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Identification of catecholamine neurotransmitters using fluorescence sensor array

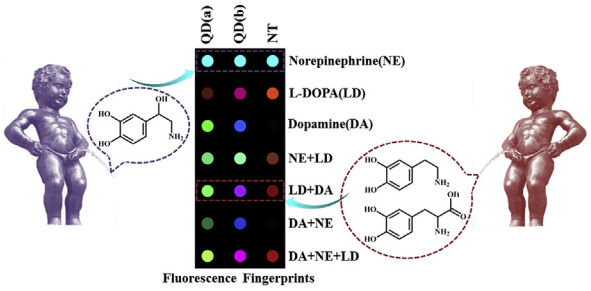
Forough Ghasemi [a], M. Reza Hormozi-Nezhad [a, b, *], Morteza Mahmoudi [c, d, **]

[a] Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran
[b] Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Tehran, Iran
[c] Department of Nanotechnology and Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 13169-43551, Iran
[d] Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305-5101, United States

HIGHLIGHTS

- We have proposed a fluorescence sensor array to detect catecholamine neurotransmitters.
- Visual differentiation of catecholamines is provided by fluorescence array fingerprints.
- Discrimination of catecholamines from each other, and from their mixture is obtained on a PCA plot.
- Proposed sensor array can be used for detection of catecholamines in urine samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A nano-based sensor array has been developed for identification and discrimination of catecholamine neurotransmitters based on optical properties of their oxidation products under alkaline conditions. To produce distinct fluorescence response patterns for individual catecholamine, quenching of thioglycolic acid functionalized cadmium telluride (CdTe) quantum dots, by oxidation products, were employed along with the variation of fluorescence spectra of oxidation products. The spectral changes were analyzed with hierarchical cluster analysis (HCA) and principal component analysis (PCA) to identify catecholamine patterns. The proposed sensor could efficiently discriminate the individual catecholamine (*i.e.*, dopamine, norepinephrine, and L-DOPA) and their mixtures in the concentration range of 0.25–30 μmol L^{-1}. Finally, we found that the sensor had capability to identify the various catecholamines in urine sample.

1. Introduction

Catecholamines (*e.g.*, dopamine (DA) and norepinephrine (NE)) play vital roles as neurotransmitters or hormones at central and peripheral levels [1,2]. DA, which is derived from the decarboxylation of 3,4-dihydroxyphenylalanine (L-DOPA (LD)) [3], is involved in fine motor activity, inspiration, blood pressure, intuition, and focus [4–6]. NE (secreted in the adrenal medulla) decreases peripheral circulation, stimulates arteriole

* Corresponding author. Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran.
** Corresponding author. Department of Nanotechnology and Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 13169-43551, Iran.
E-mail addresses: hormozi@sharif.edu (M.R. Hormozi-Nezhad), mahmoudi@stanford.edu (M. Mahmoudi).

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contraction, and affects human cardiovascular system [7,8]. Therefore, detection of catecholamine's abnormalities in the biological fluid, in terms of their concentrations and by products, is of quite importance. For example, their abnormal concentration can be related to the variety of diseases including Schizophrenia, Parkinsonism, Alzheimer, Huntington, severe head trauma, various neuroblastoma, adrenocortical carcinoma, pituitary adenoma, depression, pheochromacytoma and cancerous tumors [4,6,9–13]. Hence, monitoring of the concentrations and by products of catecholamines can be recognized as promising strategy for early detection of several catastrophic diseases.

Several techniques including spectrophotometry [14,15], chemiluminescence [16–20], electrochemistry [21–29], fluorometry [30–34], capillary electrophoresis (CE) [35–38], and high-performance liquid chromatography (HPLC) [39–41] have been employed for detection of catecholamines. However, some of the predetermined approaches have shorcomings in terms of sensitivity, cost-effectiveness, and complexity (*e.g.*, having derivatization steps). More specifically, CE and HPLC with UV detection, have low sensitivity. With regards to fluorescence detection, emission of the catecholamines is appeared in short wavelengths region and, therefore, its detection is disturbed by co-eluting compounds even after derivatization [42]. In electrochemical techniques, the redox peaks of catecholamines can have considerable overlap with each other and those of commonly coexisted compounds including uric acid and ascorbic acid [43–45].

