Controllable electrode activities of nano-carbon films while maintaining surface flatness by electrochemical pretreatment

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Abstract

A carbon film consisting nanocrystallites with mixed sp^2 and sp^3 bonds formed by using the electron cyclotron resonance (ECR) sputtering method was studied with respect to the changes in characteristics caused by electrochemical pretreatment (ECP). Unlike glassy carbon, our sputtered nanocrystalline carbon film deposited at an acceleration voltage of 75 V (ECR-75 nano-carbon film) largely retained its surface flatness after the ECP. This robust surface could be caused by an increase of 42 % in the sp^3 carbon realized by increasing the acceleration voltage during sputtering. The electrode activity of ECR-75 nano-carbon film was improved for surface sensitive species including $Fe^{3+/2+}$ unlike the boron doped diamond (BDD) electrode. This is because a sufficient quantity of surface sp^2 bonds remained and because the introduction of surface oxygen-containing groups is more efficient than with the BDD electrode. With pretreated ECR-75 nano-carbon film, the peak potential of glutathione was reduced solely due to the increase in the surface hydrophilicity with a sufficient quantity of surface sp^2 bonds, thus achieving the lowest detection limit (0.4 μM) ever obtained with carbon electrodes. We also achieved the stable measurement of 30 μM of serotonin (20 times) without the electrode surface fouling found with other electrodes.

1. Introduction

Carbon materials are widely used for the electrochemical measurement of biomolecules. Glassy carbon (GC) electrodes, which generally consist of sp^2 bonds, have been used for such electroanalysis since they exhibit a wider potential window and lower cost than metal electrodes such as Au and Pt. The electrode activity of graphite electrodes, including the GC and highly oriented pyrolytic graphite (HOPG) electrode, depends on the electrode surface properties such as surface roughness and amount of surface oxygen.[1-8] Therefore, it is important to control these surfaces. The electrochemical pretreatment (ECP) of graphite electrodes is well known to improve electrode activity.[1-8] In contrast, ECP also induces an increase in the capacitive current and reduces the potential window of these electrodes owing to an increase in surface roughness and oxygen-containing groups.[2,4-8] This is disadvantageous in terms of achieving a low detection limit in electroanalysis.

Recently, new carbon materials including boron doped diamonds (BDDs) and carbon nanotubes (CNTs) have attracted considerable interest as regards electroanalysis. BDD electrodes have excellent electrochemical properties such as a wide potential window and a low capacitive current.[10-15] However, the electron transfer rate at the BDD electrode is slower than that of a conventional carbon

electrode for several analytes (e.g. dopamine (DA)) because BDDs mainly consist of sp^3 bonds and small grain boundary layers between diamond crystals and so have few oxygen-containing groups on their surfaces.[10,14] At the same time, this heterogeneous structure prevents the ECP from enhancing the BDD electrode activity even though several groups have reported that ECP could provide the BDD electrode with a wider potential window.[8,16,17] Since the activation mechanism is different from sp^2 carbon based electrode materials such as GC, the electron transfer rate at a BDD electrode becomes slower for several analytes after ECP.[8,17,18] One reason for this is small number of surface oxygen-containing groups even after ECP. The electron transfer rates of some important redox species are known to be affected by the amount of surface oxygen.[2,4,10,13,15,19,20] The presence of a sufficient number of surface oxygen-containing groups is also important in terms of suppressing electrode surface fouling.[21] The other reason is the reduction in sp^2 carbon after the ECP since some biologically important analytes including catecholamines and nucleic acid need a π - π interaction with the electrode surface to increase electron transfer.[10,14,20,22] Improved electrode activity with a minimum increase in the background noise level including the capacitive current and maintaining the potential window is ideal for detecting biomolecules with a low

detection limit.

We recently reported a newly developed carbon film consisting nanocrystallites with mixed sp^2 and sp^3 bonds formed by the electron cyclotron resonance (ECR) sputtering method.[22-28] The sputtered nanocrystalline carbon film (ECR nano-carbon film) surface has controllable sp^3/sp^2 ratios and is extremely flat. This film electrode exhibited excellent electrochemical properties including a wide potential window, low capacitive current and low surface fouling, compared with the GC electrode.[22,24-28] The electron transfer rates of several electroactive species (e.g. DA) at the ECR nano-carbon film electrode are higher than those at the BDD electrodes since the ECR nano-carbon film electrode has more sp^2 bonds.[26] Indeed, the ECP of the ECR nano-carbon film electrode provided an increased electron transfer rate and anti-fouling property of NADPH without any large increase in the capacitive current.[28] As a result, the lowest detection limit for NADPH could be obtained electrochemically at a pretreated ECR nano-carbon film electrode (10 nM). Further investigation of the ECP of the ECR nano-carbon film electrode will be significant in extending its application to a wide variety of important analytes such as biomolecules.

