Study on the Parameters Affecting Oxygen Release Time Measurements by Amperometry

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The amperometric analysis of photosynthetic oxygen evolution in thylakoid membrane preparations of tobacco, sedimented on a large surface electrode, shows that the oxygen signal is preceded by a lag phase of 1.2 ms. As the used tobacco lamellar systems have an average diameter of 4–5 μm this lag phase is interpreted to be due to an average distance of 2.4 μm between oxygen emitting sources and the electrode surface. This interpretation of the lag phase is substantiated by the fact that photosystem I-mediated oxygen uptake in the same preparation measured with the same amperometric technique exhibits the same lag phase although the kinetic uptake parameters are considerably slower than those of oxygen evolution due to the water-splitting reaction. The analysis of the oxygen evolution signal shows that the sedimentation time *i.e.* the quality of the contact of the source with the electrode is decisive for the measured value for the oxygen release time, which with this method without much effort comes out to be certainly not longer than 1.5 ms. A computer simulation shows that the distance of the oxygen emitting layer from the electrode as well as its thickness and the actual release time are variables which influence practically all parameters (signal shape, amplitude etc.) of such amperometric signals. From this it appears that the determined apparent experimental parameters of oxygen evolution kinetics contain deformations due to the thickness of the sample and the diffusion distance from the electrode. An extrapolation calculated for a sample of infinite thinness separated from the electrode by a minimal distance of 0.001 μm with τ of 5.5 ms yields a lower limit value for the half-rise time of 0.5 ms, a value which has been experimentally determined by EPR oximetry (K. Strzałka, T. Walczak, T. Sarna, and H. M. Swartz, Arch. Biochem. Biophys. **281**, 312 (1990)).

Introduction

Since the measurements by Joliot and co-workers more than 25 years ago [1] it was accepted knowledge that oxygen evolution following charge accumulation characterized by the transition $S_0 \rightarrow S_4$ was a very rapid event. Joliot and co-workers found 0.8 ms [1]. Later measurements confirmed these values in essence, yielding always values not longer than 3 ms [2–6]. This state of knowledge was questioned by observations from the van Gorkum laboratory who found that time constants for the release of photosynthetic oxygen were in the range of 30–130 ms [7, 8]. As the importance and the implications of the observation, if correct, are considerable, it appeared legal to make a rough and quick estimate on the time constants with the now-a-days available methods. Lavergne was able to show that under anaerobic conditions photosynthetically evolved oxygen was apparently used in the respiratory chain of algae and was detected as cytochrome *c* absorption change within 3 ms [9]. Schulder *et al.* presented signal analyses which yielded half-rise times for oxygen release in the region of 1 ms [10]. Particularly short times of $\approx$ 0.4 ms were obtained by EPR oximetry measurements [11]. The relative ease with which these values are obtained seem to show that the values by Plijter *et al.* [7] cannot be correct and that the measurements by Joliot and co-workers [1] are still the correct ones. Thereafter it is temptating to just play around and look what type of lag period can be observed (if at all) with now-a-days techniques and methods before the onset of O_2 evolution and what type of parameters with respect to the source and the measuring technique would affect release time measurements. The measurable lag time before the onset of oxygen evolution detectable with our material yields a value which comes out to lie between 1.2 and

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2.1 ms. When trying to give this lag a reasonable interpretation we find that it could correspond to a diffusion distance of 2.4 μm. Knowing that the diameter of a tobacco lamellar system is a measurable 4–4.5 μm value, the lag time can obviously correspond to the time which is needed for oxygen to arrive from the source at the electrode surface. Thereafter the analysis of the oxygen evolution signal proper allows for the transition $S_4 \rightarrow S_0$ a time determination with a generous upper limit of 1.5 ms. Computer simulation shows that an infinitely thin sample in the closest possible contact with the electrode should bring the release time in the region of a lower limit value of ≈ 0.5 ms.

Materials and Methods

Plant material and thylakoid preparations

Leaves from 6 weeks old tobacco plants of *N. tabacum* var. William's Broadleaf grown under controlled conditions in a growth chamber were used for chloroplast preparation. Chloroplasts and thylakoid membranes were isolated according to Homann and Schmid [12].

