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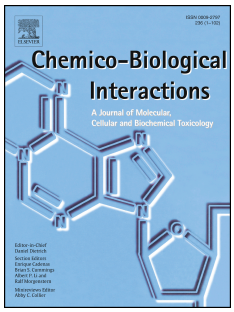

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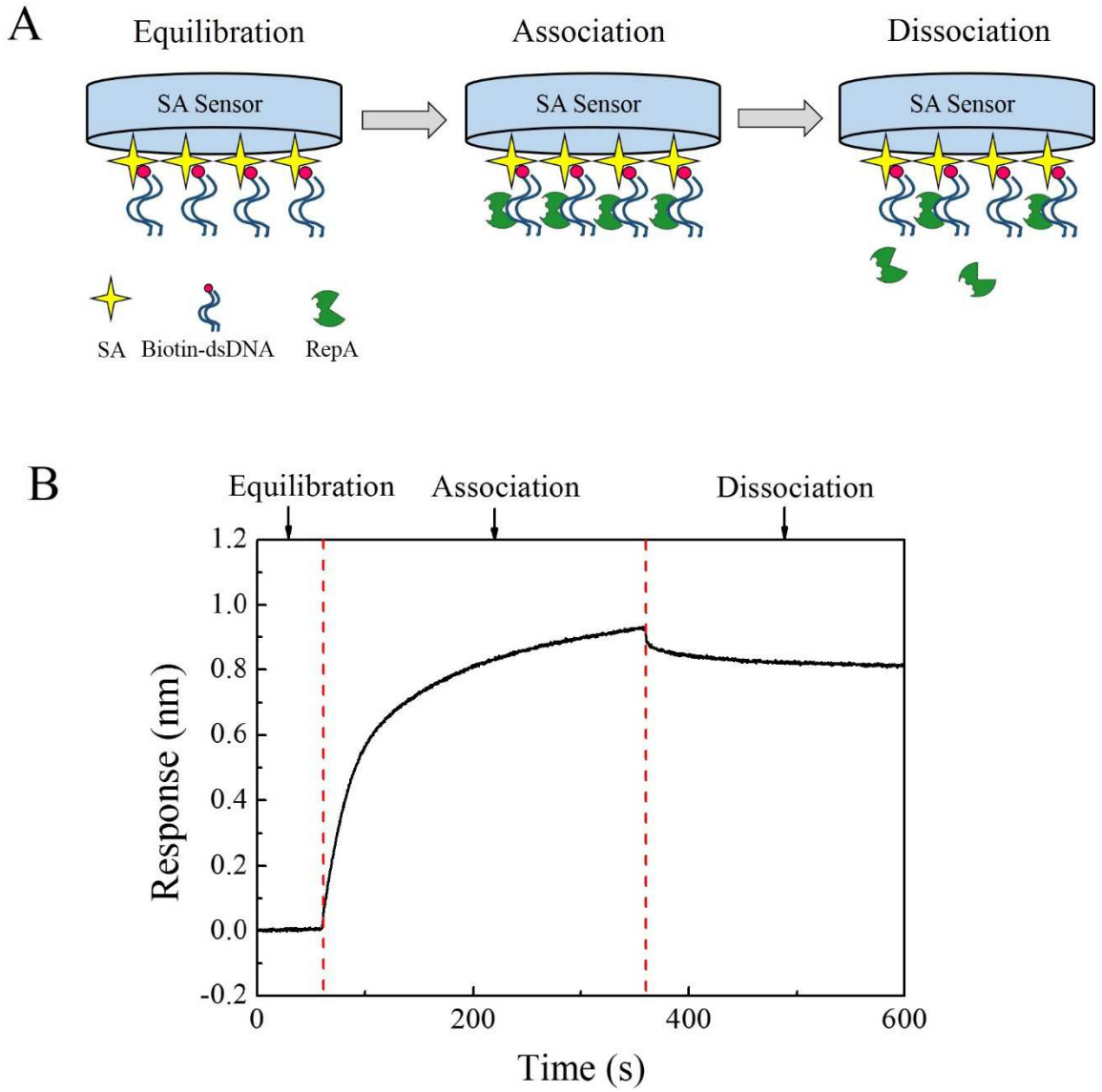

Quantitative determination of testosterone levels with biolayer interferometry

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Abstract

Natural and synthetic steroid hormones are widely spread in the environment and are considered as pollutants due to their endocrine activities, even at low concentrations, which are harmful to human health. To detect steroid hormones in the environment, a novel biosensor system was developed based on the principle of biolayer interferometry. Detection is based on changes in the interference pattern of white light reflected from the surface of an optical fiber with bound biomolecules. Monitoring interactions between molecules does not require radioactive, enzymatic, or fluorescent labels. Here, 2 double-stranded DNA fragments of operator 1 (OP1) and OP2 containing 10-bp palindromic sequences in chromosomal *Comamonas testosteroni* DNA (ATCC11996) were surface-immobilized to streptavidin sensors. Interference changes were detected when repressor protein RepA bound the DNA sequences. DNA–protein interactions were characterized and kinetic parameters were obtained. The dissociation constants between the OP1 and OP2 DNA sequences and RepA were 9.865×10^{-9} M and 2.750×10^{-8} M, respectively. The reactions showed high specifically and affinity. Because binding of the 10-bp palindromic sequence and RepA was affected by RepA–testosterone binding, the steroid could be quantitatively determined rapidly using the biosensor system. The mechanism of the binding assay was as follows. RepA could bind both OP1 and testosterone. RepA binding to testosterone changed the protein conformation, which influenced the binding

between RepA and OP1. The percentage of the signal detected negative correlation with the testosterone concentration. A standard curve was obtained, and the correlation coefficient value was approximately 0.97. We could quantitatively determine testosterone levels between 2.13 and 136.63 ng/ml. Each sample could be quantitatively detected in 17 min. These results suggested that the specific interaction between double-stranded OP1 DNA and the RepA protein could be used to rapidly and quantitatively determine environmental testosterone levels by the biolayer interferometry technique.

Keywords:

Biolayer interferometry

Comamonas testosteroni

DNA/protein interaction

Quantitative determination of testosterone

Abbreviations:

3α-HSD/CR: 3α-hydroxysteroid dehydrogenase/carbonyl reductase; BIA: biomolecular interaction analysis; BLI: Biolayer interferometry; bp, base pair; CO, control; DON: deoxynivalenol; dsDNA: double-stranded DNA; E2: 17β-estradiol; EE2: 17α-ethinylestradiol; EMSA: electrophoresis mobility shift assay; k_a: association rate constant; k_d: dissociation rate constant; K_D: equilibrium dissociation constant; LOD: limit of detection; OP1, operator 1;

OP2, operator 2; SA: streptavidin; SLS: sodium lauroyl sarcosinate; ssDNA: single-stranded DNA; T: testosterone; TbA: trenbolone acetate; UV: ultraviolet

1. Introduction

Natural and synthetic steroid hormones are continuously released into the environment from humans, livestock, and aquaculture sources [1, 2, 3, 4]. Synthetic steroids, such as the androgens testosterone (T) and trenbolone acetate (TbA) and the estrogens 17β-estradiol (E2) and zeranol, are primary growth promoters used in the United States livestock industry to increase animal growth [5]. These steroids have been widely detected in sewage treatment plants [6, 7], rivers [8], and drinking water [9].

