

Electronic Supplementary Information

Fingerprinting the Tertiary Structure of Electroadsorbed

Lysozyme at Soft Interfaces by Electrostatic Spray

Ionization Mass Spectrometry

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MATERIALS AND METHODS

Reagents. The organic phase was gelled and prepared using bis(triphenylphosphoranylidene) tetrakis(4-chlorophenyl)borate (BTPPA⁺TPBCl⁻, 10mM) and 10% w/v low molecular weight poly(vinyl chloride) (PVC) in 1,6-dichlorohexane (1,6-DCH). The BTPPA⁺TPBCl⁻ salt was prepared by metathesis of bis(triphenylphosphoranylidene)ammonium chloride (BTPPA⁺Cl⁻) and potassium tetrakis(4-chlorophenyl)borate (K⁺TPBCl⁻).¹ Lysozyme solutions were freshly prepared in 10 mM HCl (pH 2) prior to electrochemical experiments. All reagents were purchased from Sigma-Aldrich Switzerland Ltd. All aqueous solutions were prepared in purified water from an alpha Q Millipore system (Zug, Switzerland).

Electrochemical apparatus. All electrochemical experiments were performed using an Autolab PGSTAT302N electrochemical analyser (Metrohm Autolab, Utrecht, The Netherlands). The millimetre size interface was formed using a glass pipette of 0.075 cm radius. The total geometric area of the water-organogel interface was estimated to be 0.035 cm², assuming that the droplet formed is a perfect hemisphere. The electrochemical cell is summarized in Scheme 1.

Electroadsorption. A three-electrode system was employed for all measurements. Electrochemical adsorption (or pre-concentration) at fixed applied potentials for various times was implemented before ESTASI-MS analysis of the organogel. There was no electrochemical detection of the adsorbed protein by stripping voltammetry, as reported previously,² so that the protein adsorbed at the interface was retained for analysis *via* mass spectrometry.

ESTASI-MS. After pre-concentration of lysozyme at the water-organogel interface, the organogel was cut from the end of the glass pipette and fixed onto a 0.2 mm GelBond®PAG film (Lonza), as shown in Figure 1, for ESTASI-MS analysis. The gel was placed close to the MS inlet.³ A gold electrode was placed underneath the plastic layer (GelBond®PAG film). During ESTASI-MS, drops (1-2 μL) of an acidic solution consisting of 1% (v/v) acetic acid in a 50:50 water:methanol (v:v) solution were placed on top of the organogel, followed immediately with the application of high voltage (HV) pulses to start the ESTASI. Moreover, a control analysis of lysozyme in solution without organogel *via* ESTASI-MS was performed by a similar procedure, where 1 μL of lysozyme (1, 5, 10 or 20 μM) in 1 % (v/v) acetic acid in water:methanol (50:50, v:v) was deposited onto the insulating plastic film for ionization.

The HV pulses were generated by a electric circuit of one direct current HV source (9 kV, limiting current 0.5 mA) and two switches that regulating the electrospray electrode to be either connected with the HV source *via* switch 1 or grounded *via* switch 2 (Figure 1). A LabView program controlled the two switches in order to synchronize their application. The spray voltage of the internal power source of the mass spectrometer (Thermo, LTQ Velos) was set to 0 V. An enhanced ion trap scan rate (10,000 m/z per second) was selected to obtain a good spectral resolution. All the MS data were processed using the Xcalibur program (Thermo Fisher Scientific Inc., Hampton, New Hampshire, USA). In addition, mathematical deconvolution of mass spectra was performed using ProMass (Thermo Fisher Scientific Inc., Hampton, USA).

CONTROL EXPERIMENTS

The organogel, consisting of 10 mM organic electrolyte (bis(triphenylphosphoranylidene) tetrakis(4-chlorophenyl)borate, (BTPPATPBCl)) in 1,6-dichlorohexane gelled by adding polyvinyl chloride (10% w/v), was characterized by ESTASI-MS. A sharp peak at a mass-to-charge ratio (m/z) of 538.26, corresponding to the molecular mass of the BTPPA⁺ cation with a single charge, was observed in the positive mode MS analysis, while the TPBCl⁻ anion was observed at an m/z ratio of 457.16 in the negative mode (Fig. S1).

Then, different concentrations of lysozyme (1, 5, 10 and 20 μ M) in the acidic buffer solution (1% (v/v) acetic acid in a 50:50 water:methanol (v:v)) were deposited on the plastic film, and analyzed by ESTASI-MS (Fig. S2). The ESTASI mass spectra obtained for lysozyme showed four charge states of the protonated protein, dominated by one peak at an m/z of 1590 and followed by peaks at m/z values of 1787, 1430 and 2043 in order of decreasing relative intensity (Fig. S2). The four spectra show a predominant peak at m/z of 1590 for the different concentrations of lysozyme with no significant changes in the charge state distribution.

