Plasma-Treated Sputtered Nanocarbon Film Electrodes for Suppressing Electrochemical Fouling by Serotonin

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ABSTRACT

The fouling of electrodes with large molecules and/or electrochemically oxidized biomolecules limits the potential applications of bioelectroanalysis. The present work examined the effects of various plasma treatments when applied to sputtered carbon film electrodes. This was done by assessing the electrode responses to serotonin, a neurotransmitter that can be adsorbed after electrochemical oxidation. Although the oxidation current of serotonin rapidly decreases after five potential cycles with 5-minute intervals at the untreated and H2O plasma-treated carbon films, the magnitudes of the currents were almost unchanged at the films after NH3 and NH3/H2O gas plasma treatments. Cyclic voltammetric measurements of Fe(CN)63−/4− before and after serotonin oxidation revealed that there are no detectable fouling with oxidized serotonin by either the NH3 or NH3/H2O treatment, unlike the case with untreated and H2O plasma-treated carbon film electrodes. Similar to the serotonin response, both 5-hydroxyindoleacetic acid and 5-hydroxytryptophol show improved stable responses after the NH3/H2O plasma treatment. X-ray photoelectron spectroscopy data suggest that the suppression of fouling might be due to the electrostatic repulsion between the amino group of serotonin and surface charges after the NH3 and NH3/H2O treatments, in addition to the effects of hydrophilic surfaces generated by the plasma treatments.

Keywords: Serotonin, NH3/H2O and NH3 Plasma Treatments, Carbon Film Electrode

1. Introduction

Electroanalytical techniques have been widely applied in the field of bioanalysis, including in vivo and in vitro clinical tests,1−3 because these methods provide low detection limits and high sensitivity despite using very simple and inexpensive device structures. However, electrochemical detection has serious problems in measuring biological fluids because the electrode surface can become fouled with some biomolecules. In particular, large molecules such as proteins strongly adsorb on the electrode surface, especially if the surface is hydrophobic. This is a serious problem when measuring blood and serum samples, which contain high concentrations of proteins and lipids. Electrode sensitivity decreases greatly after fouling due to the electron transfer decrease. Similarly, electrodes are often fouled with small molecules, including alkylphenols and indole amines, after electrochemical oxidation, since that process can turn these molecules into hydrophobic compounds. A typical example is serotonin, a well-known neurotransmitter in the brain that works to reduce depression, regulate anxiety, and so on. Similar to catecholamines, which are also known as neurotransmitters, serotonin can be detected by direct electrochemical oxidation and has been analyzed quantitatively by high-performance liquid chromatography with electrochemical detection.4,5 In vivo and in vitro electroactive neurotransmitters, typically incorporating carbon-fiber–based microelectrodes, have been commonly used due to their small diameter, high electroactivity, and low cost. However, serotonin is electrochemically irreversible, and its oxidized product is more hydrophobic and thus readily adsorbs on electrode surfaces. Therefore, an electrode having a surface less prone to fouling is required for continuous, reproducible measurements of serotonin, particularly during in vivo measurements. Conventionally, chemically modified electrodes have been employed as a means of suppressing fouling. As an example, neurotransmitters such as dopamine and various polymers, including Nafion and oxidized polypyrrole, have been used to improve both stability and selectivity during analyses of biomolecules such as L-ascorbic acid and uric acid.10−16 However, electrode modification with such polymers slows the response time significantly and decreases sensitivity due to the much lower diffusion coefficient of the analyte in the modified film. As an alternative to film modification, electrodes having hydrophilic surfaces can be used to limit fouling when assessing hydrophobic molecules. For example, diamond-like carbon (DLC) film treated with an oxygen plasma has been reported to suppress fouling by several biomolecules compared to glassy carbon (GC), which was confirmed by peak separation (∆E) changes in surface-sensitive Fe(CN)63−/4− before and after biomolecule detection.17 Our own group has also determined that a nanocarbon film electrode formed by electron cyclotron resonance (ECR) sputtering shows superior anti-fouling properties against large molecules such as DNA18,19 and proteins such as bovine serum albumin (BSA) compared to GC and DLC electrodes thanks to its atomically flat surface.20 Moreover, since ECR-sputtered nanocarbon film contains a significant number of sp2 bonds and has high etch-resistance, its surface can be converted such that it has a
sufficiently hydrophilic surface while limiting the concurrent increase in surface roughness (from 0.07 to 0.11 nm). As a result, the ECR-sputtered nanocarbon film electrode exhibited much improved anti-fouling properties against 10 mg/ml BSA after the electrode surface was oxidized by oxygen plasma treatment. More recently, we compared the electrochemical performance of a carbon film formed by unbalanced magnetron (UBM) sputtering, which can also increase sp² content like ECR-sputtering method nanocarbon film, before and after water vapor (H₂O) and/or ammonia gas (NH₃) plasma treatments. Cyclic voltammetry with the plasma-treated carbon film generated a peak separation (ΔE) increase of less than 20 mV in trials with a 1 mM Fe(CN)₆³⁻/⁴⁻ containing highly concentrated 100 mg/ml BSA, while a large ΔE increase of approximately 600 mV was observed when the untreated carbon film was used. A carbon film exposed to an NH₃ plasma showed the lowest ΔE increase, suggesting the possibility of excellent anti-fouling properties of N-containing functional groups.

