Characterization of the substrate recognition and subunit assembly properties of homo-oligomeric human CCT subunits of TRiC

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Numerous proteins, including essential proteins such as tubulin and actin, are unable to fold to their native state in the cell without assistance from chaperones. Chaperonins are a family of chaperones that encapsulate their substrates and assist their folding in an ATP-dependent manner. The eukaryotic chaperonin, TCP-1 Ring Complex (TRiC), a hetero-oligomeric complex composed of eight different Chaperonin Containing TCP-1 (CCT) subunits has been well-characterized by Frydman and colleagues. Each CCT subunit may have distinct substrate recognition and ATP-binding properties. Additionally, mutations in CCT4 (C450Y) and CCT5 (H147R) have been identified as causing hereditary sensory neuropathies in a stock of Sprague-Dawley rats and in a Moroccan family, respectively. Our aim was to express each human CCT subunit individually in *E. coli* to investigate whether they form chaperonin-like double rings complexes. This gives us the opportunity to study the specificity and redundancy of each CCT subunit in a chaperonin context.

CCT4 and CCT5, but not the other six CCT subunits, formed high molecular weight complexes within the *E.coli* cells that sedimented about 20S in sucrose gradients. When CCT4 and CCT5 were purified, they were both organized as two back-to-back rings of eight subunits each, as seen by negative stain and cryo-electron microscopy. This morphology is consistent with that of the hetero-oligomeric double-ring TRiC purified from bovine testes and HeLa cells. Both CCT4 and CCT5 homo-oligomers hydrolyzed ATP at a rate similar to human TRiC, and were active as assayed by luciferase refolding and human γD-crystallin aggregation suppression and refolding. Thus both CCT4 and CCT5 homo-oligomers have the property of forming eight-fold double rings absent the other subunits, and these complexes carry out chaperonin reactions without other partner subunits. Using these homo-oligomeric chaperonins and human TRiC as a control, we are studying the recognition and refolding of more stringent substrates such as mutant huntingtin, tubulin, and actin. We are also studying the structure and function of the neuropathy mutations of CCT4 and CCT5 by purifying these mutants and assaying their structure and function. To understand the regulation of TRiC assembly, we are assaying the formation of hetero-oligomeric rings with all eight CCT subunits and either CCT4 or CCT5.