In-situ monitoring of H_2O_2 degradation by live cells using voltammetric detection in a lab-on-valve system†

Ilkka Lähdesmäki,* Young K. Park, Andrea D. Carroll, Michael Decuir and Jaromir Ruzicka

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This paper describes a method for monitoring the degradation of hydrogen peroxide by cells immobilized on a beaded support. The detection is based on the voltammetric reduction of hydrogen peroxide on a mercury film working electrode, whilst combining the concept of sequential injection (SI) with the lab-on-valve (LOV) manifold allows the measurements to be carried out in real time and automatically, in well-defined conditions. The method is shown to be capable of simultaneously monitoring hydrogen peroxide in the 10–1000 μM range and oxygen in the 160–616 μM range. A correction algorithm has been used to ensure reliable H_2O_2 results in the presence of varying oxygen levels. The method has been successfully applied to monitoring the degradation of H_2O_2 by wild-type cells and by catalase-overexpressing mouse embryonic fibroblasts. Since the technique allows the monitoring of the initial response rate, it provides data not accessible by current methods that are end-point-based measurements.

Introduction

A method has been developed for quantifying the rate at which hydrogen peroxide is being degraded by adherent cells. The method is based on the voltammetric detection of oxygen and hydrogen peroxide in a lab-on-valve (LOV) flow manifold.

The project originated from a need to have a method for the determination and real-time monitoring of catalase activity in live cells. Catalase decomposes hydrogen peroxide and is believed to be one of the key antioxidant enzymes protecting cells against oxidative stress.[1] In the presence of Fe^{2+}, H_2O_2 is converted *via* the Fenton reaction to a hydroxyl radical, which is highly reactive and damages lipids, proteins, and DNA. Production of H_2O_2 in response to cellular stress, and potentially as a by-product of normal cellular respiration, has been implicated in cellular apoptosis and senescence.[1,2] Mitochondrial-targeted overexpression of catalase can extend lifespan in mice, suggesting that enhancing H_2O_2 removal is physiologically important.[3] In order to better understand the cellular H_2O_2 production in response to stressful stimuli and its amelioration by antioxidants such as catalase, a better understanding of the kinetics of H_2O_2 release and removal by individual cells is required. Some parameters, such as cell viability, catalase expression level and localization, are readily characterized by existing reagents and techniques. However, monitoring the functional H_2O_2 production in response to stressful stimuli and its degradation by antioxidant enzymes, including catalase, in intact cultured cells has only been carried out using end-point-type assays that require the user to manually sample the culture solution for each data point.[2,4] These methods are laborious and prone to temporal imprecision and operator error. In addition, they do not protect the cell culture from the external environment. An automated method providing improved precision, repetitive monitoring and shielding from the environment should open new possibilities for functional assays of H_2O_2 production and catalase activity using cultured cells. This technique can be readily combined with other parameters, such as the use of fluorescent intracellular probes, to simultaneously monitor multiple cellular events in real time.

The general methods for determining catalase activity rely either on the measurement of H_2O_2 consumption or O_2 accumulation. They are based on UV-VIS absorbance,[5] fluorescence,[6] chemiluminescence[7] or electrochemical[8] detection, and perform very well for determining catalase levels in serum samples and tissue homogenates. However, their application to quantifying the catalatic activity of live cells seems to be very limited. The likely reason for such limited use is that each of the above methods falls into one or more of the following categories: (1) methods that are restricted to using an end-point determination after a fixed reaction time has elapsed (as opposed to continuous/repetitive monitoring), (2) methods that use chemical components that are harmful to live cells, (3) methods that are susceptible to interference by components in cell culture media, (4) methods that are incapable of sensing sub-mM concentrations of H_2O_2, and (5) methods that necessitate contact with ambient air.

In this work, voltammetric reduction of O_2 and H_2O_2 on a mercury (Hg) electrode was chosen as the detection method. The choice was based on the following merits: (1) O_2 and H_2O_2 can be monitored simultaneously and repetitively, (2) no reagents are required, (3) the use of a Hg electrode for O_2 and H_2O_2 measurements is well documented, (4) the cellular material will not interfere with electrochemical detection, and

Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA
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(5) the use of ambient conditions is not necessary but the growth medium and oxygen levels can be chosen to suit the cell culture.

