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Dendrimer-encapsulated silver nanoparticles as a novel electrochemical label for sensitive immunosensors

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ABSTRACT

This paper reports on the synthesis and characterization of a novel electrochemical label for sensitive electrochemical stripping metalloimmunoassays based on silver dendrimer-encapsulated nanoparticles (NPs). Silver dendrimer nanocomposites (Ag-DNCs) were synthesized from a generation 5–7 (G5–7) hydroxyl-terminated ethylenediamine-core-type (2-carbon core) PAMAM dendrimer. Several fixed ratios of Ag^+/dendrimer were prepared with the aim to obtain stable nanocomposites with maximal silver loading in the interior of a polymeric shell. Synthesized Ag-DNCs were characterized by UV–vis spectrophotometry, atomic force microscopy (AFM), transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS). The G7 Ag-DNC synthesized in 2000 molar excess of silver (1/4 ratio of tertiary amine/Ag^+) turned out a more suitable candidate for the label development. By combination of the differential pulse voltammetry (DPV) and the anodic stripping analysis (ASV) on a carbon electrode, down to $1.35 \times 10^{+10}$ of individual Ag-DNCs (LOD = 0.9 pM, 25 ml volume) was detected after the dissolution of silver nanoparticles in a diluted nitric acid. The potential advantages of proposed electrochemical label are discussed.

1. Introduction

Over the past few years, the novel highly sensitive electrochemical detection schemes of biorecognition events were presented predominantly in the area of DNA sensors. Assays based on the electrochemical stripping analysis of metal or semiconductor nanoparticle labels have attracted significant attention mainly due to their high sensitivity and their possibility of multiplexing. So far, gold (Dequaire et al., 2000; Wang et al., 2001; Liu and Lin, 2005), silver (Cai et al., 2002; Lee et al., 2003), copper (Liu and Lin, 2005) or semiconductor (Wang et al., 2002; Zhu et al., 2004; Merkoçi et al., 2007) nanoparticle labels have been studied extensively in the field of DNA biosensors. In addition to it, their application and promising fmol detection limits in case of immunosensors have also been reported (Liu et al., 2004).

Various methods of metal nanoparticle synthesis have been published. Recently, dendrimers have turned out to be an effective polymeric scaffold for the preparation of highly stable and monodispersed noble metal nanoparticles, often referred to as dendrimer metal nanocomposites (Esumi et al., 2000; Ottaviani et al., 2002). Dendrimers are highly and regularly hyperbranched oligomers synthesized from a branched reaction core by repeated cycles of synthesis. The relatively free and accessible space inside and the flexible modulation of dendrimer properties is the reason for their potential use as nanoscaffolds for targeted drug delivery and diagnosis (Kobayashi et al., 2000; Kobayashi et al., 1999). The most common process of nanoparticle synthesis is usually based on a chemical complexation of a dendrimer with metal salt and its subsequent reduction by UV irradiation (Esumi et al., 1997; Zhao et al., 1998) or, more frequently, by addition of $NaBH_4$ (Endo et al., 2005; Xu et al., 2007). The resulting metal nanoparticles are formed either in the dendrimer interior or its periphery depending on the metal used, the type of reaction core and on the peripheral functional groups of the dendrimer.

In this paper we present a synthesis of highly stable Ag-NPs in a PAMAM-OH polymer scaffold and their characterization by several analytical methods. Our research has proved that a stable silver dendrimer nanocomposite potentially represents an alternative electrochemical label for a sensitive detection of binding events in various immunoassay formats.

2. Materials and methods

2.1. Chemicals

Hydroxyl-terminated G5–7 PAMAM ethylenediamine core type (2-carbon core) dendrimers in methanol solution (5 wt.%) were pur-

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chased from Sigma–Aldrich, Inc. (Germany). Sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), nitric acid (HNO_3), and potassium chloride (KCl) were also purchased from Sigma–Aldrich, Inc. All solutions were prepared in ultrapure water with conductivity below 1 μS cm^{-2} (Goro Pharmapur system, Czech Republic).

2.2. *Preparation of dendrimer-encapsulated silver nanoparticles*

Prior to the silver nanocomposite synthesis, the PAMAM-OH dendrimers were desiccated by methanol evaporation in a dryer (50 °C, 1 h) and dissolved in distilled water to yield 0.4 μM stock solutions. For the synthesis of Ag-DNCs, dendrimers were mixed with water solution of silver nitrate in molar ratios: 1:125, 1:250, 1:500, 1:1000, 1:2000 and 1:4000 (dendrimer/Ag^+). Molar ratios 1:125, 1:250, 1:500 correspond to the presence of the amount of tertiary amines in G5, G6 and G7 (∼1:1 ratio), respectively. The synthesis was performed by mixing dendrimer/Ag^+ and 3.2 mM water solution of sodium borohydride in 1:1 volume ratio dropwise at constant speed (10 ml/min). During the synthesis, the solution was stirred slowly. The synthesis of NPs occurred immediately after dendrimer/Ag^+ and $NaBH_4$ solutions were mixed. The reaction was observed by changes in colour. Depending on molar ratios of dendrimer and Ag^+ ions, the solution mixtures stayed without or with intensive colour changes from bright yellow to dark-brown. Intensive colour changes were observed up to 2 h after the synthesis.

