Amyloid fibril formation by bovine milk α_{s2}- and κ-casein, and its inhibition by the molecular chaperones α_{s1}- and β-casein

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Table of Contents

List of Figures, Schemes & Tables

Figures

Schemes

Tables

Synopsis

The caseins (α_{s1}, α_{s2}, β, and κ) are the predominant proteins of bovine milk. While having little sequence homology, they have similar structural and behavioural characteristics, e.g. they lack well-defined tertiary structures and exhibit a strong tendency to associate with themselves and each other to form milk micelles. This thesis describes work examining the propensity of individual casein proteins, their mixtures, and their minor components, to form long, rope-like, β-sheet-rich aggregates, termed amyloid fibrils. Amyloid fibril formation is of considerable research interest because of its intimate association with a wide range of debilitating diseases, including Alzheimer's, Parkinson's and Huntington's diseases and type II diabetes. Amyloid fibrils were formed at physiological pH and temperature by α_{s2}-casein and κ-casein, but not α_{s1}-casein, β-casein, or whole casein, i.e. the mixture of all casein proteins. Fibril formation was assessed via dye binding assays, transmission electron microscopy and X-ray fibre diffraction. What sets α_{s2}- and κ-casein apart from the other casein proteins is that they both possess intra- and intermolecular disulfide linkages, giving rise to monomers and disulfide-linked dimers, respectively, or, in the case of κ-casein, larger multimeric species. Accordingly, these components of α_{s2}- and κ-casein were isolated to examine their individual fibril-forming propensity. The monomeric form of κ-casein had the highest fibril-forming propensity whereas α_{s2}-casein was most prone to fibril formation in its disulfide-linked dimeric form. Consequently, under conditions that reduce disulfide bonds, fibril formation by α_{s2}-casein was inhibited whereas κ-casein fibril formation was promoted. Differences in the overall morphology of the fibrils were also observed with α_{s2}-casein forming twisted 'ribbons' and κ-casein forming rigid 'rods'. Additionally, the ability of α_{s1}- or β-casein to inhibit fibril formation by α_{s2}- or κ-casein in a chaperone manner was investigated. In doing so, the inability of whole casein, which comprises considerable amounts of α_{s2}- and κ-casein, to form amyloid fibrils was largely accounted for by the chaperone action of α_{s1}- or β-casein. The significance of this work is further highlighted by the incidence of mammary *corpora amylacea*, an amyloid-like condition that infrequently develops within the mammary tissue of cows. The propensity of bovine α_{s2}- and κ-casein to form amyloid fibrils under conditions comparable to those found *in vivo* strengthens the possibility of involvement by these proteins and suggests a preventative role of α_{s1}- and β-casein in the development of this condition.

Declaration

I declare that the work presented within this thesis is original work that, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is given. Part of the written and practical work described in Chapters 1 and 2 was carried out during my Honours year at the University of Wollongong, but excepting this, no material contained herein has previously been submitted for the award of any other degree at this university or elsewhere.

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David C. Thorn

13 September 2010

If you're going through hell, keep going.

~Winston Churchill

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