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Electrochemical aptasensor of cellular prion protein based on modified polypyrrole with redox dendrimers

A. Miodek [a], G. Castillo [b], T. Hianik [b], H. Korri-Youssoufi [a,*]

[a] CNRS UMR-8182, Institut de Chimie Moléculaire et de Matériaux d'Orsay, Equipe de Chimie Bioorganique et Bioinorganique, Univ Paris-Sud, Bâtiment 420, 91405 Orsay, France

[b] Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 84248 Bratislava, Slovakia

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ABSTRACT

This work consists of the development of an electrochemical aptasensor based on polyprrole modified with redox dendrimers, able to detect human cellular prions PrP^C with high sensitivity. The gold surface was modified by conductive polypyrrole film coupled to polyamidoamine dendrimers of fourth generation (PAMAM G4) and ferrocenyl group as redox marker. The aptamers were immobilized on the surface via biotin/streptavidin chemistry. Electrochemical signal was detected by ferrocenyl group incorporated between dendrimers and aptamers layers. We demonstrated that the interaction between aptamer and prion protein led to variation in electrochemical signal of the ferrocenyl group. The kinetics parameters (diffusion coefficient D and heterogeneous constant transfer k_{et}) calculated from electrochemical signals demonstrate that the variation in redox signal results from the lower diffusion process of ions during redox reaction after prion interaction due to bulk effect of larger protein. The association of redox dendrimers with conducting polypyrrole leads to high sensitivity of PrP^C determination with detection limit of 0.8 pM, which is three orders of magnitude lower, compared to flat ferrocene-functionalized polypyrrole. Detection of PrP^C in spiked blood plasma has been achieved and demonstrated a recovery up to 90%.

1. Introduction

Prion proteins are responsible for the transmissible spongiform encephalopathies (TSEs), which is a group of fatal neurodegenerative diseases. This includes Creutzfeldt–Jakob disease in human and spongiform encephalopathy in animals (Collinge, 2001). The diseases are highly contagious with possible transmission from animals to humans. It is assumed, that these diseases are caused by transformation of cellular prions (PrP^C) into their infectious isoform PrP^{Sc} (Masters et al., 1981; Prusiner, 1991). PrP^{Sc} differs from PrP^C in high content of β sheets, in resistance to protease digestion and in tendency to form large aggregates that cause formation of amyloid plaques in brain of mammals (Pan et al., 1993; Prusiner et al., 1998). Detection of prion using immunological techniques (Western blot and ELISA) is one of the most accurate method (Nunnally, 2002; Ingrosso et al., 2002). However, these methods are based on rather expensive antibody–enzyme conjugates, are time consuming and require qualified staff. Therefore the development of inexpensive, label-free rapid, sensitive and easy to use method for the detection of PrP^{Sc} is crucial for early diagnostics of prion diseases. Especially useful would be point-of-care assay that can be used even by physicians, outside of specialized clinical laboratories (Fournier-Wirth et al., 2010). So far, mostly the immunodiagnostic procedure (Safar et al., 2005) and post-mortem histopathological identification of brain tissues were applied (Kawatake et al., 2006; Kuczius et al., 2007). However, in contrast with cerebrospinal fluid, the concentration of PrP^{Sc} in the blood is down to pM (Panigaj et al., 2011), which makes the measurement difficult to achieve.

Biosensor technology, which is growing substantially in recent years, can help to overcome existing difficulties. The development of biosensors requires the achievement of an efficient interface between the biomolecules and the electronic transducers. Conducting polymers (CPs) are widely used as a transducer for biological interactions. In fact, CPs are polyconjugated polymers with an electronic structure giving them intrinsic characteristics such as electrical conductivity which can be controlled by a doping/de-doping process, low ionization potential, high electron affinity and optical properties. The electronic structure of conducting polymers is very sensitive to any modification of the backbone conjugation and conformation related to the biological interaction (Tlili et al., 2005). Thus, the success of CPs as a transducer relies on their combined versatility in providing a suitable interface for grafting bioreceptors onto micron-sized surfaces (Livache et al., 1998; Grosjean et al., 2005) and in their ability to

* Corresponding author. Tel.: +33 1 69157440; fax: +33 1 69157281.
E-mail address: hafsa.korri-youssoufi@u-psud.fr (H. Korri-Youssoufi).

