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Monitoring hormone-induced oxygen consumption in the salivary glands of the blowfly, *Calliphora vicina*, by use of luminescent microbeads

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Abstract

The salivary glands of the blowfly were injected with luminescent oxygen-sensitive microbeads. The changes in oxygen content within individual gland tubules during hormone-induced secretory activity was quantified. The measurements are based on an upgraded phase-modulation technique, where the phase shift of the sensor phosphorescence is determined independently from concentration and background signals. We show that the combination of a lock-in amplifier with a fluorescence microscope results in a convenient setup to measure oxygen concentrations within living animal tissues at the cellular level.

Key words: oxygen sensor, phase-modulation, cellular respiration, blowfly, salivary gland

1. Introduction

The use of luminescent spherical microsensors (sometimes also called PEBBLEs; Probes Encapsulated By Biologically Localized Embedding), is very promising for in-vivo measurements of molecular oxygen (here simply referred to as oxygen) concentrations on the cellular level. Synthesis and application of these oxygen sensitive microsensors have been described previously [1-3]. However, few examples exist of successful applications in biological systems. In this work, oxygen-sensitive microbeads were applied to obtain quantitative information about the oxygen concentration and its changes induced by hormonal stimulation of living salivary glands. We used the simple tubular salivary gland of the blowfly (*Calliphora vicina*) as a model. The gland tubules (outer diameter ca. 100 µm) are formed by a single layer of secretory epithelial cells [4]. This functionally intact miniorgan can be isolated and easily manipulated. The hormone serotonin (5-hydroxytryptamine, 5-HT) stimulates fluid secretion [5, 6]. Since secretory activity consumes energy, a decrease in the oxygen content in the salivary gland lumen was to be expected. Polystyrene beads with an oxygen-sensitive phosphorescence dye were injected to measure the oxygen concentration in the glandular lumen by determination of the luminescence lifetime via a phase shift measurement.

2. Oxygen sensor

Entrapped into a polymer matrix, Pt-porphyrins show intense phosphorescence in the red to NIR spectral range, which is strongly quenched by oxygen [7-10]. The presence of oxygen leads to a faster depletion of the excited-state population and therefore to a decrease of lifetime and luminescence intensity. As oxygen sensor, approximately 0.3 to 1 µm diameter polystyrene beads containing Pt(II)-tetra-pentafluorophenyl-porphyrin (PtPFPP) were used [11, 12].

3. Detection technique

The oxygen dependent phosphorescence lifetime of the sensor beads was determined using a two-frequency phase modulation technique, which allows to mask background signals. As a consequence, the measurement results are completely independent of the sensor concentration. This method was described in detail previously [12]. In brief: If the sensor is excited by sinusoidally modulated light, this results in a phase shift between the excitation and emission signals [8, 10, 12-14]. The sensor lifetime τ is related to the phase shift Φ by

$$\tan \Phi = \omega \tau \tag{1}$$

in which ω is the angular frequency of the sinusoidal modulation. In the presence of background signals, such as fluorescence of the sample or remainig excitation light, superposition of the signals will take place, leading to a total light signal with an apparent phase shift Φ_{app} and only an apparent lifetime τ_{app} is yielded by Eq. (1). However, identifying the apparent lifetimes τ_{app} at two different modulation frequencies ω and ψ allows an evaluation of the actual sensor lifetime τ by using Eq. (2).

$$\frac{\tau_{app,\omega}\left(1+\omega^2\tau^2\right)}{\tau_{app,\omega}-\tau} = \frac{\tau_{app,\psi}\left(1+\psi^2\tau^2\right)}{\tau_{app,\psi}-\tau} \tag{2}$$

This technique is based on the fact that the time delays of all background signals can be assumed to be zero compared to the microseconds lifetime of sensor's phosphorescence. Finally, the oxygen concentration is evaluated from τ by using a calibration curve.

4. Measurement setup

The core of the setup was a computer controlled Zeiss UMSP 80 microscope spectrophotometer equipped with a monochromator in front of a red sensitive Hamamatsu R928 photomultiplier tube (Fig. 1). Usually, the multiplier is used to measure intensities. To determine phase shifts, the output of the photomultiplier's amplifier was tapped und

connected to a digital two phase lock-in amplifier (Stanford Research SR830). The oscillator output of the lock-in amplifier was used to sinusoidally modulate a bright cyan 509 nm LED (Luxeon Star) via a purpose-built driver circuit. Red components of the LED were blocked by a green band pass filter. The lock-in amplifier and microscope were controlled by computers, which also took charge of data acquisition and evaluation.

