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Short communication

Electrogenerated chemiluminescence behavior of peptide nanovesicle and its application in sensing dopamine

Chunxiu Huang^a, Xu Chen^{a,*}, Yanluo Lu^a, Hui Yang^b, Wensheng Yang^{a,*}^a State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, China^b Beijing Institute for Neuroscience, Capital Medical University, Beijing Center of Neural Degeneration and Repairing, Key Laboratory for Neurodegenerative Diseases of Ministry of Education, Beijing 100069, China

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ABSTRACT

The electrogenerated chemiluminescence (ECL) behavior of the bioinspired peptide nanovesicles (PNVs) was reported for the first time. The PNVs modified glassy carbon electrodes have shown a stable and efficient cathodic ECL signal with $K_2S_2O_8$ as coreactant in aqueous solution. The possible ECL reaction mechanism was proposed. Dopamine (DA) was chosen as a model analyte to study the potential of the PNVs in the ECL analytical application. It was found that the ECL intensity of the PNVs was effectively increased by trace amounts of DA. The limit of detection was estimated to be 3.15 pM ($S/N=3$). These results suggest that the PNVs could be a new class of promising materials for the ECL design and bioassays in the future due to their fascinating features, such as excellent biocompatibility, tunable composition as well as capability of molecular recognition.

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1. Introduction

Electrogenerated chemiluminescence (ECL) is an attractive analytical technique due to its high sensitivity and wide linear range. In recent years, the employment of nanomaterials has promoted the rapid development of ECL in bioanalytical applications (Ding et al., 2002; Miao, 2008; Li et al., 2012). Semiconductor nanocrystals, such as CdSe (Myung et al., 2003) and CdTe (Cheng et al., 2010), are often used in ECL studies. However, the inherent toxicity from the heavy metal elements limits their wide applications in the bioassay. To maintain a benign environment, low-toxicity silicon (Ding et al., 2002) and carbon (Zheng et al., 2009) nanocrystals (NCs) as well as Au nanoclusters (Li et al., 2011) have been tried in ECL reactions. Nevertheless, the analytical performances based on their ECL still needed to be further improved. Therefore, the development of new highly efficient, biocompatible and tunable ECL nanoemitters is highly desirable for both fundamental and bioanalytical applications (Hu and Xu, 2010; Deng and Ju, 2013).

Bioinspired nanomaterials, especially fabricated from peptide building blocks, are of increasing interest owing to their biocompatibility, well-defined structures and capability of molecular recognition (Hartgerink et al., 2001; Scanlon and Aggeli, 2008). Recently the simplest peptide building blocks of diphenylalanine

(FF), found as the structural motif for the β -amyloid associated with Alzheimer's disease, have attracted considerable attention (Reches and Gazit, 2003; Kim et al., 2010). Self-assembled FF-based nanostructures, especially peptide nanotubes (PNTs), have extraordinary mechanical strength and good chemical and thermal stability (Kol et al., 2005), which make them appealing structural elements for various applications (Yemini et al., 2005; Yan et al., 2010). Very recently, Rosenman et al. observed the quantum confinement (QC) phenomenon in PNTs (Amdursky et al., 2009a), peptide nanospheres (Amdursky et al., 2009b) and hydrogels (Amdursky et al., 2010a), which was found previously only in semiconductor crystals. Further investigation on these self-assembled peptide structures indicated that they were composed of nanocrystalline regions or quantum dots (QDs) (Amdursky et al., 2010b). These bionanostructures with QC features have exceptional electronic and photonic properties, which make them new candidates of environmentally clean optical materials for luminescence devices (Ryu et al., 2009; Yan et al., 2011; Kim et al., 2011). However, to the best of our knowledge, no research has been reported involving the FF and its derivative-based nanostructures in the ECL study.

In the present work, we at the first time explored the ECL properties of cationic dipeptide (H-Phe-Phe-NH₂·HCl, derived from FF, see Fig. 1A) self-assembled nanovesicles (PNVs). The nanospherical morphology is preferred in biosensing, labeling of biomolecules and bioimaging applications relative to long nanotubes. The cathodic ECL of the PNVs modified glassy carbon electrodes (GCE) was firstly observed in the presence of coreactant

* Corresponding authors. Tel.: +86 10 64435271; fax: +86 10 64425385.

E-mail address: chenxu@mail.buct.edu.cn (X. Chen).

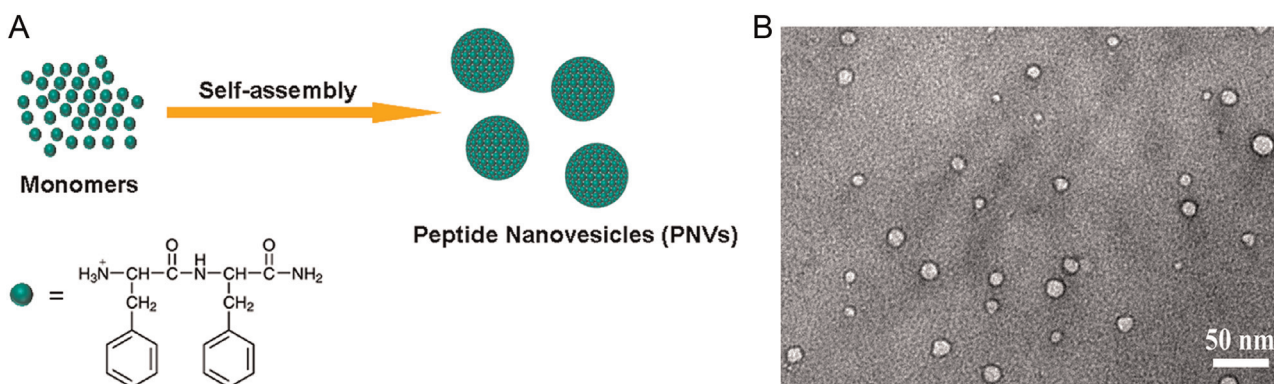


Fig. 1. (A) Schematic illustration of cationic dipeptide self-assembly into PNVs. Inset: Molecular structure of cationic dipeptide. (B) TEM image of PNVs.

