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Electronic Supplementary Information

Investigating Solvent-Induced Structural Transitions of Lysozyme in an Electrospray Ionization Source

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The Structure of Lyz in Ammonium Acetate Solutions. The R_g values of Lyz from our SAXS experiments are ~15.3 Å in native conditions (Table 1). However, we observe smaller R_g values of ~14.2 Å in case of Lyz in ammonium acetate solutions at pH 4.5, even at low methanol concentrations which are not sufficient to denature Lyz. It is of note that the R_g value of Lyz crystal structure (PDB code 2LYZ) is 14.3 Å. The higher R_g values of Lyz observed from SAXS experiments are due to its hydration layer.[1] Therefore, it can be inferred that the hydration layer of Lyz is disrupted by high ammonium acetate concentrations at pH 4.5. Additional compaction of Lyz from its native structure is less probable as the Kratky plots of Lyz in ammonium acetate structure are slightly less bell-shaped than Lyz at native state in other solutions (Figure 1c).

Hofmeister series describe the influence of different anions on the stability of proteins in solution.[2] Lyz is known to follow a reverse Hofmeister series,[3] and acetate has denaturing effect on Lyz. The fundamental aspects of Hofmeister series is still not yet clear. However, recent studies indicate a direct interaction between protein and anions to be important for the Hofmeister effect,[2] which may affect the hydration layer of Lyz to decrease its experimental R_g from SAXS experiments.

The Structure of Lyz in Acetic Acid Solutions and the Structure of Lyz Ions from Acetic Acid Solutions. Lyz in aqueous acetic acid solution at pH 2.2 maintains its native fold.[4] The results from SAXS experiments in this study show that Lyz unfolds in acetic acid solutions at pH 2.2 with 40% and 80% methanol (Figure S4). This is in contrast to the preservation of native Lyz structure in HCl and formic acid solutions with 40% methanol (Figure 1). Rather than specific effects by acetic acid, this result should be interpreted as the influence of the high acetic acid content (> 15% by volume) necessary to obtain pH 2.2 solutions. The high acetic acid contents could behave as non-aqueous solvent, which makes the total non-aqueous content in 40% methanol solution exceed

55%. The largest R_g value of Lyz observed in 80% methanol solution with acetic acid can also be explained similarly. In this condition, the non-aqueous content reach almost 100%, which would facilitate unfolding of Lyz.

However, unfolding of Lyz in acetic acid solutions is not reflected in the charge state distribution (CSD) and ion mobility (IM) spectra of Lyz ions. Figure S5 shows that shifts of CSD into higher charge states are absent for Lyz from acetic acid solutions with 40% or 80% methanol (Figure S5a). Moreover, acetic acid-induced unfolding of Lyz during ESI[4] is suppressed with addition of methanol. The unfolded Lyz conformers observed from aqueous acetic acid solutions disappear in methanol cosolutions (Figure S5b). This also supports that methanol suppresses the formation of unfolded Lyz conformers during ESI.

Figure S1. a) SAXS profiles of Lyz and b) comparison between experimental SAXS profiles of Lyz and theoretical fitting curves of *ab initio* SAXS envelopes in Figure 1d. The solution pH values are given in parentheses. (AmAc: ammonium acetate, FA: formic acid)

