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Supporting Information

Retention of Native Protein Structures in the Absence of Solvent: A Coupled Ion Mobility and Spectroscopic Study

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Experimental procedures:

Gas-phase measurements

All chemicals were purchased from Sigma-Aldrich. The proteins are dissolved at a concentration of 50 µmol/L in either a) pure water with ammonium acetate (10 mmol/L) as a buffer, b) 1/1 water/methanol or c) 1/1 water/methanol with the addition of 1% formic acid. To all solutions containing β-Lactoglobulin, 50 µmol/L of palmitic acid is added as well.

The experiments are performed using an apparatus described previously.[1] In brief, the proteins or protein complexes are transferred into the gas phase using Pd/Pt-coated borosilicate nano-electrospray ionization (*n*ESI) capillaries. Ions are accumulated in a radio frequency (RF) ion funnel and pulsed into a drift region where IMS separation occurs. After transfer to high vacuum, a small drift time window and thereby the geometrical size/charge ratio is selected, the ions are passed through a quadrupole mass/charge selector and afterwards irradiated by the tunable IR light from the FHI Free Electron Laser.[2] When multiple photons are absorbed, fragmentation can occur which is monitored by a time of flight mass/charge analyzer. Plotting the fragmentation yield as a function of IR wavelength gives then the IR spectrum of the ion. All gas-phase IR spectra in this work are the averages of at least two repeated scans.

Myoglobin has a non-covalently bound heme group attached which is easily lost for high charge states in the gas phase or when being energized. β-Lactoglobulin is able to bind hydrophobic molecules such as fatty acids.[3] To record some of the IR spectra shown, the dissociation of the ligand (heme group or palmitic acid) from the protein is used as a marker for the absorption of (multiple) photons. Compared to standard IRMPD experiments,[4] a smaller number of absorbed photons are needed to induce dissociation, thereby reducing the influence of anharmonicities and nonlinearities on the resulting spectra.

In the here presented experiments, palmitic acid is observed to bind to β-Lactoglobulin when being sprayed from aqueous solutions. The corresponding mass peaks are marked in the mass spectra of Fig. 2B as 9+', 8+' and 7+'. For the charge states 8+ and 9+, cross sections have been measured for both, the bare ions and the corresponding ions complexed with palmitic acid. Their respective differences in cross sections are small and within the dot sizes of Figure 2d. When being sprayed from water/methanol, no palmitic acid adduct is observed. For Myoglobin, the heme containing holo form is observed when being sprayed from aqueous solutions as well as from water/methanol, however not when 1% formic acid is added, in which case the apo form of Myoglobin is observed. Mass spectra for β-Lactoglobulin and Myoglobin ions when being sprayed from water methanol with 1% formic acid added are shown in Fig. S1. The mass spectra shown here and in Fig. 2 are similar to those reported by others.[3a, 5]

The IR spectra for Myoglobin 8+ and 10+ (Figure 3b, 3c and 3d and Figure S3 b) are recorded by monitoring the loss of the heme group as a function of excitation wavelength. For the 10+ charge state, several conformers are present (Figure 2c and Figure S3 a), all having cross sections

larger than what is expected for a native protein. The spectrum shown in Figure 3b results from the low cross section conformer II, however, the spectra for all other Myoglobin conformers in this charge states are essentially identical (Figure S3 b). To record the IR spectrum of the 18+ charge state, dissociation of the molecule is monitored. For β-Lactoglobulin, the IR spectrum of the 8+ charge is recorded by measuring the loss of the palmitic acid ligand. For higher charge states, fragmentation of the molecule is monitored.

Condensed-phase circular dichroism measurements

CD spectra are measured at 20 °C using a Jasco J-810 spectropolarimeter (Jasco Inc., Easton, MD, USA) equipped with a Jasco PTC-423S Peltier temperature control system and a HAAKE WKL water recirculator (Thermo Electron GmbH, Karlsruhe, Germany). Proteins are dissolved at a concentration of 50 μmol/L in either a) 10 mmol/L ammonium acetate buffer (pH ~7) or b) 1/1 water/methanol, identical conditions as used for the gas-phase experiments. A quartz glass cuvette with 1-mm path length is used to record CD spectra at 190–240 nm with 2 nm spectral resolution. Solvent spectrum is recorded under identical conditions and used for the background-correction. CD spectra for β-Lactoglobulin are measured with and without the addition of 50 μmol/L of palmitic acid.

