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Electrochemical detection of *E. coli* 16S rDNA sequence using air-plasma-activated fullerene-impregnated screen printed electrodes

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Abstract

A new DNA modified electrode for the electrochemical detection of 16S rDNA extracted from *Escherichia coli* (JCM1649) is proposed. The electrodes were fabricated by screen printing a fullerene-impregnated carbon ink onto a poly(methylmethacrylate) substrate and immobilizing a probe DNA on the surface after activating the electrode with air plasma. The results indicated a dramatic improvement in the surface coverage of the immobilized probe DNA, and of the reduction peak of the redox indicator ($Co(phen)_3^{3+}$) due to the incorporation of fullerene. By immobilizing the probe onto the fullerene-impregnated screen-printed electrodes, the PCR product of the 16S rDNA extracted from *E. coli* was directly detected without any pretreatment. A well defined signal difference was observed between the perfectly matching oligonucleotide and the mismatching one, and it was possible to detect the target at the modified electrode. This method enabled us to clearly detect the two base mismatches in the ca. 1500-bases long 16S rDNA sequence.

Keywords: Air plasma activation; Fullerene impregnated screen printed electrodes; *E. coli* 16S rDNA; Electrochemical hybridization detection

1. Introduction

The fabrication of DNA electrochemical sensors has attracted a considerable recent attention [1–5, 6(a)–6(d)]. Electrochemical detection has been used to monitor DNA hybridization due to its high sensitivity, low cost, miniaturization and compatibility with modern microfabrication and microchip devices. Electrochemical and/or piezoelectric transduction modes, aimed at detecting the hybrid between the probe and the target, have been developed [3,7–12]. As in most cases, the preparation of DNA hybridization sensors involves processes for the attachment of oligonucleotide probes, and DNA immobilization has been considered a fundamental technique in DNA biosensing. Many electrode materials, such as mercury [13], gold [14,15], carbon paste [3,16–18], glassy carbon [19,20], carbon fibers [21] and screen printed electrode [8,22–24], have been used to immobilize the DNA, and many immobilization techniques have been developed [3,10,13,14,24,25]. On the other hand, carbon nanotubes (CNT) are compounds which have attracted much interest as the materials for DNA sensors and biosensors because of their unique properties [26–28]. The voltammetric behaviors of DNA and other biologically important compounds at CNT modified electrodes have been investigated [29–33]. It has been reported that fullerene (C_{60}) plays an important role in the electron transfer of methyl pyropheophorbide-a (chlorine; synthetic chlorophyll analogue) on C_{60} modified carbon paste electrodes [34,35]. The electrochemical behavior of C_{60} and related types of carbon thus offers great promise for biosensor research.

In this study, an attempt was made to demonstrate a new procedure of immobilizing DNA onto a fullerene impregnated

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screen printed electrode (FISPE) and detecting 16S rDNA, extracted from *Escherichia coli* (JCM1649) by the surface hybridization event. The FISPE was fabricated by printing the carbon ink containing C_{60} onto a substrate. Wang's tram [3,8,17,18,22] reported that the adsorptive accumulation of DNA onto a screen printed electrode (SPE) surface was accomplished by electrochemical activation in acetate buffer (pH 5). According to this method, DNA can be easily immobilized onto carbon electrodes such as SPEs and carbon paste electrodes. The electrochemical oxidation of electrodes, which is called the "activation of electrodes" in their papers, is very important, as DNA is immobilized onto the oxidized surface [3,8,12,17,18,22,36–38]. In our present work, the activation of FISPE was achieved by exposure to air plasma, which oxidizes the electrode surface. Consequently, the amount of probe oligo-DNA immobilized onto the FISPE increased due to the plasma pretreatment and the C_{60} addition. By means of the new DNA modified FISPE, we examined the detection of the 16S rDNA amplified from a microbial DNA. The 16S rDNA, of which the size is 1500 base, is used for the identification of microbial [39,40]. The size of the target DNA, as determine by an electrochemical DNA biosensor, is usually less than 100 base-pair and the report of the detection of long DNA sequences is few. So the analysis of 16S rDNA is considered significant not only in phylogenetic research but also in electrochemical DNA sensor fields.

