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DOI
10.1016/j.elecom.2018.07.022

Publication date
2018

Document Version
Accepted author manuscript

Published in
Electrochemistry Communications

Acid phosphatase behaviour at the electrified soft junction and its interfacial co-deposition with silica

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Key words: ITIES, sol-gel process of silica, interfacial deposition, proteins, acid phosphatase

Abstract

The behaviour of acid phosphatase at the electrified liquid – liquid interface was studied in this work. It was found that only the protonated form of the protein can undergo interfacial adsorption process that is affected by pH of the aqueous phase. With ion transfer voltammetry we could detect acid phosphatase from concentrations as low as 0.1 µM. We were able to co-deposit the protein and silica at the electrified liquid – liquid interface via controlled proton transfer to the organic phase where it catalyzed the tetraethoxysilane hydrolysis, followed by polycondensation to silica.

1. Introduction

When found at elevated concentrations, acid phosphatase (AP) was recognized as one of the biomarkers indicating prostate cancer [1]. This protein can be found also at high concentrations in semen [2], and hence, is frequently used as a target analyte of presumptive tests in forensic investigations [3]. The interface between two immiscible electrolyte solutions (ITIES) emerges as a unique analytical platform with detection arising from interfacial charge transfer reactions including ions or electrons [4]. Proteins, when (positively) charged, can undergo potential dependent adsorption to the ITIES as it was found for Haemoglobin [5], Lysozyme [6], Insulin [7], Myoglobin [8], Albumin [7], Ferritin [9], amongst others. Proteins and synthetic multi-charged macromolecules (e.g. polyelectrolytes [10], dendrimers [11]) can be described with similar interfacial charge transfer characteristics. The electrochemically driven adsorption of multi-charged species to the interface facilitates the transfer of hydrophobic anions present in the organic phase to the aqueous phase, where protein – organic anion complex formation is possible [6],[12]. This was found to be affected

by the surface charge of the protein, its molecular structure – availability of the hydrophobic pockets – and the nature of the organic phase anion [13]. Voltammetric detection of proteins at the electrified liquid – liquid interface, besides a few subtle disparities, is rather obscured as it does not allow the discrimination between different species. It does however provide an elegant method for proteins pre-concentration in a defined (liquid – liquid interface) environment, and when combined with complementary technique (e.g. mass spectrometry) serves as a powerful analytical tool [14]. Silica based materials were recognised as attractive supports for proteins immobilisation [15] due to easiness of geometrical shaping and surface functionalization. Electrochemically assisted pH modulation at solid [16] or soft junctions [17],[18], provide tools to generate silica materials (originating from sol – gel processing) with controlled properties. Surfactant templated methods were also proposed to decorate ITIES with silica deposits [4].

In this work we describe the behaviour of the AP at the ITIES, and to the best of our knowledge this it is the first time this had been published as such. We investigate the effect of experimental conditions on the protein interfacial adsorption process. Moreover, we propose a method, where AP can be co-deposited together with a silica film at the ITIES. The latter arises from electrochemically controlled sol-gel process of silica. The transfer of protons from the aqueous to the organic phase allow the hydrolysis of tetraethoxysilane (from the organic phase) followed by condensation and silica film formation at the ITIES.

2. Methods and materials

AP from wheat germ (Sigma-Aldrich) was used as a model protein. HCl (1M, Merck), citric acid monohydrate (≥99%, Sigma-Aldrich), sodium phosphate monobasic monohydrate (≥99.5%, Sigma-Aldrich) were used to prepare the aqueous solutions. The pH was adjusted with HCl or NaOH (1M, Merck) whenever necessary. The organic phase electrolyte – Bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate ($BTPPA^+TPBCl^-$) was prepared from $BTPPA^+Cl^-$ (97%, Sigma-Aldrich) and K^+TPBCl^- (98%, Simga-Aldrich) according to a previously published protocol [19]. Tetraethoxysilane (98%, Sigma-Aldrich) was used as the silica precursor. All experiments were performed with an Autolab PGSTAT302N in a four electrode macroscopic glass cell[20] equipped with two reference electrodes (Ag/AgCl) and two Pt counter electrodes. Composition of the aqueous and the organic phases are given in Cell I and Cell II:

Cell I – Interfacial behaviour of AP

$$\mathrm{Ag}\left|\mathrm{AgCl}\right|\begin{array}{c}x\ \mu M\ AP \text{ in } 10\ \mathrm{mM\ HCl} \text{ or}\\ 10\ \mathrm{mM\ citric\ acid} \text{ or}\\ 10\ \mathrm{mM\ Na_2HPO_4\ (aq)}\end{array}\left|\left|10\ \mathrm{mM\ BTPPA^+TPBCl^-(org)}\right|\begin{array}{c}10\ \mathrm{mM\ BTPPA^+Cl^-}\\ 10\ \mathrm{mM\ NaCl}\\ \mathrm{(aq)}\end{array}\right|\mathrm{AgCl}\right|\mathrm{Ag}$$