The lab-on-valve (LOV) injection/detection manifold for sequential injection (SI) is a relatively new concept that so far has been combined with spectrophotometric, fluorescence, mass spectrometric, atomic absorption and ICP detection techniques.[9] This work extends the use of the LOV flow cell to electrochemical detection by integrating a three-electrode voltammetric detection cell into an LOV manifold. In that sense, it is a logical continuation of the extensive array of electrochemical detection schemes designed to operate in traditional SI setups.[10] This configuration is compatible with the bead injection concept, allowing a column of disposable microbeads that serve as carriers for live cells to be packed into the detection chamber. Besides automation, the chief advantage of this approach is that it provides a means of confining a large number of adherent cells into a small volume. Consequently, it avoids the issue of excessive dilution that often complicates accumulation/depletion measurements with adherent cell cultures. Furthermore, it eliminates biological variability within an experimental run series, since the experiments are carried out by reproducibly sampling cell/bead suspensions from the same cellular culture.

The objective of this paper is the development of a method for quantifying the H_2O_2 degradation ability of live adherent cells. The method should be able to detect H_2O_2 at levels of 10–1000 μM. This range is defined by the fact that below 10 μM catalase is no longer the predominant factor responsible for H_2O_2 elimination,[4,11] and above 1000 μM the toxic effect of H_2O_2 starts to complicate the interpretation of the results. The SI-LOV system (Fig. 1) was interfaced with a voltammetric detector and the operation of the system comprised the following steps. First, a column of Cytodex-2 beads with cultured cells was metered and packed into the flow cell. Next, a bolus of H_2O_2 solution was delivered into the cell and the flow was stopped. A voltammetric scan was recorded. A bolus of de-aerated solution was delivered to the flow cell for background signal acquisition. Finally, the bead column was discarded.

Experimental

Chemicals

Hydrogen peroxide (30% solution, reagent grade) and sodium chloride were purchased from J. T. Baker (Phillipsburg, NJ, USA). Silver wire (0.5 and 1.0 mm diameter, 99.9%) was from Alfa Aesar (Ward Hill, MA, USA). Platinum wire (0.5 mm diameter), mercury (99.9995%) and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) were from Sigma (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium for cell culture (DMEM, without phenol red) and Hank's Balanced Buffer Solution (HBSS) were from Gibco (Grand Island, NY, USA). L-Glutamine and penicillin/streptomycin were from Cambrex BioScience (Walkersville, MA, USA). Serum (Fetal Clone III) was from Hyclone (Logan, UT, USA). Glucose was from MCB Reagents (presently EMD Chemicals, Gibbstown, NJ, USA). Cytodex-2 microcarrier beads were from Pharmacia (presently GE Healthcare Bio-Sciences, Uppsala, Sweden). Sodium hydroxide solution (1 M) was from VWR (West Chester, PA, USA).

Supplies

Polishing sheets were purchased from ThorLabs (Newton, NJ, USA) and the colloidal silica and polishing cloth from Allied High Tech Products (Rancho Dominguez, CA, USA). The fluidic components (tubing, fittings, ferrules) were from Upchurch Scientific (Gig Harbor, WA, USA).

Instruments

The experiments were carried out with a Sequential Injection apparatus, consisting of a FIALab 3000 instrument (FIALab Instruments, Bellevue, WA, USA) equipped with an LOV manifold mounted on the multiport valve. A schematic representation of the system is shown in Fig. 1A. The system was configured with a 1 mL syringe pump and a 1400 μL holding coil made out of 0.75 mm i.d. stainless steel tubing. The flow cell volume was approximately 4 μL. The instrument was controlled using the FIALab for Windows 5.0 software (FIALab Instruments, Bellevue, WA, USA). Voltammetric detection was carried out with a BAS Epsilon potentiostat,