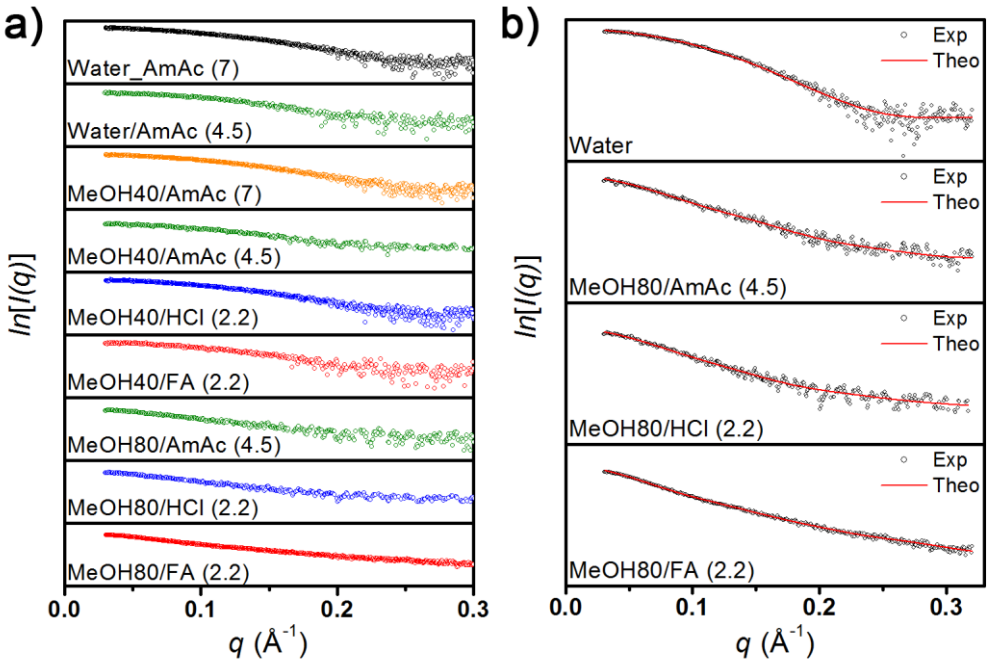

Figure S2. ESI-MS spectra of Lyz in 40% methanol solutions.

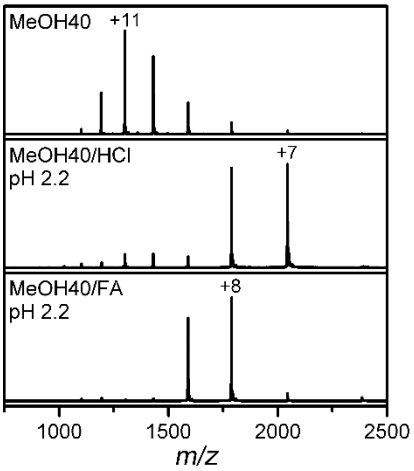

Figure S3. IM spectra of +6 to +12 charged Lyz ions from 40% methanol solutions (black lines). IM spectra of Lyz from pure water are given (red dotted lines) for comparison.

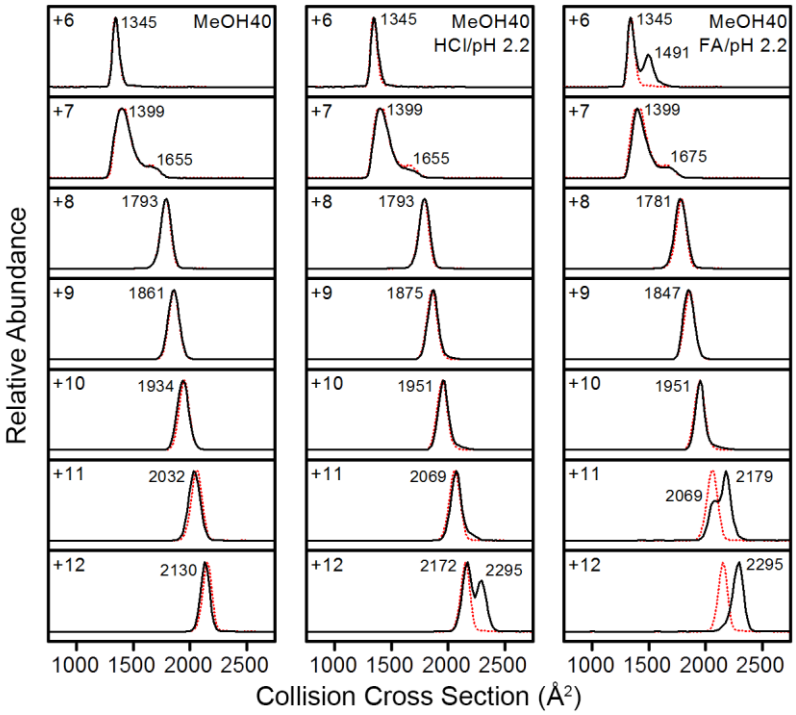

Figure S4. a) SAXS profiles, b) Guinier plots of SAXS profiles, and c) Kratky plots of SAXS profiles of Lyz in acetic acid (AA) solutions.

