Biosensors and Bioelectronics 24 (2009) 2559–2565

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Capacitive biosensor for quantification of trace amounts of DNA

Apon Numnuam[a,b], Proespichaya Kanatharana[a,b], Bo Mattiasson[c], Punnee Asawatreratanakul[a,d], Booncharoen Wongkittisuksa[a,e], Chusak Limsakul[a,e], Panote Thavarungkul[a,f,*]

[a] Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[b] Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[c] Department of Biotechnology, Lund University, Box 124, 221 00, Lund, Sweden
[d] Department of Biochemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[e] Department of Electrical Engineering, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[f] Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

ARTICLE INFO

Article history:
Received 13 October 2008
Received in revised form 6 January 2009
Accepted 6 January 2009
Available online 14 January 2009

Keywords:
Capacitive biosensor
Histone
Self-assembled monolayer
SAM
DNA-assay

ABSTRACT

A flow injection capacitive biosensor system to detect trace amounts DNA has been developed based on the affinity binding between immobilized histone and DNA. Histones from calf thymus and shrimp were immobilized on gold electrodes covered with self-assembled monolayer (SAM) of thioctic acid. Each of these histones was used to detect DNA from calf thymus, shrimp and *Escherichia coli*. The studies indicated that histones can bind better with DNA from the same source and give higher sensitivity than the binding with DNA from different sources. Under optimum conditions, both histones from calf thymus and shrimp provided the same lower detection limit of 10^{-5} ng l^{-1} for DNA from different sources, i.e., calf thymus, shrimp and *E. coli*. The standard curve for the affinity reaction between calf thymus histone and DNA shows sigmoidal behavior and two linear ranges, 10^{-5} to 10^{-2} ng l^{-1} and 10^{-1} to 10^{2} ng l^{-1}, could be obtained. The immobilized histones were stable and after regeneration good reproducibility of the signal could be obtained up to 43 times with a %R.S.D. of 3.1. When applied to analyze residual DNA in crude protein extracted from white shrimp recoveries were obtained between 80% and 116%.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

DNA quantification is critical for many biological studies since it is often used as a reference for measurements of other biologically active components in biological fluids and genetic diagnosis (Huang et al., 2002; Georgiou and Papapostolou, 2006; Prem Kumar et al., 2005). In biological and biopharmaceutical products, such as monoclonal antibodies, lymphokines and vaccines, quantification of residual cellular DNA from the host cells in the purification process is also important since they have to meet specific requirements regarding contaminating cellular DNA. Guidelines of The World Health Organization (WHO) published in 1986 recommend that the residual cellular DNA permitted in purified products contain less than 100 pg per dose (WHO, 1987). In 1997, this value was changed to 10 ng per dose (WHO, 1998).

The quantification method commonly used for residual DNA determination is real-time quantitative polymerase chain reaction (Q-PCR) (Lovatt, 2002). Other biochemical assay methods include spectrophotometry where DNA absorbs maximally around 260 nm (Samuel et al., 2003), densitometric scans of gels from agarose or polyacrylamide electrophoresis (Projan et al., 1983), chemiluminescence (Ma et al., 2004), spectrofluorimetric and resonance light scattering (RLS) methods (Li et al., 2002; Wang et al., 2005). Each method has its strengths and weaknesses in terms of sensitivity, specificity, running time, robustness, material safety/toxic waste, reagent stability and cost (Li et al., 2002; Ma et al., 2004). For example, in the case of the fluorescence method, many fluorescent reagents have been used to enhance fluorescence intensity for DNA determination, such as ethidium bromide, 4,6-diamidino-2-phenylindole, bis-benzimidazole dye Hoechst 33258. However, the preparation of reagents is inconvenient (Wang et al., 2005) and some reagents, such as ethidium bromide is a carcinogen (Link and Tempel, 1991). Therefore, development of alternative methods which are more convenient and have high sensitivity and specificity for the detection of DNA is desirable and use of affinity biosensors is an interesting approach.

Affinity biosensors are based on binding interaction between the immobilized biomolecule and the analyte of interest (Mattiasson, 1984; Wang, 2000). Affinity biosensors can be categorized as label-free (direct) and labeled. Between the two, label-free affinity biosensor is more attractive since it requires less steps. To detect the affinity binding reaction, capacitive transducers have been applied

* Corresponding author at: Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand. Tel.: +66 74 288753; fax: +66 74 212817.
E-mail address: panote.t@psu.ac.th (P. Thavarungkul).

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.01.005

and found to be very sensitive (Berggren et al., 2001, 1998; Berggren and Johansson, 1997; Bontidean et al., 2001, 1998; Hedström et al., 2005; Hu et al., 2002; Limbut et al., 2006) To detect DNA, it may be possible to apply histones as recognition element since histones are the basic proteins which are complexing with DNA *in vivo* to form the nucleosome (Helliger et al., 1988). The four core histones (H2A, H2B, H3and H4) form an octamer, which DNA is wrapped around to form the nucleosome particle and the histone H1 acts as the linker between the constituents of the nucleosome particle (Allan et al., 1981; Yoshikawa et al., 2001).

In this work we investigated the application of a flow-injection capacitive biosensor for the rapid determination of DNA based on the affinity binding of DNA to histone by immobilizing whole histone on gold electrode surface via self-assembled monolayer SAM of thioctic acid. The technique was tested using DNA preparations from various sources, i.e., calf thymus, shrimp and *Escherichia coli*. The signals of the binding reaction of DNA to white shrimp and calf thymus histones were also compared. This system was also validated with real samples by using it to determine genomic DNA contamination in crude shrimp protein preparation.