Cell II – Interfacial co-deposition of AP and silica

$$\mathrm{Ag}\left|\mathrm{AgCl}\right|\begin{matrix} x\ \mu M\ AP \\ \text{in 10 mM citric acid(aq)}\end{matrix}\Big|\Big|\begin{matrix} \text{7 mM BTPPA}^{+}\text{TPBCl}^{-} \\ \text{70\% 1,2} - \text{DCE} + \text{30\% TEOS (org)}\end{matrix}\Big|\begin{matrix} \text{10 mM BTPPA}^{+}\text{Cl}^{-} \\ \text{10 mM NaCl (aq)}\end{matrix}\Big|\mathrm{AgCl}\Big|\mathrm{Ag}$$

Potential axis given in Galvani potential were calibrated to $\Delta_{\mathrm{org}}^{\mathrm{aq}}\Phi_{\mathrm{TMA}^{+}}^{0} = 160\ \mathrm{mV}$ [21].

3. Results and discussion

The potential for all voltammograms was scanned from low to high values on the forward scan. The potential window was limited by inorganic anion transfer on the lower potential values side and the proton or inorganic cation transfer on the higher potential values side. Figure 1A displays a set of typical voltammograms recorded in the presence of AP. A number of interfacial adsorption characteristics were found and include (i) a sudden drop in current for the reverse peak; (ii) forward and reverse peak currents ratio are significantly deviating from unity; (iii) calibration curve with two linear regimes (see Figure 1B); (iv) increasing forward charge upon repetitive cycling (see Figure 1C and error bars on Figure 1B) and finally, (v) clear wrinkled protein film formation as can be seen from photograph shown in Figure 1E. The currents observed on the voltammograms in the potential range from 0.5V to 0.9V arise from the facilitated transfer of the organic phase electrolyte anions – $TPBCl^{-}_{org\rightarrow aq}$. This charge transfer characteristics could be detected for AP at concentration as low as 0.1 µM. The amount of $TPBCl^{-}$ transfer from the organic to the aqueous phase is governed by the surface excess of AP at the electrified junction. From the intersection of two linear regions recorded on the calibration curve (Figure 1B), we concluded that below [AP] = 1 – 2 µM an incomplete monolayer is present. . Monolayer formation was found for similar concentrations for other proteins [5],[7]. Further increase in [AP] and the number of voltammetric cycles lead to thicker deposit formation, and hence, a second slope is observed (Figure 1B).