Steroids can interfere with the hormone systems of humans and other organisms by mimicking physiological hormones, inhibiting signaling pathways as endocrine disruptors, interfering with normal biological responses [10].

In most fish, environmental steroids can alter gonad development even after sex differentiation has occurred, and these exogenous steroids can cause sex reversal [11]. For example, the expression of vitellogenin in fish can be induced by 17α-ethinylestradiol (EE2), even at a concentration as low as 0.1 ng/L, which can affect sex differentiation of the fish [12, 13].

To date, several reviews have been published on steroid-analysis methods [14, 15]. A variety of classical analytical techniques for specifically estimating steroids has already been reported, such as high-performance liquid chromatography (HPLC), liquid chromatography coupled with fluorescence measurement, or mass spectrometry [16, 17]. For screening

purposes, enzyme-linked immunosorbent assays are widely used [18]. Novel developments for quantitating low levels of estrogens include highly sensitive immunoassays with surface plasmon resonance systems [19] or electrochemical immune sensors [20]. Although the problem of pollution with steroids has received substantial attention, data regarding their concentrations in the environment are lacking. The methods currently used to determine environmental steroid levels are expensive and complex. Therefore, new methods for low-cost, rapid, easy steroid detection and quantification are urgently needed. The method used should be sensitive enough to detect low concentrations of steroids in the environment.

In early investigations, 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) was found to act as a key enzyme in degrading *Comamonas testosteroni* steroids. In the bacterium, the RepA protein functions as a repressor that inhibits 3α-HSD/CR transcription. RepA can bind to 2 palindromic operator sequences (OP1 and OP2), thereby blocking 3α-HSD/CR transcription. When testosterone binds to RepA, the conformation of the RepA protein changes, which leads to decreased affinities between RepA and the operator sequences, and increased 3α-HSD/CR expression in *C. testosteroni* [21]. Based on these findings, a novel, rapid and fluorescence-based screening method was generated for steroid determination using a cell-free biosensor system, and the limit of detection (LOD) was as low as 28 pg/ml for testosterone and 0.029 fg/ml for estradiol

[22].

The Biolayer interferometry (BLI) Octet RED96 system (Pall ForteBio, USA) includes a white light source, a transmission optical fiber, an optical fiber sensor, and optics spectrometers. White light is launched into the optical fiber sensor with a polymer-coated tip. After the light reaches the sensor's fiber top, some of the light is reflected into the fiber. Some light continues through the fiber and is reflected when it encounters molecules immobilized on the top of the sensor's fiber, while the rest of the light continues into the biomolecular solution. Both beams of reflected light can interfere with each other, leading to a shift in the wavelength of the detected light. The shift is related to the thickness of the layer immobilized on the top of the sensor fiber. As a result, the system essentially measures the increase in thickness on the top of the fiber as molecules bind to it [23]. The binding process can be monitored in real time, and biomolecular interaction analysis (BIA) can be achieved.

In this work, based on BLI technique, BIA of the RepA protein with the dsDNA OP1 and OP2 templates was rapidly performed in vitro. Subsequently, the quantitative determination of testosterone based on RepA and dsDNA interactions was established with the BLI technique. The new method does not require label radioactive, enzymatic, fluorescence materials, or washing steps. Microfluidic flow-through systems are not used; therefore, the potential problem of clogging the micro-channels is avoided [24].

2. Materials and methods

2.1. Bacterial strain and plasmid

The host strain *Escherichia coli* BL21 (DE3) pLysS (TransGen Biotech, Beijing, China) and the recombinant plasmid pET-RepA were used to overexpress the RepA protein. The recombinant plasmid pET-RepA containing the ampicillin-resistance gene was a gift from the Institute of Toxicology and Pharmacology, University of Kiel (Kiel, Schleswig-Holstein, Germany) [25].

2.2. Growth conditions

Bacterial cells were grown in Luria-Bertani medium (OXOID, Basingstoke, UK) at 37ºC in a 180-rpm shaker. The growth medium contained 100 μg/ml ampicillin (Amresco, USA).

2.3. Other reagents

Testosterone standards (Dr. Ehrenstorfer GmbH, Augsburg, Germany) were used to establish a standard curve. Sodium lauroyl sarcosinate (SLS) (Sigma, UK) was used to isolate the RepA protein. Ultrapure water (Millipore, Boston, USA) was used to prepare all reagents.

2.4. Preparation of dsDNA OP1 and OP2 templates

Three biotin-labeled, 38-base pair (bp) dsDNA fragments (OP1, OP2, and a control [CO] template) were synthesized (Fig. 1). OP1 and OP2 contained 10-bp palindromic sequences beginning at nucleotide positions 935 (TCAAAGCCCA) and 2568 (TGGGCTTTGA) in plasmid p6 [21]. The 38-bp CO DNA fragment was located 14 bp upstream of OP2 and was used as a control. The OP1, OP2, and CO fragments were prepared by annealing complementary biotin-conjugated primers (20 μM, Sangon, Shanghai, China). Two primers in each reaction were incubated at 90ºC for 10 min and allowed to cool down slowly to 25ºC for 1.5 h in TEN buffer (10 mM Tris-HCl, 1 mM EDTA, 0.1 M NaCl; pH 8.0) [21]. Two complementary single-stranded DNAs (ssDNAs) could be melted (90ºC) and annealed (25ºC) to form dsDNA. The reactions could be controlled by the changing of the ultraviolet (UV) spectrum, measuring the absorbance at 260 nm could prove the formation of dsDNA fragments.

2.5. Overexpression and purification of the RepA protein

To prepare the recombinant RepA protein, the pET-RepA plasmid was transferred into *E. coli* BL21 (DE3) pLysS. Recombinant bacteria were induced

with 1 mM isopropyl β-D-1-thiogalactopyranoside at 37ºC in a 180-rpm shaker for 5 h. The culture medium contained 100 µg/ml ampicillin to ensure the presence of recombinant plasmid. After induction, the cells were lysed with 100 µg/ml lysozyme and 0.5% SLS. A nickel-nitrilotriacetic acid column (Qiagen, Germany) was used to purify the RepA protein under a native condition with NPI buffer with 0.5% SLS.

