Lysozyme – Water Interactions Studied by Sorption Calorimetry

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Introduction

Sorption calorimetry is a powerful method to study interactions of organic and biological substances with water. Previously this method was used for investigation of properties of surfactants, lipids and phospholipids, DNA and other materials. The method allows simultaneous monitoring of water activity and enthalpy of mixing in aqueous systems at isothermal conditions.

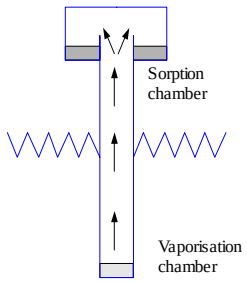
Hydration of proteins plays an important role in their functions and stability. Mobility of proteins is believed to affect their activity and is strongly dependent on degree of hydration and water content. Therefore the method of sorption calorimetry potentially can provide valuable information on thermodynamic, structural and functional properties of proteins.

Sorption calorimetry

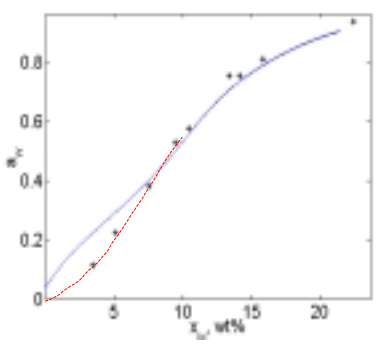
$$H_w^{mix} = H_w^{vap} + P^{sorp}\frac{H_w^{vap}}{P^{vap}}$$

$$a_w = 1 - \frac{P^{vap}}{P^{vap}_{\max}}$$

$$m_w = \frac{\int P^{vap} dt}{H_w^{vap}}$$



Sorption isotherm, 25 °C

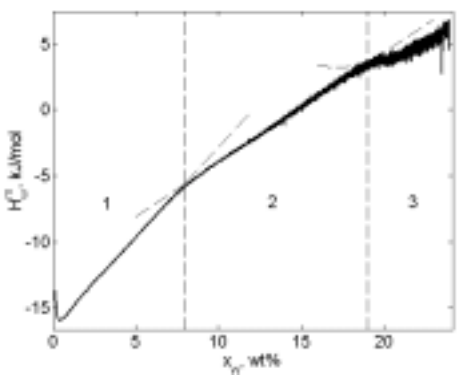


On the figure to the left stars (*) denote the points obtained by isopiestic method (by equilibration of lysozyme with water vapour provided by saturated salt solutions at 25°C). The curve presents calorimetric results. The two methods gave similar results excluding a region with very low water content (0-8 wt% of water). This indicates that in this composition region the kinetics of the process of water sorption is slow. Since the equilibration time during isopiestic experiments was much longer, the stars represent equilibrium points.

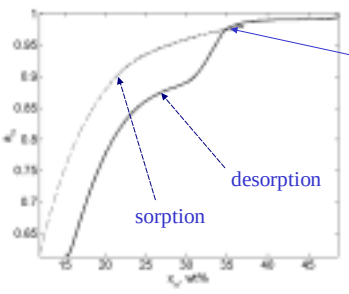
Partial molar enthalpy of mixing of water, 40 °C

Three regimes of sorption:

1. Slow penetration of water molecules to protein-protein interface. Protein molecules are in the rigid (glassy state). β-sheet content is higher than in solution.
2. Gradual glass transition:
   Rigid + nH_2O = Flex
   Occurs in a chemical reaction fashion (independently for each molecule)
3. Further water uptake with disaggregation of large protein aggregates.

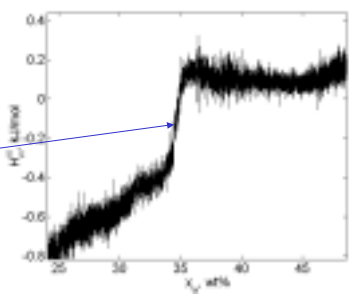


Desorption results



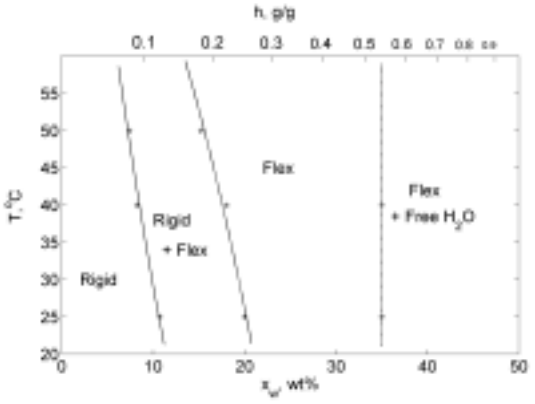
During desorption of water a_w drops at 35 wt%

At the same point enthalpy changes in stepwise fashion



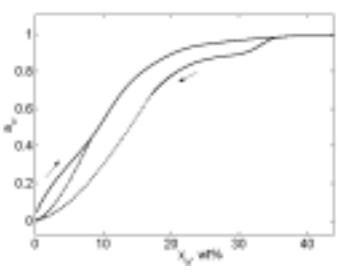
These data indicate disappearance of "free" water upon desorption.

Phase diagram



Hysteresis, aggregation and free water

We observed two hysteresis loops in the sorption isotherm.
The small loop: caused by slow kinetics of penetration of water between surfaces of highly aggregated protein molecules at low water content.
Large loop: in desorption experiments protein molecules are initially disaggregated, therefore larger surface is available for the solvent, which lowers water activity.
Free water: the amount of free water is **420** water molecules per lysozyme molecule. This is higher than the amount of unfreezing water.



Conclusions

- **Four regimes of sorption** of water on lysozyme was found:
  - •Slow penetration between unhydrated lysozyme molecules
  - •Gradual glass transition
  - •Further hydration with disaggregation
  - •Accumulation of free water
- **Two hysteresis loops** is observed. The main loop is caused by the difference in aggregation properties of lysozyme during sorption and desorption.
- **420 water molecules** are bound to each lysozyme molecule