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Kinetic study on the interaction between tannin and bovine serum albumin with a wireless magnetoelastic biosensor

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Abstract

A wireless and low-cost magnetoelastic biosensor is developed for the kinetic study on the interaction of tannin with bovine serum albumin (BSA). The measurement is based on the interaction of tannin with BSA which is coated on the magnetoelastic biosensor, producing an insoluble tannin–BSA complex which binds tightly to the sensor surface resulting in a change in the sensor resonance frequency. A pseudo first-order reaction equation is established and the reaction rate constant is calculated to be 0.119 min^{-1} based on the response curves. The biosensor demonstrates a linear shift in resonance frequency with tannin concentration ranging from 0.60 to 1.08 mM, with a detection limit of 0.10 mM at a noise level of ~20 Hz.

Keywords: Magnetoelastic; Wireless; Biosensor; Tannin; Bovine serum albumin

1. Introduction

Tannin, a polyphenolic compound, can be found in fruits and beverages such as red wines. Scientists in many fields are interested in interaction of tannin with proteins, because such interaction is closely related to leather making [1], physiological activity of herbal medicines [2,3], taste of foodstuffs and beverages, and nutritional value of feeds [4,5]. Two mechanisms of tannin–protein co-precipitation have been proposed [6]. One is a cross-linking mechanism based on association, in which one tannin molecule binds to two or more protein molecules. Another is a two-stage mechanism, which involves an initial complexation stage of tannin with protein and a subsequent precipitation of the complexes. The common methods to study the interaction of proteins with tannin include capillary electrophoresis [7], spectrophotometric assay [8], HPLC [9], colorimetric assays [10], isothermal titration microcalorimetry [11], the dye-labeled bovine serum albumin assay [12], total radical-trapping antioxidant potential [13], nephelometric study [14], FTIR spectroscopy [15], and NMR [16].

In this work a low cost, wireless magnetoelastic sensing platform was proposed for the kinetic study of the interaction between tannin and BSA using a wireless ribbon-like magnetoelastic sensor. In a time-varying magnetic field, magnetoelastic materials efficiently translate magnetic to mechanical energies, so the magnetoelastic ribbon longitudinally vibrates at a fundamental characteristic resonance frequency f_r that inversely depends on the sensor length L. With sensor width and thickness given by: much smaller than length Young's modulus E, and density ρ [17], the resonance frequency is

$$f_r = \sqrt{\frac{E}{\rho}}\frac{1}{2L} \tag{1}$$

A small mass load Δm evenly deposited on a sensor of mass m_0 shifts the measured resonant frequency by an amount [17]:

$$\Delta f = -f_r\frac{\Delta m}{2m_0} \tag{2}$$

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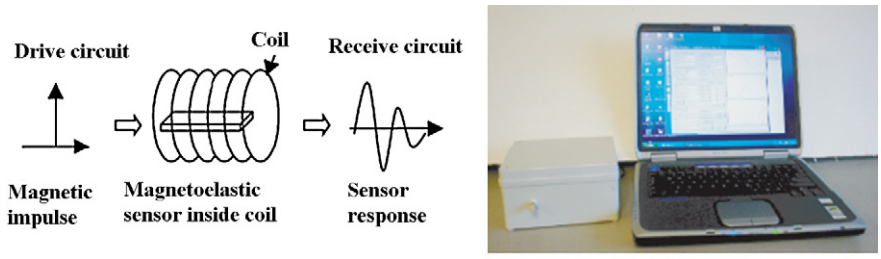

Fig. 1. Schematic showing the operation of the wireless magnetoelastic sensor (a) and the magnetoelastic sensor-reader box (b, on left) connected to a portable computer via a RS 232 port. The computer interface allows for user-control of measurement parameters, data display, and data storage.

