

Fattori della coagulazione: Biochimica e fisiologia

BEYOND IONIC STRENGTH: ANION EFFECTS ON FIBRIN CLOT FORMATION, STRUCTURE, AND MECHANICAL PROPERTIES

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Background and Aims. The role of anions in protein aggregation and gelation has long been underestimated and commonly interpreted as arising from direct macromolecule–anion interactions or from simple ionic strength effects. However, growing evidence across several protein classes, as well as in non-protein systems, supports a more general underlying mechanism. These effects correlate with the position of anions in the Hofmeister series, which reflects their physico-chemical properties and their ability to influence water structure. Within this framework, anions range from kosmotropes—including polyvalent (sulfate, phosphate, carbonate) and monovalent (fluoride, bicarbonate) species—to monovalent chaotropes (chloride, bromide, nitrate, iodide). This perspective is particularly relevant for fibrinogen, whose conversion into fibrin represents a sol–gel phase transition that underlies the final step of the coagulation cascade. This study investigates how anions modulate fibrin clot formation, structure, and functional properties, providing insight into their contribution to haemostasis and thrombosis.

Methods. Fibrin clots were prepared from desalted, purified human fibrinogen (0.085–0.50mg/mL) and thrombin (0.01–0.1U/mL) in presence of sodium salts (50–500mM) and calcium chloride (0–5mM), pH 7.5. Anion-dependent effects on clot structure and viscoelastic properties were evaluated using turbidity, confocal microscopy, and microrheology. Maximum absorbance (A_{MAX}), derived from turbidity measurements, was used as an indicator of the gel's final macroscopic structure. Fractal dimension and lacunarity, obtained from confocal microscopy image analysis, were used as quantitative descriptors of network spatial density and heterogeneity, respectively. Chloride was used as a reference anion due to its central position in the Hofmeister series and its biological abundance.

Results. At physiological concentration, A_{MAX} in turbidity experiments was 0.072±0.004 for chloride. Monovalent kosmotropes increased A_{MAX} to 0.236±0.042 ($p<0.0001$), whereas polyvalent kosmotropes and chaotropes decreased it to 0.006±0.004 ($p=0.0126$) and 0.006±0.005 ($p=0.0051$), respectively. This was reflected in confocal imaging, where chloride generated the most organized and homogenous network structure, as reflected by the highest fractal dimension (1.796±0.004) and lowest lacunarity (0.299±0.013). Changing anions induced differences in fibrin clot architecture, including fiber diameter, network density and connectivity, pore distribution, and spatial heterogeneity. Polyvalent and monovalent kosmotropes reduced fractal dimension to 1.766±0.028 ($p=0.0230$) and 1.719±0.015 ($p<0.0001$), respectively, and monovalent kosmotropes increased lacunarity significantly to 0.445±0.116 ($p=0.0076$). Chloride resulted in the stiffest clots, exhibiting the highest storage modulus (G') at 10Hz (2.327±0.676 Pa), whereas all other anions significantly reduced it by approximately 85% ($p<0.0001$), thereby decreasing clot strength.

Conclusions. Progression along the Hofmeister series yields distinct fibrin clots at the same concentration, reflecting each anion's physico-chemical profile and revealing effects beyond simple ionic strength contributions. These findings highlight the importance of the anionic environment during clot formation, as alterations in anion composition, common in many thrombosis-associated conditions, can significantly reshape clot structure, mechanical properties, and function.