Measurement of oxygen evolution

Oxygen evolution was measured at room temperature with the "Three Electrode System" described in detail by Schmid and Thibault [13]. Measurements were carried out at a polarization voltage of −680 mV. The electrode system was interfaced either with an Atari Mega ST 4 computer or with the Tracor Northern Multichannel Analyzer. Additionally the oxygen signal was viewed on the screen of a digital oscilloscope and photographed if needed. The amount of thylakoid membrane preparation applied to the electrode as well as the concentrations of the exogenous electron donors and acceptors used is specified in the "Results" section.

Light sources

For pulse illumination a flash lamp (Stroboscope 1539 A, General Radio) with a flash duration of 8 μs and saturating power (flash energy 0.5 Joule) was used. Continuous illumination with white saturating light was provided by a halogen lamp projector equipped with an electronically operated shutter.

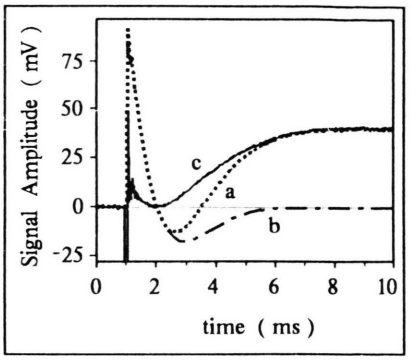

Fig. 1. Amperometric signal obtained from a dark adapted sample of a thylakoid membrane preparation from *N. tabacum* var. John William's Broadleaf after the third single-turnover flash. The curves represent: a) the convoluted oxygen + parasite signal; b) the signal of the electrical parasite recorded with heat-inactivated thylakoids (averaged from 221 light flashes); c) the oxygen signal after subtraction of the electrical parasite. The used thylakoid membrane preparation, sedimented on the electrode, corresponded to 20 μg of chlorophyll; sedimentation time was 45 min; dark adaptation 20 min; concentration of parabenzoquinone in the assay 10 μM, time resolution of the curves 10 μsec per measuring point.

Results

A typical amperometric oxygen signal obtained from a dark-adapted sample of thylakoid membranes after the third single turnover light pulse is shown in Fig. 1 (curve a). The signal represents the superposition of the oxygen signal and an electrical "parasite" and starts with a distinct electrical noise mainly caused by the discharge of the electrical circuit of the flash lamp but possibly also by some photoelectric cathode phenomenon due to flash illumination.

The parasite alone (Fig. 1, curve b) exhibits a characteristic shape. First there is an almost instant rise starting concomitantly with the light pulse, followed by a fast decrease with a minimum below the baseline then followed by a slower rise to the baseline level. The presence of such an electrical parasite complicates somewhat the precise determination of the onset of the amperometric signal related to the release of photosynthetic oxygen after the light flash. To eliminate the contribution of the parasite to the amperometric oxygen signal, the following experiments were carried out:

In the first experimental approach the contribution of the electrical parasite to the amperometric

signal was determined by analyzing different amounts of thylakoid membrane preparation on the electrode. As the size of the noise signal is independent on the amount of thylakoid membrane preparation present, the influence of the parasite on the oxygen signal can be minimized. Fig. 2 shows amperometric signals from flash illuminated samples observed with varying amounts of thylakoid membrane preparation. The contribution of the electrical noise to the overall signal decreases with rising amounts of thylakoid membrane preparation. As seen in Fig. 2 the values for the half-rise time and rise time, if measured from the signal minimum are virtually the same or very similar for samples with chlorophyll concentrations varying in the range from 25 μg/ml to 100 μg/ml. Therefore it seems very unlikely that these values are affected by the accompanying electrical noise. However, the remaining problem is whether the assumption that the signal minimum indeed corresponds to the beginning of oxygen detection by the cathode is correct.

