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Short communication

A recombinant estrogen receptor fragment-based homogeneous fluorescent assay for rapid detection of estrogens

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ABSTRACT

In this work, we demonstrate a novel estrogenic receptor fragment-based homogeneous fluorescent assay which enables rapid and sensitive detection of 17β-estradiol (E2) and other highly potent estrogens. A modified human estrogenic receptor fragment (N-His × 6-hER$_{270–595}$-C-Strep tag II) has been constructed that contains amino acids 270–595 of wild-type human estrogenic receptor α (hER$_{270–595}$) and two specific tags (6 × His and Strep tag II) fused to the N and C terminus, respectively. The designed receptor protein fragment could be easily produced by prokaryotic expression with high yield and high purity. The obtained protein exhibits high binding affinity to E2 and the two tags greatly facilitate the application of the recombinant protein. Taking advantage of the unique spectroscopic properties of coumestrol (CS), a fluorescent phytoestrogen, a CS/hER$_{270–595}$-based fluorescent assay has been developed which can sensitively respond to E2 within 1.0 min with a linear working range from 0.1 to 20 ng/mL and a limit of detection of 0.1 ng/mL. The assay was successfully applied for rapid detection of E2 in the culture medium of rat hippocampal neurons. The method also holds great potential for high-throughput monitoring the variation of estrogen levels in complex biological fluids, which is crucial for investigation of the molecular basis of various estrogen-involved processes.

1. Introduction

Naturally occurring estrogens, including 17β-estradiol (E2), estrone (E1) and estriol (E3), not only play crucial roles in the growth, development and maintenance of the reproductive system, but also affects the functions of many other systems such as immune, skeletal, and neuronal systems (Toran-Allerand et al., 1999; McEwen et al., 2001; Zhang et al., 2004; Okada et al., 2008). Despite a number of existing approaches for the detection of these estrogens in different sample matrices, rapid quantification of them in complex biological fluids remains a critical challenge. High-throughput methods for real-time monitoring of the variation of E2 and other highly potent estrogens in extracellular environment are highly desired to precisely elucidate their interactions with other molecules and understand the molecular basis of the estrogen-involved processes.

A variety of heterogeneous assays such as surface plasmon resonance and evanescent wave biosensors have been reported for the detection of E2 by using antibodies or aptamers as the recognition molecules (Miyashita et al., 2005; Yildirim et al., 2012). These methods often need multiple operation steps to immobilize the capture molecules or remove the unreacted probes, which are time-consuming and labor-intensive. By contrast, a homogeneous assay that directly responses to the variation of target concentration is more favorable for rapid monitoring of the bioactive substances.

Cell-based assays have been developed utilizing the conformational change, folding or dimerization of estrogenic receptor (ER) for screening ligands by using fluorescent proteins as the reporters (Awais et al., 2004; Michelini et al., 2004; Paulmurugan and Gambhir, 2006). But the in vitro assay based on the purified fusion proteins showed very low sensitivity. Lannigan and co-workers developed a fluorescence resonance energy transfer (FRET)-based sensor by using ER ligand-binding domain (LBD) flanked by two different fluorescent proteins for in vitro detection of ER ligands. However, the assay takes about 30 min (De et al., 2005). Alternatively, competitive receptor binding assays have also been reported by using radioactive estradiol such as [^{3}H]E2 (Shelby et al., 1996) or [^{125}I]E2 (Kuiper et al., 1997) as the labeled ligands to provide signals. However, since the labeled E2 had similar strong binding affinity to ERα with that of free E2, the reported competitive/displacement reactions were very slow (16–18 h) and high concentration of competitive free estrogens was needed.

Coumestrol (CS) is a natural fluorescent phytoestrogen which specifically binds with ERα but has a much lower affinity than E2

