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A full-automated magnetic particle-based chemiluminescence immunoassay for rapid detection of cortisol in milk

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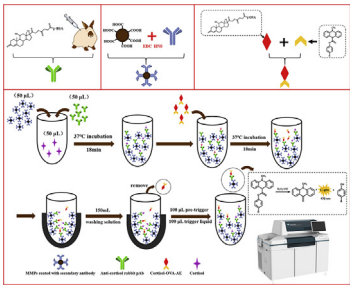
HIGHLIGHTS

- An automatic rapid assay for cortisol in milk was developed using MMPs-based CLIA.
- Cortisol detection: 0.42–72.27 ng/mL concentration range; 0.12 ng/mL detection limit.
- The proposed method showed high sensitivity, good reproducibility and stability.
- The strategy showed great potential in the fabrication of a novel cortisol test kits.

GRAPHICAL ABSTRACT



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ABSTRACT

A sensitive full-automated micromagnetic particles (MMPs) based competitive chemiluminescent immunoassay (CLIA) was developed to detect cortisol in milk. Polyclonal antibody (pAb) with good specificity against cortisol was produced. The antigen (cortisol-OVA) was labeled with acridinium ester (cortisol-OVA-AE) as signal tracer. During the detection, the free cortisol in sample will compete with cortisol-OVA-AE for binding to pAb. To capture pAb, MMPs conjugated with goat anti-rabbit IgG was added. The whole immunoassay process (exclude sample pretreatment) was performed by automatic chemiluminescence immunoassay instrument, which could consume less test time (within 40 min) and avoid error from manual operation. The method showed a good detection limit of 0.12 ng/mL, a broad linear range from 0.42 to 72.27 ng/mL for cortisol detection, negligible cross-reactivity with related analogues and satisfied recovery (84.3%–102.3%) for spiked milk samples test. Simultaneously, since the results of proposed method had no significant difference with those of LC–MS/MS, the proposed method was confirmed to have a potential applicability for rapidly monitoring cortisol in the food.

1. Introduction

Cortisol is type of glucocorticoid from steroid hormones, which is produced by the adrenal glands and plays vital roles within the multidirectional human physiological processes, such as the metabolism of carbohydrate and proteins, the regulation of blood

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pressure, the immune response and bone density. More so, cortisol is widely used in human and veterinary medicine with the function of treating inflammatory diseases, shock and stress [1]. Despite its clinical success, some glucocorticoids including cortisol often been illegally used as growth-promoting agents with the potency to increase weight gain [2], water retention [3] the fat and protein percentages in milk [4]. The usage of corticosteroids may lead to drug residues in dairy and other products of animal origin intended for human consumption, which may have negative effects on human health [5]. To date, different countries and organizations have set regulation specifying permitted maximum residue limits (MRL) of glucocorticoid for various kinds of food products. China and the European Union have provided cortisol for external use only. Moreover, Japan and the United States stipulates the maximum residue of cortisol in animal food is 10 μg/kg. Therefore, it is necessary to develop a sensitive detection method to prevent the risk of human cortisol exposure.

Up to now, a number of techniques have been proposed for determining cortisol concentration, such as HPLC [6,7], GC-MS [8], LC-MS [9], surface plasmon resonance biosensor [10], near-infrared reflectance spectroscopy [11], electrochemical sensors [12,13] and enzyme linked immunosorbent assays (ELISA) [14–16]. Unlike chromatographic techniques, ELISA is a method based on the antigen-antibody specific reactions for analyzing the target compound in samples, which have the many significant merits such as rapidity, low cost and applicability for on-site testing. However, the sensitivity of ELISA is relatively low compared with chemiluminescence immunoassays. During these years, the chemiluminescence immunoassays with good sensitivity, a broad linear range and excellent stability has received much attention from clinical analysis, environmental analysis and food analysis [17–19]. However, in traditional chemiluminescent immunoassay (CLIA), microplate was applied to immobilize antigen or antibody by physical absorption, which holds a negative effect on the performance of assay because of the low specific surface area of microplate. The use of micromagnetic particles (MMPs) have proven to be a support for addressing the shortage of traditional CLIA mentioned above [20]. The MMPs-based chemiluminescence immunoassays could improve the surface area for immobilization, capture efficiency and accuracy of the assay, thus obtaining higher sensitivity and shorter analysis time [21]. Additionally, chemiluminescence label is a crucial factor for chemiluminescence immunoassays. Acridinium ester (AE) has been widely used in bioassay technique because of its high stability, high chemiluminescence quantum yield and ease-to-use in labeling [22,23]. Moreover, in order to shorten the reaction time and improve the repeatability of the method for a large number of samples detection, automatic chemiluminescence immunoassay instrument which was widely used in clinical analysis is considered. Therefore, a full-automated MMPs-based chemiluminescence immunoassays utilizing AE as chemiluminescence label might become a new approach to detect cortisol.

