Transactions of the Materials Research Society of Japan 31[4] 1069-1072 (2006)

Biofouling Control by Phospholipid Polymer on Microchannel DNA Separation

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Several biocompatible phospholipid copolymers were evaluated as surface modification materials for capillary electrophoresis. The poly-[2-methacryloxyethylphosphorylcholine (MPC) -*co*-3-methacryloxypropyltriethoxysilane (MPTS)] (PMSi) modified surface showed the good uniformity and the negative ζ-potential around -10 mV. T4 (165.6 kbp) DNA and λ (48.5 kbp) DNA were separated by used of the capillary modified with PMSi, and the capillary with PMSi can use by repetition without heavy alkali cleaning. The chemical stability for long-time use was achieved by permanent bonding of copolymer toward the silica surface. The results of DNA separation suggested that the PMSi modified surface showed the excellent biocompatibility, namely non-biofouling on the capillary surface, and the appropriate ζ-potential for the capillary zone electrophoresis (CZE). MPC polymer with silane coupling unit has a grate potential for the DNA separation on microchip as well as a conventional CZE.
Keyword: DNA Separation, 2-methcryloxyethylphospholylcholine(MPC), Capillary Electrophoresis, Surface Modification

1. INTRODUCTION

Capillary electrophoresis (CE) has emerged as a powerful technique for separation analysis of biomolecules, including peptides, proteins, nucleic acids and drugs [1-3]. Many micro-fluidics devices for a chemical and a biochemical analysis, so-called μ-TAS (micro-total analysis systems) have been developed owing to their features, such as design flexibility, ease of fabrication, and on-chip integration [4]. From the viewpoint of DNA separation, the microchip electrophoresis (MCE) has been expected as a new separation method because of high throughput comparing with conventional CE [5,6]. However, there exist still some problems in DNA separation analysis for CE and MCE. On the conventional CE and MCE, it is necessary to wash the capillary inner wall for long time around several hours by the alkali solution and the purified water at each measurement, because DNA and biomolecules adsorb the inner wall of capillary or micro-channel, it leads to deteriorate the analytical accuracy. To avoid the complex cleaning process after finishing the analysis, the addition of properties on anti-bioadhesion and appropriate ζ-potential should be required. In this study, we have created a biocompatible interface by using several typed phospholipids polymers showing the suitable biocompatibility [7-9], to achieve the high performance DNA separation by capillary zone electrophoresis (CZE) using microchip with micro or nano channel. The modified inner wall of capillary is able to expect the effect of suppressing the adsorption of the biomolecules. To examine the optimum modified conditions, we use T4 and λ DNA as a model biomolecule. Moreover, the homogeneous coating characteristic of phospholipids polymers was evaluated by Rhodamine6G [10] which adsorbed in static electricity with the phosphorylcholine(PC) groups.

2. EXPERIMENTAL

2.1 Materials

Poly[2-methacryloxyethyl phosphorylcholine (MPC)-*co*-(*n*-butylmethacrylate(BMA)) (PMB30, MPC 30 mol%) [7], poly[2-methacryloxyethyl phosphorylcholine (MPC)-*co*-3-(methacryloxypropyl)-triethoxysilane (MPTS)] (PMSi, MPC 90 mol%)[8], and poly(potassium3-methacryloyloxypropyl-sulfonate (PMPS)-*co*-MPC-*co*-BMA(PMSB) [9] are used as biocompatible phospholipids polymers. The chemical structures of the MPC copolymers are illustrated in the Figure 1, which are synthesized by our laboratory. ζ-potentials of PMB30, PMSi, and PMSB are 0 mV± 5 mV, -10 mV±5 mV, and -20 mV±5 mV in the electrophoresis buffer (45 mM Tris-borate, 1 mM EDTA,