The purified RepA protein was identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [27]. The predicted molecular mass of RepA is 47.6 kDa (45.4 kDa + a 2.2 kDa His tag). The concentration of RepA was 0.58 mg/ml, as determined using the “Bradford” method and bovine serum albumin as a standard [28]. The purified RepA was allowed to interact with the dsDNA templates, OP1 and OP2.

The RepA protein was expressed in inclusion bodies, and 0.5% SLS was used to solubilize the protein. Electrophoresis mobility shift assays (EMSAs) [21] were performed to study binding of the isolated RepA protein to OP1 or OP2. SLS was present at a low concentration when RepA was added to the binding reaction. Under these conditions, SLS did not likely influence the binding activity of RepA to OP1/OP2. It was difficult to obtain the highly purified RepA protein, although it was sufficiently pure for the next analytical steps.

2.6. Immobilization of dsDNA fragments onto the fiber optic sensor surface

Preparation of the BLI fiber optical sensors was described previously [26]. The sensors consisted of a small length of optical fiber, with the top modified with streptavidin (SA sensors). The biotin-labeled dsDNA fragment was immobilized on the top of the sensors by immersing them in 0.2 ml diluted, biotin-labeled dsDNA solution for 5 min at 30ºC. To reduce non-specific binding, the biotin-labeled dsDNA was diluted in 10 mM phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBST). The immobilizing dsDNA sensors were freshly prepared on the day of each experiment and were equilibrated with PBST before use.

2.7. Assay of RepA protein binding to dsDNA OP1 and OP2

Three steps were required for detecting the interaction between dsDNA and RepA by BLI. During the equilibration step, the dsDNA template OP1 or OP2 was immersed in PBST for 60 s (Fig. 2A), and RepA was diluted into different concentrations of PBST. The sensors were immersed in RepA solutions for 300 s. Finally, the sensors were immersed in PBST for 240 s to remove the free RepA. All steps were performed in 0.2 ml at 30ºC and 1000 rpm.

A typical sensor model is shown in Fig. 2B. The signals from each sensor during the association and dissociation steps were monitored in real time. The kinetic parameters of the interactions between dsDNAs and RepA were

calculated using the Octet Data Analysis Software.

2.8. Quantitative determination of testosterone

A testosterone standard was dissolved to 250 μg/ml in absolute ethanol. Different concentrations of testosterone were prepared by serial dilution in PBST. Before the determination, 200 μl PBST (equilibration solution) was added to the wells of a 96-well, black microtiter plate. One hundred microliters of a standard testosterone solution and 100 μl RepA (40 μg/ml) were added to the other wells. The plate was incubated for 10 min at 30ºC.

Two steps were required during the process. The first was an equilibration step wherein the SA sensors of immobilized dsDNA were immersed in PBST for 60 s. The second was an association step wherein the dsDNA bound to the limited amount of RepA, which was not bound to testosterone. All reactions were conducted at 30ºC, 1000 rpm.

Association curves were used to calculate the testosterone concentrations in samples. To compare the maximal signal intensities among the sensors, the percentage of the signal from the blank control (BC) (without testosterone in the mixture) was used as a reference. Therefore, the standard curve was established based on the relationship between the percentage of signal response and the concentrations of the testosterone standards. The testosterone concentrations in the samples was determined by comparing the

samples to the standard curves.

3. Results

3.1. Preparation of dsDNA OP1, OP2, and CO templates

Three 38-bp, biotin-labeled dsDNA fragments (OP1, OP2, and CO) were prepared by annealing hybridization with the synthetic oligonucleotides. The G + C percentage of the dsDNA OP1, OP2, and CO fragments were calculated to be 76.3%, 52.6% and 50%, respectively (Fig. 1), and their melting temperatures were 86ºC, 75ºC, and 72.4ºC, respectively. The three dsDNA fragments were denatured at 90ºC for 10 min and allowed to cool down slowly to room temperature for 1.5 h. The wavelength-scanning results in the UV spectrum are shown in Fig. 3. The highest absorption peaks of ssDNA and dsDNA fragments were found at a wavelength of 260 nm. The dsDNA fragments were successfully obtained. However, the hypochromic effects of the OP1 and OP2 oligonucleotides were weak. Perhaps the UV spectral curves were affected by the high GC percentages of OP1 and OP2, such that dramatic changes during the transition between ssDNA and dsDNA were not observed.

3.2. dsDNA OP1, OP2 loading on the SA sensors

Three different concentrations of biotin-labeled dsDNA OP1 were loaded onto the SA sensors through non-covalent binding. The response curves of the loading process are shown in Fig. 4. The maximum response was obtained when a sensor was loaded 1 μM OP1 dsDNA. Because dsDNA OP2 and CO contain the same base pairs as OP1 dsDNA does, the best immobilization concentration was 1 μM when biotin-labeled dsDNAs (OP1, OP2, and CO) were loaded on SA sensors.

3.3. Interaction of dsDNA OP1, OP2, and RepA protein

SA sensors loading with the dsDNA OP1 or OP2 template were incubated with different concentrations of RepA diluted in PBST. The association and dissociation processes were monitored in real time. Interaction curves were obtained. Association and Dissociation steps are shown in Fig. 5A and Fig. 6A. During association, the responses were decreased in turn, while during dissociation, the binding of dsDNA OP1 and OP2 to RepA were a little bit separated. During the analysis step, the fitting of the association and dissociation curves was calculated based on the nonlinear regression fits from 1:1 global analysis of all data from line “a” to line “g” by the Octet Data Analysis Software. The kinetic parameters of the interactions between RepA and dsDNA OP1 or OP2 were obtained. The association rate (k_a) and dissociation

rate (k_d) values with errors of dsDNA OP1 and RepA interaction were $1.196 \times 10^4 \pm 38$ Ms^{-1} and $1.179 \times 10^{-4} \pm 1.6 \times 10^{-6}$ s^{-1}. The k_a and k_d values (with errors) for the dsDNA OP2–RepA interaction were $1.399 \times 10^4 \pm 74$ Ms^{-1} and $3.847 \times 10^{-4} \pm 6.4 \times 10^{-6}$ s^{-1}, respectively. The equilibrium dissociation constant (K_D) values for RepA with the dsDNA OP1 and OP2 templates were 9.865×10^{-9} M and 2.750×10^{-8} M, respectively. The global fit correlation coefficients (r^2) of the interactions between RepA and the dsDNA OP1 and OP2 templates were 0.998 and 0.995, respectively. The K_D represents the affinity of the interaction between 2 molecules. In binding reactions, similar K_D values are expected when the same palindromic OP sequence is present in different dsDNA templates, although different K_D values for the RepA interaction with OP1 and OP2 were found. Perhaps the sequences flanking the palindromic 10-bp binding site affected the results. The K_D values for RepA with OP1 and OP2 were not dramatically different; therefore, both dsDNA OP1 and OP2 can be used for detection purposes. In quantitative determinations, dsDNA OP1 and RepA was used to detect testosterone.