In order to solve this problem, we used the approach in which the electrical noise was eliminated by subtracting from the convoluted parasite-oxygen signal the signal of the parasite, recorded for a sample not evolving oxygen. This was achieved by sedimentation of the appropriate amount of thylakoid membrane preparation on the cathode in which oxygen evolution had been blocked by heat

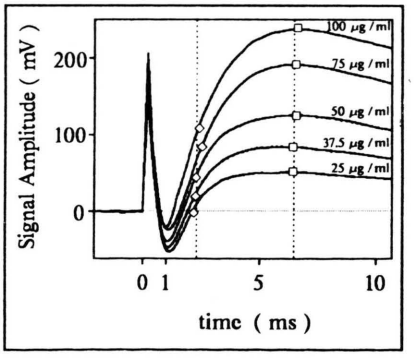

Fig. 2. Dependence of the kinetic parameters of the amperometric oxygen signal on the amount of thylakoid membrane preparation deposited on the cathode. Amount of thylakoids in the samples is given as chlorophyll content. ◇ indicates half-rise time and □ rise time of the oxygen signal. The electrical parasite is not subtracted from the oxygen signal. Sedimentation time 15 min; dark adaptation 15 min; time resolution of the curves 195 μs per measuring point.

shock. Although the general shape of the electrical parasite was practically the same from one flash to another, minor variations in the amplitude of its respective components occurred occasionally. Therefore for the subtraction of the parasite from the convoluted oxygen signal the averaged response to several hundred light pulses was used. The thus obtained oxygen signal free from contamination by the electrical parasite is shown in Fig. 1, curve c. As is clearly seen in Fig. 1c a lag phase of a duration of 1.22 ms separates the light pulse from the onset of oxygen evolution. Lag phases determined in 5 experiments (not shown) varied between 1.2 and 2.1 ms having an averaged duration of 1.69 ms. It is reasonable to assume that the observed lag phase is related to oxygen diffusion from the oxygen-evolving complex *i.e.* the source to the electrode surface. Aside from the lag phase the kinetic parameters of the oxygen signal itself, obtained by this approach yield a half-rise time of 1.7 ms and a rise time of 5.6 ms, which is in good agreement with data obtained for samples with varying amounts of thylakoid membrane preparation on the electrode (compare Fig. 2).

In order to verify the correctness of the lag phase duration determined by the subtraction of the parasite a series of experiments with continuous light was carried out. In this type of experiment no electrical parasite appears. The onset of continuous illumination was triggered by a shutter and the precise location of its beginning on the time axis was determined by means of the signal from a photodiode mounted in the optical path between the shutter and the sample. Fig. 3 shows the photograph taken from the oscilloscope screen showing again a lag phase preceding the oxygen evolution signal. The determination of its duration (1.46 ms) was achieved by simultaneous Tracor recordings having a time resolution of 20 μs per point (data not shown). Again the lag phase varied from 1.4 up to 2.1 ms, yielding an average value of 1.65 ms, which fits the values from flash light experiments.

Flash light as well as continuous light experiments were also performed in order to monitor the kinetics of PS I-mediated oxygen consumption by reduced methyl viologen. The typical shape of the PS I response induced by a continuous light pulse from heat-inactivated thylakoid membrane preparations supplemented with methyl viologen is

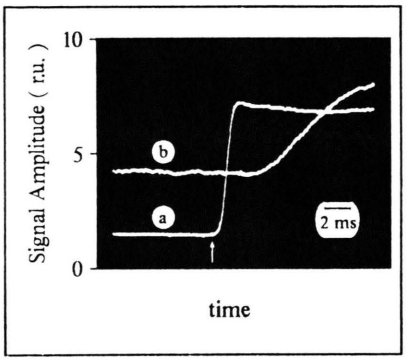

Fig. 3. Time relationship between the onset of continuous illumination, monitored by a photodiode (curve a) and the beginning of detection of oxygen evolution on the electrode (curve b). The arrow marks the opening of the shutter. The curves represent the averaged value of 200 continuous light pulses of a duration of 24.25 ms each separated by 1.85 s dark intervals. Prior to the experiment 30 preflashes were applied in order to desynchronize oxygen evolution. The amount of thylakoids in the sample corresponded to 20 μg of chlorophyll; sedimentation time was 30 min; concentration of parabenzoquinone 10 μM; time resolution of the presented curve is 20 μs per measuring point.

