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Electrochemical immunosensor for the determination of insulin-like growth factor-1 using electrodes modified with carbon nanotubes–poly(pyrrole propionic acid) hybrids

V. Serafín, L. Agüí, P. Yáñez-Sedeño *, J.M. Pingarrón

Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, 28040 Madrid, Spain

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ABSTRACT

An amperometric immunosensor for the determination of the hormone insulin-like growth factor 1 (IGF1) is reported for the first time in this work. As electrochemical transducer, a multiwalled carbon nanotubes-modified glassy carbon electrode on which poly(pyrrole propionic acid) was electropolymerized was prepared. This approach provided a high content of surface confined carboxyl groups suitable for direct covalent binding of anti-IGF1 monoclonal antibody. A sandwich-type immunoassay using a polyclonal antibody labeled with peroxidase, hydrogen peroxide as the enzyme substrate and catechol as redox mediator was employed to monitor the affinity reaction. All the variables involved in the preparation of the modified electrode were optimized and the electrodes were characterized by electrochemical impedance spectroscopy and cyclic voltammetry. Moreover, the different experimental variables affecting the amperometric response of the immunosensor were also optimized. The calibration graph for IGF1 showed a range of linearity extending from 0.5 to 1000 pg/mL, with a detection limit, 0.25 pg/mL, more than 100 times lower than the lowest values reported for the ELISA immunoassays available for IGF1 (30 pg/mL, approximately). Excellent reproducibility for the measurements carried out with different immunosensors and selectivity against other hormones were also evidenced. A commercial human serum spiked with IGF1 at different levels between 0.01 and 10.0 ng/mL was analyzed with good results.

1. Introduction

Insulin-like growth factor 1 (IGF1) is a peptide hormone containing 70 amino acid residues (Miura et al., 1992). Pituitary secretion of growth hormone (GH) stimulates IGF1 production in the liver which then acts upon peripheral tissues (Ibrahim and Yee, 2004). IGF1 has significant mitogenic effects and is involved in mechanisms of function, maintenance and repair of many tissues (Le Roith et al., 2001; Frystyk, 2004). IGF1 plays important roles in a number of human malignancies contributing to unregulated cell proliferation (Velcheti and Govindan, 2006). Moreover, this hormone exerts an acute anabolic action on protein and carbohydrate metabolism by increasing the cellular uptake of amino acids and glucose (Dimitriadis et al., 1992; Jones and Clemmons, 1995) and being involved in diabetes (Bach and Rechler, 2009; Lewitt, 1994). The normal range of IGF1 in human serum varies from 1 to 1096 ng/mL. Aging has been associated with reductions in the plasma and brain levels of IGF1 (Darnaudery et al., 2006). On the contrary, high levels of IGF1 have been associated with abdominal aortic aneurysm and aortic diameter in older men (Yeap et al., 2012).

The determination of IGF1 is usually performed by ELISA methods using optical detection. Table 1 summarizes the analytical characteristics of commercially available kits. As it can be seen, most of them involve sandwich-type immunoassays using biotinylated anti-IGF1 as detection antibody conjugated with avidin– or streptavidin–HRP and the reagent H_2O_2/TMB for the detection step. However, the ELISA assays suffer, in general, from relatively high detection limits, short calibration ranges in particular for the lower analyte concentrations and low precision levels when compared with the analytical performance that can be achieved using immunosensors. To our knowledge, only one immunosensor for IGF1 has been described in the literature. It is an impedimetric immunosensor where anti-IGF1 antibody was immobilized onto gold electrodes modified with 1,6-hexanedithiol self assembled monolayers bearing gold nanoparticles. The range of linearity reported ranged between 1.0 and 180.0 pg/mL and the limit of detection achieved was 0.15 pg/mL (Rezaei et al., 2011).

On the other hand, conducting polymers have largely demonstrated their suitability to be used in the development of electrochemical biosensors because they provide appropriate ways to immobilize biomolecules and achieve rapid electron transfer (Teles and Fonseca, 2008). Poly(pyrrole) (pPy), due to its high conductivity and stability, has been extensively employed for such purpose (Yuqing et al., 2004; Shang et al., 2009), specially for the construction of enzyme biosensors where the biomolecules are

* Corresponding author. Tel.: +34 1 3944317; fax: +34 1 3944329.
E-mail address: yseo@quim.ucm.es (P. Yáñez-Sedeño).

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Table 1
Analytical characteristics of various ELISA kits available for IGF1.

