A quasi-solid state electrochemical cell for *in situ* EXAFS measurements on biological samples

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A new "quasi-solid state" spectroelectrochemical cell for in-situ XAS measurements is described and tested using microperoxidase as reference material. The cell substantially improves conventional thin layer cells used for solution XAS spectroelectrochemistry in terms of assembling time and, more important, equilibration of the redox system under study with the applied potential. Spectra can be, in fact, recorded simultaneously during a slow scan rate cyclic voltammetric scan thus permitting correlation of the spectra and the electrochemical curve. Other advantages are the possibility to use very small quantities of material also with second-generation rings. With high intensity sources having focussed beams a further decrease of the specimen weight can be easily obtained and the acquisition time of spectra further reduced.

Keywords: quasi-solid state electrochemical cell; microperoxidase; redox center; XANES.

Introduction

In two recent papers (Ascone et al. 1999 and Giorgetti et al. 2000), we dealt with in situ XAS fluorescence measurements using spectroelectrochemical cells in which the oxidation state of biological samples, such as hydroxocobalamin or microperoxidase, were controlled in solution by a electrochemical techniques such as potential steps or low rate potential scan. The interest for in-situ XAS spectroelectrochemical measurements on different systems is testified by the growing numbers of papers describing electrochemical cells and applications (Tryk et al. 1995; Antonio et al. 1997; Melendres et al. 1998; Farley et al. 1999). The advantages of the spectroelectrochemical approach have been stressed several times. The reduction (or oxidation) products may be studied without poisoning the system with chemical reagents. In addition, any possible variation of the oxidation state due to the electrons produced by the incident beam is avoided as the electrode, under potentiostatic control, acts as a scavenger (Dewald et al. 1986; Smith et al. 1984).

For solution studies of biological compounds using first or second generation synchrotron machines, the thin-layer type electrochemical cells require relatively high concentrated solutions. Last generation high intensity sources allow lower sample concentrations however protein metal environment may change upon irradiation. A sample preparation method to reduce this phenomenon has been recently proposed (Ascone et al. 2000) but further studies are still necessary.

In this paper we describe a different approach for in-situ XAS spectroelectrochemical measurements that relies on the use of quasi-solid state electrochemistry. Instead of using aqueous solutions of the material, the active hydrated compound under study is spread on a graphoil current collector. The quantity of supporting electrolyte solution is sufficient to hydrate the compound and to assure penetration inside the graphoil current collector. The electrode, assembled in a suitable cell (see below) can be conveniently polarized to change the oxidation state of the material under investigation. Equilibration rate is fast due to the fact that graphoil acts as a diffuse electrode in intimate contact with the active material. The paper describes the cell operation and some typical results in comparison with previously reported data and outlines possible applications.

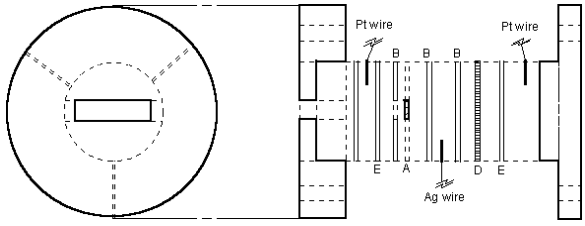


Figure 1
Schematic view of the spectroelectrochemical cell used for experiments. A: sample; B: filter paper separator; D: counter electrode; E: graphoil sheet.

Experimental

Cell

A schematic view of sandwich type three electrode cell is shown in Fig. 1. As briefly mentioned in the introduction, the working electrode is a graphoil (Goodfellow cat. C 000200/2) sheet onto which few milligrams of microperoxidase hydrated with drops of a 1 M solution of KCl are spread. The active hydrated paste was placed at the center of a graphoil sheet in correspondence of the cell kapton window. The counter electrode was a layer of Prussian Blue (PB) on a graphoil sheet. Two filter paper foils, soaked with the supporting electrolyte separated working and counter electrodes. An Ag wire, working as quasi-reference electrode, was placed between the two filter paper separators. Two teflon frames held the electrode assembly. Electrical contacts were realized using Pt wires tightly inserted into one of the teflon frame. Some filter paper, inserted through the contact hole, could be used to compensate, by capillary effect, for eventual loss of solvent.

Experimental procedure

A CHI 650 electrochemical station (CHI Instruments, Austin, Texas) was used for all the measurements. The XAS measurements were taken at the Fe K-edge during cell polarization using a 7 elements fluorescence detector at D21 beam line (LURE, Orsay) operating at 300 mA, 1.85 GeV.

