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REVIEW

Oxygen detection in biological systems

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Abstract This article presents a brief description of analytical tools for monitoring evolution and consumption of molecular dioxygen in biological organisms. Based on its nature as a gas and its physical and chemical properties of the ground state $^3\sum_g O_2$, different approaches have been developed for quantitative determinations: (i) manometry, (ii) formation of titratable sediments, (iii) solid state electrodes, (iv) EPR oximetry, (v) luminescence quenching, (vi) biological sensoring, (vii) mass spectrometry and (viii) amperometry. Among these methods mass spectrometry and amperometry are of special relevance for studies on the mechanisms of photosynthetic dioxygen evolution. Mass spectrometry is described in the article of Beckman et al. in this special issue. Therefore, the major part of this contribution focuses on amperometric methods that are currently widely used. Two different types of electrodes are described: (i) Clark-type electrode and (ii) Joliot-type electrode. The complementary advantages of both systems are outlined. A more detailed description comprises the potential of the Joliot-type electrode for mechanistic studies on the reactivity of the different redox states of the water oxidizing complex (WOC).

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Introduction

The dioxygen molecule O_2 plays a paramount role for the existence of all higher forms of life on earth. It provides the unique and indispensable oxidant for highly efficient Gibbs energy extraction from food via aerobic respiration. The "cold combustion" of these substances with O_2 enhances the specific amount of available Gibbs energy by more than a factor of 10 compared to their corresponding catabolism under anaerobic conditions (for discussion, see Nicholls and Ferguson 1982; Renger 1983). Most interestingly, virtually all molecular oxygen of the atmosphere and that dissolved in oceans and lakes originates from a single source, i.e., the light-driven water splitting of oxygenic photosynthesis (Kasting and Seifert 2002). The invention of a system that enables the light driven splitting of water into molecular oxygen and metabolically bound hydrogen occurred 2–3 billion years ago at the evolutionary level of prokaryotic cyanobacteria (Buick 1992; de Marais 2000; Xiong and Bauer 2002; Larkum 2008). This event led to the present day aerobic atmosphere (Kasting and Seifert 2002; Lane 2003) and the generation of the stratospheric ozone layer as the indispensable protective "umbrella" against deleterious UV-B irradiation (for a review, see Worrest and Coldwell 1986).

Molecular dioxygen is characterized by unique properties. Of paramount importance for the biosphere is the triplet spin multiplicity of its electronic ground state which is characterized by the term symbol $^3\sum_g O_2$. In this configuration the O_2 molecule can only form a "spin forbidden"

transition state (for details, see Atkins 2001) with most of the organic compounds, which attain a singlet spin multiplicity in the ground state and are the essential constituents of living matter. As a consequence, their thermodynamically favorable oxidation reactions with O_2 are kinetically highly retarded. This situation, however, drastically changes when O_2 is transferred to the very reactive singlet state (characterized by the term symbol $^1\Delta_gO_2$, see Atkins 2001) via a sensitized reaction or if O_2 becomes reduced to the superoxide radical $O_2^{-\bullet}$ through interaction with 1-electron donors. Even more dangerous is the potential of subsequent formation of the most reactive $^\bullet$OH radical via a Haber–Weiss-reaction that is catalyzed by transition metal ions (Haber and Weiss 1934). Under these circumstances oxidative destruction becomes rather fast. Because of its unique properties molecular dioxygen is a "Janus-face" type species for biological organisms: on the one side it offers the indispensable substance for a sufficiently high Gibbs energy extraction from food as the energetic prerequisite for the development and sustenance of all higher forms of life but on the other hand it acts as a strong poison which gives rise to serious oxidative damaging of biological systems that may ultimately lead to their death (for further reading, see Gilbert 1981).

It is therefore not surprising that the appearance of molecular oxygen in the biosphere was a catastrophic event for the existing anaerobic forms of life that were killed to a large extent. Only organisms could survive that found suitable anaerobic ecological niches or were able to develop proper defense strategies. Accordingly, organisms living under aerobic conditions are furnished with mechanisms which lead to suppression of reactive oxygen species (ROS) like singlet oxygen ($^1\Delta_gO_2$), superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), and $^\bullet$OH radical. Likewise, organisms have oxygen sensor systems (often heme proteins, for a review, see Gilles-Gonzalez and Gonzalez 2005) that enable them to respond to fluctuations of O_2 levels in the cell and their environment, e.g., via regulation of gene expression (for reviews, see articles in Bauer et al. 2009).

This short introduction illustrates that the enzymology of oxygen comprises most important physiological phenomena that are topics of high scientific relevance. Therefore, suitable analytical tools for monitoring the O_2 content and turnover are indispensable for detailed studies on this subject.

Detection methods of molecular dioxygen

A Google search which leads to almost three million (2.700000) records for "oxygen measurements" readily shows that any attempt must be hopeless to present a complete review in a comparatively short article. Therefore, only some basic principles and few selected examples can be described here. The main topic of this article will be restricted to the most widely used techniques for detection of the ground state molecule $^3\Sigma_gO_2$ evolved by light-induced oxidative water splitting of photosynthesis (for a review on this process, see chapters in Renger 2008).

The different methods of qualitative detection and quantitative determination of $^3\Sigma_gO_2$ are based on its nature as a gas and the chemical and physical properties. In the following a brief description of the underlying principles of different methods will be presented (see also van Gorkom and Gast 1996):

Manometry

The method is based on monitoring of volume changes at constant pressure and temperature due to evolution or consumption of O_2 in closed vessels containing the sample. In the middle of the last century manometric determinations of photosynthetically evolved O_2 were widely performed using a set up developed by Warburg et al. ("Warburg apparatus", see Warburg and Krippahl 1960). This method, however, which was also applied for studies on respiratory cell metabolism (Krebs 1951) permitted only limited resolution and was prone to distortion by external parameters like rapid changes of air pressure of the atmosphere. At present this technique is not used anymore.

Formation of titratable compounds

The earliest approach of this type was reported by Priestley in 1772. He used nitric oxide as reactant with O_2 thus forming NO_2, which is removed by chemical absorption. The net result is a volume decrease, which is a measure of the O_2 content in a gas mixture. With this method a surprisingly precise value of 20.4 v/v % was obtained for the percentage of O_2 in air (Priestley 1772). Alternatively, O_2 dissolved in solution can react with compounds to form sediments which are quantified by titration (e.g., the Winkler method; see Ohle 1936). This approach is very time consuming and not appropriate for studies in photosynthesis research.

Solid state electrodes

Another type of O_2 reaction is the interaction with solid metal oxide material, which leads to an electrochemical reaction with the oxide:

$$2e + \frac{1}{2}O_2 \rightleftharpoons O^{2-} \quad (1)$$

where O^{2-} is the anion of the solid state metal oxide. The electrochemical response can be used for rather precise quantitative analyses on the O_2 content in gas mixtures. In

