

Electropolymerization of Pyrrole Applied to Biosystem

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Abstract. We have been investigating electropolymerization of pyrrole in aqueous electrolyte solutions in acidic as well as in neutral conditions by *in situ* electrochemical quartz crystal oscillator method, where resonant frequency and resonant resistance can be monitored simultaneously with current-voltage measurements during electropolymerization of pyrrole. The properties of thin PPy films prepared on electrode surfaces depended strongly on the experimental variables of electrode potentials applied, solution pH, kinds and quantity of supporting electrolytes, added chemicals, and the mode of electrochemical method employed. We are applying our experience gained on electropolymerization of pyrrole to immobilizing biomolecules onto electrode surfaces to develop a biosensor system. In this work, we wish to present the results on electrochemical monitoring on electropolymerization of pyrrole in the presence of DNA and albumin in different electrochemical conditions. Additionally we will summarize our investigations on the miniaturization of biomolecules/PPy composites by means of scanning tunneling microscopy.

Key words : Polypyrrole, DNA, Albumin, EQCO, STM

1. Introduction

Since the first successful demonstration of electrochemically preparing coherent polypyrrole (PPy) films on the electrode surface and of lifting the highly conducting material from it was reported in 1979¹⁾, a large number of works were performed to understand the electrochemical growth of PPy on conducting substrates in aqueous as well as in nonaqueous solutions^{2,3)}. As pyrrole can be dissolved in water, PPy and its derivatives are most widely used for the entrapment of polyanion such as bio elements (DNA, enzymes, proteins etc.) in aqueous solutions. Recently, these conducting polymers have been successfully used for the immobilization of biological elements during the electropolymerization process or by covalent binding of biological component at the functionalized polymer network. Electrochemical immobilization within conducting polymers provides a promising alternative for the one-step loading of biological elements over a small and defined area of the transducer element^{4,5)}. In the past two decades, PPy has attracted much attention, mainly because of its relatively high environmental stability⁶⁾, electrical properties⁷⁾, ion-exchange⁸⁾ and its biocompatibility⁹⁾. PPy has unique properties such as redox chemistry and conductivity that make it suitable for the design of novel biosensors¹⁰⁾. So much, most research with PPy has tended to be directed toward technological applications for biosystem, but physical, chemical and microrheological property of PPy has been

largely neglected. Actually, it is very important that understanding of microrheological property of PPy. Because of, often, electrochemically-induced deposition of conducting polymer films on electrode surfaces, the properties of thin PPy films prepared on electrode surfaces depended strongly on the experimental variables of electrode potentials applied, solution pH, kinds and quantity of supporting electrolytes, added chemicals, and the mode of electrochemical method employed¹¹⁻¹³⁾. Understanding of microrheological property of film, help forward that the film thickness and morphology of the coatings could be adjusted by controlling the reaction conditions. Furthermore, we may be able to adjust the chemical composition, morphology, electronic transport, and bioactivity of polymer coatings on electrode surfaces on a multichannel micromachined analytical capture probe by controlling electrochemical deposition conditions.

We have been interested in investigating changes in microrheological property of PPy during electrochemical growth. *In situ* electrochemical quartz crystal oscillator (EQCO) method could be successfully applied to monitor films developed at the interface between the electrode and electrolyte solutions, when PPy grew homogeneously on the substrate electrode^{14,15)}. In a previous work, we reported that the viscoelastic behavior of PPy film prepared in various aqueous solutions was fundamentally different depending the experimental variables employed and it significantly varied as the film thickness increased^{13,16)}. We also reported that electropolymerization of pyrrole in the aqueous electrolyte solutions of potassium chloride (with 30 mM PB, pH 7.0) was investi-

