Probing the impact of protein/peptide adsorption onto solid-substrates on functional behavior and stability.

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Background: Protein adsorption refers to the accumulation and adherence of a protein to the surface of a solid, but without surface penetration occurring. Proteins can adsorb to a variety of materials that are used in bioprocess, manufacture, formulation, and storage and can have unintended consequences such as loss of expensive protein medicines. Protein adsorption to a surface is strongly influenced by the physicochemical properties of the protein, protein-formulation interactions, and protein-surface interactions. Despite increasing knowledge in the field of adsorption, a molecular-level understanding of all aspects of protein adsorption is still incomplete particularly with observed phenomena such as conformation and orientation, cooperativity, and aggregation. To elucidate the influence of protein-formulation interactions on adsorption behavior and its corresponding phenomena, our study investigates the role of buffer choice, temperature, and pH on the adsorption of Lysozyme to type I borosilicate glass.

Methods: Quantification of desorbed proteins was performed using an Agilent 1200 HPLC system equipped with a Diode Array Detector G1315D at 215 nm. Chromatographic separation was performed using a Poroshell 300SB-C8 column. Circular dichroism spectroscopy was measured with a path length of 0.1 mm in the range 185-260 nm. The average of three spectra was obtained and a 5-point smoothing algorithm applied. Isothermal titration calorimetry experiments were performed with a Microcal PEAQ-ITC microcalorimeter at 298 K. Lysozyme solution was loaded in the cell and buffer was loaded in the syringe. With a fixed stirring speed of 1000 rpm, the first drop was set to 0.4μL followed by 19 drops for subsequent 2μL injections.

Results: Lysozyme in PBS at pH 7.4 had the highest amount of protein adsorbed overall, 0.501 mg/ml for 2-5 °C and 0.587 mg/ml for 20-25 °C. The amount of adsorbed lysozyme reduced when PBS was exchanged for histidine buffer with 0.279 mg/ml for 2-5 °C and 0.426 mg/ml for 20-25 °C. Histidine also adsorbed to borosilicate with 0.323 mg/ml at 2-5 °C and 0.194 mg/ml at 20-25 °C. At pH 5.5 in histidine buffer, more lysozyme adsorbed at 2-5 °C than histidine and equal amounts adsorbed at 20-25 °C. To further understand the role of pH we studied lysozyme at pH 3.6 in citrate buffer and glycine-hydrochloride. There was a large increase in the adsorption of lysozyme at 2-5 °C for citrate buffer, 0.882 mg/ml, and a reduction was observed at 20-25 °C, 0.248 mg/ml compared to PBS. This was similar for glycine buffer however, the total amount of protein adsorbed was significantly less. We also studied citrate buffer at pH 5.5 and found that it has a similar adsorption profile to citrate at pH 3.6. The ITC data revealed that lysozyme interacts with histidine buffer at pH 7.4. At pH 7.4 histidine retains a partial positive charge and we surmise that the histidine molecules are interacting electrostatically with pockets of negatively charged residues on the surface of lysozyme. CD Spectroscopy revealed that for lysozyme in pH 3.6, lysozyme was able to adsorb without structural reorientation. For pH 7.4 and pH 5.5, lysozyme underwent conformational changes upon adsorption for all buffers, with loss of α-helix and increased random coil being observed.

Conclusions: These results suggest that pH and temperature have a strong effect on the adsorption profiles of lysozyme, but that buffer choice can aid suppression of protein adsorption to borosilicate. CD Spectroscopy indicated that lysozyme has better structural stability on adsorption at pH 3.6 than at pH 5.5 or pH 7,4.