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An Electrochemical Sensor Based on the Human Estrogen Receptor Ligand Binding Domain

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ABSTRACT

Three different conformations of the ligand binding domain of the human estrogen receptor (ER-LBD) are observed for the native state when binding an agonist and when binding an antagonist. By conjugating ER-LBD conformation specific peptides to CdS nanoparticles, the three different states can be identified by anodic stripping voltammetry. This electrochemical sensor can detect and distinguish the binding of different ligands to the human estrogen receptor.

A range of environmental pollutants, so-called endocrine disrupters, including pesticides, polychlorinated biphenyls, and/or some of their metabolites, have been reported to bind to the human estrogen receptor (ER).[1−3] Binding of these ligands to the ER induces distinct conformational changes in the ligand-binding domain of the estrogen receptor (ER-LBD) and thus disturb the hormonal pathways.[4,5] The specific conformational change in the ER-LBD are highly dependent on the structure of the ligand. The most prominent conformational change is a ligand-mediated repositioning of helix 12 (H12). It has been reported that, upon binding to the natural agonist estradiol (E2), H12 lies over the ligand-binding cavity and forms a binding surface for transcriptional co-regulators, together with H3 and H5. (Figure 1).[6] Interaction with a partial antagonist such as 4-hydroxytamoxifen (TOH) induces a conformational change, where H12 occupies the groove between H3 and H5 and prevents co-activator binding.

The existence of discrete ligand-induced conformations of the ER-LBD could be detected using conformation specific (CS) peptides that recognize different conformations of the ER-LBD. McDonnel and co-workers[4,7] used phage-displayed peptide libraries for selection of series of small peptides interacting specifically with different conformations of the ER-LBD, which were then used for the conformational analysis. In particular, they identified the three peptides $\alpha\beta$/I, $\alpha\beta$/II, and $\alpha\beta$/III, which have selective affinity for the conformation of the apo form of the ER-LBD and the conformations induced by estradiol and the antagonist 4-hydroxytamoxifen, respectively (Figure 1). Previous studies using ELISA techniques,[4,7,8,29] a piezoresistive canteliver,[9] quartz crystal microbalance,[10] or cyclic voltammetry[11,12] have successfully shown that ER agonists and antagonists can be detected and, in the setups using ELISA and cantilevers, distinguished through the selective binding of CS peptides to the ER.

Within recent years, several inventive designs for sensors based on an electrochemical readout have appeared.[13−15] The combination of electrochemistry and nanotechnology with molecular biology provides very powerful tools for the development of highly sensitive and highly specific biosensors.[16−18] Such sensors have received considerable attention in the development of enzyme-based biosensors,[19] DNA sensors,[20] and immunosensors,[21] etc. While traditional detection methodologies rely mainly on association of an immobilized structure with probes generating an optical readout, development of new setups utilizing electrochemical readouts has gained increasing interest.[22−25] Metal nanoparticles have been employed as electrochemical markers in biosensor assays for highly sensitive detection of DNA[26,27] and proteins.[28−30] The distinct redox potentials of the metal nanoparticles offer the possibility of detecting and distinguishing targets without complicating the setups significantly.

In this study, we apply cadmium sulfide nanoparticles conjugated to three different CS peptides for the electrochemical detection of the binding of ligands to the ER-LBD. For this purpose, cadmium sulfide metal nanoparticles were synthesized using the reverse micelle technique.[31] TEM images of the CdS metal nanoparticles revealed relatively monodisperse particles with an average diameter of 3 nm.[27]

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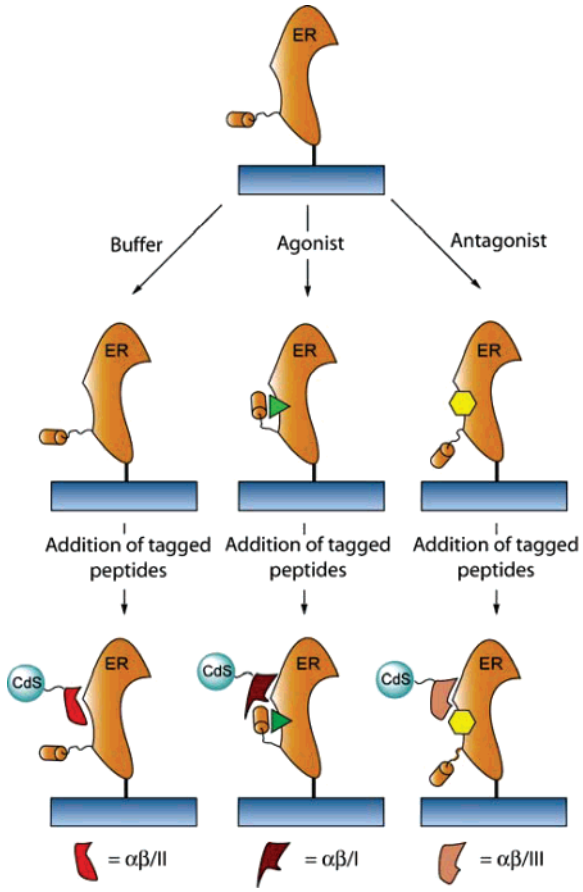

Figure 1. Schematic illustration of ER-LBD H12 positioning upon interaction with the ligands, followed by the binding to respective conformation-specific peptides. The ER-LBD was immobilized on Ni-NTA coated HisSorb Strips via a N-terminal 6xHis-modification. Addition of ligands to the immobilized ER-LBD induces a conformational change. This change enables CdS nanoparticles conjugated to conformation specific peptides to bind specifically to the ligated ER-LBD.