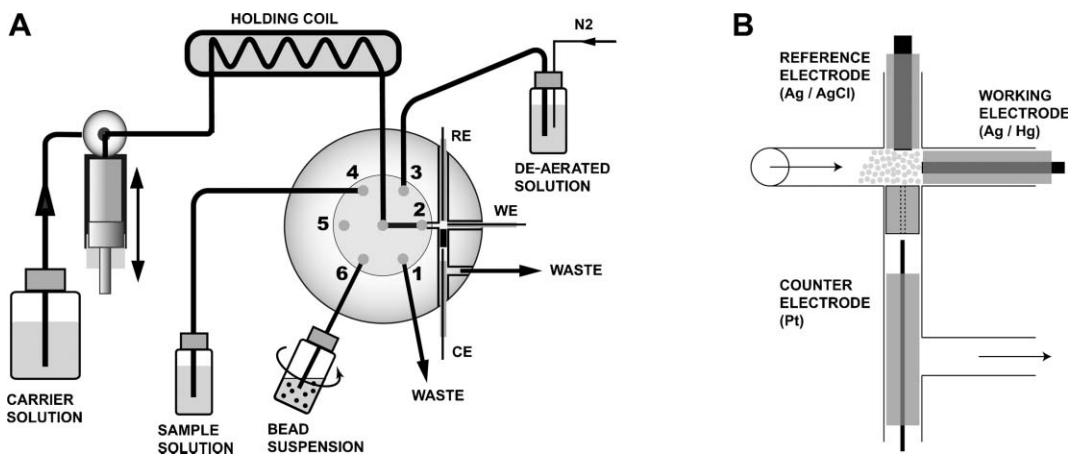

Fig. 1 (A) Instrumental setup; (B) arrangement of electrodes in the lab-on-valve flow cell.

controlled by its own software (Bioanalytical Systems, West Lafayette, IN, USA). The potentiostat was interfaced to the FIALab 3000 instrument *via* a LabJack I/O module (LabJack Corporation, Lakewood, CO, USA) in order to enable the user to trigger voltammogram scans from the FIALab software.

Electrodes and voltammetry

Working electrode. The Ag/Hg working electrode was prepared by securing a 0.5 mm diameter silver wire into a piece of 0.506 mm i.d. polyetheretherketone (PEEK) tubing with epoxy. The end of the electrode was polished on a series of successively finer polishing sheets (5, 3, 1 and 0.3 μm) to get a flat, disk-shaped silver surface of 0.5 mm diameter. The final polishing step was done using 0.05 μm colloidal silica on a polishing cloth. The electrode was then immersed in a pool of mercury, resulting in the silver surface being covered with a mercury droplet. Most of the mercury droplet was wiped off on a piece of bare polishing cloth, leaving a mercury film on the electrode surface. The use of a given working electrode was limited to three days after its preparation. This precaution was taken because it was noticed that after about one week from the electrode preparation, the amalgamation of the mercury film and the silver substrate reached the electrode surface and changed its response.

Reference and counter electrode. The Ag/AgCl reference electrode was prepared by securing a 1.0 mm silver wire into a piece of 1.02 mm i.d. PEEK tubing. The electrode was polished on 5 μm polishing sheet to get a flat, disk-shaped silver surface of 1.0 mm diameter. It was placed in a container filled with 0.15 M NaCl solution, and a potential of *ca.* +2 V was applied on it for 5 s to cover the surface with AgCl. The Pt counter electrode was prepared by securing a 0.5 mm platinum wire into a piece of 1.02 mm PEEK tubing, in such a fashion that the wire extended *ca.* 2 mm out of the tubing. A three-electrode setup was assembled in the LOV as shown in Fig. 1B.

Voltammetry. The signals for O_2 and H_2O_2 were recorded by scanning the working electrode potential from 0 to −1600 mV at a scan rate of 1000 mV s^{-1}, in stopped-flow conditions. Shortly before recording the signals, the electrode surface was conditioned by an identical scan while the solution was passing through the flow cell. Each measurement was followed by recording a background scan in de-aerated solution.

Cell culture

Wild-type mouse embryonic fibroblasts (WT), as well as mitochondrial catalase-overexpressing mouse embryonic fibroblasts (MCAT) were a kind gift of Professor Peter Rabinovitch. A more detailed description of the cell lines is provided elsewhere.[3] The cells were grown on Cytodex-2 microcarrier beads, in Dulbecco's Modified Eagle Medium supplemented with glutamine, serum and penicillin/streptomycin.

Preparation of solutions and bead suspensions

Physiological buffer solution. A bag of HBSS powder was dissolved in water. After that, HEPES was added to a final concentration of 10 mM, followed by pH adjustment to 7.3 with 1 M sodium hydroxide. The solution was then diluted to its final volume.