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gated by means of chronoamperometry and that PPy in potassium chloride electrolyte buffer solution (neutral pH) was less homogeneous and more elastic than PPy in low pH aqueous solution¹⁴. Furthermore, we were able to control the electrochemical growth of PPy to produce PPy patterns on gold electrode surface in the aqueous electrolyte solutions of potassium chloride, and the size of each dot and of triangle of dots can be controlled by change in electropolymerization time and mechanical movement, respectively. Thus PPy film dots, also grew elastic¹⁷. PPy did not grow homogeneous in certain conditions, because the film viscoelasticity strongly depended on the electropolymerization potential. Because structure and morphology of conducting polymers are known to be strongly correlated with the intrinsic electronic properties of polymers we undertook the microrheological studies of PPy during electropolymerization in aqueous electrolyte solutions and the temperature dependence studies of the four-probe resistivity with the electrochemically grown films¹⁸. We are applying our experience gained on electropolymerization of pyrrole to immobilizing biomolecules onto electrode surfaces to develop a biosensor system. PPy polymerization is a radical coupling and counter ion doping process. Electrostatic interactions were found to be the driving forces for the DNA-PPy interactions since DNA is negatively charged and PPy positively charged. Albumin, a common protein used, has the pI of 4.9-5.1, and as such it is negatively charged at neutral pH. There are other contributions to protein adsorption and protein-protein interaction or protein folding, such as those due to the van der Waals forces. PPy generally behaves as a strong Lewis acid and PPy-PPy strongly interacts in water, which leads to that of protein adsorption to albumin¹⁹. PPy-protein hydrophobic and electrostatic interactions contribute to more protein stability with PPy networks. Using in situ EQCO methods for PPy film should be adopted to be investigated in various experimental conditions to adjust microrheological properties of the PPy films. With current emphasis on the miniaturization and mass production of biosystem, the efficient and reproducible immobilization of biological elements is regarded as one of the most important development. In this work, we wish to present the results on electrochemical monitoring on electropolymerization of pyrrole in the presence of DNA and albumin in different electrochemical conditions. Additionally, we will summarize our investigations on the miniaturization of biomolecules/PPy composites by means of scanning tunneling microscopy.

2. EXPERIMENTAL

2.1. Reagents and standard solutions

All chemicals used in this work were of the best quality available from Aldrich and were used without further purification except pyrrole, which was purified by distilling under reduced pressure to produce colorless liquid. Solutions were made up with Milli-Q grade water. Also obtained from Sigma were freeze-dried human serum albumin (HSA), and bovine serum albumin (BSA). Freeze-dried DNA was purchased from Fluka.

2.2. Instrumentation

The EQCO instrumentation used in this work consisted of an EG&G model 283A potentiostat, which was coupled to a Seiko EG&G QCA 917 quartz crystal analyzer. The working electrode consisted of 0.196 cm² gold (3000Å) sputtered onto a titanium layer (500Å) on a quartz crystal and a frequency of 9 MHz AT-Cut crystal. Ag|AgCl(saturated KCl) and Pt were used as the reference and counter electrodes respectively. Each gold (working electrode) surface was pre-cleaned by immersion in piranha solution (3:1 v/v concentrated sulfuric acid: 30% hydrogen peroxide). The gold surface was then thoroughly rinsed with deionized water and subsequently dried under a gentle stream of argon.

2.3. Preparation of DNA or Albumin-containing PPy electrodes

PPy was grown by applying the constant potential applied to the electrode between +650 and +700 mV versus Ag|AgCl electrode in aqueous solutions of 0.025-0.2 M pyrrole, and 0.1 M potassium chloride unless described otherwise. To form biomolecule/PPy composites, 100~1000 ppm DNA or 100~300 ppm albumin was employed. The presented data were obtained independent of the cell location within the cell, at the bottom or side. All the experiments were performed under argon atmosphere at 23±2°C.

3. RESULTS AND DISCUSSION

3.1. Incorporated DNA in PPy

PPy films were grown potentiostatically at +650 mV vs. Ag|AgCl in aqueous solution by chronoamperometry.

In our experimental conditions, a quartz resonant frequency change of 1 Hz is equivalent to approximately 1.09 ng by Sauerbrey equation. Pyrrole was polymerized on quartz crystal Au electrode in the presence and absence of DNA (1000 ppm) solutions through chronoamperometry in various concentrations of pyrrole. Experimental observations thus far include that current-voltage response of electropolymerization of pyrrole was nearly the same in the presence and absence of DNA but that changes in both resonant frequency as well as resonant resistance during electropolymerization of pyrrole in the presence of DNA deviated from those observed in the absence of DNA. Figure 1 shows changes in resonant frequency and in resonant resistance simultaneously observed in different concentrations of pyrrole, 0.1 M KCl, and in the absence and presence of DNA for the chronoamperometric experiments at 0.65 V versus Ag|AgCl, where change in resonant frequency was set to be monitored up to 8000 Hz. The dependence of the rate of electropolymerization on monomer concentration can be seen from the slope of the resonant frequency versus time plot during electropolymerization. The deposition of PPy depended on pyrrole concentration and dopant as DNA. It can be seen that the timescale of the resonant frequencies decrease and the magnitudes of currents increase as : (a) the concentration of pyrrole is increased (b) the presence of DNA is made more rapid. Figure 1(a) shows that the resonant frequency decreased constantly in the first

