Oxidation effects in antiaggregogenic properties of Epigallocatechingallate

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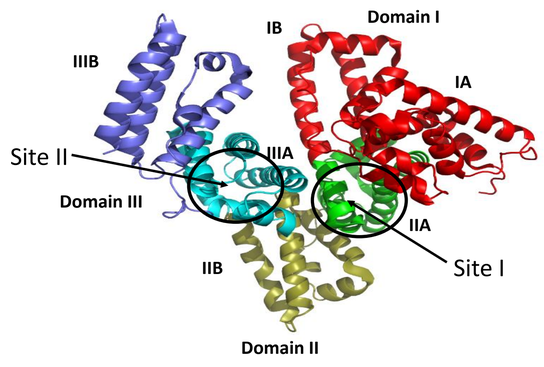
Epigallocatechin-gallate (EGCG), the most abundant flavonoid in green tea, has been extensively studied for its potential in the treatment of amyloid related disorders. This molecule was found to modulate abnormal protein self-assembly, reducing resulting cellular toxicity. EGCG is known to suppress or to slow down the aggregation processes of several proteins, thus supporting the idea that general mechanisms regulate its anti-aggregogenic effects and, interestingly, in the oxidised form it demonstrated an higher efficiency in reducing protein aggregation with respect to intact molecule.

We here investigate the effects of intact and oxidized EGCG the thermal aggregation pathway of Bovine Serum Albumin (BSA), a well-known model protein whose aggregation processes are known in details.

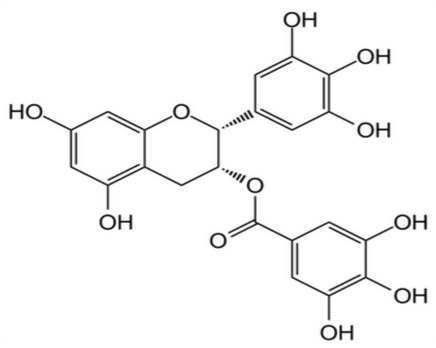
By means of different spectroscopic methods, we evaluate similarities and differences of the two molecules during protein aggregation. Different solution conditions are investigated, close and away from the isoelectric point of the protein, with the aim of eliciting the role of electrostatics in the observed EGCG-Protein interaction and in the supramolecular assembly which are dramatically dependent on solution conditions.

BSA

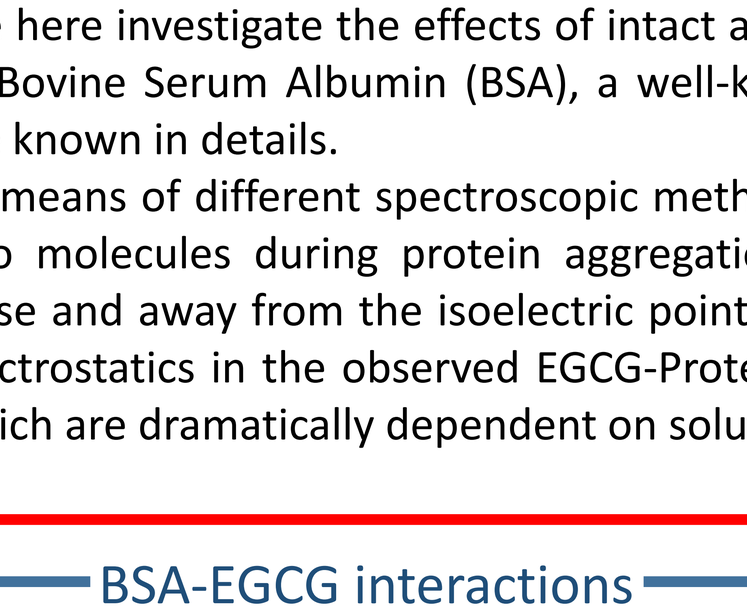


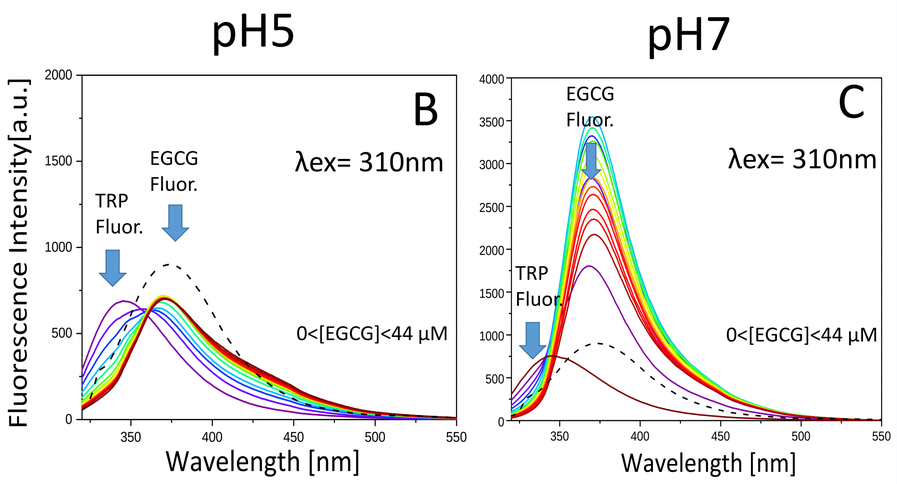
Crystal Structure
BSA 2.47 Å, PDB ID 4F5S

