Supporting Information

Electrothermal Supercharging of Proteins in Native Electrospray Ionization

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Experimental

Nano-ESI mass spectra of 20 μM bovine ubiquitin or equine cytochrome *c* in 200 mM ammonium bicarbonate, pH 7.0 (pH adjusted with acetic acid), solutions were obtained in a linear trapping quadrupole (LTQ) mass spectrometer (Thermo). For the ubiquitin experiments, the temperature of the heated metal "entrance capillary", which is the conductance-limit between atmospheric pressure and the first pumping stage of the instrument, was varied in 25 °C increments between 150 and 300 °C. To ensure any effect of the entrance capillary temperature on the charge-state distributions observed was not related to heating of the bulk nESI solution prior to droplet formation, the temperature of the air space 5 mm away from the entrance capillary, where the tip of the spray capillary was placed for these experiments, was measured using a thermocouple. At entrance capillary temperatures of 150 and 300 °C, the air space was 34 and 40 °C, respectively. The low air space temperatures were maintained by removing the glass windows on both sides of the nESI source assembly. The potential applied to the platinum wire inserted into the nESI capillary in contact with the analyte solution ("spray potential") was varied relative to instrument ground in 0.2 kV increments between 0.8 kV and 1.8 kV. Spectra were recorded in triplicate at each spray potential and at each temperature using a new nESI spray capillary for each replicate measurement. Spectra at each spray potential/temperature setting were acquired continuously for 30 s and signal-averaged. For the cytochrome *c* experiments, the solution was continuously nanoelectrosprayed while the spray potential was switched 20 times between 0.8 and 1.8 kV at a source temperature of 200 °C. Spectra were signal-averaged for 30 s at each potential before switching to the alternate spray potential.

For experiments on the 9.4 T Berkeley-Bruker FT-ICR, a 100 mM ammonium bicarbonate, pH 7.3 (pH adjusted with acetic acid), solution containing 25 μM cytochrome *c* and 10 μM ubiquitin was prepared as a mixture to eliminate tip-to-tip variability so that the extent of unfolding for these two protein could be directly compared. The ion source of this instrument has a ~18 cm heated glass capillary as the conductance-limit between atmospheric pressure and the first pumping stage of the instrument and the maximum entrance capillary temperature is 180 °C. Five spectra were obtained and signal averaged with the source temperature set to either 125 or 180 °C, with the spray voltage increased in 50 V increments between 1.05 kV and 1.4 kV. The nESI spray capillary was placed 3 mm from the entrance capillary. Triplicate measurements were obtained with three different nESI spray capillaries.

For the experiments in which ionic strength was investigated, solutions of 10 μM ubiquitin in pure water, 0.001, 0.01, 0.1, 0.5 or 1.0 M ammonium bicarbonate, pH 7.0 (pH adjusted with formic acid), were nanoelectrosprayed on the Thermo LTQ at a source temperature of 220 °C with the spray potential increased in 50 V increments between 0.75 and 1.3 kV. The nESI spray capillary was placed 5 mm from the entrance capillary. Mass spectra were acquired for 30 s. Spectra were obtained in triplicate at each spray potential using a new nESI capillary for each replicate measurement.

For the Hofmeister series experiments, 20 μM ubiquitin solutions were prepared in 10 mM ammonium acetate, bicarbonate, thiocyanate, phosphate, sulfate, and perchlorate solutions. The pH of these solutions was adjusted to 7.0 with either ammonium hydroxide or acetic acid. Higher salt concentrations were not used owing to the formation of salt clusters (for sulfate, perchlorate and phosphate) and extensive protein ion adduction that interfered with the measurements. The source temperature on the Thermo LTQ was set to 250 °C and 30 s scan

averaged mass spectra were obtained with a spray voltage of 1.2 kV. The tip of the nESI spray capillary was placed 5 mm from the entrance capillary for all of the solutions. Triplicate measurements were made with three different nESI spray capillaries for each solution.