Supporting Information

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Design of a Conformation-Sensitive Xenon-Binding Cavity in the Ribose Binding Protein

Thomas J. Lowery, Seth M. Rubin, E. Janette Ruiz,
Alexander Pines, David E. Wemmer*

Department of Chemistry, University of California at Berkeley, Berkeley, CA 94720, USA, and
Physical Biosciences Division and Material Sciences Division, Lawrence Berkeley National
Laboratory, 1 Cyclotron Rd, Berkeley, CA 94720, USA.

Experimental Methods

Cavity Volume Prediction. Cavities were designed using the PDB entries 1URP and 2DRI for the closed and open conformations of RBP.^[1,2] Calculations of cavities were made with VOIDOO^[3], using a probe size of 1.4 Å³ and a primary grid spacing of 0.5 or 1.0 Å. Volumes reported were calculated with a primary grid spacing of 0.5; calculated volumes decreased by ~25 Å³ when the primary grid spacing was set to 1.0, but the difference in calculated cavity volumes between the two L19A conformers remained ~30 Å³. Molecular visualization was done using O^[4] or PyMol.^[5]

Protien Preparation. The w.t. *E. coli* RBP gene without the periplasmic signaling sequence was a gift from Sherry Mowbray. The RBP gene was cloned into a pSKB3 plasmid, which adds an N-terminal mTEV-cleavable histidine tag. A Stratagene QuikChange Site-Directed Mutagenesis Kit was used to mutate the CTG Leu codon to the GCG Ala codon at base numbers 55-57. The presence of the mutation was verified by DNA sequencing. Proteins were expressed

in BL21* cells. To ensure that purified protein was free of endogenous ligand, wild-type and L19A RBP were purified under denaturing conditions (50 mM sodium phosphate pH 7, 0.3 M NaCl, 8 M Urea), refolded on an Qiagen Ni-NTA agarose column, as described,^[6] then dialyzed overnight against 50 mM sodium phosphate pH 7. For the urea denaturation and ^1H - ^{15}N HSQC experiments the histidine tag was cleaved by adding 0.5 mg of mTeV to the overnight dialysis solution with 50-80 mg of RBP. mTEV protease and cleaved histidine-tag were removed by passing the solution through a Ni-NTA column under native conditions. The extent of protease cleavage was greater than 95% as determined by gel electrophoresis. Protein was used either directly or desalted with an Amersham Biosciences PD-10 column, frozen, and lyophilized for later use. Typical yields were 50-80 mg/L. Protein purity was determined to be greater than 95% by gel electrophoresis. L-Methionine (~10 mg/L) was added to all lysis and purification buffers to protect against methionine oxidation. Mass spectrometry was used to confirm the molecular weights of the wild-type and mutant proteins, and the absence of methionine oxidation.

^{129}Xe Nuclear Magnetic Resonance Spectroscopy. Protein sample buffers and dilution buffer were prepared by dissolving lyophilized protein or diluting purified protein in 8.0 mL of 25 mM sodium phosphate pH 7.0, 10% D_2O . 5 mM ribose was added for assays of the closed RBP conformation. The dilute protein solution was then concentrated to ~280 μL with an Amicon-Ultra 4 or 15, 5000 MWCO, Centrifugal Filter Device at maximum speed. Concentrator flow was saved and used as matched dilution buffer for ^{129}Xe NMR assays. Laser polarized ^{129}Xe was prepared as described.^[4,5] ^{129}Xe NMR spectra were obtained on a 300 MHz (proton frequency) Varian Inova spectrometer at 25° C.^[5] A method of sequential dilutions was used to obtain the data shown in Figure 1 and 4. Within 1 minute of introducing polarized ^{129}Xe to the ~8 mL of

degassed dilution buffer, 140 μ L of said buffer was added and mixed with the $\sim$ 280 μ L of concentrated protein solution ($\sim$ 1.5 mM). The sample was immediately inserted into the magnet to obtain the ^{129}Xe spectrum with a single, broadband 90° pulse.^[5] Immediately after the measurement, the sample was ejected, $\sim$ 140 μ L was removed and saved for later analysis, and another $\sim$ 140 μ L aliquot of ^{129}Xe -pressurized dilution buffer was added. This process was repeated five or six times within a total time of ten minutes. The ^{129}Xe chemical shift in the absence of protein was measured before and after each titration by adding polarized ^{129}Xe -dilution buffer to $\sim$ 280 μ L of protein-free dilution buffer before the first and after the last protein titration points. The change in the ^{129}Xe chemical shift in dilution buffer was less than 0.02 ppm over the course of the experiment. The $\sim$ 140 μ L aliquots of sample that were removed after each ^{129}Xe NMR measurement were used to determine the protein concentration by absorbance at 280 nm ($\epsilon = 7.45 \text{ cm}^{-1} \text{ mM}^{-1}$ for His-tagged RBP; $\epsilon = 4.47 \text{ cm}^{-1} \text{ mM}^{-1}$ for cleaved RBP). Extinction coefficients were determined as described.^[7] Data shown in Figures 1 and 4 were obtained from duplicate protein preparations and ^{129}Xe NMR experiments.