In this work, we investigated the ECR nano-carbon film electrode with

respect to morphological and characteristic changes caused by the ECP. Variations in the surface properties, such as surface roughness and chemical structure, caused by the ECP were characterized by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and contact angle measurements. We studied the basic electrochemical properties of ECR nano-carbon films using cyclic voltammetry (CV) with several redox species whose electron-transfer properties differed depending on the electrode surface conditions. The surface properties, structure and electrochemical performance were compared with results obtained for other conventional GC and BDD electrodes. We also investigated using a pretreated ECR nano-carbon film electrode to measure some biologically important analytes, namely glutathione (oxidized form, GSSG) and serotonin (5-hydroxytryptamine, 5-HT), which are difficult to measure electrochemically because of their high oxidation potential or electrode stability.

2. Experimental

2.1. ECR nano-carbon film fabrication and electrode preparation

ECR sputtered nano-carbon film was deposited on highly doped silicon (100) substrates at room temperature and the ECR sputtering apparatus is shown in

Figure 1.

Figure 1. Schematic of ECR sputtering apparatus

The microwave power and DC voltage applied to the carbon target were 500 W and 500 V, respectively. The Ar gas pressure was 5.0×10^{-2} Pa. We used ion acceleration voltages of 20 and 75 V between the target and substrate to form carbon films with different quantities of sp^2 bonds, and described as ECR-20 and ECR-75, respectively. The ECR nano-carbon films were about 40 nm thick and were deposited in 8 minutes.

BDDs deposited on a conductive silicon substrate were purchased from Sumitomo Electric Industries, Ltd. (0.023 cm thick $\times$ 25 cm², SHMF-12730) The GC was obtained from Tokai Carbon Co., Ltd. (1.0 cm thick $\times$ 100 cm², GC-20SS). The

GC surface was polished using 6 and 1 μm diamond and 0.05 μm Al_2O_3 before the electrochemical measurement. We cut each carbon material (i.e. ECR-20 and ECR-75 nano-carbon films, BDD and GC) into rectangles. We then fixed a plastic tape with a 2 mm-diameter hole in it onto the ECR nano-carbon film to form a disk electrode.

2.2. Carbon characterization

XPS was performed using a Shimadzu/Kratos model AXIS Ultra (Al K_{α} 1486.6 eV) spectrometer to determine the elemental composition and the quantity of chemical bonds in the carbon electrode surfaces. AFM measurements were performed with an SPI4000 (SII NanoTechnology, Inc.) using a silicon cantilever in air at room temperature. Images were recorded in the dynamic force AFM mode at scan rates of 0.3 Hz with 128×128 pixels. The contact angle was measured with the Dropmaster Model DM 300 (Kyowa Interface Science Co., Ltd.). An ultrapure water (Milli-Q) droplet (droplet volume 5 μL) was used in the experiment to characterize the hydrophilicity of the carbon electrode surfaces.

2.3. Electrochemical measurement and electrochemical pretreatment of carbon

electrodes

Cyclic voltammograms were obtained using a three-electrode configuration with an electrochemical analyzer Model 760B (CH Instruments Inc., USA). In all the experiments, platinum wire and an Ag/AgCl electrode were used as auxiliary and reference electrodes, respectively. We used ECR-20, and ECR-75 nano-carbon films, a GC and a BDD electrode as the working electrode. The ECP was carried out using cyclic voltammetry (CV) between 0 and 2 V for the ECR-20, and ECR-75 and the GC or 0 and 2.3 V for the BDD at a scan rate of 0.1 V/s in a phosphate buffer (PB) solution (pH 7.0). There were 4 potential cycles during ECP for the ECR-20 nano-carbon film electrode and 10 for the ECR-75 nano-carbon film, the GC and the BDD electrodes, respectively. The lower number of potential cycles for the ECR-20 nano-carbon film was because we observed a reduction in the film thickness after 10 potential cycles. In contrast, ECP with higher potential was used for BDD because of its chemically inert surface with respect to electrochemical oxidation compared with other carbons. In the background voltammetry measurement, the electrolyte solution was purged with Ar for 20 min to remove the dissolved oxygen. The apparent double layer capacitance value (C^0) of the electrodes was obtained from CVs at 0.25 V vs Ag/AgCl in 1.0 M KCl in accordance with McCreery's report.[18]

2.4. Chemicals

All the chemicals were analytical grade, or better, and were used as received. Potassium ferrocyanide, DA and perchloric acid were purchased from Nacalai Tesque, Inc., (Kyoto, Japan). Hexaammineruthenium (III) chloride was obtained from Aldrich (Milwaukee, WI). Ammonium iron sulfate and 5-hydroxytryptamine were purchased from Sigma-Aldrich (St. Louis, MO). Glutathione (oxidized form) was obtained from Wako (Tokyo, Japan). KH_2PO_4 , Na_2HPO_4 and sulfuric acid were purchased from Kanto Chemical. We prepared 0.1 M PB, which contained 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4 . Ultrapure water was used in all the experiments.

