Structural Transition and Thermal Behavior of α-lactalbumin Polymers

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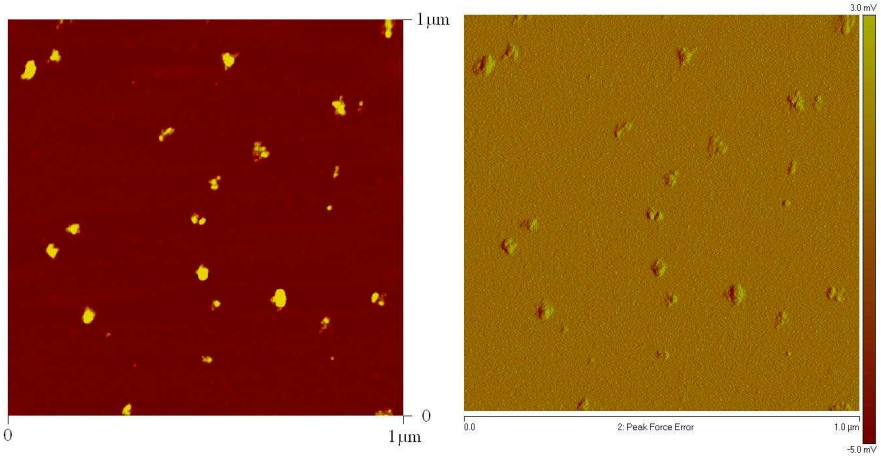

Introduction. Functional properties of food proteins are highly sensitive to the structure (secondary & tertiary structure and architecture) and aggregation state of the proteins1. While heat-treatments are the most common way of influencing the aggregation state of food proteins, there has also been considerable interest in using cross-linking enzymes. An understanding of the impact of cross-linking on functionality first of all requires a detailed characterization of the structure, architecture and thermal properties of the enzymatically cross-linked proteins. Here we provide such a characterization for the cross-linking of the tyrosine residues of apo-α-lactalbumin catalyzed by horse-radish peroxidase under the (stepwise) addition of peroxide.

Method. 10g/L apo-α-lactalbumin and 0.5g/L were mixed in 100mM NH4Ac at pH 7. Cross-linking was induced by heating to 37°C. Every 10 min, peroxide was added corresponding to a concentration change of 0.1mM.

Results. In the first stage of the reaction, the majority of the protein monomers are quickly incorporated into protein oligomers. In the next stage, remaining uncross-linked tyrosine residues on the protein oligomers are slowly cross-linked to give very large protein polymers or protein nanoparticles. AFM confirms that the particles consist of loosely coupled protein oligimers (see Fig. 1). CD shows that the dimerization via cross-linking leads to a nearly complete loss of secondary/tertiary structure. DSC shows an exothermic peak for the protein nanoparticles rather than the endothermic (unfolding) peak typically observed for native proteins. The protein nanoparticles do not aggregate under heating. Instead, prolonged heating leads to a slow decrease of their size. However, the untreated protein rapidly aggregates and precipitates in 1h below pH neutral pH. CD shows that structural transition on α-helix for protein particles are similar to the untreated proteins.

Concluding Remarks. Currently we are extending this approach to cover a wider range of protein/enzyme combinations, and by exploring the functionality of these well-characterized protein nano particles for example in foams and gels.