Soft landed protein voltammetry

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The present work illustrates a new method: soft landed protein voltammetry (SLPV); this experimental procedure is based on the coupling of ion soft landing with a voltammetric technique and allows the electrode surface to be functionalized with biologically active molecules, thus opening up numerous new perspectives ranging from molecular electronics to protein chips.

Ion soft landing is defined as the deposition at low kinetic energies of specific molecular ions on a solid surface using a suitably modified mass spectrometer. Since the discovery of the possibility of ion storage on a structurally organized self-assembled monolayer,[1] this technique has been enhanced, and has enabled organic ions,[2,3] DNA fragments[4] and proteins[5,6] to be collected on different surfaces. Furthermore, biological compounds deposited onto liquid surfaces retained their biological properties,[7] which were tested by traditional techniques after rinsing the protein spot from the surface. In this work we coupled soft landing with voltammetric detection in order to check the immobilization yield of a deposited protein, the retention of its bioelectrochemical properties as well as its native structure, without depleting it from the surface and giving some indication of the nature of the interaction between the landed material and the surface. For this purpose we used microperoxidase-11 (MP-11), an undecapeptide derived from the enzymatic cleavage of cytochrome c which retains its peroxidase activity. It maintains residues 11–21 of the protein, the heme-c group and His18 as fifth ligand at the iron atom. MP-11 is able to catalyze the oxidation of a wide range of organic substrates and to exhibit reversible electrochemistry of the heme Fe^{II}/Fe^{III} couple. In particular, the heme-group of MP-11 is not shielded by a large polypeptide, which enhances direct electron transfer.

The soft-landing of mass-selected ions was achieved using a TSQ700 triple quadrupole from Thermo Finnigan Ltd (UK). We modified the TSQ700 to allow the target surface to be positioned between the third quadrupole and the detector, just as Cooks' group did with a SSQ710C mass spectrometer.[7] For this purpose we substituted the top glass cover of the mass spectrometer manifold with a polycarbonate one onto which we mounted a direct insertion probe apparatus. The probe with the electrode attached to one end can be placed just outside the exit of the third quadrupole and allows the transfer of the soft-landing surface in and out of the mass spectrometer without disrupting the high-vacuum environment.

Sample ionization was performed in the TSQ700 ESI source by operating in the following conditions: needle voltage 4.0 kV, flow rate 20 μL min^{-1}, capillary temperature 423 K, capillary exit and skimmer voltage 40 and 90 V, respectively, hexapole dc offset −0.8 V.

Solutions of MP-11 were prepared daily in H_2O–CH_3OH 1 : 1 1% CH_3COOH at a concentration of approximately 10^{-4} M.

As previously reported,[8] the ESI spectrum of neutral solution of MP-11 is dominated by the $[MP\text{-}11 + H]^{2+}$ ion whereas less abundant intensities of $[MP\text{-}11 + 2H]^{3+}$ are present. In the experimental conditions used in this work (1% CH_3COOH) similar intensities of these two ionic species were observed (Fig. 1).

The ferric-heme moiety brings one positive charge whereas the protons, probably bound to the peptide's amino groups, are responsible for the additional positive charges. The whole unresolved isotopic pattern corresponding to $[MP\text{-}11 + H]^{2+}$ and $[MP\text{-}11 + 2H]^{3+}$ ions, respectively, was mass-selected with the first or the third quadrupole and soft-landed for different time periods, ranging from 30 minutes to 6 hours, on a screen-printed sensor. The ion kinetic energy measured by using cut-off potential can be estimated within 10–12 eV.

