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Colorimetric artificial tongue for protein identification

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ABSTRACT

Artificial tongue systems are multisensory devices which are highly desirable for the analysis and recognition of complicated composition samples. Herein, a low-cost and simple colorimetric sensor array for identification and quantification of proteins were reported. Using prophyrin, porphyrin derivatives (mainly metalloporphyrins) and chemically responsive dyes as the sensing elements, the developed sensor array of artificial tongue showed a unique pattern of colorific change upon its exposure to proteins. The composite pattern for each sample was subjected to principal component analysis (PCA), thus providing a clustering map for more practical visualization. All the pure and mixed proteins, as well as denatured proteins, gave distinct patterns, thus resulting in their unambiguous identification. The PCA analysis also suggested that the unique pattern of colorific change may be due to the change of protein conformation and local environmental pH. These results demonstrate that the developed colorimetric artificial tongue system is an excellent sensing platform for identification and quantitative analysis of protein samples.

1. Introduction

Due to the excellent performance on molecular recognition, the strategy of cross-responsive array has been widely employed to create diagnostic patterns with high accuracy (Wright et al., 2005). The cross-reactive arrays can receive complex information about local environmental changes through various receptors (Ciosek and Wroblewski, 2007). In these sensor arrays, one synthetic receptor does not possess specific binding affinity to the only one specific target analyte. Instead, it is necessary to have multiple receptors in the array bind differently to each analyte (or complex mixture), and create various signals for the pattern recognition. Thus, it is possible to analyze diverse chemical structures and complex mixtures (Lee et al., 2007; Kitamura et al., 2009; Kong et al., 2009). These diagnostic signals, which are generated from multiple differential binding interactions, provide unique patterns for the identification of individual analytes or even mixtures (Schiller et al., 2008). Recently, several groups have designed cross-reactive arrays to obtain differential responses to various analytes, such as sugars and sweeteners (Lim et al., 2008; Musto et al., 2009), amino acids (Buryak and Severin, 2005), peptides and proteins (Wright et al., 2005; Zhou et al., 2006). These methods even have been used for cellular recognition (Humston et al., 2008; Bajaj et al., 2009).

The identification of protein species and quantification of their concentrations are important processes and have many applications in pathophysiological study, medical diagnosis, and the detection of toxic pathogens (Crowley et al., 2008). Traditional methods for the identification and quantification of proteins, including high performance liquid chromatography, potentiometry, and spectroscopic analysis, are tedious and cumbersome (Xiao and Meyerhoff, 1996; Kowalska et al., 2003; Xie et al., 2005). Thus, more practical methods are highly demanded for fast detection and quantification of proteins in order to assist in routine medical diagnosis and food quality control. The cross-responsive strategy for protein identification was first illustrated by Zhou et al. (2006), in which they developed a fluorescent array to detect different metal-containing proteins. However, due to the lack of highly selective and specific binding agents, protein detecting arrays remain underdeveloped. Given that the conformational rigidity is required to offer entropic advantages and the functional surface should be readily constructed, porphyrins are the potential candidates to serve as sensitive scaffolds for protein recognition (Tsou et al., 2004).

Porphyrin–protein complexes (covalent or non-covalent modes) are important motifs in many biological systems (Urbanova et al., 2001; Huo et al., 2009a,b), and the conformational changes of protein could be caused by porphyrins (Fernandez et al., 2008). Practically, the non-covalent interaction between porphyrin and protein is one of the most frequently used sensing modes for individual porphyrin-based receptor (Urbanova et al., 2001; Yin et al., 2006; Tang et al., 2008; Ma et al., 2009). Meanwhile,

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since porphyrins display rich photophysical and electrochemical properties (Fa et al., 2009), the formation of porphyrin–protein complex can cause protein-dependent changes in the optical and photochemical properties of porphyrins. Besides porphyrins, chemical dyes may also change their optical property once they interact with proteins (Volkova et al., 2007). Therefore, prophyrins and chemical dyes could be considered as sensitive receptors in the fabrication of cross-reactive chemical sensor array for protein identification and quantification.

Unlike fluorescence-based cross-responsive array for protein identification (Zhou et al., 2006), herein we reported a rather different, but quite simple colorimetric artificial tongue for the identification of proteins and their mixtures. Based on the colorimetric detection, identification of bovine serum albumin (BSA), ovalbumin (Ova), bovine hemoglobin (BHb) and their mixtures were successfully demonstrated using an sensor array consisting of prophyrin (H_2TPP), porphyrin derivatives (mainly metalloporphyrins, include MnTPPCl, FeTPPCl, CoTPP, InTPPCl, CuTPP, EuTPPCl, H_2F_5TPP, FeF_5TPP, ZnF_5TPP, ZnTPP, TPPS, $TPPS_1$) and chemically responsive dyes (including thymol blue, fluorescein, nitrazine yellow, acridine orange, bromoxylend blue, malachite green, disperse orange, brilliant yellow, indigo carmine, cresol red, bromothymol blue, nile red, phenol red, alizarin, methyl red, methyl violet, metanil yellow, bromphenol red, bromophenol blue, chlorphenol red, congo red, bromocresol purple, and bromocresol green). A series of composite patterns generated by protein samples were then subjected to principal component analysis (PCA), and the PCA mapping converts the unique fingerprints of various samples into distinguishable clusters, thus providing the unambiguous recognition of proteins.

