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Carp vitellogenin detection by an optical waveguide lightmode spectroscopy biosensor

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ABSTRACT

A label-free carp vitellogenin sensor has a strong potential for on-site monitoring on the possible contamination of edible fish with endocrine disruptors as a sum parameter in an inland carp farm. In this study, we performed a sensitive detection for carp vitellogenin with a direct-binding optical waveguide lightmode spectroscopy-based immunosensor. Carp vitellogenin bound over the sensor surface quite specifically, judging from the sensor responses according to stepwise antibody immobilization. This was also supported by a negligible sensor response found at bovine serum albumin immobilization. When plotted in double-logarithmic scale for carp vitellogenin concentrations of 0.00675–67.5 nM, a linear relationship was found between analyte concentration and sensor response, together with the limit of detection of 0.00675 nM. The reusability of the immunosensor after the regeneration with 10 mM HCl was reasonably good, as presumed from the coefficient of variability of 6.02% for nine repetitive measurements. The model sample prepared by spiking a purified carp vitellogenin into a 10-fold diluted vitellogenin-free carp serum in 9.45 nM showed the response ratio of 96.70% against 9.45 nM of the purified carp vitellogenin. When a female and male carp sera induced with 17β-estradiol injection were analyzed, biomarker induction was even identifiable at 2000-fold serum dilution.

1. Introduction

Vitellogenin, a potent fish biomarker for estrogenic activity, is an egg yolk protein precursor and phospholipoglycoprotein in nature. It is normally present only in the blood of female fish, but males also have vitellogenin gene(s) and the estrogen receptors(s) necessary for vitellogenin regulation (Tyler et al., 2002). Therefore, the exposure of female and male fish to endocrine disruptors such as 17β-ethinylestradiol (the contraceptive pill), dioxins and PCBs can trigger vitellogenin gene expression, and as a consequence, vitellogenin accumulates in the blood abnormally (Chen, 1983; Tyler et al., 1996). Meanwhile, fish represents an important route of human contamination with endocrine disruptors because it constitutes the upper part of the food chain. Therefore, a stringent surveillance on the possible contamination of edible fresh- and salt-water fish with endocrine disruptors is required to improve human health (Kim et al., 2006). From the above facts, vitellogenin monitoring seems to be one efficient method for the assessment of fish contamination with endocrine disruptors as a sum parameter.

Until now, various analytical methods for vitellogenin have been developed. Out of them, reverse transcriptase-polymerase chain reaction methods (Celius et al., 2000; Custodia-Lora et al., 2004) determine mRNA of vitellogenin by transcriptional level. Whereas, immunoassays including enzyme-linked immunosorbent assay (ELISA) (Prakash Vincent et al., 2001; Mitsui et al., 2003) and radioimmunoassay (Norberg and Haux, 1988), and electrophoresis, Western blotting (Versonnen et al., 2004) detect vitellogenin itself by translational level. The conventional methods described above had good sensitivity, however, suffered from possible interferences from coloring substances and complexity in measurement protocol due to the use of radioisotope or enzyme label, and thus limiting them laboratory-oriented in most cases (Kim et al., 2006). Therefore, it is necessary that sensor technology for vitellogenin measurement be developed for on-site application in a fish farm to monitor the health state of fish and to afford the basis on a high-precision instrumental analysis for endocrine disruptors against the positive samples from a preliminary biosensor screening.

A label-free immunosensor based on antibody–antigen complexation is a strong candidate for simple rapid determination of fish vitellogenin due to its intrinsic high-sensitivity and specificity, together with versatility in system setup, simplicity in measurement procedure, reuse of expensive reagents like antibody and

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real-time demonstration of sensor response on computer screen (Park and Kim, 2006). Until now, however, studies on the label-free detection for fish vitellogenin are scarce. Darain et al. (2004) measured carp vitellogenin based on impedance spectroscopy using a conductive terthiophene polymer-coated glassy carbon electrode. Kim et al. (2006) developed a piezoelectric immunosensor prepared by the chemisorption of an anti-carp vitellogenin antibody thiolated with a heterobifunctional thiolation cross-linker, sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate, and measured carp vitellogenin concentration-dependently.

An optical waveguide lightmode spectroscopy (OWLS) biosensor is recently developed in the field of integrated optics and exploits the science of light guided in structures smaller than the wavelength of the light. It has been applied for studying the phenomena at solid/liquid interface such as protein–DNA interaction, cell adhesion and environmental monitoring (Vörös et al., 2002). According to the label-free nature of signal transduction which makes the analysis simple, an OWLS-based immunosensor can be a strong candidate for on-site vitellogenin monitoring, supplementing the conventional methods including ELISA (Prakash Vincent et al., 2001; Mitsui et al., 2003).