2. Materials and methods

2.1. Chemicals

Deoxyribonucleic acid (DNA) and histone from calf thymus, *N*-3-(dimethylamino-propyl-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxy succinimide (*N*-hydroxy-2,5-pyrrodinedione, NHS) and white saponin were purchased from Sigma–Aldrich (Steinheim, Germany). Thioctic acid and 1-dodecanethiol were obtained from Aldrich (Milwaukee, USA). All other chemicals were of analytical grade. Solutions and buffers used in the capacitive biosensor system were prepared with deionized water. Before use, buffers were filtered through an Albet® nylon membrane filter with pore size 0.20 μm with subsequent degassing. DNA from white shrimp (*Penaeus merguiensis)* was provided by Center for Genomic and Bioinformatic Research, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. *E. coli* DNA was obtained from Department of Biotechnology, Lund Universitiy, Sweden and purified by using Freez'N Squeeze DNA gel extraction spin column (Bio-Rad, USA). Histones from white shrimp (*Penaeus merguiensis*) erythrocyte nucleoprotein were prepared by using a slightly modified procedure from that reported by Murry et al. (1968). In brief, shrimp erythrocytes were lysed by vigorous stirring with white saponin solution (0.6% (w/v) in 0.14 M NaCl). Nuclei were collected by centrifugation at 500 × *g* for 1 h. Whole histone was then extracted from the nuclei with 0.2 M H_2SO_4 and centrifuged at 18,600 × *g* for 1 h. Ethanol was added to precipitate histones which were collected by centrifugation and washed 3 times with ethanol.

2.2. Immobilization of histone

The conditions used for immobilization of histones to gold surface modified with self-assembled monolayer of thioctic acid followed the process described by Limbut et al. (2006). In this case 20 μl of appropriate concentration of histone (see Section 3.2.1) was placed on each electrode and the reaction took place at 4 °C for 24 h. During the immobilization steps, the degree of insulation from different layers on the electrode surface is demonstrated by cyclic voltammetric measurements performed in a three electrode electrochemical batch cell containing 5 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl, at a scan rate of 0.1 V s^{-1}. The modified gold electrode was used as the working, Ag/AgCl as a reference and a platinum rod was the auxiliary electrode. The electrodes were coupled to a potentiostat (ML 160, AD Instruments, Australia) connected to a computer.

2.3. Capacitance measurements

The measurements were performed in a three electrode flow cell with a dead volume of 10 μl by applying +50 mV potential pulses, one pulse per minute to the modified gold electrode through the Powerlab system (AD Instrument, Australia) (Fig. 1). The resulting current response from each pulse was sampled with a frequency of 40 kHz. The capacitance of the electrode surface was calculated from the current response as described by Limbut et al. (2006).

2.4. Optimization of the capacitive biosensor

Operating conditions of the flow injection capacitive biosensor system were optimized for the affinity binding between DNA and histones from calf thymus. Parameters affecting the capacitive response were studied, i.e., regeneration solution, sample volume, flow rate, and carrier buffer. The parameters were optimized one by one by comparing responses obtained after injections of standard DNA solution, three replications for each test value. The optimum of each parameter was determined as a compromise between the sensitivity (slope of the calibration curve) or capacitance change and analysis time. Using the obtained optimum conditions, the performances of the system, i.e., linear range, lower detection limit, selectivity were investigated. Effects of histones and DNAs from different sources, i.e., calf thymus and white shrimp were also tested.

2.5. Real sample analysis

A crude protein extract from white shrimp was used as a representative of a real sample. This extract is used in the study of protein interaction with white spot syndrome virus in shrimp. In the preparation process, DNA was removed by DNaseI treatment and residual DNA is generally detected by UV spectrometry. To test the capacitive biosensor system, residual DNA in the extract was analyzed under optimum conditions, including the study of matrix effect and recovery.

3. Results and discussion

3.1. Insulating property of working electrode

Capacitive measurements require a proper insulation of the electrode surface in order to prevent disturbing redox reactions at the applied potential. The degree of insulation obtained by different layer in the electrode preparation was studied with cyclic voltammetry. Using the cleaned gold electrode, the reversible peaks for oxidation and reduction were clearly observed during cycling of potential. These peaks were significantly reduced with SAM modification followed by histone immobilization. After treating with 1-dodecanethiol to block any pinhole on the electrode surface, the redox peaks disappeared completely indicating that the electrode was totally insulated.

3.2. Optimization of the capacitive biosensor system

The capacitance of the binding event between histone and DNA is determined from the current response when a potentiostatic step of 50 mV is applied on the electrode (Limbut et al., 2006). The affinity binding between DNA and the immobilized histone molecules on the working electrode will result in an increase of the thickness of the layer and this would cause the total capacitance (C_{tot}) to decrease (Fig. 1). Fig. 2 shows an example of a plot of the calculated C_{tot} value with respect to time where the capacitance change (ΔC_1, ΔC_2) due to the binding can be determined. Since the interaction between DNA and histone are via non-covalent bonds, DNA could