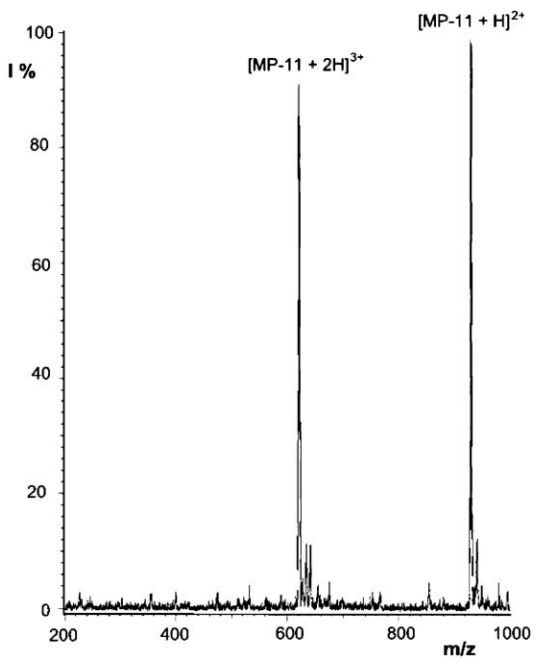

Fig. 1 Low resolution TQ-ESI mass spectrum of MP-11.

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The ion beam was collimated onto the working electrode of a screen printed electrode (cod. AC1.W1.R1) purchased from BVT Technologies (Brno, Czech Republic). The sensor was constituted by a gold surface (as working electrode: 1 mm diameter), an Ag/AgCl reference electrode (198 mV *vs.* NHE) and a gold counter electrode. The electrochemical measurements were performed in phosphate buffer solution 0.05 M pH = 7.0, with KCl 0.1 M, at different scan rates using a thermostatted electrochemical cell under a nitrogen stream with a μAutolab (from EcoChemie, Utrecht, The Netherlands).

A dc bias of −70 V was applied to the working gold electrode in order to guide the ions. The applied potential is critical in obtaining a suitable deposition on the target metal surface.

Fig. 2 shows the characterization of protein deposition on the gold surface performed using a field emission gun scanning electron microscope (FEG-SEM). Both SEM images are referred to the gold surface modified after the MP-11 soft landing procedure. The first image (Fig. 2a) is characterized by lower magnification than the second SEM image (Fig. 2b).

A thorough examination of the images affords the following observations:

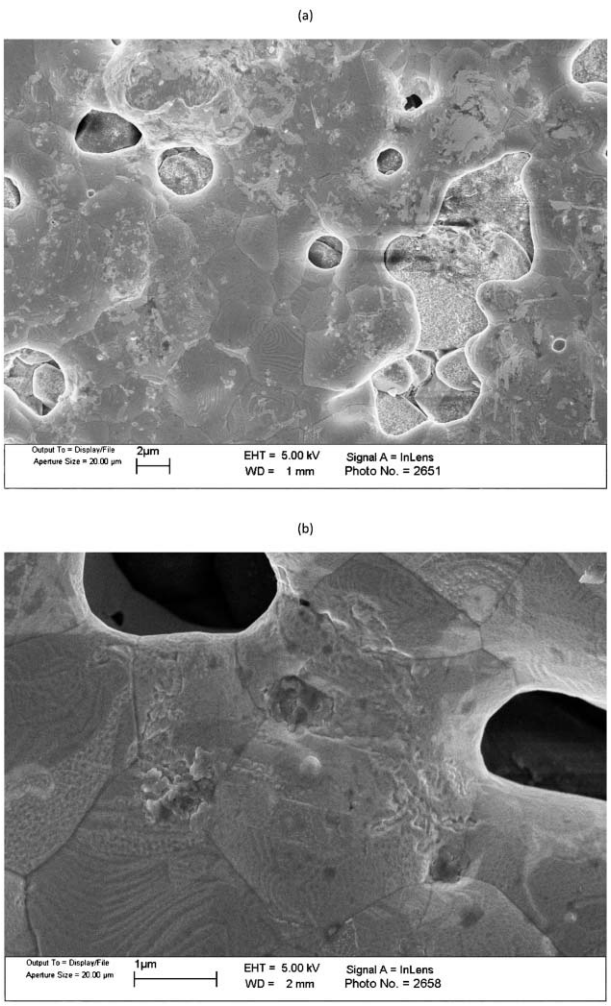

Fig. 2 Field emission gun scanning electron microscope images of MP-11 soft landed onto a gold working electrode. Image (a) is characterized by lower magnification than image (b).

