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Invited review: Caseins and the casein micelle: Their biological functions, structures, and behavior in foods[1]

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ABSTRACT

A typical casein micelle contains thousands of casein molecules, most of which form thermodynamically stable complexes with nanoclusters of amorphous calcium phosphate. Like many other unfolded proteins, caseins have an actual or potential tendency to assemble into toxic amyloid fibrils, particularly at the high concentrations found in milk. Fibrils do not form in milk because an alternative aggregation pathway is followed that results in formation of the casein micelle. As a result of forming micelles, nutritious milk can be secreted and stored without causing either pathological calcification or amyloidosis of the mother's mammary tissue. The ability to sequester nanoclusters of amorphous calcium phosphate in a stable complex is not unique to caseins. It has been demonstrated using a number of noncasein secreted phosphoproteins and may be of general physiological importance in preventing calcification of other biofluids and soft tissues. Thus, competent noncasein phosphoproteins have similar patterns of phosphorylation and the same type of flexible, unfolded conformation as caseins. The ability to suppress amyloid fibril formation by forming an alternative amorphous aggregate is also not unique to caseins and underlies the action of molecular chaperones such as the small heat-shock proteins. The open structure of the protein matrix of casein micelles is fragile and easily perturbed by changes in its environment. Perturbations can cause the polypeptide chains to segregate into regions of greater and lesser density. As a result, the reliable determination of the native structure of casein micelles continues to be extremely challenging. The biological functions of caseins, such as their chaperone activity, are determined by their composition and flexible conformation and by how the casein polypeptide chains interact with each other. These same properties determine how caseins behave in the manufacture of many dairy products and how they can be used as functional ingredients in other foods.
Key words: unfolded protein, molecular chaperone, calcium phosphate sequestration, amyloid fibril

INTRODUCTION

Caseins evolved from members of a group of secreted calcium (phosphate)-binding phosphoproteins (**SCPP**; Supplementary File, sections S1 and S2, available online at http://www.journalofdairyscience.org; Kawasaki and Weiss, 2003; Rijnkels et al., 2003; Kawasaki et al., 2004, 2011; Lemay et al., 2009). In eutherian milks, at least 3 and normally 4 gene products are found; namely, α_{S1}-, α_{S2}-, β-, and κ-CN, but in some species 2 quite different α_{S2}-CN-like genes are active, raising the total number of gene products to as many as 5. In this review, we attempt to put caseins into an evolutionary and functional context provided by comparisons with other SCPP and less closely related proteins with similar biological functions. The scope of the review is summarized in Figure 1 and covers the major events of protein association leading to the formation of the casein micelle in milk and of a casein clot in the stomach of the neonate. Subsequent events of protein digestion and absorption are excluded. The insights gained from the study of caseins as unfolded SCPP are also used in a more speculative discussion of the behavior of caseins during the manufacture of milk products.

In only a few years, the study of unfolded proteins has generated a huge body of new knowledge and understanding of this large and important group (Rose, 2002; Tompa, 2010). Our view is that unfolded proteins provide better models for describing caseins than either surfactants or globular proteins. Like many other un-

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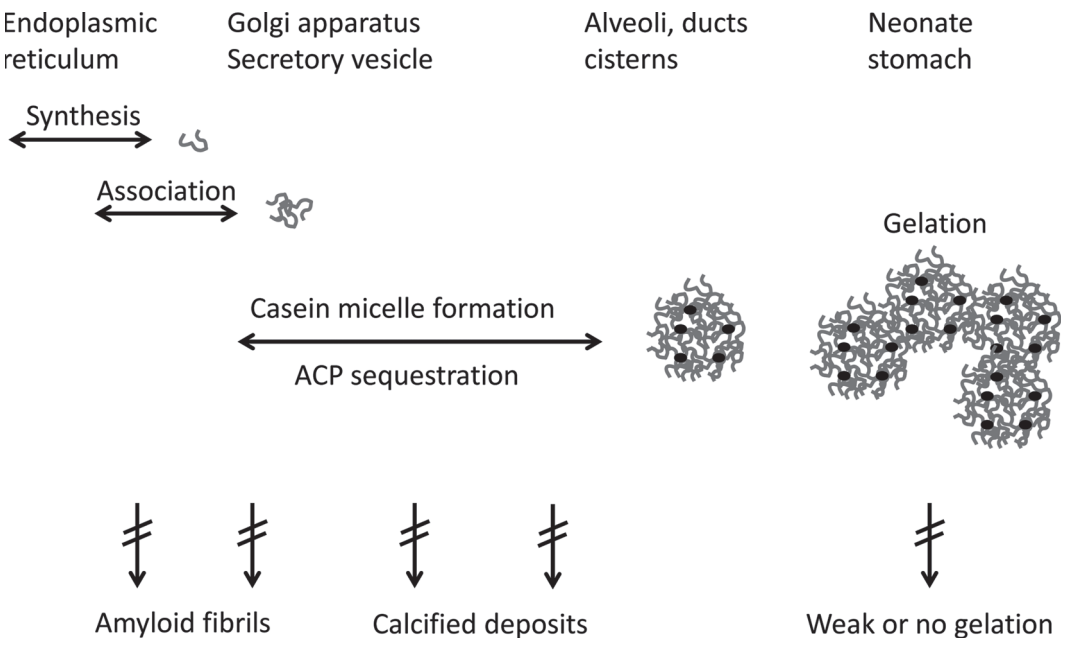

