

Casein proteins: investigating their chaperone activity and
amyloid fibril formation.

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Appendix

Abstract

Molecular chaperones are a diverse group of proteins that interact and stabilise partially folded proteins, thereby preventing improper or incorrect interactions that would result in their misfolding and aggregation under conditions of cellular stress, e.g. elevated temperature. There are two alternative and distinct routes by which the aggregation of the protein may proceed, i.e. via the formation of disordered, amorphous aggregates or ordered amyloid fibrils. The latter is of considerable interest to researchers because of its intimate association (e.g. via the formation of proteinaceous deposits or amyloid plaques) with a wide range of debilitating diseases, including Alzheimer's disease and type II diabetes. Amyloid-like plaques have also been identified in the mammary gland of various species within calcified stones known as *corpora amylacea* (CA). While the composition of the protein(s) involved in formation of these amyloid deposits has not been determined conclusively, immunoblotting and sequence analysis of peptides obtained from mammary CA indicate that fragments of several milk proteins, in particular caseins, are present.

In vitro studies have shown that α_s -, β - and κ -caseins, the major proteins in milk, are molecular chaperones, as they are able to stabilise heat-, light- and chemically-stressed target proteins by inhibiting their aggregation and precipitation. Casein chaperone-like activity is of biological importance since two of the four casein proteins, i.e. α_{s2} - and κ -casein, assemble into amyloid fibrils under physiological conditions, *in vitro*, which is inhibited by the chaperone action of the other milk caseins, α_{s1} - and β -casein. The chaperone-like activity of α_s - and β -casein is of commercial interest due to their ability to stabilise other proteins during food processing, e.g. the heat treatment of milk during pasteurisation and the production of milk-related products.

The work described in this thesis has two overall aims: (i) to further investigate caseins' chaperone-like ability and (ii) to examine the propensity of the caseins to form amyloid fibrils. As such, α_s - and β -casein, were dephosphorylated to determine the effect of phosphate groups on the ability of these caseins to act as molecular chaperones. Dephosphorylation of α_s - and β -casein resulted in a decrease in the chaperone efficiency against both heat- and reduction-induced amorphously aggregating target proteins. Circular dichroism and fluorescence spectroscopic data indicated that the loss of negative charge associated with dephosphorylation led to an increase in ordered structure of α_s - and β -casein (**Chapter 2**). The binding site of β -casein with reduced, partially folded α -lactalbumin, a milk whey protein, was explored using limited proteolysis and mass spectrometry to give insight into the mechanism of β -casein chaperone interaction with target proteins. It was concluded that the hydrophobic C-terminus of β -casein, from Ala⁹¹ to Trp¹⁴³, is involved in binding to reduced α -lactalbumin (**Chapter 3**).

Amyloid fibrils were formed from reduced and carboxymethylated κ -casein and α_{s2} -casein, and the amyloidogenic regions of both these proteins were identified using limited proteolysis and mass spectrometry. The residues from Tyr²⁵-Lys⁸⁶ and Ala⁸¹-Lys¹⁸¹ were determined to be incorporated into the core of κ -casein and α_{s2} -casein fibrils respectively (**Chapter 4**). The oxidation of methionine residues is linked to the pathogenesis of several amyloid diseases. As such, the two methionine residues in κ -casein (Met-95 and Met-106) were oxidised and its effect on κ -casein structure and fibril-formation was investigated. Oxidation increased κ -casein's fibril forming propensity and cellular toxicity. In addition, β -casein, which readily inhibits κ -casein fibril-formation *in vitro*, was less effective at suppressing fibril formation of oxidised κ -casein. As milk exists in an oxidative environment, this observation may have implications *in vivo* (**Chapter 5**).

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Tomas Koudelka and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

ACN	acetonitrile
ADH	alcohol dehydrogenase
A β	amyloid- β
AD	Alzheimer's disease
ANS	8-anilino-1-naphthalene sulphonate
CA	corpora amylacea
CD	circular dichroism
DHB	2,5-dihydroxy benzoic acid
DTT	1,4-dithiotheritol
FPLC	fast protein liquid chromatography
Glu-fib	glu1-fibrinopeptide B
MTT	methylthiazolyldiphenyl-tetrazolium bromide
MALDI	matrix-assisted laser desorption/ionisation
NMR	nuclear magnetic resonance
OT	ovotransferrin
PC	pheochromocytoma
RCM	reduced and carboxymethylated
SEC	size-exclusion chromatography
sHsps	small heat shock proteins
TEM	transmission electron microscopy
ThT	thioflavin T
TOF	time of flight
TFA	trifluoroacetic acid