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Study of casein aggregation into micelle in mammary epithelial cell

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Caseins, a family of acidic phosphoproteins which represent 78% of bovine milk proteins, interact with colloidal calcium phosphate (CCP) and aggregate to organize into a supra-molecular structure: the casein micelle. The structure of the micelle determines the functional characteristics of the milk protein fraction and has an impact on food processing. Despite the importance of the nutritional and functional value of casein micelle, the intrinsic organisation and the mechanisms of formation of these structures in the mammary epithelial cell (MEC) has not been fully established.

The aim of this work is to obtain information about the specific arrangement of the caseins in the structure and to characterize the interactions between the various components of the micelle. To understand the structural complexity of the casein micelle, we believe it is essential to gain new knowledge about the formation of this structure within the MEC in order to elucidate the role of the individual casein and of the CCP in the aggregation process. Since the aggregation of the caseins appears to be initiated in the endoplasmic reticulum (ER), and proceeds during their transport to the apical surface, the dynamic of elaboration of the micelle will be studied *in vivo*. One project is to characterize the initial micellar states formed in the ER of rat MEC. We will then follow the evolution of this basic structure in the secretory pathway. The possible role of disulfide bridges in the formation and the stabilization of the micellar states will be investigated. In addition, we will try to precise the role of the calcium in the aggregation process; we will analyse the repartition of calcium along the secretory pathway of MEC by energy-filtering transmission electron microscopy.

With this aim, we have purified microsomes using differential centrifugation techniques. Morphological observations by electron microscopy indicate that the rough ER fraction was almost pure, very few other structures such as mitochondria and casein micelles being observed. Permeabilisation of rough microsomes with saponine, at pH 6.8, induces the release of soluble resident proteins (PDI, BIP and calreticuline) of the ER but not that of the membranous chaperone calnexine. The conditions allowing the conservation of the aggregated state of the caseins will be determined. This approach is currently applied to characterize the native micelles in the ER. The micelles obtained from rough microsomes will be isolated by density sucrose gradient and their structure compared to that of the casein micelle found in rat milk. The size, homogeneity, stoichiometry of the caseins and type of interactions within the structure, as well as the occurrence of disulfide bounds will be investigated.