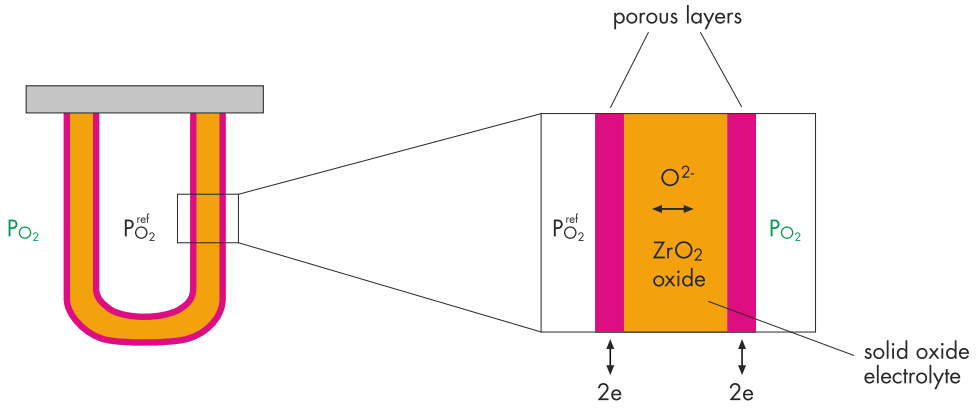

Fig. 1 Scheme of zirconium oxide electrode (*left panel*) and electrochemical reactions at the surface (*right panel*). The electrode consists of a solid state mixed oxide matrix (marked in *orange*) which is covered on both surfaces by porous ZrO_2 layers (marked in margenta) and encloses the reference chamber filled with O_2 at fixed partial pressure $P^{ref}_{O_2}$. The outer surface is in contact with the gas mixture to be analyzed

practice, systems with mixed oxides of the type ZrO_2/Y_2O_3 or ZrO_2/CaO are applied at temperatures of 500–1,000 K where O^{2-} permits ion transport in the solid state electrolyte. At the surface the electronic reactions take place. If a sheet of the heated ZrO_2 mixed solid oxide is exposed on both sides to O_2 of different partial pressure (see Fig. 1) the electrical potential difference ΔE at a concentration cell of the type $Pt,O_2(P_{O_2}(1))/ZrO_2(CaO)/O_2(P_{O_2}(2)),Pt$ (for the sake of simplicity the platinum electrodes are omitted in Fig. 1) follows the Nernst equation:

$$\Delta E = \frac{RT}{4F} \ln \frac{P_{O_2}(1)}{P_{O_2}(2)} \qquad (2)$$

where R = gas constant (8.314 JK^{-1} mol^{-1}), F = Faraday constant (96,485 $Cmol^{-1}$), $P_{O_2}(1)$ and $P_{O_2}(2)$ are the partial pressures of O_2 on both sides of the electrode (see Fig. 1).

Accordingly the sensor cell contains on one side a closed chamber filled with O_2 at fixed partial pressure as reference $\left(P^{ref}_{O_2}\right)$ and the other (open) side the gas with the O_2 content to be analyzed. This method is widely used in technical devices. However, the response time is comparatively slow. Therefore, only few examples are reported for application in photosynthesis research (Greenbaum and Mauzerall 1976, Meyer et al. 1989).

Oximety

Among different approaches the "EPR oximetry" was shown to offer a useful tool to measure the concentration of dioxygen in a liquid medium. (Subczynski and Hyde 1981; Swarts and Pals 1989; for a review see van Gorkom and Gast 1996). The method is based on the interaction of paramagnetic $^3\Sigma_g O_2$ with free radicals thus giving rise to changes of the relaxation times (T_1 and T_2) of these species, usually via a mechanism due to the Heisenberg exchange effect (see Windrem and Plachy 1980 and references therein). In practice stable free nitroxide radicals were often used (see Swartz and Pals 1989 and references therein). This method has been only rarely used in photosynthesis research (Strzalka et al. 1986, 1990).

Luminescence quenching

Molecular oxygen is a strong quencher of electronically excited states of pigments. This effect gives rise to changes of the luminescence emission from these probes. The quantum yield and in case of dynamic quenching also the kinetics of the emission depend on the concentration of the quencher (Stern Volmer equation, for details, see Lakowicz 1999) and therefore quantum yield or lifetime measurements can be used for monitoring the dioxygen content. Several approaches have been developed in particular for medical applications in analyses of cell tissues (Lippitsch et al. 1988; Klimant et al. 1999; Chodavarapu et al. 2007). However, this methodology is not often used for studies of photosynthetically evolved oxygen.

Biological sensoring

The oldest qualitative method that actually contributed to the discovery of molecular dioxygen by J. Priestley was the classical experiment with a mouse kept in a closed box either without or together with a mint plant. In this case the cytochrome c oxidase (COX) of the respiratory chain in the mitochondria of the mouse was the biosensor for oxygen (Priestley 1772). The basic underlying principle of the biosensor assay originally used by Priestley has been applied by Lavergne two centuries later for monitoring the

kinetics of flash induced oxygen evolution of algae through measuring absorption changes, which reflect the turnover of COX (Lavergne 1990).

Mass spectrometry

Gas-chromatography and mass spectrometry are very powerful tools to analyze the composition of gaseous analytes. Among different methods time resolved membrane inlet mass spectrometry (TR-MIMS) turned out to be a very useful tool in photosynthesis research. The underlying principle of mass spectrometry is the ionization of the molecules and their separation according to the ratio m/z (m = mass and z = charge of the ion). Accordingly, mass spectrometers contain an ion source, a vacuum chamber, an analyzer and a detection system. This method, which has been pioneered in photosynthesis research by Hoch, Kok, Radmer and coworkers (Hoch and Kok 1963; Radmer and Ollinger 1980a) is used to address problems of different topics like chlororespiration (Cournac et al. 2000), nitrogen fixation (Bader and Roben 1995) and oxidation of hydrazine and hydroxylamine by PS II (Radmer 1979, Renger et al. 1990). The essential functional element of a MIMS set up is the device with a semi permeable membrane, which separates the sample space from the vacuum and permits gas molecules, but not liquids, to enter the mass spectrometer. A detailed description of the MIMS technique has been recently presented in a book chapter of Konermann et al. (2008) and will also be outlined in this special issue (see article by Beckmann et al.). Of special relevance for considerations on the mechanism of the WOC were the results of measurements of the $^{18}O_2/^{16}O_2$ ratios due to $H_2^{18}O/H_2^{16}O$ exchange in the different S_i states of the WOC. The earlier studies (Radmer and Ollinger 1980b, 1986; Bader et al. 1993) were highly restricted in time resolution and therefore not able to unravel the kinetics of the exchange.

The essential breakthrough was achieved by Messinger et al. (1995) who developed a device for rapid mixing of the injected $H_2^{18}O$ thus improving the time resolution down to a few milliseconds. With this new setup the $H_2^{18}O/H_2^{16}O$ exchange kinetics were analyzed for all S_i states of the WOC and shown to be biphasic with a pronounced dependence of the rate constant on S_i (for details, see Hillier and Wydrzynski 2004; Hillier and Messinger 2005).

Polarographic methods

The underlying principle is the electrochemical reduction of dioxygen and monitoring of the diffusion limited current. This technique is currently by far the most frequently used approach for studies of oxygen evolution in photosynthesis research. It is therefore the main topic of this review article and will be described in detail in the following sections.

Measurements of photosynthetic oxygen evolution by amperometric methods

A very powerful tool turned out to be the amperometric technique as a special case of polarography. The first polarographic measurements were performed by Petering and Daniels (1938) on algal suspensions by using a dropping mercury electrode. Blinks and Skow (1938) and Blinks et al. (1950) were the first to use a static bare Pt-electrode at a fixed polarization voltage, i.e., an amperometric approach. The successful exploitation of the potential of this methodology actually led to the unraveling of the reaction sequence of oxidative water splitting in photosynthesis as will be outlined in the section "Joliot-type electrode".

Basically two different types of amperometric devices of complementary advantages are used in current photosynthesis research: (i) Clark-type electrode and (ii) Joliot-type electrode.

