

Rapid Kinetic and Spectroscopy instruments

SFM300&400 (2001 version) – Titration of protein conformation change associated with ligand binding, (updated August 13, 2009)

The experiments below demonstrate the use of the titration system for measuring the calcium induced conformation changes of Calmodulin.

Instrument used : SFM-400 in titrator mode
Spectrometer : MOS-450 AF/CD

Steady state CD spectra were first recorded at low protein concentration to extend the range of observation at the lower wavelengths. If one restricts the spectra to 200 nm it is recommended to use 5 times higher concentrations. Also TRIS concentration was reduced because of its absorbance below 210 nm.

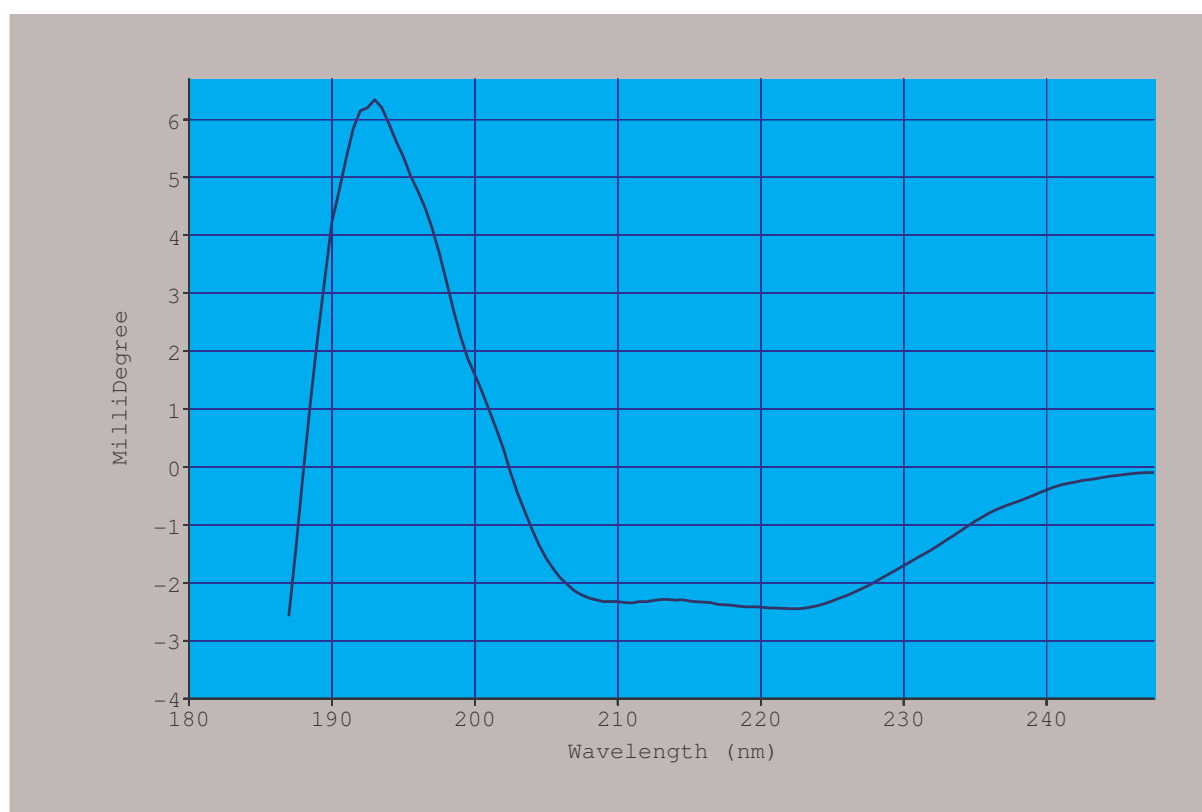


Figure 1: CD spectrum in water with 1.3 μ M protein and a 1 mm cuvette

In what follows the CD signal was recorded at fixed wavelength: 222 nm in a stirred cuvette of 1 cm light path. Additions of calcium or EGTA were first made with an Hamilton microsyringe to monitor the entire calcium induced conformational change.

Results are shown below :

