Sensors and Actuators B 227 (2016) 313–319

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Litmus-type thermochromic and solvatochromic sensors prepared with α-synuclein amyloid fibrils and polydiacetylene

Jee Eun Yang[a], Soonkoo Lee[a], Ghibom Bhak[a], Minwoo Lee[b], Dae H. Jeong[b], Seunho Jung[c], Seung R. Paik[a,*]

[a] School of Chemical and Biological Engineering, Institute of Chemical Processes, College of Engineering, Seoul National University, Seoul 151 744, Republic of Korea
[b] Department of Chemistry Education, Seoul National University, Seoul 151 744, Republic of Korea
[c] Department of Bioscience and Biotechnology, Konkuk University, Seoul 143 701, Republic of Korea

ARTICLE INFO

Article history:
Received 23 September 2015
Received in revised form
14 December 2015
Accepted 16 December 2015
Available online 24 December 2015

Keywords:
Amyloid fibrils
Colorimetric biosensor
Polydiacetylene
α-Synuclein
Litmus-type sensor

ABSTRACT

Litmus-type sensors capable of detecting temperature and organic solvents have been developed with α-synuclein amyloid fibrils and 10,12-pentacosadiynoic acid (PCDA). The PCDA molecules localized on the amyloid fibrils through either co-incubation with monomeric α-synuclein (AF/PCDA) or simple mixing with the pre-made α-synuclein amyloid fibrils (AF + PCDA) were photopolymerized with UV to exhibit blue color on paper. The paper sensors prepared with AF/PCDA and AF+PCDA showed distinctive sensitivities toward temperature and solvents. Whereas AF/PCDA were able to detect the higher temperatures between 55 °C and 90 °C by exhibiting the blue-to-red color transition on paper, the AF + PCDA-containing paper sensor was sensitive to the lower temperatures from 25 °C to 60 °C. The less tight binding of PCDA to amyloid fibrils and thus increased its molecular freedom would be responsible for the discrete thermal sensing property. The AF + PCDA paper sensor was also able to detect organic solvents with brief exposure to their vapors while the AF/PCDA sensor changed its color only after direct contact with the solvents in solution. The vapor-induced blue-to-red transition was dependent upon the duration of vapor exposure. In addition, the AF + PCDA sensor was demonstrated to successfully follow ascending temperature change by exhibiting the color transition from blue to red, which could allow the litmus-type sensor to monitor thermal history of materials in general and localize the spots previously exposed to heat. Taken together, the amyloid fibrils of α-synuclein are shown to be a decent template for PCDA to develop into the litmus-type thermochromic and solvatochromic sensors.

1. Introduction

Polydiacetylene (PDA) has been widely employed as a colorimetric sensing material to monitor external stimuli including heat, pH, and solvents [1–3]. In the previous study, we have demonstrated that 10,12-pentacosadiynoic acid (PCDA) as a PDA monomer facilitated the fibrillation of an amyloidogenic protein of α-synuclein [4], a pathological component of Parkinson's disease by constituting the Lewy bodies as the major component [5], via specific molecular interaction with a dissociation constant (K_d) of 0.29 mM. Upon co-incubation, the PCDA molecules became aligned within the mechanically stable amyloid protein nanofibrils of α-synuclein [6] comprising the highly regular secondary structure of cross β-sheet conformation [7,8]. The resulting nanocomposite material exhibited blue in color upon UV irradiation at 254 nm as the light causes topochemical photopolymerization of the regularly ordered PCDA molecules via 1,4-addition reaction which converts two triple bonds to alternating double and triple bonds (ene-yne) [9]. In the presence of the external stimuli such as heat, the blue poly-PCDA exhibited colorimetric transition to red with a fluorescence enhancement, which was due to structural relaxation of the strained ene-yne conjugations [1–3,10,11]. As a matter of fact, these unique optical properties of PCDA have been employed to develop poly-PCDA vesicles as a biosensor system capable of detecting temperature [12], pH [13], organic solvents [14,15], pathogenic bacteria [16], HCl gas [17], and antibiotics [18]. The vesicles, however, show limitations on their stability since the structures are readily sus-

Abbreviations: AF, amyloid fibrils; PCDA, 10,12-pentacosadiynoic acid; PDA, polydiacetylene.