3. Results and discussion

3.1. Effects of ECP on the surface structure and basic electrochemical properties of nano-carbon film electrodes

First, we examined the potential windows of the two kinds of ECR nano-carbon films, GC, and BDD electrodes before and after the ECP since the potential window is an important factor determining the electrode properties. The ECR-20 and ECR-75 nano-carbon film electrodes contained 21 and 42 % of sp^3

bonds, respectively.[22,25,26] Figure 2 shows the background voltammograms for the above four kinds of carbon electrodes before (dotted line) and after (solid line) the ECP.

Figure 2. Background voltammograms of the carbon electrodes before and after the ECP in 0.05M H₂SO₄ deoxygenated with Ar. Scan rate: 0.1 V/s.

The potential window is defined as the potential range between current limits that do not exceed $\pm 500 \mu\text{A}/\text{cm}^2$, as previously reported by Swain et al.[29] The potential windows of the GC (3.1 V) and ECR-20 nano-carbon film (3.4 V) electrodes narrowed to 2.1 and 3.2 V, respectively, after the ECP. This decrease in the potential windows is because the background current was increased by the increased surface

roughness, and the oxidation and reduction currents for the surface oxygen-containing groups were increased. Several researchers have already reported that the roughness and the quantity of oxygen-containing groups for the GC electrode surface was increased by the ECP and the background current was increased.[2,4-8] The ECR-20 nano-carbon film electrode also exhibited a similar tendency since it consisted of mainly sp^2 carbon. In contrast, the potential window of the ECR-75 nano-carbon film electrode had widened from 4.0 to 4.1 V after the ECP. This indicates that this electrode is very stable with respect to electrochemical oxidation compared with the GC or ECR-20 nano-carbon film electrodes despite its relatively larger quantity of sp^2 bonds (58 %). We also observed the widening of the potential window for the BDD electrode (from 3.1 to 3.5 V). Two groups reported that the widening of the potential window at the BDD after the ECP was caused by the reduction in sp^2 bonds on the surface.[8,17]

To further our investigation, we studied the changes in the surface functional groups, structure, C^0 and hydrophilicity of these electrode surfaces by using XPS, AFM, background CV and contact angle measurements. Table 1 summarizes the results of these measurements. The $sp^2/(sp^3+sp^2)$ ratios were obtained from decomposed carbon C 1s XPS spectra.

Table 1. The surface properties and the C^0 of carbon electrodes before and after ECP.

	ECR-75		ECR-20		GC		BDD	
	Before	After ^a	Before	After ^a	Before	After ^a	Before	After ^a
sp ² /sp ³ +sp ² ratio	0.581	0.450	0.788	0.736	≐1 ^b	≐1 ^b	0.040	0.054
O1s/C1s ratio	0.066	0.098	0.050	0.22	0.086	0.38	0.065	0.089
C^0 (μF/cm ²)	11.1	20.1	11.6	84.0	20.3	69.6	5.1	2.4
Average roughness (Ra) /nm	0.067	0.21	0.13	4.3	2.4	4.6	135	39
Contact angle / degree	75.5	14.0	85.8	11.6	78.0	7.7	77.9	11.9

^aECP conditions for each electrode are described in the experimental section.

^bThis value was calculated from ref. 30 because sp²-based GC includes a C-N component that makes the decomposition of C1 XPS spectra very complicated.

The peaks that appeared at 284.5 ± 0.5 eV and 285.5 ± 0.5 eV are assigned to sp² and sp³ bonds, respectively.[31-35] In the C 1s spectra of GC, the peak appearing at 285.9 eV is assigned to the C-N bond because GC is often used when fabricating polyacrylonitrile, which is unlike ECR-20, ECR-75 nano-carbon films and BDD, which are fabricated with a high-vacuum process using a pure carbon target or high purity gases.[9,36-38] As summarized in Table 1, the O1s/C1s ratios of the GC and ECR-20 nano-carbon film increased greatly after the ECP. This is because the sp² bonds on these carbon surfaces were oxidized to form C-O and C=O bonds, and there was also partial gasification (i.e. CO or CO₂) of the surface carbons, although these materials mainly consisted of sp² bonds and so the sp²/(sp³+sp²) ratios could not be measured. In contrast, the sp²/(sp³+sp²) ratio of the ECR-75 nano-carbon film was

