SUPPORTING INFORMATION

Evidence that Water Can Reduce the Kinetic Stability of Protein-Hydrophobic Ligand Interactions

Lan Liu, Klaus Michelsen, Elena N. Kitova, Paul D. Schnier, John S. Klassen

Experimental

Mass spectrometry

Bovine β-lactoglobulin (Lg, monomer MW 18281 Da), palmitic acid (PA, 256.4 Da) and stearic acid (SA, 284.8 Da) were purchased from Sigma-Aldrich Canada. For the electrospray ionization mass spectrometry (ES-MS) measurements, Lg was dissolved and exchanged directly into Milli-Q water, using an Amicon microconcentrator with a molecular weight cutoff of 10 kDa. The concentration of the Lg solution was determined by lyophilizing a known volume of the filtrate and measuring the mass of the protein. The protein stock solution was stored at -20 °C. The ligand stock solutions were prepared by dissolving each FA into 50 mM aqueous ammonium acetate. The ES solutions were prepared from aqueous stock solutions of protein and ligand. Imidazole (10 mM) was also added in order to protect the (Lg+FA) complexes against in-source dissociation. Aqueous ammonium hydroxide was added to adjust the pH of the ES solution to 8.5.

The ES-MS measurements were performed using an Apex II 9.4 T Fourier-transform ion cyclotron resonance mass spectrometer (Bruker) equipped with a modified external nanoflowES (nanoES) ion source. NanoES was performed using borosilicate tubes (1.0 mm o.d., 0.68 mm i.d.), pulled to ~5 μm o.d. at one end using a P-2000 micropipette puller (Sutter Instruments, Novato, CA). The electric field required to spray the solution in negative ion mode was established by applying a voltage of -800 V to a platinum wire inserted inside the nanoES tip. The solution flow rate was ~20 nL/min. The gaseous ions produced by nanoES were introduced

into the mass spectrometer through a stainless steel capillary (i.d. 0.43 mm) maintained at an external temperature of 66 °C. The gas flow rate into the instrument was measured to be 0.6 L min^{-1}. The ions sampled by the capillary (-50 V) were transmitted through a skimmer (0 V) and accumulated electrodynamically in an rf hexapole for 0.8 s. Ions were then ejected from the hexapole and accelerated (+2700 V) into the superconducting magnet, decelerated, and introduced into the ion cell. The trapping plates of the cell were maintained at a constant potential of -1.4 V throughout the experiment. Two flexible heating blankets placed around the portion of the vacuum tube that surrounds the ion cell were used to control the temperature of the ion cell for the BIRD experiments. The typical base pressure for the instrument was ~$5x10^{-10}$ mbar. Data acquisition was controlled by an SGI R5000 computer running the Bruker Daltonics XMASS software, version 5.0. Mass spectra were obtained using standard experimental sequences with chirp broadband excitation. The time domain signal, consisting of the sum of 50 transients containing 128 K data points per transient, was subjected to one zero-fill prior to Fourier transformation.

Surface plasmon resonance

Surface plasmon resonance (SPR) experiments were performed with a Biacore T100 instrument (GE Healthcare). Reagents used were obtained from either Sigma-Aldrich or GE Healthcare. The immobilization buffer consisted of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) (pH 7.4), 150 mM NaCl and 0.2 mM tris(2-carboxyethyl)phosphine (TCEP). Lg was immobilized onto a carboxymethyl dextran (CM5) chip using amine coupling chemistry at 25 °C. Surfaces were pre-conditioned by flowing a solution containing 100 mM HCl, 50 mM NaOH and 0.5% (w/v) sodium dodecyl sulfate (SDS) at a flow rate of 100 µl/min. The surface was activated for 7 min using a mixture of N-

Hydroxysuccinimide (NHS) and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) followed by an injection (~5 min) of 20 µg/mL Lg in 10mM sodium acetate (pH 4.0). Remaining activated groups were subsequently blocked with a 7 min injection of ethanolamine. Using this approach approximately 800-1,800 RU of Lg was immobilized. The running buffer for FA:Lg kinetic measurements consisted of 25 mM tris(hydroxymethyl)aminomethane (Tris) pH 8.0, 1mM TCEP, 0.2mM 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and 8% (v/v) methanol. Stock solutions of PA and SA were prepared and serial dilutions were performed in methanol. Diluted FAs were added to the running buffer to give a final methanol concentration of 8% (v/v). The FA's were injected over the Lg surface for 30 s association and the dissociation was monitored for 60 s at a flow rate of 80 µl/min. The sample analysis temperature was varied between 5 and 45 °C.