EGCG



BSA-EGCG interactions

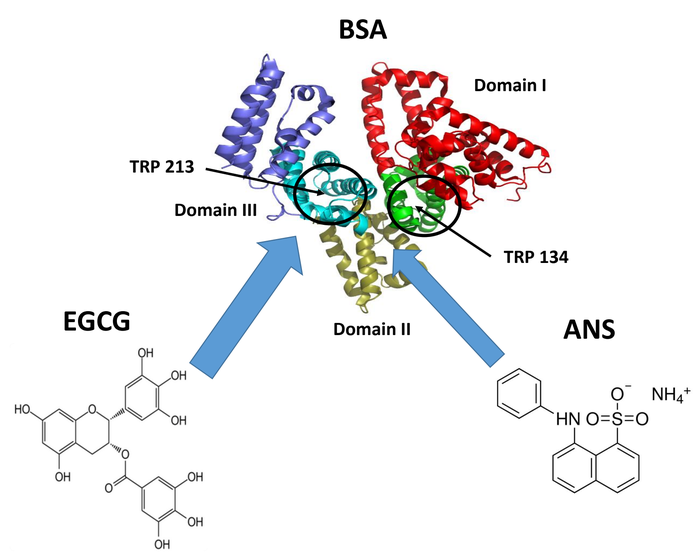




A Titration of EGCG in a 7 µM BSA solution dissolved in 0.1 M potassium phosphate buffer @270nm and @310nm.

Quenching of the two tryptophans is EGCG dose-dependent and it is the same for both solutions panel A. Increasing fluorescence intensity reported in panel C indicate a better interaction between the protein and the small-molecule at pH7. In particular EGCG interact with the hydrophobic site II of BSA near the Tryptophan 213.

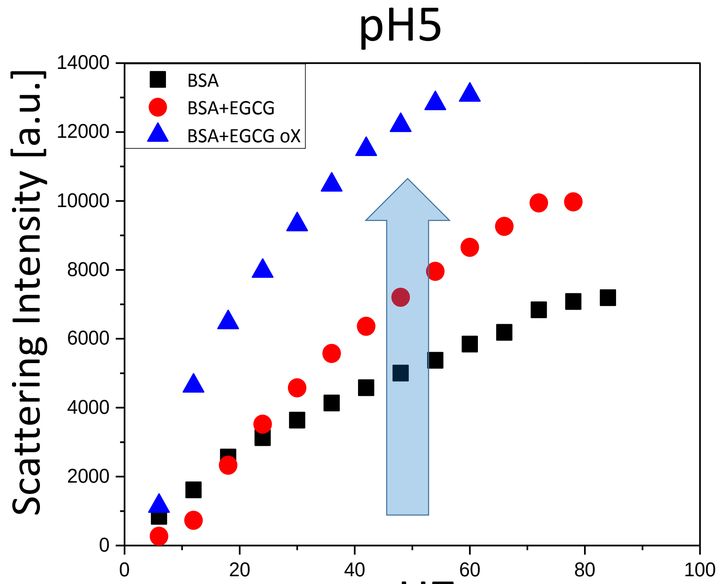
EGCG Binding

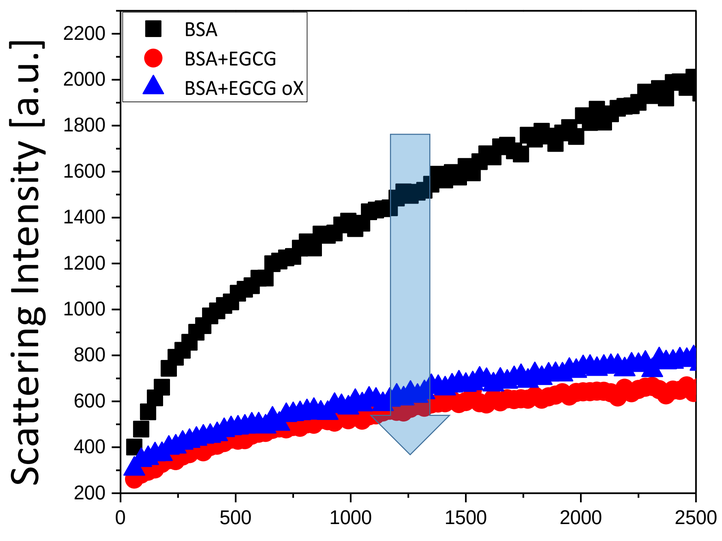


A Titration of 8-anilino-1-naphthalenesulfonic acid (ANS) in a 7 µM BSA solution in 0.1 M potassium phosphate buffer in the presence and absence of 24 µM EGCG @380nm.

There is a competitive binding process between ANS and EGCG. Fluorescence intensity in the presence or absence of EGCG is very different in particular in the presence of EGCG the intensity is much lower, this indicates a binding preference of EGCG over ANS.

Antiaggregogenic properties





Scattering Intensity growth for 7 µM BSA in 0.1M potassium phosphate buffer incubate at 60 ° C with and without EGCG or EGCG oxidized [8.5 µM].

At acidic pH, the insertion of the EGCG or EGCG ox triggers the aggregation. At neutral pH, the insertion of EGCG or EGCG oX reduces protein aggregation. This process is mediated by electrostatics which plays an important role in the process.

Conclusions

BSA-EGCG interactions were different as a function of different solution condition, better binding interaction at pH7 was reported on the blue panel. Green panel shows a competitive binding between ANS and EGCG as a function of pH value. These data confirm our hypothesis and shows a better ANS intensity in the presence of EGCG at pH7. Finally violet panel shows how the electrostatics can mediate the antiaggregogenic properties of EGCG with and without oxidation.