

## Lysozyme in water-acetonitrile mixtures: Preferential solvation at the inner edge of excess hydration

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### Abstract

© 2017 Author(s). Preferential solvation/hydration is an effective way for regulating the mechanism of the protein destabilization/stabilization. Organic solvent/water sorption and residual enzyme activity measurements were performed to monitor the preferential solvation/hydration of hen egg-white lysozyme at high and low water content in acetonitrile at 25 °C. The obtained results show that the protein destabilization/stabilization depends essentially on the initial hydration level of lysozyme and the water content in acetonitrile. There are three composition regimes for the dried lysozyme. At high water content, the lysozyme has a higher affinity for water than for acetonitrile. The residual enzyme activity values are close to 100%. At the intermediate water content, the dehydrated lysozyme has a higher affinity for acetonitrile than for water. A minimum on the residual enzyme activity curve was observed in this concentration range. At the lowest water content, the organic solvent molecules are preferentially excluded from the dried lysozyme, resulting in the preferential hydration. The residual catalytic activity is ~80%, compared with that observed after incubation in pure water. Two distinct schemes are operative for the hydrated lysozyme. At high and intermediate water content, lysozyme is preferentially hydrated. However, in contrast to the dried protein, at the intermediate water content, the initially hydrated lysozyme has the increased preferential hydration parameters. At low water content, the preferential binding of the acetonitrile molecules to the initially hydrated lysozyme was detected. No residual enzyme activity was observed in the water-poor acetonitrile. Our data clearly show that the initial hydration level of the protein macromolecules is one of the key factors that govern the stability of the protein-water-organic solvent systems.

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