ADSORPTION ON THE SURFACE OF EMULSIONS WORSEN THE ALLERGENIC POTENTIAL OF BOVINE BETA-LACTOglobULIN

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Adsorption on the surface of oil droplets resulted in significant changes in the tertiary structure of bovine betalactoglobulin (BLG). BLG is a whey protein broadly used as a food ingredients, and a major food allergen. We tested here the nature and extent of structural changes ensuing from adsorption of BLG on the surface of nano-sized oil droplets, and their consequences on physiologically relevant properties of the protein. Solid-state fluorescence spectroscopy and reactivity of selected residues was used to assess structural changes. Modifications in the proteolysis pattern (trypsin) were assessed by LC/MS, and modifications in immunoreactivity by competitive ELISA or by Western blotting. The structurally modified adsorbed protein had increased sensitivity to trypsin, and had an overall increased immunoreactivity towards specific monoclonal antibodies. In spite of extensive tryptic breakdown of emulsion-adsorbed BLG, some sequence stretches in BLG became trypsin-insensitive upon absorption on the fat droplets and were identified by mass spectrometry. As a consequence - at contrast with free BLG - proteolysis of emulsion-bound BLG did not decrease the immunoreactivity of the protein, and some of the large peptides generated by trypsinolysis of emulsion-bound BLG were specifically recognized by suitable monoclonal antibodies. Structural changes occurring in emulsion-bound BLG and their consequences are discussed in comparison with those occurring when the tertiary structure of BLG is modified by chemical agents, by physical processes, or upon interaction with solid hydrophobic surfaces. Such a comparison highlights the nature and relevance of situation-specific modifications, that in turn affect physiologically relevant features of the protein.