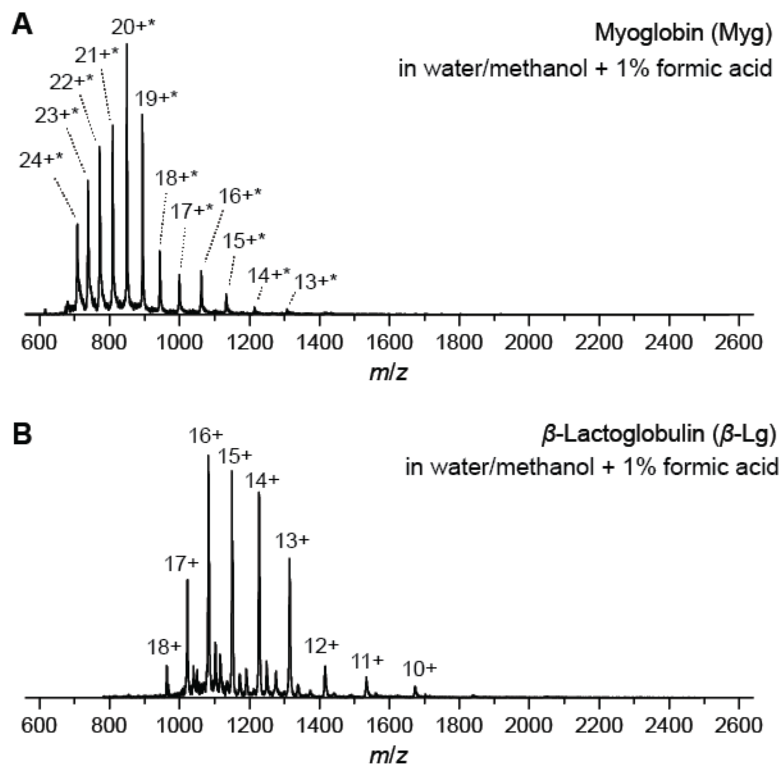

Figure S1. Charge state distribution of proteins in acidic solution. (A) Mass spectrum of Myoglobin in water/methanol with 1% formic acid. The heme is readily detached from myoglobin in the acidic water/methanol, and thus only apo-form of Myoglobin is observed. The notation *n*+* stands for [Myoglobin – heme + *n*H]$^{n+}$. (B) Mass spectrum of a 1:1 mixture of β-Lactoglobulin and palmitic acid in acidic water/methanol. Palmitic acid is not bound to β-Lactoglobulin under those solvent condition and only bare proteins are detected at high charge states. The notation *n*+ stands for [β-Lactoglobulin + *n*H]$^{n+}$.