Clark-type electrode

The disadvantage of the blank Pt electrode is its sensitivity to interference by other compounds, which are reacting with the Pt-cathode and/or inactivating it as a poison. Clark et al. (1953) solved this problem by covering the platinum with a semi-permeable membrane which prevents the contact with disturbing chemicals and water, but permits efficient diffusion of O_2. A schematic description of this Clark-type electrode is shown in Fig. 2. The anode of the system is usually Ag and the electrolyte either KCl (in the vast majority of electrodes) or KOH to give rise to a constant potential with insoluble AgCl or Ag_2O, respectively. In the case of KCl as electrolyte the following reactions take place (at the cathode H_2O_2 is formed as intermediate):

$$\text{Pt-cathode}: O_2 + 2H_2O + 4e \rightarrow 4OH^- \quad (3a)$$

$$\text{Ag-anode}: 4Ag + 4Cl^- \rightarrow 4AgCl + 4e \quad (3b)$$

In order to obtain a linear response to the O_2 concentration the system operates in the range of the diffusion limited electric current I_D. A polarization voltage of the cathode of about -0.7 V is usually applied. Under these conditions the I_D is given by the relation (see Atkins 2001).

$$I_D = z \cdot F \cdot C \cdot D \cdot \frac{A}{\delta} \quad (4)$$

where z = number of electrons participating in the reaction, F = Faraday constant (9.6485×10^4 C/mol),

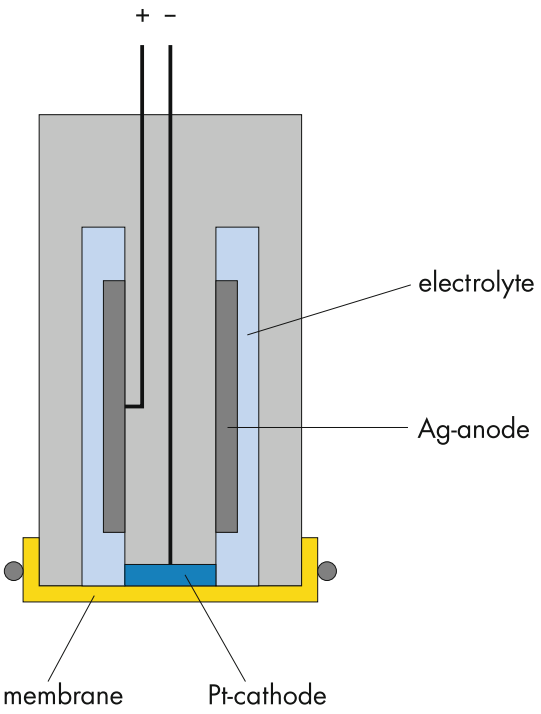

Fig. 2 Scheme of a Clark-type electrode consisting of a platinum cathode (marked in *dark blue*) and Ag/Ag_2O or Ag/AgCl anode (marked in *dark gray*) both fixed in a plastic or glass housing (marked in *light gray*) which contains the electrolyte (KOH or KCl, respectively) chamber (marked in *light blue*). The electrolyte chamber and the Pt-cathode are covered with a semipermeable membrane (marked in *yellow*). The Ag anode must be shielded to prevent its illumination in order to avoid artefacts

C = concentration of O_2 in bulk solution, D = diffusion constant of O_2 in the permeable membrane, A = cathode area and δ = thickness of the diffusion layer (determined by the membrane).

Inspection of Eq. 1 shows that the sensitivity of the system is determined by the ratio $D \cdot A/\delta$. The diffusion coefficient D of the membrane (often Teflon) depends on the temperature and therefore also the current I_D.

In photosynthesis research the Clark-type electrode is widely used for monitoring the O_2 evolution induced by saturating CW illumination of suspensions containing different types of sample material (cyanobacteria, algae, thylakoids, PS II membrane fragments, and PS II core complexes) and several types of apparatus are commercially available. In this type of experiments the oxygen evolution rate (in µmole O_2 per mg Chl and hour) is measured under defined temperature and pH conditions using a combination of a quinone (usually phenyl-para benzoquinone or di-chloro-benzoquinone at concentrations of about 100–200 µM) and $K_3[Fe(CN)_6]$ (1 mM) as artificial electron acceptors. Typically a strong projector light is employed for illumination and the Chl concentration in the 1 ml volume cell is about 10–20 µM. For thylakoids an uncoupler such as gramicidin needs to be added to avoid the acidification of the lumen that gives rise to a decreased rate (Rumberg and Siggel 1969). From the slope of the linear signal rise, which often remains constant for about 60 s and is limited by the amount of added electron acceptor and by photo-inhibition, the O_2 evolution rate $r(O_2)$ [in units of µmol (mg Chl)$^{-1}$ h^{-1}] is calculated according to:

$$r(O_2) = s_{net} \cdot \frac{[O_2]_{H_2O}}{[Chl]} \cdot \frac{1}{S_c} \cdot \frac{V_m}{V_c} \cdot 3600 \qquad (4a)$$

where $s_{net} = [dS/dt]_{net}$ is the net slope (mm s^{-1}) of the linear signal rise (taking background drifts into account if required), $[O_2]_{H_2O}$ is the concentration of oxygen in air-saturated water at given temperature and air-pressure (µmol ml^{-1}), [Chl] the chlorophyll concentration in the cuvette (µg ml^{-1}), S_c the amplitude of the calibration (mm), and V_m and V_c the relative sensitivities at which the measurement and the calibrations were performed. The calibration is done by comparing the signal of air saturated water (here tables exist which give the O_2 concentrations for various temperature, pressure and salt conditions) with the zero level created either by adding dithionite to the water in the cell (this starts a reaction that quickly consumes the dissolved oxygen) or by flushing the cell with nitrogen.

Figure 3 shows a special home built set-up, which was developed for measuring oxygen evolution under excitation with repetitive single turnover flashes (Renger 1966). In order to achieve sufficient resolution, the O_2 content of the buffer is drastically reduced by pre-flushing with nitrogen before filling the cuvette and subsequent injection

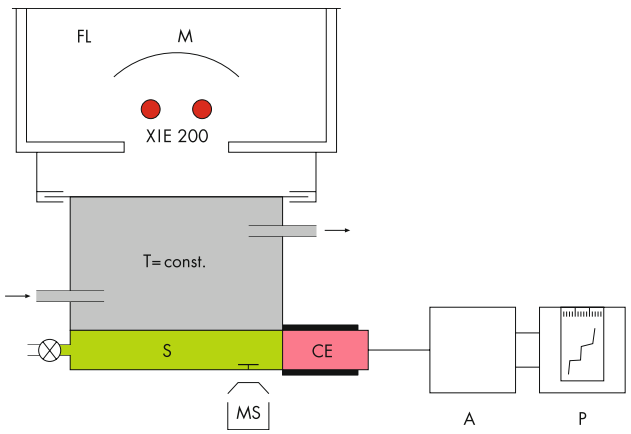

Fig. 3 Scheme of home built set-up for measuring photosynthetic oxygen evolution with a Clark type electrode (CE) under repetition flash excitation. The flash lamp holder houses two Xenon XIE200 (red circles) tubes in order to permit double flash experiments at short dark times between the two flashes, *S* sample cuvette with thermostable bath (*T* const), *M* mirror, *MS* magnetic stirrer, *A* voltage supply and amplifier, *P* recorder