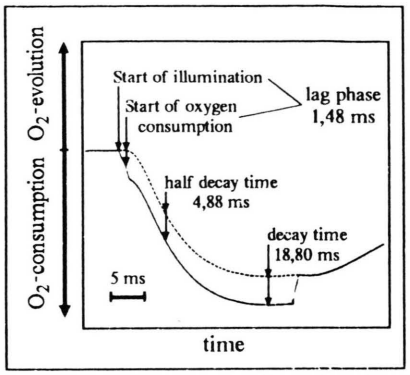

Fig. 4. Amperometric, parasite-free signal, of methyl viologen-mediated oxygen consumption after a continuous light pulse. The signal represents the averaged response to 280 light pulses, preceded by 30 preflashes in order to desynchronize the system. Pulse duration was 24.25 ms with dark intervals of 1.85 s. Thylakoids prior to the experiment were treated by heat shock (5 min at 50 °C), and corresponded to 20 μg of chlorophyll. The reaction medium contained 50 μM methylviologen and 5 mM diphenylcarbazide. Sedimentation time was 20 min and the time resolution of the given curve 40 μs per measuring point.

presented in Fig. 4. The signal is an average of 280 recordings. This signal is superimposed by a photodiode signal which marks the beginning of illumination. Again, as in the case of oxygen evolution, a lag phase of 1.48 ms precedes the onset of oxygen consumption. The duration of this lag phase is again in good agreement with the corresponding value of 1.36 ms obtained for the lag phase of the oxygen consumption signal in an experiment with flash light (data not shown). The calculated kinetic parameters for the signal, namely the half-decay time of 4.88 ms and the decay time of 18.8 ms for oxygen consumption in this pulse experiment show that the uptake reaction is considerably slower than the evolution reaction and is not much influenced by the presence of exogenous electron donors to PS II (diphenylcarbazide) or to PS I (DCPiP/ascorbate) in the reaction medium (Table I). On the other hand, it is also seen from Table I that the sedimentation time has

Table I. Effect of exogenous donors to PS I and PS II and of the sedimentation time on kinetic parameters of the amperometric signal for the methyl viologen-mediated oxygen consumption with heat-inactivated thylakoid membranes.

Acceptor	Donor	Sedimentation time [min]	Number of flashes	Decay time [msec]	Amplitude [mV]
MV	–	10	501	9.68	2.251
MV	–	25	502	8.78	5.590
MV	DPC	20	510	9.56	3.214
MV	DPC	35	500	9.20	5.340
MV	DPC	50	501	9.00	7.142
MV	DCPiP/ascorbate	20	535	10.10	5.998
MV	DCPiP/ascorbate	30	500	9.80	7.922

Time resolution 20 μs/measuring point, flash interval 300 ms, concentrations used in the assay: methyl viologen (MV), 50 μm; diphenyl carbazide (DPC), 5 mM; dichlorophenol indophenol/ascorbate (DCPiP/ascorbate) corresponding to 100 μM DCPiP (reduced by ascorbate).

a considerable influence on the presented kinetic parameters of the thylakoid membranes on the electrode. Sedimentation affects the rise time and the amplitude of the signal observed, indicating that sedimentation, that is the close contact of the preparation with the electrode surface, is an important variable affecting the shape of the measured signal and therefore should be under control of standard conditions.

