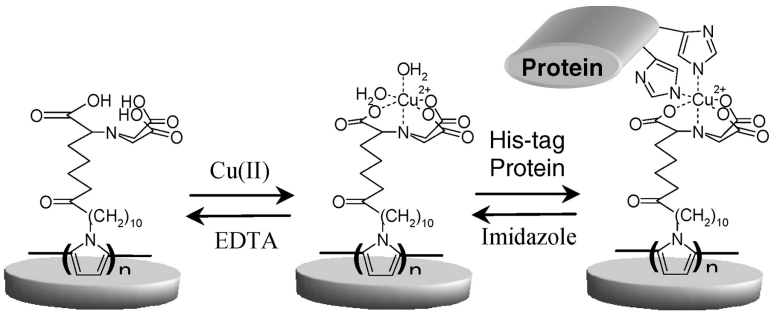
Communication

Electrogeneration of a Poly(pyrrole)-NTA Chelator Film for a Reversible Oriented Immobilization of Histidine-Tagged Proteins

Naoufel Haddour, Serge Cosnier, and Chantal Gondran

J. Am. Chem. Soc., **2005**, 127 (16), 5752-5753• DOI: 10.1021/ja050390v • Publication Date (Web): 08 March 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 10 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 03/08/2005

Electrogeneration of a Poly(pyrrole)-NTA Chelator Film for a Reversible Oriented Immobilization of Histidine-Tagged Proteins

Naoufel Haddour, Serge Cosnier,* and Chantal Gondran

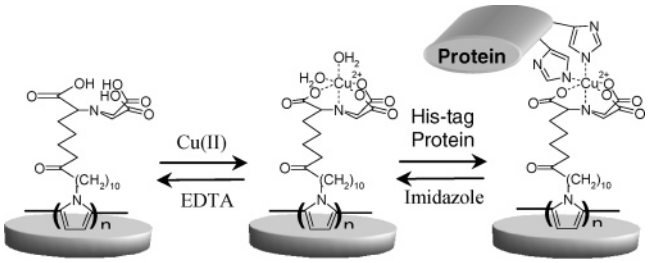
Laboratoire d'Electrochimie Organique et de Photochimie Rédox (CNRS UMR 5630), Institut de Chimie Moléculaire de Grenoble FR CNRS 2607, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France

Received January 20, 2005; E-mail: Serge.Cosnier@ujf-grenoble.fr

Deposition of biological macromolecules with the entire retention of their activity is the subject of increasing research efforts owing to its potential applications in the field of medical diagnostics and bioreactors. One such method uses transition metal complexes of nitrilotriacetic acid (NTA),[1−3] particularly in the protein purification technique known as immobilized metal-ion affinity chromatography (IMAC). This approach utilizes the NTA chelator to coordinate bivalent metal cations (e.g. Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}) leaving free coordination sites of the chelator−metal complex for the ligation of histidine-tagged proteins.[4−6] Hexahistidine tags can be easily attached to N- of C-terminus of the protein by mutagenesis and hence can be used as tools in protein localization and orientation. However, nanoengineered biosensors and biochips would require the immobilization of NTA on transducer surfaces with a controlled spatial resolution at nanoscale level.

The electrosynthesis of polymers is one of the few known methods that allow the reproducible and precise functionalization of conductive microsurfaces of complex geometry by organic molecules.[7] With the aim of developing an immobilization method combining the advantages of electrochemical addressing and affinity immobilization, this contribution describes, for the first time, the design of an electropolymerized NTA film for the reversible and oriented anchoring of histidine-tagged proteins (Scheme 1).

