

Application of DLS in the Study of Dental Enamels

Our studies are aimed to advance the understanding of the fundamental mechanisms of molecular interactions involved in the formation of dental enamel. These include enamel protein proteolysis and analysis of their structure and function.

Enamel is the hardest, most highly mineralized tissue in the vertebrate body with an extraordinary microarchitecture of carbonated hydroxyapatite crystals. Enamel formation starts from the secretion of proteins by ameloblasts. Amelogenins, which are hydrophobic in nature, constitute more than 90% of the extracellular matrix during the early stage of enamel formation. During enamel maturation the crystals of the mineral phase (carbonated hydroxyapatite) grow rapidly and this process is concomitant with the massive degradation of the organic matrix. The self-assembly of amelogenin proteins, their stepwise degradation and their interaction with hydroxyapatite crystals have been recognized to be the key factors for controlling enamel biomineralization.

Dynamic light scattering instruments (DynaPro 99E-MS/X and other models) aided the investigation of aggregation/assembly properties of native, recombinant and engineered mouse and pig amelogenins. The acquisition time for each run was set at 10 seconds. The experiment continued for 60-100 runs which corresponded to 10-15 minutes.

The size distribution of amelogenin proteins varies under different conditions, depending on amino acid sequence, solution pH, protein concentration, and temperature. Using DLS we have detected and identified particles with hydrodynamic radii of 10-30nm that we termed as "nanospheres". We have used the DynaPro to analyze particle size distributions at lower protein concentrations and after short acquisition times in order to study the subunits prior to amelogenin nanospheres assembly.

In our recent report in *Science*, using TEM, AFM and DLS we have reported the chain assembly of the nanospheres up to particles of 100-200 nm RH. These chains further assemble to form elongated particles we termed as micro-ribbons. Another aspect of assembly that was studied by DLS related to the function of proteolytic activities in controlling amelogenin assembly and structure. We showed that cleavage of certain domains within the molecule results in the change of size particle size distribution and in some cases nanospheres dis-assembly. The outcomes of our studies will contribute insight into the biomineralization mechanisms in general and will provide scientific basis for the design and development of novel biomaterials.

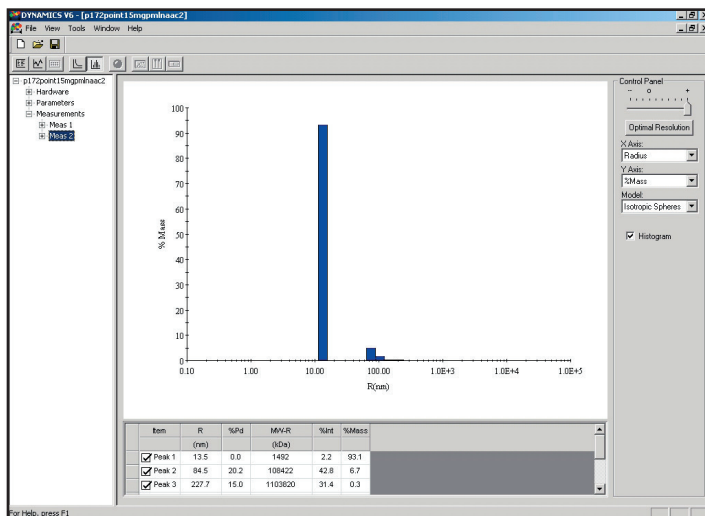


Figure 1. 0.15 mg/ml rP172 in acetate buffer (pH 4.5). Particles with hydrodynamic radii (RH) of 13.5 nm (defined as "nanospheres") and 84.5 nm were detected as major components based on mass distribution over a period of 10 minutes measurement.

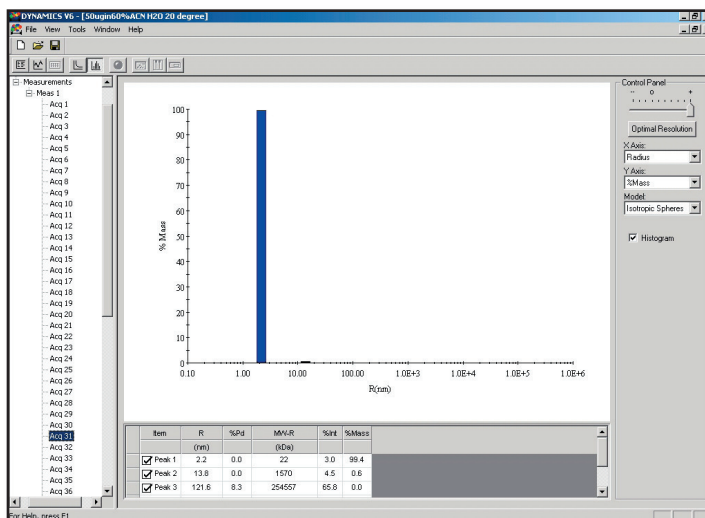


Figure 2. 0.05mg/ml rP172 in 60% aqueous acetonitrile. The smallest particle with RH of 2.2 nm corresponded to the rP172 monomer based on the molecular weight estimation was detected in an individual acquisition (10 seconds).