Carrier solution. Carrier solution was prepared by mixing 5 parts (vol.) physiological buffer solution, 1 part (vol.) DMEM culture solution and adding glucose to a final concentration of 4.6 g L^{-1}.

Standard and background solutions. Hydrogen peroxide standard solutions were prepared daily by sequential dilution of 30% H_2O_2 with carrier solution. The first of these dilutions was assayed for H_2O_2 using permanganate titration. Oxygen standard solutions were prepared by bubbling mixtures of O_2 and N_2 gas through a reservoir of carrier solution. The concentration of dissolved O_2 in the standard solutions was verified with an Extech model 407510 dissolved O_2 meter (Waltham, MA, USA). A de-aerated solution for background measurements was prepared by bubbling N_2 through the carrier solution.

Bead suspensions. Beads were prepared for experiments by mixing 1 part (vol.) of wet Cytodex-2 beads with 9 parts (vol.) of carrier solution, resulting in a 10% (v/v) suspension.

Sequential injection protocols

A typical monitoring protocol comprised the steps listed in Table 1. Details on the cell/bead handling volumes and solution flow rates are given in the full software protocols for controlling the sequential injection instrument, available as ESI.† These protocols apply to the H_2O_2 degradation experiments. The protocols used in the linearity/accuracy experiments were identical, except that only one voltammetric scan was recorded during the stopped-flow period.

Data processing

Digital filtering. An appreciable degree of 60 Hz line frequency noise was occasionally observed in the signal. This noise was removed by applying a second order Butterworth notch filter (50–70 Hz notch) on the signal. Further smoothing was performed by six-point boxcar averaging. The filtering

Table 1 An outline of the monitoring protocol

Step	Description
1	Load bead column
2	Prime sample solution inlet
3	Aspirate 500 μL sample solution
4	Deliver 200 μL sample solution onto bead column Run conditioning voltammogram
5	Stop flow. Collect first voltammogram
6	Wait 3 min, collecting a voltammogram every 1 min
7	Discard sample solution
8	Prime inlet for de-aerated solution
9	Aspirate 500 μL de-aerated solution
10	Deliver 200 μL de-aerated solution onto bead column Run conditioning voltammogram
11	Stop flow. Collect background voltammogram
12	Discard de-aerated solution
13	Discard bead column

operations were carried out using LabView 8.0 software (National Instruments, Austin, TX, USA).

Background subtraction. The background signal (recorded in de-aerated solution) was digitally subtracted from the actual signal, using LabView 8.0.

Correction for the contribution of O_2 reduction to the H_2O_2 signal. It is well known that the first step of O_2 reduction on a Hg electrode produces H_2O_2.[12] This adds an unwanted contribution to the H_2O_2 signal and needs to be corrected for. The contribution was subtracted from the H_2O_2 signal by using a correction factor, K, so that

$$(H_2O_2 \text{ peak height})_{corrected} = (H_2O_2 \text{ peak height})_{observed} - K \times (O_2 \text{ peak height})_{observed}$$

The correction factor was determined by packing a column of bare Cytodex-2 beads into the flow cell, washing them with carrier solution and recording a voltammetric scan in carrier solution, under stopped-flow conditions. After that, the bead column was discarded. The above cycle was repeated five times, and the ratio (H_2O_2 peak height)/(O_2 peak height) was calculated for each measurement. The average of the five ratios was defined as the correction factor K. A new correction factor was determined every day that experiments were carried out.

Linearity

A linearity measurement cycle was initiated by packing a column of bare Cytodex-2 beads into the flow cell. After that, a 500 μL bolus of a standard solution (H_2O_2 or O_2) was aspirated into the holding coil. From the holding coil, the bolus was transported into the flow cell, stopping the flow about half way (200 μL) through the delivery. This procedure left the bead column and the electrodes surrounded by undiluted sample solution. During the stopped-flow period (15 s), signal was acquired by triggering the potentiostat to run a voltammetric scan. A bolus of de-aerated solution was then aspirated and delivered to the flow cell, followed by background signal acquisition. Finally, the flow cell was washed with carrier solution and the bead column was discarded. The entire cycle was repeated for each individual measurement. The details of H_2O_2 linearity measurements and O_2 linearity measurements are given below.