reduced by the ECP since the sp^2 bonds on the film surface were also oxidized to form C-O and C=O bonds and gasification occurred. However, the increase in the O1s/C1s ratio on the ECR-75 nano-carbon electrode surface was much less than that observed for the GC and ECR-20 nano-carbon films. This is because the ECR-75 nano-carbon film has fewer sp^2 bonds than the GC and ECR-20 nano-carbon films, and forms an sp^2 and sp^3 nanocrystalline structure, which is very stable with respect to electrochemical oxidation. This is supported by the result for the wider potential window of ECR-75 nano-carbon film electrode. The increase in the O1s/C1s ratio was also small at the BDD since the diamond crystalline structure was stable as a result of electrochemical oxidation and there were very few sp^2 bonds on the carbon surface compared with the other carbons. This suggests that the number of sp^2 bonds on the BDD surface became even lower as a result of the ECP as previously reported by Fujishima et al. and Swain et al.,[8,14] although it was difficult to determine the $sp^2/(sp^3+sp^2)$ ratio because of the very small quantity of sp^2 bonds and the effects of other components in the C1s. We performed an AFM measurement to observe directly the surface morphological change caused by the ECP.

Figure 3 shows AFM images of carbon electrode surfaces before and after the ECP.

Figure 3. AFM images of carbon electrode surfaces before and after the ECP determined from $5 \times 5 \mu\text{m}^2$ images . (a, b) ECR-75 nano-carbon film before and after ECP, (c, d) ECR-20 nano-carbon film before and after ECP, (e, f) GC

After the ECP, the Ra had increased from 0.13 to 4.3 nm at the ECR-20 nano-carbon film and from 2.4 to 4.6 nm at the GC, indicating that the surface roughness becomes similar after the ECP in spite of an initial difference in flatness. This suggests that the ECR-20 nano-carbon film exhibits similar etching resistance to that of the GC. By contrast, the Ra of the BDD remained almost the same after the ECP since the BDD mainly consisted of inert diamond crystals for chemical etching by electrochemical oxidation. Therefore the Ra depends on the crystal size and shape. Although it has been reported that sp^2 carbon decreases on the BDD surface after the ECP, the very small sp^2 carbon content before ECP may not contribute to increasing Ra. The Ra of the ECR-75 nano-carbon film was increased from 0.067 to 0.21 nm by the ECP. However, the Ra increase was more than one order of magnitude lower than those

with the GC and the ECR-20 nano-carbon films. In a previous report by some of the present authors (APL Hirono et al.), the parallel-layered structure assigned to graphene sheets decreases and a curved and fine-closed structure becomes dominant when the acceleration voltage increases from 20 to 75 V.[23] The increase in the curved and fine-closed structure indicates increasing sp^3 content. This suggests that the structural change in the nano-carbon film could prevent deep corrosion of the surface and make it possible to maintain a smooth surface after introducing oxygen-containing groups via ECP. In fact, the relatively deep corrosion regions observed in ECR-20 nano-carbon film after the ECP (Fig.3 d) could not be found in the image of the ECR-75 nano-carbon film after the ECP (Fig.3 b), indicating that the ECR-75 nano-carbon film electrode has also highly stable structure when subjected to chemical etching by electrochemical oxidation although its surface contains of 55% sp^3 bonds after ECP. At the same time, the conductivities of the ECR-20 and ECR-75 nano-carbon films deposited on an undoped silicon wafer are 38 and 20 S/cm, respectively. This suggests that the graphite layer becomes more stable if we introduce a certain amount of sp^3 carbon with a minimum reduction in its conducting properties. We also studied the variations in apparent C^0 after the ECP at four carbon electrodes since the apparent C^0 values obtained by CV reflect surface conditions

such as surface area and the quantity of oxygen-containing groups.[2,4,5,8,19,26,39]

The C^0 values of the GC and ECR-20 nano-carbon film electrodes increased greatly from 20.3 to 69.6 and from 11.6 to 84.0 $\mu\text{F}/\text{cm}^2$, respectively, after the ECP. This was due to the increase in surface roughness and the amount of surface oxygen of these electrodes. The C^0 value of the ECR-75 nano-carbon film electrode also increased from 11.1 to 20.1 $\mu\text{F}/\text{cm}^2$, but this was much less than of the increases observed for the GC and the ECR-20 nano-carbon film electrodes. This is because the small increases in the surface roughness and amount of surface oxygen at the ECR-75 nano-carbon film almost maintained the surface structure. In contrast, the C^0 of the BDD electrode was reduced from 5.1 to 2.4 $\mu\text{F}/\text{cm}^2$ by the ECP despite the small increase in the amount of surface oxygen. This lower C^0 results from the lower charge carrier concentration and few oxygen-containing groups on the surface rather than surface roughness as reported by Swain et al.[39] The impurity bonds (mainly sp^2 carbon) on the electrode surface were etched by the oxidative gasification as several researchers have already reported that the C^0 was decreased by the ECP owing to the reduction in surface impurities such as sp^2 bonds.[8,14,16,18] In contrast, the contact angle was reduced at all the electrodes after the ECP, demonstrating that these surfaces had become hydrophilic. These surface properties