Results and Discussion

Electrochemical cell characterization

The cell has been characterized using cyclic voltammetry at different scan rates. Fig. 2 shows a typical cyclic voltammogram obtained at 0.2 mV/s. Taking into account the high after peak background, the shape of the curve closely resembles the one expected for a quasi-reversible surface wave. The cathodic to anodic

peak ratio is very close to one and the peak separation, not corrected for iR drop, is of the order of 23 mV. The peak current vs scan rate are linear up to 5 mV/s. The electrochemical curve is quite similar to those previously obtained at bare or Gold plated Reticulated Vitreous Carbon (Zamponi et al. 1990). This demonstrates that the experimental set up works as a thin-layer cell and, more important from the XAS point of view, complete electrolysis may be achieved. It should be pointed out that the choice Prussian Blue (PB) as counter electrode is not casual but dictated by at least two important considerations. First of all PB can be either reduced or oxidized to Prussian White (PW) or Berlin Green (BG) in a quasi-solid state (Kuleska et al. 1990) and, hence can accommodate either a reduction or an oxidation. Either PB or its reduced or oxidized forms are, in addition, highly insoluble products, and hence mixing of cathodic and anodic reagent is unlikely. In a preliminary experiment it has been verified that no iron fluorescence signal is present in the absence of microperoxidase sample. The cell is rather easy to assemble and, contrary to what happens in the case of spectroelectrochemical cell for XAS measurements, it requires small quantity of reactant because of the absence of liquid junctions (dead volumes) to accommodate the counter and reference electrodes. In addition all the active material is concentrated directly on the beam path resulting in a higher signal to noise ratio in the spectra.

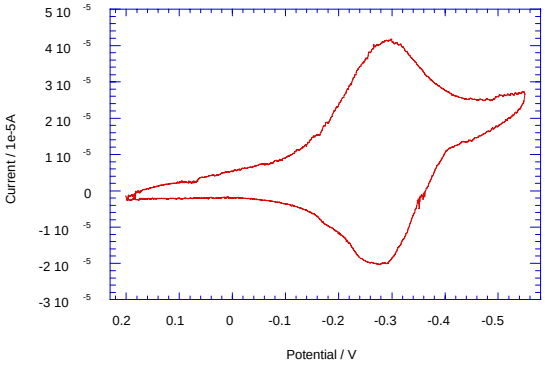


Figure 2
Cyclic voltammetry of Microperoxidase (MP-11) ; 0.2 mV/sec scan rate

In order to give a more precise idea of the advantages of this cell as compared to those described in Ascone et al. 1999, one may observe that a better signal is obtained lowering the quantity of material by a factor of about 2. The other advantage is that equilibrium in the solid state cell may be achieved rather rapidly. Using the previously mentioned cell for liquid samples, at least 30 minutes were necessary to achieve equilibrium after each potential step, with a considerable waste of beam time. With the quasi-solid state electrochemical cell, XAS spectra were recorded simultaneously with the current signal during a slow potential scan cyclic voltammogram.

XAS measurements

Fig. 3 shows two XANES spectra of oxidized microperoxidase. The first was obtained using a 6 mM solution in the thin-layer cell described in Ascone et al. 1999, and the second using the present "paste" electrode. As may be seen the spectra are practically identical. This demonstrates that the two different experimental approaches give the same result. However, in order to record a reasonable the cell for sample in solution required an acquisition time of 8 second per point, while only one second per point was sufficient for the new cell. Fig. 4 shows the initial (fully oxidized, Fe(III)), final (fully reduced, Fe(II)) and back oxidized XANES spectra obtained with the "paste" electrode. As may be seen the spectra of the oxidized and reduced form are practically identical but all XANES features are shifted of about 1 eV toward lower energy. This is the same value reported in Ascone et al. 1999 using a solution spectroelectrochemical cell. In addition, the spectra of the initially oxidized material overlaps with the one obtained after back oxidation of the reduced form. This, as expected from the electrochemical data in Fig. 2, indicates that the reaction is completely reversible and that no degradation of the sample occurs during the experiment. Fig. 5 shows a curve obtained by plotting the normalized intensities at a selected energy as a function of the applied potential during a slow potential scan cyclic voltammogram.