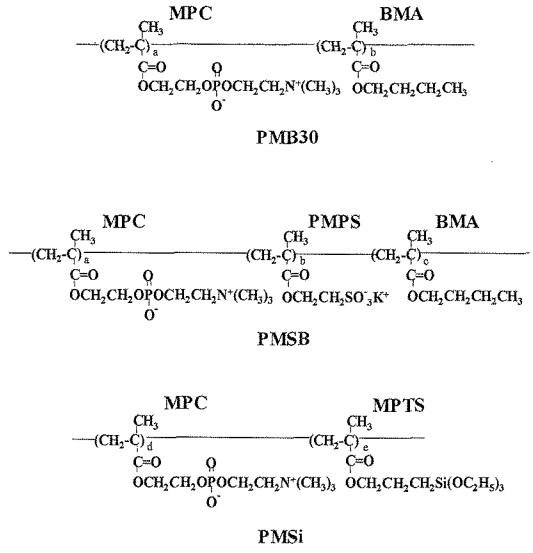

Fig. 1 The chemical structures of MPC polymers

pH 8.0; TBE). Rhodamine6G was received by Aldrich Chemical Japan and the concentration is 1 wt%. Bacteriophage λ DNA (48.5 kbp) and T4 DNA (165.6 kbp) were purchased by Nippon Gene, Tokyo, Japan, and used for DNA separation. DNA separation buffer was used TBE.

2.2 Coating procedure on the inner surface of capillary

The following procedure was performed on a 52 cm length of fused-silica capillary (Outer Diameter 375 μm × Inner Diameter 75 μm). MPC copolymers (PMB30, PMSi, and PMSB) were dissolved in ethanol to give a final concentration of 0.05 wt%. PMB30 and PMSB solutions were flowed the capillary for 3 min, after elimination of the polymer solution from the capillary, it was stored overnight in the vacuum desiccator. PMSi solution was also flowed through the capillary for 3 min. After elimination of polymer solution, the capillary was dried for 4 hr at 70 °C.

2.3 Surface characteristics

To evaluate homogeneous coating of the capillary surface modified with MPC copolymers, we used Rhodamine6G. Rhodamine6G were dissolved in purified water to give a final concentration of 200 ppm. This solution was flushed through the polymer-coated capillary for 70 sec, then it was washed out by the purified water for 90 sec. The fluorescence intensity in the capillary was observed with fluorescence microscope (Axioskop2 plus: Carl Zeiss) integrated with a CCD camera (Keyence VB-7010). In addition, the chemical composition of the capillary inner surface modified with MPC copolymers was determined by X-ray photoelectron spectroscopy(XPS) on AXIS-ULRTA (Kratos-Shimazu Analytical). The scan was taken at take-off angle of 90°.

2.4 Capillary zone electrophoresis

T4 DNA(165.6 kbp) and λ DNA(48.5 kbp) were separated by the electrophoresis with CAPI3200 (OTSUKA ELECTRONICS CO.,LTD). DNA samples were prepared as stock solutions at 0.4 mg/mL in an electrophoresis buffer (TBE) and stored at 4 °C. An antioxidant agent does not include in the separation buffer in this study. Electrophoresis was performed with a -15 kV power supply and DNA was detected from the absorbance of 260 nm. The typical field strength was 300 V/cm, leading to currents ranging from 10 to 20 μA.

2.5 Measurement of adsorbed proteins on capillary

FITC-albumin solution (0.45 g/dL in PBS) was introduced to the capillary and incubated for 60 min at 37 °C. After that, the channel was rinsed two times by fresh PBS solution. Fluorescence intensity of the capillary was observed with a fluorescence microscope.

3. RESULTS AND DISCUSSION

3.1 Coating characteristics evaluated by Rhodamine6G and XPS

The result of using Rhodamine6G of capillary surface modified with MPC copolymers (PMB30, PMSB, and PMSi) and capillary without coating were shown in Fig.2. The capillary surfaces modified with PMB30 and PMSB show strong fluorescent intensity compared with the capillary without coating and with modified PMSi. The fluorescent intensities on capillary modified with PMB30 and PMSB are very homogeneous. Therefore, the surface was completely covered with MPC copolymers with good uniformity. Though the capillary with modified PMSi does not imply the polymer coating by the fluorescent image study, XPS data indicated that PMSi was coated on the capillary surface and the data was shown in Fig.3. The small signals of P2p at 133 eV

