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Influence of binding conjugated linoleic acid and myristic acid on the heat- and high-pressure-induced unfolding and aggregation of β-lactoglobulin B

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Abstract

Heat and pressure treatment of β-lactoglobulin B (β-LG) causes it to partially unfold and aggregate. β-LG solutions at pH 7.2 were heat treated at temperatures between 40 and 93 °C for 12 min or were pressure treated at pressures between 50 and 800 MPa for 30 min. Another set of samples also contained myristic acid (MA) or conjugated linoleic acid (CLA) in a molar ratio of 1:1.1 protein:ligand. All the treated samples were analysed using polyacrylamide gel electrophoresis (PAGE) and near- and far-UV circular dichroism (CD) spectroscopy. Native- and sodium dodecyl sulfate (SDS)-PAGE showed that, at temperatures above about 63 °C, bands of lower mobility were produced. As the treatment temperature increased, the quantity of native protein decreased steadily and the quantity of higher molecular weight aggregates increased. Thus β-LG could be native (Stage I T) or non-native disulfide-bonded monomers and polymers (and their stable hydrophobic adducts) (Stage II T). Pressure treatments of 50 and 100 MPa had no discernible effect. At pressures between 150 and 350 MPa, PAGE analysis showed that only non-native and dimer β-LG were produced but that hydrophobic adducts were apparent in the native-PAGE patterns. At pressures above 500 MPa, the whole range of polymers (higher molecular weight aggregates) was discernible. Therefore three stages have been proposed for the pressure denaturation of β-LG. Thus β-LG could be native (Stage I P), non-native disulfide-bonded monomers, intermediates in the aggregation process and dimers (and their stable hydrophobic adducts) (Stage II P) or larger polymers (Stage III P). This model is consistent with the near- and far-UV CD data and with reported 8-anilino-1-naphthalenesulfonate probe data. The addition of MA or CLA to β-LG followed by heat or pressure treatment shifted the transition of native β-LG to non-native monomer or dimer by stabilizing the native structure (Stage I T or Stage I P). Pressurizing similar samples at higher pressures showed that only CLA had the ability to inhibit the transition from Stage II P to Stage III P.

Keywords: Heat; Pressure; β-Lactoglobulin B; Myristic acid; Conjugated linoleic acid

1. Introduction

Whey proteins are used as functional ingredients in many food products and their functionality can be affected by either heat treatment (Mulvihill & Donovan, 1987) or pressure treatment (Patel, Singh, Havea, Considine, & Creamer, 2005). β-Lactoglobulin (β-LG), the most abundant whey protein in milk (Farrell et al., 2004), governs the overall process-induced aggregation and gelation of whey protein products (Van Camp & Huyghebaert, 1995; Zasypkin, Dumay, & Cheftel, 1996). Heat-induced denaturation of β-LG has been reported in detail at the molecular level (Belloque & Smith, 1998; Edwards, Jameson,

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Palmano, & Creamer, 2002). The free thiol of Cys121 of native β-LG can react with the Cys106:Cys119 disulfide bond to give a free Cys119 and Cys121:Cys106. The free Cys119 reacts with the other β-LG disulfide bond, Cys66:Cys160, leading to the formation of Cys119:Cys66 and a free Cys160, which has the potential to react with the disulfide bonds of other β-LG molecules if present (Creamer et al., 2004a) to give a wide range of β-LG polymers.

The results of pressure studies (in situ) at 130 MPa led Panick, Malessa, and Winter (1999) to conclude that hydrophobic interactions are absent and that a single aggregation mechanism is operative. Considine, Singh, Patel, and Creamer (2005a) suggested that pressures below approximately 150 MPa at neutral pH and 22 °C do not allow exposure of CysH121 and therefore disulfide bond reaction cannot occur. Pressures between 150 and 250 MPa allow the reaction of Cys121 with Cys106:Cys119 to occur. Pressures greater than 250 MPa allow this exchange and the further reaction to release Cys160.

