Electrostatics regulate Epigallocatechin-Gallate effects on Bovine Serum Albumin Aggregation

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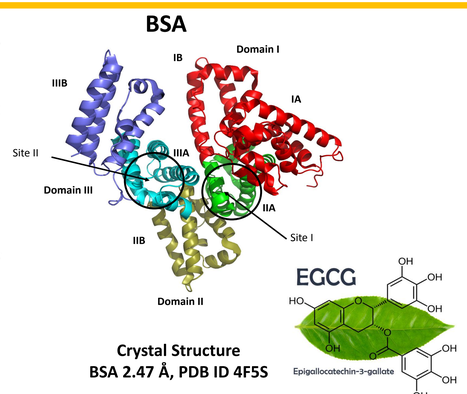
Protein aggregation processes are complex phenomena often involved in the etiology of several pathologies. It is now assessed that all proteins, in suitable conditions, may undergo supramolecular assembly. Aggregation pathways are known to be controlled by solution conditions which regulate protein-protein and protein-solvent interactions affecting binding mechanisms, morphology and inherent toxicity of the aggregate species. In this context, the presence of small molecules was indicated as a promising method to modulate protein-protein interactions reducing pathogenic aggregation.

In the light of the idea that common mechanisms regulate anti-aggregogenic properties of small molecules, we here investigate Epigallocatechin-Gallate (EGCG) effects on the thermal aggregation pathway of Bovine Serum Albumin (BSA), a well-known model protein. EGCG is a small molecule extracted from green tea, which is known to reduce aggregation of key proteins involved in neurodegenerative diseases [1]. Fundamental mechanisms which regulate EGCG effectiveness as therapeutic molecule are still not clearly elucidated.

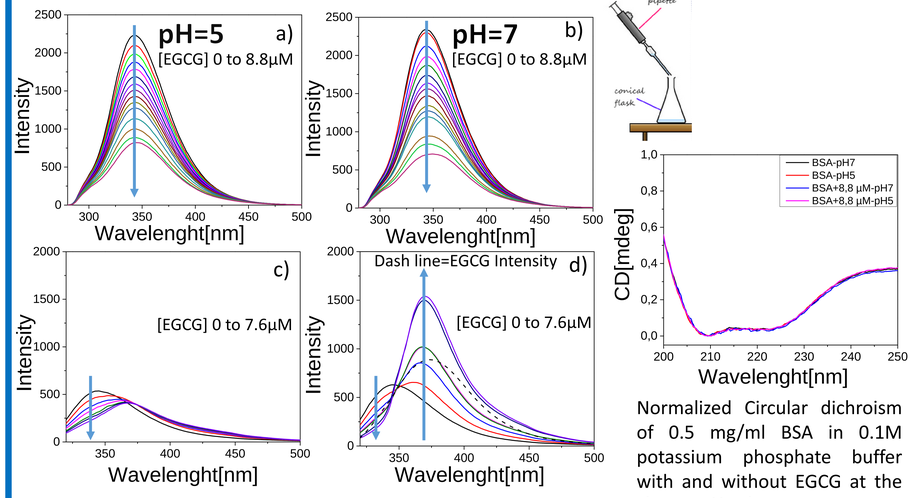
The interaction of EGCG with BSA and its effects on thermal aggregation pathway were investigated by means of spectroscopic methods and Isothermal Titration calorimetry as a function of solution conditions.

Results show that electrostatic forces modulated by pH play a key role in regulating EGCG interactions with BSA. Data shows that close to the isoelectric point of the protein, EGCG is found to promote the supramolecular assembly, whilst away from the isoelectric point, EGCG is found to reduce aggregation mechanisms increasing protein conformational stability. These results reveal the large impact of electrostatics in small molecules effects on the protein aggregation phenomena requiring larger investigation aimed at rationalizing their effects on related pathogenic mechanisms.