Accordingly, this is the first report on the development of a highly sensitive label-free OWLS-based immunosensor for the detection of vitellogenin in carp (*Cyprinus carpio*), one of the predominant freshwater species reared in Korea. We studied the performance of the above sensor operated at a direct-binding principle and subsequently applied it to vitellogenin measurement for the carp sera amplified with vitellogenin by 17β-estradiol induction. Compared to the conventional method like ELISA, the vitellogenin sensor of this study seemed to have merits on its simplicity and rapidity, together with its on-site applicability in a fish farm.

2. Experimental

2.1. Reagents and transducer

Carp vitellogenin was prepared by the serum amplification using two intraperitoneal 17β-estradiol injections into carp and, the following purification by a linear gradient DEAE, cellulose column chromatography (Moon et al., 2006). The purified carp vitellogenin thus prepared was used for the production of an anti-carp vitellogenin antibody from rabbit and the reactivity of the prepared antibody was determined by an ELISA (Moon et al., 2006). An amino-terminal silane compound, 3-aminopropyltriethoxysilane (APTS), and glutaraldehyde which were used for the silanization of an optical grating coupler (OGC) sensor chip (OW 2400, MicroVacuum Ltd., Budapest, Hungary) and the following activation of the silanized sensor chip, respectively, were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were the products of Sigma–Aldrich Chemical Co. or guaranteed reagents from various suppliers, and double distilled water was used throughout this study.

The diffraction grating of the OGC sensor chip had a surface relief depth of ~20 nm, grating periodicity of 2400 lines/mm, and grating area dimensions of ~2 mm (length) and 16 mm (width). The refractive index (n_F) and thickness (d_F) of the waveguide layer under the diffraction grating were 1.77 and 170–220 nm, respectively. The dimensions of the substrate glass slide under the waveguide layer with a refractive index (n_S) of 1.53 were 48 mm (length), 16 mm (width) and 0.55 mm (thickness).

2.2. System setup

The OWLS-based immunosensor system of this study was operated in a direct-binding mode as follows based on previous reports (Kim et al., 2007a, 2008). The system was constructed with a Reglo digital pump (ISM 832A, Ismatec SA, Glattbrugg, Switzerland), a Rheodyne injector, the main unit (OWLS 110, MicroVacuum Ltd.) composed of a sensor holder with a flow-through cuvette attached over the OGC sensor chip, two photodiodes, a beam mirror, shutter and He–Ne laser source emitting a monochrome light of 632.8 nm, plus a PC (Fig. 1). The individual parts of the system were connected with capillary tubing. During the operation, the monochrome light was diffracted by the optical grating of the sensor chip and started to propagate via the total internal reflection inside the waveguide layer. At a well-defined incident angle, the phase shift during one internal reflection was equal to zero and a guided mode excited, which generated an evanescent field penetrating into the covering medium (Vörös et al., 2002). The change in refractive index at the surface, caused by the added layer resulting from the antibody–antigen complexation, was then monitored on-line by the precise measurement of incoupling angle as a function of time with the operating software of the system.

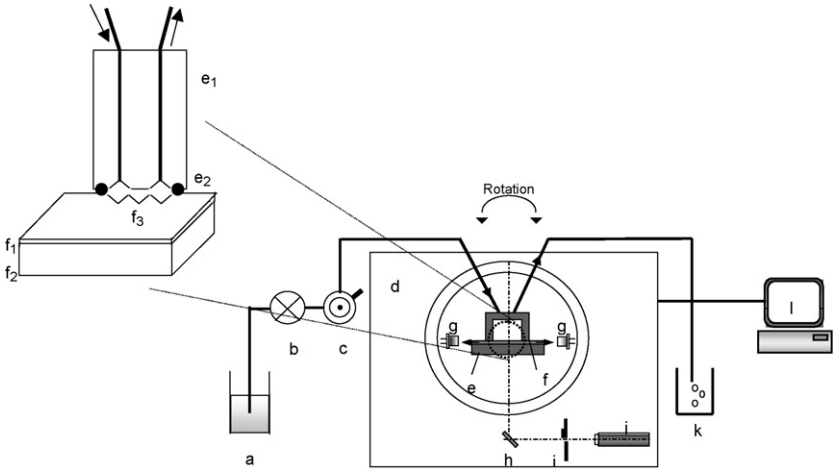

Fig. 1. Schematic diagram of the OWLS-based immunosensor system. The instrument was equipped with a flow-through cell introducing a liquid sample to the grating part of sensor surface in a reproducible manner. (a) Buffer solution; (b) peristaltic pump; (c) injector; (d) main unit; (e) sensor holder; (e1) flow-through cuvette; (e2) O-ring; (f) OGC sensor chip; (f1) waveguide layer; (f2) glass substrate; (f3) grating; (g) photodiode; (h) beam mirror; (i) shutter; (j) laser source; (k) waste and (l) PC.