2. Experimental

2.1. Chemicals and reagents

Polyguanylic acid potassium salt, polycytidylic acid potassium salt and 21-mer oligonucleotides (probe) were purchased from Sigma Aldrich (Japan). The specific oligonucleotides probe sequences were as follows: probe A: 5′-TCA GCA AAG CAG CAA GCT GCT-3′; probe B: 5′-TCA GCA AAG TGG CAA GCT GCT-3′; probe C: 5′-TCA GCA AAG TGA CAA GCT GCT-3′; probe D : 5′-TCC CGT AGA GTC TGG ACC GTG-3′; probe E : 5′-TAC TTC TTT TGC AAC CCA CTC-3′. The sequences of probe A was perfect match for a fragment of the 16S rDNA extracted from *E. coli*, probe B two base mismatches, probe C had three base mismatches and probe D had five base mismatches. The probe E sequence perfectly matched a fragment different from that matched by probe A. The underlined bases in the probe sequences are the positions mismatching the target DNA. The chemicals for the buffer solutions, background electrolytes and organic solvents were all analytical grade, purchased from Wako Pure Chemical Industries, Ltd. (Japan). All solutions were prepared by using autoclaved ultrapure water and the water was purified with Milli-Q Gradient-A10 (Millipore Co., Japan).

Pure fullerene was acquired from Tokyo Kasei Kogyo Co. (catalog no. B1641, 99.9%; Japan) and used as received. The C_{60} stock solution was prepared by dissolving C_{60} in benzene and filtering off the undissolved C_{60}. The concentration of the C_{60} solution was determined based on the UV absorbance at 335 nm [41]. Poly(methylmethacrylate) (PMMA, 0.8 mm thick) was used as the substrate of the screen printed electrodes; it was purchased from Nitoh Jushi Co. (Japan). The carbon ink was acquired from Jujoh Chemicals Co., (product no. Jelcom CH-10; Japan).

2.2. Target DNA and probes

Escherichia coli (JCM1649), provided by the Riken Bioresource Center (Japan), was cultured in a LB medium, which was composed of trypton, yeast extract and NaCl (pH 7) and purchased from Difco Co. (Luria-Bertani Broth Miller, Catalog No. 244620). The DNA of the strain was extracted from the bacterial colony and purified [39,42]. The 16S rDNA primers used were 20f (5′-TGG CTC AGA TTG AAC GCT GGC-3′) and 1510r (5′-CGC CCT CCC GAA GTT AAG CTA-3′) synthesized and purified by Sigma Aldrich (Japan). PCR amplification was performed in the reaction mixtures containing the extracted *E. coli* DNA, Taq polymerase (Ex Taq, Thermus aquaticus DNA polymerase; Takara Bio Chemicals Co., Japan), dNTP mixture (dATP, dGTP, dCTP, dTTP; Takara Bio Chemicals Co., Japan), and PCR buffer solution (Ex Taq buffer; Takara Bio Chemicals Co., Japan). The reaction was done in a thermal cycler (PCR Thermal Cycler 480; Takara Bio Chemicals Co., Japan): 28 cycles of 0.5 min at 60 °C, 2 min at 72 °C, and 0.5 min at 95 °C, with a final extension step of 72 °C for 10 min. Agarose gel electrophoresis revealed a single band of ca. 1500 bases, which was confirmed by a comparison with DNA marker bands, with no sign of the presence of any other oligomer. The 16S rDNA of the PCR product was thermally denatured by heating at 95 °C for 6 min and subsequently cooled in an ice bath. The target DNA sample solutions for the electrochemical detection were prepared by adding phosphate buffer (pH 7.0) and NaCl solutions to the denatured PCR product. The concentration of the target DNA concentration in the sample solution was ca. 30–40 ppm, as determined by the UV absorbance for adenine residue at 260 nm.

