Biosensors and Bioelectronics 51 (2014) 136–142

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Interference-free determination of ischemia-modified albumin using quantum dot coupled X-ray fluorescence spectroscopy

Yang Luo [a,*], Chaoming Wang [b], Tianlun Jiang [c], Bo Zhang [a], Junfu Huang [a], Pu Liao [d], Weiling Fu [a,**]

[a] Department of Laboratory Medicine, Southwest Hospital, the Third Military Medical University, Chong Qing 400038, China
[b] NanoScience Technology Center, University of Central Florida, Orlando, FL 32826, USA
[c] Department of Transfusion Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
[d] Clinical Laboratory Center of Chongqing, Chongqing 400014, China

ARTICLE INFO

Article history:
Received 8 July 2013
Accepted 22 July 2013
Available online 29 July 2013

Keywords:
Albumin cobalt binding
Ischemic heart disease
Ischemia-modified protein
Quantum dots
X-ray fluorescence

ABSTRACT

Ischemia-modified protein (IMA) is the most sensitive diagnostic biomarker of ischemic heart disease, but differentiation of IMA from human serum albumin (HSA), a ubiquitous serum protein, is still challenging owing to the shared antigenicity. In this investigation, we developed a rapid and interference-free approach for IMA determination using quantum dots-coupled X-ray Fluorescence Spectroscopy (Q-XRF). In a typical Q-XRF assay, serum total HSA is quantified using quantum dot-coupled sandwich immunoassay, and intact HSA (iHSA) is determined using a XRF spectroscopy, by measuring XRF intensity of Co (II) bonded to iHSA. IMA concentration is automatically determined within 30 min by calculating the difference between total HSA and iHSA. This strategy can effectively eliminate the interference from native HSA level. Results show that no significant influences have been observed from hemolysis or high levels of cholesterol (7 mg/L), triglyceride (5.2 mg/L), IgG (10 g/L), and fibrinogen (4 g/L). A linearity of 1–100 mg/mL is obtained in iHSA determination using XRF (r^2=0.979). The proposed Q-XRF assay demonstrates a lowest detection limit of 0.05 U/mL. Receiver-operating characteristic (ROC) curves reveal that Q-XRF assay provide an improved sensitivity than ACB assay (95.9% vs. 82.9%) in differentiating ischemic patients from health individuals, at an optimal cutoff point of 79.2 U/mL. The proposed approach provides a new strategy for interference-free, simple and rapid evaluation of IMA concentration by combining sandwich immunoassay and XRF spectroscopy.

1. Introduction

Ischemic heart disease (IHD) remains the leading cause of death and disability worldwide and it affects 17.6 million Americans, resulting in about 450,000 deaths in the United States annually. Rapid and accurate diagnosis of IHD at the outset of myocardial ischmia provides improved prognosis through appropriate treatments in the emergency department setting (Boden et al., 2013). Conventional diagnosis of IHD relies on the combinational results from electrocardiogram, imaging, and various biochemical biomarkers. Considering the convenience and feasibility, biochemical biomarkers such as troponin, Creatine Kinase (CK-MB), and myoglobin have been considered the most important indicators in early diagnosis of IHD (Kruger et al., 2005; Sato et al., 2009; Spindler et al., 2004). Among them, CK-MB is most popular in indicating myocardia damage when combined with other enzymes such as lactate dehydrogenase and aspartate transaminase (Li et al., 2012). But its specificity in IHD dignosis is limited because of its short duration, and the damaged skeletal muscle can also release these enzymes into blood.

Compared with CK-MB, troponin provides higher specificity in diagnozing myocardial damage, but it always takes 12 h to reach peaks after myocardial damage, thus decreasing its sensitivity in early IHD diagnosis (Jensen et al., 2007). Recently, a more sensitive and specific myocardial biomarker named by ischemia-modified albumin (IMA) has been proposed, which can recognize cardiac ischemia in minutes (reach peaks in 2–4 h) when ischemic events occur, providing the earliest detection of IHD (Bar-Or et al., 2000; Peacock et al., 2006). IMA is produced due to the cleavage of N-terminal amines acids of human serum albumin (HSA) mainly in ischemic condition. Previous mass spectroscopy analysis has

Abbreviations: ACB, albumin cobalt binding; CI, confidence interval; DTT, dithiothreitol; HSA, Human serum albumin; IHD, Ischemic heart disease; iHSA, intact HSA; IMA, Ischemia-modified protein; MI, Myocardial ischemia; QD, Quantum dots; Q-XRF, Quantum dot-coupled X-ray fluorescence; XRF, X-ray fluorescence

* Corresponding author. Tel.: +86 13594088001.
** Corresponding author.
E-mail addresses: luoyang@tmmu.edu.cn (Y. Luo), weilingfu@yahoo.com (W. Fu).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.07.046

revealed that the difference between IMA and HSA merely relies on the conformation of the last four amino acids (Asp-Ala-His-Lys) in the N-terminus (Peacock et al., 2006). So far, almost all existing immunoassays or biochemical assays fail to determine IMA concentration because IMA and HSA have no significant differences in antigenicity.