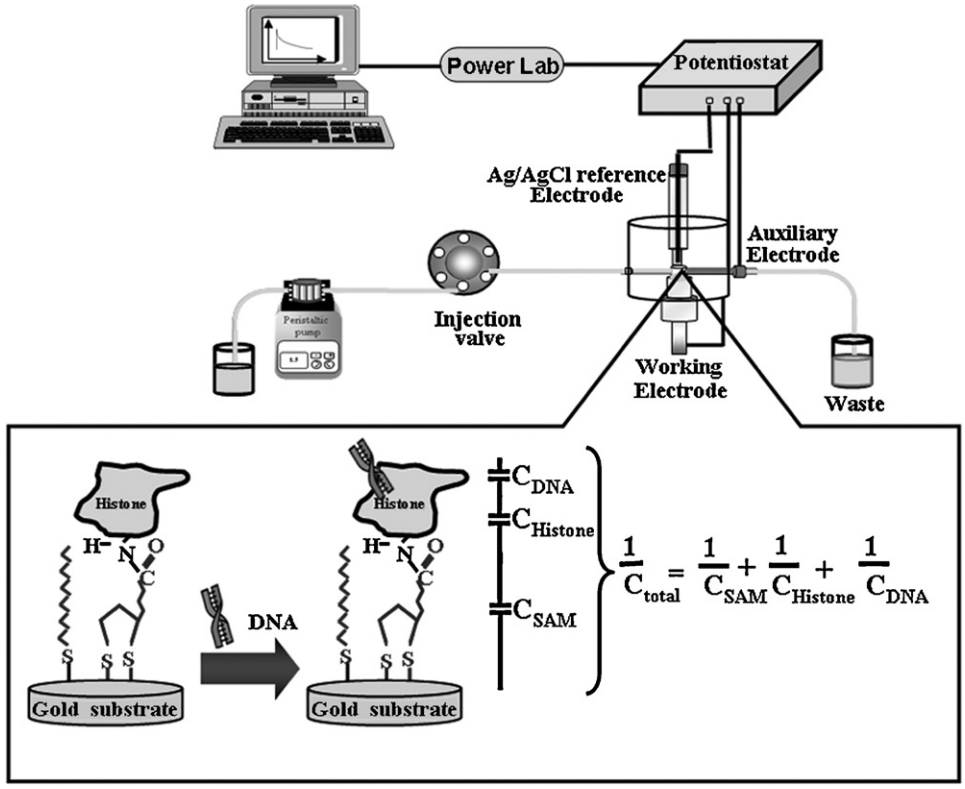

Fig. 1. Schematic diagram of the flow injection capacitive biosensor system. The total capacitance measured at the working electrode/solution interface (C_{tot}) comes from C_{SAM}; the capacitance of self-assembled thioctic acid monolayer, $C_{Histone}$; the capacitance of histone layer and C_{DNA}; the capacitance DNA analyte interaction (modified from Limbut et al., 2006).

be dissociated from the histone on the electrode surface by using regeneration solution. Optimizations were carried out as follows.

3.2.1. Concentration of histone

The effect of different concentrations of histone on the electrode response was studied. Solutions of histones (25 μg ml^{-1}, 50 μg ml^{-1}, 75 μg ml^{-1} and 100 μg ml^{-1}) were used for the immobilization process. Four electrodes were tested by injecting standard DNA solutions between 0.1 ng l^{-1} and 100 ng l^{-1}. The sensitivity (slope of calibration curve) increased with increasing histone concentration and stabilized for electrodes produced from 50 μg ml^{-1} (87 ± 2 nF cm^{-2} ng^{-1} l) and upwards. To see the specific binding of DNA on the sensor surface, an electrode without immobilized histone was tested by injecting the same range of standard solutions of DNA. The responses did not change with concentrations and were lower than the response of the limit of detection (see Section 3.4). This result indicated that the immobilized histone is specific to DNA.

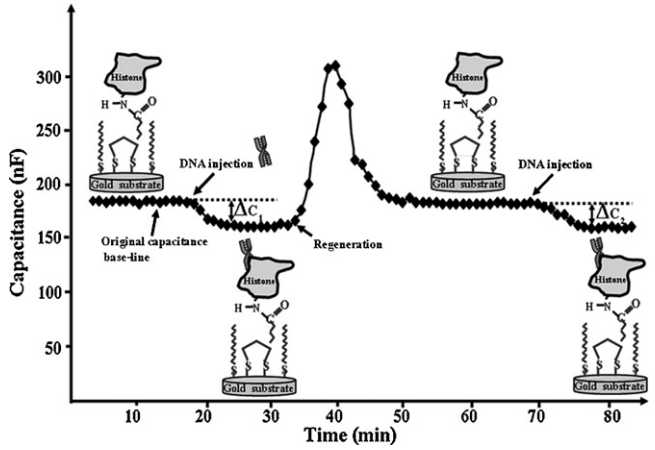

Fig. 2. An example of the capacitance (C_{tot}) plots as a function of time. The binding between histone and DNA causes the capacitance to decrease (ΔC_1) with subsequent signal increase due to dissociation under regeneration conditions. After regeneration, the system can be reused to detect DNA in a new injection (ΔC_2).

3.2.2. Regeneration solution

The biosensor needs regeneration of the sensor surface before measurement can be repeated. The goal is to break the non-covalently bonds between DNA (the analyte) and histones immobilized on the gold surface, while maintaining the activity of the histones.

