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Functionalized Single-Walled Carbon Nanotubes and Nanoparticles for the construction of high performance Biosensors

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1. Introduction :

Due to the increasing need to monitor health and environment in real time, new materials are under extensive investigations for the fabrication of reliable sensors appropriate for the detection of a wide range of target molecules. Between others, single-walled carbon nanotubes (SWCNTs), exhibit unique electrical, geometrical, and mechanical properties that make them an attractive material for the construction of ultra sensitive devices.

Due to these exceptional properties, the construction of nanotube based biosensors has become a steady growing research field. Immobilization of biomolecules around single-walled carbon nanotubes hes been realized by adsorption [1-3], covalent attachment to SWCNTs modified with carboxylic acid groups [4, 5], and incorporation in SWCNT-composite matrices [6, 7]. Particular interesting results were obtained by electropolymerization of biotin-pyrrole derivatives onto the SWCNT sidewalls to obtain highly functional nanotube composites[8]. The highly porous nanotube-polypyrrole layer provided affinity interactions with avidin. SWCNT coatings were thus functionalized with poly(biotin-pyrrole) and applied to the anchoring of glucose oxidase (GOX), modified with biotin.

Here, we report an alternative approach by using the affinity system adamantane - β-cyclodextrin (β-CD)[9], β-cyclodextrin modified gold nanoparticles could be attached onto adamantane functionalized SWCNT deposit as intermediate layer. This allows the immobilization of adamantane tagged GOX. The responses of such biosensors to glucose were measured by potentiostating the modified electrodes in order to oxidise the enzymatically generated hydrogen peroxide in presence of glucose and oxygen. Compared to similar glucose sensor setups, the highest sensitivity and maximum current density were recorded for the configuration based on β-cyclodextrin modified gold particles as intermediate layer between adamantane functionalized SWCNTs and GOX.

2. Experimental :

Single-walled carbon nanotubes, produced by the HiPco® process (Purified, CNI grade/Lot #: P0313), were purchased from Carbon Nanotechnologies, Inc. (CNI) and used as received.

Adamantyl-11-pyrrolyl-1-undecyl carboxylic acid amide, the β-CD-gold nanoparticles, and the functional enzymes were prepared as described [9, 10].

Electropolymerization and cyclic voltammetric experiments were performed with an EG&G PARC, Model 173 potentiostat equipped with a Model 175 universal programmer and a Model 179 digital coulometer in conjunction with a Kipp and Zonen BD 91 XY/t recorder. All electrochemical experiments were carried out in a conventional three-electrode cell under argon atmosphere in a glove box. The amperometric measurements were performed with a Tacussel PRG-DL potentiostat in phosphate buffer solution (0.1M, pH 7). All enzymatic electrodes were prepared on platinum disks (5mm diameter) polished with 2 µm diamond paste (MECAPREX Press PM). An aqueous saturated calomel electrode (SCE) was used as reference electrode while a Pt wire placed in a separate compartment containing the aqueous electrolyte served as counter electrode.

FE-SEM images were recorded using ULTRA 55 FESEM based on the renowned GEMENI® FESEM column with beam booster (Nano Technology Systems Division, Carl Zeiss NTS GmbH, Germany) and a tungsten gun. For nanotube containing electrodes 5 kV were applied as accelerating voltage whereas 2kV were used for the adamantane-polypyrrole coated electrode.

3. Results and Discussion :

To apply this principle for the construction of biosensor devices, the electrochemical polymerization of adamantane-pyrrole onto a deposit of carbon nanotubes was followed by incubation with β-cyclodextrin modified glucose oxidase (GOX) which serves again as enzyme model [11].

The analytical characteristics of the resulting 3D enzyme configuration towards the detection of glucose were investigated (Figure 1). Compared to the corresponding "2D" configuration using poly(adamantane-pyrrole) with β-cyclodextrin modified GOX (Figure 1C), the performance of the SWCNT-based biosensor shows a 20 fold increase (from 0.425 to 8.72 $mAM^{-1}cm^{-2}$) of the sensitivity (Figure 1B). In addition, a remarkable enhancement of the J_{max} values by a factor 3.5 was observed suggesting that the geometrical effect of nanotube leads to a larger area covered by the poly(pyrrole adamantane) film and hence an increase in the amount of anchored GOX. The design of 3D bioarchitectures was pursued by combining SWCNT coating, affinity polymer and gold nanoparticles. The construction of the SWCNT-poly(adamantane-pyrrole) biosensor was carried out using β-CD modified gold nanoparticles as intermediate layer for the specific anchoring of adamantane-tagged GOX (Figure 1A). The resulting glucose sensor exhibits an even higher sensitivity and J_{max} value.