Scheme 1. Schematic Representation of the Reversible Immobilization of Histidine-Tagged Biomolecules to an Electrogenerated Poly(pyrrole)-NTA Film



We report herein the first successful synthesis and electropolymerization of an NTA derivative and the use of the resulting poly(pyrrole) film for the successful coordination of copper ions and histidine-tagged glucose oxidase. In this study, the pyrrole monomer (**1**) functionalized with an NTA group was prepared by reaction of nitrilotriacetic acid with succinimidyl ester of 1-(11-undecanoic acid)pyrrole.[8] The pyrrolic NTA was characterized by ^{1}H NMR and by FAB mass spectrometry.[9] The succinimidyl ester was synthesized using the carbodiimide method[10] and characterized by ^{1}H NMR.[11]

The electrochemical behavior of the pyrrole NTA **1** (4 mM) was investigated in CH_3CN + 0.1 M $LiClO_4$. Upon oxidative scanning, the monomer exhibits an irreversible peak at 1.08 V corresponding to the oxidation of the pyrrole group. As expected, the oxidation potential of **1** is higher than that of regular pyrrole (Figure 1A). Electropolymerization of **1** was accomplished by repeated potential cycling over the range of 0−0.8 V (Figure 1B); the appearance and the continuous growth of reversible oxidation waves at 0.18 V clearly indicate the formation of a polymeric coating on the electrode surface. The resulting electrode was transferred with thorough rinsing to a CH_3CN + 0.1 M $LiClO_4$ solution free of monomer **1**. As expected, the cyclic voltammogram of this electrode exhibits a reversible peak system at $E_{1/2}$ = 0.18 V assigned to the oxidation of the poly(pyrrolic) matrix (Figure 1C). The apparent surface coverage of the electropolymerized **1**, $\Gamma = 4.1 \times 10^{-8}$ mol·cm^{-2}, was determined from the charge recorded under the oxidation wave of the poly(pyrrole) backbone.

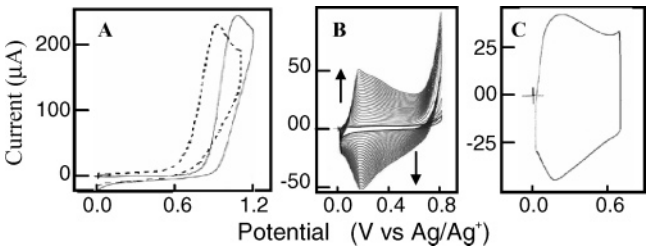


Figure 1. (A) Cyclic voltammogram recorded at a platinum electrode (diameter = 5 mm) of **1** (4×10^{-3} M, full line) and regular pyrrole (2×10^{-3} M, dashed line) in CH_3CN + 0.1 M $LiClO_4$. Scan rate = 0.1 V·s^{-1}. (B) First 25 scans in the oxidative electropolymerization of **1** (4×10^{-3} M) in CH_3CN + 0.1 M $LiClO_4$. Scan rate = 0.1 V·s^{-1}. (C) Cyclic voltammogram of the polymerized **1** ($\Gamma = 4.1 \times 10^{-8}$ mol·cm^{-2}) in CH_3CN + 0.1 M $LiClO_4$. Scan rate = 0.1 V·s^{-1}.

Initially, the chelation of copper ions into the electropolymerized poly(pyrrole)-NTA (poly **1**) was investigated by cyclic voltammetry after the poly(pyrrole) electroactivity was destroyed by electrochemical overoxidation. The coordination of Cu^{2+} ions with polymerized NTA groups was carried out by immersing the modified electrode for 15 min in sodium acetate/acetic acid buffer solution (pH = 4.5) containing 10^{-2} M of $CuCl_2$ kept under stirring conditions. After accumulation of the metal ion, the electrode was carefully washed with distilled water, and the chelated Cu^{2+} ions were then reduced at −0.9 V versus SCE for 10 s in 0.5 M aqueous NaCl. Upon oxidative scanning from −0.9 to 0.5 V, an irreversible peak and a reversible one were observed at −0.15 and 0.17 V, respectively (Figure 2A). The latter corresponds to the successive one-electron oxidation of reduced Cu(0) to Cu(I) and Cu(I) to Cu(II). The amount of immobilized copper was determined by integration of the charge under these oxidation peaks. It appears that the copper ion content increases almost linearly with the thickness of the poly **1** film (Figure 2B), providing an average value of 3.5 polymerized NTA for each Cu^{2+} center. The immersion of Cu^{2+}-doped poly **1** film in sodium acetate/acetic acid buffer solution (pH = 4.5) containing EDTA (0.2 M) for 2 h showed the