Figure 1. Scope of the review. To avoid amyloidosis through the formation of amyloid fibrils by caseins, a mixture of 3 or more different caseins associate by many alternative and nearly equivalent interactions to form amorphous aggregates known as casein micelles. To prevent calcium phosphate precipitation, nanoclusters of amorphous calcium phosphate are sequestered by the calcium-sensitive caseins within the casein micelles. In the stomach of the neonate, casein micelles can form a gel either by pH reduction to around the isoelectric point of casein or by limited and specific cleavage of κ-casein by an aspartate proteinase.

folded proteins, bovine κ- and α_{S2}-CN can each form highly structured amyloid fibrils (Farrell et al., 2003; Thorn et al., 2005, 2008; Léonil et al., 2008). Caseins also show similarities to the small heat-shock proteins (**sHsp**) in being able to act as molecular chaperones (Morgan et al., 2005). The molecular chaperone action of the caseins is facilitated by their unfolded and flexible conformation. When interacting with a fibril-forming target protein, for example, many alternative and nearly equivalent interactions of low sequence specificity compete with the sequence-specific interactions involved in forming the cross-β-sheet structures of fibrils. In consequence, an amorphous aggregate is formed, rather than a fibril.

The properties of caseins as molecular chaperones provide an insight into the nature of the protein–protein interactions involved in the self-association of individual caseins and in the formation of amorphous aggregates by mixtures of caseins. Hence, in mixtures with other caseins, the chaperone action of the caseins prevents or limits (Lencki, 2007; Thorn et al., 2008) amyloid fibril formation. The sequences responsible for casein intermolecular interactions are mainly the Pro- and Gln-rich (P,Q-rich) sequences encoded by longer exons (Supplementary File, section S2; http://wwwjournalofdairyscience.org). However, a notable exception is the C-terminal sequence of κ-CN (the macropeptide), which does not readily form inter-protein interactions because of its higher content of the hydroxy-amino acids Ser and Thr (S,T-rich) and negatively charged residues. Posttranslational modifications of the primary structure by glycosylation and phosphorylation further increase its charge density and hydrophilicity. The remainder of κ-CN (para-κ-CN) is mostly composed of P,Q-rich sequences that can bind to similar sequences in other caseins. As a result, whereas κ-CN acts to limit the size of amorphous aggregates formed by mixtures with other caseins and calcium ions, para-κ-CN causes a similar mixture to precipitate or form a gel (Waugh and Talbot, 1971). With the appropriate number of sequestered nanoclusters of amorphous calcium phosphate (**ACP**), whole casein forms the amorphous aggregate known as the casein micelle. In the casein micelle, virtually all the fibrillogenic α_{S2}-CN is bound to the nanoclusters of sequestered ACP (Holt, 2004) to further reduce its propensity to form fibrils.

Besides caseins (Holt et al., 1996, 1998; Holt, 2004; Little and Holt, 2004), a number of other SCPP and a few non-SCPP have the unfolded conformation and short, multi-phosphorylated, sub-sequences called phosphate centers (**PC**) that are needed to sequester ACP and form stable complexes (Holt et al., 2009). Solutions containing this type of sequestered calcium phosphate are thermodynamically stable provided there is a stoi-