Gas-phase Treatment Methods for Chemical Termination of Sputtered Nanocarbon Film Electrodes to Suppress Surface Fouling by Proteins

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Electrodes that suppress protein adsorption are particularly important for the development of electrochemical biosensors and electroanalysis of biological samples. We studied the electrochemical performances of carbon film electrodes before and after water vapor (H2O) and ammonia gas (NH3) plasma treatments. The H2O plasma treatment substantially increased the surface oxygen concentration and decreased the contact angle. The NH3 plasma treatment increased the surface nitrogen content to about 5 at %, but a similar amount of oxygen remained on the surface. The sp2 bond amounts decreased and the sp3 bond amounts increased after the H2O plasma treatment, whereas both amounts changed little after the NH3 plasma treatment. Cyclic voltammetry with the plasma-treated electrodes showed an increase in the peak separation (ΔE) of less than 20 mV for 1 mM Fe(CN)63-/4- containing 100 mg/mL bovine serum albumin (BSA), whereas ΔE of the untreated carbon film increased by about 600 mV. Thus, both plasma-treated electrodes strongly suppressed the protein adsorption. The NH3 plasma-treated film showed the highest electrochemical activity and lowest redox peak separation with and without BSA despite its higher contact angle value than of the H2O plasma-treated film. For both plasma-treated films, ΔE did not depend on BSA concentration.

**Keywords:** Sputtered carbon film electrode, Anti-fouling, Biomolecule, Plasma modification, Cyclic voltammetry

1. Introduction

Electrochemical measurement and sensing methods have been used for detecting various biological molecules, including clinical markers, due to their low detection limits, high sensitivity, and simple, inexpensive device structures. For example, catecholamines and indolamines, which are neurotransmitters in the brain, have been detected by an electrochemical method at the nanomolar level [1-3]. However, low-concentration analytes in blood samples are very difficult to detect because blood samples contain high concentrations of proteins and lipids, which easily foul the electrode surface and reduce the measurement sensitivity. Therefore, an electrode surface with anti-fouling properties is required for continuous and reproducible measurements of low-concentration analytes in blood samples.

A practical way to suppress electrode surface fouling is to use chemically modified electrodes. For example, modifying electrodes with ionic polymers, such as Nafion film, improves both the stability for detecting dopamine and selectivity against anionic interfering substances, including L-ascorbic acid and uric acid [4]. However, the
electrode surface modification drastically increases the response time and decreases sensitivity because the diffusion coefficient of the analytes in the anionic polymer is 1 or 2 orders of magnitude lower than that in the electrolyte solution. Self-assembled monolayers (SAMs) have been used to avoid the non-specific adsorption of proteins. SAM molecules contain the functional groups of phosphorylcholine [5-7] and polyethylene glycol [8] and combine positively and negatively charged components to create zwitterionic SAMs [9-12]. Although the molecular weight of SAMs is much smaller than that of the polymers described above, the electrode activity is still decreased by SAM formation on the electrode. This is because the electron transfer rate of redox molecules is sharply decreased as the thickness of immobilized SAMs increases, even for nanometer-order thicknesses. Hydrophilic surfaces can exhibit anti-fouling properties against large biomolecules, including proteins, without polymer and SAM modifications and without decreasing electrode activity. Goto et al. reported that a diamond-like carbon (DLC) electrode with an sp²/sp³ hybrid structure fabricated by oxygen plasma treatment exhibited good anti-fouling properties in several biomolecule solutions compared with a traditional glassy carbon (GC) electrode [13]. We also reported that oxygen plasma treatment of a nanocarbon film formed by electron cyclotron resonance (ECR) sputtering improved the biocompatibility and electrochemical properties compared with GC and DLC electrodes after the same plasma treatment. This pretreatment enabled the ECR-sputtered nanocarbon film surface to achieve sufficient hydrophilicity without greatly increasing its surface roughness (0.07 to 0.11 nm) [16,17].

2. Experimental

2.1. Chemicals

All chemicals were analytical grade or better and were used as received. Potassium chloride, potassium hexacyanoferrate (III), bovine serum albumin (BSA; IgG-free, protease-free), and iron (II) chloride tetrahydrate were purchased from FUJIFILM Wako Pure Chemical Industries, Corp. (Japan). Ultrapure water (Milli-Q) was used in all of the experiments. Perchloric acid was obtained from Nacalai Tesque, Inc. (Japan).

2.2. Carbon film deposition

Nanocarbon films were deposited on a boron-doped silicon wafer by unbalanced magnetron (UBM) sputtering without heating the substrate. The base pressure of the deposition chamber was 7.0×10⁻⁷ Pa. The Argon gas pressure was kept at 6.0×10⁻¹ Pa. The film thickness was about 40 nm. The target power and the substrate bias voltage were set at 100 W and -100 V. We reported that the average roughness (R_a) of the film was about 0.17 nm [16,17].

