Materials Science and Engineering C 70 (2017) 535–543

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Polydiacetylene/triblock copolymer nanosensor for the detection of native and free bovine serum albumin

Jaqueline de Paula Rezende [a], Guilherme Max Dias Ferreira [b], Gabriel Max Dias Ferreira [b], Luis Henrique Mendes da Silva [b], Maria do Carmo Hepanhol da Silva [b], Maximiliano Soares Pinto [c], Ana Clarissa dos Santos Pires [a,*]

[a] Department of Food Technology, Universidade Federal de Viçosa, Av, PH Rolfs, s/n, Campus Universitário, Viçosa, MG 36570-000, Brazil
[b] Department of Chemistry, Universidade Federal de Viçosa, Av, PH Rolfs, s/n, Campus Universitário, Viçosa, MG 36570-000, Brazil
[c] Institute of Agrarian Sciences, Universidade Federal de Minas Gerais, Av. Universitária, 1000, Bairro Universitário, Montes Claros, MG 39404-547, Brazil

ARTICLE INFO

Article history:
Received 6 May 2016
Received in revised form 18 August 2016
Accepted 6 September 2016
Available online 7 September 2016

Keywords:
Nanosensor
Milk protein
Fluorescence
Microcalorimetry
Zeta potential

ABSTRACT

Bovine serum albumin (BSA) has been recognized as a marker of the cow's health, milk quality, an allergenic protein and as a carrier. Its detection is important in the food, pharmaceutical and medical industries. However, traditional techniques used to detect BSA are often time-consuming, expensive, and show limited sensitivity. This paper describes properties of polydiacetylene-triblock copolymer (L64) nanosensors, synthesized to easily detect BSA. Sensor efficiency was studied as a function of nanosensor composition, polydiacetylene chemical structures, BSA conformation and hydrophobic domain availability, using spectroscopic, calorimetric, light scattering, and electrokinetic analyses. Nanosensors were sensitive to detect the average BSA concentration of milk and dairy products and discriminated between native and denatured protein through naked-eye detectable blue-to-red transition. The standard Gibbs free energy ($-10.44 < \Delta G° < -49.52$ kJ M), stoichiometry complex ($1 <$ "n" < 3), and binding constant ($6.7 \times 10^2 < K_a < 4.79 \times 10^8$ M^{-1}) of BSA-nanosensor complex formation established a direct relationship between nanosensor response and BSA-nanosensor interaction. BSA-nanosensor interaction was entropically (without cholesterol), and enthalpically driven (with cholesterol). Eugenol-BSA complex did not induce colorimetric transition. Polydiacetylene-L64 nanosensors are potential low-cost sensors for rapid detection of BSA, discriminating between native/denatured and free/bound protein.

1. Introduction

Bovine serum albumin (BSA) detection has attracted increasing attention [1,2] mainly due to the application of BSA in different fields such as food, biochemical and immunological sciences. BSA has been used as a marker of the health of the cow's mammary gland and milk quality [3], and has been recognized as a significant allergenic protein [4]. It is widely used as a protein calibrant [5], and it can be used as ligand-carrier in applications relevant to the food and pharmaceutical industry [6,7].

Traditional techniques used to detect BSA are often based on spectrometric methods [8,9]. However, these methods suffer limitations such as complicated steps, expensive equipment, limited sensitivity and narrow linear range, and commonly shortening of the detection time is required. Therefore, it is necessary to develop alternative ways to detect small amounts of BSA in different matrixes, in real-time. Recently, some sensitive techniques such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF-MS) have improved protein identification in biological samples [10,11]. Nanomaterials can also be combined with analytical chemistry to develop ultra-sensitive detection methods [12], and can also improve sensitivity of colorimetric sensors.

Polydiacetylenes (PDAs) are conjugated polymers with the unique intrinsic ability to respond to different stimuli, undergoing blue-to-red transition [13]. Using colorimetric changes, some systems based on PDAs have been constructed to detect bacteria [14], enzymes [15], viruses [16], surfactants [17], solvents [18], and others. These chromic properties makes PDA suitable for use in sensor systems, with several merits such as simple and rapid detection, easy recognition through naked eye color change, and label-free detection [16].

