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

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ABSTRACT

The behaviour of acid phosphatase at an electrified liquid–liquid interface was studied in this work. It was found that only the protonated form of the protein can undergo interfacial adsorption which is affected by the pH of the aqueous phase. With ion transfer voltammetry we could detect acid phosphatase in 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 tetraethoxysilane hydrolysis, followed by poly-condensation to silica.

1. Introduction

When found at elevated concentrations, acid phosphatase (AP) is one of the biomarkers indicating prostate cancer [1]. This protein can also be found at high concentrations in semen [2], and hence is frequently used as a target analyte 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 has been found for haemoglobin [5], lysozyme [6], insulin [7], myoglobin [8], albumin [7] and ferritin [9], amongst others. Proteins and synthetic multi-charged macromolecules (e.g. polyelectrolytes [10], dendrimers [11]) can be considered to have 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 has been found to be affected by the surface charge of the protein, its molecular structure – availability of hydrophobic pockets – and the nature of the organic phase anion [13]. Voltammetric detection of proteins at the electrified liquid–liquid interface, with a few minor exceptions, does not give a clear result as this technique does not allow discrimination between different species. It does however provide an elegant method for pre-concentration of proteins in a defined (liquid–liquid interface) environment, and when combined with a complementary technique (e.g. mass spectrometry) serves as a powerful analytical tool [14]. Silica-based materials have been recognised as attractive supports for protein immobilisation [15] due to their easy geometrical shaping and surface functionalization. Electrochemically assisted pH modulation at solid [16] or soft [17, 18] junctions can be used to generate silica materials (originating from sol–gel processing) with controlled properties. Surfactant templated methods for decorating ITIES with silica deposits have also been proposed [4].

In this work we describe the behaviour of AP at the ITIES; to the best of our knowledge this is the first time such a study has been published. We investigate the effect of experimental conditions on the protein interfacial adsorption process. Moreover, we propose a method by which AP can be co-deposited together with a silica film at the ITIES. The latter arises from the electrochemically controlled silica sol–gel process. The transfer of protons from the aqueous to the organic phase allows 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 (1 M, Merck), citric acid monohydrate (≥99%, Sigma-Aldrich) and sodium phosphate monobasic monohydrate (≥99.5%, Sigma-Aldrich) were used to prepare the aqueous solutions. The pH was adjusted with HCl or NaOH (1 M, Merck) whenever necessary. The organic phase electrolyte – bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate ($BTPPA^+TPBCl^-$) was prepared from $BTPPA^+Cl^-$