Till now, only one FDA approved assay is clinically available for serum IMA determination. This assay, namely albumin cobalt binding (ACB) test, is based on the premise that IMA will lose its ability to bind to transition metal element Co (II) when myocardial damage occurs-(Bar-Or et al., 2001). Primary ACB assay is hand-manipulated and can only get qualitative results, while improved ACB assay can be integrated into various automated biochemical analyzers with the capability of reading out results within 30 min. With the broad application of ACB assay in various laboratories and emergency departments, the diagnostic value of IMA as an ischemia indicator has been widely questioned (Beetham et al., 2006; Karahan et al., 2010; Kim et al., 2010; Sbarouni et al., 2011). Firstly, strict preanalytical requirements need to be performed to exclude any possible interferences such as chelators and hemolysis (Amirtharaj et al., 2008). In addition, interpretion of results should be cautious when HSA concentration are <20 g/L or >55 g/L; or in the presence of increased lactate and ammonia concentrations (Gaze et al., 2006; Hakligor et al., 2010). This serious situation is mainly derived from the detection principle of ACB test. In principle, an ACB test indirectly detects the IMA by measuring residual Co (II) that unbound to HSA. This kind of indirect manner is susceptible to the interferences from over high or low HSA level (Collinson et al., 2006.) Moreover, it is widely reported that the analytical characteristics of colorimetric-based assays are potentially affected by many factors such as serum proteins and lipidemia (Kotani et al., 2011). Thus, ACB test fails to discern the true IMA level in most of the clinical settings. The aim of this study is to explore an assay that can eliminate these endogenous interferences.

X-ray fluorescence (XRF) spectroscopy is an analytical technology widely used in geology for metallic elements detection in complicated samples due to its high sensitivity, specificity and simplicity (Hatzistavros et al., 2007; Mann et al., 2000). In a typical XRF spectroscopy, a primary X-ray will irradiate the target elements to emit a characteristic secondary X-rays, which can be detected using an X-ray fluorescence spectrometer. Because the secondary XRF is the inherent characteristic of the specific elements being analyzed, the amount of elements can be accurately quantified by measuring the intensity of XRF peak. Recently, our team has explored several preliminary biological applications of XRF spectroscopy in clinical sample detection using nanoscale metallic elements as transducers (Hossain and Su, 2012; Hossain et al., 2010). In these explorations, multiple nucleic acids or protein biomarkers can be detected simultaneously using metallic elements (bismuth, gold) as detection probes (Hossain et al., 2010). The selection criteria of metallic elements as the transducer depend largely on the intensity of XRF, the toxicity, and the interferences from endogenous elements existed in biological samples. In the case of IMA determination, although many transition elements such as cobalt (II), iron (II), and zinc (II) can be adopted to differentiate IMA from HSA, iron and zinc should be excluded because they are rich in serum. Thus, cobalt is the most promising candidate due to its least toxicity and solid status. In addition, cobalt has a characteristic spectrum peak at 6.93 KeV that can differentiate itself from most of other metallic elements inherently existed in the serum, providing the possibility of using XRF spectroscopy to detect the biomolecules those can specifically bind to cobalt.

In this paper, we propose a novel interference-free assay, namely quantum dot (QDs)-coupled X-ray fluorescence spectroscopy (Q-XRF), to determine true IMA value by calculating the difference between total HSA level and iHSA level. The detection procedure of Q-XRF approach is composed by two steps, which is elucidated in Fig. 1A. The total HSA is determined using a QD-coupled sandwich immunoassay, and the iHSA concentration is determined by measuring the XRF spectrum from irradiated Co (II). Then the IMA concentration can be calculated according to the difference between total HSA and iHSA. The two-step strategy effectively eliminates the interferences from over high or low albumin level, and can be accomplished rapidly and conveniently in a 96-well microplate, suggesting promising clinical applications. The proposed Q-XRF assay integrates classical microplate sandwich immunoassay, XRF spectrum assay, and QDs labeling, and can readout the IMA concentrations in an extremely rapid and accurate manner. The most significant improvement of the Q-XRF assay is that it can accurately detect the true IMA value, regardless of the interferences from extremely high or low concentration of albumin.

2. Experimental section

2.1. Materials

Purified human serum albumin, monoclonal anti-albumin antibody produced in mouse (clone HSA-9), monoclonal anti-albumin antibody produced in mouse (clone HSA-11), dithiothreitol (DTT), cholesterol, triglyceride, hemoglobin, fibrinogen, lyophilized IgG from human plasma, Phosphate buffered saline (PBS), bovine serum albumin (BSA), sodium bicarbonate, were obtained from Sigma-Aldrich (St. Louis, MO). The Qdot 565 ITK Streptavidin Conjugate Kit was obtained from Invitrogen (Gaithersburg, MD). Ultra-pure water (18.2 MΩ cm) from Milli-Q (Billerica, MA) was used for the preparation of all solutions and for cleaning of substrates. A Synergy HT Hybrid Multi-Mode Microplate Reader was obtained from BioTek (Winooski, VT) for fluorescence measurements. UV-transparent, clear-bottom microplates (96-well) were obtained from Corning (Lowell, MA). A Microplate Genie mixer from Scientific Industries (Bohemia, NY) was used for reagent mixing.

2.2. Patients

Clinical samples were collected from Southwest hospital affiliated to the Third Military Medical University between January 2011 and January 2013. All participants provided signed informed consents, and the study was approved by the Ethics Boards of the Third Military Medical University. All participants were designated into two groups: ischemic or non-ischemic. The non-ischemic group consisted of 125 individuals (74 males and 51 females) with an average age of 35 y (22 y–63 y), whose chart-reviewed diagnosis showed no evidence of MI or the participants were generally healthy.

The ischemic group consisted of 64 participants (34 males and 30 females) with an average age of 39.4 y (31 y–56 y). The inclusion criterion of ischemic group is mainly according to the reference Bhagavan et al. (2003). Patients with renal diseases were excluded from this study in advance. Clinical assessment of MI included several objective clinical indices, imaging studies, ECG studies, and serum cardiac biochemical markers, such as CK-MB and cardiac troponin I. The diagnosis of MI was based on criteria defined by the Joint European society of Cardiology/American College of Cardiology Committee-(Antman et al., 2000), which are as follows: typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following: (a) ischemic symptoms; (b) development of pathologic Q