ADSORPTION OF BIOMOLECULES ON NANOSHELL CARBON

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Introduction

Nanoshell carbon (NS) invented in our laboratory [1] has unique properties such as hollow shell like structure, size range of primary particle (20-50 nm in diameter) and high rate of electron transfer, etc. In addition, NS shows excellent dispersivity in aquenous solution without surface modification. Focused on these properties of NS, we have started the research to evaluate the applicability of NS in the field of bioscience. For the purpose of mentioned above, adsorption behavior of biomolecules such as various proteins on NS was investigated. Possible mechanisms for adsorption of biomolecules on NS surface were also discussed from physicochemical properties of biomolecules and NS.

Experimental

The carbon precursor was a phenol-formaldehyde resin. The metal complexes were phthalocyanines of cobalt and iron (Tokyo Kasei Co. Ltd.) were used without further purification. NS was prepared by the following method. A phenol-formaldehyde resin was mixed with cobalt phthalocyanine or iron phthalocyanine in acetone, and then the mixture was carbonized at 800, 1000 and 1200 ºC in a nitrogen stream for 1 h. The obtained carbons were pulverized by using a planetary ball mill followed by acid treatment with conc. HCl in order to remove the metal species appeared on the surface of the carbons. The carbonized samples are distinguished by the abbreviation of the type of complex, and the carbonization temperature. For example, the carbon prepared with 3 wt % of CoPc which was carbonized at 800 ºC was denoted as CoPc800. The proteins used as makers were bovine serum albumin (BSA, M.W. 69 kDa, pI 4.9), cytochrome *c* (cyt *c*, M.W. 12.4 kDa, pI 10.1), holo-Transferrin (holo-Tf, M.W. 77kDa, pI 5.0-5.6), lysozyme (Lys, M.W. 14.4 kDa, pI 11) and myoglobin (Myo, M.W. 17.8 kDa, pI 7.0) were purchased from Sigma (St. Louis, Mo). Horseradish peroxidase (HRP, M.W. 44 kDa, pI 7.2) was purchased from Wako pure chemical.

Transmission electron microscopic (TEM, JEM 2010, JEOL) observation was carried out for the ground NS placed on a copper grid coated with an amorphous carbon film with an acceleration voltage of 200 kV.

N_2 adsorption isotherms were obtained at liquid nitrogen temperature with an automatic apparatus (BELSORP 28SA, Japan BEL Co. LTD.). Before the measurements, the samples were evacuated at 200 ºC for 2 h. BET analysis and D-H analysis were applied to the isotherms in order to obtain the surface areas and mesopore volumes.

The slurry was prepared by ultrasonically blending the mixture of NS (5 mg) with 5 % Nafion solution (50 μl, Aldrich), ethanol (150 μl) and distilled water (150 μl) in a plastic vial. The slurry (4 μl) was then placed on the glassy carbon disc electrode (6 mm Ø). The electrolyte was 0.5 M H_2SO_4 aqueous solution, and the counter and the reference electrodes were a platinum plate and an Ag/AgCl electrode, respectively. Cyclic voltammetry was recorded by a dual-potentiostat (ALS 700, BAS Japan, Inc.).

Evaluation of the protein adsorption to NS was performed as follows. NS (10 mg) was suspended in 1 ml of 10 mM phosphate buffer (pH 7.4), incubated for 10 min, centrifuged at 15,000 rpm. Then, aliquot of 500 μl of the supernatant was removed, and equal amount of a protein solution (3 mg/ml) was added. Incubation was carried out for 1 h on a ROTATOR RT-50 (TAITEC). Supernatants were obtained by centrifugation at 15,000 rpm (twice). The amount of proteins adsorbed on NS was determined from the supernatant by using bradford method. All experiments were performed at room temperature.

Results and Discussion

NS has curved shell-like structures with laminated graphitic layers. It was supposed that this unique structure was introduced by the metal catalyst produced from pyrolyzed metal complex which was added in the preparation of NS precursor. This is a result of a kind of catalytic graphtization effects [2]. Size range of the primary particle was about 20-50 nm, which is approximately 2 to 10 times larger than spherical molecular diameter of proteins used in this study. Primary particles of NS were conglomerated each other to form larger aggregation.

In order to evaluate the extent and the behavior of the protein adsorption of NS, commercially available proteins with various isoelectric points (pI) and molecular weights (M.W.) were used as makers. CoPc800 and FePc800 were selected as the typical NSs. It was found that NSs could adsorb all kinds of proteins used in this experiment (Fig. 1). FePc800 showed better protein adsorption ability compared to that of CoPc800. Since the extents of adsorption did not depend on the pI-values of proteins, the adsorption onto the NSs was not governed by simple electrostatic interaction. Possible mechanisms for adsorption of proteins should be considered focusing on physicochemical properties of NS. In the previous works, it was suggested that hydrophobic interaction and π-π stacking are the main driving force of adsorption between biomolecules including nucleic acids and nanocarbon materials [3,4]. On the other hand, the presence of quinone group which react with amino or guanidino group of proteins [5] would be important. Considering the former case, it was known that the catalytic graphitization ability of Fe catalyst was stronger than that of Co catalyst. It was considered that graphitic structures of FePc800 were better developed than that of CoPc800. From the results observed here, it was estimated that NS carbonized at higher temperatures were suitable for protein adsorption. To verify