In order to overcome the predetermined issues for identification of the catecholamines, one strategy could be the use of 'electronic tongue' [46]. Electronic tongue approach, which was inspired by the mammalian olfactory system, uses a large number of sensor elements with a different degree of interactions to produce unique fingerprint pattern for each target analyte [47]. In design of a sensor array, cross-reactive sensor elements are more efficient that the traditional lock-and-key systems. Each sensor element shows different levels of affinity to various analytes and therefore provide unique patterns. More specifically, the response dataset of all the sensor elements goes through pattern classification algorithm to produce response patterns or fingerprints [48]. Among various type of sensing mechanisms for electronic-tongue (*e.g.*, impedance [49], color [50,51], potential [52], conductivity [53], and current [54]), fluorescent-based sensor arrays have attracted considerable attention, due to their sensitivity, simplicity, cost-effective instrumentation, and large changes in emission spectra.

Due to their superior optical properties, colloidal quantum dots (QDs) have attracted increasing attention in chemical and biological application [55–57]. Size-tunable optical characteristic, photostability, high fluorescence quantum yield, broad excitation spectra, and narrow emission band [58,59] make QDs superior to other fluorophores.

In the current study, we introduce a sensor array with capability to identify and discriminate catecholamines (*e.g.*, NE, DA and LD). CdTe QDs capped with thioglycolic acid (TGA) was synthesized in aqueous solution. Under alkaline conditions, catecholamines are oxidized to quinone derivatives and then transformed to fluorescent compounds [32,60]. Both fluorescence of oxidation products and variations of QD's emission spectra with different size in presence of oxidation products, were employed as cross-responsive sensor elements. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed to analyze the cumulative array responses.

2. Experimental

2.1. Chemicals

Dopamine hydrochloride (DA), L-DOPA (LD), norepinephrine bitartrate (NE), sodium borohydride ($NaBH_4$), tellurium powder (Te), thioglycolic acid (TGA) and cadmium chloride ($CdCl_2 \cdot 2H_2O$) were obtained from Sigma. The stock solutions of different catecholamines were prepared in phosphate buffer (100 mmol L^{-1} with pH 6.2; the detailes tegarding the pH selection are available in Supporting Information(SI)). Deionized distilled water was used throughout.

2.2. Instrumentation

Absorbance spectra were obtained with a Lambda spectrophotometer from Perkin Elmer and the use of 1.0 cm glass cell. The fluorescence spectroscopy was performed using a Cary Eclipse fluorescence spectrometer (Varian) with the use of 1 × l-cm quartz cell. All the spectra were recorded at room temperature. Size distributions of the particles were collected using Zetasizer Viscotec 802 at ambient temperature.

2.3. Synthesis of TGA functionalized CdTe QDs

TGA functionalized CdTe QDs with different emission capability were synthesized according to the procedure described previously with slight modifications [61]. Briefly, 2.87 mmol of TGA was added to 10 mL of 0.290 mol L^{-1} $CdCl_2$ solution; NaOH solution was used to adjust the pH to 9.0. The solution was then stirred under argon flow for 5 min. Te powder (0.76 mmol) was added to $NaBH_4$ solution (75 mL, 64 mmol L^{-1}) under argon at 50 °C in a three neck round bottom flask. After 20 min, 10 mL of pH-adjusted $CdCl_2$ solution was added and temperature was increased to 150 °C. TGA functionalized CdTe QDs with different emission were taken out at different reaction times and allowed to cool in ice bath. Emission spectra of as-prepared CdTe QDs with a peak centered at 532, 555, 570 and 605 nm for QD(1), QD(2), QD(3) and QD(4), respectively, are shown in Fig. 1a. Tunability in emission wavelength (from dark green to orange) was probed under UV irradiation (Fig. 1b).

2.4. Design of fluorescence based sensor elements

Fluorescence of alkali-oxidized catecholamines (sensor element O-CA): different concentrations of the analyte were added to a solution contained NaOH with a final concentration 20 mmol L^{-1}. All fluorescence spectra were recorded 6 min after addition of the analyte at the excitation wavelength of 340 nm.

Fluorescence quenching of two different size of CdTe QDs (*i.e.*, QD(1) and QD(3) named QD(a) and QD(b) in following) by alkali-oxidized catecholamines (sensor element QD(a) and QD(b)): A solution contained QD(a) with a final concentration 99 nmol L^{-1} (calculated with Beer–Lambert law [62]) and NaOH with final concentration of 50 mmol L^{-1} was prepared. After 15 min (the reason to choose the time 15 min is available in SI), the different concentrations of analytes were added as the total volume was constant for all experiments. All fluorescence spectra were recorded 6 min after the addition of analyte at the excitation wavelength of 340 nm (detailes tegarding the selection of excitation wavelength are available in SI). The same experiment was done on QD(b), but QDs were used with a final concentration 26 nmol L^{-1}.

2.5. Preparation of urine sample

Urine was obtained from a healthy in fast volunteer. The urine