– the presence of gold crystalline grains with a crystallized and porous thin film, as well as a smooth edges porosity, characterized by a quasi circular shape;

– the film is continuous (except for large porosities);

– the film is a solid obtained in a crystalline form with a growth characterized by several surface steps;

– it is possible to observe the presence of superficial nanoporosity on crystallized grains and this nanoporosity is generally normal to the smooth surface;

– the crystalline protein structures seem to be spread over most of the gold surface areas.

The cyclic voltammetry of MP-11 obtained by soft-landing $[MP\text{-}11 + H]^{2+}$ (**MP-11a**) and $[MP\text{-}11 + 2H]^{3+}$ (**MP-11b**) ions, respectively, is shown in Figs. 3a and 3b: in both cases the

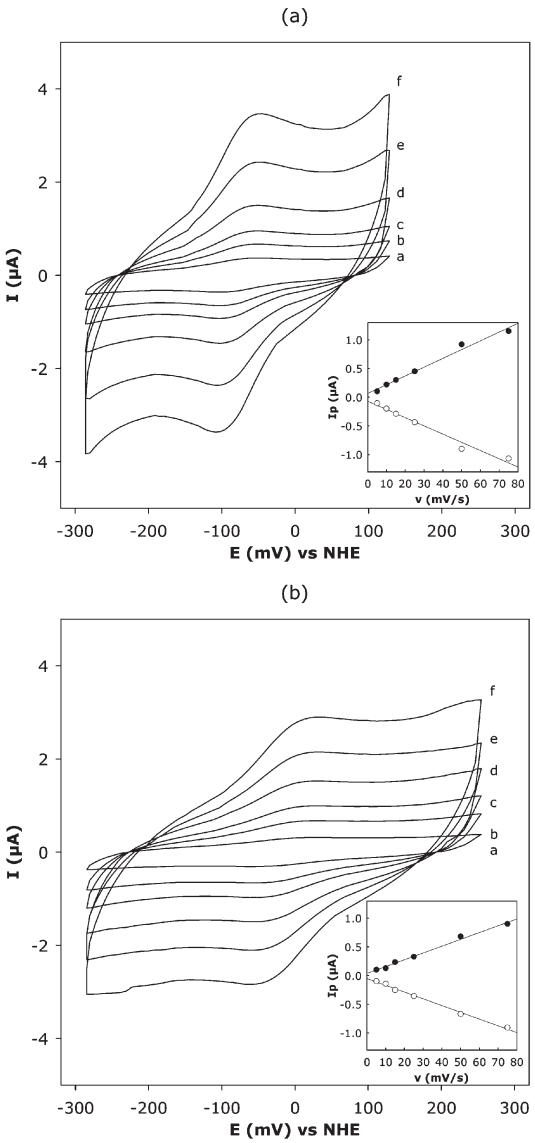

Fig. 3 (a) Voltammetric behaviour of **MP-11a**; (b) voltammetric behaviour of **MP-11b**. In the inset figure the variation of the peak current intensity (Ip) *vs.* scan rate is reported. Cyclic voltammetry experiments were performed in phosphate buffer solution 0.05 M pH = 7.0, with KCl 0.1 M, at the following scan rates: a. 5 mV s^{-1}, b. 10 mV s^{-1}, c. 15 mV s^{-1}, d. 25 mV s^{-1}, e. 50 mV s^{-1}, f. 75 mV s^{-1}.