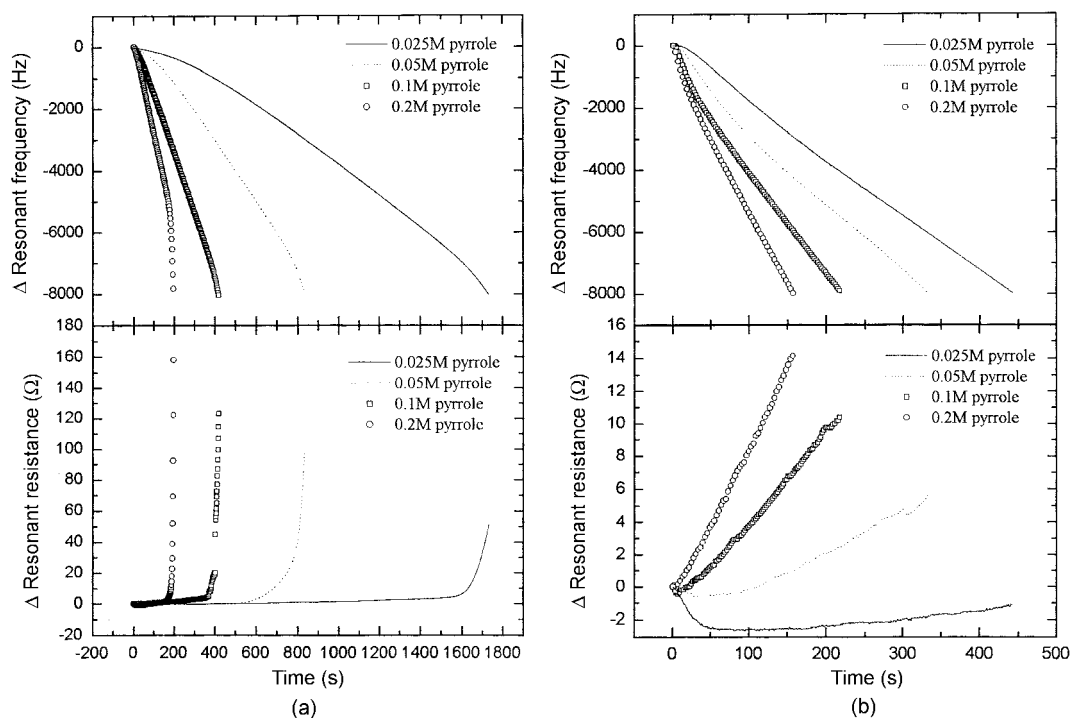


Fig. 1. Changes in resonant frequency and resonant resistance during chronoamperometric experiments at 0.65 V vs. Ag|AgCl in different concentration of Pyrrole and 0.1 M KCl. (a) in absence of DNA, (b) in presence of DNA(1000 ppm)

stage and decreased more rapidly after it. But Figure 1(b) shows the resonant frequency decreased almost constantly. The resonant resistance increased according to the decrease of the resonant frequency. The resonant resistance showed a rapid increase simultaneously with the steep frequency decrease. It reveals the change of PPy film characteristic in resonant frequency and resonant resistance. This shows the increase in the viscoelastic property of the film. In the case of a viscoelastic film deposition (Figure 1(a)), the resonant frequency change with film deposition reflects the mass increase, and also the resonant resistance increases with film deposition reflecting the viscosity of the film. According to the results shown in Figure 1, PPy films prepared in the presence of DNA is more elastic, while those in the absence of DNA is more viscoelastic films.