of the ECR-75 nano-carbon film are useful in terms of electrochemical measurement since we will be able to obtain relatively high electrode activity as with the GC electrode at high potential regions, and the BDD electrode has a low electron transfer rate since graphite carbon material exhibits better activity for many analytes than diamond material. This is particularly advantageous when measuring various organic electroactive species with high oxidation potentials. The sufficiently hydrophilic and smooth surface of the ECR-75 nano-carbon electrode is also advantageous for suppressing fouling when measuring electroactive biomolecules. A comparison of the electrochemical responses at the above carbon electrodes will be discussed in a later section.

3.2. Electrochemical responses of redox species at carbon electrodes before and after ECP

Several groups have reported that the properties of the carbon electrode surface (e.g. amount of oxygen, roughness, and amount of sp^2 bond) have an effect on the electrochemical responses of many redox systems. Since ECP greatly changes the carbon electrode surface, we studied the variations in electrochemical responses for several redox species before and after ECP.

Table 2. Peak separations (ΔE_p) of different redox species at the carbon electrodes before and after ECP.

	ECR-75		ECR-20		GC		BDD	
	Before	After ^a	Before	After ^a	Before	After ^a	Before	After ^a
$\Delta E_p \text{ Ru(NH}_3)_6^{3+/2+}$ (mV) ^b	63	60	69	63	65	71	111	106
$\Delta E_p \text{ Fe}^{3+/2+}$ (mV) ^b	657	65	628	79	614	81	861	770
$\Delta E_p \text{ Fe(CN)}_6^{3-/4-}$ (mV) ^b	94	60	83	65	80	66	96	425
$\Delta E_p \text{ DA}$ (mV) ^b	339	388	288	88	294	31	560	1001

^aECP conditions for each electrode are described in the experimental section.

^bThe supporting electrolyte for 0.1M $\text{Ru(NH}_3)_6^{3+/2+}$ and $\text{Fe(CN)}_6^{3-/4-}$ was 1 M KCl, while 0.5 mM $\text{Fe}^{3+/2+}$ and DA were prepared in 0.1M HClO_4 . The potential sweep rate for $\text{Ru(NH}_3)_6^{3+/2+}$, $\text{Fe}^{3+/2+}$ and DA was 0.1 V/s, while the CV of $\text{Fe(CN)}_6^{3-/4-}$ was carried out at 0.01 V/s.

As shown Table 2, the ΔE_p at all the carbon electrodes remained almost unchanged after the ECP owing to the high electron transfer rate and its surface insensitivity. It is well known that the kinetics of $\text{Ru(NH}_3)_6^{3+/2+}$ are mainly influenced by the density of electronic state.[9,12,14,17-19] However, the ΔE_p value of $\text{Ru(NH}_3)_6^{3+/2+}$ at the BDD electrodes employed in this experiment was slightly high, irrespective of the ECP. By contrast, the ΔE_p value of $\text{Fe}^{3+/2+}$, whose electron transfer is sensitive to surface carbonyl groups,[2,4,9,12,14,18,19] was decreased by the ECP at all the electrodes owing to the increase in the amount of surface oxygen. In addition, the surface roughness also contributes to the reduction in the ΔE_p value of $\text{Fe}^{3+/2+}$. The