$K_2S_2O_8$. The possible ECL reaction mechanism was proposed. The analytical application of the PNVs ECL was also demonstrated.

2. Experimental

2.1. Materials

The cationic dipeptide (H-Phe-Phe-NH₂ · HCl, CDP) in a lyophilized powder was obtained from Bachem AG (Switzerland). 3-Hydroxytyramine hydrochloride (Dopamine) and 1.1.1.3.3.3-hexafluoro-2-propanol (HFIP) were purchased from Sigma-Aldrich. Phosphate-buffered solution (PBS) (pH 7.2, 0.2 M) was prepared using 0.2 M Na₂HPO₄, 0.2 M NaH₂PO₄ and 0.1 M KCl. All other reagents were of analytical grade and used as received without further purification. Ultrapure water was used throughout the experiments.

2.2. Apparatus

Transmission electron microscopy (TEM) images were taken on a JEOL JEM-2100 electron microscope with an accelerating voltage of 100 kV. The ECL spectra were recorded by collecting the ECL data during the cyclic potential sweep with a series of optical filters. Cyclic voltammetric and ECL measurements were carried out with a Model MPI-E Electrochemiluminescence Analyzer (Xi'an Remex Analytic Instrument Co., Ltd., Xi'an, China). Detection was carried out in a static ECL system. The voltage of the photomultiplier tube (PMT) was set at 800 V and the scan rate was 100 mV s⁻¹ in the process of ECL detection. A conventional three-electrode system was used in the experiment with a bare or modified glassy carbon working electrode (diameter 3 mm), a Pt wire counter electrode, and an Ag/AgCl reference electrode.

2.3. Synthesis of peptide nanovesicles (PNVs)

PNVs were prepared according to a previously reported method (Yan et al., 2007). Fresh stock solutions were prepared by dissolving 5 mg lyophilized CDP powder in 40 μL HFIP. Then the stock solution was diluted to 10 mg mL⁻¹ by using a pH 7.2 PBS. Finally, the 10 mg mL⁻¹ solution was diluted to 1 mg mL⁻¹ by using ultrapure water and the self-assembled PNVs were formed. To eliminate the possible residual monomer and cytotoxic HFIP, the PNVs solution was further dialyzed for 48 h with ultrapure water. The resulting solution was used in the experiments to the characterization of the PNVs and the fabrication of the modified electrodes.

2.4. Preparation of PNVs modified electrode

Prior to use, the GCE was polished carefully with 1.0, 0.3 and 0.05 μm alumina slurry, followed by washing thoroughly with water. Then the electrode was sonicated successively in water and allowed to dry in a stream of N₂. PNVs solution (optimized 6 μL) was dropped on the surface of the GCE and dried at room temperature.

3. Results and discussion

3.1. Characterization of PNVs

Fig. 1B displays a TEM image of the PNVs. A typical spherical and vesicle structure with the diameters of 10–25 nm was observed. A typical atomic force microscopy (AFM) image (Fig. S1) of the PNVs on mica slice further confirmed the morphology of the PNVs. The cross-sectional profile shows that the PNVs are approximately 5 nm in height, indicating that they are flat (the diameter of the PNVs in two dimensions, 10–25 nm). Moreover, UV-vis spectrum of the PNVs solution showed the identical spike-like behavior (Fig. S2), indicating the existence of identical nanosize regions of QDs in the PNV structures (Amdursky et al., 2010b). Additionally, the photoluminescence (PL) spectrum of the PNVs solution exhibited two peaks under excitation at 250 nm (Fig. S3). The first peak was located at 281 nm, which is characterization of the phenylalanine residue (Mihalyi, 1968). A second peak was found at about 559 nm, which might be attributed to the assembled nanostructures (Amdursky et al., 2009a).

3.2. ECL behavior of PNVs modified electrode

Fig. 2A shows the ECL responses obtained on the bare and PNVs modified GCEs in 0.2 M PBS (pH 7.2) containing 0.1 M K₂S₂O₈ and 0.1 M KCl. Herein, K₂S₂O₈ played a coreactant role, which is generally used in ECL systems. It was found that only a weak ECL signal (line a in Fig. 2A) was observed on the bare GCE when the potential was cycled between 0 and -1.60 V. However, a strong ECL signal, about 7 times than that of the bare GCE, was obtained at -1.30 V on the PNVs modified GCE (line b in Fig. 2A). These results suggested that the ECL signal originated from the system of PNVs. Furthermore, the ECL signal remained at an almost constant value during consecutive cyclic potential scanning (Fig. 2B), indicating the good reversibility of the modified electrode. Additionally, the ECL working conditions were optimized and shown in Fig. S4. The optimal parameters were used throughout this work.

The ECL spectra were also measured by employing a series of optical filters, and a distinguished ECL spectrum peak at approximately 627 nm was observed (Fig. 2C). It was different with that

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