The frequency shift is downward with increasing mass. Fig. 1 illustrates the operating principle of the magnetoelastic sensor. A magnetic field is used to excite the sensor, causing it to mechanically vibrate at a resonance frequency which shifts in response to mass loading [18]. The mechanical vibrations of the magnetostrictive material, in turn, generate a magnetic flux that can be remotely detected using a simple pick-up coil. A frequency counting technique, previously described [19], implanted by use of microprocessor-based electronics is employed to determine the resonance frequency of the magnetoelastic sensor. No physical connections between the sensor and the detection system are required for signal telemetry, nor is precise alignment necessary per optical telemetry systems. The facile wireless capabilities of the magnetoelastic sensor platform makes it a powerful tool for *in situ* and *in vivo* analyses. Published applications include glucose [20], microbial biosensors [21], blood coagulation monitoring [22], endotoxin [23] and trypsin [24].

In this work the sensor was fabricated by first coating a magnetoelastic ribbon with a layer of polyurethane and upon it a layer of BSA. The insoluble tannin–BSA complex is bounded tightly to the sensor surface, inducing a change in sensor resonance frequency. The kinetic parameters related to the process were estimated.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA) and tannin were purchased from Sigma Co. Bayhydrol 110, an anionic dispersion of an aliphatic polyester urethane resin in water/*N*-methyl-2-pyrrolidone solution (50% w/v) was purchased from Bayer Corp. (Pittsburgh, PA). Dimethylaminopropyl-3-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich and used as received. The serially diluted standard tannin solutions in phosphate-buffered saline (PBS) at pH 4.1 were freshly prepared with 10 mM PBS (pH 4.1) and sodium hydroxide (NaOH) solution. A 28-μm-thick ribbon of Metglas alloy 2826 MB, alloy composition $Fe_{40}Ni_{38}Mo_4B_{18}$, was used as received from Honeywell Corp. The sensors in the size of 18 mm × 6 mm × 28 μm rectangles were cut from the ribbon. The resonance frequency of an uncoated sensor in air is approximately 105 kHz.

2.2. Sensor fabrication

The magnetoelastic ribbons were ultrasonically cleaned in general-purpose soap, rinsed with water and acetone, and dried in air under room temperature prior to use. About 5 μl Bayhydrol 110 was applied to both sides of the cleaned sensors by dip-coating. The polyurethane-coated sensors were dried in air and then heated at 150 °C for 2 h to form a robust protective membrane, which protects the iron-rich magnetoelastic substrate from corrosion and provides –NH for the covalent binding of BSA. The polyurethane-protected sensors were then coated with 10 μl 12.5 g/l BSA in water, which contains 0.46 g/l EDC and 0.38 g/l NHS. A thin BSA film gave a hydrophilic surface so that the insoluble tannin–BSA complex can bind to the sensor surface tightly.

2.3. Measurement

Microprocessor-based magnetoelastic sensor monitoring electronics employing a frequency counting technique [25] were used to determine the resonance frequency of the sensors. The sensor was first immersed in a pH 4.1 phosphate-buffered saline (PBS) solution. As the response was stable, the initial frequency of the sensor was measured (f_0), and 0.60–1.08 mM tannin was then added into the detection cell to start the precipitation reaction. The time-dependent frequency shift was recorded as $\Delta f = f - f_0$. The frequency shift within 30 min was used to characterize the protein-precipitating activity. All the data were the mean values of at least four parallel measurements.

3. Results and discussion

3.1. Optimization of the sensor

The optimum pH for the formation of insoluble complex of tannin–BSA was investigated in the range of pH 2.0–6.0 at 37 °C using 0.80 mM tannin. As shown in Fig. 2, the optimal pH value is 4.1, which is less than the BSA's p*I* (4.7). The tannin–BSA complex is formed by electrostatic force between electropositive BSA and electronegative tannin. When pH > 4.7, both BSA and tannin are electronegative, which is unfavorable to the complex formation.

The sensor response is also dependent on the BSA loading. Fig. 3 shows the BSA loading-dependent frequency shifts in