2. Materials and methods

2.1. Materials and preparation of analyte solutions

Protein and indicator dyes were supplied by Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. Porphyrin and its derivates were obtained from Frontier Scientific (Logan, UT, USA). The porous hydrophobic membrane was bought from Millipore Co. Ltd. (Bedford, MA, USA). Ultra-pure water was generated by a Millipore Direct-Q Water system (Molsheim, France). Protein stock solutions (10 μM) were prepared by directly dissolving appropriate amount proteins into ultra-pure water to mimic the real samples. The denaturation of protein was obtained by a thermal treatment (immerging protein samples into a boiling water bath for 20 min).

2.2. Fabrication of colorimetric artificial tongue system

The artificial tongue system was specially developed for protein detection in liquid phase using sol–gel method. With a similar fabricated procedure that described elsewhere (Rakow and Suslick, 2000; Zhang and Suslick, 2005), the disposable colorimetric arrays were created. Briefly, tetraethyl orthosilicate (TEOS), ultra-pure water, hydrochloric acid (1 mol/L), ethanol and indicator solution were mixed with a volume ratio of 25:4:3:15:10. In particular, the selected indicator solutions were added into the sol–gel solution after stirring for 2 h at room temperature (See Table S1 for the structures and placement of indicators). The pH indicators were selected with a color range around normal physiological environment (pH 7.0), and metallo-porphyrins were paying the utmost attention based on the interaction between protein and porphyrin. A quartz capillary was used to deliver approximately 0.1 μL pigment onto the surface of a porous hydrophobic membrane. Once printed, the arrays were placed in a 60 °C oven for 24 h after which the oven temperature was reduced to 35 °C and the arrays left for another 24 h. Then the arrays were placed in a nitrogen-flushed black environment where they were stored until use (minimum of 12 h).

2.3. Application of the as-prepared artificial tongue for protein detection

A fully functional prototype device for protein detection was constructed and the schematic diagram is shown in Fig. 1. Based on an inexpensive white light-emitting diode (LED) and a complementary metal-oxide semiconductor (CMOS) camera, an "initial" image was acquired before a sensor array was used. Then the array was exposed to the as-prepared protein solution. Once reaching nearly complete equilibration (5 min), the array was taken out and scanned again for a "final" image after the excess liquid on the membrane was removed by a filter paper. By comparing the "initial" and "final" images (Fig. 1) (Montes-Navajas et al., 2009), a colorful red–green–blue (RGB) difference map was obtained. To avoid subtraction artifacts at the periphery of the spots, an edge extracting process was performed. The center of each dye spot was averaged, and filled into a standardized visualized array.

3. Results and discussion

3.1. Analysis of Euclidean Distance

For a cross-reactive analysis, pattern recognition is a very powerful tool because it is possible to analyze diverse chemical structure and complex mixtures. On a typical chemosensor

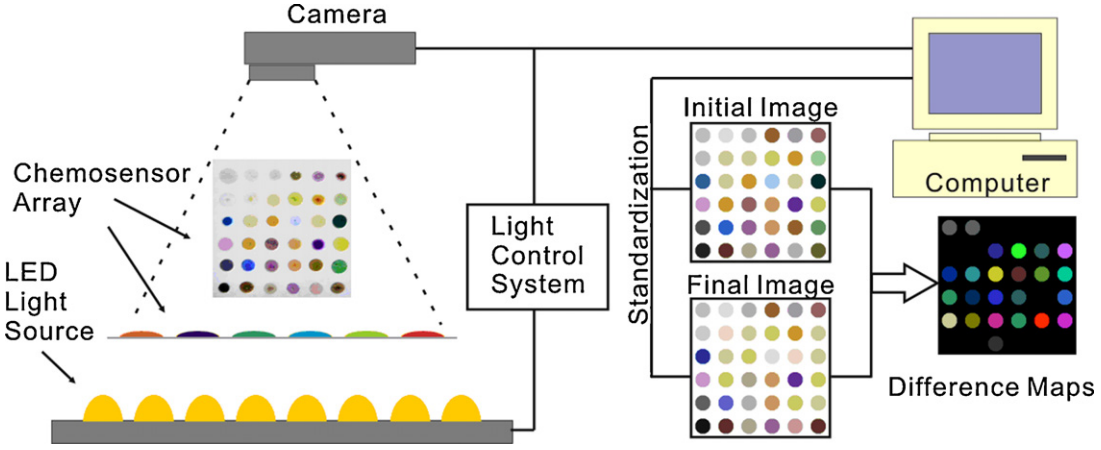

Fig. 1. 6 × 6 Colorimetric sensor array and schematic diagram of detection system.