

高アフィニティ分子鑄型高分子ナノ粒子の創製と電気化学バイオセンサへの応用

Development of molecularly imprinted polymer nanoparticle with high affinity
and its application to electrochemical biosensor¹The Univ. of Tokyo, ²Univ. of California, Berkeley, ○Youyuan Man¹, Shoichi Nishitani², Toshiya Sakata¹

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Background. Molecularly imprinted polymers (MIPs) have the potentials to be low-cost and stable alternatives to natural receptors such as enzymes and antibodies for biosensors. In our group, we have been developing enzyme-/antibody-free electrochemical biosensors with the MIP-coated electrodes [1,2]. However, the conventional polymerization methods for MIPs have the limitations such as their heterogeneities that reduce high binding affinity sites and the presence of residual templated molecules, which are detrimental to biomolecular recognition. To address these issues, we have synthesized molecularly imprinted polymer nanoparticles (nano-MIPs) with virtually free of heterogeneous templates and higher affinity for target biomolecules by using a solid-phase approach synthesis method [3]. In this study, we have focused on the biomarkers such as human serum albumin (HSA) for diabetes and then synthesized the redox-labeled HSA-templated nano-MIPs (HNMs) for electrochemical biosensor.

Methods. Derivatized glass beads, which were chemically immobilized with HSA, were used as solid phase. The polymerization mixture was composed of *N*-Isopropylacrylamide (NIPAm, 514 mg), *N*-tert-butylacrylamide (TBAm, 157 mg), Acrylic acid (AAc, 30 μ L), *N*-(3-aminopropyl)methacrylamide hydrochloride (APM, 58 mg), ferrocenylmethyl methacrylate (FMMA, 17 mg), and *N,N'*-Methylenebisacrylamide (BIS, 40 mg), which were dissolved in a mixture of 100 mL water/ethanol = 85/15 (v/v) and mixed with 30 g of derivatized glass beads. Polymerization was initiated by 2,2'-AzoBIS(2-methylpropionamidine)dihydrochloride (V50, 27 mg) and carried out at 60 °C for 12 h. The beads were then washed with distilled water at 60 °C using a fritted solid-phase extraction (SPE) cartridge. High affinity HNMs were eluted with water at 0 °C. The HNM chemical composition was measured by proton nuclear magnetic resonance analysis (¹H NMR) spectroscopy, and the hydrodynamic radius and polydispersity index (PDI) were evaluated by dynamic scattering spectroscopy (DLS). AFM images of HNMs were also collected to measure the size under dry state. Afterward, HNMs were immobilized on Au electrodes to evaluate electrochemical characteristics based on a cyclic voltammetry (CV) method. Finally, the interaction of HSA with HNMs was evaluated by surface plasmon resonance (SPR) spectroscopy.

Results and Discussion. From the ¹H NMR spectrum, the components of HNMs were expectedly confirmed. The DLS analysis shows that the hydrodynamic diameter of HNM was 154 nm, while the PDI was 0.54 (Fig. 1). The AFM image shows a smaller HNM diameter of 54 nm, which was due to the shrinkage of polymer upon drying (Fig. 2). On the basis of the SPR spectra, moreover, the dissociation constant of HNM was calculated to be 0.43 μ M (Fig. 3). The electrochemical properties of HNMs immobilized on the Au electrodes will also be reported on the day of conference.

References [1] Kajisa, T.; Sakata, T. ACS Appl. Mater. Interfaces 2018,10, 34983–34990. [2] Sakata, T.; Nishitani, S.; Kajisa, T. RSC Advances 2020, 10, 16999–17013. [3] Canfarotta, F. et al. Nat. Protoc. 2016, 3, 443455.

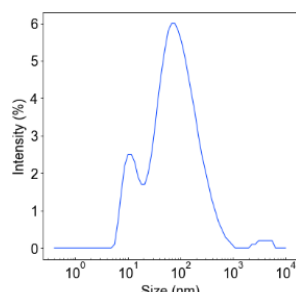


Fig. 1 DLS analysis

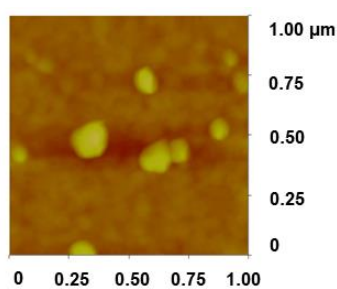


Fig. 2 AFM image

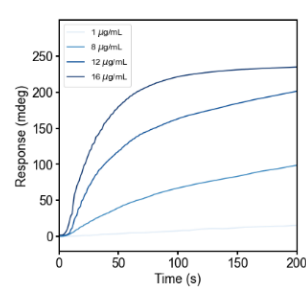


Fig. 3 SPR analysis