In addition, deconvolution of the spectra gave a molecular mass of 14,296 $\text{g}\cdot\text{mol}^{-1}$, which matches the monoisotopic molecular mass of lysozyme ($\text{C}_{613}\text{H}_{951}\text{N}_{193}\text{O}_{185}\text{S}_{10}$), 14,295.8 $\text{g}\cdot\text{mol}^{-1}$.⁴

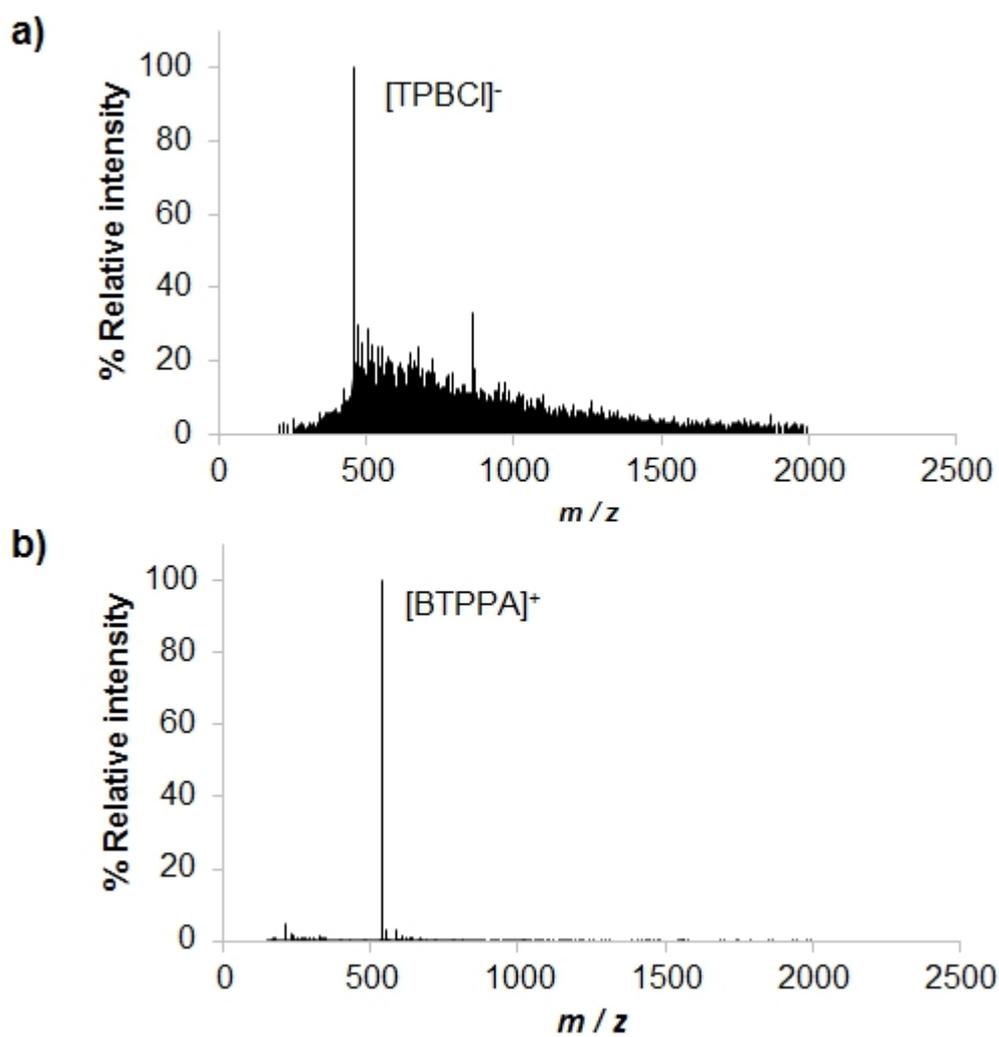


Fig. S1. Mass spectra of the organogel (10 mM BTTPATPBCl in 10% (w/v) PVC/1,6-DCH) a) in the positive ion mode and b) in the negative ion mode obtained by ESTASI-MS.

