ChemComm

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Cite this: *Chem. Commun.*, 2012, **48**, 10868–10870

www.rsc.org/chemcomm

COMMUNICATION

Ionic self-assembly of electroactive biorecognizable units: electrical contacting of redox glycoenzymes made easy†

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Received 17th August 2012, Accepted 16th September 2012
DOI: 10.1039/c2cc35949a

This work explores the synergistic combination of ionic self-assembly and recognition-directed assembly for the modification of electrode surfaces with redox glycoenzymes on the basis of electroactive glycopolyelectrolyte–surfactant complexes.

The concept of ionic self-assembly (ISA) holds unparalleled versatility for the facile creation of supramolecular materials.[1] ISA exploits the functional capabilities of ionic surfactants and oppositely charged polyelectrolytes to form supramolecular mesostructures exhibiting excellent stability and stiffness.[2] As a result this approach has found incredible resonance in the emerging field of "nanoarchitectonics"[3] provided that it offers new strategies for the bottom-up assembly of integrated supramolecular systems displaying concerted functions. Along these lines, several research groups studied the incorporation of predefined functionalities within either the polymer or the surfactant counterparts in order to attain self-assembled materials with specific functional features.[4] Current research on ISA has reached the frontiers of electrochemistry and consequently new electroactive ionically self-assembled materials came to light. Tong *et al*. reported the preparation of electroactive films constituted of sodium poly(styrenesulfonate) (PSS) complexed with *n*-alkyl (ferrocenylmethyl)ammonium bromide.[5] In a similar vein, Faul, Manners and co-workers described the preparation of redox-active mesomorphic complexes from the ionic self-assembly of cationic polyferrocenylsilane polyelectrolytes and different anionic surfactants.[6] In this context, the integration of biorecognizable units into the electroactive supramolecular material is of critical importance to further broaden the reach of ISA into the arena of bioelectrochemistry. This is particularly important if we consider that the combination of the ISA technique and redox glycoproteins with electrochemistry may offer new perspectives in the design of amperometric biosensors or biofuel cells.[7] Successful immobilization and electrical contacting of redox proteins with conducting substrates constitute the cornerstone of modern bioelectrochemistry and represent critical events in the construction of bioelectrodes.[8] Within this framework, recognition-directed biosupramolecular assembly emerged as an interesting and attractive alternative due to its simplicity and versatility, without introducing chemical modifications into the enzyme.[9] This noncovalent approach is based on the remarkable selectivity of the interaction between the constituting building blocks.[10] In the case of glycoenzymes, it has been demonstrated that this methodology allows the rapid immobilization of considerable amounts of protein on surfaces modified with lectins.[11] Thus, one of the remaining challenges in ionic self-assembly is its combination with recognition-directed assembly in order to provide almost unlimited possibilities to create supramolecular materials compatible with the preparation of bioelectrodes and other chemoresponsive electrochemical interfaces. Herein, we describe for the first time the synergistic combination of ionic self-assembly and recognition-directed assembly to immobilize and "wire" redox glycoenzymes on electrode supports using an electroactive glycopolyelectrolyte as a functional building block. To the best of our knowledge, this is the first report on a trifunctional polyelectrolyte able to: (i) form mesostructured polyelectrolyte–surfactant complexes *via* ionic interactions, (ii) expose carbohydrate ligands participating in the biorecognition-driven assembly of glycoproteins and (iii) facilitate electron transfer processes using intrafilm redox sites to generate bioelectrocatalytic signals ("redox wiring").

The glycopolyelectrolyte was synthesized by sequential modification of polyallylamine hydrochloride with Os bipyridyl complexes (redox units) and lactose moieties (biorecognizable units) (see ESI† for details). The modified polyelectrolyte (GOsPA) is soluble in water and upon mixing with a SDS solution yields a precipitate that can be readily dissolved in DMSO (GOsPA-DS) and form dimensionally stable layers firmly adhering to gold, silicon or even mica surfaces (Fig. 1). The microphase-separated morphology of GOsPA-DS complexes was probed by synchrotron-based small-angle X-ray scattering (SAXS) (LNLS, Campinas, Brazil) (Fig. 2). The presence of a narrow scattering peak corresponding to the long period of *ca.* 3.7 nm is consistent with the formation of lamellar mesostructures in the supramacromolecular assembly.[12] According to Antonietti *et al.*[13] these lamellar domains

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† Electronic supplementary information (ESI) available: Experimental details of the glycopolyelectrolyte synthesis. See DOI: 10.1039/c2cc35949a

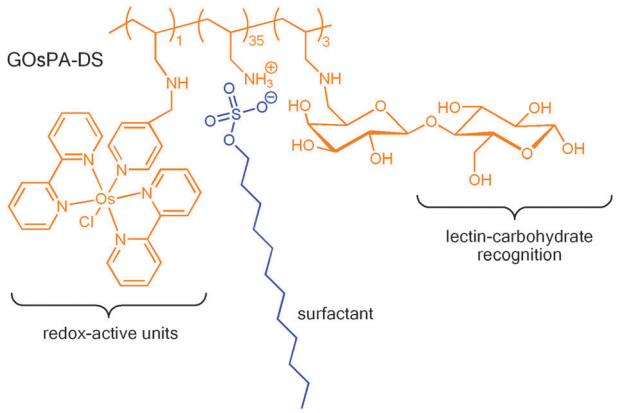

Fig. 1 Chemical structure of the GOsPA–DS supramolecular complex.

can be rationalized as alternating alkane and ionic layers where the alkane side chains of two layers are intercalated.