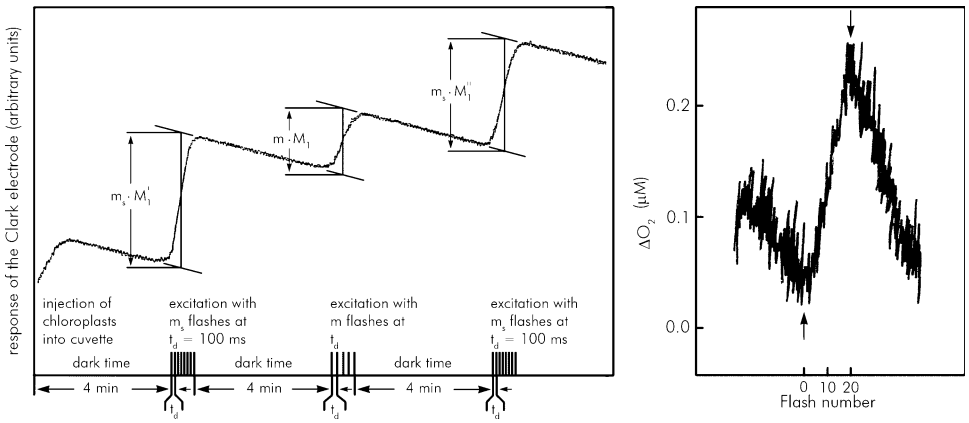

Fig. 4 Response of the Clark type electrode as a function of excitation conditions and measuring times (*left panel*) and calibrated signal induced by 20 flashes (*right panel*). The electrode response caused by the flash light is calibrated by injection of air saturated water into the cuvette (signal not shown). The time t_d between the flashes of the second illumination is variable and the signal $S_m = m \bullet M_1$, where m is the number of repetitive flashes at t_d and M_1 is the average signal per flash. For studies of the dependence on t_d, M_1 is normalized to $1/2(M_1' + M_1'')$ in order to correct for activity decrease during the measurement, where m_s is the number of repetitive flashes at $t_d = 100$ ms and $m_s \bullet M_1', m_s \bullet M_1''$ are the measured signals S_m' and S_m'', respectively. For further experimental details see Renger (1972) and for the calibrated signal (*right panel*, Renger 1969). The absolute molar O_2 evolution per flash (μM/flash) is given by: $\Delta O_2/\text{flash} = \frac{S_m}{m \bullet S_{H_2O}} \bullet \frac{V_{H_2O}}{V_C} \bullet [O_2]_{H_2O}$, where S_m and S_{H_2O} are the signal amplitudes due to illumination with m single turnover flashes and injection of air saturated water, respectively, V_c and V_{H_2O} are the volume of the cuvette and the injected water, respectively, and $[O_2]_{H_2O}$ is the molar content of oxygen in air saturated water (μM). Together with the chlorophyll concentration in the cuvette, [Chl], the number of chlorophyll molecules per PSII with a fully competent WOC, Z_{O_2}, is obtained according to: $Z_{O_2} = \frac{[Chl]}{4 \bullet \Delta O_2/\text{flash}}$ where ΔO_2 is the change of the oxygen concentration (see right panel). It must be emphasized that this simplified formula leads to meaningful values only if the probabilities of misses (α) and double hits (β) are approximately of the same magnitude thus largely canceling each other. In almost all cases, however, the values of α exceeds those of β and Z_{O_2} is an upper limit of the exact number

of the sample. Typical traces of this type of measurements are shown in Fig. 4 (Renger 1972). Calibration of the signals by injection of a small volume of air-saturated water (alternatively the difference between air saturated and nitrogen flushed solutions is used for calibration of the setup) permits a rather precise determination of the number of PS II complexes that are fully competent in oxygen evolution. Inspection of the signals in the left panel of Fig. 4 shows that oxygen yields depend (at a constant flash number) on the dark time t_d between the repetitive flashes as is illustrated in Fig. 5, top panel. At short t_d values the normalized average oxygen yield per flash increases with t_d reaching a plateau before decreasing at long t_d values. The rising part reflects the rate limitation of the electron transport chain and the decrease is an indication of the dark reduction of redox states S_2 and S_3 of the water oxidizing complex (WOC) (see section data evaluation). The latter effect is clearly illustrated by the action of ADRY (acceleration of the deactivation reactions of system Y) agents (see Fig. 5, bottom panel) that selectively catalyze the decay of S_2 and S_3 (Renger et al. 1973; Hanssum et al. 1985).

The negative slope of the base line in Fig. 4 is caused by a small O_2 consumption of the electrode. In the case of analyzing cell suspensions of cyanobacteria and algae the drift exhibits a much more pronounced negative slope due to the respiratory activity of the sample. This effect can give rise to a strict anaerobiosis in the cuvette concomitant with a blockage of light induced electron transport as a consequence of over-reduction of the plastoquinone pool in the thylakoid membrane. Increase of the O_2 content in the cuvette by injection of air-saturated water was shown to circumvent this inhibition (Renger, unpublished results).

The approach of excitation with repetitive single turnover flashes was successfully applied to determine the pH dependence of functionally competent WOCs (Renger 1969; Renger et al. 1977) and is currently used for routinely screening the intactness of the WOC (expressed in the number of chlorophyll molecules per fully competent WOC) in PS II core complexes from *Thermosynechcoccus* (*T.*) *elongates*, which is the sample material for structure analysis of PS II by X-ray diffraction crystallography (Zouni 2008 and references therein).

Excitation with repetitive double flash groups of varying dark time between the two flashes unraveled that the rate limiting step of PS II is characterized by a half life time of about 600 μs (Vater et al. 1968) and an activation energy of 10–20 kJ/mol (Renger 1969) under steady state conditions.

In spite of many advantages the conventional Clark-type electrode has also drawbacks due to its rather limited time response and a low sensitivity that does not permit the resolution of the O_2 yield emerging from the excitation with individual single turnover flashes. The resolution limit

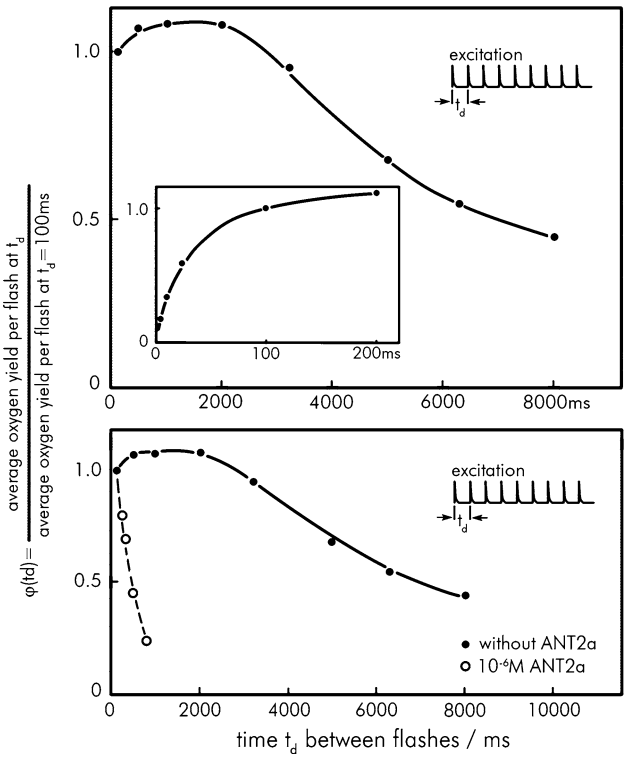

Fig. 5 Normalized average oxygen yield per flash as a function of dark time between the flashes in chloroplast suspensions in the absence (*top panel* and *filled circles* in *bottom panel*) and presence of 1 μM 2–(4-chloro) aniline–3,5–dinitrothiophene (ANT 2a, *open circles*). For further experimental details, see Renger (1972)

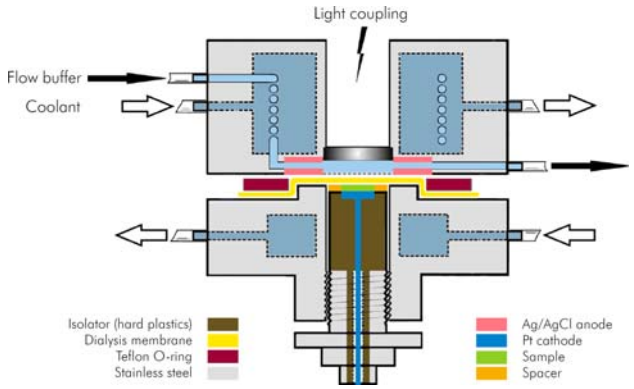

Fig. 6 Scheme (redrawn from Sheleva 2008) of a Joliot-type electrode modified by Dohnt (1984), Hanssum (1991) and Messinger (1993). For further details, see text

of the setup of Fig. 3 is illustrated by the signal shown in the right panel of Fig. 4. A significant improvement, however, was achieved by Ananyev et al. using special membranes and electrodes (Baranov et al. 2004). This device was successfully applied in studies on the process of photoactivation of the WOC (Baranov et al. 2004).

