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Surface plasmon resonance-based immunoassay for procalcitonin

Sandeep Kumar Vashist [a, *], E. Marion Schneider [b], Eberhard Barth [c], John H.T. Luong [d]

[a] Institute of Clinical Chemistry and Pathobiochemistry, Klinikum rechts der Isar der Technischen Universität München, Ismaninger Str. 22, D-81675, Munich, Germany
[b] Sektion Experimentelle Anaesthesiologie, University Hospital Ulm, Albert Einstein Allee 23, 89081 Ulm, Germany
[c] Sektion Operative Intensive Care Medicine, Department Anesthesiology, University Hospital Ulm, Albert Einstein Allee 23, 89081, Ulm, Germany
[d] Innovative Chromatography Group, Irish Separation Science Cluster (ISSC), Department of Chemistry and Analytical, Biological Chemistry Research Facility (ABCRF), University College Cork, Cork, Ireland

HIGHLIGHTS

- We developed a surface plasmon resonance based procalcitonin (PCT) immunoassay.
- It detects PCT in the range of 4–324 ng mL^{-1} with a LOD of 4.2 ng mL^{-1}.
- It is rapid, simplified and analytically-superior to the conventional procedures.
- It determines PCT in diluted serum and EDTA plasma of patients.
- It has high precision similar to that of commercial ELISA.

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ABSTRACT

A surface plasmon resonance (SPR) biosensor has been developed for rapid immunoassay of procalcitonin (PCT) with high detection sensitivity and reproducibility. The 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC)-activated protein A (PrA), diluted in 1% (v/v) 3-aminopropyltriethoxysilane (APTES) was dispensed on a KOH-treated Au-coated SPR chip, resulting in the covalent binding of PrA in 30 min. This "single-step" PrA immobilization strategy led to the oriented binding of the anti-PCT antibody (Ab) on a PrA-functionalized gold (Au) chip. The leach-proof immobilization procedure is five-fold faster than conventional counterparts, enabling high detection specificity and reproducibility. The IA detects 4–324 ng mL^{-1} of PCT with a limit of detection (LOD) and a limit of quantification (LOQ) of 4.2 ng mL^{-1} and 9.2 ng mL^{-1}, respectively. It was capable of detecting PCT in real sample matrices and patient samples with high precision. The Ab-bound SPR chips were stable for more than five weeks.

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1. Introduction

PCT, a propeptide of calcitonin, has a molecular weight of 13 KDa and 116 amino acids. It comprises an N-terminal region, a C-terminal region of katacalcin, and a middle region of calcitonin consisting of 57, 21 and 32 amino acids, respectively [1]. As first described by Bohoun in 1992, PCT was a critical parameter for inflammation and is now well recognized for acute and severe bacterial as well as viral infection, similarly to C-reactive protein (CRP). Its concentration might be up to 300 ng mL^{-1} in patients with most severe cases in septic shock (unpublished observations). Procalcitonin is the natural precursor of calcitonin produced in the thyroid gland by C-cells and proteolysis, however, multiple tissues secrete procalcitonin during the early stages of inflammation. Considering its stability [2] with a half-life of ~24 h, hyper-procalcitoninemia in systemic inflammation or infection occurs within 2–4 h and often reaches peak concentrations in 8–24 h. It is very stable [3], and persists for as long as the inflammatory process continues [4]. Numerous assays for PCT for *in vitro* diagnostic (IVD) have been developed. Indeed, it is a promising biomarker for bacterial infections [5–7] and the early diagnosis of sepsis in critically ill patients [8,9]. PCT is also a useful biomarker in discriminating infectious fever from non-infectious one in febrile diseases [10] apart from identifying low-risk febrile infants [11], even though

* Corresponding author.
E-mail address: sandeep.vashist@yahoo.com (S.K. Vashist).

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PCT is not a good infection marker in newborns [12]. With the exception of newborns, PCT serves as an important biomarker in pediatrics as its assessment is of importance in neonatal sepsis, pyelonephritis, pneumonia, cancer, bacterial infections, bone and joint infections, meningitis, and systemic inflammatory response syndrome after cardiac surgery [13].

When compared to generalized infection and sepsis, pulmonary infections do not lead to a very fulminant rise in PCT but still provides important information concerning the risk of ventilator-associated pneumonia [14] and hospital-acquired pneumonia [15]. The serial quantification of PCT can predict clinical outcomes in patients with community-acquired pneumonia (CAP) [16] apart from assessing the treatment response in such patients [17]. PCT can also differentiate between severe and very severe CAP [18] and between CAP and acute decompensated heart failure [19]. Additionally, PCT is a prognostic biomarker of early graft failure after heart transplantation [20]. It has also been associated with the prognosis of acute heart failure [21,22] and might be used as a predictor of long-term mortality in ischemic stroke patients [23]. The measurement of PCT can exclude bacterial infection and guide antibiotic treatment in congestive heart failure patients in the emergency department [24]. Longitudinal PCT measurements support the timely reduction of antibiotic treatment in intensive care units' (ICUs) patients, who are treated for suspected bacterial infections [25]. The serum PCT level is a marker of lower respiratory tract infections, which can be used for the diagnosis, prognosis and follow-up of antibiotic therapy [26]. Even in the absence of other predictors, a plasma PCT concentration of $\geq$10 ng mL^{-1} at the time of admission to ICU is a strong predictor of short-term mortality [27]. Along the same lines, elevated PCT levels have also been shown to predict mortality in critically ill neurological patients [28].