Discussion

Our data on the time resolution of amperometric measurements of oxygen evolution presents evidence that the half-time of this process measured in our experimental system comes out to be on the average 1.5 ms which is in contrast to the data presented by the van Gorkom group [7, 8]. A possible error source with measurements like ours is the possibility that the measurement, although thought to be an amperometric one, in reality is not (for example due to a working resistance in the measuring system). In this case the measured signal would in reality be a derived one. Therefore, the determined half-time of oxygen evolution is only correct, if the recorded signal is neither an integrated nor a derived one. In this context a series of control experiments have been carried out (electrode response to a rectangle signal, application of different ranges of resistance between cathode and anode etc., data not shown) which clearly showed that the electrode neither integrates nor derives the oxygen signal. The test of our measuring system shows that the oxygen signal measured as current (hence a strictly amperometric measurement!) has the same time characteristics with half-rise times of around 1.5 ms as those measured as voltages in Fig. 2. Therefore one of the possible sources for the discrepancy between the results of Plijter *et al.* [7] and those presented in this paper and by others could be an integration of the original signal by the other electrode system. As shown in Fig. 5 the computed integral of the parasite-free oxygen evolution signal after the third single turnover flash gives kinetics having the parameters close to those reported by the van Gorkom group. This is only a possibility for an explanation but does not claim to be the case.

Recently a series of papers on the oxygen release time problem has been published in which related

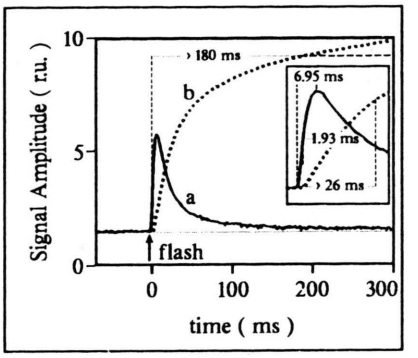

Fig. 5. Kinetics of the original parasite-free amperometric oxygen signal (curve a) and its calculated integral (curve b). Half-rise time and rise time for the original signal are 1.93 ms and 6.95 ms respectively. For the integrated signal 26 ms and 200 ms respectively. Experimental conditions for the recording of the original signal are as in Fig. 1.

or totally different methods than ours were applied. Jursinic and Dennenberg [14], using a bare platinum electrode operated under weak bias conditions, found a half-time for the rise of the oxygen signal from thylakoid membranes of 2.7 ms. A different approach to this problem was carried out by Mauzerall who used a time-resolved photoacoustic technique to monitor the kinetics of oxygen release from chloroplasts in the intact leaf [15]. Considering modifying effects on the measured kinetics of such factors as the oxygen diffusion distance and water thermal diffusion he still found that the observed rise time to the half maximum of the oxygen signal is not longer than 5 ms [15].

Lavergne in his elegant work on anaerobic algal cells was able to show that absorption changes due to the internal oxidation of the heme of cytochrome *c* oxidase by photosynthetically evolved oxygen have a half-time of 3.5 ms which clearly indicates that the oxygen liberation time has to be shorter [9].

Especially well suited for the study of oxygen evolution kinetics is nitroxide-based EPR oximetry [11, 16]. Due to a phenomenon of Heisenberg spin exchange, the oxygen molecule being paramagnetic affects the spin probe line as soon as it is released from the oxygen evolving complex. The only limiting factor is the bimolecular collision rate. This approach avoids the problem linked to oxygen diffusion which always causes a significant deformation of the real oxygen release kinetics in polarographic

methods or those based on photoacoustics. The half-time of oxygen release determined by EPR oximetry with a time resolution of 50 μs was found to be 0.4–0.5 ms [11], being the shortest time for oxygen release reported so far [11].

As Fig. 1 and 3 show, a time lag exists separating the light pulse and the detected onset of the signal. Since the response time of the oxygen electrode and that of the electronics used lies in the range of microseconds (significantly below 0.1 ms) the presence of a lag phase (≈ 1.5 ms) indicates that in the experimental system used either the reaction of oxygen release is delayed with respect to the light pulse or that the determined lag phase reflects the time which is necessary for diffusion of oxygen from the water splitting complex *i.e.* the source to the electrode surface.