Crystal Structure
BSA 2.47 Å, PDB ID 4F5S

BSA-EGCG interactions

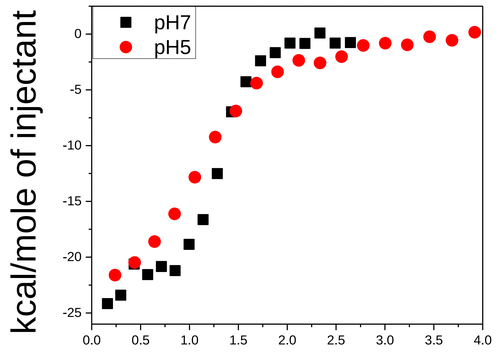


Titration of 0.5 mg/ml BSA in 0.1M potassium phosphate buffer with increasing concentration of EGCG at 270 nm and 310nm.

Normalized Circular dichroism of 0.5 mg/ml BSA in 0.1M potassium phosphate buffer with and without EGCG at the chosen pH values.

➢ The tertiary structure of the protein slightly changes, a quenching of tryptophans is noted following the insertion of the EGCG. EGCG fluorescence intensity reported in panel d indicate better protein-small molecule interactions and suggests that the EGCG is inserted into domain II of the protein.

Thermodynamic study of BSA-EGCG



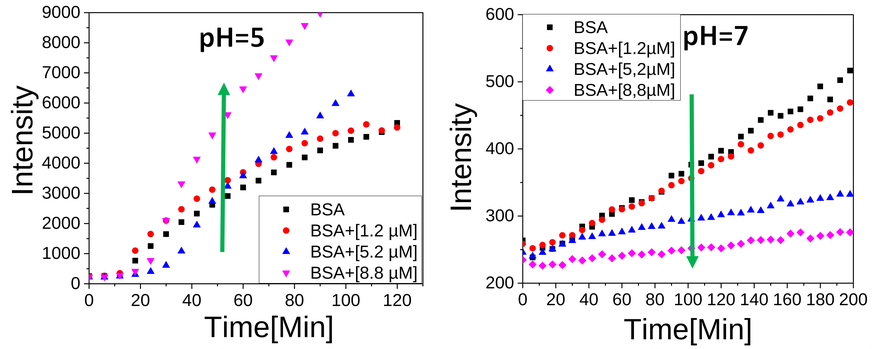
pH	N (sites)	K_d (μM)
5	1.10 ±0.02	2.35 ± 0.03
7	1.26 ±0.02	0.36 ± 0.82

Isothermal Titration Calorimetry at 25°C of 18 μM BSA in 0.1M potassium phosphate buffer with an incremental concentration of EGCG at chosen pH.

➢ Different affinities depending on the pH. The results suggest an increase in small molecule-protein affinity at pH 7.

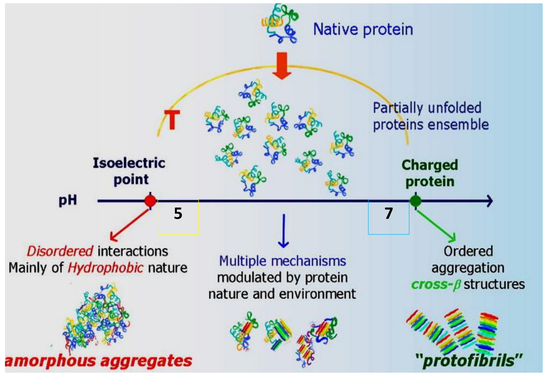
[1]Lorenzen N. et Al. J Biol Chem. 2014 1;289:21299-310.

Kinetic aggregation modulated by EGCG



Scattering Intensty growth for 0.5 mg/ml BSA in 0.1M potassium phosphate buffer incubated at 60 ° C with an increasing concentration of EGCG.

➢ At acidic pH, near the isoelectric point of the protein, the protein is not charged, the aggregation process is driven by hydrophobic interactions, the insertion of the EGCG triggers the aggregation. At neutral pH, the protein is negatively charged, EGCG insertion reduces protein aggregation.



Adapted from V. Vetri et Al. Archives of Biochemistry and Biophysics,Volume 508, Issue 1,2011,Pages 13-24.