2.3. Plasma treatment

Plasma treatments were used to make the carbon film surfaces into hydrophilic. The plasma reaction apparatus used for surface treatment was similar to that previously reported [18]. The apparatus consists of a vacuum pump, a reaction chamber, a gas supply system, and a 13.56 MHz radio-frequency oscillator circuit.

H₂O plasma treatment was performed by introducing Ar gas through the water. NH₃ plasma treatment was performed by introducing Ar gas containing 25 at. % NH₃ gas into the reaction chamber. The RF power for both plasma treatments was 100 W and treatment time was 1 minute.

2.4. Contact angle and X-ray photoelectron spectroscopy measurements

The water drop contact angle was measured with a contact angle meter (LSE-ME3, Nick Co., Japan), using a water drop with a volume of about 0.6 µL to ensure that the effects of gravity were negligible.
Surface analysis of the nanocarbon films was performed by X-ray photoelectron spectroscopy (XPS; ESCA Quantum 2000, Ulvac-phi Co., Japan) using Al Kα monochromatic X-rays (1486.6 eV) to determine the elemental composition and bonding state.

2.5. Electrochemical measurements
Cyclic voltammograms (CVs) were obtained using ALS/CHI Model 1208B and 720E electrochemical analyzer (CHI Instruments, Inc., USA). A platinum wire and an Ag/AgCl (3M NaCl) electrode (BAS, Japan) were used as auxiliary and reference electrodes, respectively.

3. Results and discussion
3.1. Surface analysis of the carbon film
Table 1 showed the contact angle, ratios of C 1s, O 1s, and N 1s, evaluated by the wide- scan of XPS spectra, and the calculated N/C and N/O values before and after H2O and NH3 plasma treatment. The sp² and sp³ bonds evaluated by the narrow-scan XPS spectra are also shown in Table 1. The contact angles of both treated carbon films were decreased substantially compared with that of the untreated carbon film and lowest value of 26 degree was obtained with H2O plasma treatment. This might be due to the introduction of a large amount of oxygen atoms, which form hydrophilic functional groups, to the film surface. The amounts of sp² bonds greatly decreased and the amounts of sp³ bonds increased after H 2O plasma treatment. In contrast, only small changes in sp³ and sp² amounts were observed after NH 3 plasma treatment, suggesting that the active species generated by H2O plasma react with the double bonds in the graphene structure. No nitrogen atoms were observed on the H2O plasma-treated carbon film, whereas similar amounts of oxygen and nitrogen atoms were detected on the NH3 plasma-treated carbon film. This is due to residual oxygen or H2O in the vacuum chamber and a higher vacuum system is needed to decrease the amount of oxygen.

3.2. Electrochemical response of redox species after plasma treatment
Figure 1(a) compares the electrochemical responses of Fe(CN)₆³⁻/⁴⁻ at the untreated and plasma-treated sputtered carbon film electrodes. The surface roughness was not changed even after this treatment. Indeed, our previous work exhibited that the surface roughness evaluated by atomic force microscopy (AFM) did not changed substantially [19]. Therefore, the change in electrochemical responses after treatment in the present study should be caused by the surface functional groups, which affect surface wettability and affinity for the analytes and interfering molecules. The ΔE value of the untreated carbon film was 162 mV, and this was decreased to 0.143 V and 0.101 V after H2O and NH3 plasma treatments, respectively. Nitrogen-containing carbon materials could have various nitrogen-containing groups, such as amino, pyridine, and pyrrole groups, in addition to nitrogen atoms incorporated in the graphene structure. Pyridine and amino groups may be positively charged at lower pH condition. The lower ΔE value for the NH3 plasma-treated carbon film compared with the H 2O plasma-treated film was due to the positive charge of the nitrogen-containing groups which possibly attracting negatively charged Fe(CN)₆³⁻/⁴⁻ caused by electrostatic interaction. This speculation is also supported by the higher ΔE of positively charged Fe²⁺/³⁺ for the NH3 plasma-treated film compared with the H 2O plasma treated film and the untreated carbon film, (data not shown), although the surface oxygen concentrations of untreated and NH 3 plasma-treated carbon films were similar to the amounts of oxygen (5.0 at. % for NH 3 treated carbon film, and 5.6 at. % for untreated carbon film) (Table 1). The other possible reason for the lower ΔE value of the NH3 plasma-treated carbon film is that NH3 plasma-treated carbon film had a higher sp² bond amounts than the H 2O plasma-treated carbon film. The H 2O plasma-treated carbon film because the NH3 plasma-treated carbon film was expected to have a higher amount of surface edge plane, which is more electroactive.