In this work, a sensitive and selective MMPs-based competitive chemiluminescent immunoassay (MMPs–based CLIA) was constructed to detect cortisol in milk. Micromagnetic particles was as the solid phase which was coated with goat anti-rabbit antibody. Cortisol and cortisol-OVA labeled to AE bind competitively with pAb of cortisol. According to the observed luminescence intensity of cortisol-OVA-AE, the concentration of cortisol was detected. The established MMPs-based CLIA method exhibited a good specificity, stability and reproducibility in milk samples test. Furthermore, the result from MMPs-based CLIA compared with those obtained by the standard LC-MS/MS showed no significant differences implying excellent precision and accuracy of the proposed method.

2. Materials and methods

2.1. Reagents

Cortisol (≥98%), bovine serum albumin (BSA), ovalbumin (OVA), N-hydroxysuccinimide (NHS), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and complete and incomplete Freund's adjuvants were obtained from Sigma (St. Louis, USA). The carboxyl micromagnetic particles (6 μm) and acridinium ester (AE) were supplied by Darui Co., Ltd. (Guangzhou, China). Some analogues of cortisol, such as progesterone, megestrol, 1, 3, 5(10)-estratrien-3-ol-17-one, meprednisone, ethynyl estradiol, dexamethasone and norethindrone were purchased from Dalian Meilun Co., Ltd. (Dalian, China). Goat anti-rabbit IgG (secondary antibody) were purchased from Boster Biotech Co., Ltd. (Wuhan, China). Anti-cortisol polyclonal antibody and antigen were synthesized in our lab. Pre-trigger and trigger liquid was supplied by Darui Co., Ltd. (Guangzhou, China). The 0.01 mol/L pH 7.4 phosphate buffer saline (PBS) was prepared with KH_2PO_4 and Na_2HPO_4. The PB buffer (pH 6.3) was used by dissolving 8.1 g $Na_2HPO_4 \cdot 12H_2O$ and 12.1 g $NaH_2PO_4 \cdot 2H_2O$ in 1 L water. Solution A (1 mol/L zinc acetate dihydrate solution containing 3 mL of glacial acetic acid per 100 mL) and solution B (0.25 mol/L potassium ferrocyanide trihydrate solution) were prepared. The TBST buffer was prepared by dissolving 0.6 g Tris, 1.8 g NaCl, 4.0 g BSA, 0.1 g NaN_3, 2.0 g trehalose and 100 μL Tween 20 in 200 mL water and adjusted pH with hydrochloric acid to 7.2. While, the binding buffer was prepared by 9.8 g MES in 500 mL water and adjusted pH with NaOH solution to 5.0. All water used in this work is double distilled water. New Zealand white rabbits that were 2–3 months old (1.5–2 kg) were provided by Guangdong Experimental Animal Center.

2.2. Apparatus

Nuclear magnetic resonance (NMR) spectra were achieved with the DRX-400 spectrometer (Bruker, Rheinstetten, Germany). Automatic chemiluminescence immunoassay instrument (Caris 200) was supplied by Darui Co., Ltd. (Guangzhou, China). The concentration of antibody was detected by NanoDrop (2000c) spectrophotometer from Thermo fisher scientific (Shanghai, China). The protein purification system (GE, America) was used to separate the cortisol-OVA labeled with AE from excess of labeling reagent. The incubation procedures for coating MMPs with (secondary antibody) were carried out using a simple mixer (Thermo fisher scientific, Shanghai, China). The incubation procedures for antigen labeled to AE were carried out using a constant-temperature shaker (Xin-jingke Biotechnology, Beijing, China). A magnetic particle concentrator (MPC-6, Dynal) was supplied by Thermo fisher scientific (Shanghai, China). The 1200 series LC system (Agilent Technologies) equipped with the Agilent 6410 Triple Quad LC-MS System was taken for LC-MS/MS analysis.

2.3. Preparation of hapten-protein conjugates

The synthesis of hapten-protein conjugates is shown in Fig. 1. The hapten of hydrocortisone was synthesized as follows. Firstly, 600 mg of cortisol was dissolved in 5 mL N, N-dimethyl formamide (DMF), then 4-dimethylaminopyridine (44.2 mg) and succinic anhydride (163.8 mg) were added. The obtained mixture was stirring for 24 h and then evaporated to dryness. The colloidal product was dissolved in chloroform, then 20 mL of distilled water was added. Amounts of light yellow crystals were obtained after stirred. The crystal was dried after filtrated and washed by distilled water. The residue was added into 100 mL saturated sodium bicarbonate solution, then the supernatant was separated after mixed and

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