To evaluate the performance of the regeneration solution, percentage of residual activity (%residual DNA-binding activity) of histone was calculated from capacitive change given by a consecutive binding between DNA (1 ng l^{-1} with sample volume 300 μl) and histone before (ΔC_1) and after ((C_2) regeneration (Fig. 2) according to the equation:

$$\%\,\text{Residual activity} = \frac{\Delta C_2 \times 100}{\Delta C_1} \quad (1)$$

Four different types of regenerating agents were compared. 50 mM KCl and high pH solution, 50 mM NaOH, were less effective than a low pH of 50 mM glycine–HCl, pH 2.4 and HCl pH 2.4. They gave low percentage residual activity, less than 70%, and required a longer regeneration time, more than 17 min, compared to 13 min for low pH. At low pH, glycine–HCl gave higher percentage residual activity (87 ± 2%) than HCl (83 ± 2%), therefore, glycine–HCl, pH 2.4 was further investigated to see the influence of its concentration (10–100 mM). At 25 mM the highest residual activity of 93 ± 3% was achieved. The effect of pH was then tested at this concentration

and 97 ± 3% residual activity was gained at pH 2.4. Therefore, 25 mM glycine–HCl, pH 2.4 was chosen for the continued experiments.

3.2.3. Buffer solution

Initially two widely used biochemical buffer types were tested, 10 mM of sodium phosphate and Tris–HCl buffer, pH 7.20, by analysing standard DNA between 0.1 ng l^{-1} and 100 ng l^{-1} in these buffers. When analysis was carried out in the two buffers, there was no difference between sensitivity (slope of calibration curve), i.e., 88 ± 2 and 89 ± 5 nF cm^{-2} ng^{-1} l for Tris–HCl and sodium phosphate buffer, respectively. However, the assays in Tris–HCl gave a higher response and a more steady baseline. Different concentrations of Tris–HCl buffer (5–100 mM) were then tested. The highest change in the capacitive signal, from injection of 1 ng l^{-1} DNA, were obtained at 5 mM (164 ± 25 nF cm^{-2}) and 10 mM (160 ± 5 nF cm^{-2}) but a more steady baseline was obtained at 10 mM so this concentration was selected. When optimizing the sensor response at different pH of Tris–HCl buffer (pH 7.00–8.00), the maximal capacitive change was found at pH 7.00. Therefore, 10 mM Tris–HCl, pH 7.00 was used in the continued experiments.

3.2.4. Flow rate

The effect of flow rate was investigated by injecting 300 μl of 1 ng l^{-1} of DNA solution at different flow-rates (50–500 μl min^{-1}). The capacitance change decreased as the flow rate increased due to the reduction of binding interaction time. The flow rate of 50 μl min^{-1} gave the highest response (169 ± 10 nF cm^{-2}), however, nearly the same response was also obtained at 100 μl min^{-1} (165 ± 3 nF cm^{-2}), but the analysis time was shorter, 14 min compared to 18 min at 50 ml min^{-1}, so 100 μl min^{-1} was chosen.

3.2.5. Sample volume

Generally, an increase in response can be achieved with an increase in sample volume. Therefore, the effect of sample volume on capacitance changes to 1 ng l^{-1} of DNA standard was studied from 50 μl to 400 μl. The change in capacitance signal increased with sample volume until 250 μl and become steady, therefore, 250 μl was chosen.

In summary the optimum conditions are: 25 mM glycine–HCl, pH 2.40 for regeneration solution, 10 mM of Tris–HCl, pH 7.00 for carrier and sample buffer solution, flow rate at 100 μl min^{-1} and 250 μl of sample volume, the analysis time was 13–15 min with another 13 min of regeneration time.

3.3. Reproducibility

To test whether the response of the histone modified electrode can be reproduced after the removal of DNA by regeneration solution, the same concentration of 1 ng l^{-1} of DNA was injected into the system with subsequent regeneration. The change in capacitance signal after regeneration was used to calculate the percentage of residual activity of the histone electrode by comparing the response to the initial capacitance change. The histone immobilized on the self-assembled monolayer method retained 95.2 ± 3.1% of its ability to bind DNA after 43 times of regeneration and the residual activity after this point dropped below 90%. The results indicate that the histone electrode can be reused with good reproducibility up to at least 40 times.

3.4. Linear dynamic range and detection limit

Standard calf thymus DNA solutions ranging from 1×10^{-7} ng l^{-1} to 1×10^{3} ng l^{-1} were injected with intermittent regeneration steps using 25 mM glycine–HCl, pH 2.4. Fig. 3 shows the calibration curve for DNA under optimal conditions. The plot between capacitance change and logarithm of DNA concentration showed a sigmoidal

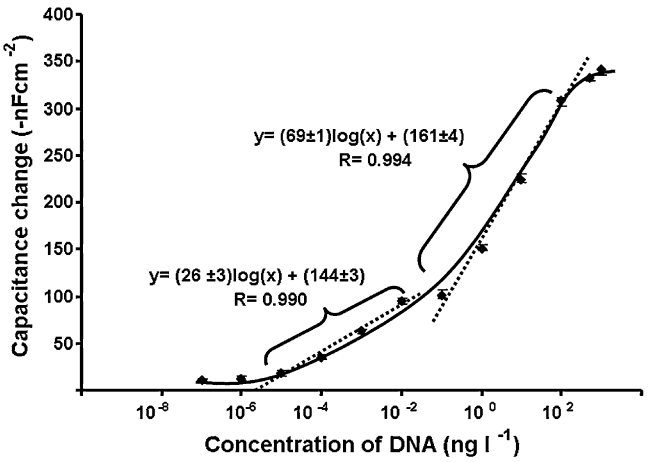

Fig. 3. Capacitance change vs. the logarithm of calf thymus DNA concentration detected by electrodes with immobilized calf thymus histone under optimum conditions (100 μl min^{-1} flow rate, 250 μl sample volume, 10 mM Tris–HCl buffer, pH 7.00).

