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Towards understanding the effect of high pressure on food protein allergenicity: β-lactoglobulin structural studies

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ABSTRACT

A number of studies were devoted to understanding an immunological effect of pressure-treated β-lactoglobulin. In our previous work we have proved that high pressure significantly modifies β-lactoglobulin conformation and consequently its physicochemical properties. Here, structure of β-lactoglobulin complex with myristic acid determined at the highest accepted by the crystal pressure value of 550 MPa is reported. Our results structurally prove that pressure noticeably modifies positions of the major β-lactoglobulin epitopes. Considering the biological impact of observed changes in epitope regions, high pressure β-lactoglobulin structure presents a step forward in understanding the pressure modification of food protein allergenicity. The conformational changes of pressurized β-lactoglobulin did not support the hypothesis that proteolytic digestion facilitated by pressure is caused by an exposure of the digestive sites. Our findings demonstrate that high pressure protein crystallography can potentially identify the most pressure-sensitive fragments in allergens, and can therefore support development of hypoallergenic food products.

1. Introduction

Approximately 2–3% of children under 2 years of age and even 1% of the adults experience allergic reactions to cow's milk (Björnsson, Janson, Plaschke, Norrman, & Sjöberg, 1996). The major allergen involved in allergy to cow's milk proteins is bovine β-lactoglobulin (LGB). β-Lactoglobulin, together with several allergens of animal origin, belongs to the lipocalin family, which share a similar tertiary structure (β-barrel) and high affinity for hydrophobic ligands (Mäntyjärvi, Rautiainen, & Virtanen, 2000). The ruminant β-lactoglobulin is a globular protein composed of 162 amino acids with a molecular weight of 18 kDa. It contains 5 cysteine residues, 4 of which are forming disulfide bridges. β-Lactoglobulin is predominantly a β-sheet protein with nine β-strands and one major α-helix (Brownlow et al., 1997). As many allergens, LGB is a transient dimer (Niemi et al., 2015). In physiological conditions β-lactoglobulin is in the form of non-covalent dimers, whereas at low pH it forms monomers that preserve its native state (Taulier & Chalikian, 2001). Additionally, β-lactoglobulin undergoes a conformational change, the so-called Tanford transition, that is also associated with pH and occurs around neutral pH (Sakurai & Goto, 2006). Dimeric β-lactoglobulin molecules exist in the open conformation at basic pH, while the closed conformation is observed at acidic pH. This open-close conformational change involves displacement of the EF-loop (residues 85–90) and regulates access to the binding pocket located in the β-barrel.

The epitopes of LGB have been characterized in many studies during the last two decades. The IgE strong epitope peptides identified by Järvinen and co-workers (Järvinen, Chatchatee, Bardina, Beyer, & Sampson, 2001) are located on the DE loop (Lys75-Asp85) and in the α-helix H3 (Glu127-Pro144). Three weaker epitope peptides were also proposed: Leu31-Pro48, Lys47-Lys60, and Leu57-Ile78. In another study, Clement et al. have characterized the IgG epitope map of LGB by using a competitive immunoassay (Clement et al., 2002). According to those studies, the most antigenic regions are located in the α helix and on the external loops, including Glu127, Asp130 and Glu134 residues, as well as in the β-strand B where a minor antigenic area Glu44-Glu45 was identified (Fig. 1). Recently, Niemi et al. reported the crystal structure of an IgE Fab fragment in complex with β-lactoglobulin

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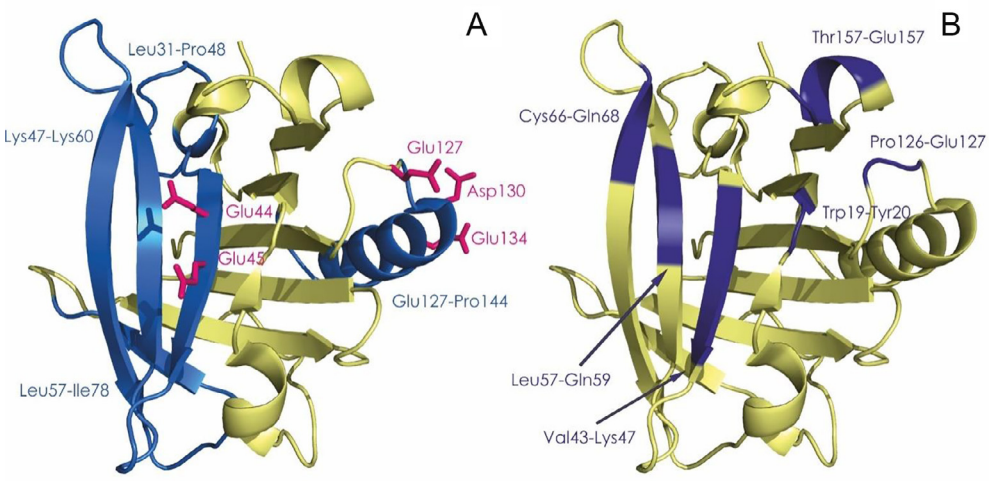

Fig. 1. LGB epitopes: (A) functional epitopes on LGB regarding interaction with IgE (blue) and IgG (pink) antibodies, according to Järvinen et al. (2001) and Clement et al. (2002), respectively; (B) structural epitopes defined by the set of LGB atoms considered to be in contact with atoms of the antibody Fab-IgE identified by Niemi et al. (2007). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Niemi et al., 2007), which revealed that 15 amino acid residues of LGB molecule form contacts with the antibody. Those interactions between LGB and an IgE fragment include 2 salt bridges and 13 hydrogen bonds. Large fragments of β-strands were reported as epitopes: Trp19-Tyr20 (β-strand A), Val43-Lys47 (β-strand B), Leu57-Gln59 (β-strand C) and Cys66-Gln68 (β-strand D). In addition, the loop between β-strand H and the following helix H3 (Pro126-Glu127), and the short C-terminal α-helix H4 (Thr154-Glu157) were reported to be a part of the epitope.

