mechanism behind its increased amyloidogenicity: an expanded tetramer which is stabilized by a lower number of H-bonds and hydrophobic interactions. Interestingly, in the presence of T4 and lumiracoxib the structure of A25T was similar to that displayed by the wt protein. These data show that an expanded A25T tetramer with a decreased thermodynamic stability is prone to aggregate forming amyloid fibrils that trigger leptomeningeal amyloidosis. Support: CNPq and FAPERJ.

163-Pos

Thermal Stability of the Extracellular Hemoglobin of *glossoscolex Paulistus*: Differential Scanning Calorimetry (dsc) and Circular Dichroism (cd) Studies

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Hemoglobin of *Glossoscolex paulistus* (HbGp) in the oxy- and cyanomet-forms was studied by circular dichroism (CD) and differential scanning calorimetry (DSC). DSC experiments were performed for protein concentration 0.5 mg/ml and scan rate of 1°C/min, at pH 7.0. CD experiments were performed in the near UV to monitor the peptide region (0.2 mg/ml of protein, 195-250 nm) as well as in the Soret band spectral region (3.0 mg/ml protein, 500-250 nm) to monitor changes in the heme group envinronment, in the pH range 5.0-9.0. Experiments were made in the range 25-70°C. Analysis of CD data, based on a two-state thermodynamic denaturation model, allowed to obtain the fraction of denatured protein, critical temperatures as a function of pH, equilibrium constants and corresponding free energies. Cyanomet-HbGp (Tm=65°C at pH 7.0) is significantly more stable as compared to the oxy-form (Tm=59°C). Our CD data suggests that the protein denaturates as a whole, loosing its secondary structure simultaneously for all domains of the oligomer. Critical temperatures are smaller as the pH increases in the alkaline range. On the other hand, DSC results suggest that the denaturation for oxy-HbGp is more complex, presenting low cooperativity since the endotherm could be fitted only for two components centered at 58.3 $\pm$ 0.2 and 60.6 $\pm$ 0.1°C. For the cyanomet-form the best fit for the endotherm corresponds to three components centered at 61.6 $\pm$ 0.2, 64.8 $\pm$ 0.2, and 67.2 $\pm$ 0.2°C. DSC data, in agreement with CD, also support the higher thermal stability of cyanomet-HbGp as compared to the oxy-form. Support: FAPESP, CNPq and CAPES Brazilian agencies.

164-Pos

On the Thermal Stability of Extracellular Hemoglobin of *glossoscolex Paulistus*: Optical Spectroscopic Studies

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Hemoglobin of *Glossoscolex paulistus* (HbGp) in the oxy- and cyanomet-forms was studied by dynamic light scattering (DLS) and optical absorption spectroscopy (OAS). At 25°C, oxy-HbGp, in the pH range 5.0-8.0, is stable presenting a mono-disperse size distribution with hydrodynamic diameter (D_h) of 27 $\pm$ 1 nm. Cyanomet-HbGp behaves in a similar way up to pH 9.0. More alkaline pH, above 9.0, induced an irreversible dissociation process, resulting in smaller D_h of 10 $\pm$ 1 nm, suggesting oligomeric dissociation. At pH 7.0, no oligomeric dissociation is observed as a function of temperature and denaturation occurs at 52°C and 57°C, respectively, for oxy- and cyanomet-HbGp. Dissociation temperatures were lower at higher pH, for both forms of HbGp. Based on the higher critical denaturation and dissociation temperatures cyanomet-HbGp is more stable than the oxy- form. Kinetic studies were performed for oxy-HbGp using UV-VIS OAS and DLS. Rate constants as a function of temperature and the activation energy (E_a) have been estimated by DLS for oxy-HbGp at pH 7.5 and 8.0, giving E_a values of 278 and 262 kJ/mol, respectively. Auto-oxidation kinetics monitored by UV-VIS at pH 8.0 in the temperature range 38-44°C is mono-exponential with an E_a value of 333 kJ/mol. Oligomeric protein dissociation promotes an increase in auto-oxidation rate and vice-versa. The present work shows that DLS is suitable to follow quantitatively the changes on the oligomerization of multisubunit proteins. Support: FAPESP, CNPq and CAPES Brazilian and FCT-MCTES Portuguese agencies.