5. Experimental Procedure

To set a zero phase shift on the apparatus, the signal of eosin dissolved in ethanol was used (τ_{eosin} = 3.1 ns [13]; eosin does not generate a detectable phase shift at the used modulation frequencies). Blowflies (*Calliphora vicina*) were taken from colonies bred at the institute. The abdominal secretory portions of the salivary glands were dissected (for details see [6]) and maintained in physiological saline (PS) containing (mM): 155 NaCl, 20 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 2.7 sodium glutamate, 10 TRIS, and 10 glucose, pH 7.2.

An isolated gland tubule was mounted in a superfusion chamber and its lumen injected with an approximately 2 % [w/w] aqueous suspension of the oxygen sensor beads under microscopical control (Fig. 2). Subsequently, the superfusion chamber was mounted on the stage of the microscope spectrophotometer and continuously superfused at a rate of ca. 1 ml / min with PS at 22 °C. A rectangular variable diaphragm in front of the photomultiplier tube allowed the area from which luminescence was collected to be limited to the area that was injected with the oxygen sensor. The injected sensor beads did not distribute throughout the tubular gland lumen but rather remained and localized to the site of injection. Thus, the optical signal reflects the local oxygen concentration at a defined point within the gland lumen. Note: Oxygen molecules to be detected have to diffuse radially from the bath through the respiring epithelial cells to the sensor beads in the gland lumen.

The high-voltage of the photomultiplier and the amplification of the voltage converted from the photomultipliers anode current were set to obtain an optimal signal to noise ratio. To separate the sensor signal from background signals, the respective apparent phase shifts at 4 and 6 kHz were measured at the beginning of the measurement. Subsequently, the apparent

phase shift at 6 kHz was read every 0.5 s and the sensor lifetime τ was evaluated by the computer. The corresponding oxygen concentration was calculated using a calibration curve. After recording the signal from the unstimulated gland, it was superfused with PS containing 100 nM 5-HT for several minutes in order to stimulate active fluid secretion and cellular respiration.

6. Results and Discussion

Fig. 3 displays the measured oxygen concentration in a resting gland and after the stimulation of fluid secretion with the hormone 5-HT. The detected signal had excellent signal-to-noise ratio and stability. 100 % air saturation denotes the oxygen content of air-saturated water, which is in equilibrium to water-vapor saturated air. At 22 °C and 1013 mbar, 100 % air saturation corresponds to 8.7 mg / L [15]. Initial oxygen concentration was found to be about 50 % air saturation (4.4 mg / L) due to resting respiratory activity of the tissue and reversibly decreased to 25 % (2.2 mg / L) within less than one min after the stimulation of fluid secretion. This drop in oxygen concentration is due to stimulated cellular respiration. 5-HT stimulation could be repeated several times. Fig. 3 shows also a gradual increase of the oxygen concentration within the unstimulated gland, because it deteriorated slowly during the experiment and therefore consumed less oxygen.

7. Conclusions and Outlook

It was shown that polystyrene beads with an oxygen sensitive dye are suitable to monitor quantitatively the oxygen content in animal tissues at the cellular level. Combining a lock-in amplifier with the optics of a microscope photometer leads to a very convenient setup, which easily allows spatially resolved oxygen measurements at an optimum signal strength. This non-invasive optical recording of tissue oxygen concentrations is less deleterious to the tissues and less awkward than e.g. the use of oxygen-sensitive microelectrodes. Further miniaturization of the sensor beads is currently in progress. We believe that we will soon be able to routinely measure oxygen consumption of single cells.

References

[1] E. Monson, M. Brasuel, M.A. Philbert, R. Kopelman, PEBBLE nanosensors for in vitro bioanalysis, in: T. Vo-Dinh (Ed.): Biomedical Photonics Handbook, CRC Press, Boca Raton, 2003 (Chapter 59).

[2] M.. Brasuel, R. Kopelman, J.W. Aylott, H. Clark, H. Xu, M. Hoyer, T.J. Miller, R. Tjalkens, M.A. Philbert, Sensors and Materials 14, No. 6 (2002) 309-338.