10.1021/ja050390v CCC: $30.25

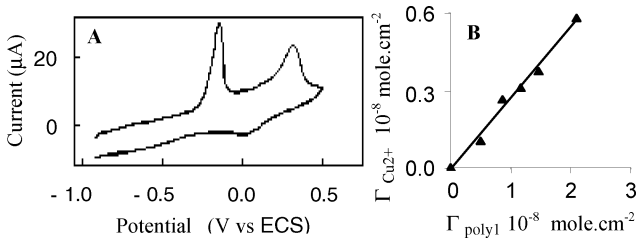


Figure 2. (A) Cyclic voltammogram of poly **1**-modified glassy carbon electrode (diameter = 3 mm, $\Gamma = 2.1 \times 10^{-8}$ mol·cm^{-2}) containing chelated Cu^{2+} in 0.5 M aqueous NaCl. Scan rate = 0.1 V·s^{-1}. (B) Evolution of the apparent surface coverage of coordinated Cu^{2+} (Γ_{Cu2+}) as a function of apparent surface coverage of polymerized NTA monomer (Γ_1).

disappearance of the oxidation peaks systems, indicating the removal of immobilized Cu^{2+} ions via EDTA coordination.

To investigate the potential affinity of metal−poly **1** film for histidine-tagged proteins, two poly **1** platinum electrodes containing coordinated Cu^{2+} ions were soaked in 0.1 M phosphate buffer, one in the presence of histidine-tagged glucose oxidase (his-GOX, 0.1 mg·mL^{-1}) and the other in the presence of regular glucose oxidase (GOX, 0.1 mg·mL^{-1}). Since this enzyme catalyzes in the presence of oxygen, the oxidation of glucose with the concomitant production of H_2O_2, its activity can be monitored via the electrochemical oxidation of H_2O_2 at the underlying electrode surface.[12] The maximum current density (j_{max}) determined at glucose saturating conditions was markedly higher for the electrode incubated with his-GOX (3.4 μA cm^{-2}) than for the electrode incubated with GOX (0.48 μA cm^{-2}). Since j_{max} is directly proportional to the enzyme loading, this difference clearly indicates that the enzyme binding was mainly due to the specific ligation process of the histidine residues to the coordinated Cu^{2+} sites of the poly **1**. In addition, the similarity between this value (3.4 μA cm^{-2}) and the J_{max} value (3.7 μA cm^{-2}) reported under the same potential and pH conditions for a close-packed monomolecular layer of biotinylated GOX immobilized via avidin−biotin bridges,[13] demonstrates the efficiency of the metal affinity immobilization system. However, it should be noted that this method cannot be applied to the fabrication of electrochemical devices working at negative potentials lower than −0.5 V because of the reduction of Cu(II) into Cu(0).

To validate the role of the metal ion, a control experiment was carried out with a pure poly **1** film incubated with his-GOX. As expected, the resulting modified electrode provided a low J_{max} value (0.78 μA cm^{-2}), reflecting solely nonspecific binding of his-GOX. Moreover, the incubation of the enzyme electrode with a high concentration of imidazole (0.2 M) for 4 h induced a drastic decrease in j_{max} (0.44 μA cm^{-2}). This phenomenon was attributed to the replacement of the histidine ligand of the five-coordinate complex by imidazole, leading thus to a quasi total release (88%) of the anchored his-GOX. The immersion of the same electrode in $CuCl_2$ solution then in his-GOX solution allows us to restore its initial enzymatic activity. Five successive cycles of enzyme release and electrode regeneration were carried out without noticeable decrease in the amperometric response of the modified electrode toward glucose. This illustrates the reversibility of this concept of protein−polymer interactions, contrary to biotinylated polymers that led to irreversible anchoring of biotinylated biomolecules via the formation of an avidin bridge.

These results clearly demonstrate the efficiency and reversibility of the oriented immobilization of histidine-tagged protein onto an NTA-poly(pyrrole) film through an affinity "lego" procedure. However, the usefulness of the method may be hampered in acidic media (pH < 4) that prevent the Cu^{2+} coordination. It is expected that this simple and promising approach of protein fixation will be useful in the development of bioanalytical nanodevices.