We also tried cyclic voltammetry with the above films to investigate the ion exchange properties of the film in electrolyte solutions. After the deposition of the PPy and PPy/ DNA electrodes on Au quartz electrode surfaces, the cell containing the polymer electrode was rinsed with copious amounts of distilled water, and the electrolyte solution of pyrrole monomer was replaced with 0.1 M KCl only. Figure 2 shows changes in resonant frequency and in resonant resistance and the current response of a 8000 Hz PPy film during sweeping the potential applied to the electrode from -500 to +300 mV versus Ag|AgCl electrode in 0.1 M KCl. PPy films prepared in the absence of DNA(Figure 2(a)) shows anion transport (Cl^-). A decrease of resonant frequency occurs commencing at -0.2 V. The resonant frequency decrease is identified as a net mass gain resulting from incorporation of anions(Cl^-) into the PPy film. On the contrary, PPy films prepared in the presence of

DNA (Figure 2(b)) shows cation transport (K^+). An increase in resonant frequency occurs commencing at -0.4 V, when the potential was scanned from -0.5 ~ +0.3 V. The initial frequency increase, corresponding to a mass loss in the film, is believed to be due to the expulsion of cations (K^+). This significant difference in ion transport behavior of these polymers reveals that the electrochemical properties of these polymers are affected by dopant ions (DNA anion versus Cl^-). This observation can be explained by the concept of electroneutrality coupling and entrapment of DNA size effect. The associated movement of anions into the PPy demonstrates the principle of the electroneutrality coupling, which requires a flux of ions into or out of the polymer film to provide internal charge compensation. PPy films differ with respect to the extent of oxidation, doping anions, and also reduction, doping cations. Such properties can be switched dynamically between anion and cation coupling or hopping through electrochemical oxidation and reduction of the PPy backbone²⁰.

We observed changes in current and passed charge during chronoamperometric experiments at 0.65 V vs. Ag|AgCl in different concentration of pyrrole and 0.1 M KCl in the presence and absence of DNA to make a comparison. Total charge value in the presence of DNA was smaller than in the absence of DNA. This represents that DNA act on dopant ion as large molecular mass during electropolymerization of pyrrole. And, since the DNA anions have a hydrophobic property, PPy films were well deposited on electrode surfaces. Consequently, PPy films prepared in the presence of DNA has smaller total charge value than in the presence of DNA.

We also used four-probe method investigated PPy/DNA conductivity. An experimental procedure was the same as

