NMR. We developed a strategy to generate acceptable NMR spectra of ill-behaved E7 and assigned 85 – 95% of its backbone $^{1}H/^{15}N/^{13}C$ spectra. The trends in ^{13}C chemical shifts locate β-strands and α-helix in C-terminal portion of E7. The poorly structured region is likely to confer much of the high affinity for pRb. (American Cancer Society)

625.4

Effect of C-terminal truncation on the molecular chaperone function of Escherichia coli trigger factor

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Trigger Factor (TF) is an important catalyst of nascent peptide folding and possesses both peptidyl-prolyl cis-trans isomerase (PPIase) and chaperone activities. TF has a modular structure, containing three domains with distinct structural and functional properties. The N-terminal domain of TF is important for ribosome binding and the M domain carries the PPIase activity. However, the function of the C-terminal domain remains unclear and the residues or regions directly involved in substrate binding have not yet been identified. To examine the chaperone function of TF and its relationship with the C-terminal domain, a number of C-terminal truncated mutants were constructed, namely: TF419, TF389, TF380, TF360 and TF344, in which the C-terminal 13, 43, 52, 72 and 88 residues were deleted respectively. Co-expressions of adenylate kinase (AK) with TF and the C-terminal truncated mutants were achieved using a plasmid pBVAT that allowed expression of TF and AK in the same plasmid under separate control. Results show that the C-terminal truncated TF mutants express about the same ability in assisting AK refolding in the co-expression system and all of the C-terminal truncated TF mutants can still bind with ribosome, indicating that the C domain of trigger factor may not be essentially important for the in vivo molecular chaperone function of TF. However, the purified C-terminal truncated TF mutants reduced dramatically the ability in assisting GAPDH refolding in vitro and almost not be able to form dimer, showing that the C-terminal of TF is necessary in in vitro molecular function and is important to form dimer.

625.5

Detection of structural changes of alpha-crystallin upon binding of metals.

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Alpha-crystallin, one of the most abundant proteins in the lens is known to be a member of the heat shock family and a molecular chaperone. This protein prevents the aggregation of other lens proteins which can occur under stressful conditions and may be a factor in cataractogenesis, a clouding of the lens. Also, α-crystallin has been shown to assist in the *in vitro* folding of other polypeptides. Many molecular chaperones require cofactors and none has been clearly identified for α-crystallin. We used the detection of conformational changes of α-crystallin upon incubation with several metals as a means for the identification of possible cofactors for this protein. Interestingly, we found that calcium significantly altered the conformation of α-crystallin as detected with tryptophan fluorescence spectroscopy. Furthermore, studies using the fluorescent probe, bisANS, indicated that these structural changes led to an increased exposure of hydrophobic surfaces on α-crystallin. Finally, as the concentration of the metal was increased to levels such as those found in cataracts, the protein was shown to aggregate as monitored by light scattering as well as thioflavinT spectroscopy.

625.6

The expression, isolation and analysis of Staphylococcus aureus ClpB

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ClpB is a member of the highly conserved Clp family of heat shock proteins that has been shown to be essential for thermotolerance in bacteria and eukaryotes. Although mutation analysis has been conducted on the *S. aureus clpB* gene, the protein has not been studied in great detail. Heterologous expression and purification of ClpB, and other heat shock proteins DnaK, DnaJ and GrpE from *S. aureus* has been performed via the addition of a 6X-histidine tags to allow separation and purification of the staphylococcal proteins from those of *E.coli*. DnaK, DnaJ and GrpE modulate the activity of ClpB and together, have been found to be essential for the suppression and reversal of protein aggregates in *E. coli*. Because it has been demonstrated in several prokaryotic systems that the DnaJ/DnaK/GrpE proteins from one species will not support ClpB activity from another, we are examining how these *S. aureus* proteins modulate the function and biochemical activities of staphylococcal ClpB. As is common to several bacterial *clp*B genes, staphylococcal ClpB protein expression results in the dual translation of two proteins of differing size (a full length product and one of lower molecular weight), and future experiments will investigate the functional significance of these forms, if any, and their interactions with other heat shock proteins.

625.7

Effects of nucleotides on the structure of alpha-crystallin, using fluorescence spectroscopy.

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Alpha-crystallin, a major protein found in the eye lens has been shown to be a member of the small heat-shock proteins and a molecular chaperone. Most molecular chaperones require for their function the binding of a nucleotide, such as ATP. However, no nucleotide(s) have yet been conclusively identified to bind α-crystallin. Therefore, we have investigated the possible nucleotide binding to α-crystallin by determining the intrinsic fluorescence of the protein while in the presence of varying concentrations of several different nucleotides. Screening of the nucleotides was performed at 25°C as well as 37°C. Of the nucleotides screened, most showed no significant changes of the proteins' fluorescence. However, fluorescence spectroscopy does seem to indicate that the nucleotides GTP and GDP interact with α-crystallin. Titration of α-crystallin with increasing amounts of GTP showed a proportional quenching of the fluorescence intensity with no significant shift of the maxima wavelength. Binding by GTP was further supported by the use of the fluorescent analog TNP-GTP whose fluorescence intensity and wavelength maxima were significantly changed upon binding to the protein.

625.8

Characterization of hsp90-binding to the the PASB domain of the Ah receptor

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The Ah receptor (AhR) is a ligand-dependent transcription factor, which regulates the biochemical and toxic effects of structurally diverse chemicals. Hsp90, one subunit of the AhR complex, appears to direct proper folding and maintenance of the high affinity ligand binding conformation of the AhR in some species. Using a structural homology model we developed for the ligand- and hsp90-binding PASB domain of the AhR to guide our mutagenesis, we have examined the physical interactions of hsp90 with the AhR and subsequent effects on AhR functionality (hsp90, ligand and DNA binding and transcriptional activation). Deletion of the PASB domain resulted in the complete loss of hsp90-binding and constitutive ligand-independent activation of DNA-binding and reporter gene transcription, suggesting that documented interactions of hsp90 with the AhR bHLH domain are insufficient on their own for hsp90 binding. Deletions within the AhR PASB domain resulted in intermediate levels of hsp90 binding and revealed that the bulk of the central PASB 5 strand β-sheet is required for the optimal hsp90-binding. PASB deletions that eliminated amino acids 339-362 resulted in ligand-independent transformation and DNA-binding, similar to full PASB deletion. Based on the AhR PASB model, these results indicate that two β-strands (Gβ and Hβ) along with the interconnecting flexible loop are essential for ligand-induced activation of AhR transformation possibly by weakening of the hsp90-AhR interactions within this region. (NIH ES07685)