2.3. Apparatus

Electrochemical measurements were performed using an electrochemical analyzer Model 623B (ALS, Japan) controlled by a PC. The three-electrode system consisted of the screen printed electrodes, prepared as described below, a platinum wire counter electrode (diameter: 1 mm) and an Ag/AgCl reference electrode (3 M-NaCl, Model RE-1; BAS), to which all potentials were referenced. All experiments were carried out in a glass cell containing 1.0–2.0 mL of the solution. The UV spectrum measurements were carried out using a spectrophotometer (UV260; Shimadzu, Japan).

2.4. Probe immobilization and voltammetry on the FISPE

C_{60} impregnated ink was prepared by mixing the carbon ink with a C_{60} benzene solution until the mixture became uniform. The 9:1 (volume ratio) mixture of the carbon ink and the 0–2 mM C_{60} benzene solution was then manually printed through a patterned screen (Nissha Printing Co., Ltd., Japan) onto a

50×100 mm PMMA plate, which has a good affinity for the carbon ink. A 1-mL benzene solution was always added to 9 mL carbon ink to adjust the volume ratio of benzene to carbon ink. Arrays of 10 FISPEs were thus printed and subsequently dried for 45 min in an oven (100 °C). After drying, an insulator layer of epoxy resin was spread on part of the printed carbon, leaving a clearly defined 3×4 mm working area and a similar area on the opposite side for electrical connection. A SPE printed with the carbon ink but no C_{60} was fabricated in a similar manner. The thickness of the FISPE in the dry state was ca. 20 μm. The new electrode strips were activated by a 3-s exposure to an air plasma, generated by a ST-7000 (Keyence, Japan), and were then immediately immersed into the probe DNA solution for 3 min in order to immobilize the probe onto the electrode surface. For the poly-G immobilization, the activated electrode was immersed into a 30 ppm poly-G solution (pH 7), and for the specific oligonucleotide, into a 1×10^{-6} M probe solution (pH 7) for 3 min. After immobilizing the probe DNA and rinsing with the buffer solution, hybridization-based sequence detection was accomplished in accordance with the procedures reported by Wang et al. [3,5,8,17,18,22,36–38]. The FISPE onto which the probe was immobilized was transferred to the stirred target DNA solution containing 0.2 M NaCl and 0.05 M phosphate buffer at a potential of +0.5 V, and the hybridization reaction was carried out at 55 °C for 15 min. After the hybridization, the electrode was rapidly cooled to below 10 °C by dipping it into a 0.05 M Tris–HCl buffer solution (pH 7.0) which was kept in an ice bath. The indicator, $Co(phen)_3^{3+}$, was accumulated into the surface hybrid when the electrode was immersed in the stirred 0.5×10^{-3} M $Co(phen)_3^{3+}$ solution of Tris–HCl buffer for 1 min while the potential was held at +0.5 V. Then the accumulated $Co(phen)_3^{3+}$ in the hybrid was measured by differential pulse voltammetry after the electrodes transferring to the Tris–HCl buffer solution (pH 7) not containing $Co(phen)_3^{3+}$. The parameters of the differential pulse voltammetry were as follows: pulse amplitude, 50 mV; pulse period, 20 ms; pulse width, 50 ms; increment potential, 4 mV; initial potential, +0.5 V and a negative scan was used. During the electrode transfer to the next solution, the surface was rinsed with a specific buffer solution. The background was measured on the air plasma activated FISPE (PA-FISPE) and the plain SPE for the corresponding blank solution without the target DNA, which means that the peak in the background voltammogram was the reduction current of $Co(phen)_3^{3+}$ adsorbed directly onto the electrode surface. Every electrochemical detection process was carried out in 0.02 M Tris–HCl (pH 7.0) at room temperature, except the hybridization and the cooling. Repetitive measurements were performed using the newly printed electrode strips.