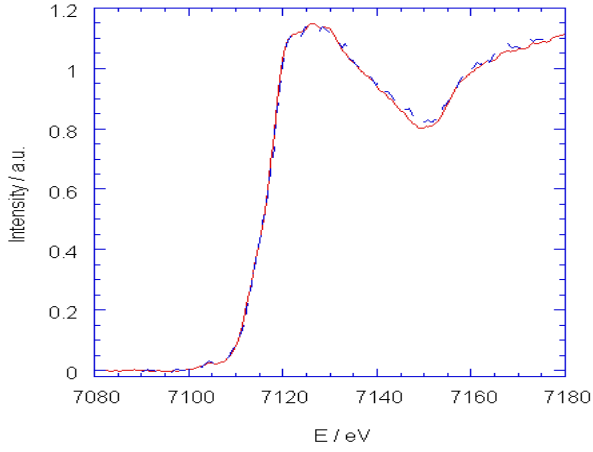


Figure 3
XANES spectra of microperoxidase : — MP-11 6 mM solution; --- hydrated MP-11 with "paste" electrode.

As each spectrum required 180 s and the scan rate was 0.2 mV, every point, representing the signal intensity at 7125 eV, is displaced of 36 mV on the potential axis.

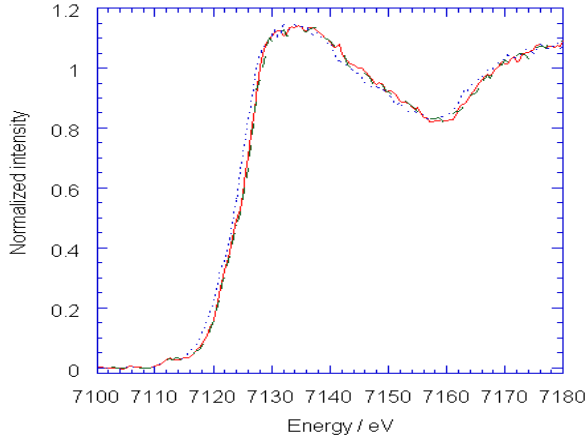


Figure 4
MP-11 with "paste"electrode: — fully oxidized; … fully reduced ; ---- back oxidized.

At constant energy, the signal intensity is correlated to the Ox/Red ratio which depends on the applied potential. By simple use of the Nernst equation, it is easy to demonstrate that in a thin-layer type

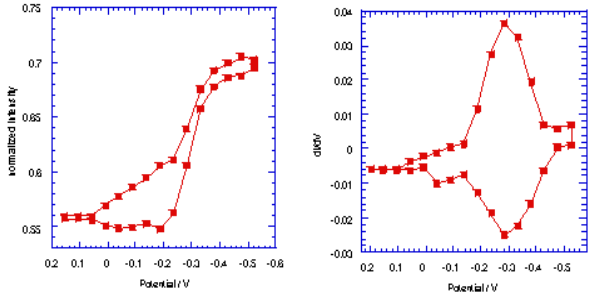
cell any spectroscopic signal related to the Ox/Red ratio may be described using an equation of the type

$$I_E = \frac{I_1}{1+P} \quad (1)$$

where I_E is the potential dependent spectroscopic signal, I_l is the limiting value (i.e the intensity signal when the sample is completely reduced) and P =exp[nF/RT(E-E°')]. The resulting curve is S shaped (see equation 5 in Zamponi et al. 1989 for a derivation in the case of UV-Vis spectroelectrochemistry). The curve in Fig. 5 panel (a) follows this equation. The intensity progressively increases, reaches a plateau at potentials corresponding to the complete MP reduction and decrease again to a constant value when the potential scan is reversed. The potential derivative of the curve in Fig. 5 results in the curve in panel (b) that has the same shape of the cyclic voltammogram as expected from the derivative of eq. 1.

The relevant result is that careful cell design permits to use XAS to reconstruct an electrochemically significant signal: the shape of the curve in Fig. 5 panel b is perfectly equivalent to the cyclic voltammogram of figure 2 (they were recorded at the same time) with the important difference that it is "cleaner": i.e. free from the side effects that distort the current in a normal experiment.

The use of more intense sources coupled with the quasi–solid electrochemical cell will permit the extension of this type of studies to other molecules and a correlation of the electrochemical signal with structural variations caused by oxidation state changes.



(a) (b)

Figure 5
Panel (a) normalized intensity of XANES spectra at E=7125 eV during slow cyclic voltammetry; panel (b) derivative of intensity as function of potential.

As concluding remark, it seems important to underline that the dimensions of the quasi-solid sample, and hence the quantity of material, can be easy adjusted to the X-ray beam spot. This means that the present cell can be used indifferently in any synchrotron radiation center.

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