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A new biotinylated tris bipyridinyl iron(II) complex as redox biotin-bridge for the construction of supramolecular biosensing architectures

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Received (in Cambridge, UK) 19th September 2003, Accepted 26th November 2003
First published as an Advance Article on the web 8th January 2004

The bioaffine immobilization of several avidin layers on an electrode modified by a biotinylated polymer was accomplished by the first biotinylated redox bridge consisted of a tris(bipyridyl)iron(II) complex bearing six pre-oriented biotin groups.

The remarkable strong affinity interactions between the glycoprotein avidin and four biotins, a vitamin (association constant K_a = 10^{15} M^{-1})[1] have been extensively used in various fields such as immunohistochemistry,[2] electron microscopy,[3] enzyme-linked immunoassay (ELISA)[4] and DNA hybridization.[5] With the aim of fabricating biosensors and biochips, the use of avidin–biotin linkage has, in particular, achieved wide acceptance in recent years for binding biological species to surfaces.[6] Among the conventional immobilization methods, the avidin–biotin technique constitutes one of the few procedures providing a high degree of control over the molecular architecture of biological assemblies. The anchoring of the protein or oligonucleotide monolayer was performed by the formation of avidin–biotin bridges between biotinylated surfaces and avidin-conjugated enzymes, or biotinylated enzymes, antibodies or oligonucleotides.[7–10] However, the concept of three-dimensional conjugation of avidin–biotin as an approach for enhancing the sensitivity of biosensors and biochips was restricted to the elaboration of multilayered enzyme electrodes based on enzyme, liposome, or polymer chemically modified by several biotin groups as macromolecular bridge.[11–13] The elaboration of DNA sensors and immunosensors was thus limited to the anchoring of a monolayer of biotinylated antibody or oligonucleotide. In order to improve the sensitivity of DNA sensors and immunosensors *via* the increase of the density of immobilised biomolecules, our aim was to create and immobilise an avidin coating on the electrode surface. As the first step toward the preparation of multilayered avidin structures, we report herein, to our best knowledge, the synthesis of the first biotinylated redox building block possessing a three-dimensional oriented distribution of biotin groups.

In this study, a novel biotin-labeled tris(bipyridyl) iron(II) complex was prepared by reaction of $Fe(II)Cl_2$ with bis(biotin)bipyridine ligand and characterized by H^1NMR and UV-visible absorption spectra as well as by EI mass spectrometry (Fig. 1).[14] The 4,4′-bis(biotin)-2,2′bipyridine ligand was prepared by esterification of 4,4′-bis(hydroxymethyl)-2,2′bipyridine[15] with biotin, using the carbodiimide method and characterized by H^1NMR and FAB mass spectrometry.[16]

The electrochemical behavior of the iron complex (1.0×10^{-3}M) was investigated by cyclic voltammetry in CH_3CN containing 0.1M n-Bu_4NPF_6. Upon oxidative scanning with a sweep rate of 0.1 V s^{-1}, the cyclic voltammogram displayed a reversible peak system (0.77V *versus* Ag/Ag^+ 1.0×10^{-2}M in CH_3CN) due to the well-known one-electron oxidation of the $Fe^{(II)}$ into $Fe^{(III)}$ (Fig. 2A).[17] Upon reductive scanning three successive reversible peak systems were observed at −1.76 V, −1.98 V and −2.2 V. As previously described for other polypyridinyl complexes of iron(II), these reductions may be assigned to the successive one-electron reduction of the bipyridyl ligand.[18] Thanks to its cationic nature, the chloride salt of the iron complex was soluble in water. Its cyclic voltammogram in aqueous 0.1M $LiClO_4$ solution, exhibited the reversible Fe^{II}/Fe^{III} oxidation system at 1.0 V *versus* an aqueous saturated calomel electrode (Fig. 2B). Sugawara *et al.* have previously reported the use of electroactive biotin derivatives for the electrochemical assay of avidin–biotin interaction in aqueous solution.[19] The availability of the biotin groups attached to the bipyridyl ligand for the binding to avidin was therefore examined *via* the influence of avidin on the electrochemical behavior of the iron complex. After the addition of avidin (1.25×10^{-5} M) to an aqueous solution containing the biotinylated complex (10^{-4} M), the peak current of the Fe^{II}/Fe^{III} redox system decreased and completely disappeared with time illustrating the formation of avidin–biotin complex (Fig. 3). A similar evolution was observed by recording the absorbance change of the iron complex with time. The rate of the absorbance decrease increased with the increase in avidin concentration indicating a cross-linking of avidin by the biotinylated iron complex (Fig. 4). The possibility of using the small biotinylated molecule as a bridge for the immobilization of several avidin layers was investigated *via* the anchoring of avidin labeled alkaline phosphatase (A-AP) on an electrogenerated biotinylated polypyrrole film.[20]