The effect of some ligands on thermal unfolding and aggregation has been examined recently (Considine, Patel, Singh, & Creamer, 2005b) and a similar study using high pressure showed some significant differences in aggregate formation (Considine et al., 2005a). The differences found explain, to some extent, the macroscopic results of the two different treatment types. It has also been shown (Swaisgood, Wang, & Allen, 2001) that a number of bioactive hydrophobic molecules, e.g. retinol and other vitamins, can bind to β-LG, which consequently increases the resistance of the protein to proteolytic degradation (Creamer et al., 2004b; Puyol, Pérez, Mata, Ena, & Calvo, 1993), thermal denaturation (Considine et al., 2005b; Puyol, Pérez, Peiro, & Calvo, 1994), pressure-induced aggregation (Considine et al., 2005a) and unfolding in urea solutions (Creamer, 1995). This has been receiving increased interest, not only as a means of controlling the denaturation pathway of β-LG but also as a method of ligand transport (Swaisgood et al., 2001). All the fatty acids were probably bound in the central calyx of β-LG (Qin, Creamer, Baker, & Jameson, 1998; Wu, Pérez, Puyol, & Sawyer, 1999).

Pérez et al. (1989) found that fatty acids were bound to β-LG in milk and that palmitic (31–35%), oleic (22–23%) and myristic (14–17%) acids predominated. The apparent binding constants of a number of saturated and unsaturated fatty acids to β-LG (Frapin, Dufour, & Haertlé, 1993; Pérez et al., 1992) were in the range of 10^{-7} M at neutral pH.

One of the most interesting groups of fatty acids, from a health and nutritional point of view, is the C18 unsaturated fatty acids. Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of octadecadienoic acid (18:2) in which the double bonds are conjugated, in comparison with the double bonds of linoleic acid, which are separated by a methylene group. The configuration of the double bonds may exhibit several possible positions, but they are found mainly at 9 and 11 or 10 and 12 (Ha, Grimm, & Pariza, 1987). Of the individual isomers of CLA, *cis*-9, *trans*-11-octadecadienoic acid has been implicated as the most biologically active because it is the predominant isomer that is incorporated into the phospholipids of cell membranes (Ip, Singh, Thompson, & Scimeca, 1994). Various studies have discussed the potential health claims associated with CLA (Kritchevsky, 2000; O'Shea, Devery, Lawless, Murphy, & Stanton, 2000; Devery, Miller, & Stanton, 2001; Haugen & Alexander, 2004; Wang & Jones, 2004).

Relatively little information on the binding of CLA to β-LG is available. The binding constants (K'_d) for linoleic acid (1.43×10^{-7} M), CLA (1.86×10^{-7} M) and CLA's methyl ester (CLAME) (1.88×10^{-7} M) were determined by changes in the tryptophan fluorescence of β-LG (Swaisgood et al., 2001). As the effect of the binding of CLA to β-LG on its aggregation behaviour during processing treatments is largely unknown, this study aims to highlight its potential role in delaying denaturation and further dairy protein aggregation.

In previous studies, we have examined the influence of a range of ligands on the early stages of heat-induced (Considine et al., 2005b) and pressure-induced (Considine et al., 2005a) changes to native β-LG and its subsequent aggregation. This study investigates the effect of two fatty acids, myristic acid (MA) and CLA, on the heat- and pressure-induced denaturation of the native state and the subsequent aggregation pathways of β-LG at pH 7.2. MA was used to further the information we have already obtained on the effect of a range of ligands (Considine et al., 2005a, 2005b). The differences in chain length (18 carbon atoms for CLA and 14 carbon atoms for MA), plus the more rigid structural properties of the molecules, may offer some insight into why these ligands can offer β-LG protection against thermal or pressure treatment.

2. Materials and methods

β-LG B was prepared as described by Manderson, Hardman, and Creamer (1998). Octadecadienoic acid, conjugated (CLA; which is a mixture of *cis*- and *trans*-9, 11- and 10,12-octadecadienoic acids) and butylated hydroxytoluene were obtained from Sigma Chemical Co., St. Louis, MO, USA. MA was obtained from Fluka Chemie AG, CH-9470 Buchs, Switzerland. All other chemicals were AnalaR grade and were from BDH Laboratory Supplies, Poole, England; Coomassie Blue R250 and the polyacrylamide gel electrophoresis (PAGE) chemicals were obtained from BioRad Laboratories, Hercules, CA, USA; Amido black 10B was obtained from Merck, Darmstadt, Germany. The water was from an artesian bore and was purified by reverse osmosis followed by ion exchange and carbon treatment using a Milli-Q system (Millipore Corp., Bedford, MA, USA). The conductivity of the water was checked routinely.