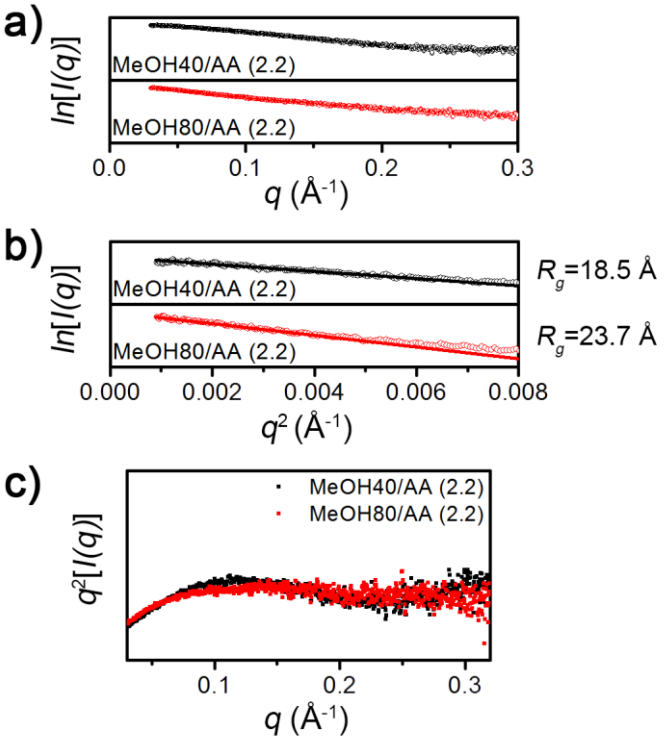

Figure S5. a) ESI-MS of Lyz in acetic acid solutions at pH 2.2, and b) IM spectra of Lyz ions from acetic acid solution at pH 2.2. IM spectra of Lyz from aqueous acetic acid solution are given (red dotted lines) for comparison.

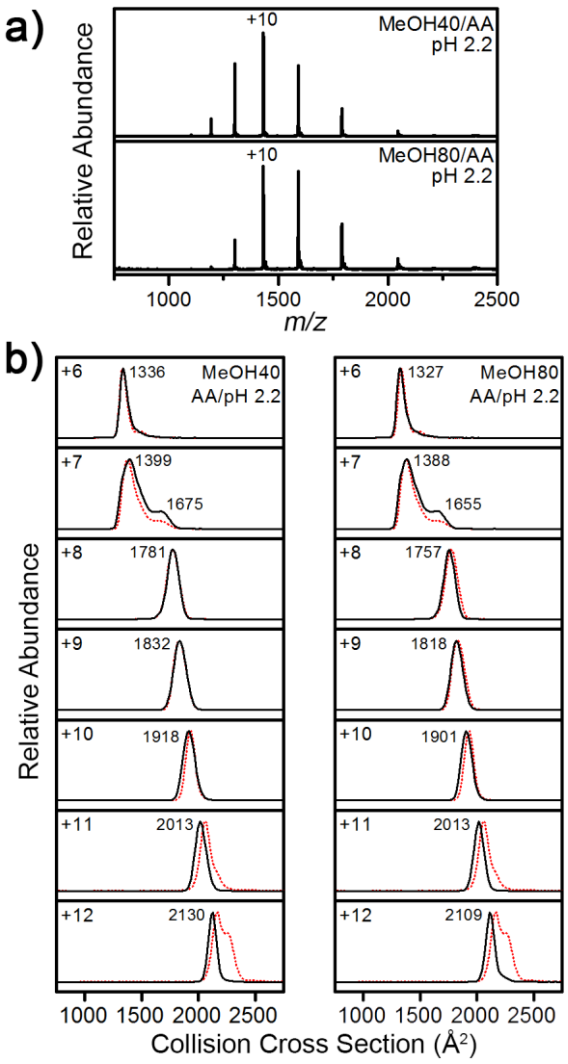

Figure S6. ESI-MS spectra of Lyz under formic acid or methanol vapor.

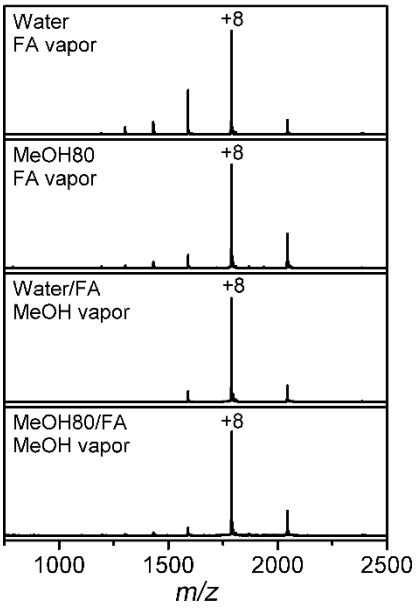

Table S1. Physical properties of solvents used in the present study.[5, 6]

Solvent	Gas-phase basicity (kJ/mol)[a]	Vapor pressure at 25 ℃ (Torr)[b]	Dielectric constant at 20 ℃[b]	Surface Tension at 25 ℃ ($mN \cdot m^{-1}$)[b]	Boiling point at 1 atm[b]	Viscosity ($mN \cdot s \cdot m^{-2}$)[b]
Water	660.0	23.76	80.20	72.14	100 ℃	0.8903 (25 ℃)
Methanol	724.5	125.03	33.0	22.07	64.7 ℃	0.544 (25 ℃)
Ethanol	746	59.70	25.3	21.97	78.3 ℃	1.074 (25 ℃)
1-Propanol	756.1	20.84	20.8	23.32	97.2 ℃	1.722 (30 ℃)
Acetonitrile	748.0	92.26	36.64	26.64	81.6 ℃	0.329 (25 ℃)

[a] from ref 5, [b] from ref 6

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