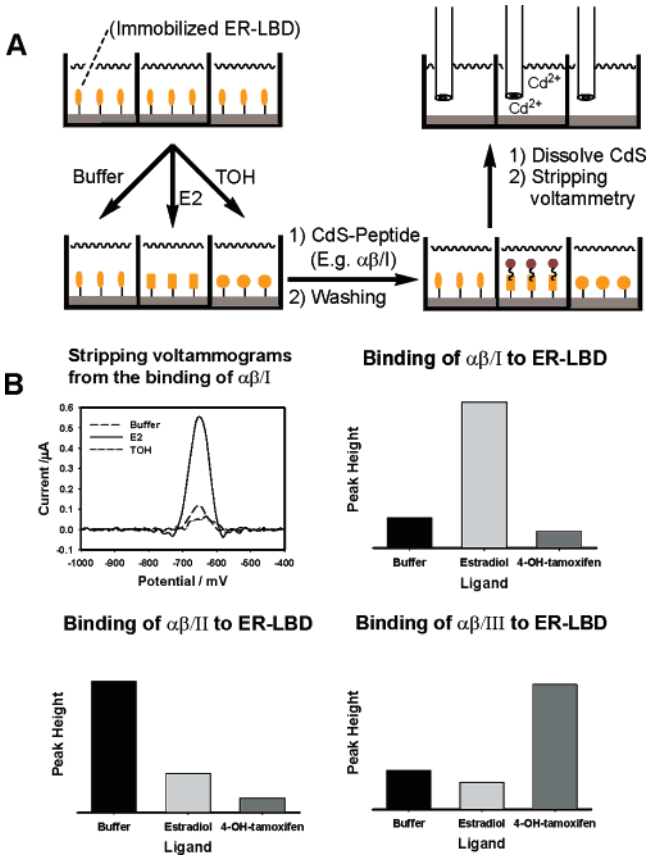

Figure 2. (A) Scematic representation of the setup. ER-LBD is immobilized via a Ni-NTA His complex, ligands are added, inducing a conformational change in the ER-LBD recognizable by the CdS nanoparticles modified CS peptides. The electrochemical detection is performed using anodic stripping voltammetry on the Cd^{2+} ions from the dissolved CdS nanoparticles. (B) Selectivity of the setup was tested by addition of 1μM of the ligands. Top left: stripping voltammograms obtained using CdS-$\alpha\beta$/I. Top right: peak heights obtained from the voltammograms using CdS-$\alpha\beta$/I. Bottom left: peak heights obtained from the voltammograms measured using $\alpha\beta$/II. Bottom right: peak heights obtained from the voltammograms using CdS-$\alpha\beta$/III.

The CdS nanoparticles were conjugated with the CS peptides $\alpha\beta$/I (SSNHQSSRLIELLSR), $\alpha\beta$/II (SAPRATISHYLMGG), and $\alpha\beta$/III (SSWDMHQFFWEGVSR) identified by McDonnell and co-workers.[7] The short synthetic peptides contain an *N*-terminal HS-$(CH_2)_2$CO-GlyGlyGly modification that allows for binding to the CdS nanoparticles via ligand exchange. As illustrated for these conjugates in Figure 1, it is expected that $\alpha\beta$/I binds to the ER-LBD ligated with and agonist, $\alpha\beta$/II to the nonligated ER-LBD, and $\alpha\beta$/III to the ER-LBD ligated with an antagonist.

The assay was prepared by immobilization of the ER-LBD on a solid support. The ER-LBD applied in this study contains six consecutive histidines at the *N*-terminal. This His-tag on the ER-LBD enables a specific complexation to a Ni-NTA modified surface (Ni-NTA HisSorb strips). The successful immobilization of the ER-LBD was verified by using an ELISA assay for detection of ligand-induced ER conformations by studying the binding of ligands to the immobilized ER-LBD.[8]

The addition of ligands such as the agonist estradiol or the antagonist 4-hydroxytamoxifen to the immobilized ER-LBD should induce a conformational change in the ER and thus enable the binding of the specific CdS nanoparticle conjugated CS peptides (as illustrated in Figure 1).