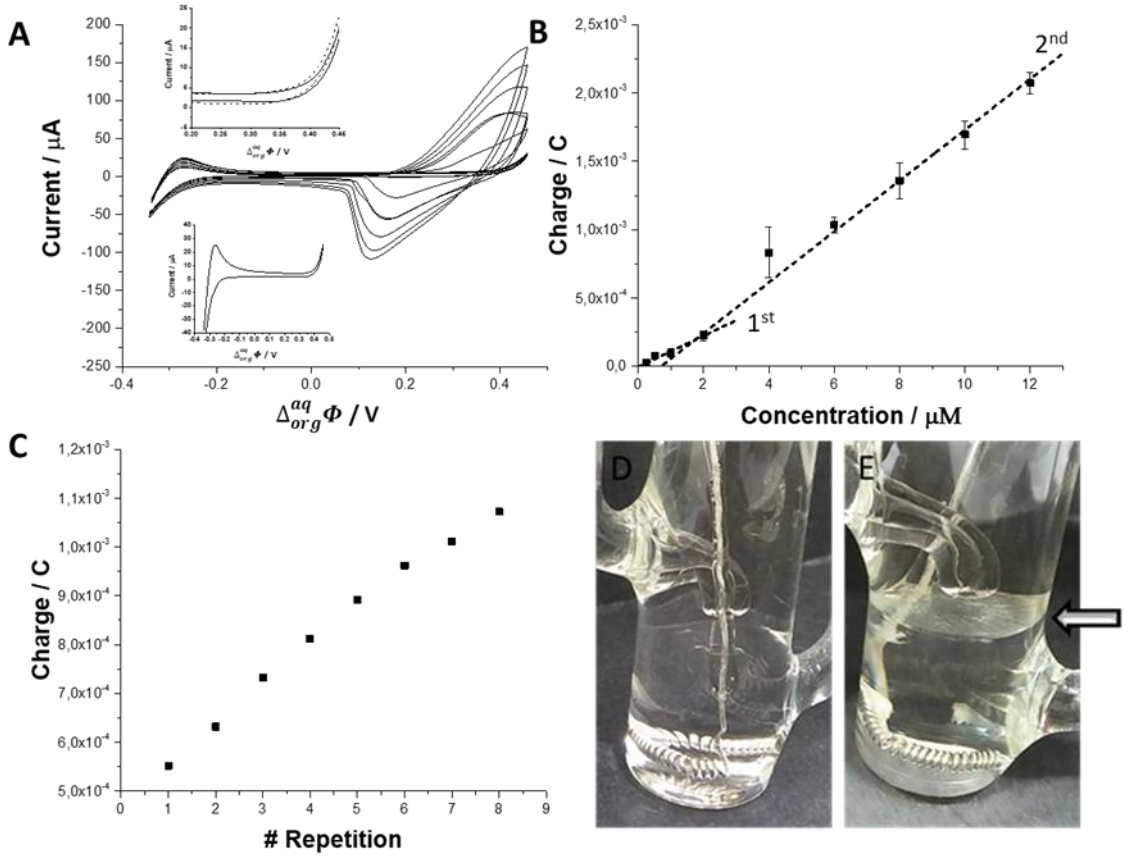

Figure 1. A – Cyclic voltammograms (8th cycle) recorded for 0.1 (upper inset dashed line, solid line correspond to blank); 0.25; 0.5; 1; 2; 4; 6; 8; 10 and 12 μM AP in 10 mM HCl at 10 mV/s. Bottom inset is the blank for [AP] = 0 μM. B – Charge transferred across the ITIES during the forward scan (average of 8 cycles) as a function of the corresponding concentration. C – Increasing charge of the forward scan for repetitive cycling recorded for [AP] = 4 μM. D and E - Photograph of the ITIES before and after protein adsorption (deposited film is marked with an arrow).

The surface charge of the AP, governed by protonation/deprotonation of the protein building blocks – amino acids- can be controlled by pH. Figure 2A – F shows the voltammograms recorded for [AP] = 4 μM at different pH values. The characteristic currents were only observed at pH < isoelectric point (pI) of a protein which for AP's usually falls within pH of its maximal activity and equals to around 5.[22] This behaviour was also observed for other proteins [5]. No signal was observed at pH higher than 5, meaning that facilitated transfer of cationic part of the organic phase electrolyte, if happens, occurs beyond available potential window.

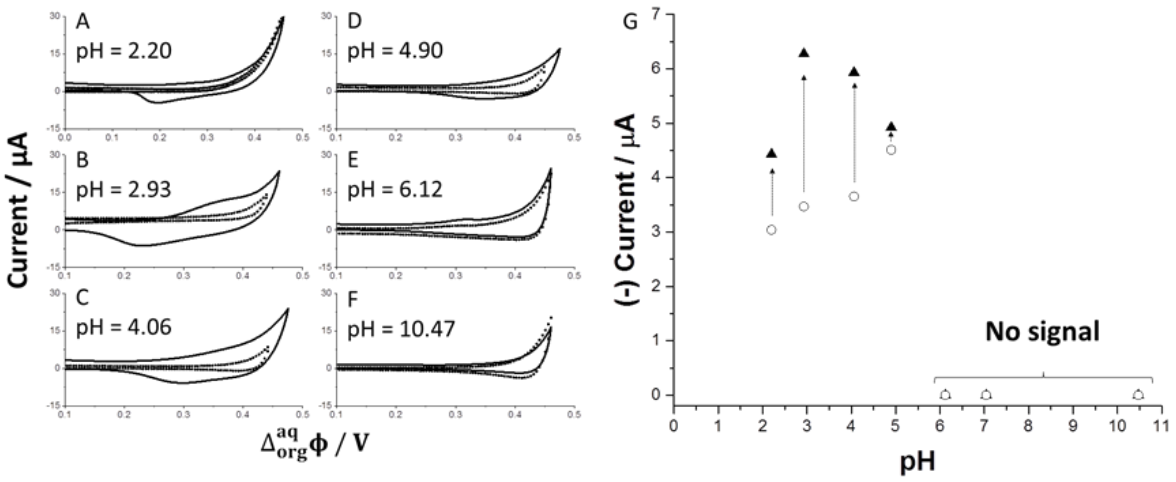

Figure 2. A – F solid line correspond to 8th cycle for [AP] = at indicated pH, dotted line is the blank. G – Is the peak current recorded for the reverse scan. Empty circles correspond to 1st whereas black triangles correspond to 8th cycles.

Neither the protein film formation was observed for pH > 6. Interestingly the magnitude of a reverse peak current (that was easier to analyse compared to forward current overlaid with the H^+ or Na^+ transfer on the positive side of the potential window) increased individually upon repetitive cycling for different pH values. As shown in Figure 2G, the most prominent current jumps were found for pH =2.8 and 4.1. At this point, we can speculate that the surface charge of AP greatly contributes to the interfacial loading (lower surface charge translates to lesser repulsive interactions between proteins) and the final amount of transferred $TPBCl^-$ is a delicate compromise between number and net charge of adsorbed proteins and exposure of hydrophobic pockets located within protein structure.

