SILICON NANOWIRE BIOSENSOR FOR STUDYING NUCLEAR HORMONE RECEPTOR AND RESPONSE ELEMENT INTERACTIONS

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ABSTRACT

Silicon nanowire (SiNW) biosensor capable of studying the interactions between human ERs (ER, α and β subtypes) and EREs (dsDNA) is described. The ERs were covalently immobilized on the SiNW surface. Various EREs including wild-type, mutant and scrambled sequences were then applied to the ER-functionalized SiNW surface. The results show that the specificity of the ERE-ERα binding is higher than that of the ERE-ERβ binding, what is more, the mutant ERE reduces the binding affinity for both ERα and ERβ. A very low concentration of 10 fM wild-type ERE was found to be able to bind to the ERα.

KEYWORDS: Silicon nanowire; Biosensor; Estrogen receptors; Estrogen response elements

INTRODUCTION

Estrogen receptors (ERs) are members of a superfamily of nuclear receptors that function as ligand-modulated transcriptional regulators. The ERs mainly function as a DNA binding transcription factor that regulates gene expression. They play a key role in many normal physiological processes, but promote aberrant growth of breast cancer cells. Ligand-activated ERs directly bind to specific DNA sequences known as estrogen responsive elements (EREs) present in estrogen-sensitive gene promoters. The sequence of all known EREs is based on a perfect inverted repeat separated by a three-base spacer, 5'-GGTCAnnnTGACC-3'. However, many naturally occurring estrogen-responsive genes have two or more imperfect core consensus sequences. As a result, a sensitive solution to differentiate between subtle differences in ER-DNA binding affinities is highly desirable.

Silicon nanowires (SiNWs) biosensor is a recently-developed technology, which is capable of label-free and highly sensitive detection of nucleic acids and proteins [1-4]. However, little, to date, is known about studying protein-DNA interactions using the SiNW biosensor. In the study, we, for the first time, characterize the interactions between ERs (ER, α and β subtypes) and EREs by using the state-of-the-art electrical SiNW biosensor platform.

EXPERIMENTAL

The SiNW sensor was prepared by self-limiting oxidation process of silicon beams patterned using conventional deep UV photolithography, which is electrically addressable on an individual basis. Nanowire chip for bio-sensing is designed with 36 clusters of 5 nanowires each. The SiNW arrays are 90 μm in length and 2 μm spacing in between two wires.

The SiNW surface was first functionalized using APTES, followed by glutaraldehyde. After which, the ER protein was covalently bound onto the SiNW. Incubation was achieved in a moist environment for 2 h. The unbounded ER proteins were then washed away using 1×PBS buffer. The ER-functionalized surface was then passivated with 1mg/ml amino-PEG to minimize the unspecific binding.

25-bp ERE sequences, wild-type ERE (wt-ERE) which possesses a consensus sequence with the palindromic GGTC half-site, mutant ERE (mut-ERE) which possesses two base-pair mismatched with one base mismatch in each of the half-sites, and scrambled ERE sequence (non-ERE), which contains the same genetic makeup as consensus sequence but in scrambled form to keep the CG content similar, were used in this work. Table 1 shows the sequences of the three kinds of DNA.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tr>
<td>Wild-type ERE (wt-ERE)</td>
<td>5'-CAACTGGGTATTCTGACCTAGAAG-3'</td>
</tr>
<tr>
<td>Mutant ERE (mut-ERE)</td>
<td>5'-CAACTGGTTATTCTGATGACTAGAAG-3'</td>
</tr>
<tr>
<td>Scrambled ERE (non-ERE)</td>
<td>5'-CAACTGAATCGTTTCTCGATAGAAG-3'</td>
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The basic sensing mechanism was performed by observing the resistance change before and after binding of the ERE to the ER immobilized on the SiNW surface. To verify this resistance change, resistance was being measured twice: before and after the addition of the ERE target. Thus, following the immobilization of the ER, resistances of 15 SiNW wires were measured by probing the two terminals, source (S) and drain (D) electrodes, with Alessi REL-6100 probe station (Cascade Microtech, Beaverton), in the presence of 0.01×SSC buffer solution. After ER-ERE binding, this bound com-
plex was sent for a second electrical measurement. The resultant change in resistance was analyzed by using the formula \(\frac{(R-R_0)}{R_0}\times100\).

RESULTS AND DISCUSSION

Figure 1 illustrates the working principle of the SiNW biosensor for studying the interactions of ER-ERE. ER is covalently immobilized on the electrically addressable SiNW surface. When wt-ERE is applied to the immobilized ER, obvious resistance change occurs because wt-ERE is capable of recognizing the ER. Nonetheless, when non-ERE is used, resistance change is minimal as the non-ERE has the sequence in the ERE arms scrambled.