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monitor the biological recognition transfer produced by probe/target interactions to a measured signal which is related to their optical (Ho et al., 2005) or electrical properties (Korri-Youssoufi et al., 1997). The most commonly used CP in sensing applications is polypyrrole (PPy) owing to its biocompatibility, high hydrophilic character and high stability in water (Ramanavičius et al., 2006). The poly(amidoamine) dendrimers of fourth generation (PAMAM G4) have been successfully applied in the fabrication of biosensors (Zhu et al., 2010). PAMAM G4 has a globular structure with diameter of about 4.5 nm and possesses 64 primary amine groups on the surface (Tsukruk et al., 1997). The amino groups with high density in PAMAM are easily functionalized by other substances that greatly extend their applications in biosensors (Shi et al., 2008). PAMAM dendrimer could be directly substituted by pyrrole monomer and then electropolymerized on surface (Şenel and Nergiz, 2012; Şenel and Çevik, 2012) or covalently attached to modified polypyrrole through amide link.

The aim of this work lies in the development of an electrochemical biosensor for real time detection of cellular prion proteins (PrP^C). A biosensor is based on covalent attachment of PAMAM G4 to the functionalized polypyrrole layer, electropolymerized on the surface of a gold electrode. High number of amine groups on the surface of PAMAM has been exploited for covalent attachment of ferrocenyl group (Fc) as redox markers. Aptamers have been selected due to their sensitivity to prion proteins (Bibby et al., 2008) and the sequence used selected from Takemura et al. (2006) was extended by a 15-mer thymine spacer in order to provide more flexibility of the aptamers for the anchoring to the layer.

2. Materials and methods

2.1. Reagents

The human prion protein, PrP^C (103-231) molecular weight 15.1 kDa was purified in INRA Jouy-en-Josas in France by Dr. Human Rezaei and Jasmina Vidic. The aptamers specific for PrP^C (103-231) with dT_{15} spacer and biotinylated on 3′ phosphoryl terminus (5′-CGG TGG GGC AAT TTC TCC TAC TGT dT_{15}-3′-Biotin) (Biopri) were provided by Thermo Fisher Scientific (Germany) and dissolved in TE buffer consisting of 10 mM tris(hydroxymethyl) aminomethane (Tris) and 1 mM ethylene di-amine tetra-acetic acid (EDTA) at pH 7.6 in double distilled water.

Pyrrole (Py) was purchased from Sigma-Aldrich and distilled in argon before use. The 3-(N-hydroxyphthalimidyl ester) pyrrole PyNHP, 1-(phthalimidylbutanoate)-1′-(N-(3-butylpyrrole)butanamide) ferrocene PyFcNHP and 1,1′-(phtalimidebutanoate) ferrocene $Fc(NHP)_2$ were synthetized according to the methods previously described (Korri-Youssoufi and Yassar, 2001; Korri-Youssoufi and Makrouf, 2001). The poly(amidoamine) dendrimers (PAMAM) of fourth generation (G4) were purchased from Sigma-Aldrich and filtered by 0.22 μM membrane filters before use. Biotin hydrazide and streptavidin were also purchased from Sigma-Aldrich. All analyses were performed in phosphate buffer saline (PBS) pH 7.4 containing 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl and 137 mM NaCl, filtered by 0.22 μm membranes and stored at 4 °C until use. Non-specific interactions were studied using Bovine Serum Albumin (BSA) (Sigma-Aldrich).

