Binding Kinetics of Calmodulin with Target Peptide of Nitric Oxide Synthase Using Surface Plasmon Resonance

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Overview

Calmodulin (CaM) is a small acidic Ca\(^{2+}\) binding protein involved in many physiological processes. CaM is able to fine-tune the orientation of its domain and residue contacts to accommodate its binding to a wide variety of target proteins and enzymes. The interaction of CaM with its target elements within these proteins is required for the activation of certain enzymes including nitric oxide synthases (NOS). OpenSPR can be used to determine the kinetic binding constants of CaM and NOS target peptide interactions. In this application note the on and off rates and affinity constant for the interaction between wild type (WT) CaM and NOS peptide are determined. Results are compared against the affinity constant obtained from a binding competition assay in the literature.

Materials & Equipment

- OpenSPR Instrument (SPR-01)
- Sensor Chip (SEN-AU-100)
- TraceDrawer Kinetic Analysis Software (TDS)
- CaM Protein (25nM - 300 nM) in PBS, 100 uM CaCl\(_2\)
- Thiolated nNOS Target Peptide (50 uM) in PBS, 100 uM CaCl\(_2\)
- PBS, 100 uM CaCl\(_2\)
- Regeneration Buffer (10 mM EDTA) in PBS, 100 uM CaCl\(_2\)
- Cysteamine blocker (1 mM) in PBS, 100 uM CaCl\(_2\)

Safety Notes

Follow the safety precautions outlined in the MSDS for all materials.

Procedure

1. Following the startup procedure found in the OpenSPR manual, setup the OpenSPR instrument and software.
**Procedure**

2. Inject nNOS thiolated (SH) peptide to functionalize OpenSPR sensor chip surface at 50 uL/min using a 100 uL sample loop. Inject 1 mM cysteamine blocker to prevent unspecific binding.

3. Inject the highest prepared concentration of CaM.

4. Regenerate the surface by injecting EDTA regeneration buffer.

5. Inject PBS, 100 uM CaCl$_2$ to flush the injection loop.

6. Inject the next concentration of CaM and continue steps 2-5 with each concentration.

7. Process data in TraceDrawer to determine the on rate, off rate and affinity. Normalize and fit the data with a 1:1 binding model.

**Results**

The magnitude of the binding curves and the association slope decreases as the concentration of CaM decreases. CaM association, CaM dissociation and surface regeneration are shown in Figure 1 for decreasing concentrations of CaM.

![Sensorgram of wt-CaM binding to nNOS](image)

*Figure 1. Raw sensorgram demonstrating the binding of various concentrations of CaM towards an immobilized –SH terminated nNOS peptide.*
Kinetic fitting of the CaM binding data produced an off rate of $3.34 \times 10^4 \text{s}^{-1}$ and an on rate of $1.56 \times 10^5 \text{M}^{-1} \text{s}^{-1}$. This gives an affinity constant ($K_D$) of 2.20 nM. Results are shown in Figure 1. These results compare well with other results reported in the literature for CaM-NOS interactions [1].

![Figure 2. Kinetic analysis of the binding between CaM and nNOS peptides on the OpenSPR instrument. Analysis performed in TraceDrawer using a 1:1 binding interaction model. $K_{on}$ of $1.56 \times 10^5 \text{M}^{-1} \text{s}^{-1}$, $K_{off}$ of $3.34 \times 10^4 \text{s}^{-1}$ and $K_D$ of 2.20 nM](image)