Isothermal Titration Calorimetry. Ribose binding for both WT and L19A RBP was measured using a Micro Cal MCS isothermal titration calorimeter at 25°C . Protein concentrations were between 80 μM and 120 μM in 15 mM sodium phosphate pH 7.2, 50 mM potassium chloride. Ribose concentrations in the injection syringe were twenty times the concentration of protein and were added to the protein solution in 7 μ L injections. Data were fit with the accompanying MicroCal Origin software to the standard single-site binding model. Reported dissociation constants, n-values, and ΔH_b are averages of duplicate experimental runs.

Urea Unfolding Experiments. Stock 0 M and 4.0 M urea solutions of 30 $\mu\text{g/ml}$ histidine-tag-free protein were prepared by adding lyophilized protein to 50 mL of stock buffer (15 mM sodium

phosphate pH 7.0, 50 mM potassium chloride, 0M or 4.0 M urea). Stock solutions were used to prepare 2 mL samples in 0.1 M urea increments ranging from 0 M to 4 M urea. The unfolding reaction was allowed to equilibrate for 24 h at 25° C. The circular dichroism signal at 222 nm was monitored for 1 minute at 25° C and averaged using an Aviv 62DS spectrometer with a Peltier temperature-controlled cell holder. The data were fitted to a two-state model, and ΔG_u was determined using a linear extrapolation.^[8]

¹H-¹⁵N HSQC Spectroscopy and Xenon Affinity Determination. Uniformly ¹⁵N-labeled histidine-tag-cleaved protein was used for all ¹H-¹⁵N HSQC experiments. HSQC spectra were obtained on a 600 MHz Bruker spectrometer as described.^[9] Xenon pressures were measured and concentrations calculated as described.^[9] All samples were 1.0-1.5 mM protein in 25 mM sodium phosphate pH 6, 10% D₂O. Ribose was added to a final concentration of 5 mM for samples of the closed RBP conformation.

Determination of $\Delta\Delta G_u$ Values. $\Delta\Delta G_u(\text{open})$ is the difference between the free energy of unfolding for the open conformations of wild-type RBP and L19A. The value of $\Delta\Delta G_u(\text{closed})$ was calculated using Equation (2),

$$\Delta\Delta G_u(\text{closed}) = \Delta\Delta G_u(\text{open}) + RT\ln(K_d(\text{WT})/K_d(\text{L19A})) \quad (2)$$

where, $\Delta\Delta G_u(\text{closed})$ is the change in free energy of unfolding for the closed conformation, and $K_d(\text{WT})$ and $K_d(\text{L19A})$ are the dissociation constants measured for L19A and wild-type RBP, respectively.

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Figure S1. a) Experimental data for the titration of 0.098 mM of L19A with 7 μL injections of 1.6 mM ribose at 25° C. b) Results of fitting the integrated data to a single site binding model. The average values of duplicate runs measured for L19A were $K_d = 0.30 \pm 0.05 \mu\text{M}$, $n = 0.8$, and $\Delta H_b = -7.3 \pm 0.7 \text{ kJ mol}^{-1}$. The values measured for duplicate runs of wild-type RBP (data not shown) were $K_d = 0.11 \pm 0.01 \mu\text{M}$, $n = 0.9$, and $\Delta H_b = -7.6 \pm 4 \text{ kJ mol}^{-1}$. The slightly decreased ribose binding affinity of L19A reflects the destabilizing effects of the L19A mutation, but confirms that in solutions of excess ribose L19A is in the closed conformation.

Figure S2. Representative portion of ^1H - ^{15}N HSQC spectra for the open conformation of wild-type RBP in the absence of xenon (black) and in the presence of 90 mM xenon (green). No wild-type resonances shift in the presence of 90 mM xenon, indicating the absence of a specific xenon binding site in the wild-type protein.

Figure S3. The fraction unfolded (F) versus urea concentration (C) as measured by circular dichroism for 30 μ M of the open conformations of L19A (O) and wild-type RBP(●). Fitting the data to a two state model^[8] yielded $\Delta G_u = 3.3 \pm 0.1 \text{ kcal mol}^{-1}$, $C_m = 0.7 \text{ M}$, and m value = 4.8 for L19A. Similarly, the values of $\Delta G_u = 5.8 \pm 0.1 \text{ kcal mol}^{-1}$, $C_m = 1.6 \text{ M}$, and m value = 3.6 were obtained for WT. The decreased free energy of unfolding for the open conformation of L19A reflects the destabilizing effects of the L19A mutation.