Immunoassay	Procedure	LOD[a] (ng/mL)	Dynamic range (ng/mL)	Sample volume (μL)	Assay time	Precision, RSD (%)	Sample
RayBio-Human IGF1 ELISA Kit (RayBio®)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-streptavidin+TMB/H_2O_2	< 0.2	0.25–60 (non-linear)	100	4 h 45 min	Intra-assay: < 10% Inter-assay: < 12%	Serum, plasma, urine
Abcam ab100545 IGF1 human ELISA Kit (Abcam®)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-streptavidin+TMB/H_2O_2	< 0.1	0.123–30 (non-linear)	100	4 h 45 min	Intra-assay: < 10% Inter-assay: < 12%	Serum, plasma, urine
Uscn E90050Hu ELISA Kit for human IGF1 (Uscn Life Science Inc.)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-avidin+TMB/H_2O_2	< 0.03	0.094–6.0 (non-linear)	100	4 h	Intra-assay: < 10% Inter-assay: < 12%	Serum, plasma, other biological fluids
Biorbyt Human IGF1 ELISA Kit (Biorbyt Ltd.)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-avidin+TMB/H_2O_2	–	0.0625–2 (non-linear)	100	4 h	–	Serum, plasma
Komabiotech Human IGF1 ELISA Kit K0332112 (Koma Biotech Inc.)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-streptavidin+TMB/H_2O_2	–	0.032–2 (non-linear)	100	4 h 30 min	–	Serum, plasma, other biological fluids
Enzo IGF1 (human) ELISA Kit ADI-900–150 (Enzo®)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+anti-IGF1+HRP-anti-IgG+TMB/H_2O_2	0.034	0.187–6 (non-linear)	100	4 h	Intra-assay: 3.6–8.9 Inter-assay: 3.4–10.9	Serum, plasma
OmniKine Human IGF1 ELISA Kit OK-0225 (OmniKine™)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-avidin+TMB/H_2O_2	–	0.031–2 (non-linear)	100	4 h 30 min	–	Serum, plasma
DRG Diagnosis IGF1 600 ELISA (DRG International Inc.)	Competitive immunoassay: anti-IGF1 immob.+IGF1+biotin -IGF1+HRP-streptavidin+TMB/H_2O_2	–	0–0.6	50	3 h 55 min	Intra-assay: 4.7–6.6 Inter-assay: 7.2–7.8	Serum
Quantiline ELISA Kit for human IGF1 (R&D Systems, Inc.)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+anti-IGF1+HRP-anti-IgG+TMB/H_2O_2	0.026	0–6	50	4 h	Intra-assay: 3.5–4.3 Inter-assay: 7.5–8.3	Serum, plasma
AssayPro Human IGF1 ELISA Kit EI1001-1 (AssayPro LLC)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-streptavidin+TMB/H_2O_2	0.7	0.75–24 (non-linear)	50	4 h 50 min	Intra-assay: 4.8 Inter-assay: 7.2	Plasma, serum
BioVendor Human IGF1 ELISA Kit RMEE20 (BioVendor R&D)	Sandwich immunoassay: anti-IGF1 immob.+IGF1+biotin-anti-IGF1+HRP-streptavidin+TMB/H_2O_2	0.09	2–50 (non-linear)	20	2 h 15 min	Intra-assay: 5.08–6.65 Inter-assay: 2.25–6.79	Serum

[a] Minimum detectable concentration.

entrapped into the polymer network (Minni et al., 2009; Cosnier, 2007; Gerard et al., 2002). However, this simple immobilization approach is not adequate for the preparation of immunosensors because the antibody–antigen binding can be strongly hindered. Therefore, different strategies to design electrode surfaces suitable for the ordered immobilization of antibodies without losing the conductive properties of pPy have been proposed. An effective alternative is the incorporation of functional groups to the polymer network allowing the covalent attachment of the biomolecules. For instance, biotinylated pPy copolymerized with pyrrole lactobioamide monomer was used for the construction of an amperometric immunosensor for the detection of cholera antitoxin immunoglobulins (Ionescu et al., 2004). An electrochemical immunosensor for the determination of D-dimer, a fibrin degradation product, has been reported by immobilization of a single-chain antibody (ScAb) on a N-alpha bis(carboxymethyl)-L-lysine (ANTA)/Cu^{2+} complex attached to a polypyrrole backbone (Chebil et al., 2010). A method to functionalize pPy with poly(propionic acid) (pPA) through copolymerization was implemented (Hu et al., 2007), and a label-free SPR immunosensor using goat IgG as a model protein was constructed by covalent immobilization of probe proteins on the resulting pPy/pPA film (Hu et al., 2008). Glassy carbon electrodes modified with electropolymerized poly (pyrrole propionic acid) (pPPA) were also used as platform for the preparation of amperometric immunosensors involving the immobilization of anti-mouse IgG and the recognition of mouse IgG by means of a sandwich configuration using a secondary antibody conjugated to alkaline phosphatase in the presence of p-aminophenyl phosphate (Dong et al., 2006). The same authors also reported a label-free SPR immunosensor using sheep anti-mouse IgG covalently immobilized on the carboxyl-containing film (Dong et al., 2008).

Recently, polymeric nanomaterials in the form of polymer nanoparticles (Xia et al., 2010) or forming hybrid materials with carbon nanotubes (CNTs) (Lahiff et al., 2010) or metallic nanoparticles (Rajesh et al., 2009) have expanded the usefulness of conducting polymers for the preparation of biosensors. For example, CNTs/polypyrrole/antibodies polymer films were synthesized on microelectrodes and applied to determine anti-goat IgG (Tam and Van Hieu, 2011). A CNT-pPPA network was also proposed for the electrochemical immunoassay of Hepatitis B surface antigen (Hu et al., 2011). Moreover, an amperometric immunosensor for the detection of neomycin was prepared by covalent immobilization of the antibody onto a glassy carbon electrode modified with gold nanoparticles and electrodeposited poly(2,5-di-(2-thienyl)-1 H-pyrrole-1-(p-benzoic acid) (Zhu et al., 2010). An electrochemical immunosensor for the hormone leptin was constructed by co-electropolymerization of pyrrole and pyrrole propylic acid in the presence of gold nanoparticles onto a glassy carbon electrode followed by the covalent attachment of protein G to capture the antibody anti-leptin IgG (Chen et al., 2010).

In this paper, we describe for the first time an amperometric immunosensor for the determination of IGF1 and its application to human serum samples. The electrochemical transducer consisted of electropolymerized pPPA onto CNTs-modified glassy carbon electrodes (Hu et al., 2011) which provided a high content of surface confined carboxyl groups suitable for direct covalent binding of anti-IGF1 monoclonal antibody. Furthermore, the porosity exhibited by the polymer coating is expected to favor the electrochemical reaction occurring on the electrode modified