165-Pos

Geometry and Efficacy of Trp-Trp, Trp-Tyr and Tyr-Tyr Aromatic Interaction in Cross-Strand Positions of a Designed β-Hairpin

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Analysis of the impact of neighboring aromatic groups on structure can lead to improved understanding of protein folding mechanisms and stability. In this study, we examined the impact of varying aromatic interactions in cross-strand positions for Trpzip2, a β-hairpin forming peptide (Cochran et al., PNAS, 2001, 98, 5578-5583), by comparison of the interactions of Trp-Trp, Trp-Tyr and Tyr-Tyr. NMR and optical spectra (ECD, FTIR) of the original TZ2 peptide and its Tyr and Val-substituted mutants were analyzed to characterize their conformations and thermal stability. Cross-strand coupled Trp-Trp and Trp-Tyr pairs show unique, strong exciton bands in ECD while the Tyr-Tyr pair doesn't show any clear exciton band. The edge-to-face cross strand interaction leads to stable β-hairpin structures when Trps are at positions 2-11 or 4-9, but the alternate coupling for Trp at positions 2-9 does not lead to a stable structure. In Trpzip2 these would correspond to a more face-to-face interaction, which may contribute to the instability. When Tyr is substituted for Trp, the Trp-Trp interaction has more contribution to the peptide stabilization than does the Tyr-Tyr pair. When Tyr is substituted into position 4 and 11, the Trp-Tyr pair also has a unique geometry. These aromatic-aromatic interactions were also compared to simple hydrophobic interaction by contrasting stabilities of peptides with Val or Tyr substituted for two interacting Trp residues. Tyr is more stabilizing than Val for such substitutions which may indicate coupling of conjugated π-electron systems dominates stability. Aromatic interactions showed a stronger effect than hydrophobic interaction for stabilization. Extended kinetic studies using laser initiated T-jumps and IR detected conformational changes have helped sort out mechanistic aspects of this folding problem (Hauser et al, JACS, 2008, 130, 2984-2992).

166-Pos

Effects of Mutations on Side-Specific Folding Mechanism of a Helix-Turn-Helix Protein

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Helix-turn-helix motifs are important super-secondary protein structural elements and excellent models for studying the mechanism of protein folding. We have been investigating folding of a *de novo* designed 38-residue helix-turn-helix motif α-t-α using IR spectroscopy with site-specific ^{13}C isotopic editing. Our preliminary site-specific thermal unfolding data revealed that α-t-α is most stable near the centers of both α-helices, and likely unfolds from the helical termini and the loose turn region. To obtain further insights into the folding mechanism, and to investigate the roles of the individual residue-residue stabilizing interactions, we have begun mutational studies of the α-t-α protein. The mutations were designed to both destabilize and further stabilize the hydrophobic core near the helical centers. Additional mutations were designed to stabilize the helical termini and the turn/loop sequence. The overall thermodynamic stability of the α-t-α was measured using CD and IR spectroscopies. The core mutations appreciably decreased or increased the overall folding stability as intended, however, stabilizing the turn and helical termini proved to be a rather challenging task. Site-specific thermal unfolding of the mutated α-t-α were probed with IR on multiple ^{13}C isotopically labeled variants of each mutant. The effects of the mutations on both the global and, in particular, local site-specific unfolding provide important clues about the stabilization of the helix-turn-helix motif by specific interactions. Although additional mutational studies are underway, thus far all the data are consistent with the proposed folding mechanism.