3. Results and discussion

3.1. Immobilization of poly-G onto the PA-FISPE

We found that DNA was immobilized onto the air plasma activated SPE surface. The air plasma caused the oxidation of the carbon ink electrode material, and the exposure period was a very important factor in the immobilization of DNA. When the FISPE was exposed to the air plasma for a long period, such as 5 s, the electrode ink became strongly oxidized and the edges of the electrode partially disappeared. But a short exposure period, such as 1 s, did not ensure sufficient surface oxidation for DNA immobilization. So we employed a 3 s exposure period. We considered that the air plasma roughened the FISPE surface through oxidation and thereby changed the effective surface area of the FISPE. The effect of plasma exposure on the electroactive surface area of the SPE was estimated from the reduction currents (E_p^c: ca. 0.2 V) on the differential pulse voltammograms of potassium ferricyanide (0.5 mM in a 0.1 M-KNO_3 solution). The 3-s plasma exposure increased the reduction current of ferricyanide by 10–20% compared to that without plasma pretreatment. So the increment of the FISPE surface area seemed to be 10–20% by the activation.

The FISPE was fabricated by using the ink mixed with a 0–2 mM fullerene solution; then, the electrode was activated and the poly-G was immobilized. The voltammograms at the poly-G modified electrodes were shown in Fig. 1. The poly-G modified electrode gave an oxidation peak of guanine residue at around 0.9 V. As the content of C_{60} in the electrode increased, the oxidation peak current became higher (Fig. 2). But, the oxidation current did not indicate the tendency to level off, when 2 mM C_{60} solution was added to carbon ink. In order to increase the content of C_{60} in a FISPE, it is necessary to add the higher concentration C_{60} benzene solution or increase the volume of that. But, it was difficult to prepare in short time a C_{60} solution whose concentration exceeded 2 mM. Also, the electrochemical characteristics of the FISPE depended on the benzene volume added as the solvent for C_{60}. When the benzene content in the ink was increased, the voltammetric peak was distorted. The effect of the benzene on the electrode reaction was small at the FISPE prepared with the 9:1 mixture of carbon ink and C_{60}

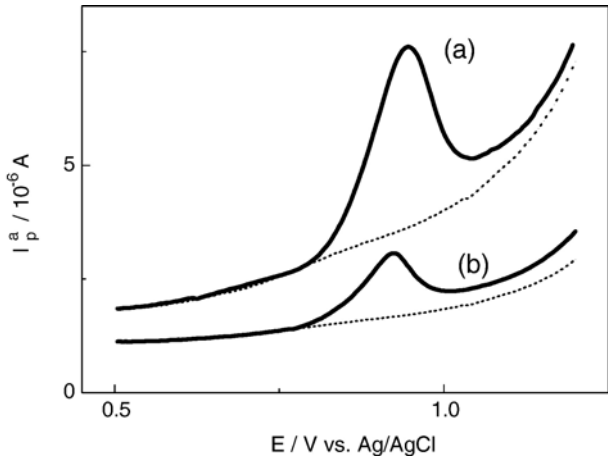

Fig. 1. Differential pulse voltammograms of the poly-G immobilized onto air plasma activated FISPE (a), the electrochemically activated SPE (b). After the FISPE activation by a 3-s air plasma exposure or SPE activation by electrochemical pretreatment for 1 min at 1.7 V, poly-G immobilization was performed in a 30 ppm ply-G solution (pH 7 phosphate buffer) for 3 min. The voltammograms were recorded in the 0.02 M Tris–HCl buffer (pH 7.0), after rinsing the poly-G modified electrode. The dotted lines were background currents at each electrodes.

