Effect of bound ligands and κ-casein on the denaturation of β-lactoglobulin

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ABSTRACT

The objective of this study was to gain greater understanding of the behaviour of bovine β-lactoglobulin (β-lg) during thermal denaturation. The first part of the study was focused on gaining more knowledge of the structural changes in β-lg during heat-induced and urea-induced denaturation and the effect of ligands and the genetic variants of β-lg. The second part of the study explored the mechanism of the heat-induced interaction of β-lg with κ-casein (κ-CN) and the effects of genetic variants.

The reversible early steps during thermal denaturation of β-lg are not readily separated from the later irreversible steps which involve sulphydryl-disulphide interchange reactions. However, it should be possible to examine the behaviour of β-lg in the early steps without reactive thiols either by using a porcine β-lg, that does not have cysteine residue equivalent to Cys-121 of bovine β-lg, by blocking the free thiol or by using solvent denaturation.

Heating to 80 °C irreversibly altered near- and far-UV circular dichroism (CD) spectra, and 1,8-anilinonaphthalene sulphonate and retinol binding fluorescence spectra for bovine β-lg, but not for porcine β-lg which unfolded reversibly. Also the examination of the induced CD spectral changes of retinol and *cis*-parinaric acid (PnA) on binding to bovine β-lg B upon heating revealed that bovine β-lg lost the ability to bind retinol and PnA in a chiral environment. In contrast, porcine β-lg did not show significantly induced CD bands upon mixing with retinol, but PnA/β-lg mixtures showed induced CD bands of low intensity. In addition to the lack of a free thiol group in porcine β-lg, the sequence differences between bovine β-lg and porcine β-lg are also likely to affect the behaviour of these β-lgs during heat treatment and the binding of ligands. Although palmitic acid does not show any absorbance in CD spectra, it enhanced the stability of the bovine β-lg and porcine β-lg mixture.

The urea-induced unfolding of bovine β-lg at neutral pH (6.7) revealed that the stability of the genetic variants followed the order, β-lg B < β-lg A < β-lg C, as observed during thermal denaturation and tryptic hydrolysis. The stabilisation effect was also

observed by adding retinol, retinoic acid and palmitic acid during urea denaturation of β-lg, and by retinol, retinyl acetate and PnA during ammonium sulphate denatuation of β-lg. Blocking the sulphydryl group of β-lg destabilised the native protein against urea denaturation through the introduction of a bulky group to the compact structure of β-lg. This result, together with the results for porcine β-lg, confirms that the sulphydryl group plays an important role in the unfolding of bovine β-lg.

In the second part of the study, an attempt was made to investigate the effect of bovine κ-CN on the established heat-induced unfolding and aggregation pathway of bovine β-lg, by adding κ-CN A or κ-CN B to native or pre-heated β-lg A, B or C and heating the mixture. The CD band intensity of the mixture of β-lg and κ-CN at 270 nm, an index of significant alteration to the disulphide bond dihedral angle, indicated increasing structural changes involving disulphide bonds during heat treatment.

The rates of loss of β-lg and the distributions of intermediate products were determined using alkaline- and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). During reaction with β-lg, some monomeric κ-CN was found by SDS-PAGE, probably as a result of disulphide bond interchanges between κ-CN and β-lg, and two-dimensional PAGE also revealed disulphide-bonded β-lg/κ-CN aggregates. In the presence of κ-CN, the loss of monomeric β-lg increased and less non-native monomer and dimer were observed compared to β-lg alone and κ-CN reacted more rapidly with β-lg that had been unfolded by prior heat treatment than with native β-lg. This suggested that β-lg probably denatured (unfolded) independently and either simultaneously or consequently and contained higher sulphydryl reactivity than native β-lg, which lead to the reactions with κ-CN via disulphide bond interchange. It is possible that the equilibrium between native β-lg and denatured β-lg shifted rapidly, because κ-CN preferred to interact with denatured (unfolded) β-lg that has higher sulphydryl reactivity than native β-lg.

The kinetics of the interaction between β-lg and κ-CN were evaluated from the heat-induced loss of alkaline-monomeric β-lg at 80 °C. The interaction between β-lg and κ-CN could not be described by any reaction order between 1.0 and 2.0. The slopes of the plots changed at about 7.5-10 min heating time and this corresponded to intensity

changes in the alkaline-monomeric, non-native monomeric and dimeric β-lg bands, which increased during the first 10 min of heating and then slightly decreased or remained relatively constant for the rest of the heating.

The loss of native β-lg in β-lg/κ-CN mixtures during heating at 80 °C was shown to be significantly influenced by the genotypes of both β-lg and κ-CN. The κ-CN B variant showed considerably higher reactivity than κ-CN A, while the β-lg B variant was the most reactive. The greatest loss of native β-lg was observed from the β-lg B/κ-CN B mixture.

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CONTENTS

PART I. ROLE OF LIGANDS AND FREE CYSTEINE IN β-LACTOGLOBULIN DENATURTION