Binding Kinetics of Carbonic Anhydrase II to Small Molecules Using OpenSPR™ High Sensitivity Sensors

Summary

- Kinetic binding of Carbonic Anhydrase II (CAII) to the small molecules Furosemide and Acetazolamide were measured using the OpenSPR™ instrument
- OpenSPR™ High Sensitivity Carboxyl Sensors were used for this study
- The K_D was determined to be 2.5µM for Furosemide and 0.025µM for Acetazolamide, which compare well to literature values
- OpenSPR™ High Sensitivity Carboxyl Sensors can be used to measure kinetics of small molecule–protein interactions

Overview

OpenSPR™ is a powerful instrument providing in-depth label-free binding kinetics for a variety of different molecular interactions. One of the most interesting areas of study are small molecule–protein interactions. OpenSPR™ High Sensitivity Carboxyl Sensors were used to measure kinetic binding between Carbonic Anhydrase II (CAII), a thoroughly studied small molecule binding protein, to furosemide and acetazolamide, small molecules with molecular weights of 331 Da and 222 Da respectively.

Materials and Equipment

- OpenSPR™ Instrument
- OpenSPR™ High Sensitivity Carboxyl Sensor
- OpenSPR™ Amine Kit
- TraceDrawer™ Kinetic Analysis Software
- Ligand: Carbonic Anhydrase II
- Analyte: Furosemide, Acetazolamide
- Running Buffer: PBS-T, pH 7.4, 3%DMSO

Procedure

1. Following the start-up procedure found in the OpenSPR™ manual, setup the OpenSPR™ instrument and software.
2. Clean the High Sensitivity Carboxyl Sensor using 10mM HCl as outlined in the technical guide.
3. Inject EDC/NHS solution from the OpenSPRTM Amine Kit to activate the COOH surface at 20 µL/min.
4. Immobilize CAII at a flow rate of 20 µL/min for a 5 minute incubation period.
5. Inject Blocking Solution from the OpenSPR™ Amine Kit to complete the immobilization. Change the sample loop to a 500µL sample loop.
6. Prepare furosemide dilutions into the running buffer at the following concentrations: 333nM, 1 µM, and 9 µM.
7. Prepare acetazolamide dilutions into the running buffer at the following concentrations: 11nM, 33nM, 111nM, and 333nM.
8. Inject the analytes above individually at a flow rate of 150 µL/min with an association time of 60 seconds and a dissociation time of 150 seconds.
9. Data from OpenSPR™ is analyzed using TraceDrawer™.

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Results and Discussion

The immobilization of CAII to the OpenSPR™ High Sensitivity Carboxyl Sensor is shown in *Figure 1* with over 4600pm of response for the immobilization. *Figure 2* shows the binding of the furosemide analyte at 3 different concentrations and *Figure 3* shows the binding of acetazolamide at 4 different concentrations. The association phase and dissociation phases are evident as is the concentration dependence. The data is fit to a one to one binding model in TraceDrawer™. The kinetic constants are shown in *Table 1*, and the fits are overlaid in *Figure 2* and *Figure 3* as solid black lines. The K_D value is determined to be 2.5µM for both of which compare well to reported values in literature.

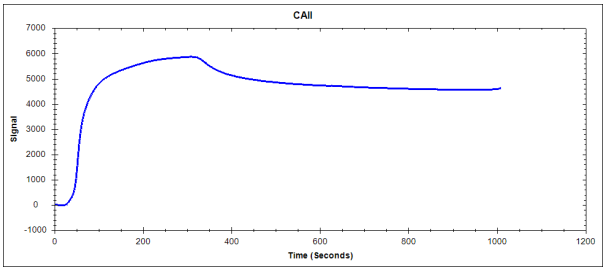

Figure 1. Ligand immobilization of CAII to the OpenSPR™ High Sensitivity Carboxyl Sensor.

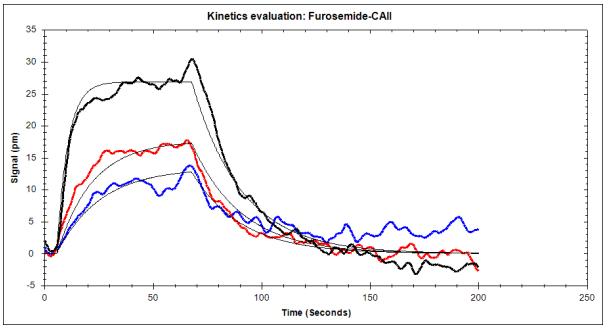

Figure 2. Binding of Furosemide at concentrations of 333nM, 1µM, and 9µM. Solid black lines are the one to one binding model fits.

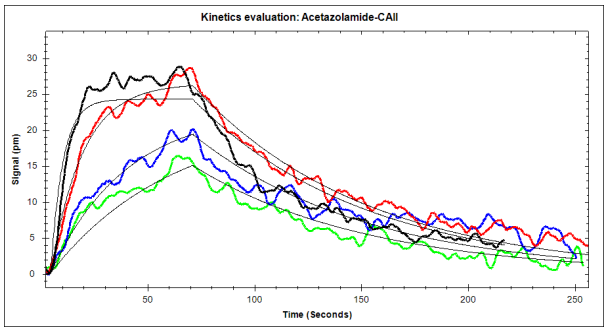

Figure 3. Binding of acetazolamide at concentrations of 11nM, 33nM, 111nM and 333nM. Solid black lines are the one to one binding model fits.

Table 1. Binding kinetics and affinity measured using OpenSPR™

	Furosemide	Acetazolamide
k_{on} [1/M*s]	1.7e4 +/-9.5e2	4.9e5 +/- 6.9e2
k_{off} [1/s]	4.4e-2 +/- 2.2e-5	1.23e-2 +/- 7.0e-6
K_D [M]	2.5e-6 +/- 1.4e-7	2.51e-8 +/- 5.0 e-11

Conclusions & Summary

This study demonstrates how OpenSPR™ can be used to measure kinetic binding interactions between proteins and small molecules. As the field of small molecule investigation grows, OpenSPR™ will provide researchers with the ability to measure the kinetics of these interactions on their own benchtop.

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