Protein structural modifications induced by physical processing

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This contribution focuses on the molecular determinants and the biotechnologically relevant consequences of structural modification of protein ensuing from: 1) physical treatments that alter the structure of solvent water (such as temperature of high pressure); 2) shear forces (as used, for example, in food processing); 3) the interaction of proteins with hydrophobic surfaces (either in liquid or in solid phases). For the sake of simplicity, most of the data to be presented and discussed relate to bovine betalactoglobulin (BLG), that offers a number of distinctive advantages for this type of studies: i) its structure is known in extreme detail; ii) it contains a number of "reporter" residues that facilitate analysis of individual unfolding steps; iii) it is a food allergen with both sequential and conformational epitopes, for which selected monoclonal antibodies are available.

The presentation will take off by considering temperature stability of the protein structure, with a focus on how unfolding can be controlled by the presence/absence of ligands and cosolutes, and on how temperature-swollen conformers of BLG may be used for selective and stable binding of hydrophobes (including bioactives and species of pharmaceutical interest) or to generate non-immunoreactive species upon selective proteolytic breakdown breakdown of sequential epitopes.

Further, focus will shift on mechanical stress, as used in generating BLG conformers trapped at the interface between polar and non-polar liquid phases, as happens when BLG is used as a stabilizing agent in emulsions. The effects of these conformational changes on the protein structure will be discussed also in terms of altered proteolytic patterns and of changes in immunoreactivity.

Finally, recent data on the unfolding of BLG upon contact with the hydrophobic surface of polystyrene nanoparticles (NPs, of various size) will be presented and discussed. Molecular dynamic (MD) studies highlighted that this "contact" unfolding is extensive and extremely fast. Structural features of the NP-bound BLG have been assessed by monitoring the reactivity and the spectroscopic features of "reporter" residues in the protein structure, as well as the immunoreactivity of NP-bound BLG and its sensitivity to proteolysis. Results confirm what predicted by MD studies, and provide also evidence of the relevance of "geometric effects" (related to the NP size) and of "molecular crowding" effects (related to the BLG/NP mass ratio).