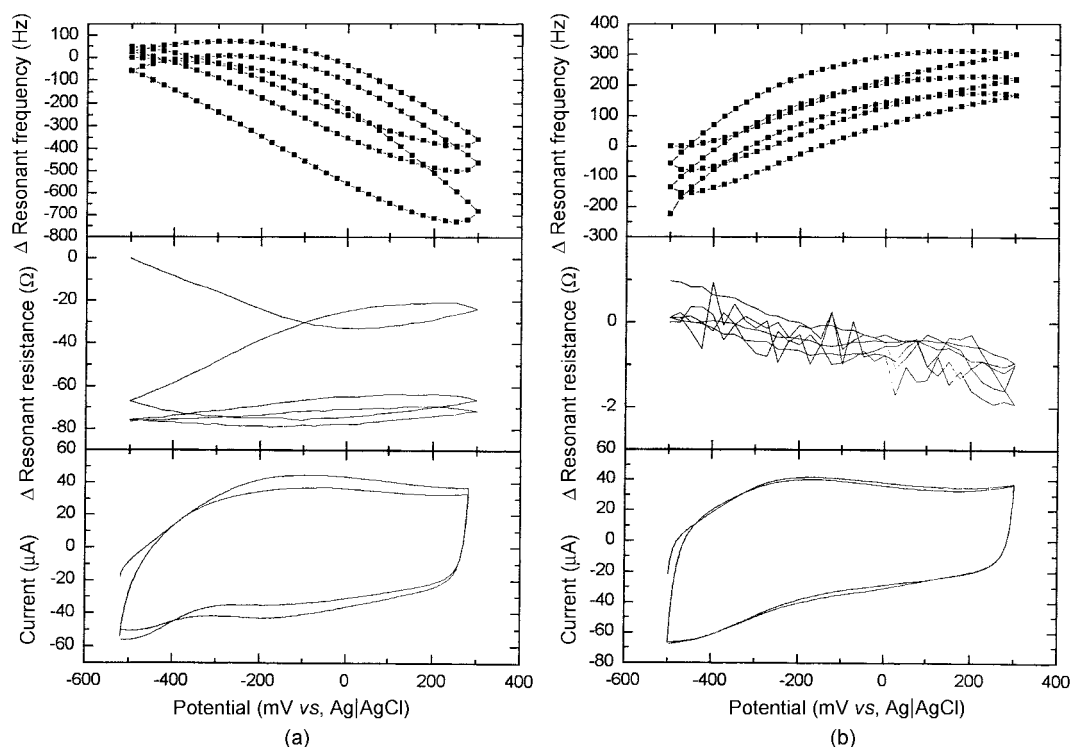


Fig. 2. Changes in resonant frequency, resonant resistance, and current response observed during potential cycling of polypyrrole electrode in aqueous solution of 0.1 M KCl. Scan rate : 25 mV/s (a) prepared in 0.2 M pyrrole, 0.1 M KCl (b) prepared in 0.2 M pyrrole, 0.1 M KCl, and DNA

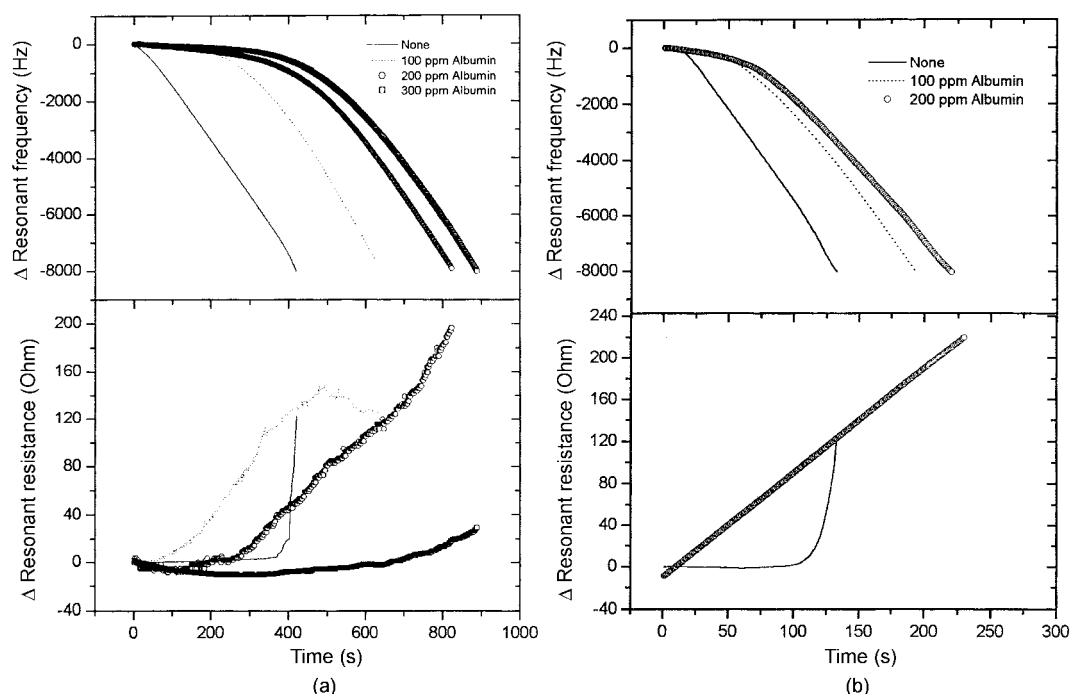


Fig. 3. (a) Changes in resonant frequency and resonant resistance during chronoamperometric experiments at 0.65 V vs. Ag/AgCl in 0.1 M pyrrole and 0.1 M KCl with different concentration of protein (0, 100, 200, 300 ppm human serum albumin). (b) Changes in resonant frequency and resonant resistance during chronoamperometric experiments at 0.7 V vs. Ag/AgCl in 0.2 M pyrrole and 0.1 M KCl with different concentration of protein (0, 100, 200 ppm human serum albumin).

was described previously¹⁷⁾. The results showed that PPy films prepared in the presence of DNA had a lower electrical

conductivity than those in the absence of DNA. This maybe due to neutralize PPy film charges using the different number

of anions. It shows that DNA acts as a dopant anion in incorporating polymerization, and in PPy entrapment of host anion. Electroactive PPy film enables the incorporation of a wide variety of counter ions into the polymer. Thus, the conductivity and viscoelasticity of PPy may be made different by doping with variable anions and PPy¹⁷⁾.

