Crystallized Protein-imprinted Polymer Chips

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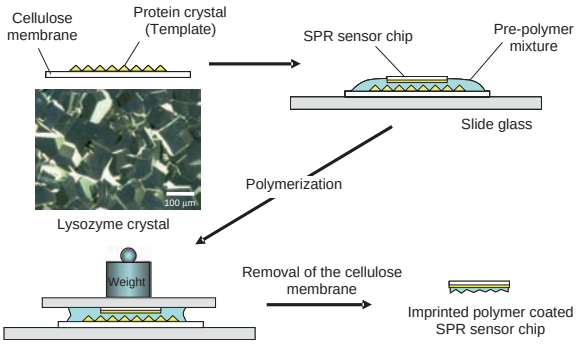
Protein-imprinted polymer thin films were prepared by using a crystallized protein as a template in aqueous solution. Motifs of surface of crystallized lysozyme can be imprinted and the resulting imprinted polymer thin films showed specific binding behaviors for the template protein.

Protein recognition materials have been of great importance in order to construct biochips for protein profiling, diagnostics of diseases, sensing elements and other applications in life sciences.[1] To date, protein recognition is conducted by using biomolecules such as antibodies, proteins, and aptamers. These biomolecule-based methods are sensitive and selective, but it is difficult to find or produce highly specific biomolecules for given targets with tailor-made fashion. They are usually expensive and short shelflife, and the immobilization is tedious and time-consuming. In contrast, synthetic polymer materials are highly stable and easily mass-produced, therefore, synthetic polymer-based protein recognition materials are highly desirable.

Molecular imprinting has been recognized as a template polymerization technique for preparing synthetic polymers bearing molecular recognition ability.[2] So far most of protein imprinting is conducted in solution,[3] however, this will not suitable for mass-production of imprinted polymer chips with wide diversity, since proteins cannot stored long time in solution due to their poor stability. In addition, proteins may be denatured and/or transformed during the radical polymerization to produce protein imprinted polymers. Therefore, we utilized a crystallized protein as a molecular template in this work to assemble functional monomers that are supposed to form the protein binding sites after the polymerization. Crystallized proteins are regularly oriented with no mobility; therefore the transcription of the surface by functional monomers interacting with the protein may be easier than conventional molecular imprinting using dissolved protein molecules with thermodynamic molecular motion. Polyurethane-based surface imprinting of living cells and crystals has been conducted in THF,[4] however, organic solvents may affect the conformation of proteins on the surfaces. Therefore, we employed aqueous solution to be able to keep the surface of proteins as it is during the imprinting process.

Crystallized lysozyme was used as a model template. After dropping a pre-polymerization mixture containing acrylic acid as a functional monomer, methylene bis(acrylamide) (MBAA) as a crosslinker, 2-methacryloylethyl phosphorylcholine (MPC) as a co-monomer, polyethylene glycol (PEG) as a precipitant and potassium peroxodisulfate as an initiator in HEPES buffer (pH 7.4), a glass chip with a gold surface treated with *N*,*N*′-bis(acryloyl)cystamine (BAC) was placed on the cellulose membrane, then polymerization was carried out (Lys-CIP).[5] A non-imprinted polymer (NIP) was also prepared without the crystal. After washing with acetone to dissolve the cellulose membrane, the chip was successively washed with 1 M NaCl to remove the possibly remained protein from the chip surface completely. PEG was added to prevent the crystal from dissolution. MPC was co-polymerized to reduce non specific binding of proteins.[6] The BAC treatment was carried out to obtain stable polymer thin films on the chips due to the co-polymerization of MBAA with covalently attached acryloyl groups of BAC on the surface. The prepared thin film was about 100–200 nm in thickness that was estimated from AFM images (See SI).[7]



Scheme 1. Preparation of Lys-CIP.

The binding behaviors were examined by surface plasmon resonance (SPR) measurements.[8] Figure 1 shows binding isotherms of lysozyme (14,400 Da, pI: 11), trypsin (23,000 Da, pI: 10.5), cytochrome *c* (11,700 Da, pI: 9.8) and chymotrypsin (25,000 Da, pI: 8.3) on Lys-CIP. The template protein, lysozyme, showed the strongest binding and an apparent dissociation constant (K_d) was estimated to be about 1.0×10^{-6} M, and the affinity decreased in the order of cytochrome *c*, chymotrypsin, and trypsin; an estimated K_d of cytochrome *c* was 2.4×10^{-4} M (See SI).[7]

The binding seems not to occur according to simple ion-

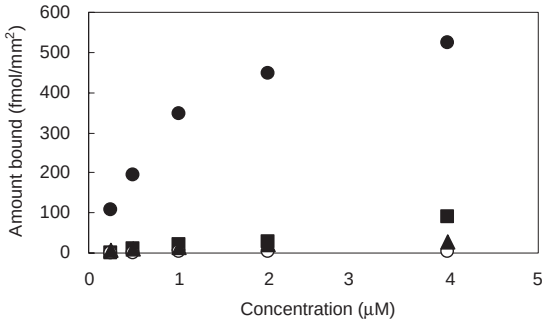


Figure 1. Binding of proteins in Lys-CIP. ●: lysozyme, ■: cytochrome *c*, ▲: chymotrypsin, ○: trypsin.