2.3. Antibody immobilization

The anti-carp vitellogenin antibody was immobilized *in situ* over the surface of the OGC sensor chip with a slight modification of the procedure by Polzius et al. (1996). The sensor chip was first dipped into piranha solution (H_2SO_4:H_2O_2 = 3:1, v/v) for 10 min with an extensive care to avoid heat generation, followed by washing with distilled water for 5 min and hydration with hot water around 90 °C for 1 h in sequence. The washed sensor chip was immersed into 10% acetonic APTS solution (pH 3.5) in a cap tube at 75 °C for 3 h and heat-treated afterward at 100 °C overnight in a convection oven. The amino-silanized sensor chip was inserted into the correct position of the sensor holder and then the flow-through cuvette was attached over the sensor chip, followed by fitting the resulting sensor holder into the main unit of the immunosensor system. Antibody immobilization was performed *in situ* as follows. After washing with distilled water for around 20 min at a flow rate of 200 μl/min, 200 μl of 2.5% glutaraldehyde was injected into the system, followed by turning off the pump for 6 min to activate the diffraction grating of the waveguide film. Then, the pump was turned on to wash the flow line with distilled water until a stable baseline was obtained. At this moment, 200 μl of the final antiserum diluted with the reaction buffer of the system, 4 mM Tris–HCl (pH 7.2), by 1:10 ratio was added into the flow line to the sensor surface at the above flow rate and eluent flow was stopped for 7 min to immobilize the antibody. After switching the system to flow mode until a new stabilized baseline, the sensor surface bound with the antibody was washed by the injection of 500 μl of 10 mM HCl. The eluent of the system was changed to the reaction buffer and a new steady-state baseline was established within 10 min. At this point, the system was ready for sample measurement. To prepare the OGC sensor chip treated with APTS only, two steps using the above glutaraldehyde and antibody solution were omitted. Likewise, the sensor chip treated with APTS plus glutaraldehyde was prepared without the flow of the antibody solution. The sensor chip immobilized with bovine serum albumin (BSA) was made by treating 100 μg/ml BSA dissolved in the reaction buffer instead of the antibody solution.

2.4. Analytical procedure

Five hundred microliters of different concentrations of carp vitellogenin were individually injected into the immunosensor system in flow mode at the above flow rate, followed by elution of the reaction buffer. A sensor response was regarded as a steady-state change in surface mass (ng/cm^2), caused by carp vitellogenin, in the reaction buffer before and after a sample addition. The change in surface coverage during a measurement was automatically displayed on the computer screen using the operating software based on the measured incoupling angles comprising the transverse electric mode (α_{TE}) and transverse magnetic mode (α_{TM}). The regeneration of the OGC sensor chip was undertaken in flow mode by the addition of 500 μl of 10 mM HCl after each measurement.

2.5. Sample analysis

Sample analysis was performed by two different aspects. First, a vitellogenin-free carp serum was prepared from a male carp according to the method of a previous study (Moon et al., 2006). Then, a model sample was prepared by spiking the purified carp vitellogenin, into a 10-fold diluted vitellogenin-free carp serum, in 9.45 nM. The sensor response for the model sample was compared with that of the diluted vitellogenin-free carp serum to correct the matrix effect. On the other hand, the sensor responses of a female and male carp serum, amplified with vitellogenin by 17β-estradiol induction, were measured after the dilution of the corresponding sera by 2000-fold and compared with those of a diluted female and male control serum.

3. Results and discussion

The possibility of carp contamination with endocrine disruptors due to the high burden of organic pollutants that are often discharged into rivers and lakes is a serious concern. In turn, this also implies that excessive levels of vitellogenin may be present in the blood of carp in the inland water (Chen, 1983; Tyler et al., 1996) and necessitates the development of a new analytical device for this protein. As an effort, a rapid and sensitive OWLS-based immunosensor was made to detect vitellogenin in carp serum in this study.