[3] Y.-E.L. Koo, Y. Cao, R. Kopelman, S.M. Koo, M. Brasuel, M.A. Philbert, Anal. Chem. 76 (2004) 2498-2505.

[4] M.J. Berridge, J.L. Oschman, Transportig epithelia. Academic Press, New York, London 1972.

[5] M.J. Berridge, The role of 5-hydroxytryptamine and cyclic AMP in the control of fluid secretion by isolated salivary glands. J. Exp. Biol. 53 (1970) 171-186.

[6] M.J. Berridge, W.T. Patel, Insect salivary glands: stimulation of fluid secretion by 5-hydroxytryptamine and adenosine 3`,5´-monophosphate. Science 162 (1968) 462-463.

[7] I. Klimant, M. Kühl, R.N. Glud, G. Holst, Sensors and Actuators B, Chemical 38-39 (1997) 29-37.

[8] D.B. Papkovsky, Methods in optical oxygen sensing: Protocols and critical analyses, in: C K. Sen, G.L. Semenza (Eds.), Methods in Enzymolgy, vol 381,.Elsevier Academic Press, San Diego, London, 2004, pp.715-735 (Section X).

[9] D.B, Papkovsky, Luminescent porphyrins as probes for optical (bio)sensors, Sensors and Actuators B, Chemical 11 (1993) 293-300.

[10] J.N. Demas, B.A. DeGraff, P.B. Coleman, Anal. Chem. News & Features 71 (1999) 793A- 800A.

[11] Y. Amao, T. Miyashita, I. Okura, Platinum tetrakis(pentafluorophenyl)porphyrin immobilized in polytrifluoroethylmethacrylate film as a photostable optical oxygen detection material. Journal of Fluorine Chemistry, 107 (2001)101-106.

[12] E. Schmälzlin, J.T. van Dongen, I. Klimant, B. Marmodée, M. Steup, J. Fisahn, P. Geigenberger, H.-G. Löhmannsröben, An optical multifrequency phase-modulation method using microbeads for measuring intracellular oxygen concentrations in plants, Biophys. J. 89 (2005) 1339-1345.

[13] J.R. Lackowicz, Principles of Fluorescence Spectroscopy, 2nd edition, Kluwer Academic/Plenum Publishers, New York, Boston, Dordrecht, London, Moscow, 1999.

[14] G. Holst, R.N. Glud, M. Kühl, I. Klimant, Sensors and Actuators B, Chemical 38-39 (1997) 122-129.

[15] B.B. Benson, D. Krause, Limnol. Oceanogr. 25 (1980) 662-671.

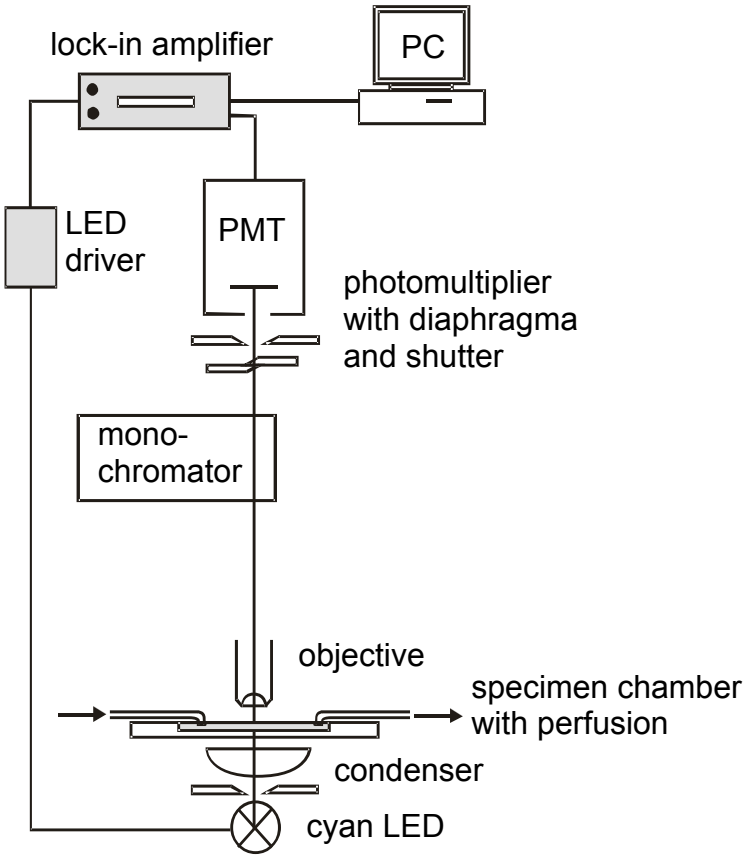

Fig. 1: Experimental setup.

