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Short communication

A homogeneous fluorescent sensor for human serum albumin

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ABSTRACT

Human serum albumin is the most abundant protein in the body and is an important biomarker used for disease-related diagnosis. Although the traditional enzyme-linked immunosorbent assay (ELISA) approach can precisely measure the concentration of human serum albumin, the multi-step procedure and time-consuming preparations of ELISA limit its diagnostic applications, preventing accurate point-of-care testing, for example. Herein, we report the recent development of an antibody-based albumin sensor that allows for a homogeneous measurement of albumin concentrations in saliva, urine and serum, in which this type of sensor is validated for the first time. The assay only requires simple mixing, and relies on time-resolved (TR) fluorescence resonance energy transfer (FRET) to produce robust, sensitive signals. The whole process, from sample preparation to final read-out, is expected to take less than 1 h and requires only a standard plate-reader, thus making the sensor a convenient and cost-effective tool for albumin analysis.

1. Introduction

Human serum albumin (HSA) is a negatively charged, non-glycosylated globular protein with a molecular weight of 67 kDa [1]. Reported to be the most abundant protein in the body, HSA accounts for 60% of the total proteins in plasma [2], and is synthesized exclusively in the liver, primarily in the polysomes of hepatocytes [1]. Possessing several low- and high-affinity ligand binding sites, HSA is able to bind ligands such as metal ions, pharmaceutical compounds, fatty acids, as well as metabolites [1,3]. Besides serving as a carrier, HSA displays a variety of properties such as antioxidation, reactive oxygen/nitrogen species (ROS/RNS) scavenging and anti-inflammation [1].

Clinically, albumin was used in past decades to maintain vascular volume in patients with cirrhosis due to its regulation of oncotic pressure [1,4]. Today, combined with other therapeutic approaches, the volume-expanding properties of albumin are still believed to be beneficial for patients with cirrhosis [1]. Further, administration of albumin has been shown in small studies to help resuscitate patients from hemorrhagic shock [5], treat intradialytic hypotensions [6], and prevent ovarian hyperstimulation syndrome [7].

Besides its applications in therapy, HSA is regarded as a standard biomarker, with its levels in serum, urine and saliva serving as diagnostic and prognostic criteria [2]. The normal concentration of HSA in blood serum is 35–50 g/L [2,8]. In diseased conditions, however, low levels of albumin in serum (hypoalbuminemia < 30 g/L) may reveal malnutrition, liver disease, nephrosis, gastrointestinal protein loss, shock, edema and cardiovascular disease [2]. On the other hand, high serum levels of albumin (hyperalbuminemia > 55 g/L) are accompanied by dehydration and increasing body weight or body fat [2]. The reference range of albumin in urine is 2.2–25 mg/L [2,9]. Albumin higher than 25 mg/L in urine is normally filtered through the glomerulus, and reabsorbed or catabolized by the proximal tubules. However, albumin loss increases once the renal glomeruli become more permeable due to diabetes or renal damage [2]. The severe leakage of the glomerular filtration mechanism can lead to either micro-albuminuria or macro-albuminuria, depending on the amount of albumin lost (detectable by a simple urine test) [10]. While the normal concentration of HSA in saliva is less than 0.5 g/L [2], a higher concentration of saliva albumin usually indicates type-2 diabetes mellitus [11], or, for some cancer therapy patients, the potential for stomatitis [12].

Currently the most common method used to determine albumin levels is the enzyme-linked immunosorbent assay (ELISA), which is accurate but time-consuming. Heyduk et al. previously reported a novel antibody-based sensor technology that allows homogenous detection of target proteins based on simple fluorescence [13,14]. With this platform, a series of homogenous sensors have been developed. They include sensors for cardiac troponin I [15], C-reactive protein [15], insulin [16], C-peptide [16], and pathogenic bacteria [17]. Herein, we describe a similar sensor design that can be adapted to rapidly determine albumin concentrations in biological