H_2O_2 response. First, correction factor K was determined as described above. After that, the linearity of the H_2O_2 response was evaluated by measuring the signal for 0, 10, 100, 500 and 1000 μM H_2O_2. Five replicate measurements were carried out for each level. The raw voltammograms were subjected to background subtraction and digital filtering. The H_2O_2 signal was then measured as the peak height at *ca.* −1.45 V (see Fig. 2A). After that, the contribution of O_2 was subtracted using the correction factor K. The corrected peak height values were then evaluated by linear regression analysis.

O_2 response. The linearity of the O_2 response was evaluated by measuring the signal for 160, 300, 490 and 616 μM dissolved O_2 as described above. (Note: ambient dissolved O_2 concentration was measured to be 260 μM.) Three replicate measurements were carried out for each level. After digital filtering and background subtraction, the data were subjected to linear regression analysis.

Accuracy

The main factor perceived to possibly compromise the method accuracy was the constancy of the correction factor K at different levels of dissolved O_2. To evaluate whether K stays constant, the data from the O_2 linearity experiments were used to calculate the ratio (H_2O_2 peak height)/(O_2 peak height) as the concentration of dissolved O_2 was varied from 160 to 616 μM.

H_2O_2 degradation experiments

First, the correction factor K was determined. After that, the actual H_2O_2 degradation experiments were carried out as described below.

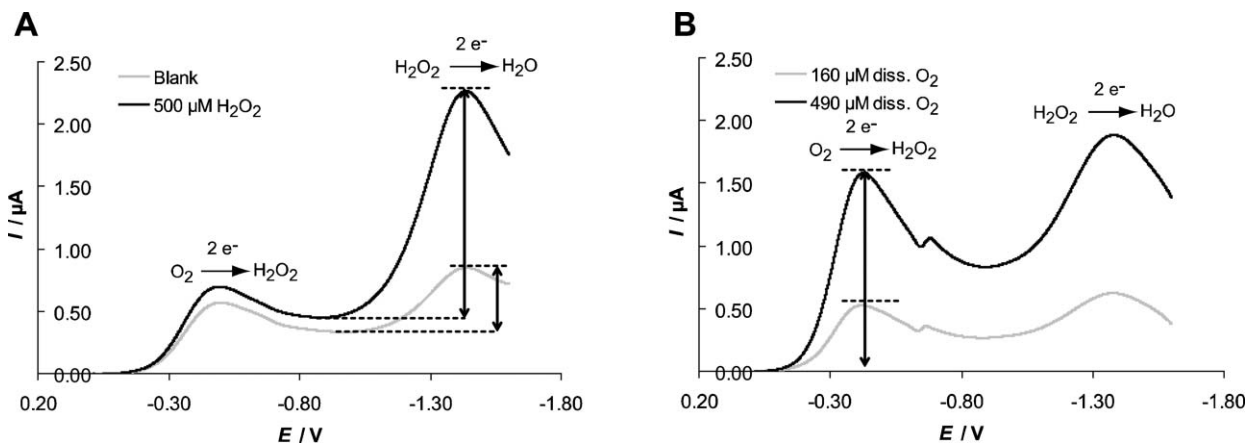

Fig. 2 Voltammetric response curves for the linearity experiments. (A) Processed H_2O_2 responses in a blank carrier solution (gray trace) and in the presence of 500 μM H_2O_2 (black trace). (B) Processed O_2 responses in the presence of 160 μM (gray trace) and 490 μM (black trace) dissolved O_2. The dashed lines and arrows show the measurement of peak heights. Note the use of the polarographic convention where reduction currents are shown as positive values.

The degradation measurement cycle was initiated by packing a column of Cytodex-2 (bare beads or beads with cells on them) into the flow cell. This was followed by aspirating a bolus of 500 μM H_2O_2 solution, delivering it into the flow cell and stopping it there. A voltammetric scan was recorded immediately after stopping the flow, and three additional scans were recorded at 1 min intervals, while keeping the flow stopped. A bolus of de-aerated solution was then aspirated and delivered to the flow cell, followed by background signal acquisition. Finally, the flow cell was washed with carrier solution and the bead column was discarded. The entire cycle was repeated for each individual measurement.