In addition, the dsDNA CO template did not show binding to RepA on the SA sensors, indicating that the binding between dsDNA OP1 or OP2 and RepA was specific.

3.4. Quantitative determination of testosterone

Xiong et al. [21] reported that testosterone can bind RepA, which causes the conformation of RepA to change and decreases the binding affinity between RepA and dsDNA OP1. The SA sensors were loaded with biotin-labeled dsDNA OP1 and immersed into a mixture of RepA and testosterone. The interaction curves obtained are shown in Fig. 7. The response of line “a” was the maximum because no testosterone was added to the mixture. dsDNA binding to RepA decreased with increasing testosterone concentrations (going from line “b” to line “h”).

Finally, the concentrations of testosterone could be tested, using a standard curve with testosterone standards ranging from 2.13 to 136.63 ng/ml (Fig. 8). The percentage of signal detected negatively correlated with the testosterone concentration. The standard curve showed good linearity, with a correlation coefficient value (r^2) of ~0.97, and was described by the equation $y = 102.57 – 48.10x$.

4. Discussion

The BLI technique is a novel optical fiber approach for reflectometry interference spectroscopy of BIA. The advantage of this new method is that optical fiber can be used as a biosensor. The method is simple to perform, involves lower cost than other methods, and provides high sensitivity and reliability. A single bioprobe can be easily prepared as a BIA biosensor for

direct real-time monitoring of association and dissociation processes in various kinds of molecular interactions, such as in immunoassays [26].

The BLI technique has been widely used for BIA in previous studies, such as for the quantitative determination of deoxynivalenol (DON) in whole wheat flour. The BLI system was first described in 2011 by Chris M. Maragos [29]. The detection principle was based on immunoassays, with competition between DON and immobilized DON-BSA for binding to limited quantities of an anti-DON antibody. The limit of detection (LOD) was 0.1 mg/kg. After signal amplification using colloidal gold was introduced, the LOD increased to 0.09 mg/kg [30]. Furthermore, Wallner et al. [31] used the BLI system to analyze different liposomes and protein interactions. The results demonstrated that the interaction between the rh-Epo protein and selected liposomes was strongly related to the biophysical properties of the membranes. The analysis technique for studying protein and liposome interactions was simple and rapid.

In a previous study conducted by Xiong et al., EMSA results demonstrated that RepA could specifically bind to dsDNA OP1, OP2, and testosterone [21]. EMSA is a classical method for detection protein–DNA interactions, but the method is time-consuming and complex. Previously, a cell-free *C. testosteroni* mutant strain CT-GFP5-1 biosensor system was generated and used as a sensitive fluorescence-based biosensor for steroid determinations in the environment [22]. However, the cytosol, enzymes, and DNA in the cell-free system were unstable. In addition, CT-GFP5-1 fluorescence biosensor

detection is not very stable at present.

In this study, RepA interactions with dsDNA OP1 and OP2 were easily detected using the BLI technique, and the process only required 10 min. The results showed that the binding of the dsDNA OP1 and OP2 templates with RepA occurred in a sequence-specific manner, in accordance with the EMSA results by Xiong et al. [21]. In addition, the kinetic parameters of dsDNA and protein interactions were obtained. In the future, instead of the classic EMSA approach, the BIA of BLI technique might be used to determine specific DNA–protein binding interactions. The technique could be also used to detect protein–protein, glucan–protein, RNA–protein, and protein–ligands interactions, as well as antibody binding to viruses.

Quantitative determination of testosterone using the BLI technique was demonstrated. The assay is easily performed and does not require radioactive, enzymatic, or fluorescent labels. Excluding the dilution, equilibrium, and SA sensor immobilization steps, an individual quantitative determination of testosterone requires approximately 17 min to complete. We could determine testosterone in a range between 2.13 and 136.63 ng/ml. The sensitivity of the quantitative determination is approximately the same as that of HPLC.

The most common steroid pollutants in the environment are estrogens, such as estrone, 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) [32]. In the future, we hope to establish a novel quantitative BLI assay for determining estrogen concentrations, using a specific estrogen-binding protein.

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Legends

Fig. 1. Location of 2 palindromic sequences, operator 1 (OP1) and OP2, and the control (CO) sequence. The location and orientation of the RepA gene and 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) gene in a 5.2-kb EcoRI fragment of *C. testosteroni* ATCC11996 chromosomal DNA are indicated by the arrows. The sequences of 3 oligonucleotides (OP1, OP2, and CO) used to study dsDNA–protein interactions are underlined. Two palindromic, 10-nucleotide sequences are in boldface. Open circles represent the locations of the 2 palindromic sequences, separated by 1.6 kb.

Fig. 2. (A) Schematic representation of the mechanism used for detecting dsDNA–RepA protein interactions. (B) Three steps in a sensorgram. Equilibration, association with RepA, and dissociation were performed in PBST.

Fig. 3. Three 38-base pair (bp), double-stranded DNA (dsDNA) OP1, OP2, and CO fragments were prepared by annealing hybridization. The hypochromic effect was measured as the absorbance value of ssDNA and dsDNA at 260 nm by ultraviolet spectrophotometry. (A) ssDNA and dsDNA OP1. (B) ssDNA and dsDNA OP2. (C) ssDNA and dsDNA CO.

Fig. 4. Different concentrations of dsDNA OP1 were loaded onto the streptavidin (SA) sensors. dsDNA OP1 (1, 0.5, or 0.25 μM; lines a–c, respectively) were loaded on the SA sensor in separate reactions.

