ELECTRONIC SUPPLEMENTARY INFORMATION

Ionic Self-Assembly of Electroactive Biorecognizable Units: Electrical Contacting of Redox Glycoenzymes Made Easy.

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Experimental

Reagents and Materials

Sodium dodecyl sulfate (SDS), poly(allylamine) (PA, MW: 65000), concanavalin A (Con A, *Canavalia ensiformis* from Jack bean), horseradish peroxidase (HRP, Type VI), lactose, galactose oxidase (GO, from *Dactylium dendroides*) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma Aldrich. The redox polymer $Os(bpy)_2ClpyNHpoly(allylamine)$ (OsPA) was synthetized as previously reported.[1] The synthesis of redox-active Con A (Os-Con A) was achieved by labeling Con A with $[Os(bpy)_2Clpy]^+$ by using a 336 Da MW polyethylene glycol (PEG) spacer following a procedure described in the literature.[2] A ratio of 12 redox probes per protein molecule was obtained. All other reagents were analytical grade.

Synthesis of the redox glycopolyelectrolyte (GOsPA)

36 mg of lactose were dissolved in 2 mL of HEPES buffer (100 mM, pH 6.5). The solution was saturated with oxygen and 200 U of galactose oxidase were added under stirring. The mixture was left to react under oxygen atmosphere at 37 °C for 48 hours with stirring. Afterward, the mixture was cooled to room temperature and centrifuged to remove any remaining aggregate. The modified lactose present in the supernatant was purified using a series of two Hitrap desalting columns (GE Healthcare, 5 mL each) with HEPES buffer (15 mM, pH 7.4, containing 15 mM NaCl) at an elution rate of 5 mL/min. For this purpose we used a FPLC system (Fast Protein Liquid Chromatography) Model ÄKTA Explorer 10 (Amersham GE). The product was then lyophilized. The fraction of modified and lyophilized lactose was dissolved in 1.5 mL of HEPES buffer (50 mM, pH 7.4) and this solution was added to a solution of OsPA (0.2 mM, 2mL) in the same buffer and treated with an excess of sodium cyanoborohydride. The resulting mixture was stirred overnight at room temperature. Afterwards, the mixture was treated with an excess of sodium borohydride and stirred for 1 hour at room temperature. The product was then dialyzed for 48 h against Milli-Q water using a 3500 MWCO membrane and lyophilized. ^{1}H NMR (500 MHz, D_2O): the δ values between 3.25 and 4.65 ppm are consistent with lactose, indicating the introduction of the glycosidic fragment in the structure of the polyelectrolyte. The 1:10 lactose:allylamine ratio and 1:35 osmium complex:allylamine ratio were determinated by integration of the signals.

Synthesis of the supramolecular material GOsPA-DS

400 μL of SDS 1% in Milli-Q water were added to a 200 μL of GOsPA (0.2 mM). The mixture immediately generated a precipitate (GOsPA+DS), which was easily separated by centrifugation. The precipitate was dissolved in 500 μL of DMSO and sonicated for 15 minutes to facilitate complete dissolution of the solid.

Construction of the chemically modified electrodes

The construction of the molecular assemblies was achieved by using silicon coated with 15 nm of Ti, 20 nm of Pd and 200 nm of gold by evaporation. The first step of the substrate modification was the application of a uniform layer of GOsPA+DS by spin coating. Afterwards, the electrode was left at room temperature for 1 h to allow complete evaporation of the solvent. Then, it was rinsed with Milli-Q water and dried with N_2. The incorporation of the subsequent building blocks was achieved through a series of sequential steps. Protein building blocks such as Con A, Os-Con A and HRP were incorporated by incubating the modified electrode for 1 h in 1 μM solutions in 0.05 M HEPES buffer, (pH 7.4) containing 0.5 mM $CaCl_2$ and 0.5 mM $MnCl_2$. The same buffer was used to rinse the electrode after each assembling step. To immobilize another layer of GOsPA onto the protein-modified surface, the electrode was incubated for 1 h in 0.2 mM GOsPA solution in 0.05 M HEPES buffer (pH 7.4). Afterwards, the electrode was rinsed with the same buffer. All steps were carried out at room temperature (*ca.* 22 °C).

Electrochemical measurements

Cyclic voltammetry experiments were carried out using a purpose-built potentiostat (TEQ-02) using a three-electrode teflon electrochemical cell equipped with a platinum mesh counter electrode and an Ag/AgCl reference electrode. Unless otherwise stated, all electrochemical experiments were performed at room temperature (ca. 22 °C) in a 0.05 M HEPES, 0.1 M KNO_3 buffer solution at pH 7.4.

References

[1] Danilowicz, C.; Corton, E.; Battaglini, F. *J. Electroanal. Chem* **1998**, *445*, 89-94.
[2] Pallarola, D.; Queralto, N.; Knoll, W.; Ceolín; Azzaroni, O.; Battaglini, F. *Langmuir* **2010**, *26*, 13684-13696.