A total of three replicate measurements were performed for each scenario investigated (no cells/WT cells/MCAT cells). After digital filtering, background subtraction and O_2 contribution correction, the H_2O_2 signal was plotted as a function of time.

Results and discussion

Linearity

Responses to H_2O_2 standard solutions are shown in Fig. 2A. The responses exhibited linear behavior over the entire range examined (10–1000 μM H_2O_2). The resulting linear fit calibration equation was $Y = 0.002304 \times X - 0.004842$, where Y is the corrected H_2O_2 peak height in μA and X is the H_2O_2 concentration in μM. The correlation coefficient (r^2) was 0.9999.

Responses to O_2 standard solutions are shown in Fig. 2B. The responses exhibited linear behavior over the entire range examined (160–616 μM dissolved O_2). The resulting linear fit calibration equation was $Y = 0.00304 \times X + 0.0511$, where Y is the O_2 peak height in μA and X is the O_2 concentration in μM. The correlation coefficient (r^2) was 0.996.

Accuracy

Ratios of H_2O_2 and O_2 peak heights at different concentrations of dissolved O_2 are shown in Table 2. The ratio is close to constant across the region of 160–490 μM dissolved O_2. At higher concentrations, though, the ratio was no longer constant. It should be pointed out that dissolved O_2 concentrations above 490 μM were never encountered in any of the cell-based measurements. In conclusion, the results support the use of the H_2O_2/O_2 peak height ratio as a correction factor – as long as the dissolved O_2 level remains at or below 490 μM.

Table 2 Accuracy of the correction factor. Values of the ratio (H_2O_2 peak height)/(O_2 peak height), recorded in carrier solution containing varying concentrations of dissolved O_2 and no added H_2O_2. Each data point represents an average of three replicate experiments (n = 3)

Dissolved O_2/μM	Peak height ratio (H_2O_2/O_2)	Standard deviation of peak height ratio
160	0.682	0.0073
300	0.671	0.0070
490	0.664	0.0005
616	0.724	0.0065

Limit of detection

An estimate for the limit of detection (LOD) for H_2O_2 can be calculated based on the standard deviation of the blank signal ($LOD = 3.3 \times \sigma_{blank}/m$, where σ_{blank} is the standard deviation of five blank signals and m is the slope of the calibration equation). This yields an LOD of 9 μM.

The above estimate is valid given that the level of dissolved O_2 stays constant. However, in conditions where dissolved O_2 shows variation, it is also necessary to take into account uncertainty in the correction factor K. In this case, an estimate for the limit of detection can be calculated using the formula

$$\mathrm{LOD} = \frac{3.3 \times \sqrt{\sigma_{\mathrm{blank}}^2 + (S_{O_2} \times K \times \sigma_K)^2}}{m}$$

where S_{O_2} is the O_2 peak height and σ_K is the uncertainty in the correction factor K. Substituting average values for K and σ_K from Table 2, as well as a representative value for S_{O_2} yields an estimated LOD of 13 μM.

H_2O_2 degradation by cells

The method was applied to monitoring the rate at which cells break down H_2O_2, with the aim to explore differences between various cell lines. The results of the degradation monitoring experiments (carried out as blind tests to identify two different cell lines), are summarized in Fig. 3 and Fig. 4. They show that the method allows the initial rates of degradation to be monitored while the entire experiment is completed within as little as two minutes. SI-LOV voltammetry can reliably and rapidly distinguish the aggressive degradation ability of the MCAT cells from the slower removal rate of the wild-type cells. It should be noted, however, that for quantitative comparison of differences in cellular degradation rates, the results should be normalized to cell abundance.