Joliot-type electrode

The cornerstone in exploitation of the full inherent potential of amperometric detection of photosynthetically evolved oxygen was the development of a bare platinum electrode system by Joliot and co-workers (Joliot 1967; Joliot and Joliot 1968). A detailed mathematical analysis (Joliot et al. 1966) on the basis of the equations for light induced generation and diffusion of O_2 in thin layers of the sample material sedimented on the Pt-cathode and the electrochemical reduction revealed that the monitored electrical signal is proportional to the oxygen yield per flash. However, a calibration in absolute values of the evolved oxygen cannot be achieved, in contrast to the above described approach using repetitive flash excitation and a Clark-type electrode (see Fig. 3).

A modern setup of a Joliot type electrode is shown in Fig. 6. The Ag/AgCl anode and platinum cathode are located in a stainless steel housing consisting of two parts. The top "anode part", hermetically closed up by a glass window, contains an entry path for excitation light (symbolized by a flash sign). The excitation source itself, which is either a modulated CW light beam, a Xenon flash or a laser pulse, can be coupled to the cuvette directly or via an optical fiber system. Below the optical window a silver disc with a slit chamber forms the anode, flooded by flow buffer. The lower side of this slit chamber (indicated by a dashed line) is sealed by a dialysis membrane (shown on top of the cathode part in yellow) that prevents direct flow contact between both parts but permits ion exchange and therefore flow of an electrical current.

The bottom "cathode part" consists of a bare platinum electrode shaped like the optical window. A 50–100 μm tape spacer on top of the cathode holder together with the dialysis membrane (marked in yellow) creates a closed small compartment for photosynthetically active sample material (marked in green) sedimented onto the platinum surface. A cathode support made of hard plastics is used as an electrical isolator and mechanical guidance for experiment assembly. Top and bottom part of the stainless steel holder has appropriate cavities for the passage of a thermostated liquid, thus allowing for temperature dependent measurements in the range from −4° to 60°C (Messinger et al. 1993; for measurements above 40°C special membranes have to be used and the signal to noise ratio is lower, Hanssum (unpublished results)).

The general measuring conditions closely resemble those of the Clark type electrode setup (see former section), e.g., a polarizing voltage −0.7 V with a resulting diffusion limited current flow (see Eq. 4).

The electronic detection circuit must be able to separate the steady-state current signal (resulting from O_2 contained in the sample buffer and from the O_2 that diffuses from the

flow-buffer through the dialysis membrane into the sample) from the transients produced by the WOC due to excitation with either single turnover flashes or modulated CW light. A low loss, low noise inductive transformer converts all time-varying currents into a voltage at its secondary winding that is typically amplified by a factor of 10^5, filtered and stored for data analysis.

The insert of Fig. 7 shows a typical oxygen release transient induced by excitation with single turnover flashes either from a Xenon discharge lamp (FWHM typically of the order of 10 μs) or laser pulses (FWHM typically few ns). As the sample material forms a loose sediment layer on the platinum surface, the rise of the signal is determined by the WOC release kinetics convoluted with the time profile of diffusion from the photosynthetic source inside the sample compartment to the Pt-cathode surface, while the decay reflects the O_2 consumption by the electrode. The negative undershoot of the signal is caused by the counter electromotive force of the transformer inductance. The time course of the flash-induced signal can be used for determination of the O_2 release kinetics (Schulder et al. 1992). A most suitable approach is the tight layering of the sample material onto the platinum surface by centrifugation, thus minimizing the diffusion distance (for details, see Clausen et al. 2004).

An analysis of the O_2 release pattern in the sense of absolute quantities is impossible due to the lack of information on how many WOCs are contributing to the overall signal. On the other hand, as all signals follow the same time course, analysis can be done if a comparable normalizing quantity is at hand. The transient oxygen release quantity is determined by the overall charge reduced at the cathode surface after excitation:

$$[O_2] = \text{const.} \bullet Q = \int_0^t I(t)\mathrm{d}t \tag{5}$$

Electrical charge is the integral of a current flow in time. Rise and fall kinetics are uniform, so that the peak amplitude is proportional to the area and hence peak determination allows assignment of relative O_2 release quantities. Under excitation with a train of single turnover flashes a steady state is reached where each flash gives rise to about the same oxygen yield, as is shown in Fig. 7 for dark adapted thylakoids. Therefore, the average signal amplitude per flash is used for normalization of the oscillation pattern.

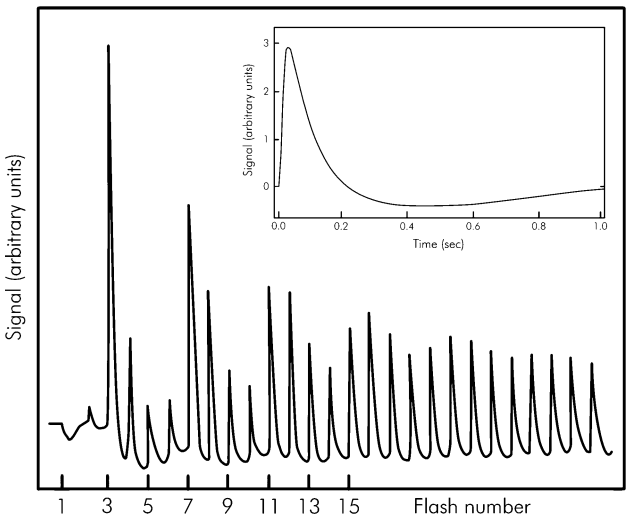

Fig. 7 Typical pattern of electric signals induced at the Joliot-type electrode by a train of single turnover flashes on dark adapted spinach chloroplasts (*inset*) and signal of the third flash at higher time resolution

Data evaluation

A flash-induced oscillation pattern of oxygen yield (FIOP) of the type shown in Fig. 7 was first reported by Joliot et al. (1969). It is characterized by a pronounced period four which reflects the population probability of the redox states S_i of the WOC in the ensemble of PS II complexes of the sample. Under normal conditions the WOC almost entirely populates redox state S_1 in the dark (Vermaas et al. 1984; Messinger and Renger 1993) and cycles through the states S_0, S_1, S_2, and S_3 and the transient S_4 (referred to as Kok-cycle, see Kok et al. 1970).