The comparison with the performance of the corresponding similar configuration without SWCNT (sensitivity 0.980 $mAM^{-1}cm^{-2}$ and J_{max} 75 μAcm^{-2}) (Figure 1C) highlights the beneficial role played by the nanotube coating. Moreover, the increase in J_{max} values by a factor 4.6 seems to indicate a combined synergetic effect of SWCNTs and gold nanoparticles that provides a higher amount of immobilized enzyme. The higher immobilized enzyme activities postulated for the two SWCNT-based biosensors were supported by the lower K_M^{app}

values determined for SWCNT and SWCNT-gold nanoparticles configurations, namely 8 and 5 mM, respectively. High density of immobilized GOX led to biosensor kinetic limited by the oxygen concentration with K_M^{app} values comprised between 1-5 mM while lower immobilized amount like a GOX monolayer exhibited K_M^{app} values of 10-25 mM [12, 13]. In addition, the comparison of the J_{max} values between SWCNT and SWCNT-gold particles configurations, clearly confirms the higher amount of immobilized enzyme due to the presence of β-CD gold nanoparticles.

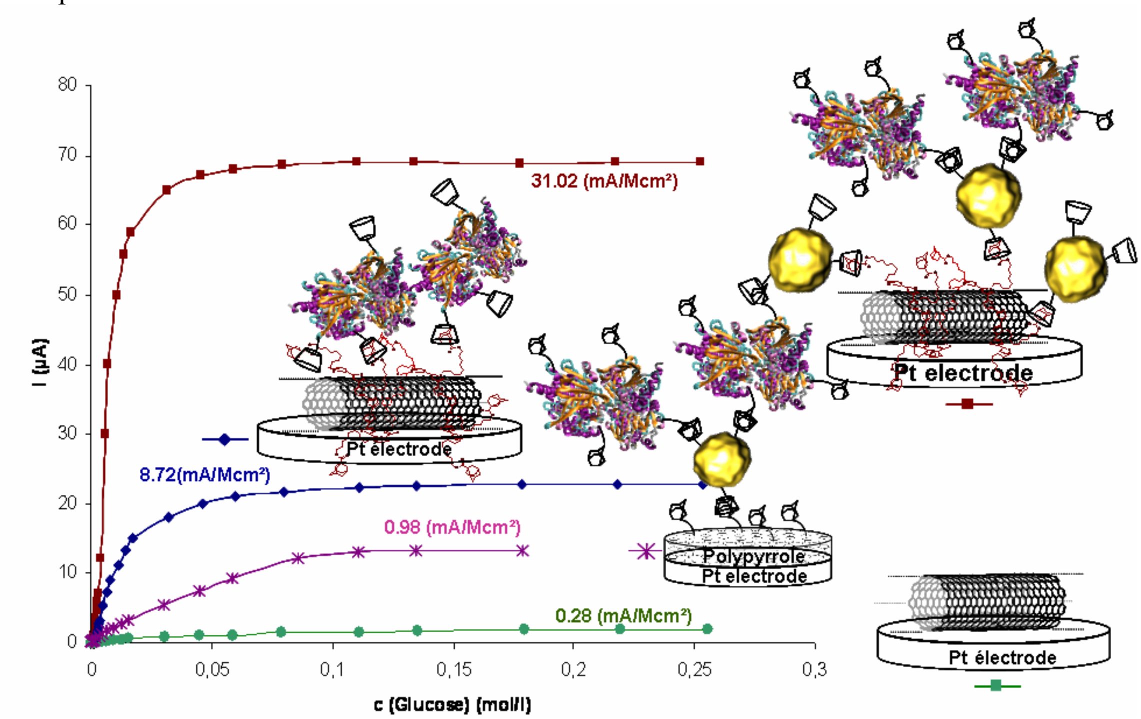

Figure 1: Calibration curves reflecting the sensitivities of the constructed glucose sensors using A) SWCNT- poly(adamantane-pyrrole) – β-CD gold nanoparticles and adamantane modified GOX, and B) SWCNT- poly(adamantane-pyrrole) and β-CD modified GOX. Calibration curves of reference experiments using C) poly(adamantane-pyrrole) – β-CD gold nanoparticles and adamantane modified GOX, and D) adamantane free SWCNTs - β-CD gold nanoparticles and adamantane modified GOX.

The influence of non-specific binding was investigated by similar cross experiments. A representative calibration curve (Figure 7D) of these control experiments show sensitivity and J_{max} values (0.28 $mAM^{-1}cm^{-2}$ and 15 μAcm^{-2} respectively) that are markedly lower than those reported for both SWCNT-based biosensors with and without gold nanoparticles as intermediate layer. The K_m^{app} value is 44mM, confirming the low amount of non-specifically adsorbed GOX.

4. Conclusion :

The described electropolymerization of adamantane-pyrrole monomer onto a deposit of single walled carbon nanotubes combined with affinity interactions represents an elegant approach for the construction of high performance amperometric biosensors. The combination of SWCNT coating with adamantan modified or biotinylated polymers offers a high specific surface with an excellent accessibility for the direct anchoring of glucose oxidase. In particular, the use of β-cyclodextrin gold nanoparticles as an intermediate layer for the specific anchoring of adamantane-tagged enzyme has led to efficient amperometric glucose biosensors. The possible successive anchoring of gold nanoparticles and enzyme molecules may constitute an attractive way for the elaboration of multilayered enzyme assemblies. Moreover, the combination of SWCNT coating with affinity polymer and gold nanoparticles offer a high specific surface with an excellent accessibility for the anchoring of proteins via affinity interactions. It is expected that such 3D composite configuration will be useful for the development of immunosensors and DNA sensors.

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