behavior (within limit of the experimental error) two linear ranges with different sensitivities, i.e., from 10^{-5} ng l^{-1} to 10^{-2} ng l^{-1} and 10^{-1} ng l^{-1} to 10^{2} ng l^{-1} can be obtained. The lower concentration range (10^{-5} ng l^{-1} to 10^{-2} ng l^{-1}) was employed for further study (see Sections 3.5 and 3.6). This sigmoidal curve might be explained by the model of cooperative binding of ligands to multimeric protein (Nelson and Cox, 2000). In this work, whole histones of calf thymus were used (H1, H2A, H2B, H3 and H4) which bind to DNA with different affinity (Cui et al., 2005), with H1 having a higher affinity to DNA than the other four histones (Cui et al., 2005). In this case H1 would be the major histone that binds to DNA at low concentration. The binding between H1 and DNA may change the conformation of DNA which will then drive the cooperative binding of other lower affinity histone onto DNA molecule at higher concentration. At concentration higher than 10^{2} ng l^{-1} the binding of DNA by histones on the electrode becomes saturated, so the plateau curve was obtained. From the results, the detection limit was found to be 10^{-5} ng l^{-1} based on IUPAC Recommendation 1994 (Buck and Lindner, 1994).

3.5. Selectivity

Since different sources of DNA or histones may affect the binding and, hence, the response of the capacitive system, it is important to investigate this. First, the effect of DNA source was studied by using immobilized calf thymus histone to detect DNA from calf thymus, white shrimp and *E. coli*. The calibration curves were investigated between 10^{-5} ng l^{-1} and 10^{-2} ng l^{-1} and these are shown in Fig. 4a. The sensitivity (slope of calibration curve) obtained with calf thymus DNA was about fourfolds higher than the one obtained from white shrimp DNA, and the lowest sensitivity was obtained from *E. coli* DNA. This is probably due to the size of DNA, longest DNA is from calf thymus, followed by shrimp and *E. coli* (qualitative comparison of sizes was tested by using agarose gel electrophoresis). The layer on the electrode surface with a longer DNA should be thicker and this would cause C_{tot} to decrease more than when a shorter DNA was bound, so calf thymus DNA would give the highest response. Although the sensitivity of the system to each DNA is different, the lower limit of detection is the same. That is, when the system was used to detect the DNA of calf thymus, white shrimp, or *E. coli* the lower limit of detection is 10^{-5} ng l^{-1}.

Histones from a different source, i.e., white shrimp, were then immobilized on gold electrodes at the same concentration as that of calf thymus histones (50 μg ml^{-1}) and studied was car-

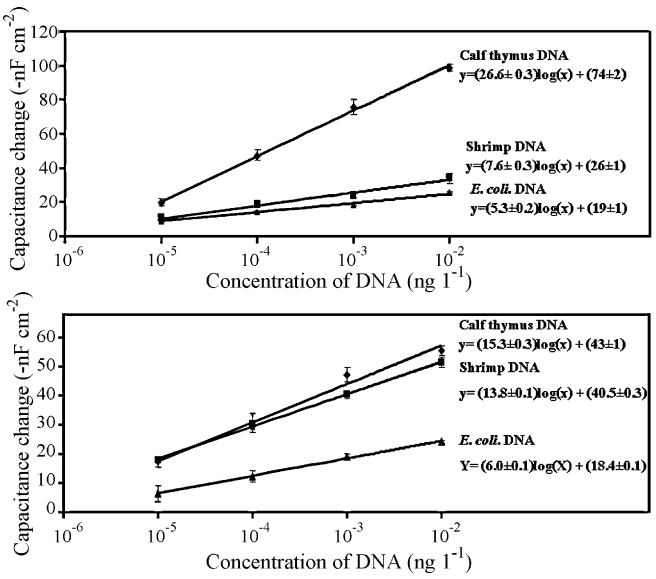

Fig. 4. Calibration curve for DNA from calf thymus, white shrimp and *E. coli* with (a) immobilized calf thymus histone and (b) white shrimp histone.

ried out by injecting DNA from calf thymus, white shrimp and *E. coli* (Fig. 4b). The results show that shrimp histone can also bind with DNA from all three sources with the same lower limit of detection of 10^{-5} ng l^{-1}. That is, neither calf nor shrimp histone is selective for DNA from its own species. Comparing between Fig. 4a and b, it can be seen that when using shrimp histone (Fig. 4b) the sensitivity to shrimp DNA can be increased from 7.6 ± 0.3 nF cm^{-2} ng l^{-1} to 13.8 ± 0.1 nF cm^{-2} ng l^{-1} while the sensitivity to calf thymus DNA decreased form 26.6 ± 0.3 nF cm^{-2} ng l^{-1} to 15.3 ± 0.3 nF cm^{-2} ng l^{-1}. That is, histone can bind better with DNA from the same source. The relatively high sensitivity to calf thymus DNA when using shrimp histone is probably due to the longer DNA of calf thymus as discussed in the previous paragraph.