decrease in ΔE_p at the BDD electrode after the ECP was much smaller than that at the other electrodes, although the O1s/C1s ratio was similar to that of the pretreated ECR-75 nano-carbon electrode (ECR-75: 0.098, BDD: 0.089). Notsu et al. reported that the ΔE_p of the $\text{Fe}^{3+/2+}$ at an anodically treated BDD electrode is larger than that at an anodically treated GC electrode since the carbonyl groups formed after the ECP on the BDD surface have strictly aliphatic characteristics (i.e. ketone type) unlike that of the graphite carbon surface (i.e. quinone type).[40] The large decrease in the ΔE_p of the $\text{Fe}^{3+/2+}$ at the ECR-75 nano-carbon film electrode suggests that the carbonyl groups on the surface are quinone type, the same as on the GC surface, due to the large amount of surface sp^2 bonds, which is unlike BDD. In addition, the ΔE_p of $\text{Fe}^{3+/2+}$ at the pretreated ECR-75 nano-carbon film electrode was comparable to or slightly lower than that of the pretreated ECR-20 nano-carbon film or GC electrode despite the large difference in roughness of their carbon surfaces. The results support the view that the ECR-75 nano-carbon film electrode depends mainly on the surface carbonyl groups. The ΔE_p values of $\text{Fe}(\text{CN})_6^{3-/4-}$ at the GC, ECR-20, and ECR-75 nano-carbon film electrodes were decreased by the ECP. On the other hand, the ΔE_p at the BDD electrode was increased greatly from 96 to 425 mV. By taking account of the fact that the electron transfer rate of $\text{Fe}(\text{CN})_6^{3-/4-}$ at graphite electrodes is

dependent on the amount of exposed edge plane on sp^2 carbon but not the amount of surface oxygen as reported by several groups,[1,10,13,20] the amount of edge plane increased at the surfaces of the ECR-20 and ECR-75 nano-carbon film electrodes after the ECP as found with the GC electrode. In contrast, the electron transfer rate of $Fe(CN)_6^{3-/4-}$ at the BDD electrode was predominantly dependent on the amount of surface oxygen. Indeed, the pretreated BDD surface was more negatively charged by an increase in the hydroxyl or carboxyl groups although the increase in the quantity of surface oxygen-containing groups was less than those of the other electrodes and so the negatively-charged $Fe(CN)_6^{3-/4-}$ was repelled. Swain et al. also reported the same ECP effect at a BDD electrode.[18]

The ΔE_p values of DA at the GC and ECR-20 nano-carbon film electrodes were decreased from 294 to 31 mV and from 290 to 90 mV, respectively, after the ECP. This is because the ΔE_p of DA is highly dependent on the surface adsorption, which is attributed to the surface microstructure, the amount of surface oxygen, and the sp^2 bonds on the surface caused by the π - π interaction between the sp^2 bonds of the surface and polar aromatic DA.[10,20] The oxidation potential of DA was negatively shifted from 662 to 647 mV by the ECP at the ECR-75 nano-carbon electrode. This indicates that the electron transfer rate of the DA oxidation was

improved since the electrode surface has a sufficient number of sp^2 bonds for the DA oxidation even after they were reduced by the ECP. However the reduction potential of DA was further negatively shifted from 323 to 259 mV **despite the increase in the surface roughness**. This might be because, although the surface was hydrophilized by the ECP, the DA (oxidized form) became more hydrophobic as a result of oxidation. Therefore, the ΔE_p of DA was increased from 339 to 388 mV. On the other hand, the oxidation and the reduction potentials were positively and negatively shifted, respectively, by the ECP at the BDD electrode. (from 747 to 897 mV and from 187 to -104 mV, respectively) This is because the electron transfer rate of both oxidation and reduction of DA were decreased since the surface are induced by further decreasing the essentially low amount of the surface sp^2 bond.[8,10,16,18] Thus the ΔE_p of DA was increased greatly from 560 to 1001 mV compared with that of the ECR-75 nano-carbon electrode. From these results, we concluded that only the pretreated ECR-75 nano-carbon film electrode has a sufficiently high electron transfer rate for surface sensitive species such as $Fe^{3+/2+}$ and $Fe(CN)_6^{3-/4-}$ with only a small increase in C^0 and without decreasing the potential window. These unique properties of the film could be because the sp^2 bond is stabilized by the hybrid structure with sp^3 carbons. This is supported by the TEM image of our nano-carbon

film as described in the previous section since the layered structure of graphene apparently decreases in the ECR-75 nano-carbon film.

3.3. Application for measuring biologically important analytes

Since ECR-75 nano-carbon film maintains its wide potential window and shows high electrode activity for many species with very little increase in C^0 after the ECP, we employed the film electrode to detect biomolecules such as GSSG and 5-HT. These compounds are biologically important and low detection limits are required for practical applications. However, both compounds are difficult to use for electrochemical measurements in terms of their high oxidation potential or electrode stability.

The tripeptide glutathione (GSH) is the most prevalent thiol in many living cells, and is known as an antioxidant that protects cells from oxidative stress by the oxidation of GSH to GSSG.[41-43] Thus, it is important to measure the GSH and GSSG concentrations in cells to understand cell redox states. The GSSG concentration in a cell is usually two orders of magnitude lower than that of GSH. For the electroanalysis of GSH and GSSG, it is known that both overpotentials are very high at a conventional carbon electrode.[42,43] Since GSSG exhibits higher

overpotential than GSH, it is more difficult to measure GSSG than GSH. Therefore, several groups have reported the electrochemical measurement of GSSG by using either a carbon electrode with an electron mediator or a BDD electrode in an acidic medium.[42,43] We studied electrochemical responses of GSSG using carbon electrodes after the ECP in order to measure GSSG even in a neutral pH medium. Figure 4 shows the effect of ECP on the electrochemical oxidation overpotential of GSSG at carbon electrodes.

