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Novel Prostate Specific Antigen plastic antibody designed with charged binding sites for an improved protein binding and its application in a biosensor of potentiometric transduction

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ABSTRACT

This work shows that the synthesis of protein plastic antibodies tailored with selected charged monomers around the binding site enhances protein binding. These charged receptor sites are placed over a neutral polymeric matrix, thus inducing a suitable orientation the protein reception to its site. This is confirmed by preparing control materials with neutral monomers and also with non-imprinted template. This concept has been applied here to Prostate Specific Antigen (PSA), the protein of choice for screening prostate cancer throughout the population, with serum levels >10 ng/mL pointing out a high probability of associated cancer.

Protein Imprinted Materials with charged binding sites (C/PIM) have been produced by surface imprinting over graphene layers to which the protein was first covalently attached. Vinylbenzyl(trimethylammonium chloride) and vinyl benzoate were introduced as charged monomers labelling the binding site and were allowed to self-organize around the protein. The subsequent polymerization was made by radical polymerization of vinylbenzene. Neutral PIM (N/PIM) prepared without oriented charges and non imprinted materials (NIM) obtained without template were used as controls.

These materials were used to develop simple and inexpensive potentiometric sensor for PSA. They were included as ionophores in plasticized PVC membranes, and tested over electrodes of solid or liquid conductive contacts, made of conductive carbon over a syringe or of inner reference solution over micropipette tips. The electrodes with charged monomers showed a more stable and sensitive response, with an average slope of -44.2 mV/decade and a detection limit of 5.8×10^{-11} mol/L (2 ng/mL). The corresponding non-imprinted sensors showed lower sensitivity, with average slopes of -24.8 mV/decade. The best sensors were successfully applied to the analysis of serum, with recoveries ranging from 96.9 to 106.1% and relative errors of 6.8%.

1. Introduction

According to recent data from World Health Organisation (WHO), prostate cancer is the commonest form of cancer in men in Europe [1]. Accurate and early detection of prostate cancer is very important in this context, attributing early diagnosis a major role in the successful treatment of the disease. A successful early diagnosis means that early alterations in the body should be looked for. A non-invasive approach is important in this context, allowing its application throughout the male population. So far, the only medical recommendation for prostate cancer early screening suggests monitoring the levels of Prostate Specific Antigen (PSA) in serum, in men over 45 years old. Monitoring PSA levels to follow up the evolution of prostate cancer disease is also recommended, especially for being a non-invasive procedure.

PSA is a glycoprotein with a molecular mass of approximately 33 kDa, produced by the secretory epithelium of human prostate [2]. A total PSA level in the blood <4 ng/mL indicates that prostate

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cancer is improbable, while PSA levels >10 ng/mL mean cancer is likely [3]; values ranging from 4 - 10 ng/mL are in a gray zone. Currently, the standard methods available for PSA screening are immunoassays, like Enzyme-Linked Immunosorbent Assay (ELISA). These methods are highly sensitive and specific for the detection of PSA, but are also very expensive because they require specific natural antibodies, with special handling and storage conditions. In addition, there is a great affinity between the antibody and its antigen, being impossible to dissociate these two biological components after binding. This feature turns the method of irreversible nature and of single use.

Biosensors have emerged in recent years as an attractive tool to carry out quick and local clinical analysis [4], being today an alternative concept to some ELISA methods. Several biosensors for PSA have also been reported, with detection limits ranging from 0.1 to 8 ng/mL. Although they use different transduction modes, varying from electrochemical [3,5–8], optical [9–13], frequency [14] to mass [15–17] detection methods, the recognition element is always an antibody. The selection of an antibody as biological receptor confers the biosensor a selective response for PSA, but the drawbacks mentioned before for the ELISA methods also apply to these.

As an alternative, a new PSA biosensor could use artificial antibodies instead of the natural ones. These are synthetic materials offering higher chemical/thermal stability than their natural counterparts [4], and that promote a reversible response, thus enabling an 'infinite' reuse of the material. Protein plastic antibodies are PIM typically obtained by surface imprinting procedures [18], where the polymeric matrix is grown around the protein and the protein extracted afterwards from it, in order to generate the binding site [19].

However, proteins have been a tricky material to carry out such tailoring processes, because they undergo conformational changes quite easily and have multiple charge locations varying with the specific conformation they exhibit. These critical points under the preparation of PIM may be avoided by using mild conditions, preferably close to those in the native environment of the protein. This includes room temperature polymerization procedures and use of compatible materials. In addition, a way to improve protein binding to the synthetic material is to label the binding site with charged monomers. This procedure was found successful on the preparation of PIM [18], but the effect of the charged labels on this binding site is yet to be proven.