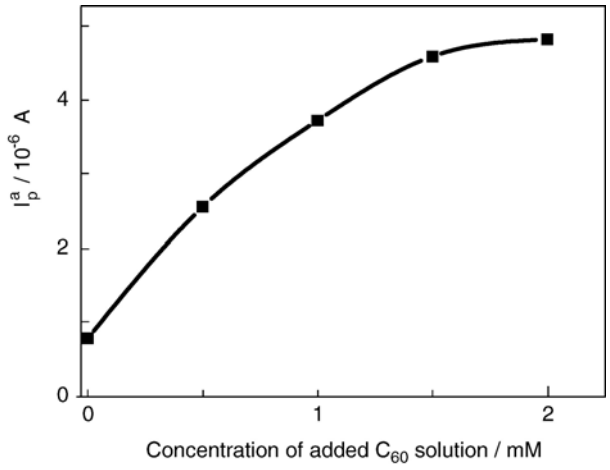

Fig. 2. Dependence of oxidation peak currents for guanine residues at FISPE on the concentration of C_{60} solution added to screen printing ink. FISPE was fabricated by printing the 9:1 (volume ratio) mixture of the carbon ink and the 0–2 mM C_{60} benzene solution onto the substrate. The poly-G immobilization procedures and other conditions were same as those in Fig. 1.

solution. In this study, only FISPE printed with a 9:1 mixture of carbon ink and a 2 mM C_{60} solution was subjected to voltammetry. The residual current at the PA-FISPE became higher than that at the electrochemical activated SPE, especially in the positive potential range (dotted lines in Fig. 1). This current increase was caused by not only an increase in the surface area but also surface modification with air plasma exposure. The oxidation peak current at the poly-G immobilized PA-FISPE was about three times that at the plain SPE immobilized ply-G by the electrochemical activation. Mixing of the C_{60} into the carbon ink and the subsequent plasma exposure contributed to the increase in the amount of poly-G immobilized onto the electrode surface. Electrochemical activation of the C_{60}-impregnated SPE did not improve the immobilization of poly-G. Probably C_{60} was not oxidized electrochemically in the activation step, because C_{60} in organic solvent shows oxidation peak around 2 V vs. Ag^+/Ag electrode [43]. When the immobilization of ply-G on SPE was carried out by an air plasma activation (in other words, the FISPE added 0 mM C_{60} solution; the plotting in the left end of Fig. 2), the oxidation peak of guanine residue was smaller than that at the poly-G modified SPE activated electrochemically by 30–40% (see Figs. 1 and 2). We considered that since the air plasma activation led the surface of carbon ink to oxidize more strongly than the electrochemical activation, the amount of poly-G immobilized by an air plasma activation was decreased. So, poly-G was not attached onto C_{60} molecules, but C_{60} played the important role in the oxidative activation step. C_{60} moderately suppressed the oxidation reaction of carbon ink by air plasma and gave the oxidative condition suitable for adsorption of poly-G onto the oxidized carbon ink surface.

3.2. Relationship between air plasma activation and the hybridization response at the FISPE

The intensity of hybridization responses at the DNA modified electrodes was affected by the amount of DNA immobilized onto the electrode surface. The effectiveness of the activation method was verified by detecting 16S rDNA of *E. coli* using the electrodes onto which the perfectly matching probe A was immobilized. The differential voltammograms of $Co(phen)_3^{3+}$ accumulated in the hybrid are displayed in Fig. 3. The indicator response on the PA-FISPE was better than that on the electrochemically activated SPE. The reduction peak current increased by 150%, although the surface area of the PA-FISPE increased only by ca. 10–20% (as mentioned above). We attributed this improvement of the double stranded DNA response to the addition of C_{60} to the carbon ink and the air plasma activation. Although the exact role of C_{60} in this phenomenon is not fully understood, the plasma oxidation of the electrode surface is clearly important for the immobilization of the probe DNA. The PA-FISPE gave a well-defined reduction peak of $Co(phen)_3^{3+}$ accumulated in the formed hybrid, and the signal was 2.5 times stronger than that given by the electrochemically activated SPE. As the peak current of $Co(phen)_3^{3+}$ reflects the amount of the indicator accumulated in the hybrid, we think that the probe DNA was immobilized onto the PA-FISPE surface in a high concentration.