Figure 4. The effect of ECP on the electrochemical oxidation overpotential of GSSG at carbon electrodes. CVs in 0.1 M pH 7.0 PB with (solid line) and without (dotted line) 0.1 mM GSSG. (A) ECR-75 nano-carbon film (a) before and (b) after ECP, (B) GC (a) before and (b) after ECP, (C) BDD (a) before and (b) after ECP.

The dotted and solid lines indicate the absence and presence of GSSG, respectively.

The oxidation peak was not observed at the untreated ECR-75 nano-carbon film electrode at potentials below 1.6 V as shown Figure 4A-a. However, the oxidation

peak appeared clearly at 1.35 V for the pretreated ECR-75 nano-carbon film electrode (Figure 4A-b). This result indicates that the GSSG peak potential for the ECR-75 nano-carbon film electrode was reduced by the ECP due to the increase in the surface hydrophilicity with a sufficient quantity of sp^2 bonds on the surface for GSSG oxidation. In addition, the background current of the pretreated ECR-75 nano-carbon film in a high potential region (over 1.0 V) was much lower than that of the untreated electrode since the potential window becomes wider after the ECP. as described above. In the same way, the oxidation peaks at the GC electrode before and after ECP were observed at 1.39 and 1.35 V, respectively (Figure 4B). However, the peak for the pretreated GC electrode was not clear since the background current was increased greatly by the ECP. Although a clear peak was obtained at the untreated GC electrode, the signal to background current (S/B) ratio was much lower than that of the pretreated ECR-75 nano-carbon film electrode owing to the larger background current in the high potential range (pretreated ECR-75: 7.1, untreated GC: 2.3). The oxidation peaks were not observed at the BDD electrode before and after the ECP at potentials below 1.8 V. (Figure 4C). Although the background current was decreased after the ECP, the peak potentials of GSSG at both of the BDD electrodes were much higher than those of the other carbon electrodes due to small quantity of sp^2 bonds at

the surfaces. As a result, by using only the pretreated ECR-75 nano-carbon film electrode, we obtained a well-defined GSSG oxidation peak with a much higher S/B ratio than those of the GC or BDD electrodes, indicating that the electrode constituted a suitable material for achieving a low GSSG detection limit. In fact, we obtained a low detection limit of 0.4 μM (signal to noise ratio =3) at the pretreated ECR-75 nano-carbon film electrode in a background subtracted CV measurement. In contrast, we could not obtain such a low detection limit at the untreated GC electrode owing to higher background current and electrode surface fouling by irreversible adsorption for the GSSG oxidation product. This result reveals that the pretreated ECR-75 nano-carbon film is a particularly advantageous electrode material as regards the highly sensitive measurement of GSSG at pH levels at which cells can survive.

Next, we investigated the stability as regards the electrochemical detection of 5-HT at carbon electrodes before and after the ECP. 5-HT is a monoamine neurotransmitter in mammalian brain, and is related to various mental diseases such as panic disorder and depression.[44-47] The electrochemical 5-HT measurement has been carried out with conventional carbon electrodes since 5-HT is electrochemically oxidized at a low potential (typically 400 mV at pH 7.4).[45-47] However, electrode

surface fouling by the irreversible adsorption of oxidized 5-HT makes it difficult to employ for long-term measurements.[47,48] It is very important to detect 5-HT by liquid chromatography with an electrochemical detector since it complicates the procedure if the electrode incorporated in the flow-through cell must be frequently polished. Figure 5 shows the variation in the anodic peak current of CVs for 5-HT at carbon electrodes. The Y-axis in Fig. 5 shows the ratio of the 5-HT response at each electrode to the first measured peak current of the 5-HT.

Figure 5. The variation in the anodic peak current of CVs for 5-HT in 0.1 M pH 7.0 PB at carbon electrodes before and after the ECP. (a, b) the ECR-75 nano-carbon film before and after ECP, (c, d) GC before and after ECP, (e, f) BDD before and after ECP. Each measurement was carried out for 5 min. The potential sweep rate was 0.1 V/s.