3.2. Incorporated Protein (albumin) in PPy

Electropolymerization is a simple and attractive technique to obtain well-adhering PPy films on the anode surface either by a galvanostatic, potentiostatic, multi-sweep or pulse technique deposition procedure used for entrapment of biomolecules⁴⁾. In this investigation the potentiostatic mode was used for protein/PPy film and microrheological properties of protein/PPy were probed during electropolymerization in various aqueous electrolyte solutions. Figure 3 shows changes in resonant frequency and resonant resistance during electrochemical growth of PPy at different potential, 0.65 and 0.7 V vs. Ag/AgCl applied in aqueous solution of 0.1~0.2 M pyrrole and 0.1 M KCl aqueous solution in the presence of different albumin concentration. In the presence of albumin, the PPy films were viscoelastic and showed a slow growth rate during polymerization. Then protein/PPy films grew more viscoelastic on anode surfaces. Also, the protein/PPy growth rate is dependent with the potential applied to anode. In previous works we reported about electropolymerization PPy in neutral aqueous solution. Thus the electropolymerization potential

appears to be an important experimental variable in influencing morphological property of PPy during electropolymerization, as was observed in neutral aqueous solutions¹³⁾. In this study, we work biomolecules/PPy in neutral aqueous solutions and biological buffer electrolyte. Figure 4 shows resistance change plotted against frequency change for various PPy films prepared during electropolymerization. Before potentiostatic electropolymerization of protein/PPy, we studied EQCO response during electropolymerization and the entrapment of protein by using cyclic voltammetry (data not shown), and we found that the protein inhibited pyrrole nucleation step of polymerization. Figure 4(a) shows EQCO results obtained during electropolymerization of pyrrole in the presence of albumin; as albumin concentration increased, initial resistance in electropolymerization increased with a delay in decrease in resonant frequency and also film viscoelasticity increased. Figure 4(b) shows influence of aqueous solution for PPy growth by potentiostatic electropolymerization. Local changes of the pH value as a result of the liberation of protons during the polymerization reaction have to be taken into account, and then situation changes bring the confirmation change of protein. In this situation, it seems that hydrophobic interaction PPy-Protein is van der Waals force rather than Lewis acid-base type. DNA and albumin produce the different results in forming biomolecule/PPy composites and main reason may be in the difference of driving force during electropolymerization¹⁹⁾.