As previously reported, a monolayer of A-AP was prepared by simple interaction with the polymerized biotin groups.[21] The subsequent alternate deposition of the biotinylated iron complex

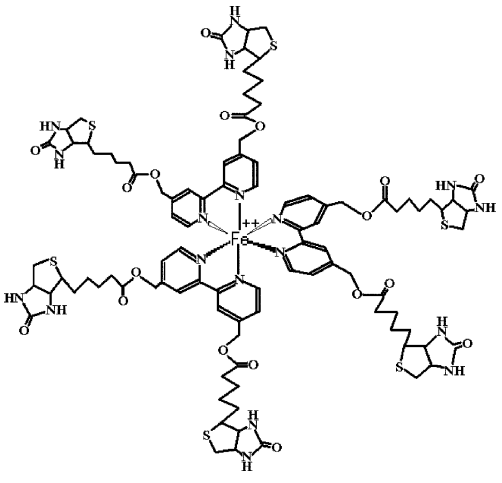

Fig. 1 Structure of biotin-labeled tris(bipyridyl) iron(II) complex.

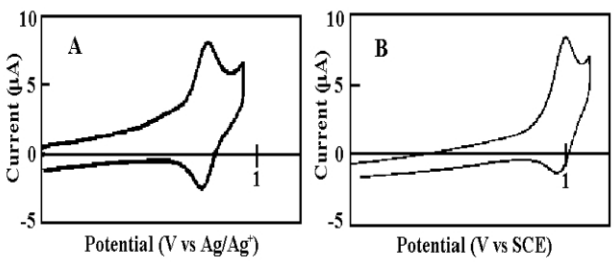

Fig. 2 Cyclic voltammograms recorded at platinum electrode (diameter 5 mm) of biotin-labeled tris(bipyridyl) iron(II) complex (1 mM) A: in CH_3CN + 0.1M n-Bu_4NPF_6 B: in H_2O + 0.1M $LiClO_4$, scan rate 0.1 V s^{-1}.

DOI: 10.1039/b311566f

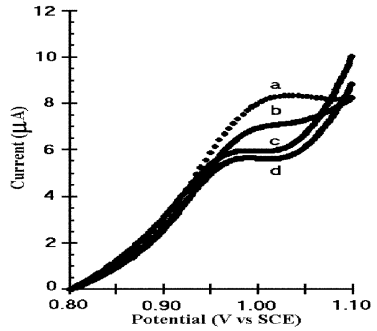

Fig. 3 Evolution of differential pulse voltammograms of the iron complex (10^{-4} M) with time in presence of 1.25 10^{-5} M avidin in 0.1M Tris buffer (pH 7); a) t = 0; b) t = 5 min; c) t = 15 min; d) t = 30 min; scan rate: 10 mV s^{-1}, step: 1 mV, pulse amplitude: 50 mV. platinum electrode (diameter 5 mm).

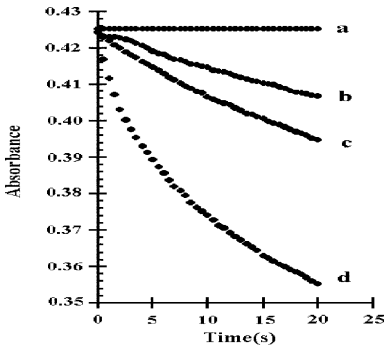

Fig. 4 Absorbance of the iron complex (0.1 mM) at λ = 533 nm as a function of the time in absence (a) and presence of : (b)1/8 eq of avidin; (c) 1/4 eq of avidin; (d) 1/2 eq of avidin.

and A-AP was carried out to elaborate two and three enzymatic layers.[22] Owing to the phosphohydrolytic activity of alkaline phosphatase, the enzymatic activity of the electrodes modified by 1, 2 and 3 A-AP layers can be determined by following at 410 nm the absorbance of *p*-nitrophenol produced from *p*-nitrophenyl phosphate. It appears that the enzymatic activity and hence the amount of immobilized enzyme increases almost linearly with the theoretically immobilized number of the A-AP layers, namely 420, 735 and 1015 mU cm^{-2} for 1, 2 and 3 layers. The presence of the immobilised enzyme was also detected through the electrochemical oxidation of *p*-nitrophenol at the underlying electrode surface potentiostated at 0.7 V *vs* SCE. The amperometric sensitivity to nitrophenyl phosphate was 41 and 85 μA M^{-1} cm^{-2} for electrodes modified by one and two layers of A-AP respectively, illustrating thus the efficient link between avidin molecules held by the iron complex.

The results described herein demonstrate that the biotin-labeled iron(II) complex constitutes an efficient small building block for the reproducible immobilization of several avidin layers by affinity interactions.

The authors thank the ACI "Nouvelles méthodologies analytiques et capteurs 2002" for financial support.

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