Despite a wide range of IA formats for the detection of PCT, the clinically-accredited IAs are still based on enzyme-linked immunosorbent assays (ELISA) [29,30], electrochemiluminescent IA [31] and immunoturbidimetric assay [32]. This adoption is mainly due to the high precision and high sensitivity of these formats, and the prominent role that it plays in the clinical diagnosis and decision-making in the healthcare settings. However, most of these conventional IA formats can take a few hours, which substantiate the critical need for rapid IA formats that enable PCT detection in minutes. Of importance is the development of a label-free SPR [33] has emerged as a potential rapid IA format, which enables label-free and real-time detection of numerous analytes. A wide range of SPR IA formats has been developed and used in bioanalytical settings. However, most of these formats employ expensive commercial SPR chips that are functionalized with carboxymethyl dextran, streptavidin, nitriloacetic acid, long chain alkanethiol molecules or lipophilic groups to facilitate antibody bioconjugation. Various immobilization chemistries [34–41] have also been developed for the binding of antibodies to the SPR Au chip, which directly impacts the bioanalytical performance of the assay [34,39]. In general, the selection of an optimal Ab immobilization strategy plays a critical role in assay development.

This paper advocates the use of a rapid Ab immobilization strategy, which involves the initial covalent binding of an intermediate Fc binding protein (PrA), followed by the oriented immobilization of Ab. The EDC-crosslinked PrA, prepared in APTES, was dispensed onto KOH-treated SPR Au chip and left incubated for 30 min at room temperature (RT). This strategy provides the covalent crosslinking of free amino groups of APTES to the carboxyl groups on capture Ab, and the simultaneous binding of the alkoxy groups of APTES to the hydroxyl groups present on the KOH-treated chip. The analytical performance of the developed PCT IA was compared with that of a conventional SPR IA procedure using a commercial carboxymethyl (CM5) dextran chip. The assay precision for the detection of PCT in EDTA-plasma samples of patients was evaluated and corroborated with that of a commercial sandwich ELISA. Other evaluated bioanalytical parameters include interferences, inter-day and intra-day variability, fabrication reproducibility and storage stability. Table 1.

2. Materials and methods

2.1. Materials

3-Aminopropyltriethoxysilane (APTES, purity 98%, w/v), Tween 20, H_2O_2 (30%, v/v), stop solution, PrA, protein G (PrG), protein A/G (PrA/G), EDC, *N*-hydroxysulfosuccinimide (sulfo-NHS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, while 2-(*N*-morpholino)ethane sulfonic acid (MES, pH 4.7) was from Thermo Fischer Scientific. The components of human PCT IA comprising the Duoset PCT kit, consisting of mouse anti-human PCT capture Ab, biotinylated sheep anti-human PCT detection Ab, recombinant human PCT standard, and streptavidin-HRP, were purchased from R&D Systems. The nonspecific control proteins, such as recombinant human serum albumin (HSA), CRP, human fetuin-A (HFA), human lipocalin 2 (LCN2), interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α, were obtained from RnD Systems. All buffers and solutions were prepared in 18 MΩ Milli-Q ultrapure water (UPW) filtered through a 2 μm filter. The SPR Au chips (SIA kit), CM5 dextran (CMD)-functionalized Au chips, ethanolamine hydrochloride (1 M, pH 8.5), HBS-EP (0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v surfactant P20), and glycine-HCl (10 mM, pH 2.0) were procured from GE Healthcare. The running buffer for BIAcore and all sample dilutions was HBS-EP. SPR was performed on the BIAcore 3000 from GE Healthcare. The conventional sandwich ELISA procedure was performed using the guidelines provided by the supplier. The anonymized left-over EDTA plasma samples of patients treated by the ICU at the University Hospital Ulm, Germany were provided by Dr. Eberhard Barth in order to validate the developed PCT IA. The PCT spiked human plasma and serum samples were prepared by spiking various concentrations of PCT in a fixed dilution (1:10) of human plasma. The EDTA-plasma samples from the patients were diluted 1:10 using the standard sample preparation guidelines for clinical IA. This enables the unknown PCT concentration within the samples to fall within the detection range of the developed IA.

2.2. Immobilization of capture anti-PCT Ab

The immobilization procedure involves the induction of hydroxyl groups on the SPR Au chip by treatment with 90 μL of 1% (w/v) KOH for 5 min and washing extensively with UPW. 990 μL of PrA (200 μg mL^{-1}) was mixed with 10 μL of EDC (4 mg mL^{-1} in 0.1 M MES, pH 4.7) and incubated for 15 min at RT. The resulting EDC-activated PrA was diluted in 1% (v/v) APTES and subsequently, 90 μL of the resulting solution was dispensed onto the hydroxylated SPR chip and left incubated for 30 min at RT in a fume hood (Fig. 1). The PrA-functionalized chip was washed extensively with HBS and docked into the BIAcore 3000 system. The chip was primed and 50 μL of anti-human PCT capture Ab (100 μg mL^{-1}) was injected at 10 μL min^{-1}, enabling the oriented Ab immobilization onto the PrA-functionalized SPR chip. This was followed by the injection of 20 μL of 2% (w/v) BSA at 10 μL min^{-1}, to block non-specific protein binding sites on the chip. For comparison, the PrG and PrA/G-functionalized SPR chips were prepared by the same procedure except that PrA was replaced by PrG and PrA/G, respectively. The details of various Ab immobilization procedures used for the

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