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Functionalized carbon nanotubes for bioelectrochemical applications: Critical influence of the linker

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ABSTRACT

A series of covalently bonded ferrocene derivatives to carbon nanotubes via either alkyl or poly(ethylene glycol) (PEG) spacers has been prepared and deposited onto glassy carbon electrodes using chitosan as a binder. Evaluation of the effect of spacer arms revealed that the hydrophilic and flexible PEG chain greatly facilitates electron transfer reactions. When adding diaphorase in the composite, effective electrocatalytic oxidation of NADH was achieved. The PEG spacer also permitted an efficient dispersion of the functionalized carbon nanotubes, without additives. In this way, they can be easily incorporated in water-based sol–gel matrices, as illustrated for bioelectrochemical applications with co-immobilized glucose dehydrogenase, diaphorase and NAD^+ cofactor.

1. Introduction

The efficient electron transfer communication between an electrode surface and the enzyme active centres is a prerequisite for constructing amperometric biosensors and biofuel cell elements. To this end, the use of electron relay units that transport the electrons between the enzyme redox centre and the conducting surface is essential [1,2]. One could anticipate two general approaches to establish such electronic communication [3]. The first one comprises of so-called "electroenzymes", in which the protein itself is modified by covalently bonded redox mediators. Chemical modification of proteins by electron relay has been successfully applied in oxidative pathway, e.g., with glucose oxidase or dehydrogenase and lactate oxidase [4–6]. A second approach concerns the covalent attachment of the redox mediator via a long and flexible spacer arm leading to electrical wiring between an enzyme active site and the electrode surface [3,7], and hence effective mediated electron transfer can be expected [8].

Electrochemical oxidation of nicotinamide adenine dinucleotide (NADH) has received considerable attention due to its significance for designing novel biosensors because NAD-dependent dehydrogenases constitute one the largest group of redox enzymes [9]. Both direct electrochemical NADH oxidation and NAD^+ reduction reactions require the application of high overpotentials, often leading to electrode poisoning; moreover, the direct electrochemical reduction of NAD^+ leads to non-enzymatically active species [10,11]. These drawbacks can be overcome by using electron transfer mediators as the electrocatalysts that are able to lower the overpotentials usually observed for the NADH/NAD^+ redox couple. Diaphorase (DI) is an enzyme utilized to enhance electron transfer reaction rates and is a suitable counterpart in bioelectrocatalysis for the oxidation of NADH and for the reduction of NAD^+ in presence of a suitable redox mediator [12–14]. A variety of redox mediators, such as quinones, methylviologen, and flavinic compounds [12,14–17] have been used for this purpose. Hence, diaphorase-catalysed oxidation of NADH is an interesting example of mediated electron transfer for development of electrochemical devices based on NADH recycling, e.g. biofuel cells or for electrosynthesis [18,19].

Carbon nanotubes (CNTs) are widely used to improve the electron transfer between enzyme redox sites and an electrode surface and they also constitute promising platforms for immobilization of various redox mediators [20,21]. CNTs have received growing interest in the field of biosensors owing to their attractive features in terms of enhancing the sensitivity and/or selectivity towards chemical or biological compounds [22,23]. The study of the oxidation of nicotinamide adenine dinucleotide using CNTs has been the subject of numerous studies related to the development of amperometric biosensors [24–27]. For example, glassy carbon electrodes modified with CNT coatings (dispersed in a solution of

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concentrated sulfuric acid) revealed decrease in the overvoltage for the NADH oxidation (490 mV) and also eliminated surface fouling effects [26]. Facilitation of NADH oxidation at CNTs treated by microwaving has been described by Wooten and Gorski [27].

One has to be aware that carbon nanotubes tend to aggregate together in almost all kind of aqueous or organic solutions due to the van der Waals forces between the tubes. This fact imposes strong difficulties in preparing homogenous CNTs dispersions and thus greatly hampers electrochemical studies and their electroanalytical applications [21,28–30]. In general, getting an electrochemical sensor modified with a CNT-dispersion consists of casting the electrode, usually glassy carbon or gold, with a drop of the given dispersion, followed by a drying step under appropriate conditions. Different media (solutions/binders) such as dimethylformamide (DMF) [31], nafion [21,32], chitosan [33], polyethylenimine [34] or surfactants [35–37] have been used for CNTs dispersion. Significant progress has also been made in covalent and non covalent functionalization of CNTs since those strategies can improve solubilizing the CNTs and further facilitating their manipulation [38,39]. However, methods for the preparation of CNTs-modified electrodes from an electrochemical point of view are still needed [21].

Ferrocene derivatives are often employed to modify bioelectrodes since they act as redox mediator between the electrode and the redox active centre of an enzyme [40]. To prevent any loss of mediator in solution, different matrices for ferrocene encapsulation (e.g., chitosan, conducting polymers, hydrogels, sol–gel, etc.) have been used in literature [41–44]. Besides, some attempts of covalent or non-covalent attachment of ferrocene derivatives to CNTs have been described [45–49]. In general, π-stacking or aryldiazonium reduction were used to modify either single-walled or multi-walled carbon nanotubes.