PDAs may exist in self-assembled form in different structures, such as liposomes or vesicles [19,20], films [21], and nanocomposites [17], based on the preparation method, and/or chemical nature of the molecules used in synthesis [22]. In addition, colorimetric transition in these sensor systems is dependent on PDA aggregation form [21,23].

* Corresponding author.
E-mail address: ana.pires@ufv.br (A.C.S. Pires).

http://dx.doi.org/10.1016/j.msec.2016.09.009
0928-4931/

The application of PDA vesicles in the detection of different molecules in suspension has been widely studied. However, they have been found to show some limitations, such as low stability for long periods [24], and restricted colorimetric transition for some conditions, that could make difficult the visual detection of color change. It would therefore be useful to develop new PDA-based templates for the efficient use of this polymer as a sensor to detect different molecules. Gou et al. [25] showed that PDAs are able to interact with amphiphilic molecules such as triblock copolymers (TCs). These molecules are formed by arrays of ethylene oxide and propylene oxide units (symbolized as $(EO)_n(PO)_m(EO)_n$, where "n" and "m" mean the number of ethylene oxide and propylene oxide unit segments, respectively) [26]. These polymers are able to form micelles, and the self-assembly of TC has been used to produce nanostructures for many applications [27,28].

Therefore, in this study, to overcome limitations of PDA vesicles, nanoaggregates formed by a mixture of two polymers (PDA and triblock copolymer L64) were used to detect BSA. Nevertheless, to develop efficient nanosensors for future application in food, biomedical and/or pharmaceutical areas is essential to understand the mechanisms of BSA-nanosensor interaction that leads to protein detection in different conditions. Thus, besides our attention in detecting the protein we were also interested in determining the thermodynamic parameters associated with nanosensor-protein interaction, and in investigating the effects of nanoaggregate composition, protein conformation, and presence of protein-ligand on the colorimetric transition of polydiacetilenic nanosensors.

2. Materials and methods

2.1. Materials

BSA (98% wt. pure), α-lactalbumin (85% wt. pure), β-lactoglobulin (90% wt. pure), lactoferrin (85% wt. pure), α_{S1}-casein (70% wt. pure), β-casein (98% wt. pure), 10,12-pentacosadiynoic acid (PCDA, 97% wt.), 10,12-tricosadiynoic acid (TCDA, 98% wt.), cholesterol (99% wt. pure), eugenol (99% wt. pure), sodium chloride (NaCl, 99.5% wt. pure), calcium chloride ($CaCl_2$, 96% wt. pure), potassium chloride (KCl, 99% wt. pure), sodium carbonate sodium chloride (Na_2CO_3, 99% wt. pure) and sodium phosphate sodium chloride (Na_3PO_4, 99% wt. pure) were purchased from Sigma-Aldrich (USA). Glucomacropeptide (80% wt. pure) was acquired from Davisco (USA). Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) TC L64 [$(EO)_{13}(PO)_{30}(EO)_{13}$], with average molar mass (M_m) of 2900 $g \cdot mol^{-1}$, acquired from Aldrich (USA), was used. Polyvinylidene difluoride (PVDF) syringe filters (0.33 mm of diameter and 0.45 μm pore) were purchased from Millipore (USA), and Millipore water, were used in all experiments ($R \geq 18.2\ M\Omega \cdot cm$).

2.2. Nanosensor production

Nanosensors of PCDA and L64 were prepared by solubilizing L64 in water, at concentration of 1.0% (w/w). PCDA (1 mM) was dissolved in this TC solution, the mixture was sonicated for 10 min (Sonics & Materials Inc., Model VC750, USA), and immediately filtrated through PVDF filter. The suspension was kept overnight at 4 °C, with the aim of orientating PCDA monomers in order to promote polymerization. Next, photopolymerization was carried out by exposing the suspension to UV radiation (254 nm), for 6 min, until the suspension turned blue in color.