Enzymatic decomposition of H_2O_2 can occur by two mechanisms:[13,14] the so-called catalatic (= catalase-like) mechanism where H_2O_2 undergoes disproportionation

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

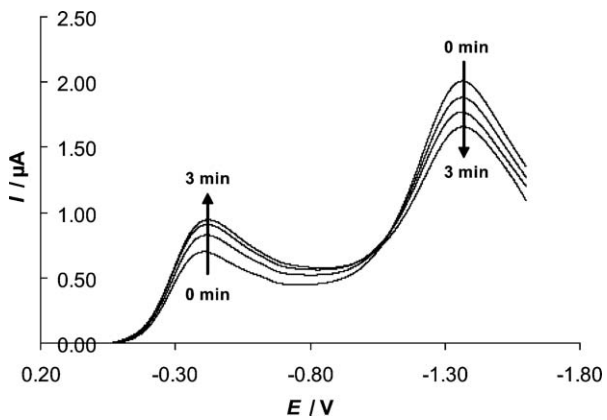

Fig. 3 Monitoring the degradation of H_2O_2. A bolus of 500 μM H_2O_2 was stopped on a column of wild-type mouse embryonic fibroblast cells on beads. The figure shows the processed H_2O_2 responses at different points of the stopped-flow period (0, 1, 2 and 3 min).

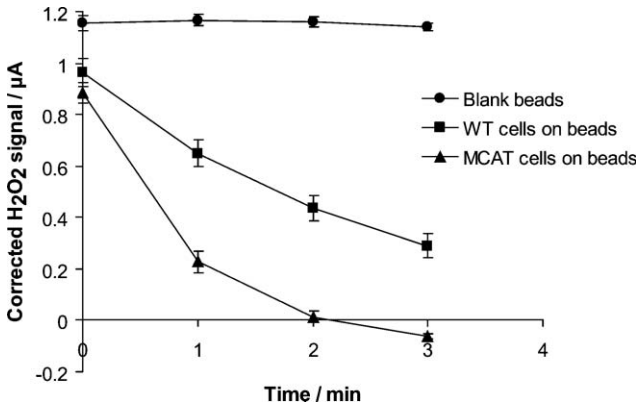

Fig. 4 H_2O_2 degradation profiles. The figure shows the H_2O_2 level as a function of time after a bolus of 500 μM H_2O_2 is stopped on a column of blank beads (●), wild-type cells on beads (■) or MCAT cells on beads (▲). Each data point represents an average of three replicate experiments (n = 3).

or the so-called peroxidatic (= peroxidase-like) mechanism where H_2O_2 undergoes reduction

$$H_2O_2 + AH_2 \rightarrow 2\,H_2O + A \quad (AH_2 = \text{a hydrogen donor})$$

The ability of SI-LOV voltammetry to detect H_2O_2 and O_2 simultaneously reveals that O_2 accumulates over the course of the monitoring period (Fig. 3). This suggests that the degradation of H_2O_2 proceeds through disproportionation (the catalatic mechanism), as opposed to reduction (the peroxidatic mechanism). Furthermore, the accumulation of O_2 indicates that the decomposition of 500 μM H_2O_2 generates O_2 faster than the cellular respiration consumes it. The aggressive consumption of H_2O_2 by MCAT cells may also be the reason why the MCAT degradation profile appears to reach negative H_2O_2 values at its final stages. If the rate of cellular consumption of H_2O_2 becomes comparable to the potential scan rate, the cells consume some of the H_2O_2 generated by the electrode and the correction factor K becomes slightly too large.

Conclusions

The developed method makes it possible to monitor cell-induced H_2O_2 decomposition within a very short time scale, automatically and within an enclosed system isolated from ambient atmosphere. This allows, for the first time, the monitoring of degradation rates and interdependence of O_2 and H_2O_2 variations. The ability of SI-LOV voltammetry to perform these tasks is due to the configuration of the microfluidic system, which enables the measurements to be made in an enclosed and confined space where external influences are eliminated. The detection capabilities are further enhanced because the cell culture in contact with H_2O_2 is held stationary within the flow cell during the monitoring period. Stopping the flow during the monitoring period ensures that the flux of electrochemically active species to and from the electrode surface is entirely controlled by diffusion (as in classical voltammetry) and that the target analyte is not diluted by the surrounding solution by hydrodynamic effects. Stopped-flow conditions, combined with locating the sensing element right next to the monitored cells, enhance the speed of response and optimizes the sensitivity of measurement.