In this work, we have followed an original strategy for covalent bonding of ferrocene derivatives to carbon nanotubes (CNTs-Fc) via either alkyl (CNT-$(CH_2)_n$-Fc) or poly(ethylene glycol) (CNT-$(EtG)_n$-Fc) linkers (Figs. 1B and 1C) and we have studied their behaviour towards the electrocatalytic oxidation of NADH. To the best of our knowledge, the role of a linker between CNTs and ferrocene (CNTs-Fc) on its electrochemistry connected with an enzymatic reaction was not considered so far in the literature. At first, glassy carbon electrodes modified by CNTs-Fc (in a chitosan matrix) were tested for diaphorase-catalyzed NADH oxidation. In a second step, CNTs-Fc were successfully incorporated in silica-based gels for co-immobilization with glucose dehydrogenase, diaphorase and NAD^+ cofactor, in the goal to evaluate their interest in reagentless bioelectrochemical devices.

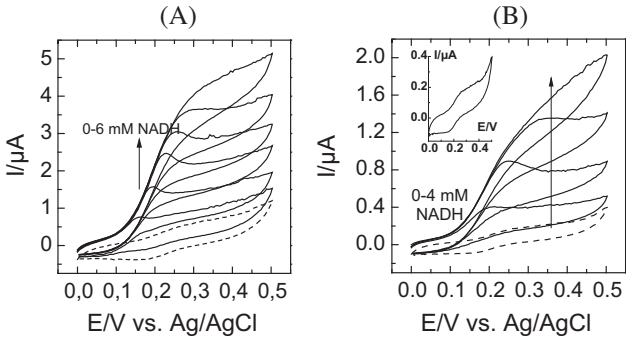

Fig. 2. Cyclic voltammetry responses to NADH measured at GCE modified with (A) chitosan/CNT-$(EtG)_2$-Fc and chitosan/DI overlayer and (B) chitosan/CNT-$(EtG)_8$-Fc and chitosan/DI overlayer. Inset in (A) shows the electrochemical response measured with CNT-$(EtG)_8$-Fc in the absence of NADH. All measurements have been performed in 0.1 M Tris–HCl buffer (pH 9.0) at a scan rate of 20 mV s^{-1}.

2. Experimental

2.1. Chemicals and materials

Carboxylic acid-functionalized single-walled carbon nanotubes (D1.5L1-5-COOH) were purchased from Nanolab. Diaphorase (DI, from *Bacillus stearothermophilus*, 1020 units mg^{-1}, Unitika, Japan.), ß-nicotinamide adenine dinucleotide, reduced dipotassium salt (NADH, 97 wt%, Sigma), ß-nicotinamide adenine dinucleotide (NAD^+, 98 wt%, Sigma), D-glucose (99 wt%, Acros), glucose dehydrogenase (GDH, from *Pseudomonas* sp., 200 units mg^{-1},

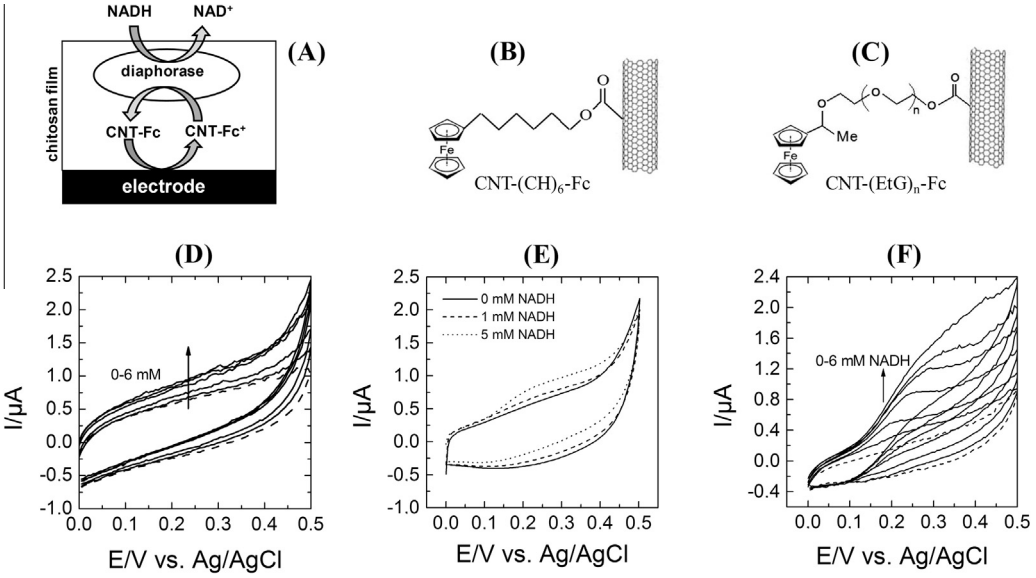

Fig. 1. (A) Schematic illustration of glassy carbon electrode (GCE) modified by chitosan/CNTs-Fc with chitosan/diaphorase overlayer for NADH oxidation. (B) Pictorial representation of carbon nanotubes covalently modified with ferrocene via alkyl: $(CH_2)_6$. (C) Pictorial representation of carbon nanotubes covalently modified with ferrocene via polyethylene glycol: $(EtG)_n$ linker. (D) Cyclic voltammetry responses to NADH measured at GCE modified with chitosan/CNT and chitosan/DI overlayer. (E) Cyclic voltammetry responses to NADH measured at GCE modified with chitosan/CNT-$(CH_2)_6$-Fc and chitosan/DI overlayer. (F) Cyclic voltammetry responses to NADH measured at GCE modified with chitosan/CNT-(EtG)-Fc, and chitosan/DI overlayer. All measurements have been performed in 0.1 M Tris–HCl buffer (pH 9.0) at a scan rate of 20 mV s^{-1}.