3.3. Detection at the PA-FISPE of 16S rDNA extracted from E. coli

In addition to the accumulation of oligo-DNA onto the PA-FISPE, it was also mentioned that 16S rDNA, extracted from *E. coli* and amplified by PCR, was directly detected using the PA-FISPEs onto which probe DNA was immobilized. In most cases

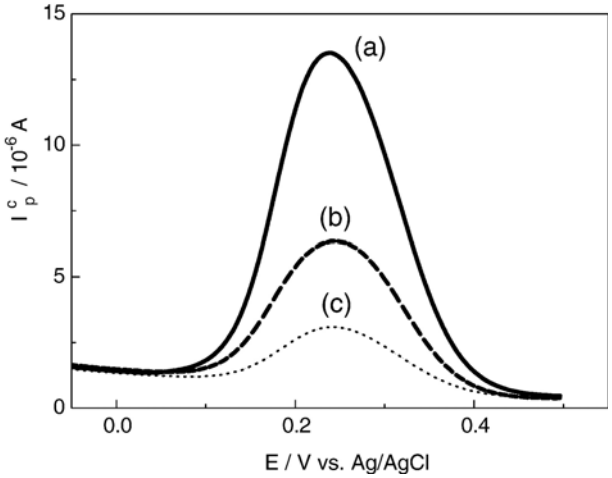

Fig. 3. Effect of the probe immobilization method and fullerene on the reduction peak of $Co(phen)_3^{3+}$ accumulated in the hybrid between the 16S rDNA extracted from *E. coli* and probe A on the electrode. Solid line (a): the hybridization signal at the PA-FISPE onto which probe A was immobilized; broken line (b): at the SPE onto which probe A was immobilized by electrochemical activation in acetate buffer (pH 5), dotted line (c): background. The target solution was prepared by adding 0.05 M phosphate buffer (pH 7) and 0.2 M NaCl to the PCR product. The hybridization step was carried out at 55 °C for 15 min and the other steps were carried out at room temperature. The indicator accumulation in the hybrid was performed in the stirred 0.5 M $Co(phen)_3^{3+}$ solution of Tris–HCl buffer (pH 7) and the differential pulse voltammograms were measured in the Tris–HCl buffer solution after electrodes transfer.

of electrochemical sensing of DNA hybridizations, the target DNAs are less than 100 bases long [37], but we attempted to detect 16S rDNAs with much longer sequences. Fig. 4 shows the dependence of the indicator peak currents on the sequences of the probe oligonucleotides immobilized onto the electrode. The typical electrochemical responses at the electrodes modified with probe A (perfect match for a fragment of the *Escherichia coli str.* 16S rDNA), probe B (two base mismatches) and probe D (five base mismatch) were presented. The hybridization step was performed in the solution, which was prepared from adding the buffer and NaCl to the PCR product. After hybridization at 55 °C and accumulation of the indicator into the hybrid, the reduction peak current of $Co(phen)_3^{3+}$ was significantly enhanced on the probe A modified electrode, but the hybridization responses on the electrodes modified with probes B, and D, did not increase much, remaining close to the background current. This phenomenon was due to the indicator accumulation in the hybrid between the target and the probe. By means of the signal differences between the perfect matching probe and the mismatching ones, the target DNA can be detected with the modified electrodes (Fig. 4). It became obvious that PA-FISPE onto which probe A was immobilized was able to detect 16S rDNA extracted from *E. coli str.* without pretreatment of the PCR product. The sample solution was prepared by denaturing the double stranded DNA, given as the PCR product of *E. coli str.* DNA, and contained two kinds of DNA with mutually complementary base sequences at the same concentration. But no interference was observed at the hybridization and detection steps. When the hybridization step was performed at room temperature, we could not detect signal differences between the perfectly matching probe and the others. As the melting points of the four probes used in this study were 65–73 °C, it is considered that a hybridization temperature higher than 55 °C is suitable. But high solution temperatures led to increased surface roughness and to an increase in the noise level of the voltammograms. For the best compromise between selectivity and sensitivity, we employed the following conditions in this work: hybridization at 55 °C for 15 min.