Like most food allergens, LGB is very stable at low pH and resistant to degradation (Bu, Luo, Chen, Liu, & Zhu, 2013). Therefore, different strategies for destroying or modifying β-lactoglobulin allergenicity, as well as digestibility including heat treatment (Bu, Luo, Zheng, & Zheng, 2009), proteolysis (Chicón, Belloque, Recio, & López-Fandiño, 2006) and high-pressure treatment (López-Expósito, Chicón, Belloque, López-Fandiño, & Berin, 2012) were investigated. High pressure is known to affect the structure of proteins. Typically, at pressure up to 200–300 MPa, conformational changes are observed while few hundred MPa pressure can lead to protein denaturation. Several trials have been performed to identify structural consequences of high pressure on the structure of LGB, mostly in order to correlate them with LGB allergenicity and digestibility. Studies performed by Chicón, Belloque, Alonso, and López-Fandiño (2008) showed that high-pressure LGB treatment (200 and 400 MPa) did not affect its binding to IgE. Contrary to those findings, Kleber et al. indicated that high-pressure conditions (200–600 MPa) could enhance the antigenicity of LGB (Kleber, Maier, & Hinrichs, 2007). Other scientists suggest that allergenic activity of LGB depends on the pressure that was applied and can be higher (at mild conditions) or lower (at elevated pressure) (Zhong et al., 2012).

Previous studies that reported high-pressure LGB structure with dodecane have revealed interesting findings concerning the influence of pressure on LGB adsorption process (Kurpiewska et al., 2018). Structural changes in β-lactoglobulin molecule introduced by the pressure of 430 MPa were interpreted as early signs of dimer dissociation and conformational changes characteristic for partially unfolded intermediate. In this study, we have investigated the influence of pressure as high as 550 MPa on LGB molecule. Crystal structure of LGB complex with myristic acid (MYR) determined at high pressure allowed to observe unique and important structural response of the protein molecule to the pressurization. Detailed analysis revealed that at 550 MPa noticeable positional shifts of the digestive sites, as well as LGB epitopes can be identified. Present analysis supports our previous findings concerning the high-pressure modification of β-lactoglobulin conformation. Current results give a deeper insight into structural origins of changes in LGB biochemical features and allergenicity caused by elevated pressure.

2. Materials and methods

2.1. Chemicals

The isoform B of β-lactoglobulin was purchased from Sigma (Sigma-Aldrich Co, St. Louis, Mo) and used without purification. All the other chemical reagents used were of analytical grade.

2.2. Crystallization

Crystals were grown at room temperature using the hanging drop vapor diffusion method according to Wu, Dolores Pé Rez, Puyol, and Sawyer (1999). Lyophilized protein was dissolved in ultra-pure water to the final concentration of 20 mg/ml. A crystallization drop containing 2 µl of the protein, 0.5 µl of myristic acid (10 mM in EtOH) and 6 µl of reservoir solution was equilibrated against 0.5 ml of reservoir solution containing 0.1 M Tris-HCl, 1.34 M sodium citrate, pH 7.5 (298 K, atmospheric pressure). An average size of LGB-MYR crystals was 0.25 × 0.40 × 0.50 mm. Size and quality of obtained crystals were suitable for experiments using Merrill-Bassett type of diamond anvil cell (DAC).

2.3. Data collection and processing

X-ray diffraction data for LGB-MYR single crystal mounted inside the DAC were collected at ambient (ap) and high pressure (hp) conditions. Steel gaskets (0.3 mm thickness) with a hole diameter of 0.6 mm were used according to the procedure previously described in Kurpiewska and Lewiński (2010). Protein crystals were transferred into the chamber previously filled with the crystallization mother liquor (Fig. 2). Determination of pressure was performed at ambient temperature using ruby fluorescence method (Kurpiewska et al., 2018). Data were collected at room temperature using SuperNova (Rigaku-Oxford Diffraction) four-circle diffractometer with a microfocus MoKα radiation source (λ = 0.7107 Å) and multilayer X-ray optics. The data sets were processed with *CrysAlis^{Pro}* software (Oxford Diffraction (2006) CrysAlis^{Pro} Oxford Diffraction Ltd, Abingdon, England, Version 1.171.36.20) and *AIMLESS* (Evans & Murshudov, 2013). Preliminary experiments allowed to determine the unit cell parameters for crystals compressed to 0.1, 170, 370 and 650 MPa. Complete data collections were performed for LGB-MYR crystal at ambient pressure (ap LGB-MYR) and at 550 MPa (hp LGB-MYR).

For more detailed information of structure solving, refinement and analysis please see the Supplementary data.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.07.104.