The effect of a mixture of DNA from different sources was also studied by using immobilized calf thymus histones to detect the mixture of DNA from calf thymus, white shrimp and *E. coli* at different concentrations. The measurement was first done for each DNA at 1 pg l^{-1}, 2 pg l^{-1} and 3 pg l^{-1}. The response from calf thymus gave the highest response at every concentration (Fig. 5). Mixtures of DNA from different sources at different concentrations were then

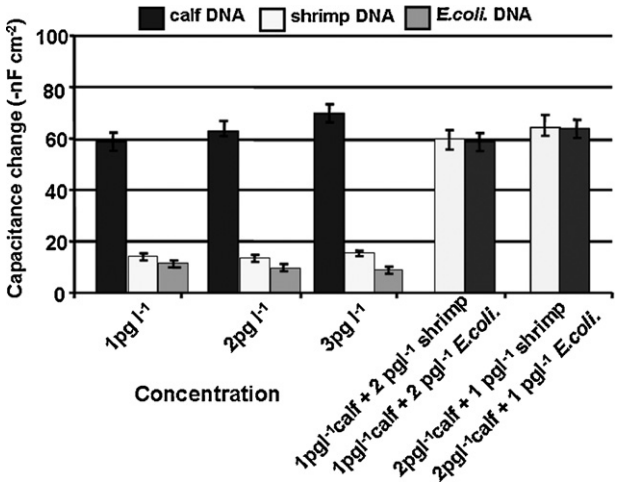

Fig. 5. Effect of DNA from calf thymus, shrimp, *E. coli* and a mixture of them on the binding with calf thymus histone.

studied, i.e., 1 pg l^{-1} of calf thymus DNA + 2 pg l^{-1} of shrimp or *E. coli* DNA, 2 pg l^{-1} of calf thymus DNA + 1 pg l^{-1} of shrimp or *E. coli* DNA. The results obtained from the mixture were less than the sum of individual but slightly higher than calf thymus DNA alone. That is, the data reflect the presence of calf thymus DNA and that calf histone can provide more selective binding to calf DNA than shrimp and *E. coli*.

3.6. Determination of residual shrimp DNA

Determination of residual DNA was carried out using a particle free homogenate of shrimp protein as real sample. This extract is normally used in the study of protein interaction with white spot syndrome virus. Since the protein in the extract may interfere with the detection of residual DNA via non-specific binding to the surface of the modified electrode, bovine serum albumin (BSA) was used to test this influence. Using the electrode with immobilized calf thymus histone, the responses due to BSA at the concentration range 0.01–100 ng l^{-1} were almost constant and were much lower than the responses from DNA. It can be suggested that non-specific binding from proteins do not play an important part in the response.

When analyzing real samples, the matrix may also cause some interference to the response. The influence of the matrix in real sample was studied by using immobilized shrimp histone. Crude shrimp protein sample without DNA (prepared by adding excess DNaseI to digest residual DNA) was spiked with known amount of shrimp DNA at 0.1–1.5 pg l^{-1} and these were used to perform the matrix matched calibration assays. A calibration curve was also obtained when analyzing standard solution in the same concentration ranges (standard calibration curve). The assay for each concentration was carried out in triplicate and the average sensor response and standard deviation were calculated. The slope of standard calibration curve and matrix matched calibration curve were compared by two-way ANOVA (analysis of variance) calculated by R solfware (R Development Core Team, 2006). The results showed that the slope of regression lines of standard and matrix matched calibration curves were significantly different ($P < 0.05$), indicating that there was effect of matrix interference on the sensor response (IUPAC Technical Report, 2002). One way of reducing the matrix effect is by dilution. Since the LOD of this system is very low (10^{-5} ng l^{-1}), it is possible to dilute the sample several times. This will be appropriate when the concentration of DNA is high, e.g., when DNA is product of process. However, when the detection is for residual DNA only trace amount will exist, in this case matrix matched calibration should be employed.

3.6.1. Matrix matched calibration

Matrix matched calibration was used for the detections of the trace amount of DNA. The response when injecting crude shrimp protein sample ($n = 3$) were used to calculate the concentration of residual DNA from the matrix matched calibration curve and no DNA was detected.

To further validate the capacitive detection system with real samples, the recovery of spiked DNA (0.1–1.5 pg l^{-1}) in crude shrimp protein samples were tested. The analysis was carried out with immobilized shrimp histones. The responses obtained from spiked shrimp protein sample were used to calculate the concentration from the matrix matched calibration curve. Recovery percentage was evaluated by the following equation:

$$\%\,\text{Recovery} = \frac{C_1 - C_2}{C_3} \times 100 \quad (2)$$

where C_1 is the concentration of analyte measured in the spike sample, C_2 is the concentration of analyte measured in the blank and C_3 is the concentration of analyte spiked into the sample (Taverniers et al., 2004). From the results (Table 1), percentage of recovery and rel-

Table 1
Recovery of shrimp DNA from spiked solutions of crude shrimp protein ($n = 3$); using matrix matched calibration (Section 3.6.1) and dilution methods (Section 3.6.2).

Spiked concentration	Immobilized shrimp histone				Immobilized calf thymus histone			
	Sample 1		Sample 2		Sample1		Sample 2	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Matrix matched calibration ($pg\,l^{-1}$)								
0.10	80 ± 18	22	–	–	–	–	–	–
0.50	116 ± 15	13	–	–	–	–	–	–
1.0	107 ± 7	7	–	–	–	–	–	–
1.5	100 ± 6	6	–	–	–	–	–	–
Dilution ($ng\,l^{-1}$)								
10	73 ± 2	3	88 ± 23	26	77 ± 6	8	95 ± 17	18
50	109 ± 5	5	98 ± 17	17	115 ± 15	13	117 ± 22	19
100	103 ± 4	4	113 ± 4	4	101 ± 6	6	116 ± 18	16
150	110 ± 1	1	108 ± 5	5	106 ± 7	7	101 ± 9	9

ative standard deviation of all spiked DNA (in $pg\,l^{-1}$ range) in crude shrimp protein are acceptable. Since the acceptable recovery in the $\mu g\,l^{-1}$ level is 40–120% and R.S.D. is 30–45.3% (Taverniers et al., 2004).

