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Electronic Supplementary Information

Hofmeister Series Reversal for Lysozyme by change in pH and Salt Concentration: Insights from Electrophoretic Mobility Measurements

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Experimental Section

Chemicals. Lysozyme from hen egg white (70,000 units/mg; 62971), sodium chloride (≥ 99%; S3014), sodium thiocyanate (≥ 98%; 251410) and sodium phosphate dibasic (≥ 99.5%; S0876) were purchased from Sigma-Aldrich. Sodium bromide (≥ 99%; 205130010), sodium iodide (≥ 99.5%; 203180010), sodium perchlorate (≥ 99%; 197120010) and sodium nitrate (≥ 99%; 205960010) were purchased from Acros Organics. Tris (hydroxymethyl)aminomethane (≥ 99.8%; 1610719) was from Biorad. All samples were prepared by using purified water (conductivity ≤ 0.054 mS cm^{-1}), prepared by means of a Millipore water purification system (Millipore, UK). In order to remove any interference on pH by carbonic acid, CO_2 was removed by bubbling argon for two hours before the preparation of each solution.

Electrophoretic mobility measurements. Electrophoretic mobility (μ_E) measurements of salt-lysozyme solutions were carried out by means of a Zetasizer nano series (Malvern Instruments). Lysozyme solutions (1 mg/mL) in 20 mM Tris (hydroxymethyl)aminomethane buffer (pH 9.4) and 20 mM phosphate buffer (pH 12.4) were prepared. The buffer at pH 9.4 was used to coincide with

the experimental conditions of Zhang and Cremer.[1] We chose another buffer (pH 12.4) in order to obtain a set of experiments at the same pH difference (about 1.5 pH units) from the p*I* of lysozyme (~ 11). We were constrained to choose HPO_4^{2-}/PO_4^{3-} species (pK_{a3}= 12.62) in order to achieve the best buffering capacity. In so doing we assume implicitly that the buffer species modulate electrophoretic mobility only by changing the protonation of the charged groups at the protein surface. In fact, it is very likely that also a specific effect due to buffer choice could affect the lysozyme mobility.[2-4]

Lysozyme concentration was chosen after some preliminary experiments which gave a good level of reproducibility. Different sodium salts (NaCl, NaBr, $NaNO_3$, NaI, $NaClO_4$, NaSCN) were dried overnight at 110°C and cooled at room temperature in a desiccator. For each series of measurements, different amounts of salt were added to 100 mL of a lysozyme dispersion in the buffer solution obtaining a concentration range 1 mM - 200 mM. After each salt addition the pH was measured. Then a small volume of the resulting solution was put in a thermostated (25°C) scattering cell for the measurement of electrophoretic mobility. The possible denaturation of lysozyme molecule due to the alkaline pH 12.4 (cystine and arginine oxidation) were hopefully minimised by taking electrophoretic measurements within 1-2 hours by the preparation of the sample. Each series of experiments was repeated 3-5 times. Each value of mobility is the average of 5-7 measurements for each salt concentration. Standard deviations were calculated and displayed as error bars in Figures 2 and 3.

Limitations of equations used to calculate zeta potentials and protein-protein interaction energies.

The zeta potential (ζ) is considered to be the potential at the shear plane assumed to reside at a certain distance, say about one ionic diameter, away from the particle surface which has a surface

potential, say ψ_0. Based on the conventional electrokinetic theory the Henry equation allows the calculation of the zeta potential ζ of a colloidal particle from electrophoretic mobility data as:[5]

$$\mu_E = \frac{2\varepsilon\zeta}{3\eta} f(\kappa a) \qquad (1)$$

Here ε and η are the permittivity and the viscosity of the electrolyte medium respectively. *f(κa)* is the Henry function which can assume the value 1 (Hückel equation). Here we have a difficulty due to the fact that Henry's equation is derived by the purely electrostatic double layer theory which ignores both hydration and dispersion forces. Nevertheless, here, since no complete theory is available we are constrained to take Henry's equation as a rough guide to calculate the zeta potentials of the lysozyme dispersions. This assumption is necessary to provide an estimate of the protein-protein interaction energy, *F(D)*, between two lysozyme molecules, relevant to the Hofmeister cloud point phenomenon. A simple approximation to the interaction energy between two spheres of radius *R*, may be derived from electrostatic Gouy-Chapman theory (a linearised Poisson-Boltzmann solution):[6]

$$F(D) = (64\pi kTRN_A C_0 1000\gamma^2/\kappa^2)e^{-\kappa D} \qquad (2)$$

where, *k* is the Boltzmann constant, *T* the absolute temperature, *R* the average radius of lysozyme ($1.65\ 10^{-9}$ m), c_0 is the bulk salt concentration, κ is the inverse of the Debye length (m^{-1}), determined from the simple electrolyte (i.e. protein charges are not counted in the Debye length. Their impact has been considered elsewhere[7,8] but requires a an independent determination of protein surface charge at the given pH and electrolytic environment, which is not available here).

D the distance between two interacting spherical particles, and $\gamma = \tanh(q\psi_0/4kT)$ in the presence of a monovalent electrolyte.

Here we assume that ζ is equivalent to an effective surface potential ψ_0 that combines the electrostatic, the bulk ($\mu_i^{bulk}=-kT\ln c_0$) and the dispersion contributions to the total chemical potential $\mu_i^{surf.}$ of an ion at the surface:

$$\mu_i^{surf.}=q\zeta \sim q\psi_0 = q\psi_i^{electrostatic} + \mu_i^{bulk}+F^{dispersion} \qquad (3)$$

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