In the present work, we studied the electrochemical responses of UBM carbon films to serotonin before and after plasma treatments in order to confirm the effects of these treatments on the analysis of small hydrophobic molecules generated by electrochemical oxidation. Treatments were applied using an NH₃, H₂O, or NH₃/H₂O plasma, and changes in surface functional groups were evaluated by X-ray photoelectron spectroscopy (XPS).

2. Experiment

2.1 Chemicals

All chemicals were analytical grade or better and were used as received. Serotonin was obtained from Sigma-Aldrich (Tokyo, Japan), while 5-hydroxytryptophol was purchased from LKT Laboratories, Inc. (St. Paul, MN) and 5-hydroxyindole-3-acetic acid, potassium hexacyanoferrate (III) (K₃[Fe(CN)₆]), potassium chloride (KCl), sodium dihydrogen phosphate (NaH₂PO₄), and disodium hydrogen phosphate (Na₂HPO₄·H₂O) were purchased from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan). A 0.1 M phosphate buffer solution (pH 7.0) was prepared from NaH₂PO₄ and Na₂HPO₄·H₂O. Ultrapure water (Milli-Q) was used in all experiments.

2.2 Carbon film deposition

Carbon film electrodes were deposited on doped silicon wafers using UBM sputtering at room temperature with a base pressure of 7.0 × 10⁻⁷ Pa in the deposition chamber. Argon (Ar) gas was applied during processing at 6.0 × 10⁻³ Pa. The target power was set at 100 W and the substrate bias voltage was maintained at −100 V during sputtering. The resulting film thicknesses were approximately 40 nm, with an average roughness of 0.17 nm.

2.3 Plasma treatment

H₂O vapor plasma, NH₃ gas plasma, and an NH₃/H₂O vapor plasma were used to treat the carbon films. All plasma treatments were performed with a low-pressure inductively coupled plasma system. The plasma reaction apparatus consisted of a vacuum pump, a reaction chamber, a 13.56 MHz radio-frequency copper coil, and a source of either pure Ar gas or Ar gas containing ammonia at 25 at.%. The equipment setup is shown in Fig. S1, and the details of the system have been previously reported.

During the H₂O plasma treatment, H₂O vapor was generated by bubbling Ar through a pure water reservoir, after which this vapor was introduced into the reaction chamber. Similarly, an NH₃/H₂O vapor mixture was obtained by bubbling Ar through a 10 w/v% solution of ammonia in water (KENEI Pharmaceutical, Osaka, Japan). The NH₃ plasma treatment was performed with a flow of a 25 at.% ammonia gas mixture into the reaction chamber. In each case, the RF power was 100 W and the treatment time was 1 min.

2.4 X-ray photoelectron spectroscopy and contact angle measurements

The surfaces of the carbon film electrodes were characterized by X-ray photoelectron spectroscopy (XPS: ESCA Quantum 2000 with Al Kα 1 radiation at 486.6 eV, Ulvac-phi, Chigasaki, Japan) to determine the elemental compositions before and after each plasma treatment. The contact angle was measured with a contact angle meter (LS-E-ME3, NicK, Saitama, Japan). Ultrapure water droplets (droplet volume 0.6 µl) were applied to characterize the wettability of the electrode surfaces.

2.5 Electrochemical measurement

Cyclic voltammograms were obtained using ALS/CHI 720E and 1208B electrochemical analyzers (CHI Instruments, Austin, TX, USA). In each trial, an Ag/AgCl (3 M NaCl) reference and a platinum wire auxiliary electrode were used. The potential sweep rate was 100 mV/s in most experiments.