* Corresponding author at: School of Chemical and Biological Engineering, Seoul National University, 1 Gwanak-Ro, Gwanak-Gu, Seoul 151 744, Republic of Korea. Fax: +82 2 888 1604.
E-mail address: srpaik@snu.ac.kr (S.R. Paik).

http://dx.doi.org/10.1016/j.snb.2015.12.060
0925-4005/

ceptible to chemical and physical deterioration caused by aging [19,20] and oxidative damage [21,22]. On the contrary, our PCDA sensor prepared with amyloid fibrils has been demonstrated to be much more stable than the vesicular PCDA against mechanical stresses such as sonication [4]. In this paper, we have introduced a litmus-type biosensor prepared with the pre-made α-synuclein amyloid fibrils and PCDA after simply mixing them together, which demonstrates a practical and convenient use of the colorimetric nanocomposites.

Paper-based sensors have been considered as an attractive sensing platform because papers are flexible, portable, inexpensive, and disposable [23,24]. In fact, a diverse set of paper-based sensors have been developed to detect, for example, heavy metals with phenanthroline and chlorophenol red [25–27], neurotoxin [28] and pathogen [29] with enzymes such as acetylcholinesterase and β-galactosidase, respectively, DNA via DNA hybridization [30,31], and glucose with the complex of glucose oxidase and quantum dot [32]. PDA including poly-PCDA vesicles has also been employed to prepare litmus-type sensors to take advantage of its colorimetric and fluorometric responses toward temperature and volatile organic compounds. The poly-PCDA vesicles were, however, difficult to be adsorbed onto paper surface since the vesicles tend to aggregate on the surface. Some surfactants were thus added in order to stabilize the vesicles on paper [33]. The PCDA molecules were also chemically modified to improve their solubility for the vesicle preparation and prevent their subsequent aggregation on the paper [13,34]. Herein, we have introduced a facile procedure for preparing PCDA-based litmus-type colorimetric sensor by employing amyloid nanofibrils of α-synuclein in the form of either amyloid fibril-PCDA composites or a simple mixture with PCDA. The resulting litmus-type sensors have been demonstrated to be capable of monitoring thermal history of materials as well as their exposure to solvent vapors.

2. Material and methods

2.1. Materials

Human recombinant α-synuclein expressed in *Escherichia coli* was purified according to the procedure previously described [35]. 10,12-Pentacosadiynoic acid (PCDA), 2-(*N*-morpholino) ethanesulfonic acid (MES), dimethyl sulfoxide (DMSO), benzene, toluene, and xylene were purchased from Sigma–Aldrich. Methanol, ethanol, acetone, chloroform, and methylene chloride were from Thermo Fisher Scientific.

2.2. Preparation of litmus-type sensors with amyloid fibrils and PCDA

The amyloid fibril and PCDA composites (AF/PCDA) were prepared by incubating monomeric α-synuclein (70 μM) for 70 h at 37 °C in the presence of PCDA at 1 mM under agitation at 200 rpm [4]. The resulting AF/PCDA were collected via centrifugation at 16,100 × *g* for 30 min. The mixture between pre-made amyloid fibrils and PCDA (AF + PCDA) was obtained by incubating the pre-made amyloid fibrils of α-synuclein and PCDA (1 mM) for 15 min at ambient temperature. The AF + PCDA were also precipitated with the centrifugation. The collected pellets of AF/PCDA and AF + PCDA were resuspended with 200 μL of 20 mM Mes (pH 6.5). Aliquots (10 μL) of AF/PCDA and AF + PCDA solutions were dropped onto Whatman filter paper and air-dried for 2 h. The spots of AF/PCDA and AF + PCDA on the filter paper turned to blue in color after UV exposure at 254 nm for 1 min.