In order to evaluate the contribution of the oxygen diffusion time on the measured kinetic parameters of the signal in the system used a mathematical model has been built (see Appendix). The model permits the simulation of an electrode signal in response to oxygen when parameters such as the distance of the oxygen evolving source from the electrode surface, the thickness of the sample as well as the time constant (τ) of the oxygen release process are varied. The influence of these variables on computer simulated electrode signals calculated for an oxygen diffusion coefficient in an aqueous medium of $D = 2{,}3 \cdot 10^{-5}\,cm^2\ sec^{-1}$ is shown in Fig. 6 and 7.

An increasing distance of the oxygen emitting layer from the electrode surface assuming its infinite thinness and non limiting τ value (0.01 ms) has a distinct effect on the shape of the electrode signal and its kinetic parameters such as the lag phase and the maximal amplitude. It also has an influence on the rise and half-rise time as seen with curves a–d in Fig. 6. If we assume that the average diffusion distance between the electrode and the lower surface of the thylakoid layer deposited on the electrode is approx. 2 μm, as calculated earlier [10], a lag phase of 1.72 ms duration is observed (Fig. 6, curve d) before the electrode starts to detect the released oxygen. This theoretically calculated value is in very good agreement with the measured experimental data (1.69 ms).

The presented model also indicates that the real time constant (τ) of oxygen release has a great impact on the kinetics of the oxygen signal observed with the electrode. Fig. 7A and B shows the dependence of the electrode signal on the increased τ value of an oxygen emitting source under the assumption of its infinite thinness and localization either in intimate contact with the electrode surface (distance 0.001 μm) or at a distance of 2 μm respectively. In both cases a considerable influence of the actual τ value on the half-rise time as well as on the rise time of the electrode signal is observed.

Another parameter influencing the kinetics of the electrode signal is the thickness of the sample. Under the assumption of a very short not limiting τ value (0.01 ms) it is seen that with increasing thickness of the oxygen emitting source layer a distinct increase in half-rise time, rise time and the maximal amplitude of the oxygen signal is observed (Fig. 7, C and D). The time scale of these changes is dependent on the distance of the sample from electrode surface. These results are supported by the experimental data given in Table I. Samples differing in sedimentation time differ in fact in the thickness of the oxygen emitting layer and its distance from the electrode. This, as shown, clearly leads to changes in the calculated kinetic parameters. Therefore, the kinetic parameters of the oxygen release process reported by Plijter *et al.* [7] using a suspension of PS II membranes (a sample of "semi-infinite thickness") brings problems.

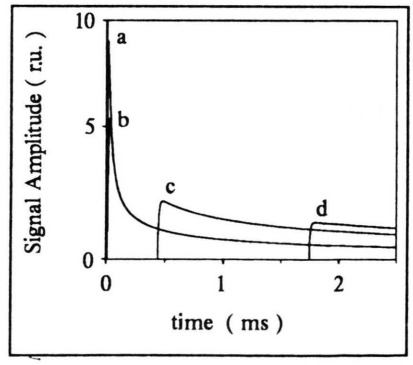

Fig. 6. Effect of the distance of the oxygen emitting source from the electrode surface on a computer-simulated electrode signal. The curves were generated for a sample of infinite thinness and short (non-limiting) τ values (0.01 ms), separated from the electrode by the following distances: curve a) 0.001 μm (this could correspond to a sample in direct contact with the electrode); curve b) 0.1 μm; curve c) 1 μm and curve d) 2 μm. Due to the drastic drop in signal amplitudes with increasing distances of the source curves b, c and d have been multiplied by a factor of 50, 200 and 500, respectively.