167-Pos

Investigating Conformational Ensembles in Alanine Based Peptides Using Vibrational and Ecd Spectroscopy

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Short alanine based peptides are of interest to the protein community, due, in part to their departure from the statistical coil model. These peptides are too small to assume major secondary structures, but rather, have been found to adopt an ensemble of conformations in aqueous solution, with a predominance of PPII. However, experimental evidence suggests that the presence of charged residues might induce the sampling of multiple turn conformations, thus leading to a more compact structure of the peptide. To check this further, we measured the amide I profiles of the FTIR, Raman and VCD spectrum of H-(AAKAAW)-OH, and subsequently simulated the vibrational spectra using an excitonic coupling model, with NMR coupling constant and end-to-end distance constraints. We included multiple conformations: PPII, ß-strand, $_R$ helix, $_L$ helix, and turns. The alanine residues experienced a high propensity for PPII structure, ~70%, while ~20% for ß-strand conformations and smaller percentages for other coil structures. Lysine, however showed a larger propensity for ß-strand ~30% than the alanine residues, but the PPII content for lysine is still high (~42%). We obtained an end-to-end distance of 10Å, which is in accordance with FRET measurements of the end to end distance of

H-Dbo-AAKAAW)-OH, (Dbo: 2,3-diazabicyclo[2.2.2]oct-2-ene-labeled asparagine). This distance is indicative of a rather compact peptide sampling many different coil structures, including a high PPII content, as well as turn structures. The charge lysine residue results in more turn structures being sampled by the succeeding alanine residue. UV circular dichroism (UV-CD) spectra of H-(AAKAAW)-OH and H-(AAAAAW)-OH indicate a higher PPII content for the latter peptide. These data show that the incorporation of lysine yields indeed a more compact conformation.

168-Pos

A Comprehensive Approach To Protein Thermal Stabilization

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Engineering proteins for higher thermal stability is an important and difficult challenge. We describe a comprehensive and multidisciplinary approach incorporating various individual methods to redesign proteins to be more stable. First, we identified mutations for thermal stabilization of our model, adenylate kinase by applying a variety of experimental and computational techniques individually and separately. The used techniques include X-ray crystallography, molecular dynamics simulation, domain-swapping and structural bioinformatics. Then, we designed variants by combining the individual stabilizing mutations together. The resulting variants have mutations for extra electrostatic interactions by newly added ion pairs, additional hydrophobic interaction and/or optimized local structural entropy. In the experiment using differential scanning calorimetry, the redesigned proteins displayed considerable increases in their thermal stabilities. Our results highlight the importance of a comprehensive approach in protein thermal stabilization.

169-Pos

Triple-Helix Folding Around Interruptions in the Collagen Repeating Sequence

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The collagen triple-helix consists of three supercoiled chains, each with a polyproline II-like conformation. This structure requires Gly at every third residue, and fibrillar collagens are composed of perfect $(Gly\text{-}Xaa\text{-}Yaa)_n$ repeats throughout their length. Non-fibrillar collagens, such as network-forming type IV collagen, contain interruptions in the repeated sequence that range in length from 0 to 60 residues. Folding of all collagens begins with trimerization of a terminal globular domain and is followed by propagation of the triple helix in a zipper-like manner. The effect of interruptions on this folding process is not well understood, but some delay is expected since type IV collagen, with more than 20 interruptions, folds more slowly than type I collagen, which contains no interruptions. The bacterial collagen Scl2 from *Streptococcus pyogenes* consists of a globular trimerization domain and a triple helix domain with 79 Gly-Xaa-Yaa repeats, and is an ideal system for investigating the effect of interruptions on triple helix formation. Scl2 can be expressed in *Escherichia coli*, allowing for insertion of interruptions with various lengths and sequences. Currently, we have expressed a control construct containing two collagen-like domains in tandem, and a construct with a 4-residue interruption, Ala-Ala-Val-Met, between the two collagen-like domains. The kinetics of triple-helix folding, as monitored by temperature jump experiments using circular dichroism spectroscopy and trypsin digestion/SDS-PAGE, show similar third order reaction rates for both the control protein and the protein containing the 4-residue interruption. Constructs containing larger interruptions are being expressed for future experiments. If folding is stalled at such sites, the process of renucleation around the interruptions will be characterized. In addition, fluorescence anisotropy will be used to study kinetics on a local scale and rotary shadowing electron microscopy will be used to examine single molecules.