2.3. *UV–vis spectral analysis*

UV–vis absorbance spectra were recorded by a double-beam spectrophotometer (Unicam UV 500, Thermo Spectronics, USA) within λ = 180–900 nm in the transmission mode. The data were analyzed by the Vision$^{\text{Pro}}$ spectral analysis software. UV–vis spectra of silver nanocomposite samples were recorded before, immediately after, 1 h after, 3 days after and 14 days after the synthesis. For kinetic measurements, the absorbance change at a fixed wavelength λ = 410 nm corresponding to the development of a stable plasmonic peak was recorded and plotted against time. In this case, the synthesis of NPs was performed in 1 ml volume directly in the spectrophotometer cuvettes.

2.4. *Atomic force microscopy (AFM) analysis*

AFM characterizations were performed with an atomic force microscope Integra Probe Nanolaboratory (NT-MDT, Russia) with the close-loop sample scanning arrangement and the semi-contact resonance mode. The samples were prepared by dropping of 10 μl of isopropanol/water (10/1) solution of dendrimers or Ag-DNCs (10^{-8} M) over mica and by a subsequent solvent evaporation at room temperature. The analysis was performed in air and at the ambient temperature and humidity. High accuracy noncontact composite (HA_NC) ETALON silicon tip cantilevers with a resonant frequency 280 kHz, a tip radius 10 nm and a constant force 11.5 N/m provided from NT-MDT (Russia) were used. The results were analyzed with the Nova$^{\text{TM}}$ image processing software. In case of the grain analysis, a scan, which yielded min 400 counts at 3 nm threshold was analyzed. The *z* dimension of individual particles was used for the histogram analysis and the average particle diameter calculation.

2.5. *Transmission electron microscopy (TEM) analysis*

TEM observations were performed (at the Institute of Cell Biology and Pathology, 1st Faculty of Medicine, Charles University in Prague) at room temperature on a Tecnai G2 Sphera 20 microscope equipped with a LaB6 cathode and a 2048 × 2048 Gatan Ultrascan 1000 CCD camera of a pixel size 14 μm. The microscope was operated at the accelerating voltage 200 kV. All images were recorded at a defocus close to the Scherzer focus with magnification 150,000. Samples were prepared on ultrathin carbon support films without staining.

2.6. *X-ray photoelectron spectroscopic (XPS) analysis*

Concentrated water solution (10^{-7} M) of dendrimer/Ag^+ complex and Ag-DNCs was spread (20 μl drop) over a polished silica substrate (1 cm^2) and dried on air at room conditions. The samples were analyzed for their chemical composition by a custom made X-ray photoelectron spectroscope (X-ray source: Specs XR 50, input power 200 W, Al/Mg anode, without monochromator; Electron energy analyzer: Specs PHOIBOS, hemispherical, single channeltron; System's ultimate pressure: <10^{-8} Pa).

2.7. *Differential-pulse anodic stripping analysis (DPASV)*

DPASV measurements were performed on an Autolab PGSTAT 12 potentiostat (EcoChemie, Netherlands) with a saturated calomel reference, a platinum auxiliary and a homemade pencil carbon-working electrode (diameter ∼0.7 mm) fixed in an electrochemical glass cell (25 ml). The measured data were processed with the General Purpose Electrochemical System (GPES) Autolab software.

Firstly, to acquire the silver calibration curve, the analyzed amount of $AgNO_3$ was dissolved in 0.1 M HNO_3 and the silver ions were electrochemically detected by ASV. Secondly, the Ag-NPs were dissolved in 0.1 M HNO_3 and the released silver ions were electrochemically detected. The ASV calibration curves were also acquired. The amount of the added nanocomposite was calculated by spectroscopic measurements. UV–vis calibration was based on the known amount of the dendrimer prior to the synthesis and the optical absorbance of freshly (but stable) prepared dilutions of the silver nanocomposite.

Before every measurement, electrodes (electrochemical cell) were washed twice in 0.1 M HNO_3. During each washing step, DPV strips from −0.45 to 0.45 V at 49.5 mV/s scan rate were applied. Then, the working electrode was amperometrically treated for 2 min with the working potential 0.2 V. In the following step, the electrode system was washed again in nitric acid and the anodic strips were applied until the measured baseline was flat. During the DPASV measurements, diluted silver ions were first deposited at −0.5 V for 600 s and further detected by an anodic DPV strip starting from −0.45 to 0.45 V at 49.5 mV/s scan rate. All data were collected with the following settings: equilibration time: 15 s; modulation time: 0.05 s; interval time: 0.1 s; step potential: 0.00495 V; modulation amplitude: 0.12 V. The limit of detection (LOD) was statistically calculated as $\text{LOD} = (b + kS_b)/m$, where b is the intercept of the linear approximation of the calibration curve, $k = 3$, S_b is the standard deviation of the regression line and m is the slope of the calibration curve.

3. Results and discussion

3.1. *Synthesis and UV–vis characterization of Ag-DNCs*

Among the other metals, silver is quite useful for the anodic stripping analysis due to its low stripping potential (∼0.2 V vs. SCE), easy dissolution under mild acidic conditions and low occurrence in biological material. Therefore, we concentrated our efforts on the synthesis, characterizations and exploitation of Ag-DNCs in the electrochemical stripping analysis although similar results could certainly be obtained with other metals as well. As reported by others, stable Ag-NPs may be prepared inside of PAMAM dendrimers