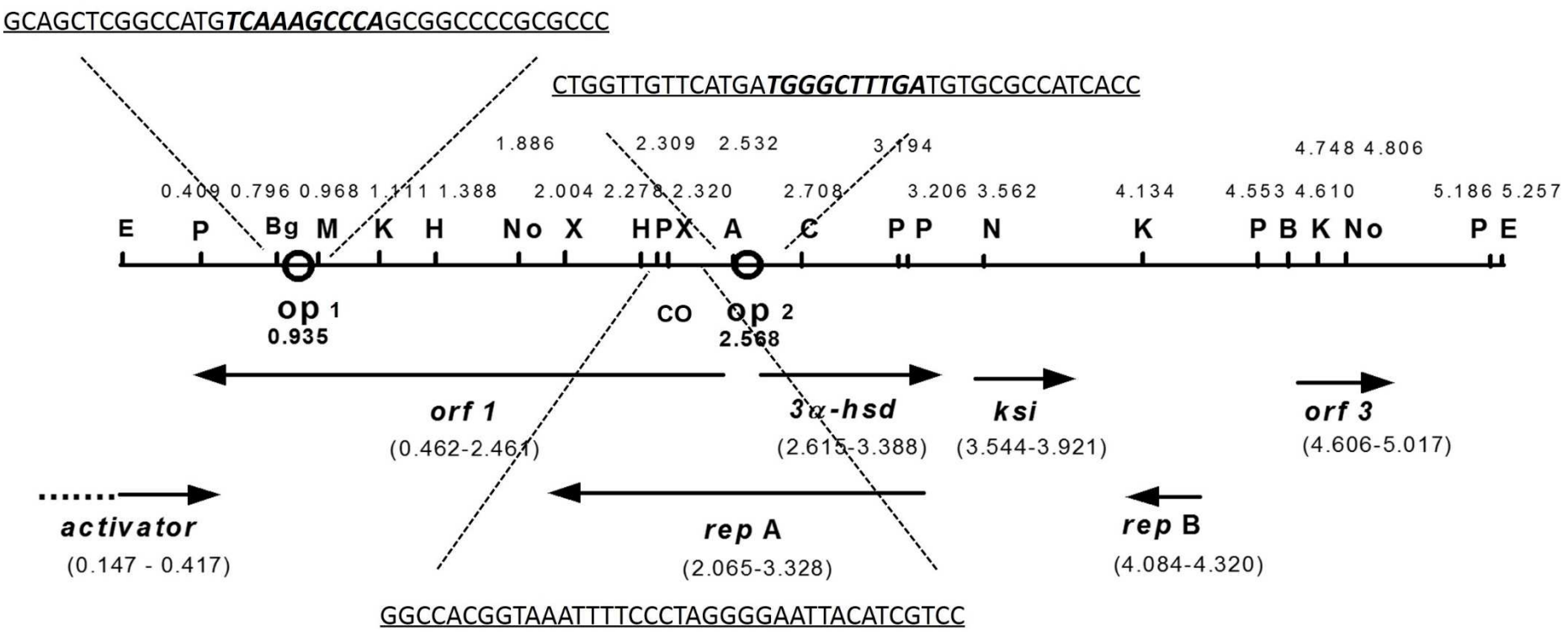
Fig. 5. Kinetics analysis of the interaction between RepA and the dsDNA OP1 template. (A) Interaction curves, lines a–g: the SA sensors were loaded with dsDNA OP1 (1 μM) and immersed into the indicated concentrations of RepA. The RepA concentrations ranged from 92 to 1.4 μg/ml. Line h represents the negative control (NC). The SA sensor loaded with 1 μM dsDNA CO was immersed into 92 μg/ml RepA. Line i is the reference curve. The SA sensor loaded with 1 μM dsDNA OP1 was immersed into PBST. (B) Kinetic parameters of the dsDNA OP1–RepA interaction are shown.

Fig. 6. Kinetics analysis of the dsDNA OP2–RepA protein interaction. (A) Interaction curves. Lines a–g: SA sensors loaded with 1 μM dsDNA OP2 were immersed into the indicated concentrations of RepA. The concentrations of RepA ranged from 92 to 1.4 μg/ml. Line h represents the negative control (NC), where the SA sensor was loaded 1 μM dsDNA CO and immersed into 92 μg/ml RepA. Line i is the reference curve, where the SA sensor was loaded 1 μM dsDNA OP2 and immersed into PBST. (B) Kinetic parameters of the dsDNA OP2–RepA interaction are shown.

Fig. 7. The signal at the end of the association step was calculated to generate a standard curve for quantitative determinations. Line a represents a blank control (BC), where no testosterone was present in the reaction mixture comprised of 100 µl RepA and 100 µl PBST. Lines b–h: the testosterone concentrations in the mixture ranged from 2.13 to 136.63 ng/ml (RepA and testosterone were present in the mixture at a 1:1 ratio [v/v]). Line i is the reference curve. The association step was performed in PBST.

Fig. 8. Generation of a quantitative standard curve with the BLI technique. The experimental data are represented as the percentage of the signal response relative to the blank control (no testosterone in the mixture). The data are shown as the mean of 3 replicates ± 1 standard deviation. The relative signal responses correlated negatively with the testosterone concentration. The testosterone concentrations are shown on a logarithmic scale. The standard curve was analyzed by linear regression, which revealed an r^2 of 0.9765.

Fig. 1.


GCAGCTCGGCCATGTCAAAGCCCAGCGGCCCCGCGCCC
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1.886
2.309
2.532
3.194
4.748
4.806
0.409
0.796
0.968
1.111
1.388
2.004
2.278
2.320
2.708
3.206
3.562
4.134
4.553
4.610
5.186
5.257
E
P
Bg
M
K
H
No
X
H
P
X
A
C
P
P
N
K
P
B
K
No
P
E
op 1
0.935
co
op 2
2.568
orf 1
(0.462-2.461)
3α-hsd
(2.615-3.388)
ksi
(3.544-3.921)
orf 3
(4.606-5.017)
activator
(0.147 - 0.417)
rep A
(2.065-3.328)
rep B
(4.084-4.320)
GGCCACGGTAAATTTTCCCTAGGGGAATTACATCGTCC

Fig. 2.