The sequence specificity was investigated by applying these three EREs to the ER\(\alpha\)-functionalized SiNW, and the resistance change was shown in Figure 2a. An obvious change was seen when 1 pM wt-ERE was used, whereas only a negligible change was obtained when 1 pM non-ERE was applied to the SiNW device. A smaller change was observed when 1 pM mut-ERE was utilized. Similar trend was found by applying different EREs to the ER\(\beta\)-functionalized SiNW surface (Figure 2b). Interestingly, ER\(\alpha\) shows a higher specificity than ER\(\beta\) as the bindings of ER\(\beta\) with mut-ERE and non-ERE, respectively, cause a bigger change in resistance, which implies difficulty in distinguishing sequence.

Sensitivity was studied by ER\(\alpha\)-wt-ERE interactions. To do so, various concentrations of wt-ERE ranging from 1 pM to 1 fM were applied to the ER\(\alpha\)-functionalized SiNW biosensor. As shown in Figure 3, blank control, in which no wt-ERE in the buffer was applied to the functionalized SiNW surface, showed negligible change in resistance. Likewise, another control, in which BSA was immobilized on the SiNW surface in place of ER\(\alpha\), also exhibited negligible change in resistance. A descending trend (~26.0% for 1 pM wt-ERE, ~16.3% for 100 fM wt-ERE, ~6.4% for 10 fM wt-ERE, and ~3.3% for 1 fM wt-ERE, respectively) could be observed for decreasing concentrations of wt-ERE. Based on the signal-to-noise ratio obtained from the ER\(\alpha\)-ERE binding, the detection limit for the binding of ER\(\alpha\)-ERE is 10 fM.

Figure 1. Schematic illustration of interactions of ER-ERE studied by the SiNW biosensor. ER is covalently immobilized on the SiNW surface, and ERE is bound to the specific ER. The ERE brings intrinsic negative charges to the SiNW surface, giving rise to increase in resistance in the n-type SiNW biosensor.

Figure 2. Interactions of various EREs with ER\(\alpha\) and ER\(\beta\) immobilized on the SiNW surface, respectively. (a) Specificity of various EREs including wt-ERE, mut-ERE and non-ERE to the immobilized ER\(\alpha\). (b) Specificity of various EREs including wt-ERE, mut-ERE and non-ERE to the immobilized ER\(\beta\).

Figure 3. Sensitivity of the SiNW biosensor for studying ER-ERE binding.
To demonstrate the effect of salt concentrations on ER-ERE binding with the SiNW biosensor, 1 pM wt-ERE in various concentrations of buffer solutions ranging from 10×SSC to 0.01×SSC was incubated with the ERα-modified SiNW surface, and the response was obtained by measuring the resistance change before and after the binding of wt-ERE to the immobilized ERα in 0.01×SSC solution. Figure 4 shows varying response versus different buffer solution used for ERα-wt-ERE binding. Results show that a decreased salt concentration from 6×SSC to 0.01×SSC disrupts the DNA binding to the protein, meanwhile an increased salt concentration from 6×SSC to 10×SSC also interrupts the binding. The pH of the buffer solution used is 7.0, so ERα (pI: ~8.3) is positively charged and on the contrary DNA is negatively charged. The formation of protein-DNA is mainly dependent on three factors, electrostatic, hydrogen bond and van der Waals force. At a higher ionic strength, the interactions are mainly disturbed by the salt ions competing with DNA for the electrostatic binding to protein. As a result, the increased salt concentrations from 6×SSC to 10×SSC depleted wt-ERE binding by ~22.7% to ERα. In the case of a lower ionic strength, the protein-DNA complex is primarily formed through hydrogen bond and van der Waals force. As the salt concentrations decrease, the hydrogen bond and van der Waals force play a weaker role in binding, thus depleting the interactions. As shown in Figure 4, the decreased salt concentration from 6×SSC to 0.01×SSC depleted wt-ERE binding by ~25.7% to ERα.

**CONCLUSION**

We demonstrated the capabilities of the SiNW biosensor for studying ER-ERE interactions. The SiNW-based biosensor allows for a sensitive determination of wt-ERE as high as 10 fM, as well as a comprehensive understanding on specificity between ERs and various EREs involving wt-ERE, mut-ERE and non-ERE. It is believed that the developed SiNW biosensor will benefit the studies in hormone receptor biology to rapidly and sensitively screen the binding sites of estrogen-responsive genes with the receptors.

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**REFERENCES**


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