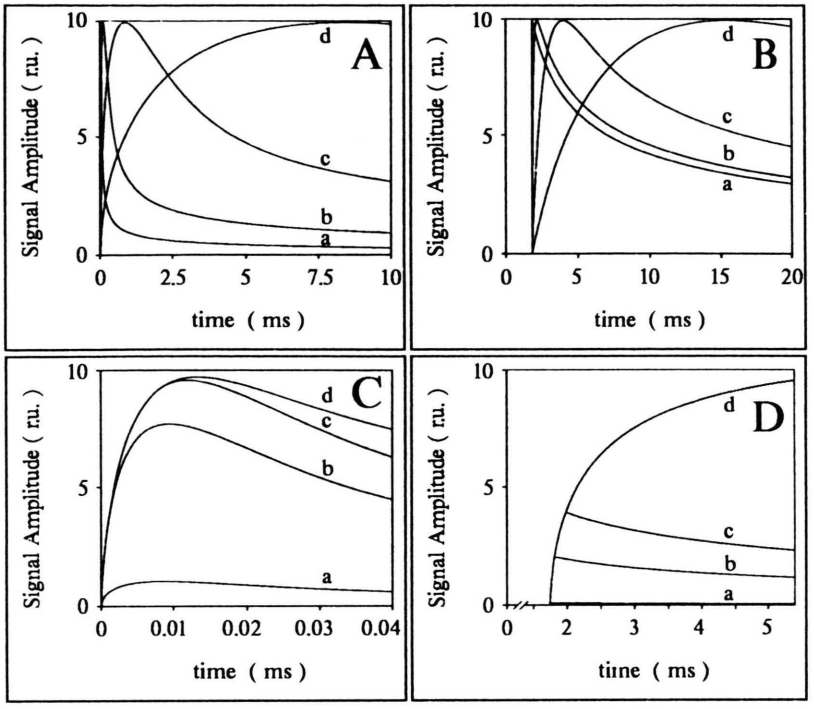

Fig. 7. Effect of τ (A, B) and of the sample thickness (C, D) on the computer-simulated electrode signal. a, b, c and d denote curves obtained for the respective τ values of 0.01, 0.1, 1 and 10 ms for samples of infinite thinness being in "direct contact" with the electrode surface (distance of 0.001 μm) (A) or separated from it by the distance of 2 μm (B). The amplitudes of the curves in the figure portion A and B are normalized to the same value. In figure portion C and D a, b, c and d denote curves obtained for a sample thickness of 0.001, 0.005, 0.01 and 0.1 μm, respectively separated from the electrode surface by a distance of 0.001 μm (C) and 2 μm (D) for the constant τ value of 0.01 ms.

The presented model has also been used for an estimation of the τ value which would allow the fit of the simulated electrode signal into the experimental curve obtained under standard conditions (thickness of the sample 5 μm and the diffusion distance of its lower surface from the electrode 1.9–2 μm). The best fit was obtained for a τ value of 5.5 ms (Fig. 8A).

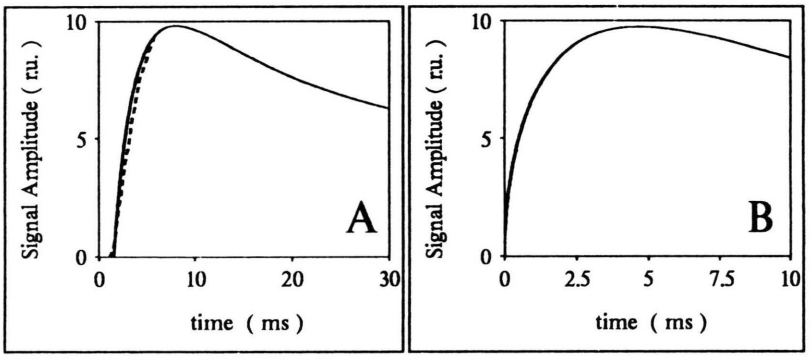

Fig. 8. Part A represents the experimental curve (dashed line) obtained for a sample of 5 μm thickness with an average distance from the electrode of 1.9 μm. The solid line represents the computer simulated curve with the same parameters and a τ value of 5.5 ms. Part B gives the simulated oxygen signal extrapolated for the sample conditions of infinite thinness, separated from the electrode by a distance of 0.001 μm and a τ value of 5.5 ms.