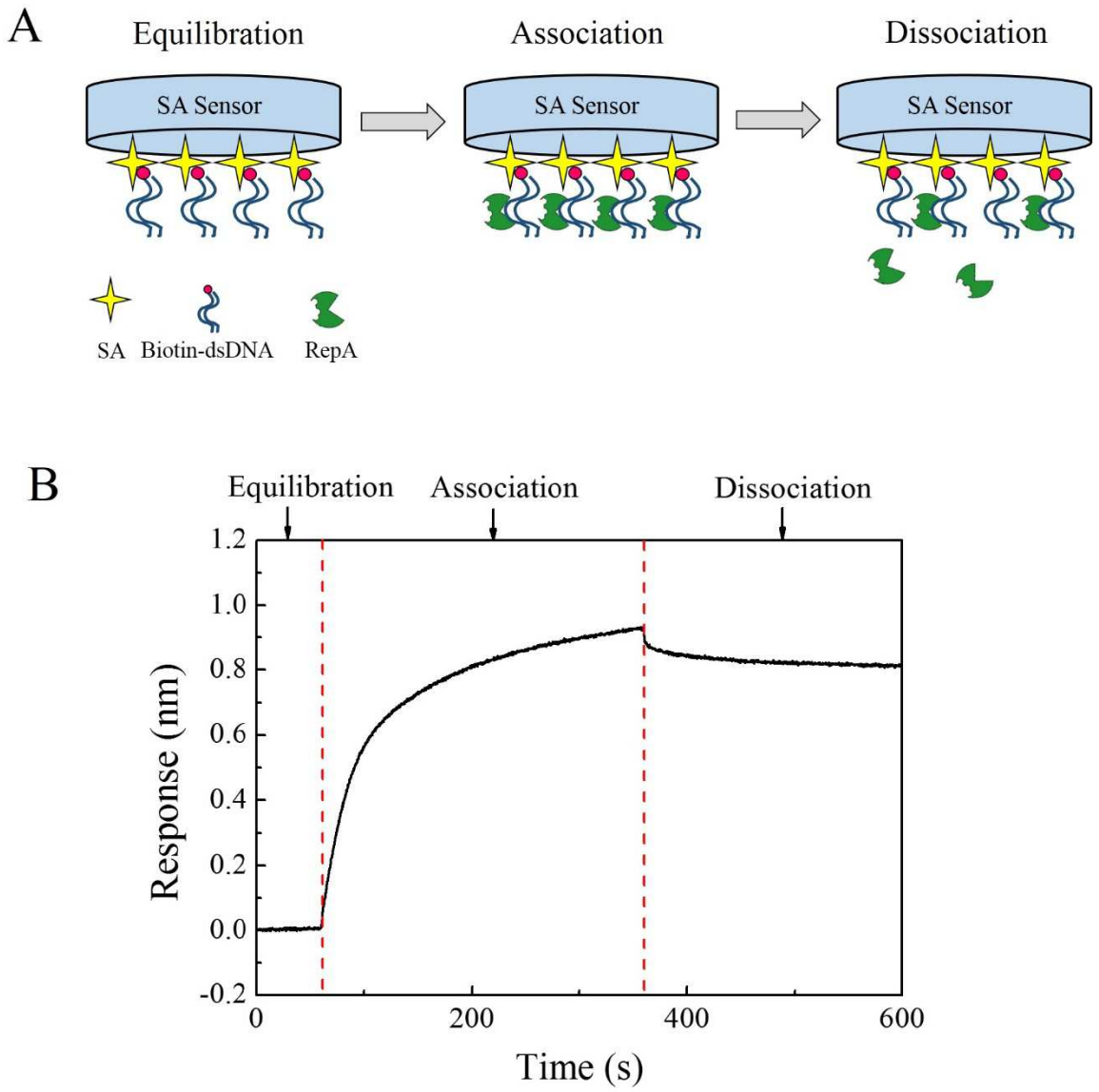

A
Equilibration
Association
Dissociation
SA Sensor
SA Sensor
SA Sensor
SA
Biotin-dsDNA
RepA
B
Equilibration
Association
Dissociation
Response (nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
200
400
600
Time (s)

Fig. 3.

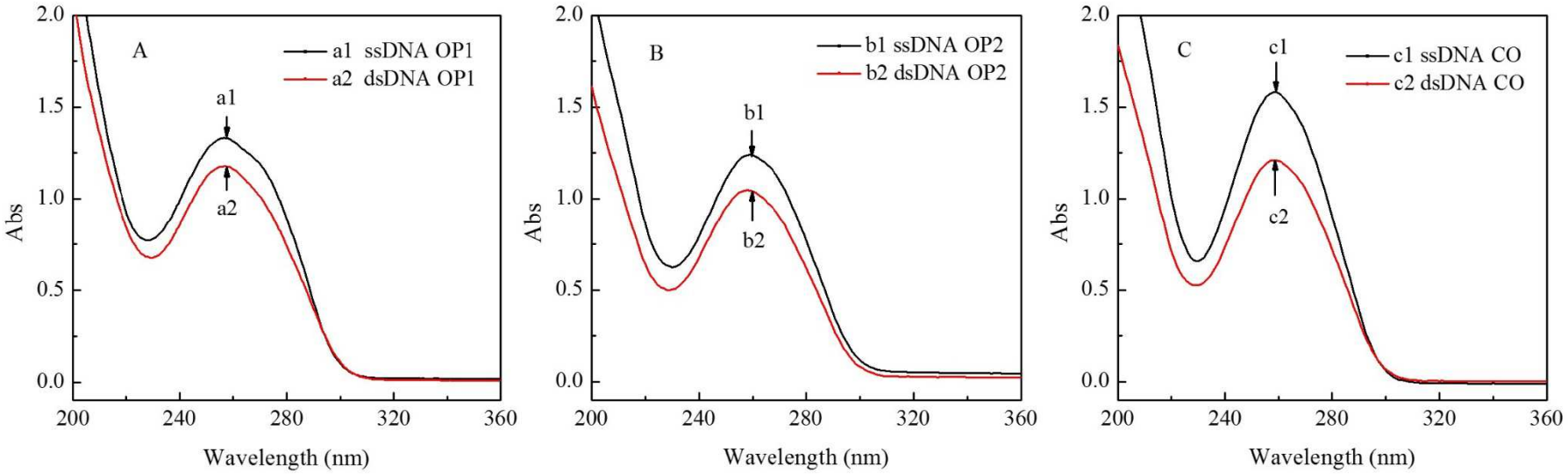

A
a1 ssDNA OP1
a2 dsDNA OP1
a1
a2
B
b1 ssDNA OP2
b2 dsDNA OP2
b1
b2
C
c1 ssDNA CO
c2 dsDNA CO
c1
c2
Abs
Wavelength (nm)
2.0
1.5
1.0
0.5
0.0
200
240
280
320
360

Fig. 4.

