Analysis of the Different Structures of Pulmonary Surfactant Collectin SP-D by Atomic Force Microscopy

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We have performed a structural and quantitative characterization of recombinant human pulmonary surfactant protein D (rhSP-D), a C-type (Ca2+-dependent) lectin belonging to the collectin family. It is found mainly in alveolar spaces, participating in the innate immune defense of the lungs. SP-D monomer contains four structural domains: an N-terminus domain, a collagen region, a α-helical coiled-coil neck and a C-terminus carbohydrate recognition domain (CRD). Monomers form trimers through folding of the collagenous region into triple helices and the assembly of a coiled-coil bundle of α-helices in the neck region. These trimers are stabilized by two disulfide bonds in the cysteine-rich N-terminal domain. Trimers associate into higher order oligomers whose size and conformation is sensitive to environmental factors and the conditions during purification and storage. Despite extensive studies carried out to characterize the oligomerization process of SP-D, the pathway and type of interactions involved in the formation of large oligomers, are not clearly understood.

In the current study, the protein produced in mammalian CHO cells, was analyzed by Atomic Force Microscopy (AFM) and electrophoresis. The goal has been the determination of the distribution of oligomeric forms, the exploration of the possible oligomerization pathway and the description of the conformational diversity of rhSP-D. AFM experiments revealed that rhSP-D is a mixture of trimers, hexamers, dodecamers, and larger oligomers ("fuzzy balls"), with the most abundant structure being the dodecamer under the conditions of these experiments. Same kind of structures were found in human SP-D, used as a control. Moreover, we have developed a cross-linking protocol to detect the presence of SP-D dodecameric forms by PAGE-SDS, in which dodecamer is only visualized after chemical crosslinking and in the presence of denaturing agents, indicating the importance of hydrophobic interactions in dodecamer formation.