These current ratios gradually decreased at all the electrodes except for the pretreated ECR-75 nano-carbon film electrode during the measurement period at intervals of 5 min. The reduction in these currents is caused by electrode surface fouling caused by

the adsorption of oxidized 5-HT. Sarada et al. reported that a stable measurement was achieved for 10 μM of 5-HT after three measurements with stirring by using the BDD electrode.[47] However, in our case, we did not obtain a stable 5-HT response even at the BDD electrode. This could be because our present study was conducted under more severe conditions (concentration; 30 μM and measuring times; 20) without stirring. In contrast, we obtained a stable current response of 30 μM 5-HT after 20 measurements at only the pretreated ECR-75 nano-carbon film electrode. This is because the hydrophilicity and the number of oxygen containing groups on the ECR-75 nano-carbon electrode surface increased without losing much of its surface flatness or many of its sp^2 bonds after the ECP. The hydrophilicity of the BDD electrode surface was also increased by the ECP. However, the surface flatness was initially high and the number of surface sp^2 bonds became very low after ECP. This makes the electron transfer rate of 5-HT very low as can be confirmed from the broader peak shape in the supplementary information. (Fig. S1(e)) The low electron transfer rate at the pretreated BDD surface was influenced by slight surface changes such as fouling, and this makes the electron transfer rate much lower. This can be clearly confirmed by the large peak potential shifts induced by potential cycles. (Supplementary Information)

Therefore, the pretreated ECR-75 nano-carbon film electrode exhibited a good anti-fouling property against 5-HT while maintaining a relatively high electron transfer rate. This result reveals that the pretreated ECR-75 nano-carbon film electrode is a significant material as regards the further development of electrochemical devices such as detectors for liquid chromatography, capillary electrophoresis and neurotransmitter sensors.

4. Conclusion

We investigated the ECP effect on the surface structures and electrochemical properties of ECR nano-carbon film electrodes compared with other carbon materials. At an ECR-75 nano-carbon film electrode, the ECP was effective as regards introducing oxygen-containing groups while largely maintaining the surface flatness (from 0.067 to 0.21 nm) and sufficient sp^2 content (from 58 to 45%). These properties result from the fact that this film has an sp^2 and sp^3 nanocrystalline structure that exhibits high stability against electrode oxidation. Such properties cannot be observed with the electrodes of lower sp^3 content such as ECR-20 nano-carbon film and GC electrodes.

As a result, high electrode activity was obtained by the ECP while largely maintaining a wide potential window (from 4.0 to 4.1V) and low C^0 (from 11.1 to 20.1 $\mu\text{F}/\text{cm}^2$), which is unlike conventional GC and BDD electrodes. This ECP of the ECR-75 nano-carbon film electrode was also effective for measuring biologically important analytes such as GSSG and 5-HT. For GSSG, the detection limit was improved at pH levels at which cells can survive since a sharp peak was obtained with a low background current due to the increase in the surface hydrophilicity with a sufficient number of sp^2 bonds and by widening the potential window. For 5-HT, we obtained stable current responses of 30 μM 5-HT during 20 measurements without stirring. This is because the surface hydrophilicity was increased without losing very much surface flatness. The pretreated ECR-75 nano-carbon film is a suitable electrode material for further improving the measurement of biomolecules, which ensures the construction of an electrochemical biosensor with higher sensitivity and stability.

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Figure captions

Figure 1. Schematic of ECR sputtering apparatus.

Figure 2. Background voltammograms of the carbon electrodes before and after the ECP in 0.05M H₂SO₄ deoxygenated with Ar. Scan rate: 0.1 V/s.

Figure 3. AFM images of carbon electrode surfaces before and after the ECP determined from $5 \times 5 \mu\text{m}^2$ images . (a, b) ECR-75 nano-carbon film before and after ECP, (c, d) ECR-20 nano-carbon film before and after ECP, (e, f) GC before and after ECP, (g, h) BDD before and after ECP.

Figure 4. The effect of ECP on the electrochemical oxidation overpotential of GSSG at carbon electrodes. CVs in 0.1 M pH 7.0 PB with (solid line) and without (dotted line) 0.1 mM GSSG. (A) ECR-75 nano-carbon film (a) before and (b) after ECP, (B) GC (a) before and (b) after ECP, (C) BDD (a) before and (b) after ECP.

Figure 5. The variation in the anodic peak current of CVs for 5-HT in 0.1 M pH 7.0 PB at carbon electrodes before and after the ECP. (a, b) the ECR-75 nano-carbon film before and after ECP, (c, d) GC before and after ECP, (e, f) BDD before and after ECP. Each measurement was carried out for 5 min. The potential sweep rate was 0.1 V/s.

Tables

Table 1. The surface properties and the C^0 of carbon electrodes before and after ECP.

Table 2. Peak separations (ΔE_p) of different redox species at the carbon electrodes before and after ECP.

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