

SUPPORTING INFORMATION

Generic pathways to stability in concentrated protein mixtures

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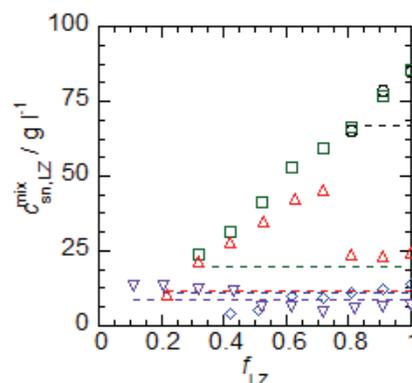
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Saturation limit of pure lysozyme solutions

To estimate the saturation limit of lysozyme S_{LZ} at the pH of 7.8 used in our experiments we exploit the data obtained by Retailleau et al, who determined S_{LZ} as a function of pH and salt concentration for $3.3 \leq \text{pH} \leq 8.7$ and $0 \leq c_{\text{NaCl}} \leq 1.2$.¹ An interpolation to $\text{pH} = 7.8$, yields: $S_{LZ} = 238.5 \text{ g l}^{-1}$ at $c_{\text{NaCl}} = 0 \text{ mM}$, $S_{LZ} = 66.8 \text{ g l}^{-1}$ at $c_{\text{NaCl}} = 100 \text{ mM}$, $S_{LZ} = 19.5 \text{ g l}^{-1}$ at $c_{\text{NaCl}} = 200 \text{ mM}$, $S_{LZ} = 11.7 \text{ g l}^{-1}$ at $c_{\text{NaCl}} = 300 \text{ mM}$, $S_{LZ} = 10.7 \text{ g l}^{-1}$ at $c_{\text{NaCl}} = 400 \text{ mM}$, and $S_{LZ} = 8.8 \text{ g l}^{-1}$ at $c_{\text{NaCl}} = 500 \text{ mM}$.

Saturation limit in pure lysozyme solutions and mixtures with α -lactalbumin

We compare the saturation limit of lysozyme, as estimated from the data of Retailleau et al,¹ to the concentration of lysozyme in the supernatant $c_{\text{sn},LZ}^{\text{mix}}$ of our α -lactalbumin / lysozyme systems with $c_{\text{NaCl}} = 100, 200, 300, 400$ and 500 mM after an equilibration time of ~ 2 months in Fig. S1. To estimate $c_{\text{sn},LZ}^{\text{mix}}$ we assume that the precipitates contain only lysozyme, the α -lactalbumin remaining entirely in the supernatant, such that $c_{\text{sn},LZ}^{\text{mix}} = c_{\text{sn}}^{\text{mix}} - c_{i'\alpha}^{\text{mix}}$, where we determine $c_{\text{sn}}^{\text{mix}}$ in UV-Vis experiments (see Experimental section in the paper). For $c_{\text{NaCl}} = 400 \text{ mM}$ and 500 mM , we find a good agreement between $c_{\text{sn},LZ}^{\text{mix}}$ and S_{LZ} both in the mixtures ($0.1 \leq f_{LZ} \leq 0.9$) and in the pure lysozyme systems ($f_{LZ} = 1$, $c_{i'\alpha} = 0 \text{ g l}^{-1}$). This demonstrates that the presence of α -lactalbumin hardly affects the saturation limit of lysozyme and that at these salt concentrations the crystallization process is complete after an equilibration time 2 months. By contrast, for $c_{\text{NaCl}} \leq 300 \text{ mM}$ $c_{\text{sn},LZ}^{\text{mix}}$ significantly exceeds S_{LZ} for both, the mixtures and the pure lysozyme systems. This indicates that the crystallization process is strongly delayed at these conditions, consistent with previous work reporting that the rate of lysozyme crystallization decreases with decreasing NaCl-concentration and supersaturation.^{2,3}



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Fig. S1 Dependence of lysozyme concentration in the supernatant on lysozyme mole fraction f_{LZ} . The data points are obtained for our α -lactalbumin / lysozyme mixtures at $\text{pH} = 7.8$ after an equilibration period of ~ 2 months at a temperature of $T \sim 22^\circ\text{C}$ with $c_{\text{NaCl}} = 100 \text{ mM}$ (black \circ), 200 mM (green \square), 300 mM (red Δ), 400 mM (blue \diamond) and 500 mM (purple ∇). To estimate $c_{\text{sn},LZ}^{\text{mix}}$, we assume that α -lactalbumin remains entirely in the supernatant, such that $c_{\text{sn},LZ}^{\text{mix}} = c_{\text{sn}}^{\text{mix}} - c_{i'\alpha}^{\text{mix}}$. The saturation limit of pure lysozyme solutions estimated from the data of Retailleau et al¹ are indicated as dashed lines.

Notes and references

1. P. Retailleau, M. RiesKautt and A. Ducruix, *Biophysical Journal*, 1997, **73**, 2156-2163.
2. G. W. He, V. Bhamidi, R. B. H. Tan, P. J. A. Kenis and C. F. Zukoski, *Crystal Growth & Design*, 2006, **6**, 1175-1180.
3. Y. X. Liu, X. J. Wang and C. B. Ching, *Crystal Growth & Design*, 2010, **10**, 548-558.