Having in mind the conclusions resulting from the presented mathematical model on the effect of the sample thickness and its distance from the electrode on the measured kinetic parameters of the simulated amperometric signal, it becomes obvious that the determined apparent experimental parameters of oxygen evolution kinetics contain deformations due to the thickness of the sample and the diffusion distance from the electrode. If we extrapolate these experimentally obtained parameters to the situation of an infinitely thin sample localized directly on the electrode surface, the real rise time as well as the half-rise time might be still shorter. An extrapolation calculated for a sample of infinite thinness separated from the electrode by the minimal distance of 0.001 μm with a τ value of 5.5 ms (see Fig. 8 B) yields a corresponding oxygen signal half-rise time of 0.44 ms *i.e.* a value which is in a very good agreement with the data obtained by time-resolved EPR oximetry [11].

It should be also emphasized that in the proposed model all oxygen molecules which arrive at the electrode are eliminated from further calculation and cannot contribute any more to the computed oxygen signal. This situation corresponds very well to our experimental conditions where at the polarization voltage of −680 mV all oxygen molecules reaching the electrode surface are reduced and therefore eliminated from the system. If we have well understood in the case of the experimental conditions used by Plijter *et al.* [7] using a cathode potential of −100 mV only about 1% of the oxygen molecules hitting the cathode are reduced creating the complicated situation in which the effect of newly arrived oxygen molecules is superimposed (combined) upon the activity of molecules still diffusing randomly from the electrode surface. The model permits also the simulation of electrode responses for samples of finite thickness separated from the electrode by a certain distance. These factors do not seem to have been taken into account neither in the one-dimension diffusion analysis [7] nor in the exponential analysis [14] models.

Appendix

Our model for oxygen diffusion from the emitting source to the electrode surface is a geometrical one. The system consists of an active (oxygen emitting) layer of finite thickness deposited on the electrode. Since the thickness of the layer is many orders of magnitude smaller than the transversal dimensions of the system, we simply assume that the transversal dimensions are infinite.

In response to a light flash some centers in the active layer release oxygen with an average intensity described by the function of time $\varrho(t)$. Every oxygen emitting point of the active layer can be treated as a source for a spherical wave the average propagation of which is determined by the diffusion law. A simple geometrical analysis leads to the following equation expressing the current detected on the cathode:

$$I(t) = \int_1^{\infty} \mathrm{d}y \int_{T_1}^{T_2} \mathrm{d}T\, \varrho(t - Ty^2). \tag{1}$$

T_1 and T_2 stand respectively for the time in which the cathode is reached by oxygen released at the surface of the sample facing the electrode and the surface distant to it. T_1 and T_2 are the characteristic times for the geometry of the system used and are calculated from the dependence: $\langle x^2 \rangle = 2 \cdot T \cdot D$, where D is the diffusion constant.

It is reasonable to assume that oxygen release is a first order process with the time constant τ. Therefore the electrode response function is given by:

$$\varrho(t) = \Theta(t) \exp(-t/\tau) \tag{2}$$

where $\Theta(t)$ is the Heaviside step function.

The cathode signal from the infinitely thin sample is described by the equation:

$$I(t) = \int_1^{\infty} \varrho(t - T_0)\, \mathrm{d}y \tag{3}$$

where ϱ means the same as in Eqn. (2) and T_0 denotes the time necessary for oxygen transport from the source to the cathode.

In case the oxygen source has suitable dimensions *i.e.* those of chloroplasts or cells the electrode response function is:

$$I(t) = \begin{cases} \int_1^{\sqrt{t/T_1}} dy \left[\exp\left(\frac{T_2 y^2 - t}{\tau} \right) - 1 \right] & \text{for } T_1 < \tau < T_2 \\ \int_1^{\sqrt{t/T_1}} dy \left[\exp\left(\frac{T_2 y^2 - t}{\tau} \right) - 1 \right] - \int_1^{\sqrt{t/T_2}} dy \left[\exp\left(\frac{T_1 y^2 - t}{\tau} \right) - 1 \right] & \text{for } \tau > T_2. \end{cases} \quad (4)$$

This equation is obtained by substituting explicite ϱ_t from Eqn. (2) into Eqn. (1). It gives the best results when τ, T_1 and T_2 are of the same order of magnitude as is the case in our measurements. To fit the simulated curve into the experimental one, the equations should be multiplied by a constant, which can be assigned experimentally.

Acknowledgements

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