Thermodynamic and structural consequences of amino acid substitutions in the β-lactoglobulin's binding pocket

β-lactoglobulin (BLG) is a major whey protein found in milk of cows and other animals. It has a characteristic tertiary structure. Its key component is a β-barrel, which constitutes a binding pocket for hydrophobic and amphiphilic ligands. The binding affinity is affected by loops located around the binding site. Amino acid substitutions in the loops can lead to obtaining protein variants with practical applications as well as deepening the knowledge of the binding process. The aim of this study was to understand the role played in the binding by the leucine residues at positions 39 and 87 and lysine residues at positions 60, 69, and 70. To achieve this goal, a set of BLG variants was designed with the following amino acid substitutions: L39A, L39S, L39F, L39Y, L39I, L87A, L87S, L87I, K60A, and K69A/K70A.

Circular dichroism measurements indicate that the introduced substitutions modify the protein's structure only to a small extent and that the variants under study remain stable in the temperature range of further experiments. Furthermore, based on the isothermal titration calorimetry (ITC) experiments, the substitutions do not cause significant changes in dimerization constant.

At pH below 7, BLG (the basic variant, WT) exhibits lower association constants with ligands due to the obstruction of the binding site entrance by one of the flanking loops. Earlier studies showed that the L39Y substitution leads to an increase in the association constant at pH 6.5, so that it reaches a value close to that at pH 7.5. Amino acid substitutions at position 39 were intended to aid in understanding this effect. The results of ITC experiments showed an increase in the association constant of sodium dodecyl sulphate (SDS) to all variants with substitutions at position 39. A similar effect was observed for L87A and L87S variants. In the case of L87A, the association constant was even higher at pH 6.5 than 7.5. On the other hand, the effect of L87I substitution was small. Moreover, thermodynamic parameters indicate that, except for L39F variant, the binding mechanism differs depending on pH even if the association constants are similar. Based on the obtained results it can be inferred that the substitutions affect the position of the loop.

K60A and K69A/K70A substitutions were aimed at estimating the importance of electrostatic interactions between the substituted residues and charged ligands. ITC measurements confirmed stronger binding of a negatively charged ligand (SDS) than a positively charged one (dodecyltrimethylammonium choride, DTAC) to BLG WT. The association constant of SDS was shown to be higher in the presence of lysine residues at positions 60, 69, and 70. On the contrary, DTAC is bound with higher association constant by K60A and K69A/K70A variants than by WT. These results point to the significance of electrostatic interactions. Ionic strength has a minor influence on the association constants of both studied ligands. If electrostatic interactions played the main role in binding, the increase of ionic strength would lead to a significant change in the association constant as a consequence of the reduction of electrostatic interactions. However, this is not the case, which is in accordance with the generally accepted view that the main contribution to ligand binding by BLG is from hydrophobic interactions.

In conclusion, the results show that the leucine residues at positions 39 and 87 are essential for the observed difference in the association constants of SDS at pH 6.5 and 7.5, while the electrostatic interactions between a charged ligand and lysine residues at positions 60, 69, and 70 have a significant but not dominant role in the binding process.