To investigate the effects of diacetylene monomer, nanosensors containing TCDA (1 mM) instead of PCDA, and L64 1.0% (w/w) were synthesized following the same steps as described above.

To evaluate the effect of a lipid insertion on color transition caused by BSA, nanosensors containing cholesterol (CHO) were manufactured. CHO (1, 2 or 3 mM) was dissolved into L64 solution (1% wt.) and the mixture was sonicated for 5 min; then PCDA or TCDA (1 mM) was added and the synthesis followed as described above for nanosensors without CHO.

2.3. Colorimetric response (CR)

In order to study the interaction between BSA and PCDA/L64 or TCDA/L64 nanosensor, BSA solution (0.75 mM) was prepared by solubilizing the protein in L64 1% (wt.). This procedure aimed did not dilute nanosensors suspension, maintaining L64 concentration constant. Aliquots of BSA solution were added to the nanosensor suspension, at increasing concentrations up to a final concentration of 0.20 mM. The mixtures were stirred for 30 s, and maintained at 25 °C for 1 h, to allow the system to achieve thermodynamic equilibrium. Spectra were obtained between 350 and 900 nm (Shimadzu UV-2550, Japan), at 25 °C. To quantify the percentage of blue-to-red conversion of polydiacetylenes, a parameter termed "colorimetric response (CR)" was calculated using Eq. 1 [29].

$$CR\ (\%) = \left(\frac{\left(\frac{A_{blue}}{A_{blue}+A_{red}}\right)_b - \left(\frac{A_{blue}}{A_{blue}+A_{red}}\right)_a}{\left(\frac{A_{blue}}{A_{blue}+A_{red}}\right)_b} \right) \times 100 \quad (1)$$

where A is the absorbance of blue (λ ~ 640 nm) and red components (λ ~ 540 nm), determined by UV–Vis spectroscopy. The terms "blue" and "red" are related to material appearance, and the indices "b" and "a" represent the absorbance before and after protein addition, respectively.

As we were interested in evaluating the effect of protein conformation, and bound protein on nanosensor CR, the procedures described above were repeated using the same concentration range, but changing BSA, denatured BSA, and eugenol-bound BSA, respectively.

In order to evaluate the stability of colorimetric nanosensors to pH change, the pH of suspensions was adjusted between 1.0 and 9.0 (at one-unit intervals) and CR was determined for each pH. We also evaluated the effect of interfering molecules (proteins, peptide and salts) on CR of nanosensors at concentration and double concentration that they are found in milk.

2.4. Light scattering and electrokinetic measurements

Size and zeta potential of nanostructures were measured at 25 °C, with a Zetasizer nano ZS90 (Malvern, UK). Samples were diluted 20 times to avoid blue color interference on the measurements. Each experiment was repeated 3 times, and each result was presented as the average of 10 measurements.

2.5. Fluorescence experiments

Fluorescence spectra of protein were recorded in a CaryEclipse Fluorescence Spectrometer (Agilent, USA), using a 1/cm path length quartz cuvette. Nanosensor aliquots were added to the protein (BSA or denatured-BSA) solution (0.75 mM), and the fluorescence emission spectra were recorded between 296 and 500 nm, at the excitation wavelength of 295 nm, which is specific for tryptophan residue excitation.

2.6. Isothermal titration microcalorimetric (ITμC) experiments

Titration analyses were performed in an isothermal titration microcalorimeter (ITμC), model CSC 4200 (TA Instruments Inc., USA), controlled by ITCRun software. The microreaction system (1.8-mL stainless steel vessels for sample and reference), containing PCDA/L64 or TCDA/L64 nanosensors, was maintained under constant stirring at 300 rpm, and analyzed in titration mode. When thermal equilibrium between the vessel and the heat sink was reached, 10-μL aliquots of protein (BSA, denatured-BSA or eugenol-bound BSA) solution were