Under excitation conditions where the time between the flashes is long compared to the rate limiting step of the linear electron transport chain, but short enough to exclude effects due to the decay of S_2 and S_3, and if the number of functionally fully competent WOCs does not change during the experiment, the distribution of the S_i-states after the *n*th flash is given by the matrix equation

$$\mathbf{S}_n = \mathbf{T}\mathbf{S}_{n-1} \tag{6}$$

where the state matrix **S** and state transition matrix **T** are

$$\mathbf{S} = \begin{pmatrix} [S_0] \\ [S_1] \\ [S_2] \\ [S_3] \end{pmatrix} \text{ and } \mathbf{T} = \begin{pmatrix} \alpha_0 & 0 & \beta_2 & \gamma_3 \\ \gamma_0 & \alpha_1 & 0 & \beta_3 \\ \beta_0 & \gamma_1 & \alpha_2 & 0 \\ 0 & \beta_1 & \gamma_2 & \alpha_3 \end{pmatrix} \tag{7}$$

with $[S_i]_n$ = population probability of redox state S_i ($i = 0, -3$) after flash number n and α_i, γ_i and β_i for $i = 0, \ldots 3$ are the probabilities that the actinic single turnover flash does not lead to oxidation of S_i (probability of misses), gives rise to transitions $S_i \rightarrow S_{i+1}$ (single hit probability) and $S_i \rightarrow S_{i+2}$ (double hit probability), respectively, where $\alpha_i + \gamma_i + \beta_i = 1$

The oxygen yield due to excitation with the *n*th single turnover flash of the sequence, Y_n, is obtained by the relation

$$Y_n = (1 - \alpha_3)[S_3]_{n-1} + \beta_2[S_2]_{n-1} \quad (8)$$

The first matrix analysis of FIOPs has been performed by Lavorel (1976) who clearly showed that Eqs. 6–8 cannot be solved in the generalized form. Therefore, for practical use reasonable approximations are required. The simplest approach already introduced by Kok et al. (1970) and still most widely used is the assumption that α_i, γ_i and β_i are independent of the redox state S_i. Considerations on the possible origin of the parameters revealed that the value of α_i depends on redox equilibria of both the donor and acceptor side of PS II (Renger and Hanssum 1988). This idea has been elaborated by Shinkarev who performed numerous analyses of different FIOPs and presented several solutions of the problem (for a review on the different models of data evaluation, see Shinkarev 2005 and references therein). In addition the value of α_i contains a significant contribution due to the kinetic competition between the dissipative recombination reaction of $P680^{+\bullet} Q_A^{-\bullet}$ and the reduction of $P680^{+\bullet}$ by Y_z (see Christen et al. 1999). There exist different lines of experimental evidence for S_i-state dependent α_i values (for a recent discussion, see Isgandarova et al. 2003). As a general conclusion gathered from studies on different sample material, the α_i values are small (or negligible) for $i = 0$ and 1 and significant for $i = 2$ and 3. This effect can be rationalized by taking into account the dependence on redox state S_i of the reduction kinetics of $P680^{+\bullet}$ by Y_z (Gläser et al. 1976; Brettel et al. 1984; Eckert and Renger 1988) which compete with $P680^{+\bullet} Q_A^{-\bullet}$ recombination (vide supra). Interestingly, the fit quality is in most cases only marginally higher than for use of equal misses $\alpha_i = \alpha$ ($i = 0,\ldots 3$).

The probability of double hits was shown to originate mainly from a second stable charge separation during the flash (Kok et al. 1970) and therefore depends on its duration and the rate constant of $Q_A^{-\bullet}$ reoxidation by $Q_B(Q_B^{-\bullet})$ (see Messinger et al. 1993). Accordingly, double hits should be negligibly small when the samples are excited with a train of short laser flashes (see also Jursinic 1981; Hillier and Messinger 2005; Jablonsky and Lazar 2008).

In addition to the values of α_i and β_i ($\gamma_i = 1 - \alpha_i - \beta_i$) the FIOPs can also be influenced by a decrease of the percentage of the WOCs during the flash sequence but this effect is of minor relevance under most of the experimental conditions.

The conventional Kok model has to be extended for the existence of "superreduced "S_{-i} states ($i = 1$, 2 and 3) which are formed by exogenous reductants like NH_2OH and NH_2NH_2 (see Messinger et al. 1997 and references therein) or populated in the course of photoactivation of the WOC in vivo (Higuchi et al. 2003). In this case matrices **S** and **T** of Eq. 7 have to be extended by $[S_{-3}]$, $[S_{-2}]$, $[S_{-1}]$ and α_{-i}, γ_{-i}, β_{-i} with ($i = 1$, 2, and 3), respectively.

Measurements of FIOPs are most appropriate for precise determination of the kinetics of S_i ($i = 2$ or 3) decay and of S_0 oxidation by Y_D^{ox} (Messinger and Renger 1993). For analyses of the S_i decay dark-adapted samples are illuminated by one or two short saturating (single turnover) flashes to populate the states S_2 and S_3, respectively. After different dark times the FIOPS are monitored and data evaluation provides the corresponding decay kinetics of S_2 and S_3 (see Messinger and Renger 1994; Isgandarova et al. 2003). Likewise, preillumination with three single turnover flashes leads to maximal population of S_0 and FIOPs measured after various dark times reflect the slow reoxidation of S_0 (for further reading, see Messinger and Renger 2008 and references therein).

Furthermore, the signals of the Joliot-type electrode also allow the determination of the kinetics of O_2 release from the sample. First results were obtained by excitation of algae with modulated CW light. Data analysis revealed that O_2 is released with a rate constant of about 800 s^{-1} (Joliot 1967). Similar values were obtained by Sinclair and Arnason (1974). These numbers fit with rate constants gathered from data obtained by other methods like EPR oximetry (Strzalka et al. 1990) and the COX assay (Lavergne 1990). Attempts to use the time course of the signal induced at the Joliot type electrode with a single turnover flash were first reported by Lavorel (1992) and Schulder et al. (1992) and similar rate constants were obtained. Likewise, Razeghifard and Pace (1999) also used the signal to determine the O_2 release kinetics. A detailed analysis showed that the signal of the inset of Fig. 7 is the composite of the rates of O_2 evolution and consumption. The resolution was improved by pelleting a thin sample layer upon the bare platinum electrode by centrifugation (Clausen et al. 2004).

As a result of all measurements and the comparison with time resolved EPR signals due to Y_Z^{OX} reduction by the WOC in redox state S_3 (Razeghifard and Pace 1999) and UV absorption changes reflecting the corresponding redox step in the WOC (Velthuys 1981; Renger and Weiss 1982) it can be concluded that the kinetics of this reaction coincides with that of dioxygen release. The implication of this phenomenon for the mechanism of oxidative water splitting in photosynthesis has been discussed elsewhere (see Renger and Renger 2008 and references therein).

Concluding remarks

Methods for detection of ground state molecular dioxygen $^3\sum_g O_2$ are powerful tools in photosynthesis research. The advanced MIMS techniques in mass spectrometry and the

highly developed Joliot-type electrode offer most reliable methods for studying the reactivity of the intermediary redox states of the WOC. They do not require complicated model based data evaluation but provide rather direct information. In combination with the potential of widely used modern techniques of genetic engineering in providing suitable sample material the advanced methods of sensitive and time resolved monitoring of $^3\sum gO_2$ formation remain indispensable for studies on the mechanism of photosynthetic water splitting leading to dioxygen release.

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References

Atkins PW (2001) Physical chemistry. Oxford University, Oxford Press

Bader K, Roben A (1995) Mass spectrometric detection and analysis of nitrogen fixation in *Oscillatoria chalyba*. Z Naturforsch C50: 199–204

Bader KP, Renger G, Schmidt GH (1993) A mass spectroscopic analysis of the water-splitting reaction. Photosynth Res 38: 355–361

Baranov SV, Tyryshkin AM, Katz D, Dismukes GC, Ananyev GM, Klimov VV (2004) Bicarbonate is a *native* cofactor for assembly of the manganese cluster of the photosynthetic water oxidizing complex. Kinetics of reconstitution of O_2 evolution by photoactivation. Biochemistry 43:2070–2079

Bauer CE, Setterdahl A, Wu J, Robinson BR (2009) Regulation of gene expression in response to oxygen tension. In: Hunter CN, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria. Springer, Berlin, pp 707–725

Blinks LR, Skow RK (1938) The time course of photosynthesis as shown by a rapid electrode method for oxygen. Proc Nat Acad Sci 24:420–427

Brettel K, Schlodder E, Witt HT (1984) Nanosecond reduction kinetics of photooxidized chlorophyll-a_{II} (P-680) in single flashes as a probe for the electron pathway, H^+-release and charge accumulation in the O_2-evolving complex. Biochim Biophys Acta 766: 403–415

