The average β-sheet intensity of amyloid fibrils prepared under different conditions is studied by Fourier transform infrared (FT-IR) and sum-frequency generation (SFG) spectroscopy and varies between about 40 and 90%. Tip-enhanced Raman spectroscopy (TERS) is used to determine with nanometer resolution the structure and amino acid content of single fibrils, which shows that the surface of the fibrils is heterogeneous in terms of its molecular structure. Bending dynamics of single, fluctuating fibrils enable us to correlate the secondary structure of fibrils to their morphology and mechanical properties. Together, our results show that although the fibrils are composed of the same peptides, they are highly polymorphic and their shape and mechanical rigidity is strongly correlated with the underlying molecular structure.

2844-Plat

Distinct Quaternary Structures of Lon Protease Control Substrate Degradation

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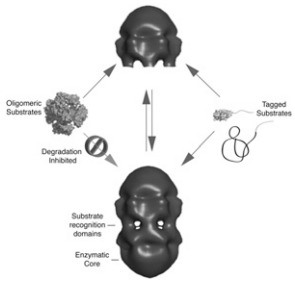
The Lon protease controls cell division in response to stress and is the major protease that degrades misfolded and damaged proteins. Previous studies indicated that Lon is a ring-shaped homo-hexamer. using biophysical techniques we now demonstrate that E. coli Lon also exists as larger oligomers at physiological protein concentrations. The structure of these large complexes, reconstructed by electron microscopy, reveals that Lon assembles into a dodecamer in which the protease cores are on the distal ends and the two hexamers are linked by a matrix formed by the N domains. Importantly, the Lon hexamer is ~10-fold faster ATPase and is ~20-fold more active in degrading some classes of substrates than is the dodecamer. This difference in degradation rate is not observed with small degron tagged substrates. We propose a model in which the Lon dodecamer uses a gating mechanism to exclude some classes of proteins allowing the enzyme's substrate profile to be altered by its assembly state.



2845-Plat

Sum of the Parts: Bacterial Biofilms by Solid-State NMR

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Bacteria harbor dedicated machinery to assemble amyloid fibers at their cell surface. Curli, produced by E. coli, are among the most well-studied bacterial amyloids and play a significant role in adhesion and in the assembly of bacterial biofilms. Curli interact with secreted polysaccharides to form a biofilm and play a crucial role in determining biofilm morphology, yet the exact composition of curli-integrated biofilms is poorly understood. The determination of biofilm composition by routine methods, such as amino acid hydrolysis and mass spectrometry, is complicated by incomplete dissolution, as well as the perturbative nature of degradative and enzymatic changes. Solid-state NMR can provide valuable information about intact biofilms and is uniquely suited to provide a quantitative perspective on biofilm composition and structure.

To dissect the contributions and interactions of curli and polysaccharides in the biofilm framework, we have purified and obtained 13C CPMAS spectra of: (i) the wild-type UTI89 extracellular matrix (ECM); (ii) the curli-free ECM produced by the curli mutant strain UTI89ΔcsgA; and (iii) purified curli. These spectra indicate that the UTI89 ECM has two major components, curli and cellulose, each in a quantifiable amount. The curli and polysaccharide regions of the spectrum also harbor unique chemical-shift contributions that allow us to examine curli-polysaccharide interactions and the modes of action of biofilm inhibitors. Our data define quantitatively the composition of the intact extracellular matrix, including cellulose and amyloid fibers, which impacts bacterial physiology. We are also working to transform cartoon representations of the curli amyloid fiber into a molecular model using selective labeling and REDOR NMR.