Once confirmed that the supramolecular assembly exhibits a microphase-separated lamellar morphology we proceeded to its electrochemical characterization. Cyclic voltammetry of thick GOsPA-DS films (~1 μm in thickness as measured by profilometry) reveals a remarkable quasi-reversible electrochemical behavior, indicating a notable electrical communication between redox centers that allows a fast intrafilm electron transfer process. The electroactive GOsPA-DS film exposing carbohydrate moieties was conjugated with redox-active concanavalin A (Os-Con A). The redox-tagged Con A layer acts not only as a "biosupramolecular glue"[14] to facilitate the robust and easy attachment of the glycoenzyme without affecting its catalytic activity but also as an electro-conducting phase to enable the

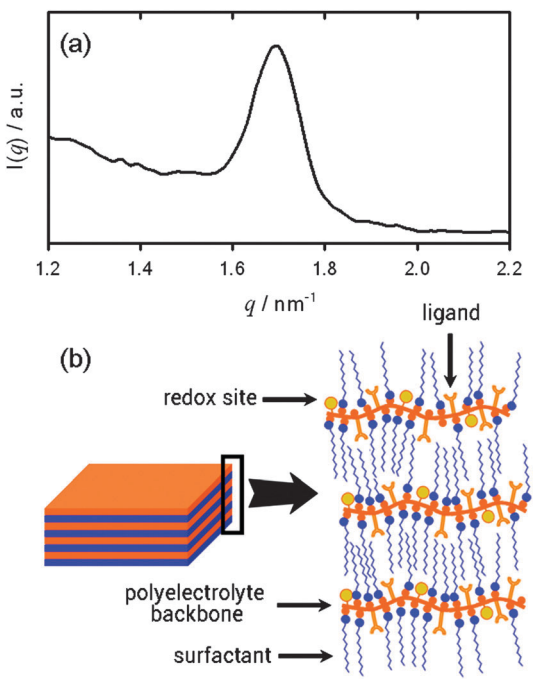

Fig. 2 (a) SAXS plot (raw data) for the GOsPA–DS supramolecular complex in the solid state. (b) Structure model for the solid state structure of the GOsPA–DS complex. A sequence of ionic layers (redox-active glycopolyelectrolyte and ionic head groups) and alkane layers (hydrophobic tails) is assembled in a lamellar fashion.

electrical communication between the prosthetic groups of the enzyme and the electroactive sites within the ionically self-assembled GOsPA-DS layer. Sequential assembly of the bioelectrochemical architecture achieves completion through the recognition-directed assembly of the glycoenzyme horseradish peroxidase (HRP) enzyme on the Au/GOsPA-DS/Os-Con A-modified electrode *via* carbohydrate–lectin interactions (Fig. 3a). Recognition-driven assembly of Os-Con A practically does not produce significant changes in the voltammogram, and its contribution to the total charge density of osmium centers is negligible (not shown). However, by adding H_2O_2, *i.e.* the enzyme substrate, an outstanding catalytic wave evolves as it