The selectivity of the designed sensor system was tested by treating the immobilized ER-LBD in three separate wells with TBST buffer (apo form), estradiol (E2), or 4-hydroxytamoxifen (TOH), respectively (Figure 2A). Subsequently, the desired CS peptide−nanoparticle conjugate was added. To eliminate signals originating from nonspecific binding of the CS peptide−nanoparticle conjugates to the surfaces, the samples were subjected to a thorough washing procedure prior to dissolution of the particles and electrochemical detection. For the detection, the metal nanoparticles binding to ER-LBD at the surface were dissolved in 0.1 M HNO_3, resulting in the release of Cd^{2+} to the solution. Quantification of the dissolved CdS nanoparticles was performed using anodic stripping voltammetry at an in situ generated mercury electrode. This method has proved highly sensitive in previous reports[19,26−29] because it contains a built-in amplification of the electrochemical signal. For each CS peptide that binds to ER-LBD, thousands of Cd^{2+} ions are generated

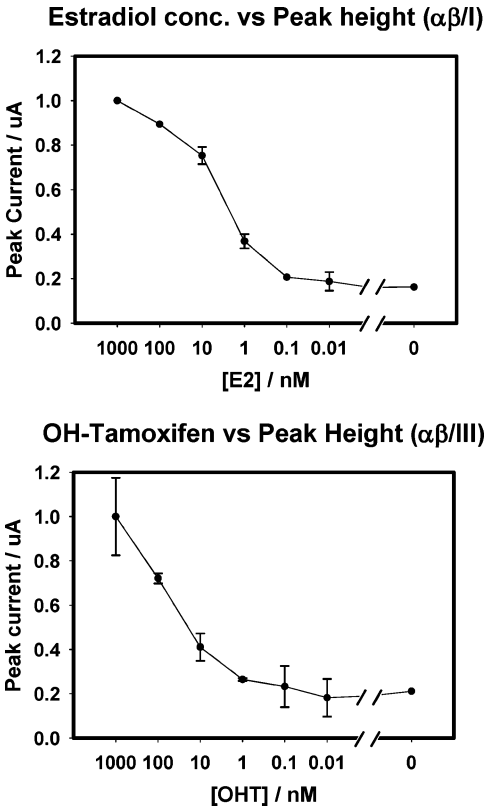

Figure 3. Ligand concentration vs peak height calibration curves reveals a sensitivity of 0.1−1 nM for estradiol and 1−10 nM for 4-hydroxytamoxifen.

and available for reduction and preconcentration as Cd in the mercury film at the electrode. Hence, detection in the attomol region was reported for DNA sensors based on this technique.[27]

After dissolution of the nanoparticles at the surface, the cadmium in solution was detected electrochemically. As seen in Figure 2B, binding of the CdS peptide conjugates to the ER-LBD is highly ligand-specific. The binding of $\alpha\beta$/I−CdS to the ER-LBD ligated with estradiol results in a significant electrochemical response compared to the signals obtained from the nonligated or the 4-hydroxytamoxifen ligated ER-LBD. The CdS-$\alpha\beta$/II binds specifically to the nonligated ER-LBD, and only a low signal is obtained for ER-LBD ligated with estradiol and an insignificant signal is obtained for 4-hydroxytamoxifen. The same trend is observed for the binding of CdS-$\alpha\beta$/III, which as expected only results in a significant signal for the binding of ER-LBD ligated with 4-hydroxytamoxifen.

The ligand sensitivity of the designed sensor system was evaluated by conducting a series of experiments in which the concentration of estradiol or 4-hydroxytamoxifen varied between 10 pM and 1 μM (Figure 3). The baseline peak height was determined in an experiment using the nonligated ER-LBD. The peaks obtained during the stripping voltammetry experiments were normalized against the maximum peak height at 1 μM of the ligand.

The distinct S-shape of the calibration curve for E2 is in good accordance with what would be expected. At high ligand concentrations (above 1 μM), saturation of the system occurs, and thus a flattening of the curve is observed. At low ligand concentrations, the concentration profile levels off, yielding a detection limit of estradiol at 0.1−1 nM, and at 1−10 nM for 4-hydroxytamoxifen (see Figure 3). The difference in sensitivity for estradiol and 4-hydroxytamoxifen corresponds well with previously observed binding affinities of the ligands.[32]

The detection limits for this setup are better than what have previously been reported in a setup using cantilevers,[9] where detection limits between 2.5 nM and 20 nM were obtained and the original ELISA detection setup had detection limits in the 10−100 nM range.[4,7]

In conclusion, we have developed a sensor based on the human estrogen receptor in which three different states of the ER-LBD could be identified and distinguished. The three different conformations result from the native state of ER-LBD, binding of an agonist, or binding of an antagonist. The electrochemical readout is reliable and highly sensitive, and the detection limit is determined by the binding constant the ligands to the ER-LBD. This sensor setup could probably be applied for monitoring the influence and affinity of potential endocrine disruptors on the human estrogen receptor.

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Supporting Information Available: Experimental details. Stripping voltammograms for concentration profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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