3.1. Specificity of the immunosensor

The specificity of the OWLS-based immunosensor of this study was checked from two aspects. In the first aspect, the sensor responses were measured during each step of antibody immobi-

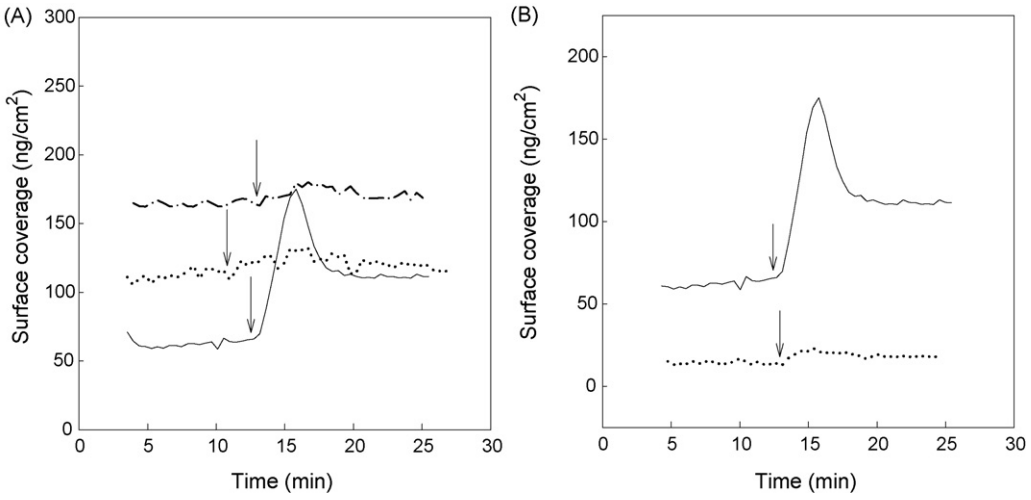

Fig. 2. Comparison of time-dependent surface coverage profiles of the OWLS-based immunosensor according to immobilization steps (panel A) and immobilized molecules (panel B). In panel A, the OGC sensor chip was treated with APTS only (···), APTS plus glutaraldehyde (– · –) and APTS, glutaraldehyde, plus anti-carp vitellogenin antibody (—). In panel B, the sensor chip was immobilized with bovine serum albumin (···) and anti-carp vitellogenin antibody (—). Arrows indicate points of sample injection.

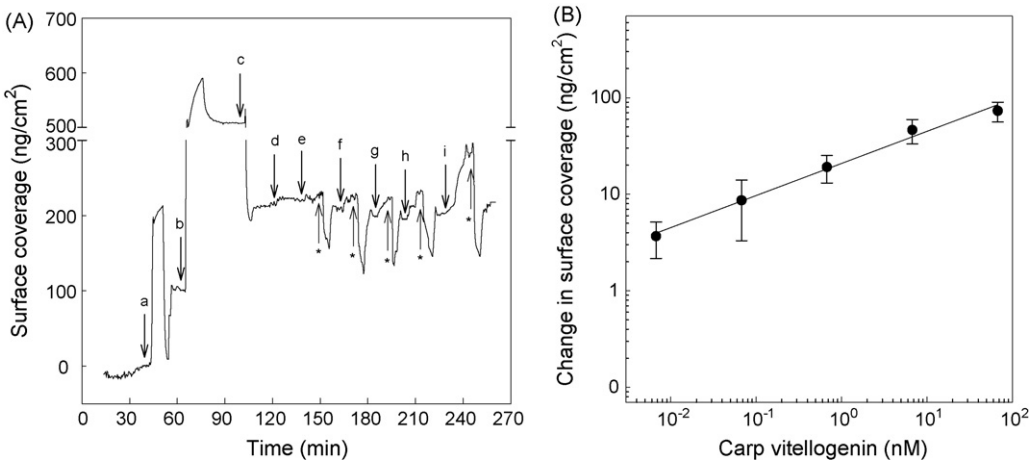

Fig. 3. Concentration-dependent responses of the OWLS-based immunosensor for carp vitellogenin (panel A) and the resulting calibration curve (panel B). In panel A, the surface of the sensor chip was sequentially treated with 0.25% glutaraldehyde (a), anti-carp vitellogenin antibody (b), 10 mM HCl as the regenerating solution (c) and the reaction buffer (d), followed by the additions of carp vitellogenin at the concentrations of 0.00675 (e), 0.0675 (f), 0.675 (g), 6.75 (h) and 67.5 nM (i) after the baseline stabilization following 10 mM HCl treatment. Arrows with asterisk in panel A indicate the points of regenerant addition. In panel B, five values of change in surface coverage at adjacent measuring time were averaged and error bars were inserted.

