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Structural insights into the stability perturbations by N-terminal variations in human and goat α-lactalbumin

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Introduction

α-Lactalbumin is a major component of milk protein and it has been used as a model protein for folding study because it forms molten globule state under mild denaturation conditions at equilibrium, which is identical with a kinetic folding intermediate. It has been well known that the N-terminal portion of α-lactalbumin significantly contributes to the stability[1,2]. The addition of an extra methionine residue at the N terminus for recombinant expression in bacteria results in a significant stability loss. This reduction of the stability hampers the mutational study of α-lactalbumin, such as the phi-value analysis in folding study, because the recombinantly expressed α-lactalbumin is largely destabilized with a single mutation and difficult to determine its free energy precisely. We have overcame this stability loss by mutating the first residue (Lys and Glu in human and goat α-lactalbumin, respectively) to methionine. Here, we show that the crystal structures of the N-terminal mutants of human and goat α-lactalbumin. The crystal structures of the mutants suggests the importance of a hydrogen bond between the N-terminal amide nitrogen atom and an Oγ atom of Thr38.

Results

Crystal structure of recHLA and HLA-K1M

We determined the crystal structures of recHLA and HLA-K1M at a resolution of 1.80 Å and 1.61 Å, respectively. The asymmetric unit contains one monomer for the recHLA crystal and two monomers for the K1M crystal (mol A and mol B). The data collection and refinement statistics are summarized in Table 2. The final model contains 121 residues for recHLA (total 124 residues) and 120 residues for K1M (total 123 residues) and the last three residues cannot be modeled in the both structures because of the poor electron density as same as the GLA-E1M structure. Every monomers in the structures contain a calcium ion. The overall structure of recHLA and HLA-K1M is similar with the autHLA structure, which has been reported previously.

Crystal structure of GLA-E1M

The crystal structure of GLA-E1M was determined at a resolution of 1.60 Å. Two monomers are included in the asymmetric unit. The crystal is isomorphous with the authentic structure which belongs to $P2_1$ space group (PDBID: 1HFY). The final model contains 120 residues (1-120 residues out of 1-123 entire sequence) and the last three residues are disordered. The each monomer contains a calcium ion. The root mean square deviation (RMSD) of Cα atoms between two molecules in the asymmetric unit (mol A and mol B) is 0.45 Å, indicating that the two structures have an almost identical conformation. Thus, from here on, we focus on mol A as a representative structure of GLA-E1M.

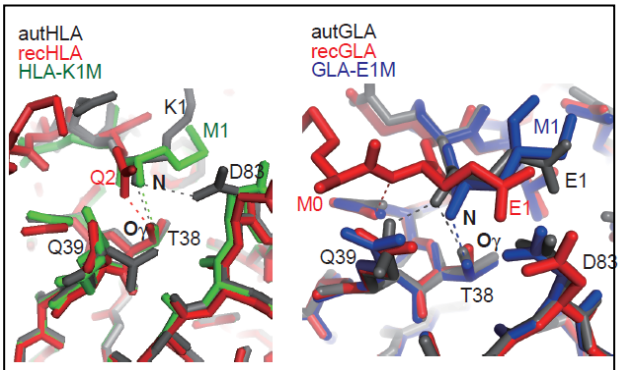

Figure: Crystal structures of the N-terminal region of human alpha-lactalbumin (left) and goat alpha-lactalbumin (right). Structure of the authentic forms (red), the recombinant forms (red), HLA-K1M (green), and GLA-E1M (blue) are shown with the stick representation.

The striking feature of the HLA-K1M and GLA-E1M structures are the recovery of the position of an backbone N atom at Met1 (Figure). The N atom of Met1 forms hydrogen bond with Oγ of Thr38 as the same way with authentic forms. Thus, we conclude that the disruption of the H-bond in the recombinant forms results in the destabilization.

References

[1] S. E. Veprintsev et al., *Proteins.* 37, 65 (1999).
[2] T. K. Chaudhuri et al., *J. Mol. Biol.* 285, 1179 (1999).