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Real-time Analysis of the Interaction between Calmodulin and Melittin by SPR Spectroscopy

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Abstract: The dynamic interaction process of calmodulin with an immobilized peptide melittin was investigated in real time by surface plasmon resonance spectroscopy, and dissociation constant of the complex was calculated to be 3.37×10^{-6} mol/L.

Keywords: Calmodulin, melittin, surface plasmon resonance.

Surface Plasmon Resonance (SPR) is a new technique for the biomolecular interaction analysis developed from 1990. Compared with traditional methods of analysis, SPR has its obvious advantages: fast-speed, no-labeling, real-time and micro sample requiring, *etc.*. In this case, it has been applied extensively to the studies in life research[1,2].

Calmodulin (CaM) is a ubiquitous calcium-binding protein in eucaryotic cells and serves as a multifunctional regulator in variety of cellular processes, such as energy, biosynthetic metabolism, cell motility, muscle contraction, *etc.*. It regulates the activity of more than thirty target enzymes or proteins[3]. Melittin, which is a small amphiphilic peptide with 26 amino acid residues purified from bee venom, usually competitively binds to calmodulin in a calcium-dependent manner with calmodulin's target enzymes and is regarded as an antagonist to CaM. We previously studied the interactions of CaM with melittin by Fluorescence and FT-IR spectroscopy[4]. In this paper, the dynamic process of interaction between CaM and melittin was investigated in real time by SPR, and the dissociation constant was calculated at the same time.

SPR is a technique that responds to change in the refractive index close to a gold surface coupled with dextran matrix. A reactant (named as ligand) is coupled to a sensor chip firstly, then, another reactant (named as analytical reagent) is injected and flows through the sensor chip surface. Once the ligand and analytical reagent bind to each other, SPR signals will be changed resulting from the change of molecular mass on the chip. **Figure 1** illustrates sensorgram of the immobilization of melittin to a sensor chip CM5. Dextran matrix was firstly activated by a mixture of N-hydroxysuccinimide and N-ethyl-N′-(dimethylaminopropyl)carbodiimide **a**, then, melittin was immobilized to

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the activated matrix by a method of amine coupling **b**, next, ethanolamine was added to remove excess peptide and to deactivate unreacted N-hydroxysuccinimide esters **c**. After immobilization, the chip's refractive index was changed with an increase of SPR signal of 1702.3 RU, corresponding to an immobilized melittin content of 1.7 ng/mm^2.

Based on the immobilization of melittin, buffer containing CaM was injected to the flowing cell. **Figure 2** displays the whole dynamic process of calmodulin binding to melittin. Calmodulin in buffer bound to melittin **a**, gradually approached an equilibrium state **b**, then, the complex was dissociated by buffer with calcium chelator **c**. A series of dynamic curves were obtained at different concentrations of CaM, the resonance signals are proportional to the concentrations of calmodulin binding to melittin, so all dynamic results were analyzed by a simulation software, and the dissociation constant was calculated to be 3.37×10^{-6} mol/L.

Figure 1 Sensorgram of melittin immobilization

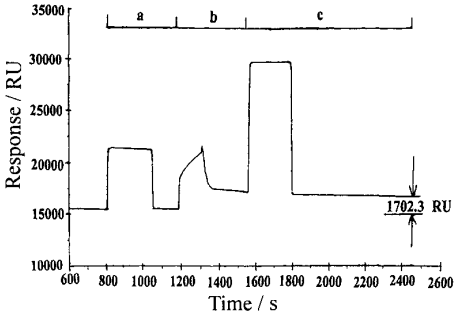
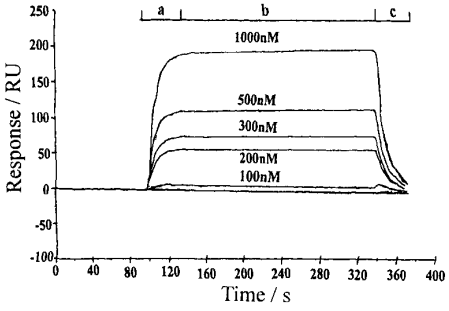


Figure 2 Sensorgram of CaM binding to melittin at different concentrations



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