Data was processed using the Biacore T100 analysis software and Igor Pro (Wavemetrics Inc). Sensograms were corrected for systematic noise and baseline drift by subtracting the response of the reference spot, which was activated but not exposed to protein. The average response from blank injections was used to double-reference the binding data. The dissociation portion of the sensograms were de-spiked, binomial smoothed and fitted to an exponential function to determine the dissociation rate constants.

Table S1. Dissociation rate constants measured by SPR for the (Lg + FA) complexes in aqueous solution at pH 8.

FA	T (℃)	k (s^{-1}) [a]
PA	5	0.42 ± 0.10
PA	12	0.60 ± 0.15
PA	18	0.82 ± 0.20
PA	25	0.90 ± 0.12
PA	32	1.20 ± 0.14
PA	38	1.35 ± 0.20
PA	45	1.6 ± 0.2
SA	5	0.023 ± 0.005
SA	12	0.032 ± 0.006
SA	18	0.043 ± 0.009
SA	25	0.051 ± 0.010
SA	32	0.061 ± 0.015
SA	38	0.072 ± 0.018
SA	45	0.092 ± 0.020

a. Errors correspond to one standard deviation.

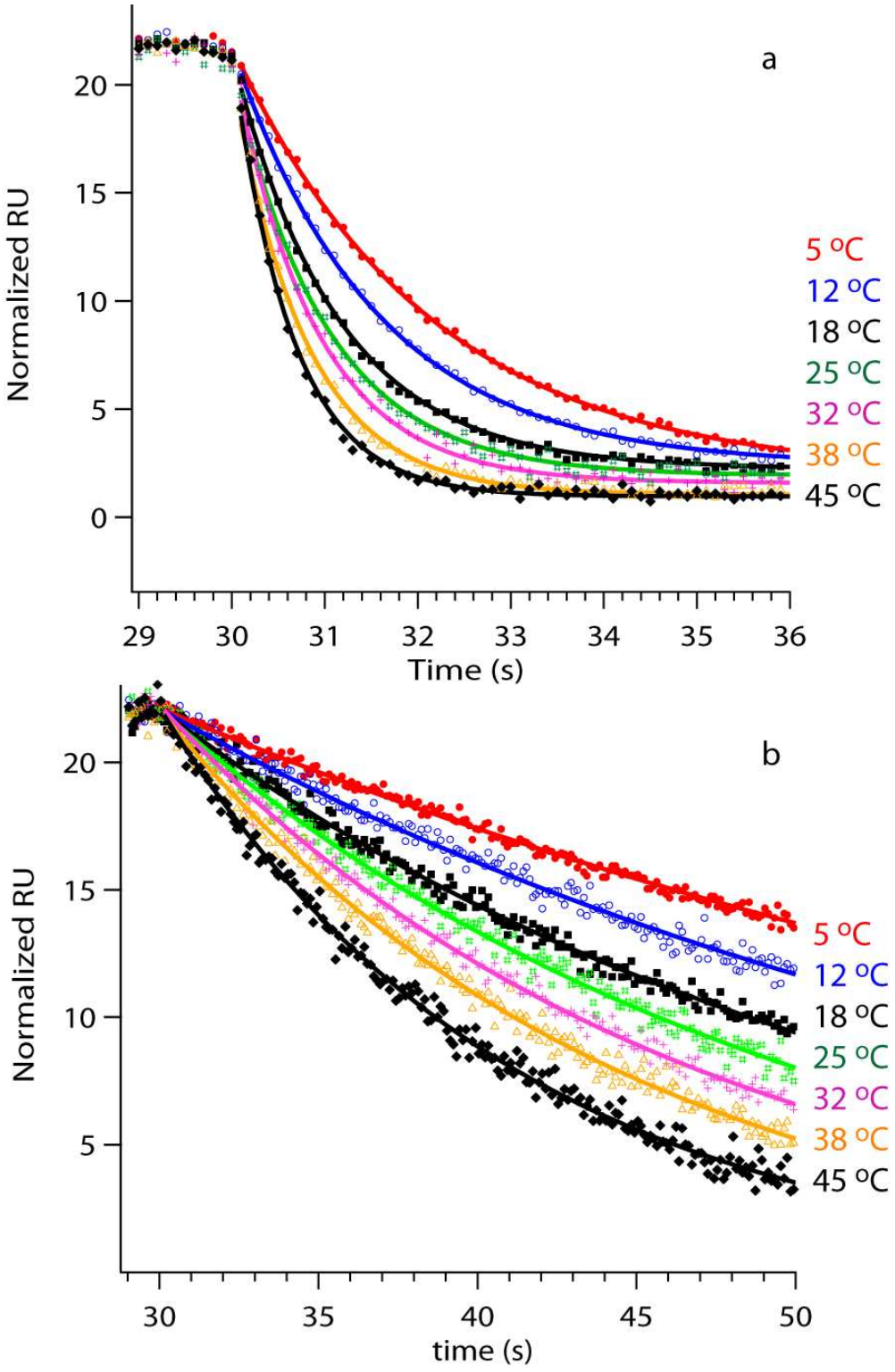

Figure S1. SPR kinetic plots for (a) (Lg+PA) and (b) (Lg+SA) at the temperatures indicated.

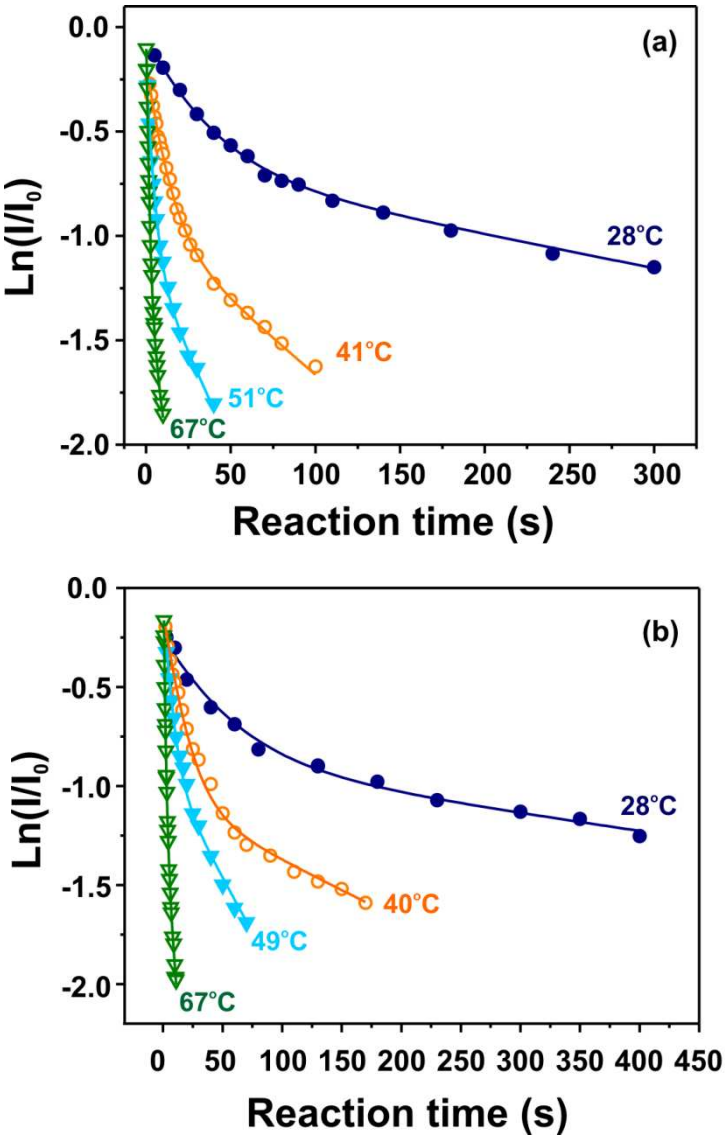

Figure S2. Plots of the natural logarithm of the normalized intensity (I/I_o) of (a) $(Lg + PA)^{6-}$ and (b) $(Lg + SA)^{6-}$ versus reaction time at the temperatures indicated.