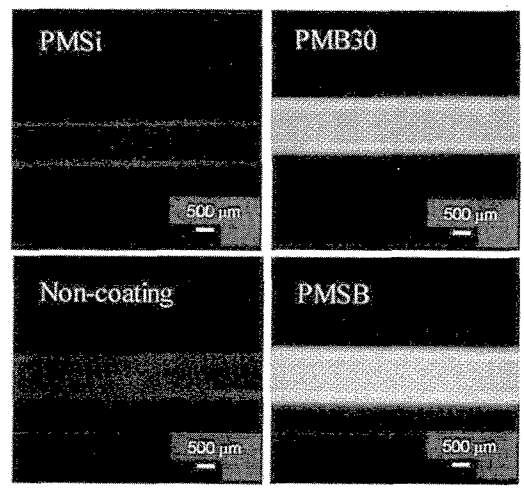

Fig.2 Fluorescent microscopy image by Rhodamine6G of capillary surfaces modified with MPC polymers.

attributed to phosphorus, and N1s at 402 eV attributed to nitrogen from the phosphorylcholine(PC) groups in PMSi are appeared. The data suggests the existence of PMSi on the inner capillary surface. The big difference on fluorescent intensity between PMB30, PMSB and PMSi is not clear, however, it is thought the one reason is film thickness of polymers. In our previous study, the film thickness of PMB30 with the concentration of 0.3wt% copolymer is around 30~50 nm, while the thickness of PMSi is several nm [8].

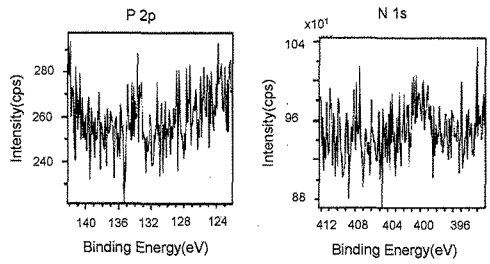

Fig. 3 XPS spectra on the capillary inner surface modified with PMSi.

3.2 Capillary Zone Electrophoresis (CZE)

To evaluate the MPC polymer coating effect such as non-biofouling property on CZE, T4 DNA and λ DNA were separated using unmodified, and MPC polymer modified silica capillary. When using the unmodified capillary having large negative surface ζ-potential around -60 mV, the separation times of T4 and λ DNA were 3.5min and 4.5min, respectively. It takes several hours for alkali cleaning to remove the adsorbed DNA on the capillary surface before the next measurement.

As for the capillary modified with PMB30, the detection times of T4 and λ DNA became short at 20sec, and both DNA was observed at the same time. The cause of the result of the capillary modified with PMB30 is suggested the small ζ-potentials almost zero and the effective electro-osmosis flow (EOF) is not achieved. When using the capillary modified with PMSB having ζ-potentials of -20 mV±5 mV, the T4 DNA and λ DNA did not separate completely, like the capillary modified with PMB30. Moreover, the separation times using the capillaries modified with PMB30 and PMSB showed large variation at every measurement. The reason on the instability of detection time at every measurement was thought the removing of polymer film on the silica surface in the condition of a high electric field during electrophoresis. Certainly the separation times of T4 DNA and λ DNA after several measurements without any alkali cleaning were shifted to values of the standard silica capillary. Thus, the separations of T4 DNA and λ DNA by CZE with capillary surfaces modified with PMB30 and PMSB are not carried out sufficiently.

PMSi modified surface is expected to prepare the permanent polymer coating on the silica surface through silane coupling reaction. The DNA separation will achieve by using PMSi coated capillary due to negatively surface ζ-potential around -10 mV. The result of CZE by the capillary modified with PMSi was shown in Fig.4. T4 DNA can detect at 3.2 min, λ DNA can detect at 3.6 min, respectively. As seem in Fig.4(B), the detection times of T4 DNA and λ DNA are almost same at 3.1 min, and 3.5min, respectively. The second measurement data was obtained using a simple cleaning process in which the purified water for 30 min was performed after first DNA separation measurement. When CZE is performed by use of the DNA buffer which is stored for 2 hours at room temperature, several detection peaks are newly appeared from 3 min to 4 min as shown in Fig.4(C). It is considered that the results come from DNA fragment by oxidation, because antioxidant agents does not include into the DNA separation buffer. However the small