3.6.2. Dilution

Crude shrimp protein samples were spiked with shrimp DNA at $10\,ng\,l^{-1}$ to $150\,ng\,l^{-1}$ and diluted 10,000 times (following Thavarungkul et al., 2007) with 10 mM Tris–HCl buffer, pH 7.0. These were analyzed ($n = 3$) and the calibration curve was compared to that of standard solution. The results showed that there was no matrix effect for the diluted samples, that is, the matrix effect can be reduced by dilution. Therefore, the standard curve could be used to determine the residual DNA contamination in diluted real sample.

The recovery of spiked shrimp DNA at $10\,ng\,l^{-1}$ to $150\,ng\,l^{-1}$ in crude shrimp protein sample was tested. The spiked samples were then diluted 10,000 times with Tris–HCl buffer, pH 7.0 to reduce the matrix effect. The analysis was carried out by two capacitive biosensor systems, one with immobilized shrimp histone and the other with calf thymus histone. The capacitance changes obtained from the capacitive systems were used to calculate the concentrations from the calibration curve of standard obtained prior to the test. From the results (Table 1) percentage of recovery and relative standard deviation of all spiked DNA (in $ng\,l^{-1}$ range) in crude shrimp protein are acceptable (Taverniers et al., 2004) (acceptable recovery in the $\mu g\,l^{-1}$ level is 40–120% and R.S.D. is 30–45.3%). These results show that the developed capacitive biosensor is suited for quantification of DNA.

The spiked samples were also measured by UV spectrometry at λ_{260}. However, no response was obtained from any of spiked samples (DNA at 10–150 $ng\,l^{-1}$) since the detection limit of this method is $5\,\mu g\,ml^{-1}$ (Noites et al., 1999). That is, the developed capacitive system can detect DNA at a much lower concentration.

4. Conclusions

The results demonstrated the possibility of using the capacitive biosensor system for direct assay of affinity binding between DNA and histone protein on self-assembled thioctic acid monolayer modified electrode. For the system using calf thymus histone to detect calf thymus DNA, it provides a lower detection limit of $10^{-5}\,ng\,l^{-1}$ and wide linear ranges 10^{-5}–$10^{-2}\,ng\,l^{-1}$ and 10^{-1}–$10^{2}\,ng\,l^{-1}$. Since histone from one source can also detect DNA from other sources, this system is suitable for screening DNA contaminants independent of their origin. However, if the source of DNA is known a calibration curve can be constructed and this system can be used to quantify the amount of DNA which will be useful for a number of biotechnology process. This method can be used to investigate DNA in samples with different matrices. In case of trace DNA level, matrix matched calibration curve can be applied, for example, residual DNA in biological or biopharmaceutical products. For quantification of high concentrations of DNA, such as in samples after cell lysis, the matrix interference can be reduced by dilution where the standard curve could be used to determine the DNA in diluted real sample. Comparing to other commonly used methods for determination of DNA concentration, this system is more advantageous. For example, spectrophotometric method (absorbance at 260 nm, or the A_{260} method), although this method is accurated and reproducible, it is relatively insensitive and can only measure DNA concentrations that is greater than $5\,\mu g\,ml^{-1}$ (Noites et al., 1999) while the develop capacitive system can detect DNA as low as $10^{-8}\,ng\,ml^{-1}$. In another method, densitometry scan of gel electrophoresis, DNA quantification between $15\,ng\,ml^{-1}$ and $700\,ng\,ml^{-1}$ can be determined (Projan et al., 1983). However, gel electrophoresis is not a very accurate quantification method, with an error range of around 10–20% (Levy et al., 2000; Projan et al., 1983). For spectrofluorometric method, although this technique is very sensitive and can detect DNA with concentration between $15\,ng\,ml^{-1}$ and $250\,ng\,ml^{-1}$ (Noites et al., 1999), it needs fluorescence dye to stain DNA such as ethidium bromide or other fluorescence stains that are powerful carcinogen. In addition, analysis time of this technique (13–15 min) was much shorter than other DNA quantitation methods (30 min to 5 h) (Projan et al., 1983; Schmidt et al., 1996). A slight drawback of the capacitive system is the time required to modify the electrode surface, i.e., 2 days. Therefore, future investigation into a more rapid procedure would be interesting. Although, the preparation of electrode requires some time, one electrode can be reused up to 40 times by using the appropriate regeneration solution and this helps to reduce the cost of analysis.

Acknowledgements

This project was supported by The Thailand Research Fund (TRF) (project BRG4980023); The Royal Golden Jubilee PhD-Program supported by The Thailand Research Fund; Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand; VR (The Swedish Research Council)-SIDA (The Swedish International Development Cooperation Agency) research links; Trace Analysis and Biosensor Research Center, Graduate School and Faculty of Science, Prince of Songkla University, Hat Yai, Thailand. We thank Assoc. Prof. Dr. Amornrat Pongdara, Dr. Warapond Wanna and Ms. Alisa Nakkaew, Center for Genomic and Bioinformatic Research, Faculty of Science, Prince of Songkla University for their help and discussion on DNA purification. We also thank Assoc. Prof. Dr. Prapaporn Utarabhand from Department of Biochemistry, Faculty of Science, Prince of Songkla University for her discussion on extraction and purification of shrimp histone.

