Supporting Material

Measuring pK_a Values in Protein Folding Transition State Ensembles by NMR Spectroscopy

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Sample preparation. Wild-type and His7Ala mutant drkN SH3 domains were expressed and purified as described.[1] NMR samples were 1.0 mM in protein, 50 mM sodium phosphate, 92% H_2O/8% D_2O. All experiments were performed at 15ºC on a Varian UNITY spectrometer with a room temperature triple resonance probehead at 500 MHz (^{1}H frequency).

Kinetic folding and unfolding measurements by longitudinal magnetization exchange NMR spectroscopy. Microscopic folding and unfolding rate constants for the drkN SH3 domain were determined by simultaneously fitting the experimental data from a series of 2D ^{1}H-^{15}N exchange correlation spectra with mixing times ranging from 11 to 451 ms to analytical expressions describing the time course of longitudinal magnetization including chemical exchange between two sites.[2] In order to account for differential relaxation of magnetization associated with folded and unfolded states, the data were recorded and analyzed as described previously,[3] yielding residue-specific rate constants that are uniform for well-resolved residues to within ~10%. Averages of residue-specific values of k_f and k_u were employed in all analyses and errors in rates estimated on the basis of the standard deviation of the values over the residues that were included in the average. Only residues for which all auto- and cross-peaks are well-resolved at all pH values were used in the analysis (residues 5, 18, 22, 43, 46 and 50). Kinetic data were obtained for wild-type protein at pH values of 2.0, 2.2, 2.4, 2.6, 2.8, 2.9 and 3.2 and for the His7Ala mutant SH3 domain at pH values of 2.0-3.0 in steps of 0.2. The pH value of each sample was measured in the NMR tube and values were not corrected for the isotope effect on the pH electrode,[4] and the protein concentration was kept constant throughout the titration experiment. The folding of a number of proteins has been shown to be solvent viscosity dependent (ref. 5 and references therein). Translational diffusion constants for the drkN SH3 domain were determined using a pulsed-field-gradient NMR experiment[6] and are invariant between pH 2.0 and pH 3.2, indicating that solvent viscosity changes in this pH range are negligible. Experimental kinetic data were therefore not corrected for the effect of solvent viscosity.

Analysis of the kinetic data. Both microscopic folding and unfolding rate constants, k_f and k_u, are obtained simultaneously from a single experiment; errors in the rates were estimated as described above and subsequently propagated to errors in $\Delta G_{U\rightarrow F}$, $-RT\ln k_f$ and $-RT\ln k_u$. Values of pK_a(‡) can be extracted directly from fits of kinetic data recorded on samples under non-denaturating, equilibrium conditions using eqs 1,2 of the text, along with known pK_a values for the titrating groups.[7] Experimental uncertainties in pK_a(‡) values for Asp8 in wild-type and His7Ala drkN SH3 domains were estimated via a Monte Carlo approach[8] in which 1000 synthetic profiles of $-RT\ln k_f$ and $-RT\ln k_u$ *vs.* pH were generated using the extracted pK_a(‡) values, the known pK_a values for Asp8 of the U and F states and the experimental errors in the rate values. The errors quoted in the paper are standard deviations in fitted pK_a(‡) values.

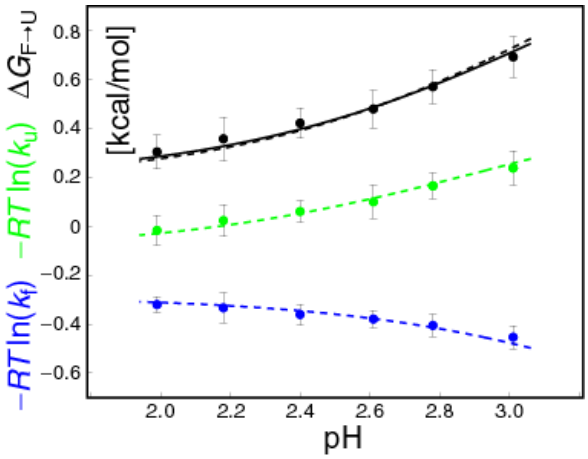

Figure S1. Figure showing $\Delta G_{F\rightarrow U}$ (black), $-RT\ln k_f$ (blue) and $-RT\ln k_u$ (green) profiles of the His7Ala drkN SH3 domain as a function of pH, 15ºC. Details are as in the legend to Figure 2.

References

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