this hypothesis, correlation between carbonized temperature and adsorption amount of protein was investigated using CoPc1000, CoPc1200 and FePc1000. Cyt *c* was selected as a maker. As expected, the adsorption amount of cyt *c* was directly proportional to carbonization temperature in this experimental condition (Fig. 2). NSs with higher carbonization temperature showed better adsorption. It was considered that higher carbonization temperature was associated with a higher degree of graphitization. It was considered that proteins would be interacted with NS surfaces via hydrophobic interaction and π-π stacking between aromatic residues of proteins and graphite structure of NS.

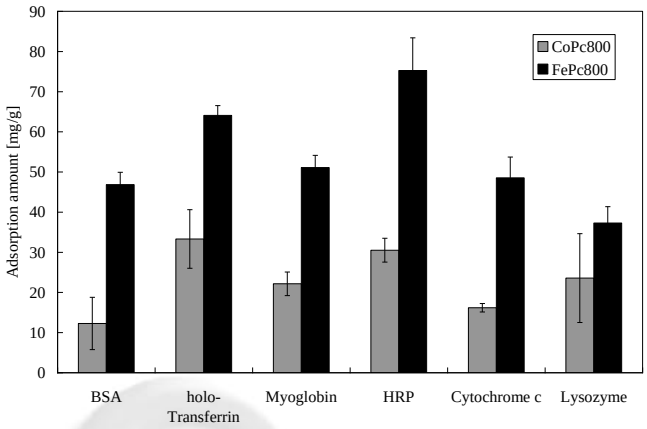

Fig. 1 Adsorption of various proteins on CoPc800 and FePc800. Error bars represent the standard deviation for three replicates.

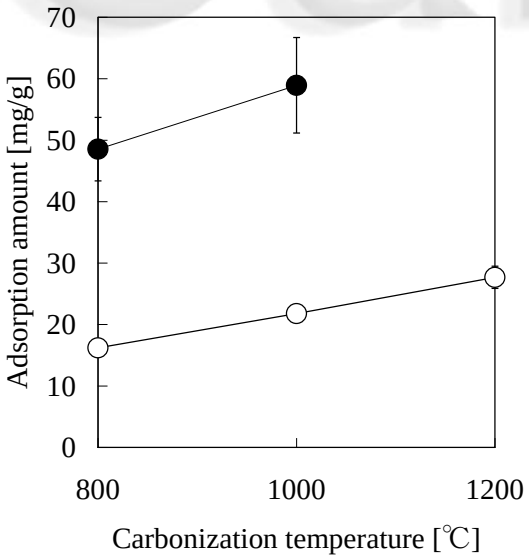

Fig. 2 Correlation between carbonization temperature of NS and cyt *c* adsorption amount: ●; FePc series, ○; CoPc series. Error bars represent the standard deviation for three replicates.

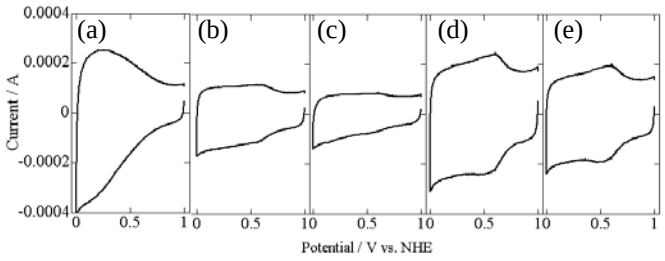

Fig. 3 Cyclic voltammograms of (a) CoPc800, (b) CoPc1000, (c) CoPc1200, (d) FePc800 and (e) FePc1000. Scan rate was 50 mVs^{-1}.

Concerning to the conformation of the qunone group on the surface of NS, the CV measurement was performed (Fig. 3). The CV of FePc series showed the well-defined redox wave around 0.6 V vs. NHE, which was attributed to the hydroquinone-quinone redox. Interestingly, in the case of CoPc1000 and CoPc1200, the redox current around 0.6 V was slightly observed even though it was not confirmed in the case of CoPc800. The result obtained here was consistent with the increase of cyt *c* adsorption amount shown in Fig. 2. The difference in the adsorption amount of cyt *c* compared with FePc series and CoPc series observed here would be dependent on the amount of quinone group. Adsorption mechanism would be based on hydrophobic interaction, π-π stacking and reaction between the quinone group of NS and the amino group or guanidino group of proteins.

Conclusions

NS have the ability for the adsorption of various proteins used in this study. The results obtained here suggest that NS have the potential for use as novel carrier for immobilization of proteins.

Further work is in progress for example, the effective method for recovery of adsorbed protein and investigation for the activity of immobilized protein on NS surface.

References

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