dependence of anodic and cathodic peak currents *vs.* the potential scan rate confirms the immobilisation of the redox protein. The formal potential value for **MP-11a** ($E^0 = -80 \pm 2$ mV *vs.* NHE) falls very close to the same value for MP-11 ($E^0 = -100$ mV *vs.* NHE) reported in the literature;[9] conversely, the E^0 for **MP-11b** is shifted 60 mV towards more positive values ($E^0 = -20 \pm 3$ mV *vs.* NHE). Moreover, the peak separation (ΔE_p) of **MP-11a** ($\Delta E_p = 58$ mV) is higher than for **MP-11b** ($\Delta E_p = 45$ mV), indicating a more reversible electrochemical behaviour of **MP-11a**. These results suggest a different interaction between the two MP-11 ions and the electrode surface, probably due to amino groups. Their key role in the interaction between several molecules and the gold surface has already been reported.[10] In particular, in the case of MP-11-derived ions, the interaction with the gold surface should occur at the level of protonated amino groups addressed by the negative voltage applied to the electrode. It is reasonable to assume that the MP-11-derived ions were discharged by proton transfer in the collision with the gold surface, otherwise the soft landing of a substantial amount of material would be prevented by strong coulombic repulsion. Hence, since the number of charged amino groups for $[MP\text{-}11 + H]^{2+}$ and $[MP\text{-}11 + 2H]^{3+}$ ions is different (one and two, respectively), we can assume that **MP-11a** is immobilized on the gold surface by means of one amino group per molecule, instead of the two of **MP-11b**. The **MP-11a** should therefore be characterised by a lower reorganisation energy,[11–13] which could explain the more reversible electrochemical behaviour observed. Electrochemical experiments performed with a bare screen printed electrode in an MP-11 solution show no evidence of interactions between the enzyme and the gold working electrode; hence, reactive soft-landing[14] could be the key factor that allows the strong interaction between the surface and the protein not observed in solution.

The amount of deposited electroactive protein (after 3 h of deposition time) was also evaluated by integration of the anodic and cathodic peaks; the values obtained from at least five deposition experiments were $4.8(\pm 0.2) \times 10^{-11}$ mol cm^{-2} for **MP-11a** (corresponding to about 90% of electrode coverage) and $3.4(\pm 0.1) \times 10^{-11}$ mol cm^{-2} for **MP-11b** (corresponding to about 64% of electrode coverage). The lower amount of coverage obtained in the case of **MP-11b** seems to confirm the assumption of ion discharging by proton transfer upon collision with the surface, a process that may reasonably be favoured in the case of the loss of only one proton.

The stability of the MP-11 bioelectrochemical properties was demonstrated by several subsequent experiments performed at different scan rates (from 5 to 1000 mV s^{-1}) as well as over different potential ranges: the electrochemical signal was observed to be stable for more than one week.

The retention of MP-11 (both **MP-11a** and **MP-11b**) biological activity is confirmed by its catalytic response to H_2O_2 as substrate, with the cathodic current intensity *vs.* hydrogen peroxide concentration showing a linear behaviour within the range: 10 μM–90 μM.

In conclusion, soft landing offers the possibility of functionalizing surfaces with biologically active molecules. This result is of fundamental importance in molecular nanotechnologies and opens up many new perspectives ranging from molecular electronics to protein chips.

Moreover, mass spectrometry can easily separate target molecules from complex samples, leading to a simplification of the sample preparation procedures for the purification of the proteins to be immobilized as well as in a reduction in the cost of the overall process.

The results obtained confirm that the coupling of soft landing with the voltammetric technique represents a powerful method for obtaining fast, accurate and exhaustive information about the immobilization yield of the deposited protein, the retention of its bioelectrochemical properties and of its native structure, as well as of the nature of the interaction between landed material and the surface.

The most immediate predicted benefit thereof consists in the possibility of realizing specific applications such as biosensors and biofuel cells.

A more complete and detailed characterization of this method involving the deposition of different redox proteins, different electrode materials, electron transfer kinetics, the influence of deposition time and of the applied acceleration potential, will be treated in a forthcoming paper.

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