lization (panel A of Fig. 2). When a solution having 6.75 nM of the purified carp vitellogenin was added into the reaction cell attached with the OGC sensor chip without antibody immobilization (treated with APTS only, and APTS plus glutaraldehyde), the changes in surface coverage of 4.48 ± 0.76 and 5.63 ± 0.43 ng/cm^2 ($n = 2$) were obtained, respectively. These false positive values were possibly caused by non-selective binding of the analyte (Pyun et al., 1998). They, however, seemed to be negligible considering that the response of 51.47 ± 0.54 ng/cm^2 ($n = 2$) attained with the sensor chip treated with APTS and glutaraldehyde, plus immobilized with the anti-carp vitellogenin antibody was bigger than them by 9.14–11.49-fold. From this fact, we concluded that a meaningful sensor response only occurred in the case of the antibody-coated sensor chip (Park and Kim, 1998; Kim et al., 2007a). In the second aspect, the responses of the OGC sensor chips immobilized with the anti-carp vitellogenin antibody and BSA, a serum protein irrelevant with the immune response of this study, were compared by adding 6.75 nM of the purified carp vitellogenin into the system (panel B of Fig. 2). As expected, virtually no sensor response approximating to the change in surface coverage of 2.88 ± 0.18 ng/cm^2 ($n = 2$) was found in the case of BSA immobilization. But, the response of the antibody-coated sensor chip was evident, amounting to 48.47 ± 0.54 ng/cm^2 ($n = 2$), thereby confirming a high specificity of the current immunosensor to carp vitellogenin binding (Kim et al., 2007a).

3.2. Concentration-dependent responses of the immunosensor

The sensorgram of the OWLS-based immunosensor showed that the surface coverage values of baseline increased steadily during each step of the immobilization procedure including glutaraldehyde activation, antibody binding plus surface regeneration, and eluent change from distilled water to the reaction buffer (panel A of Fig. 3). This fact clearly indicated that antibody immobilization was performed properly (Adányi et al., 2007). When a fixed concentration of carp vitellogenin was injected into the immunosensor system, an abrupt increase and following stabilization in surface mass was observed. Further dissociation of bound carp vitellogenin was not found even at prolonged elution of the reaction buffer (Polzius et al., 1997). This type of kinetics is quite similar to the typical binding profiles found for label-free optoelectronic devices like surface plasmon resonance (SPR)-based biosensor which measures the complexation between ligand–receptor or antibody–antigen (Leonard et al., 2004). As shown in panel A of Fig. 3, a sharp dip in surface coverage occurred after every regeneration step, which seemed to indicate the transient change in refractive index of the eluent due to the mixing between the reaction buffer and the regenerating solution, 10 mM HCl (Adányi et al., 2007). A newly established baseline surface coverage slightly moved downward after the washout of the regenerating solution. This unavoidable minute change, however, did not affect on vitellogenin quantification as shown by an extremely high correlation between analyte concentration and sensor response (panel B of Fig. 3). When various concentrations (0.00675, 0.0675, 0.675, 6.75 and 67.5 nM) of carp vitellogenin were successively injected into the system, the sensor responses of 5.01 ± 0.31 to 71.58 ± 0.67 ng/cm^2 ($n = 5$) were obtained. Whereas, the change in surface coverage obtained by the injection of the reaction buffer itself as a negative control was 0.12 ng/cm^2. Furthermore, the three-fold value against the standard deviation of baseline drift that is routinely accepted as the criterion for the determination of a meaningful sensor signal was 1.14 ng/cm^2. Based on these values, we presumed the limit of detection (LOD) of the present immunosensor as 0.00675 nM. The immunosensor showed an improved sensitivity compared to a direct-binding quartz crystal microbalance immunodevice which had a LOD of 0.4864 nM for carp vitellogenin (Kim et al., 2006). Also, the sensitivity obtained in this study was even better than that for flatfish vitellogenin by 10-fold (Kim et al., 2007b), which seemed to represent the difference in binding affinity during the immune response (Kim et al., 2004).

The relationship between biomarker concentration and sensor response was plotted in double-logarithmic scale to obtain a linear equation, as reported previously (Kuhlmeier et al., 2003; Volotovsky et al., 1997). For the linear response in panel B of Fig. 3, the regression equation of $Y(\log_{10}$ sensor response$) = 0.3327X$ ($\log_{10}$ carp vitellogenin$) + 1.3192$ was obtained, together with the correlation coefficient (r) of 0.9904. The linearity like this has also been reported in the label-free OGC and SPR immunosensor which normally measure sensor signals in a wide concentration range of target analytes (Homola et al., 2002; Kuhlmeier et al., 2003).