2.2. Instrumentation

Electrochemical polymerization and characterization were performed using a potentiostat–galvanostat Autolab PGSTAT 12 controlled by GPES software. The three-electrode cell was purchased from BASi and consists of a platinum mesh as a counter-electrode, gold disc (surface 2.01×10^{-2} cm^2) as a working electrode and Ag/AgCl as a reference electrode. After polymerization and each step of construction of the biosensor, the modified surface was analyzed in 10 mM PBS buffer pH 7.4 by the CV (cyclic voltammetry) method. CV was performed by cycling the potential from −0.4 to 0.4 with the scan rate of 100 mV s^{-1}. DPV (differential pulse voltammetry) was performed in the range of potential from −0.6 to 0.4 with conditioning time of 120 s and modulation amplitude of 50 mV.

Electrochemical impedance measurements (*EIS*) were carried out within 10 mM PBS buffer pH 7.4. All impedances were obtained at 0.15 V vs. Ag/AgCl at DC potential of 10 mV in a frequency range from 100 kHz to 0.1 Hz.

Chronoamperometric measurements were performed in PBS with 5 mM/5 mM $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ at the potential of 0.2 V. The current was measured for 90 s.

Scanning Electron Microscopy (*SEM*) images were acquired using a ZEISS SUPRA™ 55VP GEMINI®. The copolymer film and the different steps of construction of the biosensor, for SEM analysis, were prepared by electropolymerization on the plate gold surface according to the method described in the Section 2.3.

2.3. Electropolymerization of copolymers PPy-PyNHP and PPy-PyFCNHP

The copolymer film consisting of two monomers, pyrrole (Py) and pyrrole functionalized by an active ester (PyNHP), was grown on gold surface immersed in acetonitrile containing 0.5 M $LiClO_4$ by cycling the potential from −0.4 to 1.2 V vs. Ag/AgCl at the scan rate of 100 mV s^{-1}. The reaction was stopped when a current intensity corresponding to redox signal of polypyrrole reached 13 μA. During the electropolymerization the working and counter-electrodes were separated in a small volume cell (BASi) containing the solution of two monomers. The ratio of Py to modified pyrroles PyNHP or PyFcNHP used during reaction was 8:2 mM according to the optimal conditions described previously (Lê et al., 2010a, 2010b). The copolymer film of PPy-PyFcNHP was grown under the same conditions by cycling the potential from −0.4 to 0.95 V vs. Ag/AgCl and the reaction was stopped when a current intensity corresponding to ferrocene reached 30 μA to obtain the same thickness as obtained previously. After polymerization, the copolymer films were analyzed in PBS buffer by the CV method (see Section 2.2 for conditions).

2.4. Construction of the biosensor

Covalent bonding of dendrimers PAMAM to the modified polypyrrole PPy-PAMAM was performed by immersing the modified electrode in 70 μM of aqueous solution of PAMAM for 2 h at room temperature followed by washing the electrode with distilled water and PBS buffer for removing the non-linked residues. Then, ferrocene modified by two phtalymidyl $Fc(NHP)_2$ groups was associated with the surface. Reaction was performed by immersing the electrode in 1 mM solution of ferrocene for 1 h at room temperature. Non-bonded residues were washed by acetonitrile and double distilled water. Subsequently, the electrode was dipped in 40 μl of solution of 2 mg mL^{-1} biotin hydrazide in PBS buffer for 45 min at room temperature. Then non-bonded residues were removed by washing the electrode with distilled water and PBS buffer. Afterwards, the electrode was incubated for 45 min in solution of 100 μg $ml^{-1}$of streptavidin in PBS at room temperature followed by washing the electrode with distilled water and PBS buffer. Aptamer attachment on the surface was obtained by dipping the electrode in 2 μM biotinylated aptamers solution in PBS buffer for 45 min at room temperature. Then the biosensor was carefully washed with distilled water and PBS buffer and stored in PBS buffer at 4 °C during 1 night. After each step of construction of the aptasensor, the surface modifications were controlled by the CV method (see Section 2.2 for conditions).