

Thermodynamic Analysis of the CBS – CA II Interaction

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Introduction

Sensiq Pioneer is an automated SPR-based instrument for kinetic and affinity analysis. Here we demonstrate a comprehensive thermodynamic analysis of the interaction between the sulfonamide inhibitor, Carboxybenzene sulfonamide (CBS), and the enzyme Carbonic anhydrase isozyme II (CA II).

Experimental

The running buffer for all experiments was HBS-T buffer, pH 7.4, containing 20 mM HEPES, 150 mM NaCl and 0.005% Tween-20. The system temperature was 10, 20, 30 and 40°C in four kinetic experiments, respectively. The sample racks were cooled to 15°C. Prior to use, the buffer was sterile filtered using a 0.2 µm pore vacuum filter. Biotinylated CA II was captured onto channel 1 of a COOH5 sensor chip pre-immobilized with NeutrAvidin. The specific and reference channels were exposed to free biotin to block all unoccupied biotin-binding sites.

Kinetic Assay

A protocol was configured to complete all four kinetic assays, unattended, and in a single run. Automatic dilution preparation, a thermal equilibration period of 3 hours between each kinetic assay and triplicate sampling in random order was performed. The four data sets corresponding to each analysis temperature are shown in Figure 1.

As expected, the binding affinity is higher at lower temperatures while the saturation response decreases with the thermal expansion of matter at elevated temperatures. All binding curves were included in the analysis and all were of high quality and without air spikes. A kinetic 1:1 model was fitted to the overlaid and double referenced response curves. The kinetic rate constants and the Rmax were fitted globally. The standard deviation of the residuals was < 1 RU for all data sets.

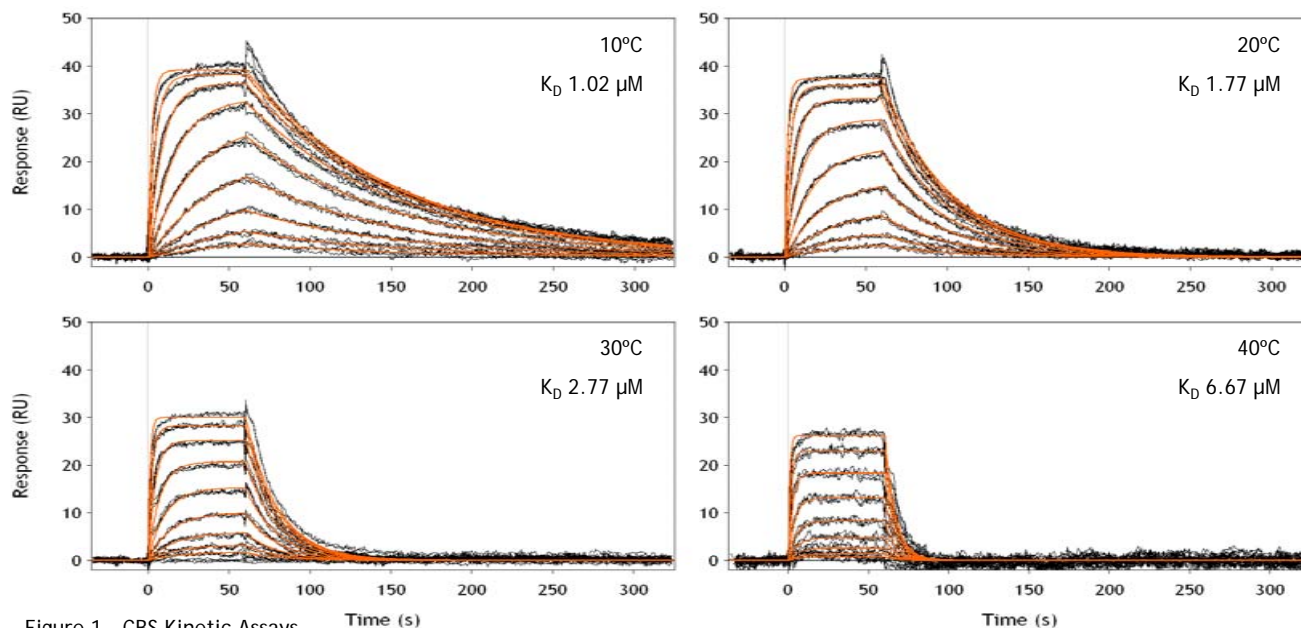


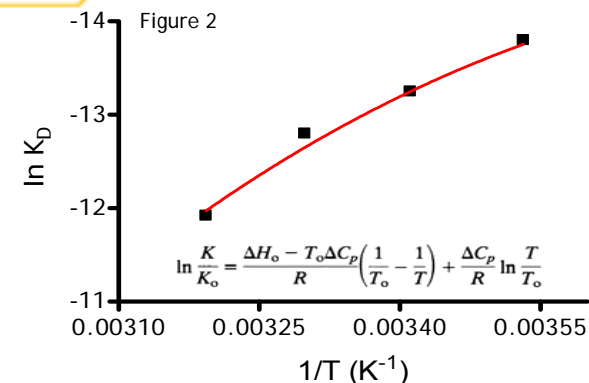
Figure 1 - CBS Kinetic Assays

Interaction	ΔG^\ddagger (kcal/mol)	ΔH^\ddagger (kcal/mol)	ΔS^\ddagger (cal/mol K)
Association	11.60	3.96	-26.1
Dissociation	19.32	14.63	-16.0

¹ Sturtevant et al. PNAS **92** (1995) p. 5597-5599

² Myszkka, D.G. Meth. Enzym. **323** (2000) p.325-340

³ Myszkka et al. J.Biomol.Tech. **14.4** (2003) p. 247-269

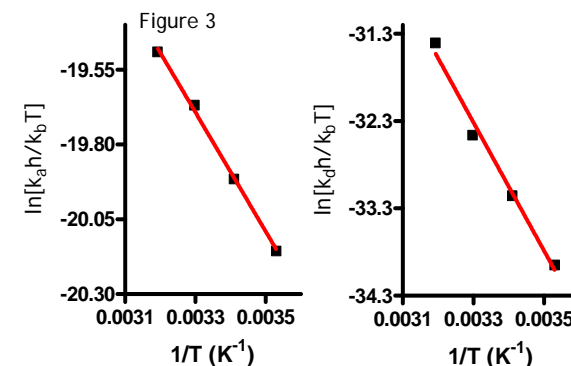


van't Hoff Analysis

The conventional linear approximation of the van't Hoff equation² was fit to the plot of $\ln K_D$ vs. $1/T$ giving a ΔH of -10.6 kcal/mol. The integrated form of this equation¹ (Figure 2), which accounts for change in heat capacity was also fitted, giving a ΔH of -9.5 kcal/mol.

Eyring Analysis²

The temperature dependence of the rate constants were plotted to extract the enthalpic and entropic contributions to the free energy of the transition state. Figure 3 below shows these analyses. The thermodynamic values for the interaction are shown in the table below.



Conclusion

Sensiq Pioneer has been demonstrated as an effective tool for thermodynamic analysis of biomolecular interactions. The data quality was maintained even at higher temperatures and the results are in agreement with published data³.