One could justifiably argue that the method may not be monitoring the H_2O_2 degradation rate in a manner that represents the entire cell population held within the flow cell, as the transport of the target analytes is hindered by cell/bead culture and controlled by diffusion. Yet, since the degree of scatter in all the data sets (Fig. 4) is quite reasonable, one may assume that the voltammetric response is a representative sample of the entire cell population or, rather, representative enough for the intended application of this technique. Finally, it should be pointed out that the stopped-flow mode has several advantages that may not be obvious at first glance. First, it makes it possible to monitor a true degradation profile instead of a final degradation level obtained by the end-point techniques currently in use. Second, the stopped-flow technique is not sensitive to the variation in the length of the cell/bead column as long as the flow cell is fully packed.

Fast scanning voltammetry allows multiparameter measurements to be carried out on a suitable time scale. In this work, simultaneous monitoring of a substrate (H_2O_2) and a reaction product (O_2) provides a novel insight into cellular activities. Voltammetry in the SI-LOV format has an additional valuable feature, since the detection volume is isolated from ambient atmosphere. It was easy to carry out measurements in solutions initially saturated with oxygen as well as in de-aerated solution. In the present work, measurements in de-aerated solutions were utilized to correct for the large charging current component associated with fast scanning. In the future, the same feature will allow the monitoring of cell cultures under dissolved gas concentrations corresponding to actual physiological conditions (high CO_2, low O_2) rather than the ambient atmosphere.

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References

1 N. J. Linford, S. E. Schriner and P. S. Rabinovitch, *Cancer Res.*, 2006, **66**, 2497–2499.
2 X. Chen, H. Liang, H. Van Remmen, J. Vijg and A. Richardson, *Arch. Biochem. Biophys.*, 2004, **422**, 197–210.
3 S. E. Schriner, N. J. Linford, G. M. Martin, P. Treuting, C. E. Ogburn, M. Emond, P. E. Coskun, W. Ladiges, N. Wolf, H. Van Remmen, D. C. Wallace and P. S. Rabinovitch, *Science*, 2005, **308**, 1909–1911.
4 N. Makino, Y. Mochizuki, S. Bannai and Y. Sugita, *J. Biol. Chem.*, 1994, **269**, 1020–1026.
5 B. Chance and D. Herbert, *Biochem. J.*, 1950, **46**, 402–414; W. G. Yasmineh, M.-Y. Li and J. I. Caspers, *Clin. Biochem.*, 1992,

25, 21–27; M. R. Slaughter and P. J. O'Brien, *Clin. Biochem.*, 2000, **33**, 525–534.
6 J. G. Mohanty, J. S. Jaffe, E. S. Schulman and D. G. Raible, *J. Immunol. Methods*, 1997, **202**, 133–141; M. Wu, Z. Lin and O. S. Wolfbeis, *Anal. Biochem.*, 2003, **320**, 129–135.
7 S. Mueller, H.-D. Riedel and W. Stremmel, *Anal. Biochem.*, 1997, **245**, 55–60.
8 H. Ukeda, Y. Adachi and M. Sawamura, *Anal. Sci.*, 2004, **20**, 471–474.
9 J. Ruzicka, *Analyst*, 2000, **125**, 1053–1060; A. D. Carroll, L. Scampavia and J. Ruzicka, *Analyst*, 2002, **127**, 1228–1232; Y. Ogata, L. Scampavia, J. Ruzicka, C. R. Scott, M. H. Gelb and F. Turecek, *Anal. Chem.*, 2002, **74**, 4702–4708; J. H. Wang and E. H. Hansen, *Trends Anal. Chem.*, 2003, **22**, 225–231; H. Wang, E. H. Hansen and M. Miro, *Anal. Chim. Acta*, 2003, **499**, 139–147.
10 R. Pérez-Olmos, J. C. Soto, N. Zárate, A. N. Araújo and M. C. B. S. M. Montenegro, *Anal. Chim. Acta*, 2005, **554**, 1–16.
11 K. Sasaki, S. Bannai and N. Makino, *Biochim. Biophys. Acta*, 1998, **1380**, 275–288.
12 D. C. Harris, *Quantitative Chemical Analysis*, Freeman, New York, 4th edn, 1995, ch. 18, p. 494.
13 H. E. Aebi, in *Methods of Enzymatic Analysis*, ed. H. U. Bergmeyer, J. Bergmeyer and M. Graßl, Verlag Chemie, Weinheim, 3rd edn, 1983, vol. 3, ch. 3.9, p. 275.
14 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 3rd edn, 1999, ch. 3.7, p.134.