Buick R (1992) The antiquity of oxygenic photosynthesis; evidence from stromatolites in sulphate-deficient Archaean lakes. Science 255:74–77

Chodavarapu VP, Shubin DO, Bukowski RM, Titus AH, Cartwright AN, Bright FV (2007) CMOS-based phase fluorometric oxygen sensor system. IEEE Transactions on Circuits and Systems Part I: Regular Papers 54:111–118

Christen G, Seeliger A, Renger G (1999) $P_{680}^{+\bullet}$ reduction kinetics and redox transition probability of the water oxidising complex as a function of pH and H/D isotope exchange in spinach thylakoids. Biochemistry 38:6082–6092

Clark LC JR, Wolf R, Granger D, Taylor Z (1953) Continuous recording of blood oxygen tensions by polarography. J Appl Physiol 6:189–193

Clausen J, Debus RJ, Junge W (2004) Time-resolved oxygen production by PS II: chasing chemical intermediates. Biochim Biophys Acta 1655:184–194

Cournac L, Redding K, Ravenel J, Rumeau D, Josse E-M, Kuntz M, Peltier G (2000) Electron flow between Photosystem II and oxygen in chloroplasts of Photosystem I-deficient algae is mediated by a quinol oxidase involved in chlororespiration. J Biol Chem 275:17256–17262

de Marais DJ (2000) Evolution. When did photosynthesis emerge on Earth? Science 289:1703–1705

Dohnt G (1984) PhD thesis, Technical University Berlin (in German)

Eckert H-J, Renger G (1988) Temperature dependence of $P680^+$ reduction in O_2-evolving PS II membrane fragments at different redox states S_i of the water oxidizing system. FEBS Lett 236: 425–431

Gilbert DL (ed) (1981) Oxygen and living processes: an interdisciplinary approach. Springer, New York

Gilles-Gonzalez M-A, Gonzalez G (2005) Heme-based sensors: defining characteristics, recent developments, and regulatory hypotheses. J Inorg Biochem 99:1–22

Gläser M, Wolff C, Renger G (1976) Indirect evidence for a very fast recovery kinetics of chlorophyll-aII in spinach chloroplasts. Z Naturforsch 31c:712–721

Greenbaum E, Mauzerall DC (1976) Oxygen yield per flash of chlorella coupled to chemical oxidants under anaerobic conditions. Photochem Photobiol 23:369–372

Haber F, Weiss JJ (1934) The catalytic decomposition of H_2O_2 by iron salts. Proc Roy Soc London Biol A 147: 332–351

Hanssum B (1991) PhD thesis, Technical University Berlin (in German)

Hanssum B, Dohnt G, Renger G (1985) On the mechanism of ADRY agent—interaction with the PS II donor side. Biochim Biophys Acta 806:210–220

Haxo FT, Blinks LR (1950) Photosynthetic action spectra of marine algae. J Gen Physiol 33:389–422

Higuchi M, Noguchi T, Sonoike K (2003) Over-reduced states of the Mn-cluster in cucumber leaves induced by dark-chilling treatment. Biochim Biophys Acta 1604:151–158

Hillier W, Messinger J (2005) Mechanism of photosynthetic oxygen production In: Wydrzynski T, Satoh K (eds) Photosystem II. The light-driven water: plastoquinone oxidoredutase, advances in photosynthesis and respiration, vol 22. Springer, Dordrecht, pp 567–608

Hillier W, Wydrzynski T (2004) Substrate water interactions within the photosystem II oxygen evolving complex. Phys Chem Chem Phys 6:4882–4889

Hoch G, Kok B (1963) A mass spectrometer inlet system for sampling gases dissolved in liquid phases. Arch Biochem Biophys 101: 160–170

Isgandarova S, Renger G, Messinger J (2003) Functional differences of photosystem II from Synechococcus elongatus and spinach characterized by flash induced oxygen evolution patterns. Biochemistry 42:8929–8938

Jablonsky J, Lazar D (2008) Evidence for intermediate S-states as initial phase in the process of oxygen-evolving complex oxidation. Biophys J 94:2725–2736

Joliot P (1967) Oxygen evolution in algae illuminated with modulated light. In: Olson JM, Hind G, Siegelman HW (eds) Energy conversion by the photosynthetic apparatus. Upton, New York, pp 418–433

Joliot P, Joliot A (1968) A polarographic method for detection of oxygen production and reduction of Hill reagent by isolated chloroplasts. Biochim Biophys Acta 153:625–634

Joliot P, Hofnung M, Chabaud R (1966) Etude de l'émission d'oxygène par des algues soumises a un éclairement modulé sinusoidalement (in French). J Chim Phys 63:1423–1441

Joliot P, Barbieri G, Chabaud R (1969) Un nouveau modèle des centres photochimiques du système II (in French). Photochem Photobiol 10:309–329

Jursinic P (1981) Investigation of double turnovers in Photosystem II charge separation and oxygen evolution with excitation flashes of different duration. Biochim Bioiphys Acta 635:38–52

Kasting JF, Seifert JF (2002) Life and the evolution of earth's atmosphere. Science 296:1066–1067

Klimant I, Ruckruh F, Liebsch G, Stangelmayer A, Wolfbeis OS (1999) Fast response oxygen micro-optodes based on novel soluble ormosil glasses. Microchimica Acta 131:35–46

Kok B, Forbush B, McGloin M (1970) Cooperation of charges in photosynthetic O_2 evolution. Photochem Photobiol 11:457–476

Konermann L, Messinger J, Hillier W (2008) Mass spectrometry-based methods for studying kinetics and dynamics in biological systems. In: Aartsma TJ, Matysik J (eds) Biopysical techniques in photosynthesis. Springer, Dordrecht, pp 167–190

Krebs HA (1951) The use of 'CO_2 buffers' in manometric measurements of cell metabolism. Biochem J 48:349–359

Lakowicz JR (1999) Principles of fluorescence spectroscopy. Kluwer/Plenum, New York

Lane N (2003) Oxygen—The molecule that made the world. Oxford University Press, Oxford

Larkum AWD (2008) The evolution of photosynthesis. In: Renger G (ed) Primary processes of photosynthesis: basic principles and apparatus, Part II reaction centers/photosystems, electron transport. Royal Society Chemistry, Cambridge, pp 491–521

Lavergne J (1990) Detection of photosynthetic oxygen through mitochondrial absorption changes in algal cell. In: Baltscheffsky M (ed) Current research in photosynthesis, vol 1. Kluwer, Dordrecht, pp 845–848

Lavorel J (1976) Matrix analysis of the oxygen evolving system of photosynthesis. J Theor Biol 57:171–185

Lavorel J (1992) Determination of the photosynthetic oxygen release time by amperometry. Biochim Biophys Acta 1101:33–40

Lippitsch ME, Pusterhofer J, Leiner MJP, Wolfbeis OS (1988) Fibre-optic sensor with the fluorescence decay time as the information carrier. Anal Chim Acta 205:1–6

Messinger J (1993) Untersuchungen über die reaktiven Eigenschaften der verschiedenen Redoxzustände S_i der Wasseroxidase Höherer Pflanzen (in German). PhD Thesis, Technical University Berlin

Messinger J, Renger G (1993) Generation, oxidation by Y_D^{OX} and possible electronic configuration of the redox states S_0, S_{-1} and S_{-2} of the water oxidase in isolated spinach thylakoids. Biochemistry 32:9379–9386

Messinger J, Renger G (1994) Analysis of pH-induced modifications in the period four oscillation of flash-induced oxygen evolution reveal distinct structural changes of the photosystem II donor side at characteristic pH values. Biochemistry 33:10896–10905

Messinger J, Renger G (2008) Photosynthetic water splitting. In: Renger G (ed) Primary processes of photosynthesis: basic priniciples and apparatus, vol. II: reaction centers/photosystems, electron transport chains, photophosphorylation and evolution. Royal Society Chemistry, Cambridge, pp 291–349