Table 1
Hybridization responses for the *Escherichia coli* (JCM1649) 16S rDNA on the FISPE and SPE onto which various probes were immobilized

Hybridization response (ΔI)/μA (signal change: $\Delta I/\Delta I_P$)					
Probe	Probe A	Probe B	Probe C	Probe D	Probe E
Plasma activated FISPE	9.23	1.68 (0.18)	1.34 (0.15)	1.24 (0.13)	9.04 (0.98)
Electrochemically	3.69	2.06 (0.56)	0.53 (0.14)	0.25 (0.07)	3.47 (0.94)

After immobilizing various probes onto the electrode, it was immersed into the target solution for the 15-min hybridization process and then dipped into the indicator solution for 1 min, followed by the voltammetric measurement in the Tris–HCl buffer after the medium exchange. Probe-A has a sequence which perfectly matches a fragment of the *E. coli* 16S rDNA, probe E perfectly matches another fragment, probe B has two base mismatches, probe C has three base mismatches and probe-D has five base mismatches. ΔI is the hybridization response, and ΔI_P is that for the electrode onto which probe A was immobilized. The sequences of the probes are presented in the Experimental section of the article.

Table 1 shows an assessment of the selectivity of the PA-FISPE and the electrochemically activated SPE as DNA sensors. The hybridization responses ($\Delta I = I_{red} - I_B$; the background current (I_B) is subtracted from the reduction current (I_{red})) and the signal changes ($\Delta I/\Delta I_P$; the ratio of ΔI, the response for the mismatching probe, to ΔI_P, the response for probe A) were calculated from the $Co(phen)_3^{3+}$ reduction currents at the electrodes modified with probes A, B, C, D and E after hybridization with the target DNA. The probe A modified PA-FISPE yielded a signal 2.5 times larger than the

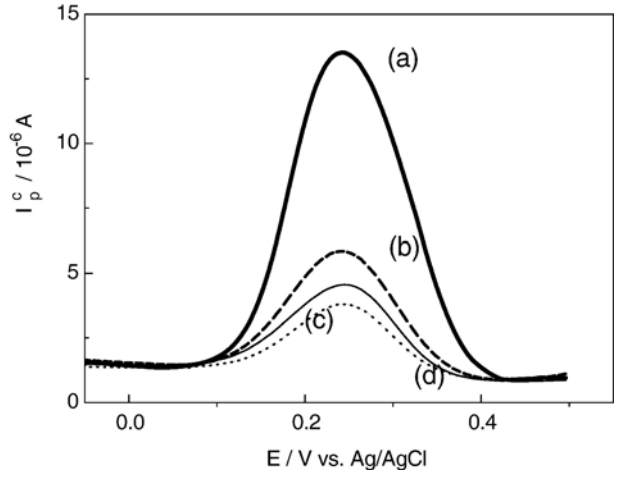

Fig. 4. The hybridization responses for the target DNA at the PA-FISPE onto which probe A was immobilized: the thick solid line (a); probe-B: broken line (b); probe-D: thin solid line (c) and background: dotted line (d). Probe A was a perfect match for a fragment of the target 16S rDNA of *E. coli*, probe B had two base mismatches and probe-D five base mismatches. The sequences of the probes are presented in the Experimental section of the article. The other conditions were same as those for Fig. 3.