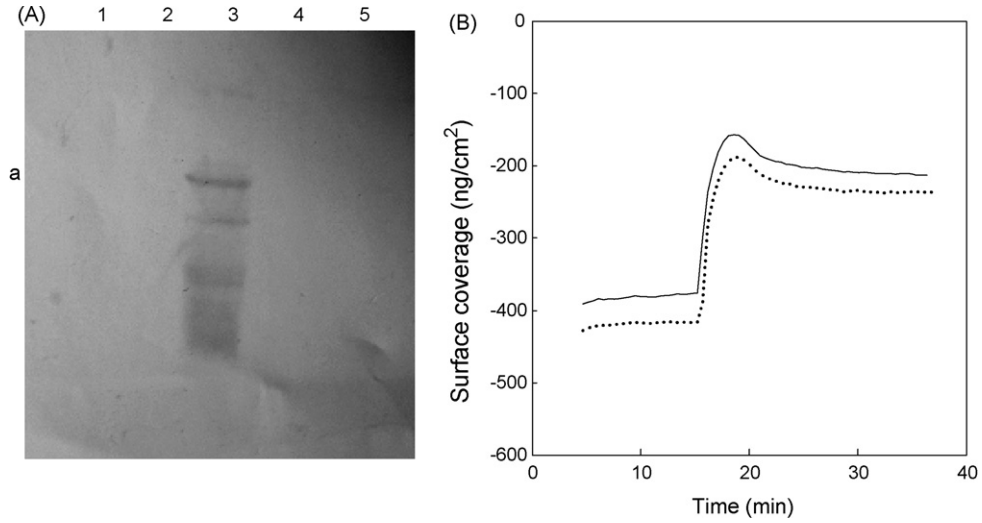

Fig. 4. Western blotting for vitellogenin in carp sera (panel A), and the sensor responses of the 10-fold diluted vitellogenin-free carp serum and model sample (panel B). Panel A: lanes 1–5, Western blotting using the antibody against carp vitellogenin; lanes 1 and 5, standard mixture of molecular mass markers; lanes 2–4, 80-fold diluted sera from male carp 1–3 without the induction with 17β-estradiol, respectively. Panel B: (—), 10-fold diluted vitellogenin-free carp serum; (···), model sample.

3.3. Reusability of the immunosensor

From the previous reports on immunosensor development, it has been recognized that the major source of error during a repetitive measurement is the possible loss of immobilized antibody after regeneration (Pyun et al., 1998; Kim et al., 2004). To evaluate the reusability of the current immunosensor, a carp vitellogenin solution with the concentration of 6.75 nM was added into the immunosensor system nine times after the regeneration procedure (Table 1). The mean change in surface coverage during the whole course of reaction was $44.21 \pm 2.66\ ng/cm^2$ ($n = 9$), representing 95.61% of the sensor response obtained at the first cycle measurement. This fact showed that the antibody layer was almost secured during sensor reuse, which might imply economic aspect of the present immunosensor compared to commercial kit assays like ELISA (Kolosova et al., 2000). The coefficient of variability, as an index of repeatability according to sensor reuse, was 6.02%. This precision seems to be reasonably good considering that the immunosensor of this study will find its applicability to an initial screening for the presence of estrogenic compounds like endocrine disruptors in the inland and coastal water, and edible fish (Chen, 1983; Tyler et al., 1996; Kim et al., 2006). We expect that the repeatability of measurement for the detection of carp vitellogenin will be improved by using nine new OGC

Table 1
Sensor responses according to repetitive use after the regeneration procedure with 10 mM HCl

Measurement number[a]	Sensor response (ng/cm^2)
1	46.42
2	42.05
3	48.31
4	42.96
5	43.57
6	41.76
7	45.32
8	40.56
9	46.96
Mean ± S.D. ($n = 9$)	44.21 ± 2.66

[a] In each instance, 6.75 nM of the purified carp vitellogenin was used as the analyte.

