peptide-to-lipid molar ratios (P/L) of 1:200 or above, the ^{19}F dipolar coupling of all labeled positions exhibits the maximum possible value of +16 kHz, indicating that at high concentration KIGAKI self-assembles into immobilized β-sheets, which lie flat on the membrane surface, which is also supported by CD and OCD spectra. Transmission electron microscopy images reveal that the aggregated KIGAKI forms amyloid-like fibrils, with less propensity for the CF_3-D-Bpg labeled peptides to aggregate, compared with the CF_3-L-Bpg labeled peptides. This aggregation difference is also reflected in the biological activity of the differently labeled peptides. At low peptide concentrations, on the other hand, all labeled positions show dipolar couplings of +8 kHz, indicating that their time-averaged alignment is still parallel to the bilayer normal, but the mobility of the peptides increases drastically, with monomeric peptides being the most likely state. Thus, flexible β-strands float on the membrane surface and undergo motional averaging in the membrane plane, similar to intrinsically unstructured proteins in solution. This is the first example of a concentration dependent transition of a flexible β-strand to an amyloid-like fibril on membrane surfaces that has been directly observed by solid state NMR.

Protein Folding & Stability I

2031-Pos Board B50

Conversion of the Sulfhydryl Oxidase Augmenter of Liver Regeneration into a Selenoprotein

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Augmenter of liver regeneration (ALR) is a flavin-dependent sulfhydryl oxidase with roles in mitochondrial oxidative protein folding and cellular signaling. To study ALR's reaction mechanism we have prepared a form of the enzyme in which sulfur was replaced with selenium. The selenium-rich ALR is catalytically active, thermally stable, and its structure is almost identical to that of the native ALR. The presence of selenium in the active site leads to the formation of a charge-transfer complex during turnover, as detected by visible spectroscopy. To further demonstrate the role selenium plays in ALR's active site, we have utilized E. coli's selenium insertion machinery to introduce selenium in a site-specific manner. using this method we are able to convert ALR's redox active CxxC motif to a selenocysteine containing CxxU motif. Our results demonstrate that the selenocysteine proximal to the FAD cofactor is sufficient to cause a charge-transfer complex during turnover. In addition, 77Se NMR spectroscopy was used to probe locations typically occupied by sulfur - an insensitive nucleus that is not amendable for NMR studies of proteins. Biological 77Se NMR has so far been underutilized due to the challenges of isotopically enriching protein with 77Se. Here, we have developed a method to introduce 77Se by heterologous expression in E. coli. We report the NMR spectra of ALR bound to oxidized and reduced FAD. An unidentified resonance appears only in the presence of the reducing agent and disappears readily upon exposure to air and subsequent reoxidation of the flavin. Hence, 77Se NMR spectroscopy can be used to directly probe the chemical environment surrounding the sulfur/selenium sites as a function of their redox state.

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Comparative Characterization of Apo-, Reconstituted- and In Vivo-Folded forms of a Durum Wheat Metallothionein

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Durum wheat metallothionein (dMT), a plant type 1 metallothionein, with a long ''hinge'' region between metal coordinating cysteine clusters, is efficient cadmium (Cd) chelator. In this work, biophysical features of purified recombinant holo-dMT, its demetallated form (apo-dMT) and the reconstituted Cd_5-dMT are compared to obtain insight into the structure and metal binding features of this protein. Results show that the purified holo-dMT is polydisperse and has 5.3 ± 0.5 Cd^{2+} ions per molecule. Demetallation followed by size exclusion chromatography yields homogeneous apo-dMT which can be reconstituted with Cd^{2+}. Synchrotron small angle X-ray scattering (SAXS) demonstrates that apo-dMT, at pH 2.0, is flexible and extended in solution. According to UV-vis, CD and native-PAGE data conformation of apo-dMT is sensitive to pH changes in the range 2.0 to 8.0. Reconstitution of the apo-protein at pH 8.0, with Cd^{2+} appears to take place in two phases during which first the monomer is folded to accommodate 5 Cd^{2+} ions and then reorganization into oligomeric forms allows incorporation of further metal ions. SAXS data indicate that holo-dMT has limited flexibility in structure, but its conformation is significantly more compact than that of apo-dMT. Results of UV-vis and circular dichroism spectroscopy show that the in vitro folded protein is structurally different from the purified holo-dMT with the same number of Cd^{2+} ions.