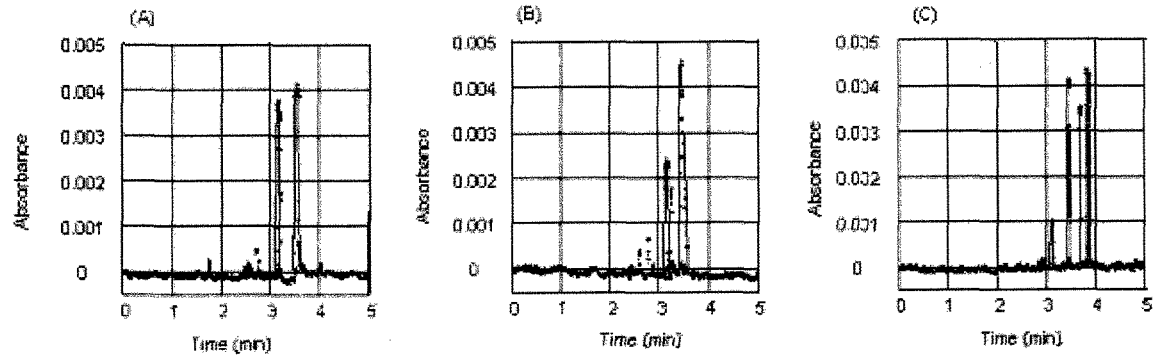

Fig. 4 DNA separation results obtained by CZE using the PMSi modified capillary. (A) first measurement, (B) second measurement, (C) data using the DNA buffer stored for 2 hours under room temperature condition.

peaks around 2.5 min, and different absorbance intensity between first and second measurements are still unclear, DNA can be separated with PMSi modified capillary. Therefore the stable interface between PMSi and silica substrate was constructed by the chemically modification. And the heavy alkali cleaning does not need for DNA separation by CZE.

Finally, we clarify the non-biofouling property of PMSi modified capillary. An amount of adsorbed protein was evaluated by FITC-labeled albumin. The intensity of FITC is significantly high comparing with that of the PMSi modified capillary as seen in Fig. 5. Thus, the PMSi modified capillary shows high resistive surface toward the protein adsorption.

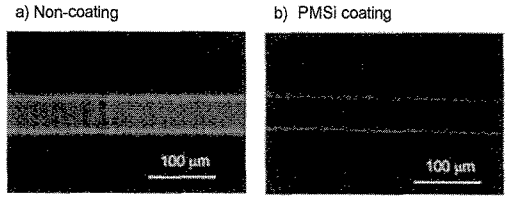

Fig. 5 Fluorescence images of a) bared and b) PMSi modified capillary after immersed FITC-labeled albumin.

As the results of DNA separation with PMSi modified capillary, DNA separation ability of capillary modified with PMSi is greater than those of PMB30, and PMSB. The effect of the EOF is not so large comparing with the standard silica capillary, but the ζ-potentials of PMSi is enough for the DNA separation. The biggest advantage of modified surface is non-biofouling property. The capillary can use several times without heavy alkali cleaning, it allows for shorter operation time. And the instability showing the MPC copolymers (PMB30 and PMSB) has improved by using the PMSi. The suitable duplicate measurement on PMSi modified capillary is obtained with the design of silane coupling unit in the MPC copolymer, which is bonded chemically on the silica surface.

4. CONCLUSION

MPC copolymers (PMB30 and PMSB) used in study were coated homogeneously in inner wall of capillary evaluated from fluorescent intensity of Rhodamine6G. PMSi was coated to the capillary determined by the result of XPS. The MPC polymers (PMB30 and PMSB) adsorbed on the silica surface physically, are easy to remove from the surface under the condition of electrophoresis with applied high voltage. While the PMSi constructed useful coating for the separation of T4 DNA and λ DNA by CZE due to an appropriate ζ-potentials on the surface produced by an effective EOF. The capillary with PMSi can be used DNA separation for repeatedly without any cleaning process. There are two reasons; one is the excellent non-biofouling property (it means to inhibit biomolecule adsorption on the substrate surface such as DNA, protein, etc.) due to MPC polymer, the second is the stable bonding between PMSi and silica substrate.

In conclusion, the MPC polymer with silane coupling unit has a great potential as suitable modification material of capillary to separate of many different sized DNA by CZE. It is believed that the MPC polymer is a candidate for separation analysis of DNA by MCE, and any microfluidic devices handled biomolecules.

5. ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for scientific Research (B) from Ministry of Education, Culture, Sports, Science and Technology, Japan (16310084).

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(Received December 10, 2005;Accepted August 3, 2006)