3. Results and Discussion

3.1 Surface characterization of carbon film electrodes

Table 1 shows the elemental composition ratios calculated with C1s, O1s, and N1s signals and the wettability of the electrode surfaces evaluated by the wide scan of XPS spectra and water droplet contact angle measurement, respectively. Untreated carbon film contained 5.9% oxygen and 0.5% nitrogen species, which could be derived from the remaining air and/or adsorbed water in the chamber. The water contact angle at the untreated carbon film surface is 80°. This hydrophobicity of the surface implies that the oxygen functional groups introduced to the film surface are speculated to be relatively nonpolar and/or derived from adsorbed molecules such as H₂O or O₂. When H₂O plasma was exposed to the carbon film, the oxygen concentration increased by 5% without changing the nitrogen concentration, and the contact angle decreased sharply to 13°. These results indicate that hydrophilic polar oxygen functional groups including hydroxyl and carboxyl groups were mainly introduced onto the carbon film surface. In contrast, a 5.4% nitrogen concentration was detected after NH₃ plasma treatment without changing the oxygen concentration (5.6%). However, the contact angle was 36°, which is high relative to that exposed to H₂O plasma. This result suggests that nitrogen-containing groups were successfully introduced onto the surface, thus providing a moderately hydrophilic surface, probably due to the lower polarities of nitrogen functional groups compared to those of oxygen functional groups. Figure 2S shows XPS narrow scan of N1s spectrum of the carbon film after each plasma treatment and Table S1 summarizes surface chemical states obtained by deconvoluting the peak.

| Elements | untreated | H₂O plasma | NH₃ plasma | NH₃/H₂O plasma |
|----------|-----------|------------|------------|---------------|
| C1s (%)  | 90.9      | 86.3       | 86.7       | 87.2          |
| O1s (%)  | 5.9       | 10.8       | 5.6        | 6.8           |
| N1s (%)  | 0.5       | 0.5        | 5.4        | 3.7           |
| Ar2p (%) | 2.5       | 1.8        | 1.9        | 1.7           |
| Si2p (%) | 0.2       | 0.6        | 0.4        | 0.6           |
| N/C      | 0.0055    | 0.0058     | 0.062      | 0.042         |
| N/O      | 0.085     | 0.046      | 0.96       | 0.54          |

Contact angle (degrees): 80° (untreated), 13° (H₂O plasma), 36° (NH₃ plasma), 46° (NH₃/H₂O plasma).
In fact, the peak deconvolution results of the N 1s spectra imply the preferential introduction of amino groups onto the film surface, which has lower polarity than the hydroxyl group (Table S1). After NH3/H2O plasma exposure, the surface oxygen and nitrogen concentrations were 6.8% and 3.7%, indicating that both nitrogen and oxygen functional groups were introduced. Interestingly, the contact angle of the NH3/H2O plasma-treated carbon film was 46°, which is higher than that of NH3 plasma-treated carbon film despite the higher concentration of oxygen functional groups at the former film surface. Although further investigation is required to reveal this mechanism in detail, the reactions between both NH3 plasma- and H2O plasma-based active species might change the ratio between surface hydrophilic and hydrophobic groups, which could be partially supported by fewer hydrophilic amino groups (also slightly decreased pyridinic groups) and increased NHC=O groups with less-hydrophilicity on the NH3/H2O-treated surface (Table S1).

This result could be due to the N-doping effect on the electrode surface, but further investigation may be required to reveal this mechanism in detail.

3.2 Inhibition of the adsorption of serotonin

The continuous detection of serotonin is challenging because the reaction products adsorb onto the bare electrode surface. In the present work, we measured this phenomenon by acquiring CV data from 100 µM serotonin solutions, obtaining a total of five voltammograms at 5-minute intervals. Figure 1(a–d) shows the CVs generated by carbon films with and without plasma treatments. Figure 1(e) summarizes the dependence of peak current densities versus recorded time. We confirmed that the oxidation peak current produced by the untreated electrode rapidly decreased as the number of scans increased. Even after the electrodes were treated with H2O plasma, the current decreased slowly following each potential cycle despite its hydrophilic surface. This result indicates that hydrophilicity is not a sufficient factor to realize a stable response to 100 µM serotonin. In fact, carbon film after UV/O3 treatment, which provided a highly hydrophilic surface (contact angle: 10°), also exhibited lower stability toward repeated CVs of 100 µM serotonin, as shown in Fig. S2(a). In contrast, the oxidation peak was almost unchanged after five potential cycles in the case of electrodes treated with an NH3 or NH3/H2O plasma.