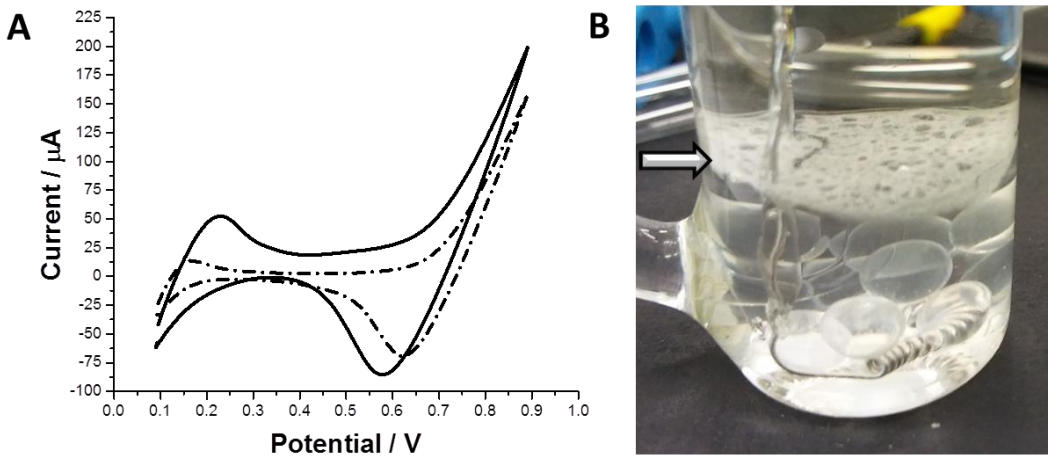

Figure 3. A – 1st (dash-dot line) and 50th (solid line) cycle recorded at the ITIES composed from: the aqueous phase 4 µM Hb, pH = 2.84; the organic phase: 30% TEOS in 1,2-DCE. **B** – Photograph of the protein in silica deposit (marked with an arrow) recorded after 50 voltammetric cycles.

The adsorption of charged proteins and the transfer of $H^+_{aq\rightarrow org}$ happens at the positive side of the potential window. We harvested this fortunate property to co-deposit protein and the silica material at the ITIES. Electrodeposition was performed in cell 2. Silica precursor – TEOS – was present in the organic phase. The increasing positive current recorded from around 0.65V (see Figure 3A) arises from cumulative protein adsorption and $H^+_{aq\rightarrow org}$. The latter, once present in the organic phase, catalyse the hydrolysis of the hydrophobic TEOS to hydrophilic silanols that further polycondensate at the liquid – liquid interface to form silica. Figure 3B shows the photo recorded after 50 consecutive cycles. Clearly, white with two distinctive phases deposit was formed and further collected. By means of infra-red spectroscopy (data not shown, νSi-O-Si at 1200 cm^{-1}) we confirmed the presence of silica material whereas the Pierce™ BCA Protein Assay Kit (data not shown) suggested the likely presence of proteins. Moreover we found that the addition of TEOS significantly affects current – voltage characteristics of the electrochemical cell. (i) The potential window was reduced to about 0.5V, due to the facilitated transfer of proton to the organic phase. (ii) Upon repetitive cycling the capacitance currents increase (see Figure 3A) and this is surely due to the presence of charged deposit at the electrified interface (we cannot neglect any effect of the ethanol formed during hydrolysis and polycondensation reactions, which will increase the mutual solubility of 1,2-DCE and water). (iii) Currents on the positive side of the potential window are high, and hence, overlaid the adsorption finger-marks of the protein. Nevertheless, *in situ* formed silica material gives a rigid support to the adsorbed multi-charged species, which can be further processed and analysed.

4. Conclusions

AP was found to undergo potential dependent adsorption to the ITIES. A number of characteristic electrochemical and visual characteristics of adsorption were detected and described. The charge transfer reaction was recorded for AP concentration as low as 0.1 µM. We found that the pH strongly affects the charge transferred across the electrified liquid – liquid interface, which was attributed to the interfacial AP packing density. Furthermore, we combined potential dependent protein adsorption to the interfacial region with proton assisted interfacial deposition of silica material. The latter was pursed by electrochemically controlled transfer of $H^+_{aq\rightarrow org}$ were it catalysed hydrolysis of tetra ethoxy silane to silanols, which further polycondensate to form silica. The ITIES is a truly unique system to facilitate protein pre-concentration. When co-deposited with an inert and rigid supporting

material, it forms a platform that can be subjected to selective protein analysis, as we currently investigate.

Conflict of Interest

Authors declare no conflict of interests

Acknowledgements

L.P. is grateful to the ChemE, TUDelft for his postdoctoral fellowship.

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