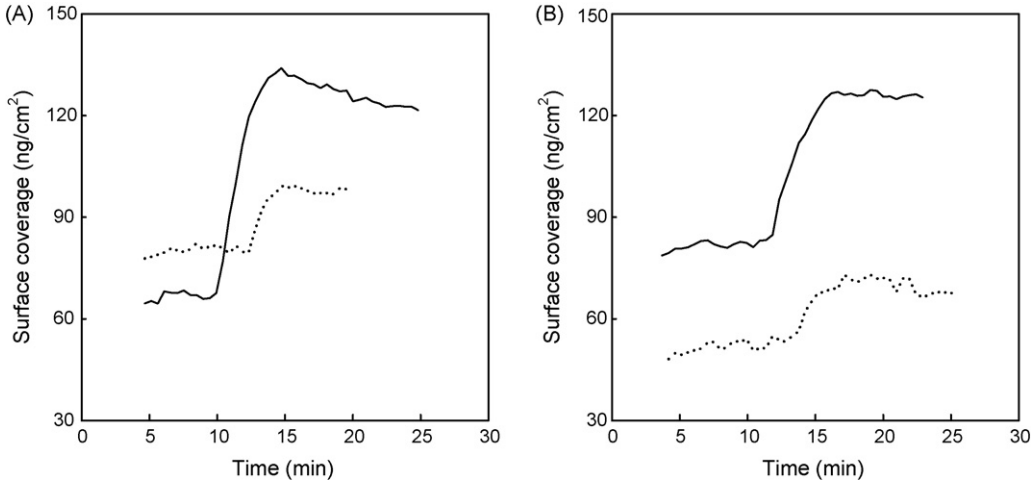

Fig. 5. Sensor responses of the 2000-fold diluted induced and control sera from female (panel A) and male (panel B) carp. (···), control sera; (—), induced sera with 17β-estradiol.

sensor chips immobilized with the anti-carp vitellogenin antibody.

3.4. Matrix effect and model sample analysis

As the OWLS-based immunosensor of this study measures the change in refractive index during an affinity-based process, antibody–antigen complexation (Kim et al., 2007a), the interference caused by sample matrix might be present. Therefore, a vitellogenin-free carp serum was first prepared to evaluate this effect. As male fish have the dormant vitellogenin gene(s) and the estrogen receptor(s) necessary for vitellogenin induction, this biomarker protein is normally not present in the blood of male fish (Tyler et al., 2002). Based on this fact, we prepared the blood sera from three male carp and analyzed the presence of vitellogenin by Western blotting. As depicted in panel A of Fig. 4, the serum in lane 3 showed the band of carp vitellogenin (band a), which indicated that it could not be used for our experiment. As the carp serum in lane 2 did not show the corresponding band, we used it as the vitellogenin-free serum to elucidate possible matrix effect. The 10-fold diluted vitellogenin-free carp serum and model sample prepared as described in Section 2.5 were determined for the time-dependent sensor response using the immunosensor system of this study (panel B of Fig. 4). As expected, a significant surface mass change was also found in the case of the diluted vitellogenin-free carp serum. To compensate for the false positive value, the change in surface coverage of the diluted vitellogenin-free carp serum was subtracted from that of the model sample and the difference was regarded as the sensor response of the model sample. When this value was compared with that of 9.45 nM of the purified carp vitellogenin, the relative ratio (response of the model sample/response of the purified carp vitellogenin $\times$ 100, %) was 96.70%. Whereas, the relative ratio determined for 9.45 nM carp vitellogenin in the model sample against the same concentration of the purified carp vitellogenin with a commercial ELISA kit was 134.60%.

3.5. Detection of 17β-estradiol-induced amplification of vitellogenin in carp serum

From a previous experiment by the SDS-PAGE employing 7% acrylamide gel, we observed that vitellogenin was greatly induced in the blood of a female and male carp, intraperitoneally injected with 17β-estradiol in the ratio of 1 mg/100 g body weight (Moon et al., 2006). To evaluate the efficiency of the present OWLS-based immunosensor as a screening tool for induced vitellogenin, the vitellogenin-induced sera prepared from the above carp were diluted with the reaction buffer by 2000-fold and the sensor responses for the corresponding sera were measured (Fig. 5). The changes in surface coverage obtained with the induced female and male carp sera were 58.70 ± 1.90 and 45.00 ± 1.10 ng/cm^2 ($n = 5$), respectively. These values were considerably bigger than those (15.20–17.50 ng/cm^2) of the 2000-fold diluted sera from control female and male carp. Therefore, we presumed that the label-free immunosensor of this study might be used for an extremely sensitive and rapid evaluation for the vitellogenin induction in fish owing to the contamination with estrogenic compounds such as 17β-estradiol and nonyl phenol in a real-time scale (Tyler et al., 2002). Considering its intrinsic sensitivity and data convergence to the calibration curve as shown earlier, the immunosensor might also be used efficiently for vitellogenin quantification in the blood of the reared inland and coastal fish.