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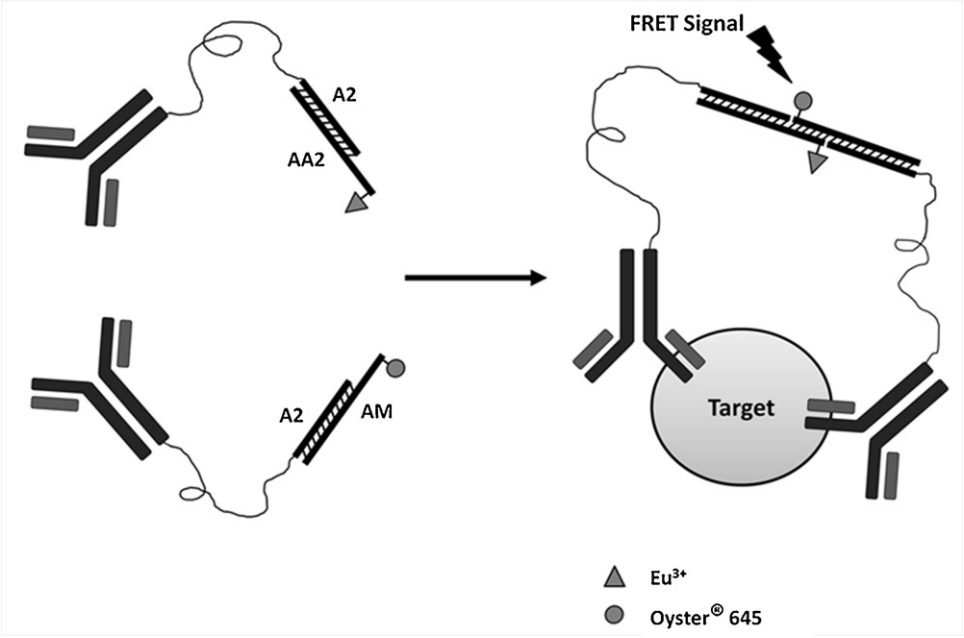

Fig. 1. Mechanism of the sensor. A pair of antibodies that recognize the target antigen is labeled with oligos that have complementary oligonucleotides at the ends. Both oligonucleotides are labeled with fluorophores that can be paired as donor and receptor. The presence of the target is expected to drive the annealing of the probe-labeled DNA, bringing the donor and receptor in close enough proximity to generate FRET signals.

samples. Using simple time-resolved fluorescence (TRF), the *in vitro* detection of human serum albumin can reach 3.9–1000 ng/mL. Most importantly, we demonstrate here for the first time the validation of this type of sensor in saliva, urine and serum, with the measured concentrations matching the results obtained with the traditional ELISA method. This validation confirms the sensor as a sensitive, reliable and convenient tool for albumin analysis.

2. Materials and methods

2.1. Sensor design

This assay consists of two human albumin-specific antibodies which recognize different epitopes of human albumin (Fig. 1). Each antibody is conjugated with short duplex DNA with overhangs complementary to each other. In the presence of human albumin, the two overhangs associate to form a duplex. Time-resolved fluorescence resonance energy transfer (TR-FRET) is initiated between the two fluorophores (labeled at the end of the two oligonucleotides) once they are brought into close proximity. The intensity of the FRET signal is proportional to the concentration of human albumin in the samples.

2.2. Materials

The Traut's reagent, NHS-$(PEG)_{12}$-maleimide, protein bicinchoninic acid (BCA) test kit, and human IgG (catalog number: 31154) were from Pierce, Thermo Scientific (Rockford, IL). Ethanol, glycogen, Tris (hydroxymethyl) aminomethane, sodium chloride, pH 7.4 PBS pouch, as well as human insulin (catalog number: I0908) and bovine serum albumin (catalog number: B6917), were all purchased from Sigma–Aldrich (St. Louis, MO). The human albumin standard (catalog number: J80310072), albumin antibodies (monoclonals, catalog numbers: 6501-100063, 6502-100064), and human C-reactive protein (catalog number: J81610) were obtained from Biospacific (Emeryville, CA). The 384-well low-volume black microplates were from Corning (Lowell, MA). The Synergy plate reader (Synergy 4, BioTek Instruments, Winooski, VT), was equipped with a TR-FRET function and a 330 nm excitation filter (40 nm band pass), as well as a 620 nm (20 nm band pass) and a 665 nm (8 nm band pass) emission filters. The albumin sandwich ELISA kit was purchased from Assaypro (St. Louis, MO) and the ELISA was conducted exactly according to the instructions.

All oligonucleotides were synthesized and purified by Integrated DNA Technologies (Coralville, IA). The following oligonucleotides were used in the sensor experiments:

A2: 5′-amino-GCAGCCGATTCGACTTGC-3′
AA2: 5′-*GCTCAT*-GCAAGXCGAATCGGCTGC-3′ (X = T modified with Europium at the C6 position)
AM: 5′-*AYGAGCG*-GCAAGTCGAATCGGCTGC-3′ (Y = T modified with Oyster645 dye at the C6 position)
AA2 and AM contain the 5′- overhang sequences (italicized) that are expected to anneal to each other and generate TR-FRET signals in the presence of the antigen (human albumin).

2.3. Antibody modifications

The antibody modification and purifications were based on previously published procedures [15,16]. For a detailed description of experimental procedures, please see Section S1.1 in Supplementary Material.

2.4. Human sample collections

Urine and saliva samples were randomly collected from apparently healthy personnel (including males and females) and stored at −20 °C, which were later on thawed only once before the immediate measurements. The human serum samples were collected by Innovative Research (Novi, MI), from people between the ages of 18 and 65 under FDA regulations. Due to the limited number of serum samples, they were randomly processed after purchasing, by either concentrating or diluting to make their albumin concentrations in a diverse range. The processed serum samples were then stored at −20 °C, and thawed only once before the immediate