The Effects of Alkali Ions on the Aggregation and Interfacial Adsorption of β-Lactoglobulin

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We report a combined experimental and computational study of the whey protein β-lactoglobulin (BLG) in different electrolyte solutions. Vibrational sum-frequency generation (SFG) and ellipsometry were used to investigate the molecular structure of BLG modified air-water interfaces as a function of LiCl, NaCl and KCl concentrations. Molecular dynamics (MD) simulations and thermodynamic integration provided details of the ion pairing of protein surface residues with alkali-metal cations. Our results at pH 6.2 indicate that BLG at the air-water interface forms mono- and bilayers preferably at low and high ionic strength, respectively. Results from SFG spectroscopy and ellipsometry are consistent with intimate ion pairing of alkali-metal cations with aspartate and glutamate carboxylates, which is shown to be more effective for smaller cations (Li^+ and Na^+). Ion pairing has several consequences: macroscopically, charge neutralization and even overcharging of the protein can be observed, while microscopically the local chemical environment of a solvated carboxylate is dramatically changed by complexation with a cation. MD simulations show not only carboxylate-alkali-metal ion pairs, but also ion multiplets with the alkali-metal ion in a bridging position between two or more carboxylates. Consequently, alkali-metal cations can bridge carboxylates not only within a monomer but also between monomers, thus providing an important dimerization mechanism between hydrophilic surface patches. Using MD simulations, we have investigated three protein surface areas involved in the formation of dimers, and identified one that is rich in carboxylate groups and thus likely to be involved in the formation of alkaline-bridged dimers.