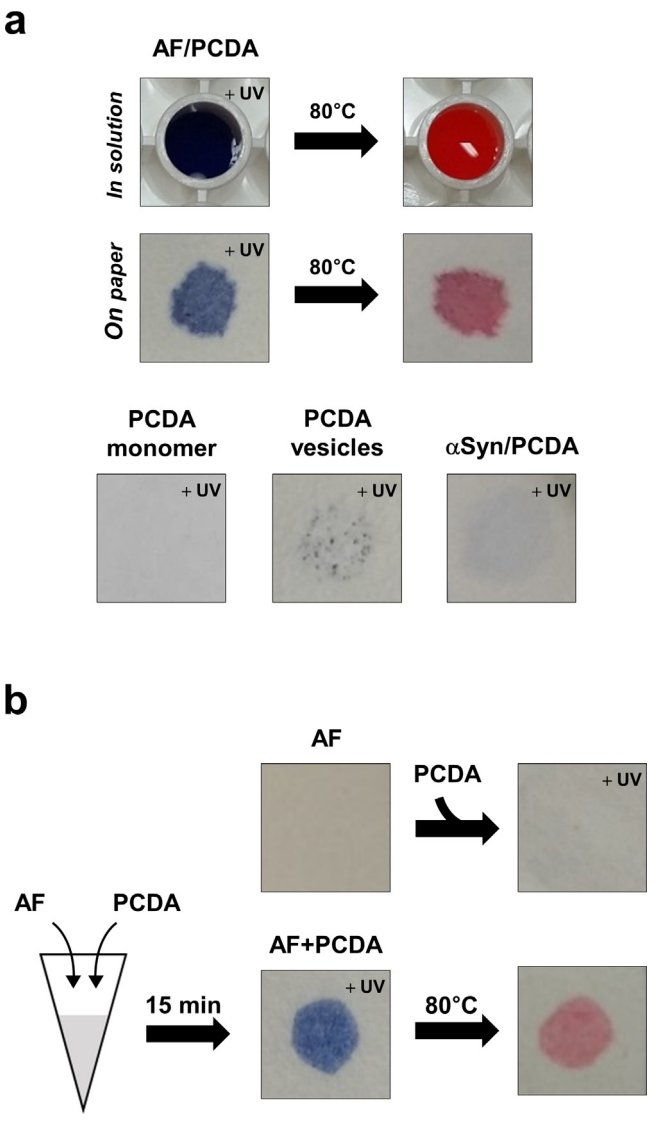

Fig. 1. Colorimetric litmus-type sensor developed with α-synuclein amyloid fibrils and PCDA. (a) Colorimetric responses of the amyloid fibril and PCDA composites (AF/PCDA) in solution and on paper to UV exposure and heat treatment. AF/PCDA were prepared via coincubation of α-synuclein (1 mg/ml) and PCDA (1 mM) for 70 h at 37 °C. PCDA monomers, PCDA vesicles, and PCDA monomers with monomeric α-synuclein (αSyn/PCDA) were spotted on paper for negative controls. (b) Colorimetric responses of the mixture of pre-made amyloids fibrils (AF) and PCDA (AF + PCDA) on paper. AF and PCDA were either separately spotted on paper in succession (upper row) or in the form of AF + PCDA obtained after 15 min of incubation between AF and PCDA (lower row). Both papers were subjected to UV exposure followed by heating.

2.3. Colorimetric and fluorescent responses of the litmus-type sensors to temperature and organic solvents

Litmus-type sensors prepared with AF/PCDA and AF + PCDA were either incubated at different temperatures from 25 °C to 90 °C for 5 min or exposed to various organic solvents in vapor or liquid. The colorimetric change of litmus-type sensors was recorded with digital camera and the fluorescence enhancement was analyzed with confocal laser scattering microscopy (CLSM, LSM 710, Carl Zeiss) with excitation at 405 nm. In order to quantitatively analyze the fluorescence enhancement, the fluorescence intensity of the spots was evaluated with microscope and imaging software provided by Carl Zeiss.