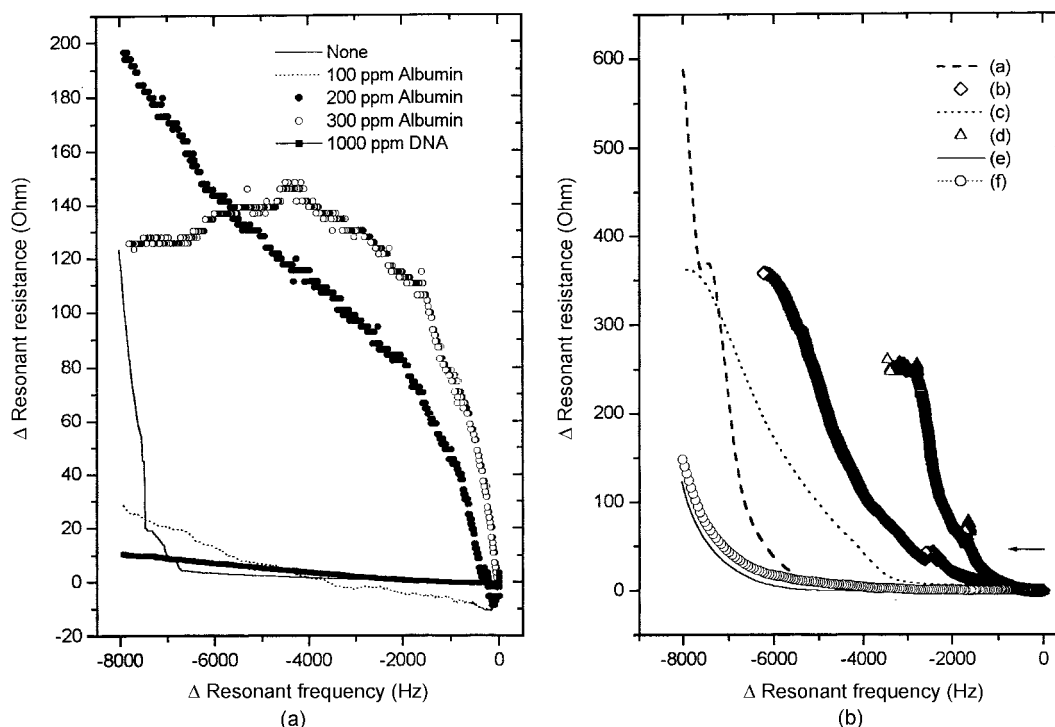


Fig. 4. Resistance change plotted against frequency change for various prepared PPy films on Au quartz crystal working electrode (a) PPy films were grown chronoamperometric experiments at 0.65 V vs. Ag/AgCl in 0.1 M Pyrrole and 0.1 M KCl with different concentration of protein (0, 100, 200, 300 ppm HSA, 1000ppm DNA). (b) PPy films were grown chronoamperometric experiments at 0.7 V vs. Ag/AgCl in 0.2 M Pyrrole and variable neutral aqueous solutions with different concentration of protein. (a) in 0.1 M NaCl, (b) 500ppm BSA and 0.1 M KCl contain 30 mM PB, (c) in 10 mM PBS contain 140 mM NaCl, (d) 500 ppm BSA and 10 mM PBS contain 140 mM NaCl, (e) in 0.1 M KCl, (f) 200 ppm BSA and 0.1 M KCl

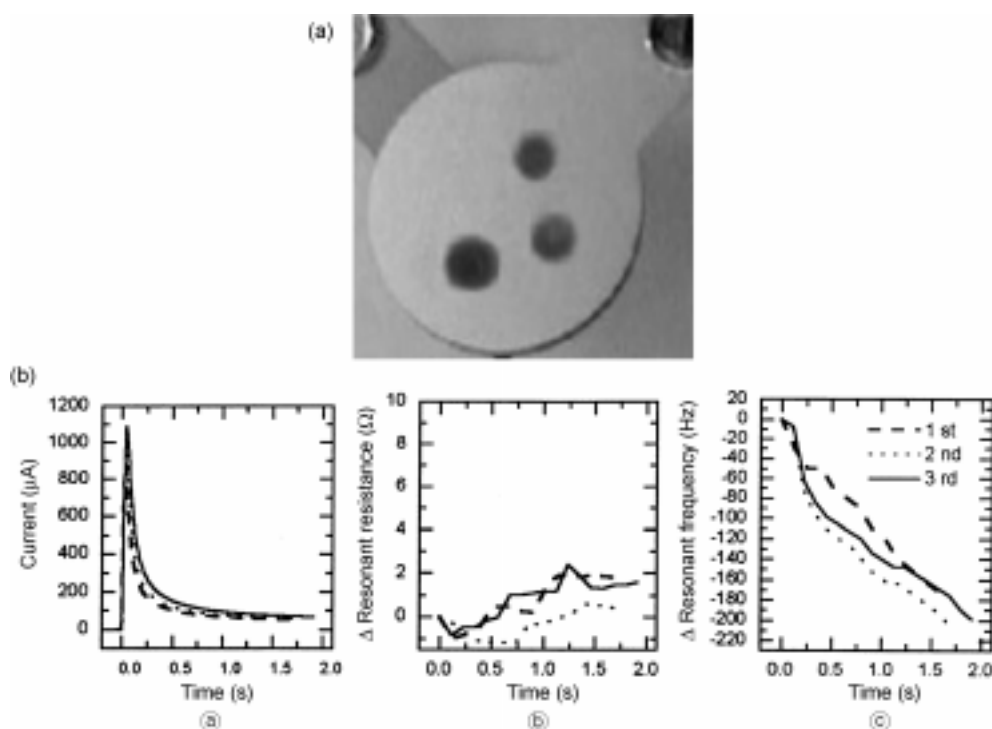


Fig. 5. (a). *Ex situ* optical microscopic images of DNA/PPy patterns of three dots formed. (1st dot : 0.32, 2nd dot: 0.30, and 3rd dot: 0.39 mm formed) (b). Chronoamperometry curves (a) and changes in resonant resistance (b) and resonant frequency (c) observed during electropolymerization of pyrrole at 0.65 V vs. Ag/AgCl

3.4. The miniaturization of biomolecules/PPy composites and localized pattern control

In a previous work, we reported that we were able to control the electrochemical growth of PPy to produce PPy patterns on gold electrode surface¹⁷⁾. And now, we are able to control the electrochemical growth DNA/PPy pattern. DNA/PPy grew on bare gold electrode surface during electropolymerization of pyrrole at +0.65 V versus SCE in the aqueous solution of 0.2 M pyrrole, 1000 ppm DNA, 0.1 M potassium chloride, and 0.03 M phosphate buffer solution at pH 7. The deposition of DNA/PPy was strongly dependent on the shape and location of counter electrode, potential imposed and the concentration of pyrrole and supporting electrolyte.