Acknowledgment. The authors thank the Commission of the European Communities Research Directorate for their support under the ELISHA project Contract No. NMP-A-CT-2003-505485-1, and the ACI 031 "Nouvelles methodologies analytiques et capteurs 2002" for partial funding. The authors also thank Drs. Charles Mioskowski and Cyrille Richard for initial synthetic attempts and fruitful discussions.

Note Added after ASAP Publication. At the request of the corresponding author, an addition was made to the Acknowledgment of the version published ASAP on March 8, 2005. The corrected version was posted on March 28, 2005.

Supporting Information Available: Experimental procedures, characterization data, and amperometric response of the enzyme electrodes. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sigal, G. B.; Bamdad, C.; Barberis, A.; Strominger, J.; Whitesides, G. *Anal. Chem.* **1996**, *68*, 490.
(2) Schmid, E. L.; Keller, T. A.; Dienes, Z.; Vogel, H. *Anal. Chem.* **1997**, *69*, 1979.
(3) Wegner, G. J.; JinLee, H.; Marriott, G.; Corn, R. M. *Anal. Chem.* **2003**, *75*, 4740.
(4) Schmitt, L.; Dietrich, C.; Tampé, R. *J. Am. Chem. Soc.* **1994**, *116*, 8485.
(5) Lu, Z.; DiBlasio-Smith, E. A.; Grant, K. L.; Warne, N. W.; LaVallie, E. R.; Collins-Racie, L. A.; Follettie, M. T.; Williamson, M. J.; McCoy, J. M. *J. Biol. Chem.* **1997**, *69*, 2043.
(6) Hart, S. R.; Waterfield, M. D.; Burlingame, A. L. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 1042.
(7) Bidan, G. *Sens. Actuators B* **1992**, *6*, 45.
(8) To a solution of succinimidyl ester (265 mg, 0.76 mmol) in DMF (2 mL) was added a solution of nitrilotriacetic acid (200 mg, 0.76 mmol) in a mixture of DMF (1 mL), TEA (500 μL), NaOH (91 mg, 2.28 mmol), and water (300 μL). The solution was stirred for 24 h at 60 °C and concentrated in vacuo. The crude was dissolved in water (3 mL), and hydrochloric acid 1 N was added dropwise. The precipitate was filtered and washed with diethyl ether. After drying, **1** was obtained as a white powder, 210 mg (56% yield).
(9) ^{1}H NMR (250 MHz/NaOD) data for the pyrrole-NTA: δ (ppm) = 6.71 (s, 2H), 5.95 (s, 2H), 3.83 (t, 2H), 3.60−3.20 (m, 5H), 2.97 (m, 4H), 2.01 (t, 2H), 1.80−1.40 (m, 8H), 1.35−1.10 (d, 14H). FAB/MS(NBA): *m*/*z* = 518 [MH + Na] $^+$.
(10) To a solution of 1-(11-decanoic acid)pyrrole (1 g, 2.8 mmol) and *N*-hydroxysuccinimide (324 mg, 2.8 mmol) in THF (10 mL) was added a solution of 1,3-dicyclohexylcarbodiimide (576 mg, 2.8 mmol) in THF (5 mL). The mixture was stirred for 8 h at rt and concentrated in vacuo, and CH_2Cl_2 (10 mL) was added. The crude was filtered, and the resulting solution was evaporated to give 1.1 g (80% yield) of the succinimidyl ester.
(11) ^{1}H NMR (250 MHz/CD_3CN) data for the succinimidyl ester: δ (ppm) = 6.62 (s, 2H), 6.12 (s, 2H), 3.83 (t, 2H), 2.80 (s, 4H), 2.54 (t, 2H), 1.80−1.60 (m, 4H), 1.40−1.20 (d, 14H).
(12) After incubation with GOX (0.1 mg/mL) phosphate buffer solution (0.1 M), the poly **1** electrodes were thoroughly rinsed with 0.1 M phosphate buffer. The amperometric measurements of glucose were carried out in stirred air-saturated 0.1 M phosphate buffer (pH 7) by potentiostating the modified electrodes at 0.6 V versus an aqueous saturated calomel electrode.
(13) Anzai, J.; Takeshita, H.; Kobayashi, Y.; Osa, T.; Hoshi, T. *Anal. Chem.* **1998**, *70*, 811.

JA050390V