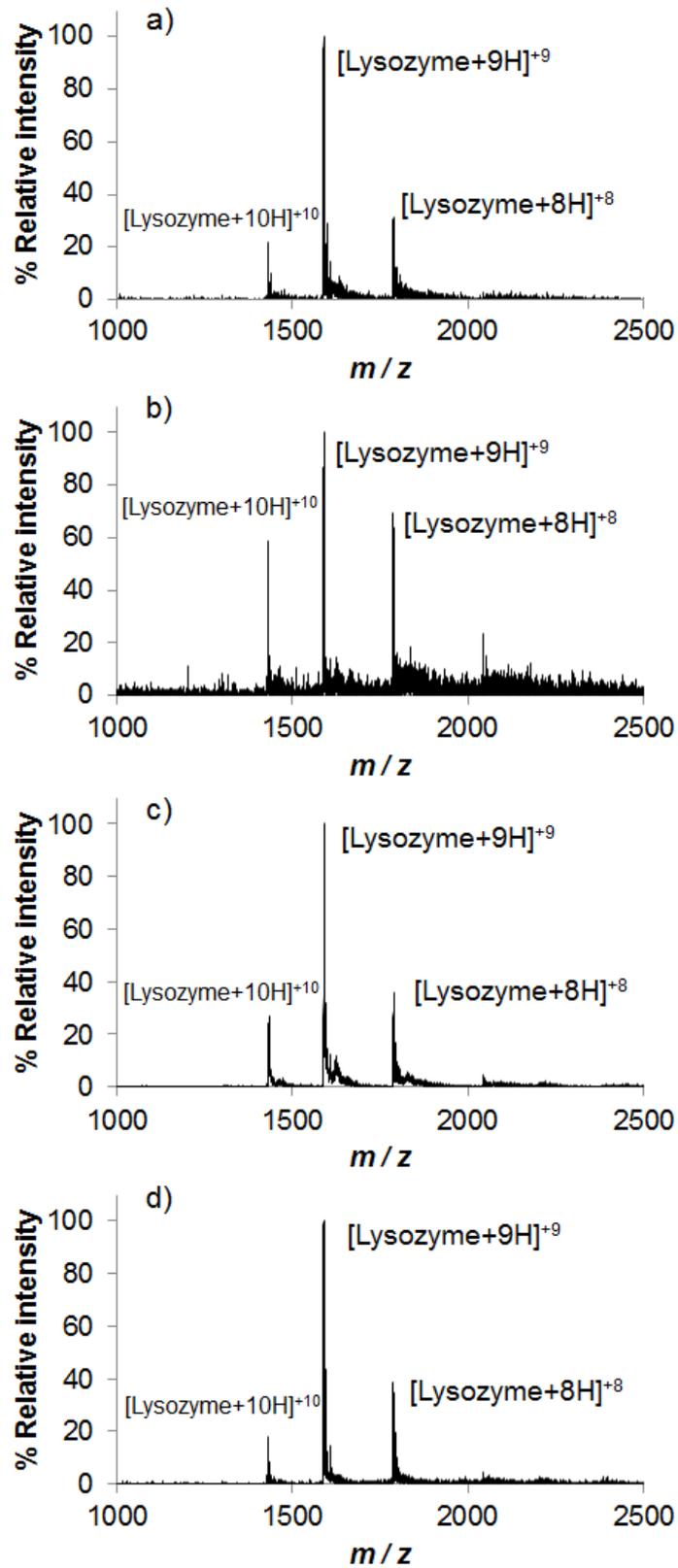


Fig. S2. Mass spectra of 1 μl a) 1 μM , b) 5 μM , c) 10 μM and d) 20 μM lysozyme in 1% acetic acid in water:methanol (50:50, v/v) deposited on the plastic film obtained by ESTASI-MS.

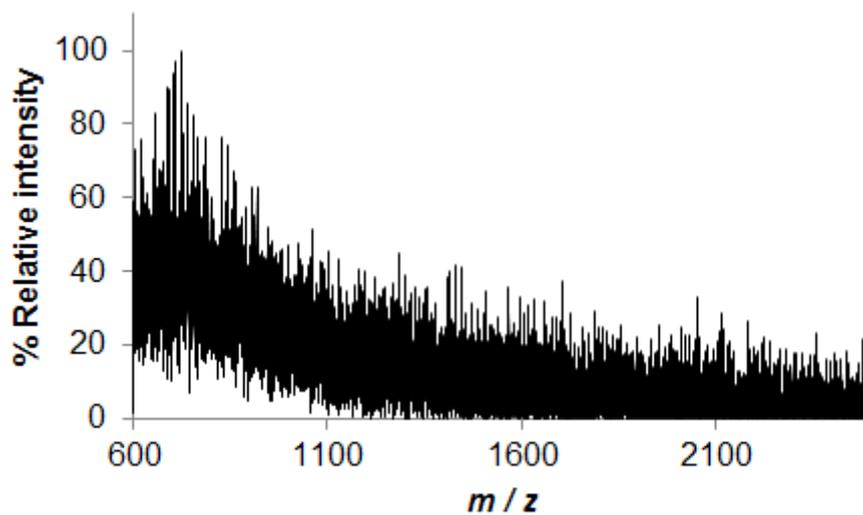


Fig. S3. Mass spectrum obtained by ESTASI-MS from the organogel (10 mM BTPPATPBCl in 10% (w/v) PVC in 1,6-DCH) after incubation in a 10 μ M lysozyme acidic solution (10 mM HCl) for 30 minutes under open-circuit conditions.

SUPPLEMENTARY REFERENCES

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