These results suggest that the N-containing functional groups play crucial roles in realizing the stable response of serotonin. In contrast, the peak potential during the first cycle with the untreated electrode was approximately 0.4 V, while the values for the electrodes exposed to the NH3 and NH3/H2O plasmas were 0.50 V and 0.56 V, respectively. Also, the oxidation currents of NH3 and NH3/H2O plasma treated electrodes start to increase about 0.1 V higher potential than those of other electrodes. The lower initial currents obtained from electrodes treated with either the NH3 or NH3/H2O plasma and the positively shifted peak potentials for these same specimens suggest that the electrostatic repulsion between the electrode surface and serotonin suppressed the adsorption of oxidized products. That is, the positive shift of oxidation peak and starting potentials at the electrodes treated with NH3 and NH3/H2O plasmas may be caused by slower electron transfer, possibly owing to electrostatic repulsion between amino functionalities of serotonin and the electrode surface. In contrast, the interactions between the electrode exposed to H2O plasma and amino groups of the serotonin molecules may have increased the adsorption, even in the case of a hydrophilic electrode surface. In addition, the untreated electrode surface would be exposed to strong adsorption of oxidized serotonin via hydrophobic interactions. In addition, double layer capacity capacitive at each electrode is slightly different as shown in Fig. 1(a–d), and the improved stability could be affected by the difference of electrode surface area, which is related to the magnitude of the double layer capacity. However, the NH3 and NH3/H2O treated carbon films with stable responses have lower double layer capacity compared with those of others. This also supported that the difference of surface functional groups are major reason to suppress the fouling.

3.3 Evaluation of fouling with Fe(CN)6^{3-/-4-} response

In order to evaluate the fouling after five measurements of serotonin, we studied differences in electrochemical responses of 1 mM potassium hexacyanoferrate (III) [Fe(CN)6^{3-/-4-}]. Figure 2 shows voltammograms of 1 mM Fe(CN)6^{3-/-4-} before and after 100 µM serotonin measurements at the carbon films without treatment (a) and with NH3/H2O plasma (b). A significant increase in ΔE was observed at the untreated carbon film after serotonin measurement, whereas only a slight increase in ΔE was observed at the NH3/H2O plasma-treated carbon film. Table 2 shows the ΔE and oxidation current decreases before and after serotonin measurements at carbon films with various plasma treatments compared to the film without treatment. It is obvious that the ΔE increases and peak current decreases after serotonin measurement were greatly suppressed after the plasma treatments. However, among these treatments, the NH3/H2O plasma and NH3 plasma treatments shows lower increases in ΔE and lower decreases in peak current compared to the films with the other treatments. Particularly, the NH3/H2O
plasma-treated carbon film shows only a 31 mV $\Delta E$ increase and 1% peak current decrease, indicating that the NH$_3$/H$_2$O plasma-treated film best suppresses oxidized serotonin. This also well reflects the results of the peak current decreases in serotonin by continuous measurements shown in Fig. 1.

### 3.4 Inhibition of other indole compounds

In order to study the effects of functional groups, two indole compounds, 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophol (5-HTOH), were assessed and the resulting electrochemical responses were compared to those obtained with serotonin. It was reported that 5-HIAA turns to quinone form after electrochemical oxidation. In case of 5-HTOH, relatively hydrophilic dimer is formed by chemical reaction after one electron oxidation or further electrochemical oxidation and formation of 5-HTOH with additional hydroxyl group to the ortho position after chemical reaction were reported in acidic solution.24

Figure 3 shows the variation in oxidation peak currents of 100 µM 5-HIAA and 5-HTOH at the carbon films treated by NH$_3$/H$_2$O plasma compared to that without treatment. In the case of 100 µM 5-HIAA and 5-HTOH at the carbon plasma compared to that without treatment. In the case of 100 µM 5-HTOH and 5-HIAA, although electrostatic repulsion between adsorbed oxidized 5-HIAA and that in solution.

Surprisingly, the NH$_3$/H$_2$O-treated carbon film exhibited high stability to repeated CVs of 5-HIAA, although electrostatic attraction between the positively charged electrode surface and negatively charged 5-HIAA was expected. Based on the above results, we speculate that, unlike serotonin detection, hydrophilicity is an important factor for realizing stable responses. This hypothesis is also supported by the results when the electrode was used after UV/O$_3$ treatment. As shown in Fig. S3(a, b), the decreases in the oxidation peak currents of 5-HTOH and 5-HIAA were smaller at the UV/O$_3$-treated carbon film compared to the untreated carbon film, which is not the case with serotonin detection. Therefore, we concluded that both the relative hydrophilicity and the positively charged surface at the electrode surface treated with NH$_3$/H$_2$O plasma exhibited superior detection stability to all the indole compounds regardless of their charges.

### Supporting Information

The Supporting Information is available on the website at DOI: https://doi.org/10.5796/electrochemistry.20-64072.

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