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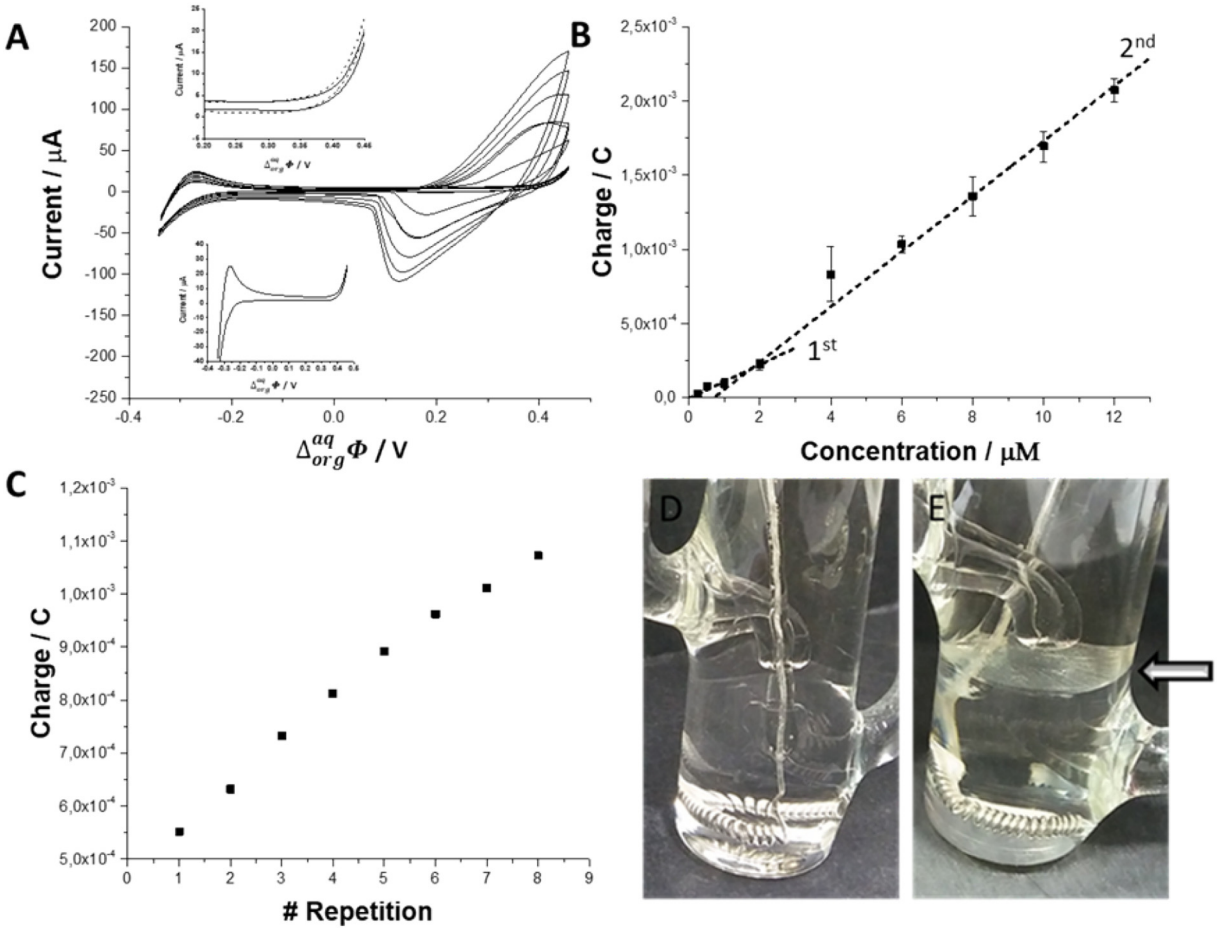

Fig. 1. A: Cyclic voltammograms (8th cycle) recorded for 0.1 (upper inset dashed line, solid line corresponds to the blank); 0.25; 0.5; 1; 2; 4; 6; 8; 10 and 12 μM AP in 10 mM HCl at 10 mV/s. Bottom inset is the blank for [AP] = 0 μM. B: Charge transferred across the ITIES during the forward scan (average 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 indicated by an arrow).

(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. The compositions of the aqueous and organic phases are given in Cell I and Cell II:

Cell I – Interfacial behaviour of AP

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

Cell II – Interfacial co-deposition of AP and silica

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

The potential axis given as the Galvani potential was calibrated to $\Delta_{org}{}^{aq}\Phi_{TMA^+}{}^0 = 160$ 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 value side and the proton or inorganic cation transfer on the higher potential value side. Fig. 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) the ratio of the forward and reverse peak currents deviates significantly from unity; (iii) a calibration curve with two linear regimes (see Fig. 1B); (iv) increasing forward charge upon repetitive cycling (see Fig. 1C and error bars on Fig. 1B) and finally, (v) clear wrinkled protein film formation, as can be seen from the photograph shown in Fig. 1E. The currents observed in the voltammograms in the potential range from 0.5 V to 0.9 V arise from the facilitated transfer of the organic phase electrolyte anions – $TPBCl_{org\rightarrow aq}{}^-$. This charge transfer characteristic could be detected for AP at concentrations as low as 0.1 μM. The amount of $TPBCl^-$ transferred from the organic to the aqueous phase is governed by the surface excess of AP at the electrified junction. From the intersection of the two linear regions recorded on the calibration curve