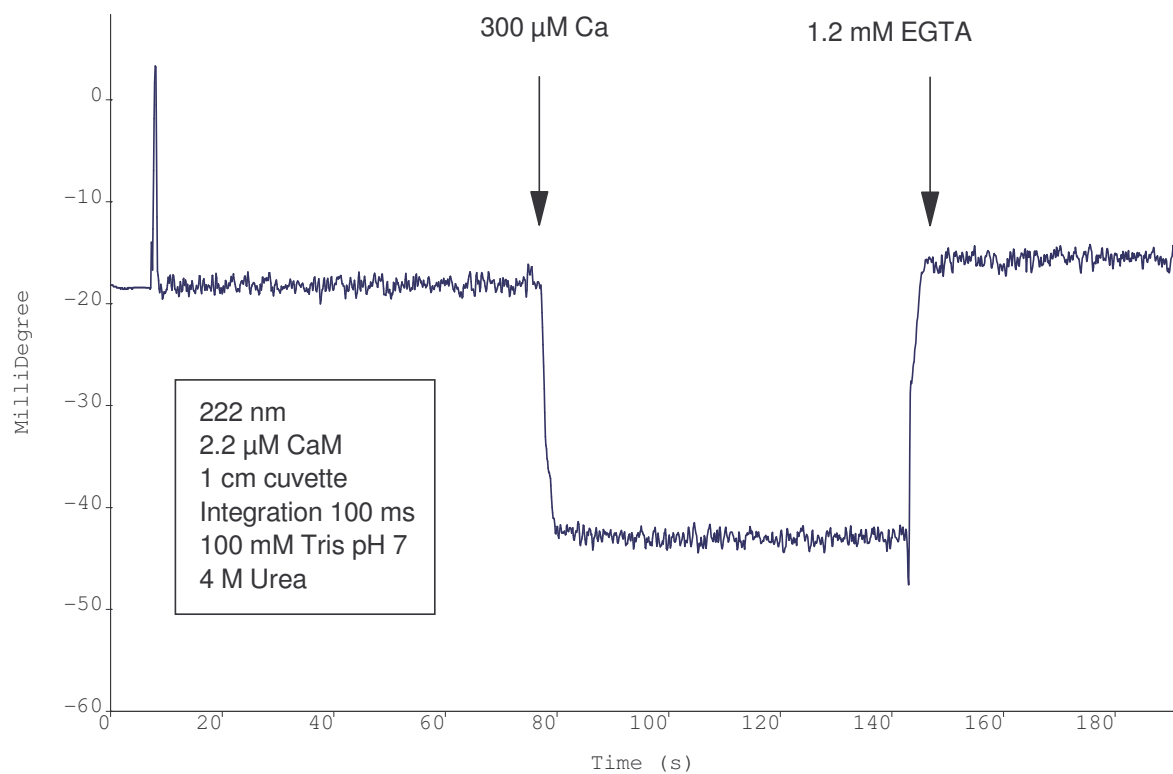


Figure 2: In the presence of 4 M urea. Switch from contaminant Ca to excess Ca and back to no calcium condition. Values of Ca or EGTA correspond to their final concentration in the cuvette.

Integration time constant was of 100 ms to try to detect any slow transition.

Next, injections were made automatically by the titrator accessory in 1 to 8 μL amounts to control the free calcium concentration.

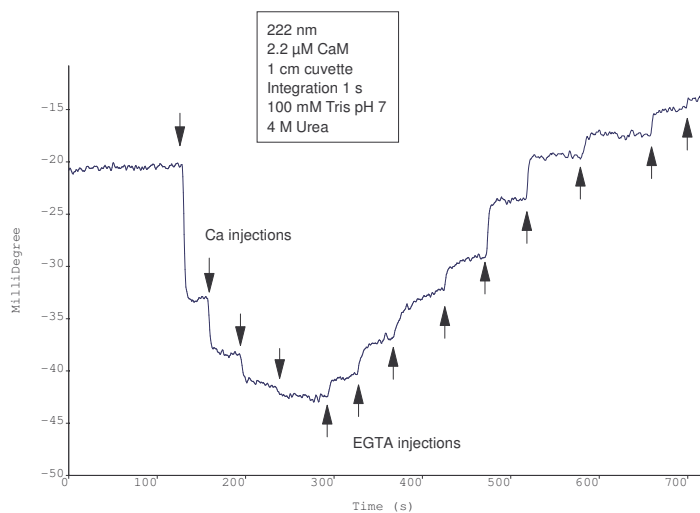


Figure 3: Same as above but with repeated injections of lower Ca and EGTA concentrations to titrate the calcium concentration dependence of the CD signal. Analysis of the data is given in the Figure 4. Integration time constant was increased to 1 s

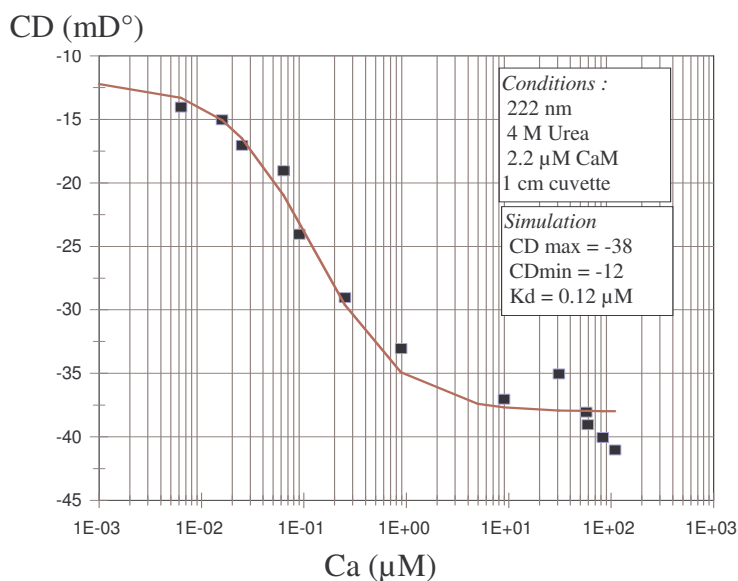


Figure 4: Analysis of the data of figure 3. It shows an apparent dissociation constant for calcium of 0.12 μM