Specificity of [i]β-lactoglobulin[/i] variants for spontaneous assembly with lactoferrin

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Formation of self-assembled structures by spontaneous association of oppositely charged proteins has increasing interest for the design of reversible encapsulation devices for food, cosmetic and pharmaceutical applications. The mixing of lactoferrin (Lf), a basic protein, with β-lactoglobulin, an acidic protein, resulted in their spontaneous assembly into well-organized microspheres under specific experimental conditions. Optimal conditions were found at pH 5.5 and at a low ionic strength. Despite a perfect similarity of their tertiary structure, β-lactoglobulin A and B variants (βlg A and βlg B) exhibited specific assembly behavior with Lf.

At constant protein concentration and at a molar ratio Blg/Lf of 10, increasing the proportion of βlg A with respect to βlg B resulted in an increase of protein association. Formation of microspheres was detected only with high proportion of βlg A over βlg B. Also, the size of formed microspheres increased with increasing the proportion of βlg A. No microspheres were detected in solution with low proportion of βlg A. This is in agreement with the proportion of protein recovered in the microspheres: 60 % of the protein was recovered in the solution containing βlg A, against 30 % in solution having an equimolar mixture of both variants and only 4 % in solution containing βlg B.

The total protein concentration is a highly significant parameter for controlling the formation of microspheres. Even if βlg A was more prone than βlg B to form microspheres, mixture of βlg A and Lf did not form spheres at low total protein concentration (< 0.01 mM). In contrast, at high total protein concentration (> 0.4 mM), it was possible to form microspheres with a mixture of βlg B and Lf. This demonstrated that βlg A and βlg B have specific threshold concentration for forming microsphere with Lf.

The interaction parameters (Ka, n) between βlg A and Lf and between βlg B and Lf, as determined by Isothermal titration calorimetry (ITC) and Thermophoresis, were not found to be significantly different. Further investigations are presently conducted to understand the origin of the specific association of βlg variant with Lf, in particular the role of amino-acid substitutions from Asp and Val (βlg A) to Gly and Ala (βlg B).