Messinger J, Schröder WP, Renger G (1993) Structure—function relations in photosystem II. Effects of temperature and chaotropic agents on the period four oscillation of flash induced oxygen evolution. Biochemistry 32:7658–7668

Messinger J, Badger M, Wydrzynski T (1995) Detection of one slowly exchanging substrate water molecule in the S_3 state of photosystem II. Proc Nat Acad Sci 92:3209–3213

Messinger J, Seaton GR, Wydrzynski T, Wacker U, Renger G (1997) The S_{-3} state of the water oxidase in Photosystem II. Biochemistry 36:6862–6873

Meyer B, Schlodder E, Dekker JP, Witt HT (1989) O_2 evolution and Chl a_{II}^+ (P-680$^+$) nanosecond reduction kinetics in single flashes as a function of pH. Biochin Biophys Acta 974:36–43

Nicholls DG, Ferguson SJ (1982) Bioenergetics, vol 2. Academic Press, London

Ohle W (1936) Zur Vervollkommnung der hydrochemischen Analyse. II. Bestimmung des im Wasser gelösten Sauerstoffs (in German). Angew Chem 49:778–784

Petering HG, Daniels F (1938) The determination of dissolved oxygen by means of the dropping mercury electrode, with applications in biology. J Am Chem Soc 60:2796–2802

Priestley J (1772) Observations on different kinds of air. Phil Trans 62:147–264

Radmer R (1979) Mass spectrometric determination of hydroxylamine photooxidation by illuminated chloroplasts. Biochin Biophys Acta 546:418–425

Radmer R, Ollinger O (1980a) Measurements of the oxygen cycle: the mass spectrometric analysis of gases dissolved in a liquid phase. In: San Pietro A (ed) Meth enzymol, vol 69. Academic Press, New York, pp 547–560

Radmer R, Ollinger O (1980b) Isotopic composition of photosynthetic O_2 flash yields in the presence of $H2^{18}O$ and $HC^{18}O_3^-$. FEBS Lett 110:57–61

Radmer R, Ollinger O (1986) Do the higher oxidation states of the photosynthetic O_2-evolving system contain bound H_2O? FEBS Lett 195:285–289

Razeghifard MR, Pace RJ (1999) EPR kinetic studies of oxygen release in thylakoids in PS II membranes: a kinetic intermediate in the S_3 to S_0 transition. Biochemistry 38:1252–1257

Renger G (1966) Diploma thesis, Technical University Berlin (in German)

Renger G (1969) PhD thesis, Technical University Berlin (in German)

Renger G (1972) The action of 2-anilinothiophenes as accelerators of the deactivation reactions in the water splitting enzyme system of photosynthesis. Biochim Biophys Acta 256:428–439

Renger G (1983) Biological energy conservation. In: Lohmann W, Markl H, Ziegler H, Hoppe W (eds) Biophysics. Springer, Berlin, pp 347–371

Renger G (ed) (2008) Primary processes of photosynthesis: basic principles and apparatus, part I and II. Royal Society Chemistry, Cambridge

Renger G, Hanssum B (1988) Studies on the deconvolution of flash induced absorption changes in the difference spectra of individual redox steps in the water oxidizing enzyme. Photosynth Res 16: 243–259

Renger G, Renger T (2008) Photosystem II: the machinery of photosynthetic water splitting. Photosynth Res 98:53–80

Renger G, Weiss W (1982) The detection of intrinsic 320 nm absorption changes reflecting the turnover of the water splitting enzyme system Y which leads to oxygen formation in trypsinized chloroplats. FEBS Lett 137:217–221

Renger G, Bouges-Bocquet B, Delosme R (1973) Studies on the ADRY—agent induced mechanism of the discharge of the holes trapped in the photosynthetic water splitting enzyme system. Biochim Biophys Acta 292:796–807

Renger G, Gläser M, Buchwald HE (1977) The control of the reduction kinetics in the dark of photooxidized chlorophyll-a_{II}^+ by the inner thylakoid proton concentration. Biochim Biophys Acta 461:392–402

Renger G, Bader KP, Schmid GH (1990) Mass spectroscopic analysis of N_2 formation by flash induced oxidation of hydrazine and hydroxylamine in normal and Tris treated tobacco chloroplasts. Biochim Biophys Acta 1015:288–294

Rumberg B, Siggel U (1969) pH changes in the inner phase of the thylakoids during photosynthesis. Naturwiss 56:130–132

Schulder R, Burda K, Strzalka K, Bader KP, Schmid GH (1992) Study on the parameters affecting oxygen release time measurement by amperometry. Z Naturforsch 47c:465–473

Sheleva D (2008) PhD thesis, Technical University Berlin

Shinkarev V (2005) Flash induced oxygen evolution and other oscillatory processes. In: Wydrzynski T, Satoh K (eds)

Photosystem II: the water: plastoquinone oxido-reductase in photosynthesis. Springer, Dordrecht, pp 539–565

Sinclair J, Arnason T (1974) Studies on a thermal reaction associated with photosynthetic oxygen evolution. Biochim Biophys Acta 368:393–400

Strzalka K, Sarna T, Hyde JS (1986) Electron-spin resonance oxymetry—measurements of photosynthetic oxygen evolution by spin-probe technique. Photobiochem Photobiophys 12:67–71

Strzalka K, Walczak T, Sarna T, Swartz HM (1990) Measurement of time-resolved oxygen concentration changes in photosynthetic systems by nitroxide-based EPR oximetry. Arch Biochem Biophys 281:312–318

Subczynski WK, Hyde JS (1981) The diffusion-concentration product of oxygen in lipid bilayers using the spin-label T_1 method. Biochim Biophys Acta 643:283–291

Swartz HM, Pals MA (1989) Measurements of intracellular oxygen. In: Miguel J, Weber H, Quintanilha H (eds) Handbook of biomedicine of free radicals and antioxidants. CRC Press, Boca Raton, pp 141–151

van Gorkom HJ, Gast P (1996) Measurements of photosynthetic oxygen evolution. In: Amesz J, Hoff AJ (eds) Biophysical techniques in photosynthesis. Kluwer, Dordrecht, pp 391–405

Vater J, Renger G, Stiehl HH, Witt HT (1968) Intermediates and kinetics of the water splitting part of photosynthesis. Naturwiss 55:220–221

Velthuys B (1981) Spectroscopic studies of the S-state transitions of Photosystem II and of the interaction of its charged donor chains with lipid-soluble anions. In: Akoyunoglou G (ed) Photosynthesis II. Electron transport and photophosphorylation. Balaban, Philadelphia, pp 75–85

Vermaas WCJ, Renger G, Dohnt G (1984) The reduction of the oxygen-evolving system in chloroplasts by thylakoid components. Biochim Biophys Acta 764:194–202

Warburg O, Krippahl G (1960) Weiterentwicklung der manometrischen Methoden (Carbonatgemische) (in German). Z Naturforsch 15b:364–367

Windrem DA, Plachy WZ (1980) The diffusion-solubility of oxygen in lipid bilayers. Biochim Biophys Acta 600:655–665

Worrest RC, Caldwell MM (eds) (1986) Stratospheric ozone reduction, solar ultraviolet radiation and plant life. Springer, Berlin, pp 171–184

Xiong J, Bauer CE (2002) Complex evolution of photosynthesis. Annu Rev Plant Biol 53:503–521

Zouni A (2008) From cell growth to the 3.0 Å resolution crystal structure of cyanobacterial photosystem II. In: Renger G (ed) Primary processes of photosynthesis: priniciples and apparatus, part ii reaction centers/photosystems, electron transport chains, photophosphorylation and evolution. Royal Society Chemistry, Cambridge, pp 193–236