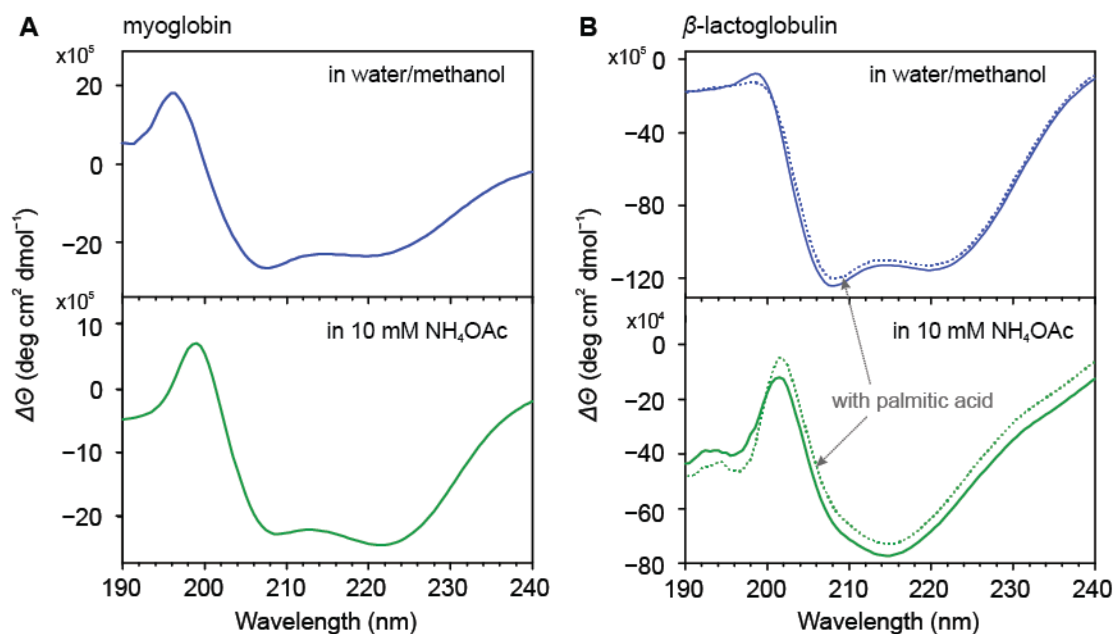

Figure S2. Circular dichroism spectra. (A) Myoglobin and (B) β-Lactoglobulin either in water/methanol (blue) or buffered water (green). Dotted lines denote CD spectra of 1:1 mixtures of β-Lactoglobulin and palmitic acid.

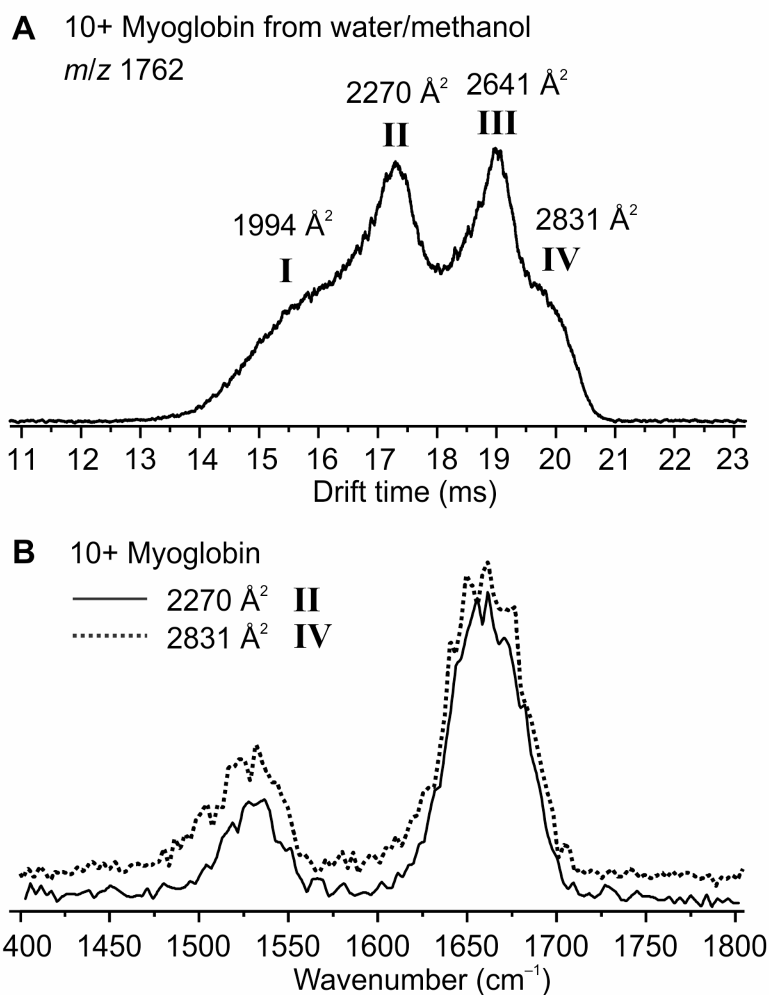

Figure S3. Arrival time distributions and IR spectra of two conformers of Myoglobin 10+. (A) Arrival time distribution of 10+ Myoglobin sprayed from water/methanol. At least four different conformers (I–IV) with vastly different CCS values (1994, 2270, 2641, and 2831 $Å^2$) are observable. (B) Gas-phase IR spectra of two different conformers (solid line for II and dotted line for IV) of Myoglobin 10+.

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