Reichert Surface Plasmon Resonance (SPR) SR7000DC Dual Channel System Application Note II

Small Molecule Assay: 4-Carboxybenzenesulfonamide (201 Da) Binding to Carbonic Anhydrase II

Introduction

Carbonic anhydrase II (CAII) is an enzyme that catalyzes the reversible hydration of carbon dioxide to form bicarbonate with the release of a proton. CAII has important physiological functions such as pH regulation, bicarbonate metabolism and control of intracellular osmotic pressure.[1] CAII activity is strongly inhibited by a variety of aromatic and heterocyclic sulfonamides. This application note presents the SPR binding experiment between CAII and an inhibitor, 4-carboxybenzenesulfonamide (4-CBS); a small molecule with a molecular weight of 201 Da.

Experimental

The experimental conditions for this assay are summarized below:

Ligand	Analyte	Analyte Concentrations	Association Time	Dissociation Time	Regeneration Solution
CAII	4-CBS	20, 6.7, 2.2, 0.74, 0.25, 0.082 µM	1 min	3 min	None

Results

Figure 1 presents the kinetic results from this small molecule binding experiment. The inset represents the Langmuir binding isotherm where the equilibrium binding response is plotted as a function of concentration. Each concentration is injected at least twice to verify reproducibility. The kinetic data is fit to a simple bimolecular model using Scrubber (Biologic Software) (red lines) and the equilibrium data in the inset of **Figure 1** is fit to a Langmuir binding isotherm model (solid line). This small molecule binding experiment was carried out on four separate occasions and **Table 1** summarizes the results.

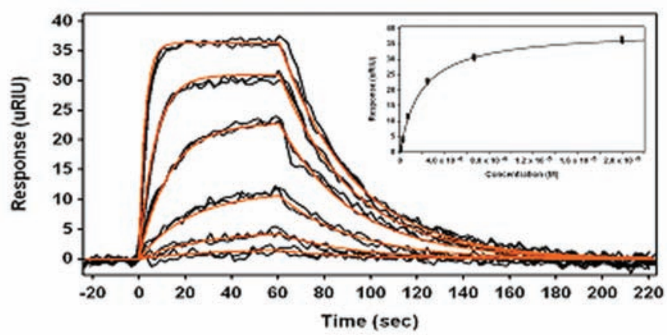

Figure 1: Normalized response versus time plots of 4-CBS binding to CAII fit (red lines) to a simple bimolecular model in Scrubber. The inset is the corresponding Langmuir binding isotherm.

Table 1 presents the association and dissociation rate constants determined from fits to a simple bimolecular model in Scrubber along with the equilibrium dissociation constants (K_D) calculated from the kinetic and equilibrium data, respectively. The results show that the system is highly reproducible and the K_D values determined through the kinetic and equilibrium analysis are in very good agreement with each other and correlate very well with that reported in the literature.[2]

Table 1: Summary of the results from four separate experiments

	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (µM) (Kinetic)	K_D (µM) (Equilibrium)
Run 1	$2.9e^{4}$	$2.9e^{-2}$	0.96	1.2
Run 2	$2.8e^{4}$	$2.9e^{-2}$	1.03	1.2
Run 3	$3.2e^{4}$	$3.2e^{-2}$	1.01	1.1
Run 4	$3.6e^{4}$	$4.2e^{-2}$	1.19	1.2

1 Maren, T.H.; Conroy, C.W. *J. Biol. Chem.* **1993**, *268*, 26233.
2 Papalia, *et. al. Anal. Biochem.* **2006**, *359*, 94.

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