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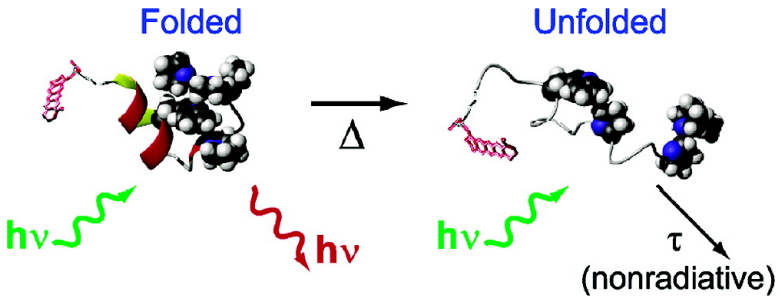

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Conformational Change in Unsolvated Trp-cage Protein Probed by Fluorescence

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Characterization of the structure and dynamics of biopolymers is of paramount importance to understanding life processes at the molecular level. Measurements of these molecules in the absence of a solvent or surface are useful for elucidating the effects of the solvent environment on the native structure and dynamics. Mass spectrometry-based measurements of ion mobility[1] and ion−molecule[2] and dissociation[3,4] reactions have been used to detect global conformational change in unsolvated biopolymers. We have been applying fluorescence spectroscopy to unsolvated biomolecule ions to take advantage of the unique ability of this technique to directly probe the local molecular environment surrounding the fluorophore.[5,6] Here, we report the first fluorescence measurements of protein unfolding in the gas phase.

Trp-cage is an actively studied 20-residue protein[7] that folds cooperatively[8] in 4 μs and presents a tractable size for calculations.[9] The native structure includes a Trp residue "caged" by three Pro residues, a feature that makes this protein especially suitable for the detection of unfolding in the gas phase by intramolecular fluorescence quenching. As the protein is heated to induce conformational change, the Trp will be released from its cage and become more exposed to intramolecular collisions with a covalently attached fluorescent dye, BODIPY TMR. Such collisions quench the dye fluorescence so that conformational changes will be directly correlated with the fluorescence intensity.

Studies of fluorescence quenching in solution[10−14] indicate that quenching occurs through contact formation between Trp and the dye during a collision (interaction distance[14] ∼5 Å). We have performed solution-phase measurements of the fluorescence lifetime of BODIPY TMR-labeled Trp-cage. The fluorescence decay was fit closely by a monoexponential model and yielded quenching rates defined by Arrhenius parameters. These quenching measurements compare very closely with those obtained in solution for similar BODIPY dyes.[12] Stern−Volmer analysis of bimolecular quenching of BODIPY TMR and Trp indicates that dynamic quenching is approximately twice as efficient as static quenching. In the gas phase, we assume contact formation continues to be the dominant quenching mechanism, however the intramolecular collision rates will not be constrained by diffusion-limited kinetics. The temperature dependence of gas-phase quenching will be the subject of planned investigations. The increased collision rate in the gas phase is an essential condition for probing Trp-cage unfolding dynamics with collisional fluorescence quenching.

Protein ions are generated by nanoelectrospray (nanoES) and injected into a custom-built quadrupole ion trap which can be heated to a maximum temperature of ∼445 K. Ions are thermalized by collisions with a background He gas at 3 mTorr and maintained in thermal equilibrium throughout the trapping cycle.[15] The mass spectrum shown in Figure 1 was obtained by ejecting ions into a Channeltron detector and displays the 2+ and 3+ charge states of BODIPY TMR-labeled Trp-cage. Resolution is sufficient to positively identify the desolvated ions, and no evidence of dissociation was observed over the temperature range studied. Ions are irradiated at the frequency doubled wavelength 532 nm by a diode-pumped Nd:YAG laser. Laser-induced fluorescence from the ions is collected by a triplet lens closely coupled to a trap aperture and passes through a dichroic beam-splitter to a two-channel detection system (GaAs photomultipliers) which isolates the fluorescence bandwidths of different fluorescent dyes. Careful design of the optical beampath reduces the detection of background-scattered laser radiation, enabling fluorescence measurements from ∼200 ions to be obtained in 100 ms with a laser intensity of ∼130 W/cm^2. There was no indication of heating during irradiation.

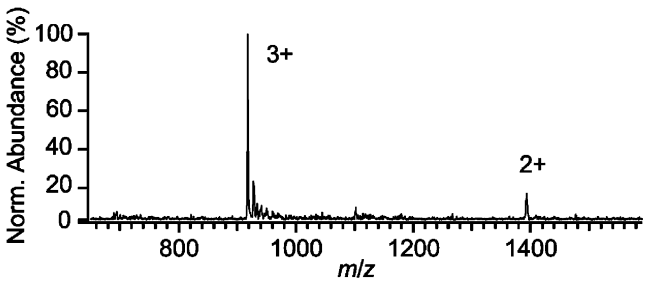

Figure 1. Representative charge state distribution of Trp-cage-(BODIPY TMR) (10 μM) ions formed from an aqueous solution by nanoES.

