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Review

Structural dynamics and folding of β-lactoglobulin probed by heteronuclear NMR

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ABSTRACT

Bovine β-lactoglobulin (βLG) has been one of the most extensively studied proteins in the history of protein science mainly because its abundance in cow's milk makes it readily available to researchers. However, compared to other textbook proteins, progress in the study of βLG has been slow because of obstacles such as a low reversibility from denaturation linked with thiol–disulfide exchange or monomer–dimer equilibrium preventing a detailed NMR analysis. Recently, the expression of various types of recombinant βLGs combined with heteronuclear NMR analysis has significantly improved understanding of the physico-chemical properties of βLG. In this review, we address several topics including pH-dependent structural dynamics, ligand binding, and the complex folding mechanism with non-native intermediates. These unique properties might be brought about by conformational frustration of the βLG structure, partly attributed to the relatively large molecular size of βLG. We expect studies with βLG to continue to reveal various important findings, difficult to obtain with small globular proteins, leading to a more comprehensive understanding of the conformation, dynamics and folding of proteins.

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1. Introduction

Bovine β-lactoglobulin (βLG) has been one of the most extensively studied proteins in the history of protein science [1–4]. The major reason for this is simply its abundance in cow's milk: the concentration of βLG in milk is about 0.2 g/100 ml, next to casein (2.9 g/100 ml) [5], making βLG easily accessible to researchers. For the same reason, sperm whale myoglobin [6,7], hen egg lysozyme [8,9], and lactate dehydrogenase [10,11] are examples of proteins on which extensive studies were performed in the 1950s, leading to the determination of their 3D structure and an understanding of structure–function relationships. Extensive studies with βLG started in the 1950s and have provided important information regarding structure and function. These included the pH-dependent conformational transition known as the Tanford transition [12] and the binding of various hydrophobic ligands [13], making βLG an excellent model for the molecular study of proteins as reviewed previously [2–4,14–17].

However, compared to other textbook proteins, detailed structural studies on βLG were slower because of several obstacles. Medium resolution X-ray crystallographic structures of βLG were reported only in the late 1980s [18], followed by a series of high resolution structures, some including ligands [19–21]. Low reversibility from denaturation linked with thiol–disulfide exchange prevented the detailed study of protein folding with βLG [22], though such exchange is relevant to gelation [23,24] and advantageous for the food applications. The X-ray structures suggested the mechanism of the Tanford transition [20] and ligand binding [19,21]. However, details in solution have remained unknown because heteronuclear NMR analysis, a powerful technique often used for other proteins, has been difficult to apply to βLG at neutral pH.

Recently, obstacles preventing the detailed molecular understanding of the structure and dynamics of βLG in solution have been cleared by expressing wild-type [25–31] and suitable mutants [32,33] or by choosing appropriate experimental conditions as well as employing advanced analytical techniques, in particular heteronuclear NMR. Consequently, we now understand the molecular basis of the unique properties of βLG indicated in earlier studies. Moreover, supported by heteronuclear NMR, we believe that βLG will continue to be one of the most important targets of protein science for several reasons. First of all, it is a typical globular protein of moderately large molecular size. So far, detailed studies of folding in solution have been performed with relatively small proteins, typically less than 100 amino acid residues, creating the concept of rapid folding without folding intermediate or complex folding funnel [34–36]. However, studies with larger proteins, e.g. apomyoglobin prepared by the removal of heme from myoglobin [37,38], suggest important roles for folding intermediates in productive folding [39]. It is likely that βLG will provide a wealth of information about the roles of such key intermediates. On the other hand, the binding of ligands to βLG continues to be an intriguing topic. From a more practical viewpoint, aggregation of βLG is closely related to food processing properties of milk. Taken together, it is increasingly important to obtain further understanding of the structure, dynamics, and ligand binding of βLG. In this review, we overview recent advances focusing on studies with

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NMR. For the physico-chemical properties of βLG, readers should also refer to other reviews [4,14,15].

2. Structure of βLG

βLG consists of 162 amino acid residues (18 kDa), containing two disulfide bonds (Cys 66–Cys 160 and Cys 106–Cys 119) and a free thiol (Cys 121). Structures of βLG have been reported by several groups with X-ray crystallography [19–21] and solution NMR [29,40,41] (Fig. 1A). It is a predominantly β-sheet protein. The β-barrel, or so-called calyx, is conical and is made of two β-sheets: the B–D strands and N-terminal half of the A strand (denoted A^N) form one sheet, and the E–H strands and C-terminal half of the A strand (denoted A^C) form the other (Fig. 1B). On the outer surface of the β-barrel, between the G and H strands, is the 3-turn α-helix. The loops that connect the β-strands at the closed end of the calyx, BC, DE, and FG, are generally quite short, whereas those at the open end, AB, CD, EF, and GH, are significantly longer and more flexible [19]. In the calyx, there is a large central cavity which is surrounded by hydrophobic residues and is accessible to solvent. This cavity provides the principal ligand-binding site. βLG contains two tryptophan residues, Trp 19 on the A strand and Trp 61 on the C strand. The former is buried in the hydrophobic core whereas the latter is exposed to the solvent in the native structure, making them useful probes for monitoring site-specific conformational changes. In addition, studies on the monomer–dimer equilibrium [30,32,42,43] and the reactivity of the thiol group of Cys121 deeply buried between the α-helix and H strand [44–48] revealed other important properties of βLG.

