Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2011

Artificial-Epitope Mapping for CK-MB Assay

Dar-Fu Tai,* Yi-Fang Ho, Cheng Hsin Wu, Tzu-Chien Lin, Kuo-Hao Lu, and Kun-Sian Lin

Department of Chemistry, National Dong-Hwa University, Hualien, Taiwan,

Fax: 886-3-8633570; Tel: 886-3-86335709; E-mail: dftai@mail.ndhu.edu.tw

Experimental section

$(Boc\text{-}L\text{-}Cys)_2$, acrylic acid, acrylamide, tyramine, and Boc-L-His were obtained from Sigma-Aldrich (St. Louis, MO, USA). The creatine kinases CK-BB, CK-MM and CK-MB used for the evaluation procedure was also purchased commercially from Sigma-Aldrich (St. Louis, MO, USA). The peptides derived from CK were synthesized by a peptide synthesizer (Discover SPPS, USA) using microwave. Acrylation of tyramine afforded *N*-Acr-tyramine, Boc-L-His-NHBn was synthesized from Boc-L-His and $(N\text{-Acr-L-Cys-NHBn})_2$ was synthesized from $(Boc\text{-L-Cys})_2$. The buffer used for all experiments was a PBS (20 mM NaH_2PO_4, pH 7.5). The QCM was obtained from Tai-Tien Electronic Co. (Taipei, Taiwan) with a reproducibility of ± 1 Hz. The QCM consisted of an 8 mm diameter disk made from an AT cut 10 MHz quartz crystal with gold electrodes (diameter: 4.2 mm) on both sides of the crystal.

Molecular imprinting of the lineal epitope of CK-MB proteins on chip: The synthesized epitope peptides of CK proteins is used as a template. Various amino acid monomers are copolymerized in the presence of template on the surface of a chip containing a linker to obtain protein-like polymer. After the template is washing out, we obtain a molecularly imprinted polymer on chip. The diluted samples containing CK proteins

This journal is © The Royal Society of Chemistry 2011

were adsorbed on the chip and detected by QCM.

Biosensor system. The quartz crystal microbalance (QCM) is a kind of bulk-acoustic wave (BAW) resonator. Because of the reverse-piezoelectric effect, when a quartz crystal places in an alternating electric field, it will oscillate, and the oscillation mode and fundamental frequency is related to the material characteristics. When a mass load is applied to this crystal, the frequency will change, and the relationship between the frequency shift and mass load is linear.

The flow injection system contained a HPLC pump (Model L7110, Hitachi, flow rate = 0.1 ml min^{-1}), home-built flow cell, sample injection valve (Model 1106, OMNIFIT), home-built oscillation circuit (including oscillator and frequency counter) and a personal computer. The polymer coated QCM was fixed between two O-rings and inserted into the flow-cell. Only one side of the QCM was in contact with the liquid. Sodium phosphate buffer (20 mM, pH 7.5) was used for circulating, washing and testing. To equilibrate the newly imprinted chips quickly, 100 µl solutions, including alkaline (pH 9 PBS), neutral (distill water) and acidic (5% acetic acid in distill water), were injected into the flow cell during circulating.

Kinetics Studies.

The data obtained were then plotted using the Scatchard analysis for specific binding:,

The data obtained were plotted using an equation $B = Bmax \cdot c/(K_d + c)$, $B = H/M_w$, derived from the Scatchard analysis $([RL]/[L] = (B_{max} - [RL])/K_d)$.

Where c is the concentration of protein, B is the fraction of sites bound, *B*max

This journal is © The Royal Society of Chemistry 2011

represents the apparent maximum number of binding sites, and K_d is the dissociation constant of the chip to analyte, M_w is the molecular weight of the analyte and H is the frequency shifts in the QCM.

From the binding experiments, the polymers imprinted with an epitope-peptide efficiently recognized the template. The frequency shifts of these QCM chips were showing a tendency of saturation in all cases near the concentration of 500 μg/mL.