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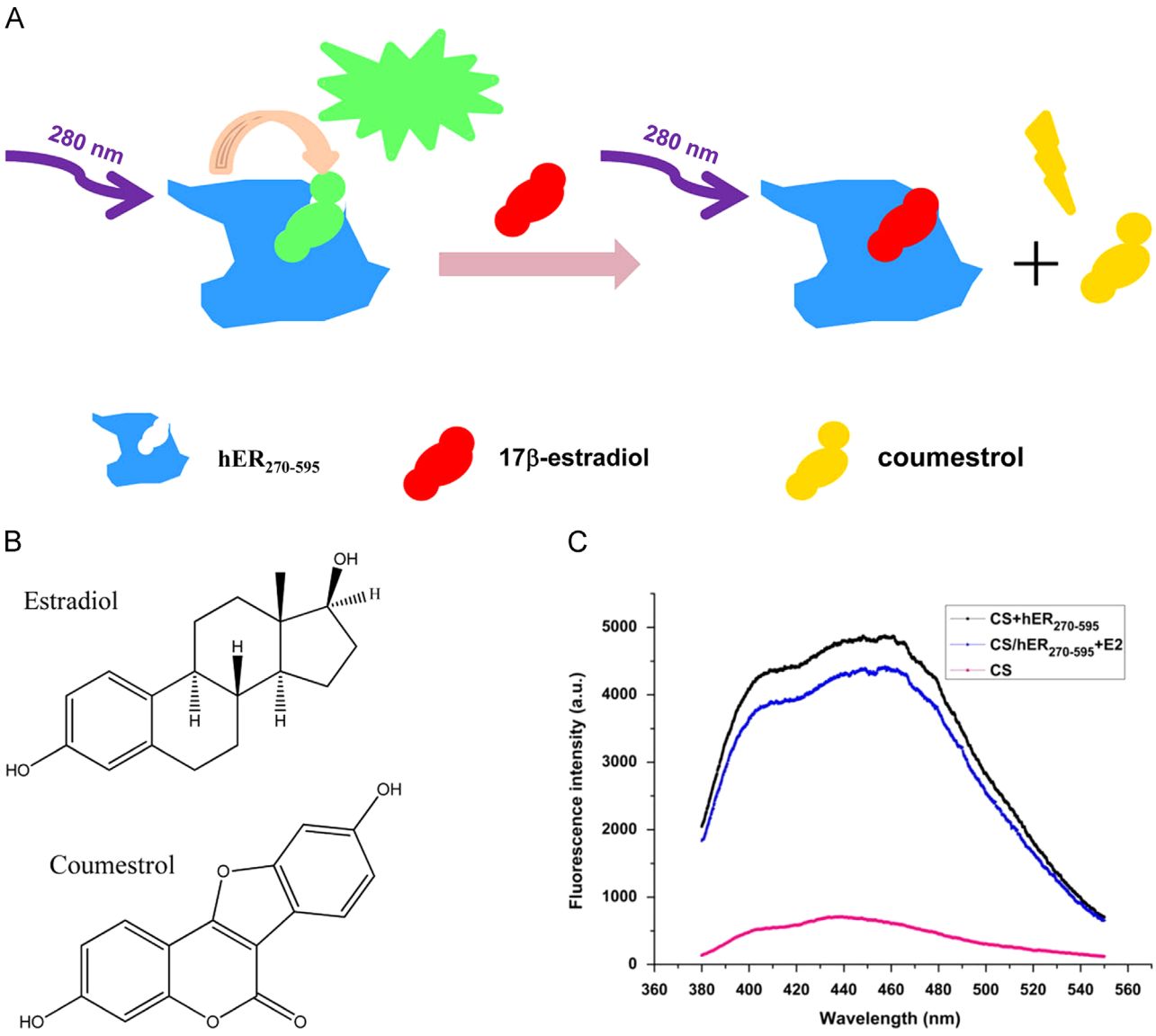
(Lee et al., 1977). ERα binds estrogenic compound in a hydrophobic pocket and provides a stable hydrophobic environment, which result in a dramatic increase of the fluorescence quantum yield of CS (Brzozowski et al., 1997). Besides, the emission spectrum of tryptophan (Trp) in ERα overlaps the absorption spectrum of CS. These features make CS an ideal fluorescent analog reporter in the development of a FRET-based CS/ERα fluorescent sensor for the detection of E2 and other highly potent estrogens via competitive displacement reactions.

Eukaryotic expression of the full-length human receptor protein is costly and labor-intensive. Moreover, the intact receptor proteins have limitations of bulky mass and low stability, which are disadvantages for construction of FRET-based homogeneous assay. To address the issue, we first focused our efforts on establishing an easy-to-operate procedure to produce the recombinant receptor protein fragments by prokaryotic expression. A modified hER fragment that contains amino acids 270–595 of wild-type human ERα ($hER_{270–595}$) is designed that consists of partial domain D (hinge), the whole domain E (LBD) and domain F. Two specific tags, 6 × His and Strep tag II (Trp–Ser–His–Pro–Gln–Phe–Glu–Lys), were added to the N and C terminus of the $hER_{270–595}$, respectively to facilitate the purification and later application of the recombinant protein. In combination with CS, a CS/$hER_{270–595}$ complex-based fluorescent assay has been developed that allows direct response to the presence of E2 with the detection limit as low as 0.1 ng/mL. The assay has been successfully applied for rapid detection of E2 in the culture medium of rat hippocampal neurons.

2. Experimental

2.1. Rapid detection of estrogens by using the CS/$hER_{270–595}$ complex solution

The basic principle of the assay is shown in Scheme 1. To a solution of 200 ng/mL CS in PBS pH = 7.4, 0.5% Tween-20, $hER_{270–595}$ was added to a final concentration of 30 μg/mL. The obtained CS/$hER_{270–595}$ complex solution was added to E2 standard solution



Scheme 1. Schematic illustration of the principle of the CS/$hER_{270–595}$-based assay for detection of 17β-estradiol (E2). (A) Working principle of the assay. (B) Chemical structures of E2 and CS. (C) Fluorescence spectra of free CS and the CS/$hER_{270–595}$ complex in the absence and presence of E2. The concentrations of CS, $hER_{270–595}$ and E2 are 20 ng/mL, 3.0 μg/mL and 1.0 ng/mL, respectively.