βLG belongs to the lipocalin family, a subclass of the calycin superfamily [49]. There are two other subfamilies in the calycin superfamily; the fatty acid-binding protein (FABP) family and the avidin family. The functions of most lipocalins, such as tear lipocalin [50], and FABPs, such as I-FABP [51], are in the transportation of ligands. However, some lipocalins, such as L-PGDS [52] and glycodelins [53], etc., have other specific functions. It is suggested that these proteins originated from the same gene, but now have different genes and functions after gene duplication and evolution [16,53]. Indeed, the behavior of βLG described above is shared by other members of the family.

3. Molecular mechanism of the Tanford transition

3.1. Equilibrium transition

Although βLG exists in a native state over a wide range of pH values, it shows slight conformational changes during a change of pH [54]. Among the pH-dependent conformational changes of βLG, the Tanford transition is the most important because it is thought to be related to the function of βLG. Tanford et al. [55] observed a change in optical rotatory dispersion at pH 7.0 representing a certain conformational change. Subsequently, they found that this conformational change is accompanied by a deprotonation of a carboxyl group with an anomalous pK_a of 7.5 [20,55].

Qin et al. [20] reported an important observation about the Tanford transition from crystal structures solved at pH 6.2 and 7.1. By comparing these two crystal structures, they found that the EF loop,

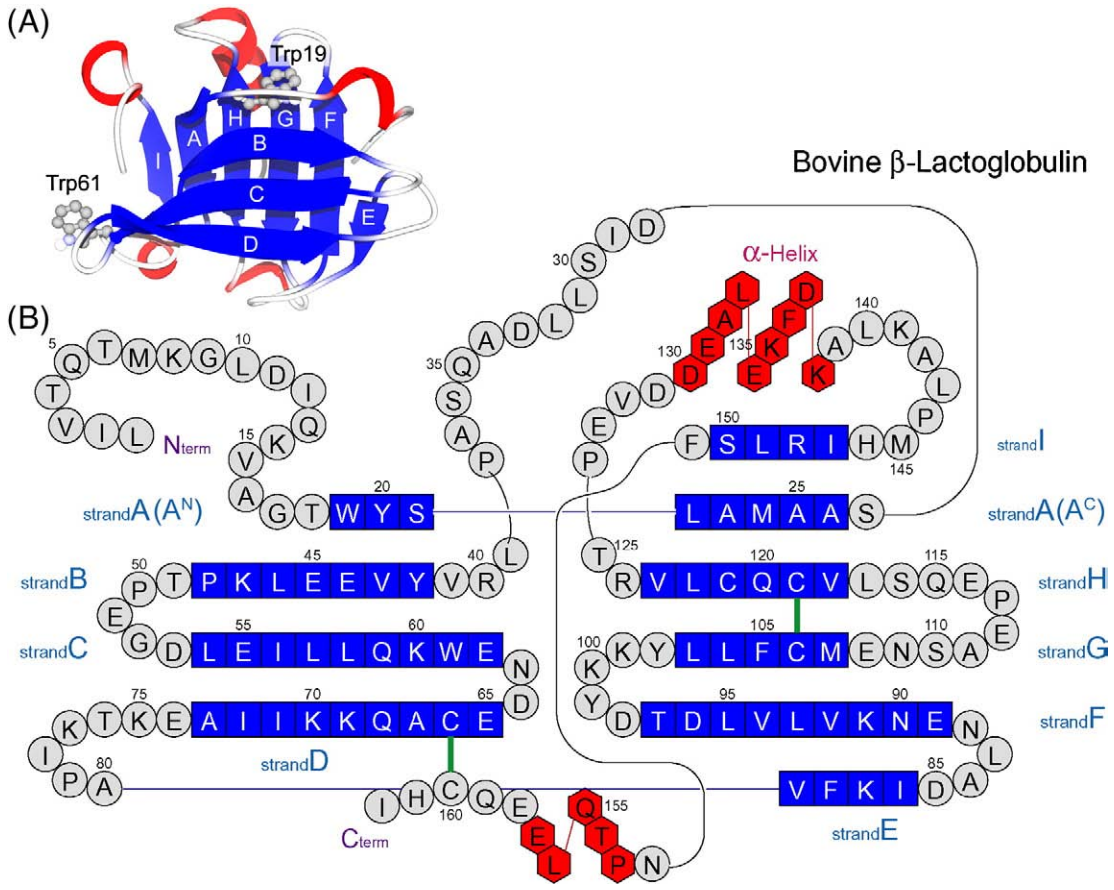

Fig. 1. The 3D structure and amino acid sequence of bovine βLG. (A) Ribbon diagram of a single subunit of bovine βLG lattice X, whose pdb code is 1BEB [19]. The β-strands are labeled. Trp residues are represented as balls and sticks. The diagram was produced using the program MolFeat (FiatLux, Tokyo, Japan). (B) A schematic representation of the amino acid residues of the βLG sequence. Residues making up the α-helix, β-sheet, and loop are represented by hexagons in red, squares in blue, and circles in grey, respectively. Green lines indicate the positions of disulfide bonds. It is seen that βLG has two β-sheets; The B–D strands and N-terminal half of the A strand (denoted A^N) consist of one and the E–H strands and C-terminal half of the A strand (denoted A^C) consist of the other.

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