The biosensor device integrating PIM for PSA detection should be coupled to simple and low cost procedures/apparatus, such as those of the potentiometric kind, one approach that has been proven successful [20]. Potentiometric sensors offer the advantage of selectivity, simplicity, being of good overall precision and accuracy [21]. The corresponding devices may be of very low cost when assemble using disposable syringe bodies and micropipette tips. This last approach has only been recently tested for an organic compound [22], and never been applied to monitor complex target analytes such as proteins.

Considering that the PIM material will be integrated in a biosensor device of electrical nature, it is reasonable to expect that it should be assembled on a compatible and low cost material support of good overall electrical performance. So the surface imprinting was made on graphene sheets, a 2D structure of special electrical features and low electrical noise [23]. Its large surface area is also expected to provide high rebinding capacity to the final PIM structure. Protein molecules will be located at the surface of the graphene sheets with high surface-to-volume ratio, thus generating an improved kinetics and accessibility to the generated binding sites and an extended template removal [24]. These features correlated to an improved accessibility of the target species to the binding site, as well as reduced binding times [25]. The use of graphene as support for protein imprint was only most recently report [26].

Thus, the present work proposes a novel PIM for PSA, supported by graphene and displaying charged labels on the binding site. N/PIM and NIM were used as control materials to check the effect of the charged labels upon the material performance. All the prepared materials were used as ionophores in membranes of conventional solid-contact carbon electrodes and the resulting biosensors evaluated in terms of binding features, calibration slopes, dynamic linear range, limit of detection, effect of pH and selectivity. The best membrane composition was used to prepare micropipette tip-based electrodes of very low detection limit and to analyse serum samples.

2. Experimental

2.1. Reagents and solutions

De-ionized water (conductivity <0.054 μS/cm at 25 °C) was employed. All chemicals were of analytical grade and used without further purification.

Graphite (nanopowder <500 nm and mean pore size of 137 Å), potassium permanganate, sulphuric acid 95-97%, hydrogen peroxide 30%, hydrochloric acid 37%, sodium chloride and sodium hydrogen carbonate were obtained from Merck. Human Prostate Specific Antigen (PSA), *N*-ethyl-*N*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), trypsin, 2-Aminoethyl methacrylate hydrochloride 90% (AMH), vinyl benzoate (VB), acrylamide (AA), *N,N*-methylenebis(acrylamide) (NMAA), creatinine, human hemoglobin, bovine serum albumin (BSA), urea and glucose were obtained from Sigma–Aldrich. Potassium nitrate, benzoyl peroxide (BOP) and tetrahydrofuran (THF) were obtained from Riedel–deHäen. *o*-Nitrophenyloctyl ether (oNPOE), poly(vinylchloride) (PVC) of high molecular weight, and *N*-hydroxysuccinimide (NHS) were obtained from Fluka, and (vinylbenzyl)trimethylammonium chloride 97% (VTA) was purchased to Acros Organics.

Stock solutions of PSA 2.5×10^4 ng/mL were prepared in Hepes 1×10^{-4} mol/L (pH ~5.2) and less concentrated standard solutions were prepared by suitable dilution in the same buffer. The effect of pH was studied by changing the pH of a 50 mL PSA solution with a concentration of 7 ng/mL. The pH alteration was achieved by little additions of either concentrated hydrochloric acid or saturated sodium hydroxide solution, freshly prepared. Selectivity studies used creatinine (130 mg/L), urea (1900 mg/L), glucose (10.5 g/L), human hemoglobin (150 g/L) and BSA (50 g/L) solutions, prepared in Hepes buffer.

2.2. Apparatus

All potentiometric measurements were made in a Crison pH-meter GLP 21 (±0.1 mV sensitivity), at room temperature, and under constant stirring. The simultaneous reading of multiple potentiometric devices was enabled by a home-made commutation unit with six ways out. The assembly of the potentiometric cell using the solid-contact support was as follows: conductive graphite | PSA selective membrane | buffered solution (Hepes buffer 1×10^{-4} mol/L, pH 5.2, or artificial serum, pH 7.3) || electrolyte solution, KCl |AgCl(s) | Ag. The reference electrode was an Ag/AgCl electrode of double-junction from Crison, 5240.

The pH of solutions was measured by a Crison GLP 21 combined glass electrode connected to the above pH meter. An SBS vortex, MVOR 03, was used to grant a good mixing of the reacting solutions. Insoluble materials were suspended in a Sonorex digitec sonicator.