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Molecularly imprinted polymers for the recognition of sodium dodecyl sulfate denatured creatine kinase

Chung-Yih Wang [a,*], Yi-Chen Chen [a], Dey-Chyi Sheu [a], Tse Chuan Chou [b]

[a] Tatung University, Department of Bioengineering, Taipei 104, Taiwan
[b] Tatung University, Department of Chemical Engineering, Taipei 104, Taiwan

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ABSTRACT

Micro-contact imprinting methodology was used to prepare the molecule recognition film for denatured creatine kinase-MM (CK-MM), which was denatured by sodium dodecyl sulfate. Interpretation of thermo-calorimetry measurements suggested the use of poly(ethylene glycol) 400 dimethacrylate (PEG400DMA) as a crosslinking molecule. The selected functional monomer, methyl methacrylate (MMA), produced the highest imprinting factor from a panel of five candidates. The molecularly imprinted polymers (MIPs) formed with 5% MMA, 95% PEG400DMA and denatured CK-MM showed excellent imprint recognition, with the imprinting factor of 8.66. The dissociation constant calculated from Scatchard plot was 3.25×10^{-8} M. MIPs had little affinity with non-template proteins, such as native CK-MM, a native and denatured form of human serum albumin (HSA) and immunoglobulin G (IgG). The competitive study in a two-protein environment showed that the selectivity of MIP was 96.8% and 98.7% for denatured CK-MM/denatured HSA and denatured CK-MM/denatured IgG, respectively. The results suggested that MIPs prepared in this work recognize not only the protein sequences but also the protein secondary structure.

1. Introduction

Biomolecules, such as proteins and enzymes, attain meta-stable states when dissolved in a physiological solution. Recently, researchers found that some misfolded proteins are responsible for the interconversion of protein secondary structures from α-helix to the protease resistant β-sheet conformation [1]. The accumulation of misfolded proteins or their fragments may induce neuron degenerative processes and the generation of amyloid plaques in brain [2]. Because those misfolded proteins have the same amino acid sequence as normal proteins, the detection of misfolded proteins relies on time-consuming analytical methods, such as conformational dependent immunoassay, Western blotting, immunohistochemical analysis, and animal bioassay [3–5]. To reduce the cost and labor, there is still a need to develop a more efficient method to differentiate proteins with different secondary structure.

Molecularly imprinted polymers (MIPs) have been successfully used to separate and purify small organic compounds [6]. Small molecules can form stable template–functional monomer complexes, thus we can exploit this ability, to create recognition cavities using many polymerization processes [7]. The development of molecularly imprinted technologies and their applications in the field of solid-phase extraction have been extensively reviewed [8–10]. Contrary to the success of preparing MIPs for small molecules, the labile nature of proteins makes the polymerization process difficult. Until recently some researchers have made progress in fabricating protein-imprinted films [11–13]. To improve the selectivity of protein imprinted polymers, Takeuchi *et al.* assembled protein imprinted polymer array to classify proteins [14]. Bonini *et al.* prepared surface imprinting beads for human serum albumin with an imprinting factor of 1.91, which was barely enough to recognize the target protein [15]. Rick and Chou prepared a protein-imprinted electrode using a conductive polymer, and generated cyclic voltammetry output in response of protein binding [16]. Turner *et al.* formed protein imprints using self-assembling lipids at the air–water interface, and transferred a monolayer to a hydrophobic substrate, thus enabling a study of protein rebinding [17]. Molecularly imprinted polymers were also synthesized on the surface of magnetic nanospheres and used to probe proteins in urine and other physiological samples [18,19]. The breakthroughs into the imprinting of bio-macromolecules and the advantage of inexpensive processes make MIPs become a practical means to achieve bio-macromolecular recognition. However, there is no discussion regarding how the protein secondary structure influences the protein binding to the MIP.

* Corresponding author. Tel.: +886 225929265; fax: +886 225854735.
E-mail address: cywang@ttu.edu.tw (C.-Y. Wang).

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The model protein selected to develop protein structure-specific MIP is creatine kinase (CK). CK is an intracellular enzyme that catalyzes the formation of adenosine triphosphate from adenosine diphosphate and creatine phosphate. CK is a dimeric molecule composed of M (muscle) and/or B (brain) subunits [20]. CK is able to exist as three distinct isoenzymes, CK-MM, CK-MB, and CK-BB. A recognition film has been prepared by the micro-contact imprinting technology for native CK-MM [21]. Non-covalent molecular imprinting technology creates recognition structures with cavities that are specific to native CK-MM. To investigate how to differentiate the secondary structure of native CK-MM and denatured CK-MM, this study used denatured CK-MM as a template to prepare MIP. Denaturation of CK-MM has been studied with urea, guanidine hydrochloride, sodium dodecyl sulfate (SDS) or heat [22–25]. The high concentration of denaturants or aggregation of thermally denatured CK-MM makes them inappropriate to be applied as template for MIP. The SDS–CK-MM complex was selected as template because it retained a portion of epitopes that appear on native CK-MM, and without the problem of aggregation. We tested the denatured CK-MM MIP with various proteins (native form and denatured form) in non-competitive and competitive rebinding experiments. The denatured CK-MM MIP may reject the binding of native CK-MM and other non-template proteins. Our approach to form a protein-recognizable MIP provides a simple, yet effective method to analyze protein with different primary and/or secondary structure.