Figure 2 shows the fluorescence intensity per ion for the 2+ and 3+ charge states of the dye-labeled Trp-cage, denoted as Trp-cage-(BODIPY TMR), formed from an aqueous, buffered nanoES solution. Each fluorescence data point represents the average counts per second of ∼10^3 ions, and identical experimental parameters yield reproducible data taken weeks apart. The fluorescence from both charge states is initially independent of temperature, suggesting a compact conformation in which the Trp residue is well shielded from intramolecular collisions with the dye. The fluorescence intensity of 3+ decreases by a factor of ∼3 more than that of 2+ (Figure 2). Molecular dynamics simulations[16] of the 2+ charge state indicate the conformation is not very different from the native state (2−4 Å C_α RMSD) at temperatures up to 420 K. The rapidly decreasing portion of the fluorescence data of the 3+ ion toward a lower plateau in Figure 2b is presumably due to an increasing exposure of the Trp residue with temperature that leads to an increased quenching rate. Heating ions of BODIPY TMR (without the protein attached) over the same temperature range did *not* result in a change in fluorescence within experimental error (data not shown). This indicates that the decrease in fluorescence observed upon heating the labeled Trp-cage is due to the conformational dynamics of the protein and not the intrinsic photophysics of the dye. The larger decrease in fluorescence observed upon heating the 3+ charge state (Figure 2b) compared with that of the 2+ (Figure 2a) is consistent with the 3+ being more readily unfolded due in part to greater Coulombic repulsion in the higher charge state.[1,4,17]

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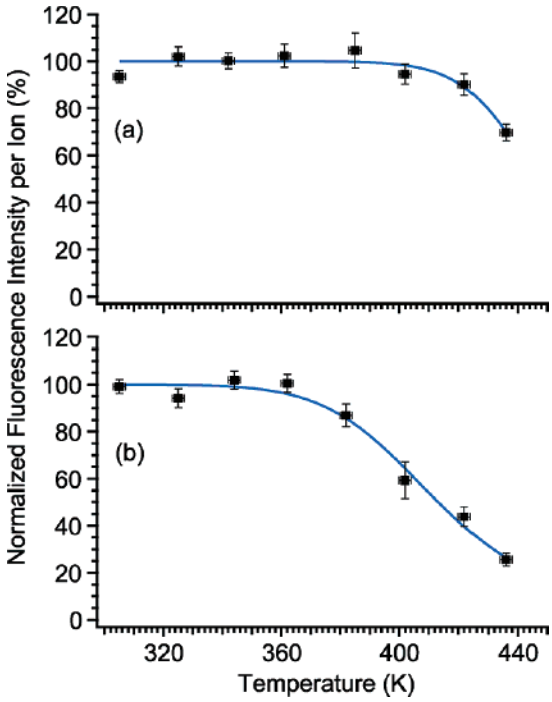

Figure 2. Normalized fluorescence intensity per ion versus temperature for (a) $(M + 2H)^{2+}$ and (b) $(M + 3H)^{3+}$ ions of Trp-cage-(BODIPY TMR) formed from an aqueous solution. Measured data points are denoted by square markers, and fits to the data by the model are delineated by blue curves. Error bars are ± one standard deviation from the mean. Plots are normalized to the values at 303 K.

Table 1. Thermochemical Parameters for Conformational Change of Trp-cage-(BODIPY TMR) Ions

charge state	ΔH (kJ/mol)[a]	ΔS (J/mol·K)[a]
2+	134 ± 52	301 ± 119
3+	75 ± 11	183 ± 28
solution[b]	~48.6	~155

[a] Stated errors are ± one standard deviation from the mean. [b] From ref 8.

Fits to the data by a fluorescent model incorporating intramolecular collisions and a two-state model of protein unfolding are represented by solid curves in Figure 2. The enthalpy and entropy changes derived from the fit for the 3+ charge state are 1.5 and 1.2 times, respectively, as large as the values measured in solution. The thermodynamic parameters derived for the 2+ are also larger than the solution values, albeit with greater uncertainties (Table 1). McLafferty and co-workers[4] obtained thermochemical parameters of unfolding for ubiquitin ions (6+ to 9+) using electron capture dissociation (ECD). In this case, the enthalpy changes for the ubiquitin ions were lower than those measured in solution, a result that was attributed to Coulombic, structural, and/or entropic effects.[4] Clearly, the effects of Coulombic repulsion do not dominate the unfolding dynamics in the lower charge states of Trp-cage, thus providing an improved opportunity to study the contributions of individual interactions, such as hydrogen bonds, to the stability and dynamics of the folded structure. For example, hydrogen bond donor and acceptor groups located on the surface of the folded protein, and exposed to solvent in solution, may form new intramolecular hydrogen bonds upon desolvation, thus contributing to an increased enthalpy of unfolding.[18] Also, the strength of the salt bridge involving Lys8, Asp9, and Arg16 and that of the hydrogen bonds in the α-helix (residues 2−8) may effectively increase upon removal of water. The total Coulombic energies of the 2+ and 3+ charge states were estimated using a point charge model (effective dielectric polarizability = 1.3),[19] the published structure of Trp-cage,[7] and charge locations derived from the ECD measurements of Zubarev and co-workers.[17] On the basis of published estimates of the energies of hydrogen bonds (~25−42 kJ/mol)[18] and salt bridges (~67 kJ/mol)[20] in unsolvated proteins, the unfolding enthalpy for gas-phase Trp-cage (for both 2+ and 3+ charge states) can be accounted for by the salt bridge plus three to six intramolecular hydrogen bonds. The higher ΔS in gas phase may be due to increased order in the folded state as a result of solvation of charges by the backbone and/or increased hydrogen bonding. In addition, hydrophobic effects have not explicitly been considered.

To better understand the changes in enthalpy and entropy, we will perform fluorescence measurements of single-point Trp-cage mutants which constrain both hydrogen bond formation and charge location. These measurements will be compared with molecular dynamics simulations to correlate measured changes in fluorescence with unfolding dynamics. In general, the instrumentation obtains reproducible results rapidly and with excellent signal-to-noise ratios. Moreover, the uncertainty in the fit parameters will be significantly reduced by increasing the temperature range of the ion trap to enable the observation of plateaus in fluorescence at higher temperatures.

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Supporting Information Available: Materials and methods, solution melting curve, Coulomb energy, and quenching model. This material is available free of charge via the Internet at http://pubs.acs.org.

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