4. Conclusions

In this research, we studied the performance of an OWLS-based immunosensor operated by a direct-binding principle and subsequently applied it to vitellogenin screening for the carp sera amplified with vitellogenin by 17β-estradiol induction. Based on its high sensitivity, reusability and real-time presentation of sensor response, it might be used as a good screening tool for the initial monitoring on possible contamination with estrogenic compounds such as 17β-estradiol and nonyl phenol in a real-time scale in a fish farm. We think that further research with the developed system should include the establishment of the normal range of sensor response in control fish, followed by checking the objects with abnormally high sensor responses. Then, the screened objects might be further confirmed for the presence of individual estrogenic compounds by instrumental analyses. Simultaneously, an effort for the detection of vitellogenin induction according to the exposure to estrogenic compounds differing in molarity and chemical structure should also be paid.

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References

Adányi, N., Levkovets, I.A., Rodriguez-Gil, S., Ronald, A., Váradi, M., Szendrő, I., 2007. Biosens. Bioelectron. 22, 797–802.
Celius, T., Matthews, J.B., Giesy, J.P., Zacharewski, T.R., 2000. J. Steroid Biochem. Mol. Biol. 75, 109–110.
Chen, T.T., 1983. Can. J. Biochem. Cell Biol. 61, 802–810.
Custodia-Lora, N., Novillo, A., Callard, I.P., 2004. J. Exp. Zool. 301A, 15–25.
Darain, F., Park, D.-S., Park, J.-S., Shim, Y.-B., 2004. Biosens. Bioelectron. 19, 1245–1252.
Homola, J., Dostálek, J., Chen, S., Rasooly, A., Jiang, S., Yee, S.S., 2002. Int. J. Food Microbiol. 75, 61–69.
Kim, N., Kim, D.-K., Kim, W.-Y., 2008. Food Chem. 108, 768–773.
Kim, N., Park, I.-S., Kim, D.-K., 2004. Sens. Actuators B: Chem. 100, 432–438.
Kim, N., Park, I.-S., Kim, W.-Y., 2006. J. Korean Soc. Appl. Biol. Chem. 49, 254–258.
Kim, N., Park, I.-S., Kim, W.-Y., 2007a. Sens. Actuators B: Chem. 121, 606–615.
Kim, N., Ryu, H.-S., Kim, W.-Y., 2007b. J. Microbiol. Biotechnol. 17, 1445–1451.
Kolosova, A.Y., Samsonova, J.V., Egorov, A.M., 2000. Food Agric. Immunol. 12, 115–125.
Kuhlmeier, D., Rodda, E., Kolarik, L.O., Furlong, D.N., Bilitewski, R., 2003. Biosens. Bioelectron. 18, 925–936.
Leonard, P., Hearty, S., Quinn, J., O'Kennedy, R., 2004. Biosens. Bioelectron. 19, 1331–1335.
Mitsui, N., Tooi, O., Kawahara, A., 2003. Comp. Biochem. Physiol. C 135, 305–313.
Moon, D.-K., Kim, N., Kim, W.-Y., 2006. J. Korean Soc. Appl. Biol. Chem. 49, 196–201.
Norberg, B., Haux, C., 1988. Fish Physiol. Biochem. 5, 59–68.
Park, I.-S., Kim, N., 1998. Biosens. Bioelectron. 13, 1091–1097.
Park, I.-S., Kim, N., 2006. Anal. Chim. Acta 578, 19–24.
Polzius, R., Dießel, E., Bier, F.F., Bilitewski, U., 1997. Anal. Biochem. 248, 269–276.
Polzius, R., Schneider, Th., Bier, F.F., Bilitewski, U., Koschinski, W., 1996. Biosens. Bioelectron. 11, 503–514.
Prakash Vincent, S.G., Keller, R., Subramoniam, T., 2001. Mar. Biotechnol. 3, 561–571.
Pyun, J.C., Beutel, H., Meyer, J.-U., Ruf, H.H., 1998. Biosens. Bioelectron. 13, 839–845.
Tyler, C.R., Van Aerle, R., Nilsen, M.V., Blackwell, R., Maddix, S., Nilsen, B.M., Berg, K., Hutchinson, T.H., Goksøyr, A., 2002. Environ. Toxicol. Chem. 21, 47–54.
Tyler, C.R., Van der Eerden, B., Sumpter, J.P., Jobling, S., Painter, G., 1996. J. Comp. Physiol. 166, 418–426.
Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Environ. Pollut. 128, 363–371.
Volotovsky, V., Nam, Y.J., Kim, N., 1997. Sens. Actuators B: Chem. 42, 233–237.
Vörös, J., Ramsden, J.J., Csúcs, G., Szendrő, I., De Paul, S.M., Textor, M., Spencer, N.D., 2002. Biomaterials 23, 3699–3710.