2. Materials and methods

2.1. Reagents

Creatine kinase (CK-MM) from rabbit, FITC labeled human serum albumin (HSA), FITC labeled immunoglobulin G (IgG), methacrylic acid (MAA), and Tween 20 were obtained from Sigma. 3-(Triethoxysilyl)propyl methacrylate (98%), Poly(ethylene glycol) dimethacrylate (mol. wt. 550, PEG400DMA), poly(ethylene glycol) dimethacrylate (mol. wt. 875, PEG600DMA), and pentaerythritol tetraacrylate (PTTA) were provided by Aldrich. Styrene and sodium hydroxide were obtained from Showa, Japan. Acrylamide and sodium dodecyl sulfate (SDS) were purchased from J.T. Baker. Tetra ethylene glycol dimethacrylate (TEGDMA) and divinyl benzene (DVB) were obtained from Fluka. The Kanto Chemical Co. Inc., Tokyo, Japan supplied methyl methacrylate (MMA). 2,2-Dimethoxy-2-phenylacetophenone (DMPAP) was purchased from TCI. Potassium chloride, sodium dihydrogen phosphate-2-hydrate, sodium chloride, and hydrochloric acid were purchased from Riedel-de-Haën. Goat polyclonal antibody to CK-MM (ab26223, concentration not determined) and goat IgG secondary antibody-H&L (ab 6741, with horseradish peroxidase conjugates, 2 mg/mL) were obtained from Abcam (UK). Amersham Biosciences supplied enhanced chemiluminescence (ECL) reagents.

2.2. Approach to select crosslinker and functional monomer

The schematic principle of micro-contact imprinting method is demonstrated in Fig. 1. The denatured CK-MM was prepared by mixing 3.5 μM CK-MM and 2.9 mM SDS solution for 1 h and the conformation change of denatured CK-MM was monitored with a circular dichroism spectropolarimeter (Jasco J720, Japan). A micro-contact approach was adapted to form the imprint sites for denatured CK-MM. A cover and substrate glasses were cleaned and prepared as described in the previous study [21]. The substrate glass was further modified by immersion in a solution composed of 69 μL glacial acetic acid, 769 μL 3-(triethoxysilyl)propyl methacrylate (0.4%) at 80 °C for 4 h, followed by drying in a stream of nitrogen gas.

The candidate crosslinker compounds, TEGDMA, PEG400DMA, PEG600DMA, PTTA, and DVB were combined with 2% DMPAP and polymerized by UV irradiation for 10 min using a photochemical reactor (Panchum Scientific Corp., Taiwan). Each polymer was then titrated with 100 μL of denatured CK-MM and the heat change monitored by thermal activity monitor (Thermometric 2277 thermal activity monitor from Thermometric AB, Sweden). The cross-link compound with minimum binding energy was selected to react with various candidate functional monomers.

The clean cover glasses were immersed in a solution of denatured CK-MM (0.35 μM) at 37 °C for 2 h prior to drying in a nitrogen stream. The selected crosslinkers and functional mono-

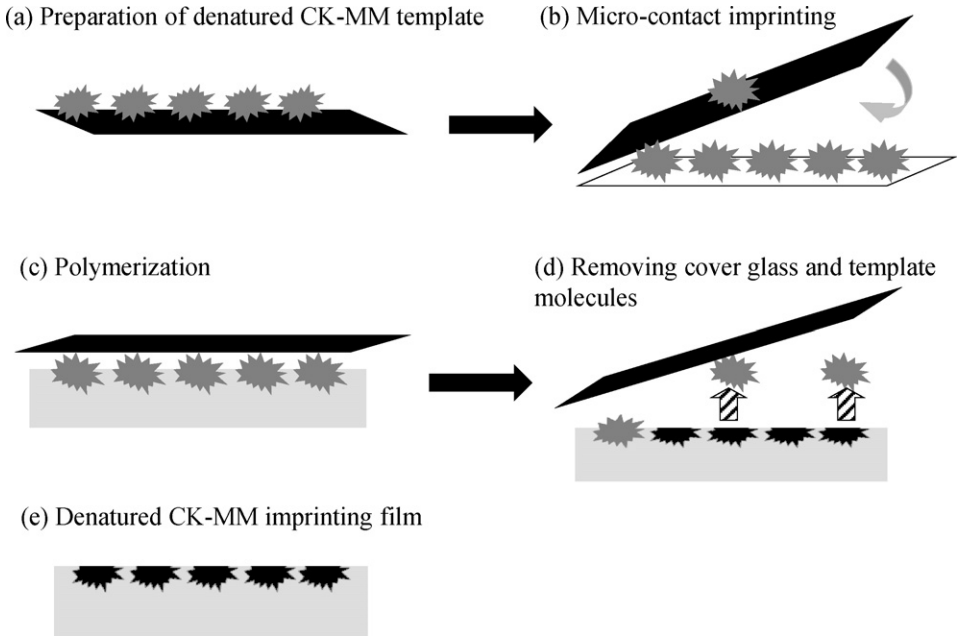

Fig. 1. The preparation of denatured CK-MM imprinted polymer. (a) Adsorption of denatured CK-MM on cover glass; (b) addition of crosslinker, functional monomer and initiator to activated substrate glass (bottom) and the imprinting with cover glass (top); (c) initiating polymerization reaction with UV irradiation; (d) removing cover glass and denatured CK-MM; (e) formation of recognition cavities.