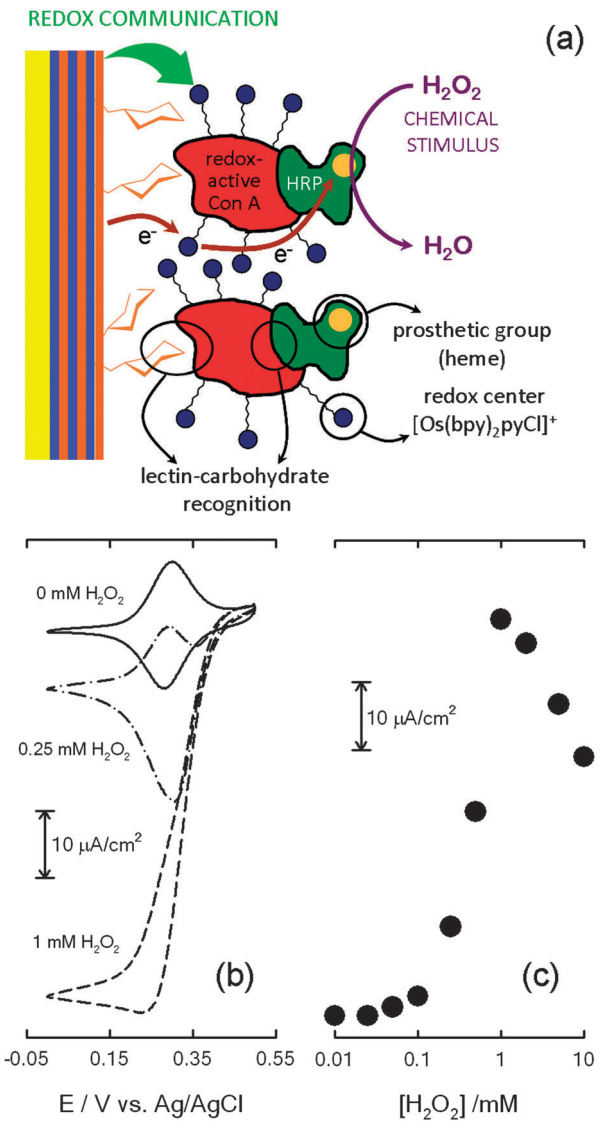

Fig. 3 (a) Illustrative schematic of the H_2O_2-responsive supramacromolecular thin film generated through the combination of ionic self-assembly and molecular recognition processes. The figure describes the constituting building blocks participating in the generation of the bioelectronic signal in the presence of H_2O_2: redox-active Con A and HRP. (b) Cyclic voltammograms describing the electrochemical response of a Au/GOsPA-DS/Os-ConA/HRP assembly in the absence and in the presence of H_2O_2. (c) Bioelectrocatalytic currents measured on Au/GOsPA-DS/Os-ConA/HRP assemblies as a function of substrate concentration. Error bars are comparable to symbol size.

can be observed for cyclic voltammograms in 0.25 and 1 mM H_2O_2. The response at 0.25 mM shows an important increase in the cathodic current, then the current decays to a plateau and a small oxidation peak is observed at the reverse scan. When the concentration is further increased to 1 mM the electrochemical response corresponds to a classic electrochemical catalytic mechanism (EC mechanism),[15,16] achieving a plateau and displaying a small hysteresis in the backward direction (Fig. 3b).

This reveals that the whole *bio*supramacromolecular assembly is electrically connected to the gold electrode. Or, in other words, the ionically self-assembled interfacial architecture incorporates functional biorecognition features and is able to transmit a signal in response to the presence of H_2O_2 in solution. It is worth mentioning that blank experiments conducted on similar ionically self-assembled systems but lacking the presence of carbohydrate units (OsPA-DS) failed to assemble the lectin and the glycoenzyme atop the redox film and no bioelectrocatalytic activity was detected. This indicates that the presence of biorecognizable units in the functional polyelectrolyte scaffold proves critical to assemble the glycoenzyme conjugate. The H_2O_2-responsiveness of the biosupramolecular enzymatic electrode is eloquently illustrated by the magnitude of the bioelectrocatalytic currents detected in the presence of increasing amounts of substrate (Fig. 3c). The catalytic current increases upon raising the substrate concentration. Then, upon further increase of the H_2O_2 concentration, the catalytic current gradually decreases, which indicates the progressive formation of the inactivated form of HRP (oxyperoxidase), similarly to that previously observed by Savéant and co-workers.[17] However, notably, the crossover of the catalytic activity takes place at 1 mM H_2O_2. This behavior is strikingly different from that reported by Savéant *et al.*[18] and our group[9b] in which inhibition by H_2O_2 was detected at 0.1 mM. These results suggest that in the ionically self-assembled film the osmium : HRP ratio is higher than that involved in previous reports, and as a consequence this type of (bio)interfacial architecture contributes to minimizing the formation of oxyperoxidase. From the functional viewpoint this interesting feature promotes an improvement by over one order of magnitude in the operation range of the bioelectrode prior to reaching the crossover of biocatalytic activity.

In conclusion, in this work we have introduced a new method to construct electrically contacted glycoenzyme electrodes by using ionically self-assembled thin films bearing electroactive and biorecognizable units. These functional building blocks are easily processed by spin-coating to produce durable and dimensionally stable layers firmly adhering to the electrode support. Charge density derived from cyclic voltammograms is 111 μC cm^{-2} which implies that 6.9×10^{14} redox sites per cm^2 are connected to the underlying gold electrode, an impressive current propagation regarding self-assembled systems.[19] The introduction of glycolytic moieties allows us to associate/integrate/connect glycoenzymes on electrode supports by a mild method preserving their activities, exemplified here by HRP, an enzyme not able to be incorporated through ionic self-assembly with this type of polyelectrolyte. The control of the inhibition of HRP at higher concentration opens new perspectives for HRP application; for example in biofuel systems. These results will further broaden the range of possibilities to design "soft" heterosupramolecular films[20] compatible with the integration of bioactive elements on electrode surfaces.

M.L.C. and D.P. gratefully acknowledge CONICET for a postdoctoral fellowship. This work was supported by Universidad de Buenos Aires, CONICET, ANPCyT (PICT Bicentenario 2010-2554 and PICT-PRH 163/08), the Max Planck Society (O.A.) the Alexander von Humboldt Stiftung (O.A.) and Laboratório Nacional de Luz Síncrotron (LNLS, Brazil) (D10A-XRD2-11639 and D11A-SAXS2-10737). M.C., O.A. and F.B are CONICET fellows.

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