Physicochemical Characterization and Colloidal Stability of Bovine Fibrinogen: The Role of Ionic Strength

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Understanding the colloidal stability and interactions of fibrinogen with nanoparticles as a function of physicochemical environment is of fundamental importance. Bovine serum albumin (BSA) acts as dysopsonin by forming a protective coating on nanoparticles, which prevents non-specific protein binding and delays blood clearance. On the contrary, fibrinogen acts as a prominent opsonin, making it essential to understand the biological balance between opsonin and dysopsonin proteins; in fact, when fibrinogen adsorbs onto the nanomaterial surface, it undergoes conformational changes that trigger the body's immune response, leading to the recognition of the material as extraneous, thus enhancing body clearance. [1] In this work, a comparative multi-technique approach comprising dynamic and electrophoretic light scattering (DLS and ELS), potentiometric and turbidimetric titrations, and surface tension measurements are employed. The above-mentioned techniques were used to correlate the structural features, charge, and interfacial properties of bovine fibrinogen at physiological pH (7.4) across different electrolyte concentrations namely 10, 50 and 100 mM in NaCl or phosphate buffer (PB). Under these conditions, the hydrodynamic behavior of the macromolecule was investigated via DLS in PB at pH 7.4, determining the diffusion coefficients from 15 °C to 50 °C at protein concentrations ranging from 0.1 to 0.5 mg/mL. This method permits the extrapolation of the diffusion coefficient at infinite dilution D_0 and to evaluate how the apparent diffusion coefficient D changes as a function of protein concentrations. The data reveal that the diffusion behavior is strongly modulated by ionic strength, as reflected by the variations in D_0 and the interaction parameter k_D at 37.5 °C. Specifically, at 100 mM PB, the system remains highly stable with minimized macromolecular interactions due to strong electrostatic screening, yielding a D_0 of 32.11 x 10^{-12} m^2/s and a slightly positive k_D of 0.004. As the ionic strength decreases, the repulsive forces are unscreened and intermolecular interactions become progressively more pronounced; this is highlighted by the shift to negative k_D values, reaching -0.102 at 50 mM PB, D_0= 33.11 x 10^{-12} m^2/s and becoming markedly more negative at 10 mM PB, where k_D drops to -0.320, D_0 = 35.08 x 10^{-12} m^2/s. To elucidate the electrostatic origin of these modulated interactions, electrophoretic and potentiometric profiles mapped the protonation state and net proton charge respectively. In parallel, turbidimetric analysis monitors the system's aggregation. Both techniques were performed at a protein concentration of 1 g/L, yielded matching isoelectric point (pI) values. In 1 mM NaCl, both methods identified a pI of 6.2. As the ionic strength increased, electrostatic screening shifted the pI toward more acidic values, reaching 5.8 in 10 mM NaCl and approximately 5.4 in 50 mM NaCl. This trend was further confirmed in 100 mM NaCl, where the pI stabilized at around 5.1 probably due to the specific binding and progressive saturation of chloride ions on the macromolecule, which modifies its net charge profile. Simultaneously, interfacial activity was quantified by surface tension measurements across a protein concentration range from 0.1 to 1 mg/mL at the same three ionic strengths. The results demonstrate that, starting from a baseline value of 70 mN/m for the buffer alone, higher salinity enhances the drop in surface tension before reaching a plateau, in 10 mM PB, the tension decreases to 64 mN/m, at 50 mM, a more pronounced reduction down to 62 mN/m is observed whereas at 100 mM, the most significant decline is recorded, reaching 58 mN/m. This profile highlights that increased ionic strength promotes fibrinogen adsorption at the air-water interface. The present comprehensive characterization contextualizes fibrinogen behavior at physiological pH, and demonstrates how increased ionic strength drives electrostatic screening, directly affecting diffusion coefficients and shifting the apparent isoelectric point. Ultimately, establishing a detailed physicochemical baseline provides the mandatory framework to decipher protein-nanoparticle

interactions, offering a rational pathway to predict and manipulate the opsonization dynamics of nanomaterials. [2]

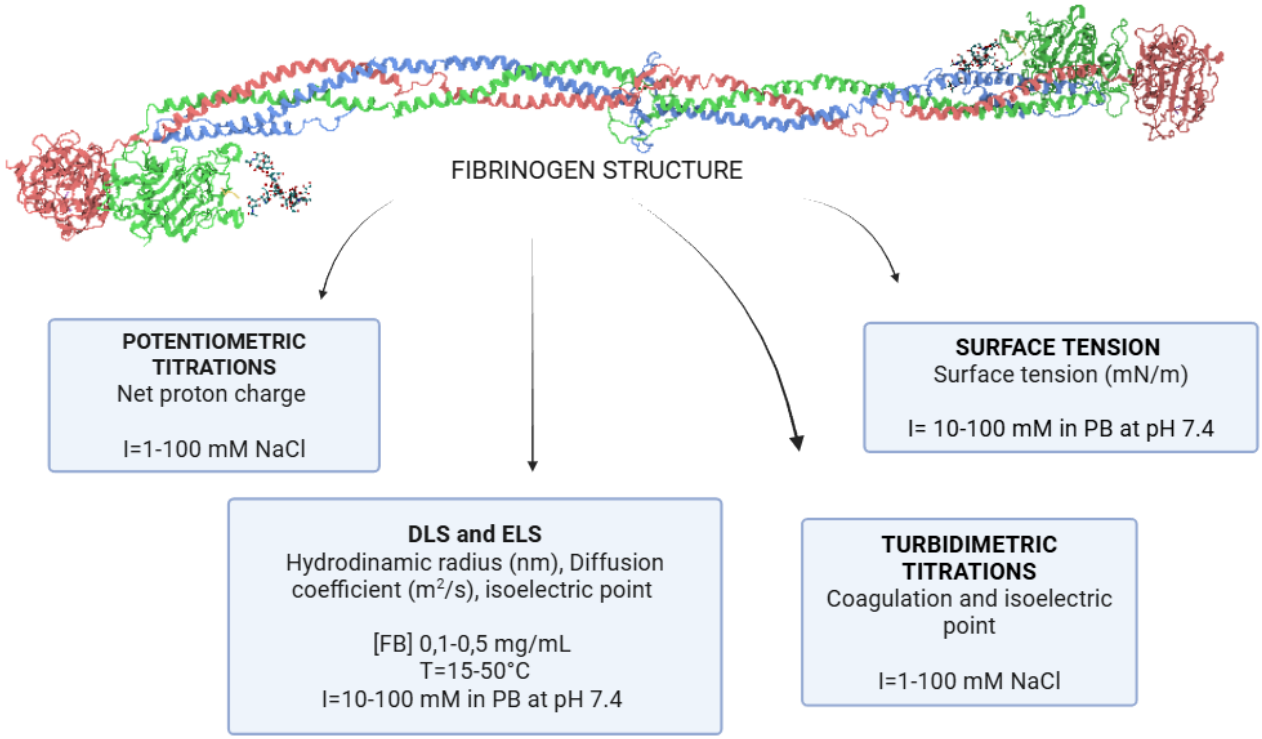

Figure 1: Experimental workflow for the physicochemical characterization of bovine fibrinogen.

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