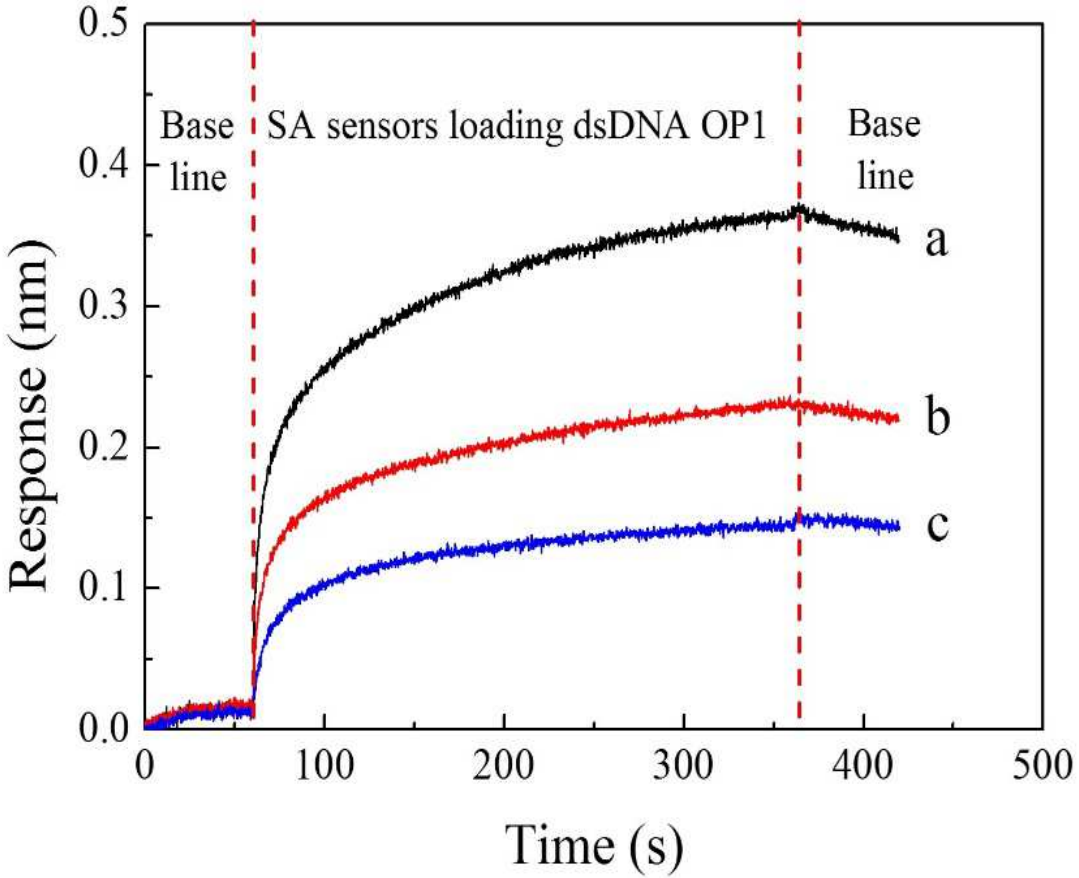
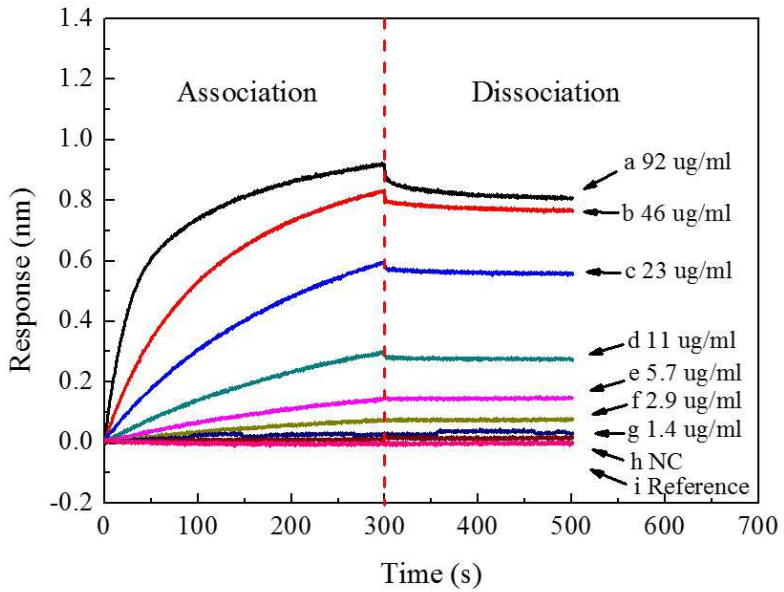

Base line
SA sensors loading dsDNA OP1
Base line
a
b
c
Response (nm)
Time (s)
0.0
0.1
0.2
0.3
0.4
0.5
0
100
200
300
400
500

Fig. 5.

A

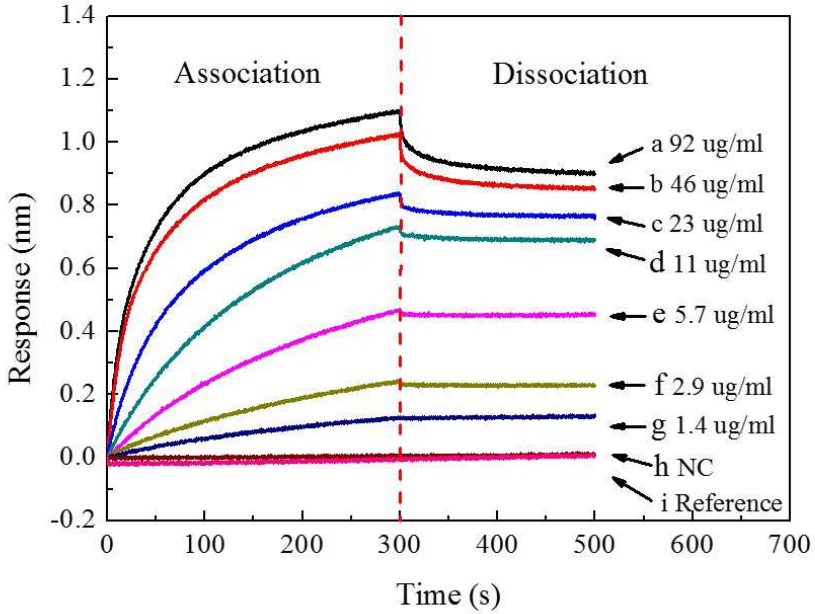


B dsDNA OP1 and RepA interaction

Sensor	Sensor a	Sensor b	Sensor c	Sensor d	Sensor e	Sensor f	Sensor g
RepA Conc. (μg/ml)	92	46	23	11	5.7	2.9	1.4
Response (nm)	0.927	0.828	0.583	0.292	0.129	0.065	0.016

Fig. 6.

A



B dsDNA OP2 and RepA interaction

Sensor	Sensor a	Sensor b	Sensor c	Sensor d	Sensor e	Sensor f	Sensor g
RepA Conc. (μg/ml)	92	46	23	11	5.7	2.9	1.4
Response (nm)	1.094	1.039	0.839	0.732	0.466	0.232	0.118

Fig. 7.

