Salt-dependent complex formation in lysozyme-alginate mixture

Asna Vakeri, Adeline Boire, Said Bouhallab, Denis Renard

Salt-dependent complex formation in lysozyme-alginate mixture

Asna Vakeri[1], Adeline Boire[1], Antoine Bouchoux[2], Said Bouhallab[3], and Denis Renard[1]

[1] *UR 1268 Biopolymères Interactions Assemblages, INRAE, 44316, Nantes, France*
[2] *TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France*
[3] *UMR 1253, STLO, INRAE, Agrocampus Ouest, F-35042 Rennes, France*

The associative interaction between oppositely charged macromolecules proceeds through either liquid-liquid phase separation (LLPS) forming complex coacervates or liquid-solid phase separation (LSPS) forming aggregates. In this work, we investigated the assembly of the basic protein lysozyme (LYS) with the negatively charged polysaccharide alginate (ALG) at pH 7 under varying conditions of mixing ratios, total concentrations, and ionic strengths.[1]

The droplet-based millifluidic device coupled with optical microscopy gave an extensive qualitative analysis of the phase behaviour of the system by probing different experimental conditions.[2] Grey level analysis associated with the droplet millifluidic experiment allowed both the quantification and the qualitative definition of phase separation. We constructed a three-dimensional phase diagram, incorporating salt, LYS, and ALG concentrations as coordinates, offering a detailed depiction of monophasic regions, liquid-solid and liquid-liquid phase separation domains, and areas of coexistence of both solid and liquid phases. The thermodynamic characterization of the formation of different LYS/ALG assemblies was carried out using isothermal titration calorimetry (ITC) where distinct ITC profiles were associated with coacervation and aggregation. The interaction affinity (Ka) for aggregation was three orders of magnitude higher than for coacervation, without significant change in binding stoichiometry. Structural differentiation of various assemblies in the nanometer range was achieved through small-angle X-ray scattering (SAXS) experiments. To gain deeper insights into the mechanisms underlying both LSPS and LLPS processes, further investigation of additional polyelectrolyte couples is needed, facilitating a more comprehensive understanding of these phase separation phenomena.

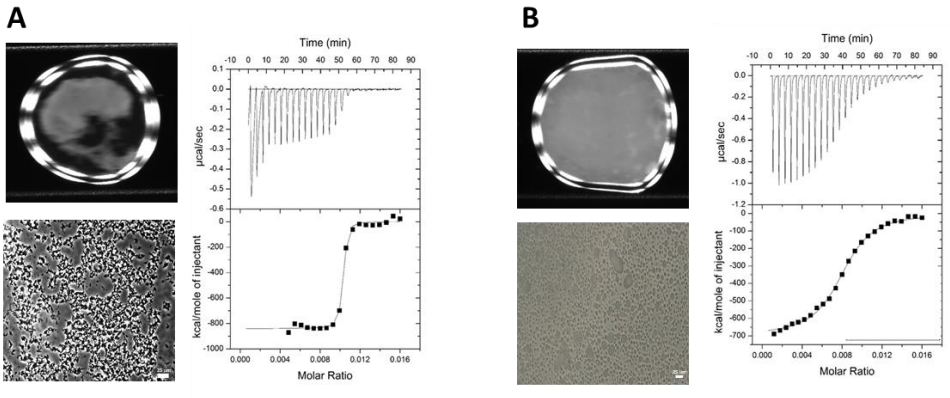

Figure 1. Droplet image, optical microscopy image, and ITC thermogram obtained for aggregation (A) and coacervation (B).

[1] Vakeri et al., *Food Hydrocolloids,* **2024,** submitted**.**

[2] Amine, C. *et al*, *Food Hydrocolloids*, **2017**, 70, 134-142; **2019**, 92, 94-103.