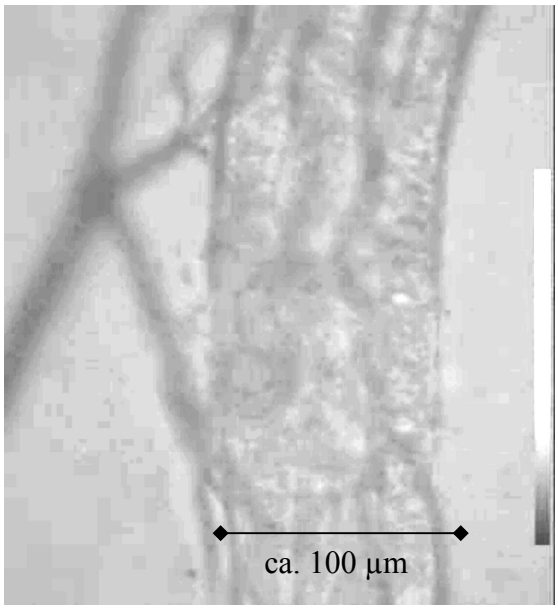

Fig.2a: Salivary gland of the blowfly (bright field)

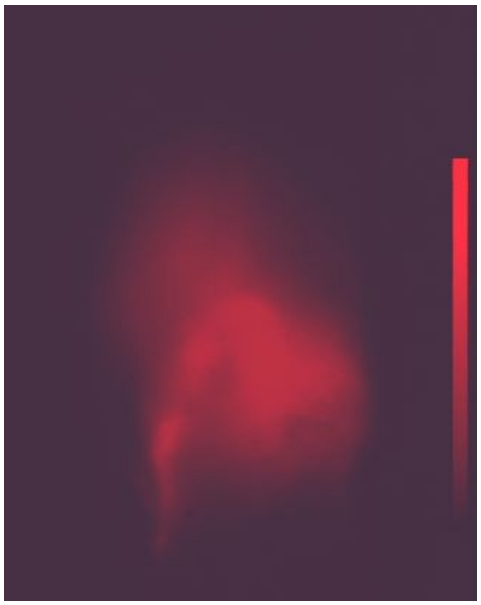

Fig. 2b: Luminescence of injected oxygen sensor

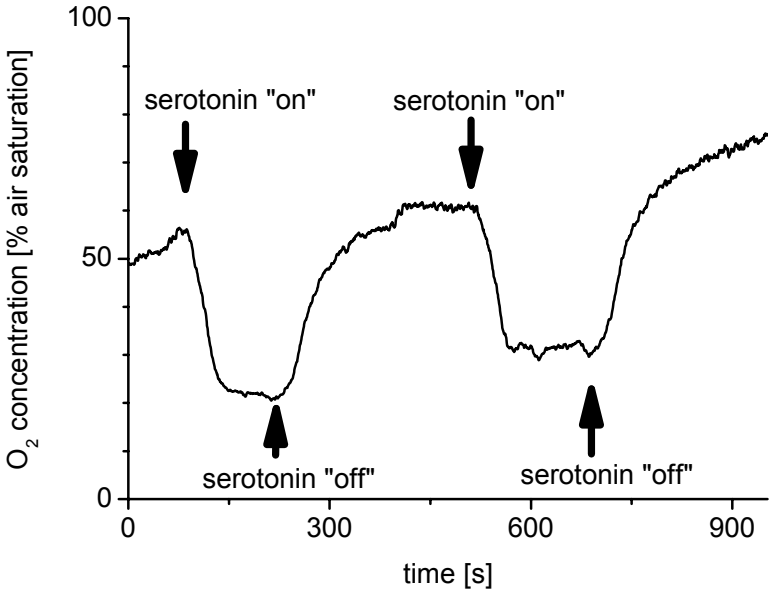

Fig. 3: Time dependent oxygen concentration within the lumen of the tubular salivary gland. The arrows mark the times of 5-HT wash-in and wash-out.