References

Allan, J., Cowling, G.J., Harborne, N., Cattini, P., Craigie, R., Gould, H., 1981. J. Cell. Biol. 90 (2), 279–288.
Berggren, C., Bjarnason, B., Johansson, G., 2001. Electroanalysis 13 (3), 173–180.
Berggren, C., Bjarnason, B., Johansson, G., 1998. Biosens. Bioelectron. 13 (10), 1061–1068.
Berggren, C., Johansson, G., 1997. Anal. Chem. 69 (8), 3651–3657.
Bontidean, I., Kumar, A., Csöregi, E., Galaev, I.Yu., Mattiasson, B., 2001. Angew. Chem. Int. Ed. 40 (14), 2676–2678.
Bontidean, I., Berggren, C., Johansson, G., Csoregi, E., Mattiasson, B., Lloyd, J.R., Jakeman, K.J., Brown, N.L., 1998. Anal. Chem. 70 (19), 4162–4169.
Buck, R.P., Lindner, E., 1994. Pure. Appl. Chem. 66, 2527–2536.
Cui, Z., Yang, F., Li, A., Yang, X., 2005. Anal. Biochem. 342 (1), 173–175.
Georgiou, C.D., Papapostolou, I., 2006. Anal. Biochem. 358 (2), 247–256.
Hedström, M., Galaev, I.Y., Mattiasson, B., 2005. Biosens. Bioelectron. 21 (1), 41–48.
Helliger, W., Lindner, H., Hauptlorenz, S., Puschendore, B., 1988. Biochem. J. 225, 23–27.
Hu, S.-Q., Wu, Z.-Y., Zhou, Y.-M., Cao, Z.-X., Shen, G.-L., Yu, R.-Q., 2002. Anal. Chim. Acta 458 (2), 297–304.
Huang, C.Z., Fan, M.K., Li, Y.F., 2002. Anal. Chim. Acta 466 (2), 193–200, IUPAC Technical Report, 2002. Pure. Appl. Chem. 835–855.
Levy, M.S., Lotfian, P., O'Kennedy, R., Yim, M.Y.L., Shamlou, P.A., 2000. Nucleic Acids Res. 28, 1–7.
Li, Y.F., Huang, C.Z., Li, M., 2002. Anal. Chim. Acta 452 (2), 285–294.
Limbut, W., Kanatharana, P., Mattiasson, B., Asawatreratanakul, P., Thavarungkul, P., 2006. Biosens. Bioelectr. 22 (2), 233–240.
Link, A., Tempel, K., 1991. J. Cancer Res. Clin. 117 (6), 549–555.
Lovatt, A., 2002. Rev. Mol. Biotechnol. 82 (3), 279–300.
Ma, Y., Zhou, M., Jin, X., Zhang, Z., Teng, X., Chen, H., 2004. Anal. Chim. Acta 501 (1), 25–30.
Mattiasson, B., 1984. Trends Anal. Chem. 3 (9), 245–250.
Murry, K., Vidali, G., Neelin, J.M., 1968. Biochem. J. 107, 207–215.
Nelson, D.L., Cox, M.M., 2000. Lehninger Principle of Biochemistry, 3rd ed. Worth Publishers, New York.
Noites, I.S., O'Kennedy, R.D., Levy, M.S., Abidi, N., Keshvarz-Moore, E., 1999. Biotechnol. Bioeng. 66 (3), 195–201.
Prem Kumar, A.A., Mani, K.R., Palaniappan, C., Bhau, L.N.R., Swaminathan, K., 2005. Microb. Infect. 7 (9–10), 1110–1116.
Projan, S.J., Carleton, S., Novick, R.P., 1983. Plasmid 9 (2), 182–190.
R Development Core Team, 2006. R: A Language and Environmental for Statistical Computing. R Foundamental for Statistical Computing. R Development Core Team, Veinna, Austria.
Samuel, M., Lu, M., Pachuk, C.J., Satishchandran, C., 2003. Anal. Biochem. 313 (2), 301–306.
Schmidt, T., Friehs, K., Flaschel, E., 1996. J. Biotechnol. 49, 219–229.
Taverniers, I., De Loose, M., Van Bockstaele, E., 2004. TrAC Trends Anal. Chem. 23 (8), 535–552.
Thavarungkul, P., Dawan, S., Kanatharana, P., Asawatreratanakul, P., 2007. Biosens. Bioelectron. 23 (5), 688–694.
Wang, J., 2000. Analytical Electrochemistry, 2nd ed. VCH, New York.
Wang, R.-Y., Gao, X., Lu, Y.-T., 2005. Anal. Chim. Acta 538 (1–2), 151–158.
W.H.O., 1987. Acceptability of cell substrate for production of biological. WHO, Geneva (WHO Technical Report Series, No. 747).
W.H.O., 1998. Requirements for the Use of Animal Cells as in vitro substrates for the production of biologicals (Requirements for biological susbstances no. 50). Biologicals 26, 175–193.
Yoshikawa, Y., Velichko, Y.S., Ichoda, Y., Yoshikawa, K., 2001. Eur. J. Biochem. 268 (9), 2593–2599.