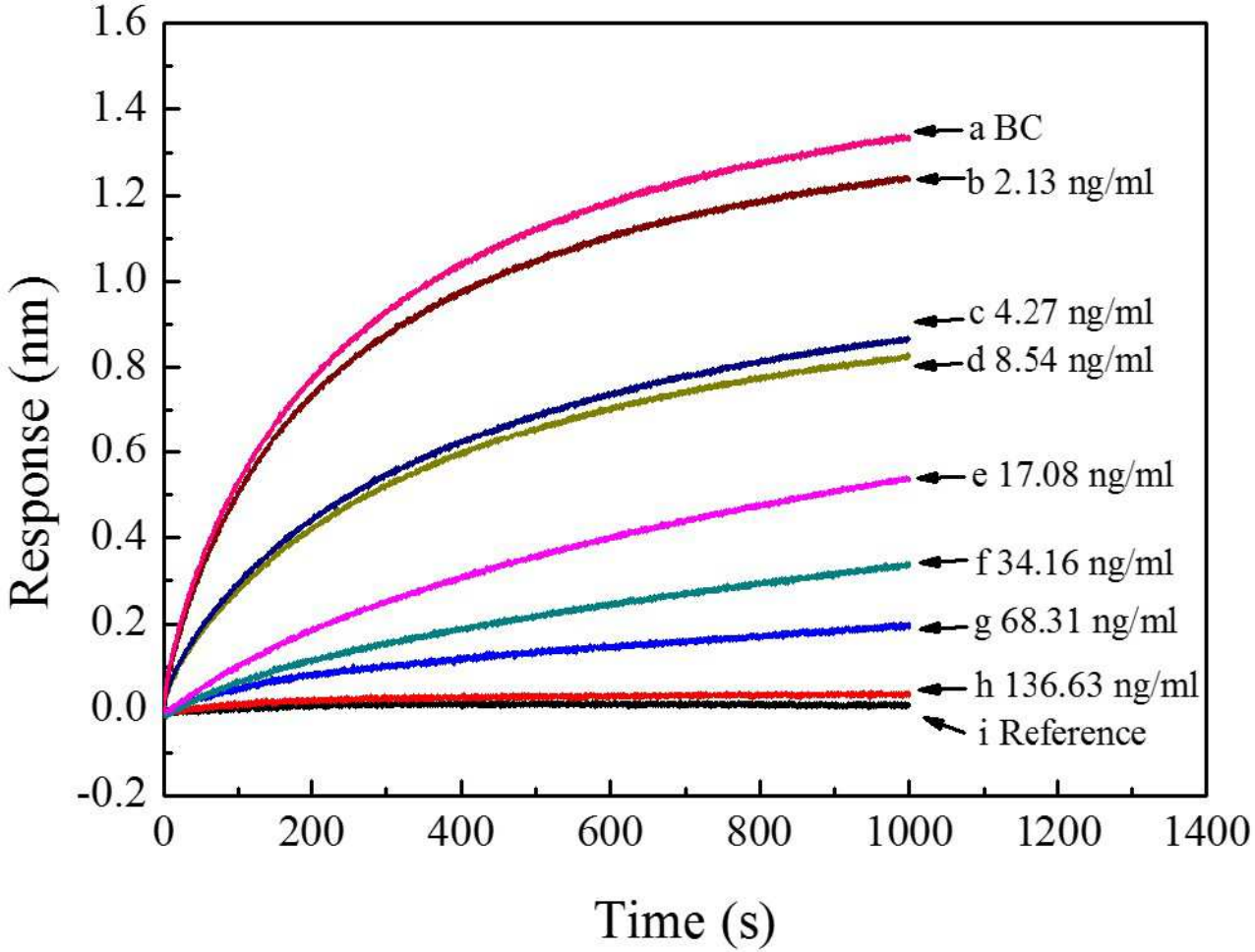
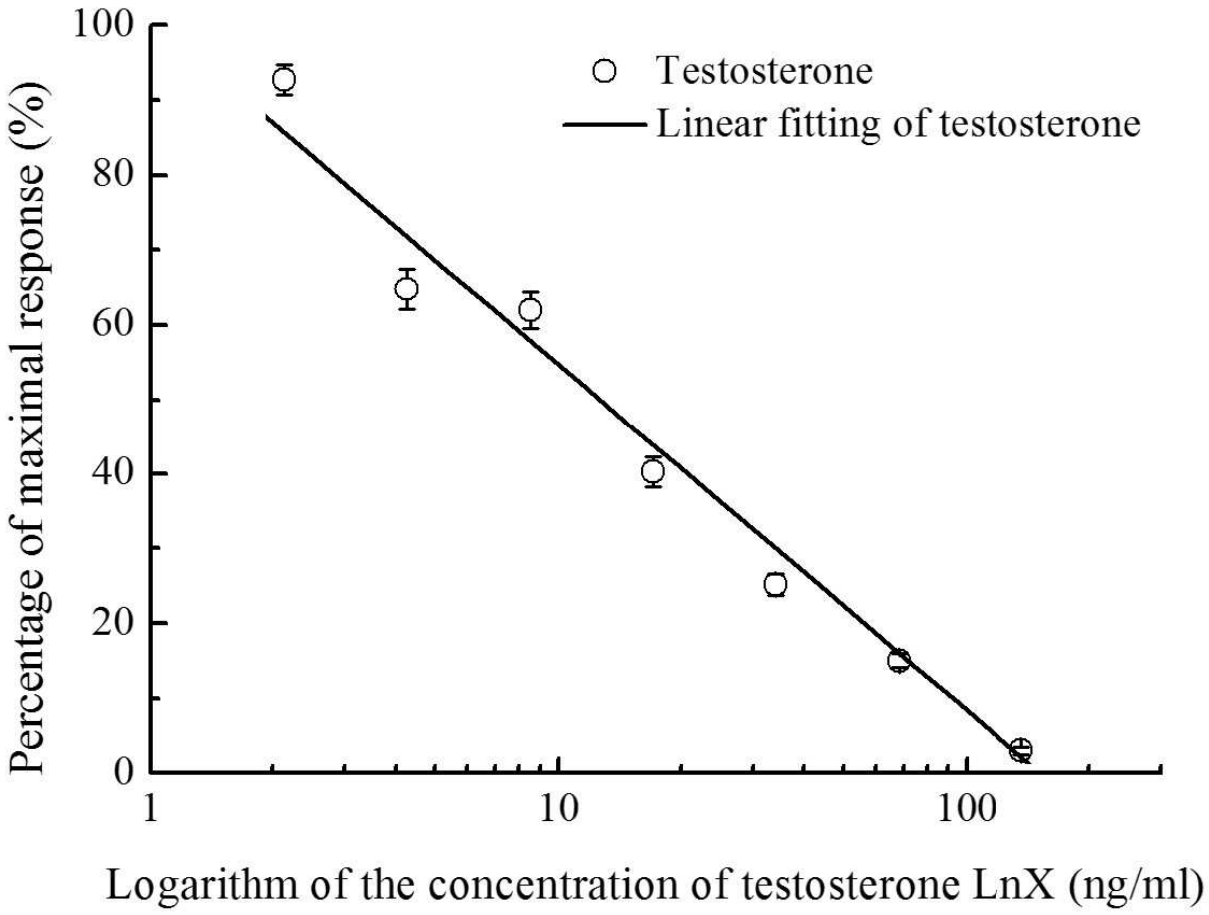


Fig. 8.



Highlights

- We developed a biolayer interferometry method to determine environmental steroids.
- In the method, DNA and protein interaction was detected easily.
- Changes in the interference of white light from the fiber surface enable detection.
- Monitoring binding does not require radioactive, enzymatic, or fluorescent labels.