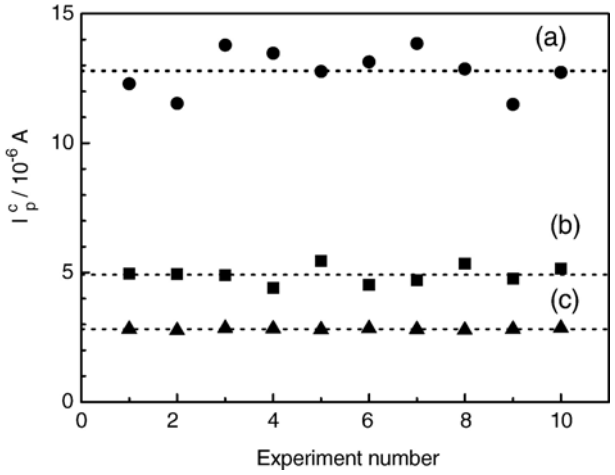

Fig. 5. The repeatability of the hybridization responses for the 16S rDNA at the PA-FISPE onto which probe A (a, ●) and probe-B (b, ■) were immobilized. Each measurement was performed on the new PA-FISPE modified probe and other experimental conditions and procedures were the same as those for Fig. 3. The dashed lines represent the averages of the responses and the triangles (c, ▲) denote the background currents.

electrochemically activated SPE. All probes, except probe B, gave signals, and the signal changes at the PA-FISPE surpassed those at the SPE; this phenomenon was caused by the increase in the quantity of the probe molecules (mentioned above). Also, the PA-FISPE gave better results than the SPE with respect to the selectivity for mismatches between the target DNA and the probe. Although the PA-FISPE and SPE modified with probe C and probe D showed a similar tendency, there was a large difference between the signal changes observed at the PA-FISPE onto which probe B (two base mismatches) was immobilized and the SPE modified with the same probe. The PA-FISPE was more sensitive to and selective for the DNA sequence with two base mismatches. It seems that the high concentration of probe DNA modified onto electrodes enabled not only a high sensitivity but also a high selectivity. The hybridization responses at the PA-FISPE onto which probe E was immobilized and at the SPE were almost equal to those measured at the probe A modified electrode. This result suggests that the indicator reduction current was independent of the 16S rDNA fragment where the perfectly matching probe might hybridize. In other words, it is easy to design the proper probe sequence because the exact location of the unique region in the target sequence does not have to be determined.

The repeatability of the signals at the newly fabricated PA-FISPE is shown in Fig. 5. The relative standard deviations of the indicator reduction currents were 7.1% for the probe A modified PA-FISPE, 6.5% for probe B modified PA-FISPE and 4.2% for the background. Since the differences in the signal changes between the perfectly matching probes and the mismatching ones were much bigger than the relative standard deviation, it was possible to confirm the existence of the target DNA in the sample solution clearly and easily. So, using a PA-FISPE onto which the proper probe DNA was immobilized, the PCR product of the *E. coli* 16S rDNA could be detected electrochemically.

4. Conclusions

In this paper, we described a new method of electrochemically detecting 16S rDNA amplified from the nucleic acid extracted from *E. coli*. The detection of long DNA sequences, such as that of 16S rDNA, is more difficult than that of short DNA sequences. But 16s rDNA is often used to identify microbial species. In phylogenetic studies of pure cultures, the analysis of microbial DNA is performed using gel electrophoresis, capillary electrophoresis and other electrophoresis techniques. However we believe that the electrochemical detection method described in this paper is very attractive for analyzing the DNA of mixed bacterial species. Although the exact role of C_{60} remains unclear, we speculate that since the amounts of the probe DNAs immobilized onto the PA-FIPSE increased when fullerene was impregnated into the electrode carbon ink and the electrode was activated with air plasma, the selectivity for the target DNA was drastically improved by this procedure. Plasma oxidation is performed in dry conditions, and electrode activation is more effective and faster in dry than the wet conditions. We believe that the present work represents a significant step toward the fast and easy identification of microbial species. A separate report on the identification of various *E. coli* species based on this method will be published elsewhere in the near future.

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