exchange process, because the order of affinity is not in the order of pI. Numbers of basic amino acid residues of lysozyme and cytochrome *c* are 18 and 24, respectively. This means that a similar number of ionic interaction points with acrylic acid may exist in lysozyme and cytochrome *c*, however, the affinity of cytochrome *c* is less than that of lysozyme. Surface hydrophobicity of proteins was estimated by calculating a ratio of the exposed water-accessible surface area of hydrophobic residues to the total exposed water-accessible surface area of the tested protein (See SI).[7] Lysozyme is less hydrophobic (0.431), compared to cytochrome *c* (0.502), trypsin (0.474), and chymotrypsin (0.492). Therefore, the apparent binding may not be derived from hydrophobic interaction. These results strongly suggest that crystallized lysozyme was successfully imprinted during the radical polymerization process in the presence of the crystal, yielding the acrylic acid-based imprinted gel bearing the specific binding sites for lysozyme, although it is difficult to confirm directly that the cavities complementary to the shape and the chemical property of lysozyme are generated by the proposed crystal imprinting.

A conventional solution-based imprinted polymer was prepared on the BAC-treated SPR chip using dissolved lysozyme as a template (Lys-MIP).[9] A simple comparison on the binding capacity between two polymers is difficult because an availability of the protein as the template molecule during the imprinting process may not be equal in the two methods. Thus, the relative amount bound (ratio of a bound amount of a tested protein to a bound amount of lysozyme) was employed to evaluate the selectivity (Figure 2). Among the three polymers: Lys-CIP, Lys-MIP, and NIP, obviously Lys-CIP showed highest selectivity. Therefore, it appears that the use of crystallized lysozyme as the template is superior to the conventional dissolved protein template for the preparation of protein-imprinted polymers. It may be explained that thermodynamic motion interferes with the imprinting process and rigid templates such as crystals allow for protein imprinting being better.

The use of crystals has merits of the better stability of target proteins than dissolved proteins and low chance of contamination and denaturation during a storage period. Moreover, it can be easy to apply dry processes for mass-production of protein recognition chips including array chips. Consequently synthetic material-based protein chips may be able to be provided by this method. In addition, proposed crystallized protein-imprinted polymers may have an ability of facilitating protein crystallization, since directed nucleation of calcite at a crystal-imprinted polymer surface has been reported.[10]

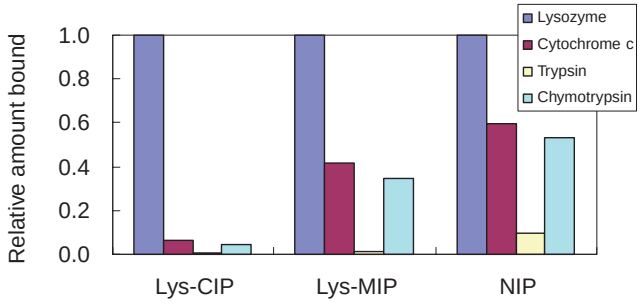


Figure 2. Selectivity of prepared polymer thin layers on the SPR chips.

The authors thank Prof. Kazuhiko Ishihara, Department of Materials Engineering, University of Tokyo (Japan), for kindly providing MPC. We also gratefully acknowledged Prof. Kazuyuki Moriwaki, Kobe University (Japan) for the AFM measurement.

References and Notes

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5 Prelaration of Lys-CIP: The cellulose membrane with the grown crystal was drawn carefully from the solution and the membrane was soaked for 1 h in an aqueous solution containing acrylic acid and PEG-3500. After removing the solution, a pre-polymerization mixture (50 mM HEPES buffer pH 7.4, 3.0 mL) containing acrylic acid (50 µmol), MBAA (500 µmol), MPC (150 µmol), PEG-3500, and potassium peroxodisulfate was immediately dropped on the membrane. A glass chip with a gold surface treated with BAC was placed on the membrane and the polymerization was initiated by heating at 36 °C.
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7 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
8 The binding behaviors were examined by SPR measurements using BIACORE Q (Biacore international AB) with a binding buffer of 10 mM HEPES buffer (pH 7.4) containing 80 mM NaCl (20 µL/min) and a washing buffer of 10 mM HEPES buffer (pH 7.4) containing 1 M NaCl. Binding amounts were calculated from the signals after 10 seconds from the end of the sample introduction period (2 min).
9 A pre-polymerization mixture (50 mM HEPES buffer pH 7.4, 3 mL) containing lysozyme (10 µmol), acrylic acid (50 µmol), methylene bis(acrylamide) (500 µmol), MPC (150 µmol), PEG-3500 (35% w/v), and an initiator, potassium peroxodisulfate was dropped on a cellulose membrane. A glass chip with a gold surface treated with BAC was placed on the membrane and the polymerization was initiated by heating at 36 °C.
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Published on the web (Advance View) August 12, 2006; doi:10.1246/cl.2006.1030