3.3. Electrochemical response of redox species in the presence of protein
For bioanalytical chemistry, preventing protein
adsorption is important. Surface fouling is a fatal problem in electrochemical biosensors, and suppression of non-specific adsorption is crucial in immunoassay or DNA sensors [7,20-22]. Modification with SAMs or thin polymer film is effective for suppressing the fouling on the electrode surface, but this drastically decreases sensitivity due to the much lower diffusion coefficient in the modified layer. In contrast, the effect of the surface treatments on diffusion coefficient is limited, although these treatments change the electron transfer rate between the redox species and the electrodes. Figure 1(b) shows voltammograms of 1 mM Fe(CN)$_6^{3-/-4}$ in the presence of 100 mg/mL BSA in 1 M KCl. Compared with voltammograms without BSA (Fig. 1(a)), $\Delta E$ of the untreated carbon film increased from 0.162 V to 0.843 V. Fouling by protein adsorption is worse on the hydrophobic surfaces; thus, the increased $\Delta E$ value was mainly due to the lower hydrophilicity of the untreated carbon film. In contrast, the effect of protein adsorption on $\Delta E$ was much smaller at the plasma-treated carbon films.

$\Delta E$ of the NH$_3$ and H$_2$O plasma-treated carbon films increased by only 20 mV and 7 mV, respectively. Fig. 1. (a) Cyclic voltammograms for 1 mM Fe(CN)$_6^{3-/-4}$ in 1 M KCl. Scan rate, 0.1 V/s. (b) Cyclic voltammograms for 1 mM Fe(CN)$_6^{3-/-4}$ obtained 100 mg/mL BSA in 1 M KCl. Scan rate, 0.1 V/s.

To investigate the effects of BSA concentration, we changed the BSA concentrations from 1 to 100 mg/mL and evaluated the $\Delta E$ values and magnitudes of the oxidation currents. The variation of the $\Delta E$ value of the plasma-treated and untreated carbon film electrodes are shown in Fig. 2. As expected, $\Delta E$ of untreated carbon film was much higher than those of the NH$_3$ and H$_2$O plasma-treated carbon films, which greatly increased with the existence of only 1 mg/mL of BSA. No clear dependence of the $\Delta E$ value on BSA concentration was observed for the NH$_3$ and H$_2$O plasma-treated carbon film electrodes (inset of Fig. 2). This may be due to the difference in BSA concentrations between the bulk and electrode surface, because the BSA concentration near the electrode surface should increase after a certain time due to the interaction between the electrode surface and BSA. Surprisingly, the NH$_3$ plasma-treated carbon film exhibited the lowest $\Delta E$ value at BSA concentrations (100 mg/mL) despite

![Fig. 2. Variation in peak separations of Fe(CN)$_6^{3-/-4}$ at nanocarbon film electrodes with increasing BSA concentration.](image)

![Fig. 3. Variation in oxidation peak currents of Fe(CN)$_6^{3-/-4}$ at nanocarbon film electrodes with increasing BSA concentration.](image)
its substantially higher contact angle (42 degrees) than the H₂O plasma-treated carbon film (26 degrees). The BSA should be repulsed from the surface of H₂O plasma-treated carbon film because the electrode surface has negatively charged hydroxyl and carboxyl groups and BSA with isoelectric point of 4.9 was also negatively charged in the pH 6 potassium chloride solution (KCl) used for measurements. In the NH₃ plasma-treated film, the nitrogen-containing groups and oxygen-containing groups were modified, which may contribute to suppressing the fouling. However, further studies should be performed, including examining the effect of changing the plasma gas concentration and treatment conditions N/O ratio.

We also evaluated the effect of on the peak current (Fig. 3). In contrast to the small dependence of the ΔE value on BSA concentration, the magnitude of the current decreased with increasing BSA concentration. This was due to the reduction of the diffusion coefficient of Fe(CN)₆³⁻/⁴⁻ because the viscosity of the measurement solution increased with increasing BSA concentration. The peak decreases were much larger for the untreated carbon film due to the deformation of the peak shape by the large increase in ΔE.

4. Conclusion

We electrochemically investigated anti-fouling properties of the H₂O plasma- and NH₃ plasma-treated sputtered nanocarbon film electrode by using the typical redox molecules (Fe(CN)₆³⁻/⁴⁻ and Fe²⁺/³⁺) and protein (BSA). H₂O plasma treatment decreased the contact angles from 74 to 26 degrees by introducing the 10 at. % of oxygen functional groups without significant change of the O₁s concentration, results in less hydrophilicity (42 degrees). Cyclic voltammograms of 1 mM Fe(CN)₆³⁻/⁴⁻ and Fe²⁺/³⁺ imply that the NH₃ plasma-treated carbon film formed a positively-charged surface, possibly derived from amino- and pyridine groups. In spite of less hydrophilicity, NH₃ plasma-treated carbon film exhibited the best anti-fouling properties against BSA compared with untreated and more hydrophilic H₂O plasma treated carbon film, where the ΔE of 1 mM Fe(CN)₆³⁻/⁴⁻ is not almost affected even by BSA addition with extremely high concentration (100 mg/mL). This might be derived from the both effects of oxygen and nitrogen functional groups, but further investigation is needed to reveal these mechanisms.

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