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N-Propanol Based Solubilization Buffer Enhances Refolding Yield of Inclusion Body Protein by Populating Intermediates to the Folding Pathway

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Most of the times, high level expression of recombinant proteins in bacteria results in accumulation of recombinant proteins into inclusion body (IB) aggregates. To obtain native protein from these aggregates, it is necessary to solubilize these aggregates followed by refolding of solubilized protein by appropriate refolding method. Conventionally, high concentration of denaturant like urea or guanidinium chloride (GdmCl) is used for solubilization of inclusion bodies which often results into aggregation of protein during refolding process. In the present study we have evaluated a novel solubilization method using n-propanol in presence of low concentration of urea. n-Propanol based solubilization agent was compared with traditional solubilization agents like 8 M urea and 6 M GdmCl for solubilization efficiency, structure and stability of the solubilized model protein, recombinant human growth hormone (hGH). hGH IBs were found to be tough and were only solubilized efficiently in presence of high concentration of denaturants (8 M urea or 6 M GdmCl). 4 to 6 M n-propanol in combination with 2 M urea was sufficient for the efficient solubilization of hGH IBs. Aggregation during refolding was also studied and it was found that solubilization with n-propanol based buffer resulted into bioactive hGH without aggregation giving significantly higher refolding yield in comparison to those obtained with urea and GdmCl based buffers which resulted in aggregation of hGH during refolding. From the results obtained, it can be concluded that solubilization of hGH IBs in n-propanol based buffer results in a partially folded folding intermediate of hGH which readily folds into native form on dilution with reduced chances of protein getting into aggregation pathway.

2034-Pos Board B53

Structural Stability of Tandem Calponin-Homology (CH) Domains Originates from their C-Terminal CH2 Domain

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Tandem CH domains form a major class of actin binding domains. In general, the N-terminal CH domain (CH1) weakly binds to F-actin whereas the C-terminal CH domain (CH2) does not bind to actin. However, when CH2 is linked to CH1, the actin binding efficiency increases by more than ten times, which implies a functional cooperativity between the two CH domains. The structural cooperativity underlying this functional cooperativity and the physical mechanism by which CH2 domain enhances the actin-binding efficiency is not understood. In this study, we examined the relative stabilities of the two CH domains of utrophin and dystrophin. The isolated CH1 domain of utrophin does not exist as a stable structure; it is more like a destabilized ''molten globule'' state. However, its CH2 domain folds to a stable structure, as evident from its alpha-helical spectrum and cooperative melt. Similar to utrophin, the CH2 domain of dystrophin is a well-structured protein, and has similar stability as that of the full-length tandem CH domain. The CH1 domain of dystrophin is quite unstable and aggregates severely. These results indicate that the CH1 domain requires CH2 for its folding, or in other words, CH2 acts as a template for CH1 folding. These stability experiments support the earlier hypothesis proposed based on cryo-EM studies that the major role of CH2 might be to stabilize the tandem CH domain. Additional support comes from the literature: no molecular structures are available for the CH1 domains alone, suggesting that they might be unstable in the absence of CH2. In contrast, structures have been determined for numerous CH2 domains. These experimental observations indicate that the CH2 domain enhances the actin-binding function by imparting structural stability to the tandem CH domains.

2035-Pos Board B54

Calcium-Induced Folding and Secretion of Alkaline Protease (Apra) from Pseudomonas Aeruginosa

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Alkaline protease (AP) is a known virulence factor secreted from Pseudomonas aeruginosa (Pa), which causes serious infection in patients with cystic