In all three experiments, chronoamperometric curves have similar features with the maximum current near 0.1 second after the potential applied. The rising and decaying transients are those expected for the nucleation and growth of a new conducting phase on an inert electrode surface. Resonant frequency decreased (about 200 Hz) monotonically with little change in resonant resistance, suggesting that DNA/PPy film grew elastic. The dots of DNA/PPy films formed in the above three chronoamperometric experiments are shown in Figure 5. All the optical microscopic images were taken *ex situ*. PPy films prepared in the presence of DNA is more elastic, compared to previous work in the absence of DNA.

For more miniaturization of biomolecules/PPy patterns on electrode surfaces, EC-STM(Electrochemical Scanning Tunneling Microscopy) was used to control the electrochemical growth of PPy to produce PPy nano patterns on electroactive

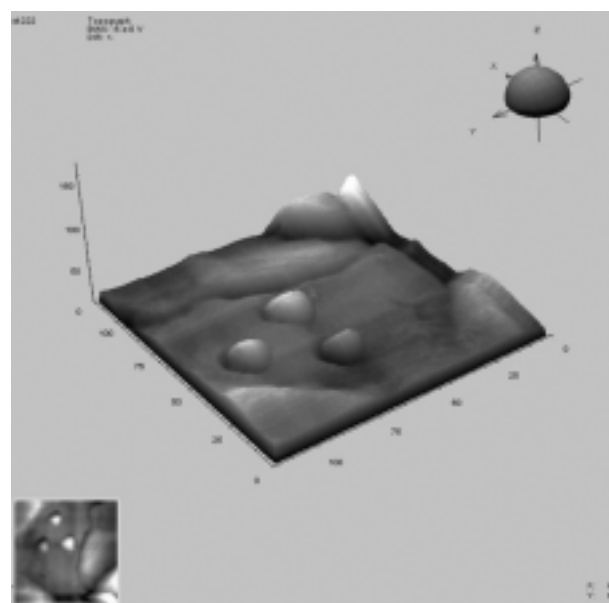


Fig. 6. *Ex situ* STM images of localized polypyrrole nano patterns of three dots formed on 125 nm × 125 nm area in bare electrode.

substrate. Figure 6 shows EC-STM results produced in 125 nm × 125 nm area on bare electrode and growth of three PPy nano dots on electrode. Using EC-STM is not only to control nano level growth of PPy, but also to obtain naked molecule information image²¹⁻²³⁾. Using electropolymerization of pyrrole at conducting substrate under ambient laboratory

conditions may be applied to miniaturization of biosystem. In most biosystem, environmental condition is based on humidity or solution reaction, and charge transfer across the thin interfacial water layer originates mostly from electron tunneling²⁴. Careful consideration of environmental factors, in particular the relative humidity is essential for the full exploitation of STM in studies of biomolecular structure and function²² in biomolecules/PPy biosystem.

4. Conclusion

Electropolymerization of pyrrole applied to biosystem has many advantages in the surface modification of bio-electric device. This method can be used on many electrically conductive surface, whereas chemical attachment techniques usually are limited to a few reactive substrate. But, electrochemically-induced deposition of conducting polymer films on electrode surfaces and the properties of thin PPy films prepared on electrode surfaces depended strongly on the experimental variables of electrode potentials applied, solution pH, kinds and quantity of supporting electrolytes, added chemicals, and the mode of electrochemical method employed. Not only these problems, but also influence of cations and anions, as well as the contribution of solvent to the analytical signal remain unclear in the present time. We have been investigating changes in microrheological property of biomolecules/PPy during electrochemical growth of PPy in aqueous electrolyte solutions and were able to control the electrochemical growth of PPy to produce PPy nano patterns on gold electrode. *In situ* EQCO studies on biomolecules/PPy may be very useful for electropolymerization of pyrrole applied to biosystem for further developments.

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