_2 the lifetime is about 1 ms. These quenching effects follow Stern-Volmer kinetics

$$(\phi_p'/\phi_p) = (\tau_p'/\tau_p) = 1 + k_Q[O_2]\tau_p' \quad \text{(i)}$$

where ϕ_p' and ϕ_p (or τ_p' and τ_p) refer to phosphorescence yields (or lifetimes) recorded in the absence and presence of O_2, respectively, and k_Q is the bimolecular rate constant for quenching the triplet excited state by O_2. Standard values representing the absence of O_2 are obtained by saturating the solution with N_2 and all other solutions were saturated with known mixtures of O_2/N_2(6). The concentration of dissolved O_2 in an unknown solution is determined simply by comparing the observed τ_p and ϕ_p values with the calibration curve.

In aqueous solution τ_p was found to be independent of the concentration of PdP,

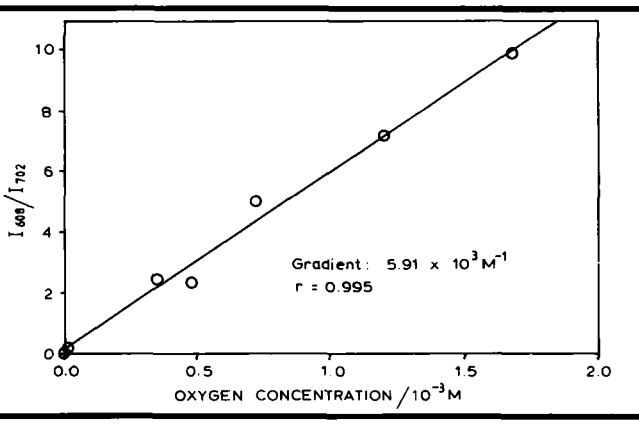


Fig. 4 The plot of the observed ratio of fluorescence to phosphorescence intensities versus the concentration of dissolved oxygen in water at room temperature

solution pH, ambient temperature, ionic strength and light intensity over wide ranges. The phosphorescence lifetime, therefore, gives an accurate measure of the concentration of dissolved O_2. By contrast, ϕ_p is not an absolute value but depends markedly upon concentration of PdP and light intensity. These same parameters affect fluorescence and phosphorescence to an equal degree, however, so that the ratio of the yields of phosphorescence and fluorescence can be used to determine accurate concentrations of dissolved O_2, since the fluorescence yield can be used as an internal standard.

Biological Environments

The above studies were extended to include determination of the concentration of dissolved O_2 in organic solvents, micelles, liposomes, vesicles, viscous media and inside the pockets of serum albumins. These studies used a range of PdP derivatives of differing hydrophobic/hydrophillic character. The luminescence properties of each PdP were determined, as above, and their reaction with molecular O_2 was quantified using laser flash photolysis methods. The effects of PdP concentration, temperature, medium, added reagents, O_2 concentration and dye stability were monitored in order to establish the ability of the technique to determine meaningful O_2 concentrations under such conditions.

The PdP derivatives were used subsequently to stain a variety of biomaterials, including membranes, mitochondria, macromolecular proteins, DNA, and both healthy and infected intact cells. The in-situ measurements were rendered difficult by the high levels of light scattering and the poor light transmitting properties inherent with such samples. It is desirable, therefore, to synthesise porphyrin derivatives that absorb and emit at long wavelengths where biological tissue is relatively transparent and light scattering is minimised. Thus, the luminescence properties of palladium(II) phthalocyanines, naphthalocyanines and "expanded porphyrins", which should absorb and emit in the near infrared region, will be evaluated in further studies. Also, it is important to ensure that the probe molecules do not perturb the biomaterial or induce photodestruction of the medium. These studies will be described in full in a later paper.

Acknowledgements

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Progress in Catalytic Technology in Japan

A review of the developments in catalytic technology made in Japan in recent years has been prepared by M. Misono and N. Nojiri (*Appl. Catal.*, 1990, **64**, (1–2), 1–30). It is suggested that the close co-operation between academic and industrial chemists is one of the factors that has contributed to the progress. Another is the effective application of new catalyst materials, such as heteropoly acids, new zeolites, bimetals and chiral transition metal complexes. One representative of each type, which is now used in a newly industrialised process, is described in some detail.

These include a novel palladium-tellurium on active carbon catalyst that has been developed for use during the diacetoxylation of 1,3-butadiene to 1,4-diacetoxyl-2-butene, under mild conditions, while a new asymmetric process for *l*-menthol production depends upon the use of optically active rhodium-BINAP to catalyse the enantioselective isomerisation of geranyldiethylamine.