Preventing effect of antioxidant (-)epicatechin in the development of amyloid fibrils from β-lactoglobulin

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Formation of amyloid fibrils is a key event in the development of several neurological disorders (Alzheimer, Parkinson, Huntington and Creutzfeldt-Jakob diseases), type II diabetes and some cancers. In spite of growing research in this field, structural and biophysical properties of fibrils of different origin in relation of their toxicity have not completely clarified so far [1]. Amyloid fibrils show proteins arranged in β-strands perpendicular to the elongation axis [2]. It has recently been demonstrated that bovine serum albumin, β-lactoglobulin (BLG), concanavalin A, lysozyme and insulin, even if not involved in diseases, are able to form under proper conditions amyloid fibrils *in vitro* identical to those associated with pathological diseases. BLG represents a valuable model protein for studying mechanism of development of amyloid fibrils since it can build up network amyloid structures with well-defined fibrils. Moreover, as a member of the lipocalin family, BLG is able to bind fatty acids, retinol, α-tocopherol and polyphenols (catechins, quercetin, rutin, resveratrol) in different sites of the protein molecule [3]. Stoichiometric binding of (-)epicatechin (EC), resulting in a specific conformational change involving protein dissociation, has been demonstrated [4]. Evidence that dietary antioxidant and anti-inflammatory polyphenols with health promoting physiological effects, such as flavonoids, might exert protection towards development of neurodegenerative diseases through inhibition of amyloid fibril formation, are limited [5]. In the present study, BLG was used as a model system of *in vitro* fibrillation under controlled conditions (pH 2, 80°C). Aggregation and fibril formation from BLG, and the effect of EC at a 1:1 EC-BLG molar ratio, were monitored measuring thioflavin T (THT) binding by fluorescence spectroscopy and secondary structure by Fourier transform infrared (FTIR) spectroscopy. As evidenced by FTIR spectroscopy, thermal-induced increase in β-sheet content of the protein (relative spectral weight of the band at 1630 cm^{-1}) at the expense of unordered conformation (band at 1660 cm^{-1}), that is associated with fibrillation in acidic conditions, was hampered by EC. Furthermore, the fluorescence results indicate that EC slows down the formation of fibrils as demonstrated by the difference THT intensity behaviour and the changes in the total diffusion coefficients. The results indicated that stoichiometric binding of EC to BLG prevents fibril formation by interfering with specific changes in the protein structure that lead to development of the process. The information provided by the present study might help to understand behaviour of polyphenols during fibrillogenesis and to plan use of natural compounds in nutraceutical preventive approaches.

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Figure 1. The relative spectral weights of the β-sheet (full dots) and the unordered (open dots) secondary structures. Panel a: BLG sample, panel b: (-)epicatechin-BLG complex. Stars are the sum of the two contributions.

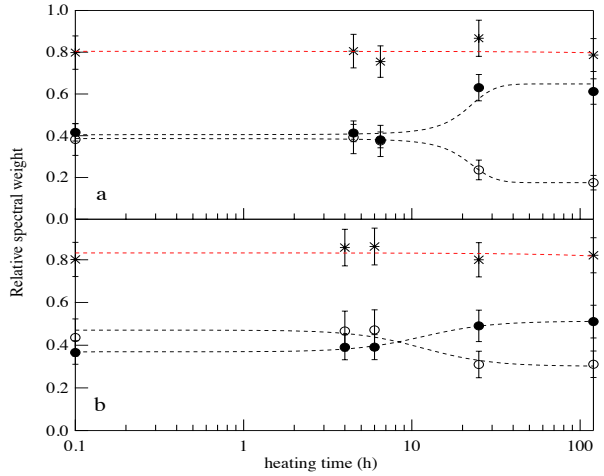

Figure 2. Panel a: fluorescence intensity of THT bound to BLG alone (open symbols) or in the presence of epicatechin; Panel b: Autocorrelation curves of BLG-THT complex at pH 2.0 (blue) and after 24 h heating time ((-)epicatechin, black and (+)epicatechin, red).