170-Pos

Contributions of Aromatic Residues to the Folding and Stability of Human γd-Crystallin

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Human γD-crystallin (HγD-Crys), one of the most abundant proteins in the eye lens, exhibits two crystallin domains, each containing two Greek key motifs. HγD-Crys must remain folded and soluble throughout the human lifetime. Aggregation of crystallins leads to cataract.
14 tyrosines and 6 phenylalanines reside in the 173-amino-acids HγD-Crys. 16 out of these 20 residues have aromatic partners within ~4 Å. ''Tyrosine corner'' is a structural element that bridges β-strands by hydrogen bonding the tyrosine hydroxyl group and a backbone carboxyl group. Also found are interacting tyrosine/phenylalanine pairs located at the β-hairpins. These aromatic structural elements may be important in the stability and/or the folding pathways.
Site-specific mutants of the all the tyrosines and phenylalanines to alanines were constructed. Equilibrium and kinetic experiments were performed to assess stability and unfolding/refolding rates.
For stability, all the N-terminal domain (N-td) mutants had the N-td destabilized, but C-td unaffected, with increased population of the single-domain-folded intermediate, although the extents of destabilization were different. All the C-td mutants had both the N-td and C-td destabilized, showing a more cooperative folding process that was best fit to a two-state model, and similarly, the degrees of destabilization varied. Selected tyrosines were mutated to phenylalanines with very little effect on the N-td or the C-td stabilities. For kinetics, C-td mutants had accelerated unfolding rates, while N-td mutants had no effect. Y45A, Y50A Y133A, Y138A had slower refolding rates, while other mutants had no effects.
The results were consistent with a sequential unfolding pathway, in which the N-td of HγD-Crys unfolds first, followed by the C-td. The aromatic residues were almost all important in the mature stability of HγD-Crys, while a subset of these aromatic residues were important determinants of unfolding/refolding rate.

171-Pos

Role of Proline in the Folding of Conotoxins

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Evaluation of the role of disulfide bridges plays an important part in understanding the concept of protein folding. We are investigating how slight changes - the presence *vs.* the absence of the cyclic amino acid proline in a certain position of the peptide-chain - in the sequence of small peptides influence their folding properties. The present studies focus on the folding of a group of small peptides found in *Conus* snails, α-conotoxins SI, SIA (found in *Conus Striatus*), and GI (*Conus Geographus*), under two different oxidizing conditions. Each peptide has two disulfide bridges leading to three possible regioisomers, only one of which is found in nature. Our results indicate that peptides containing the cyclic amino acid proline had very high selectivity for the natural isomer, suggesting that this amino acid enforces a structural rigidity on the peptides.
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172-Pos

Investigation of W121 on the Conformation and Functional Properties of the Human Acidic Fibroblast Growth Factor-1

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Fibroblast Growth Factor-1 (FGF-1) is a 16kDa heparin binding protein, which has been associated with a variety of important functions including angiogenesis and wound repair. In order for FGF-1 to enter the cell it must interact with the FGF-1 receptor on the cell surface. One vital residue involved in the binding of FGF-1 to the receptor is tryptophan 121 (W121). This study aims to examine the role of W121 on the conformation and functionality of FGF-1. Site-directed mutagenesis will be used to incorporate mutations at position 121. The effect of these mutations will be characterized using various biophysical techniques including fluorescence, CD, ITC, and mutli-dimesional NMR spectroscopy. As FGFs are involved in many crucial cellular processes, the gain from this study is expected to provide useful information on the regulation of the FGF signaling process.

173-Pos

Characterization of the Minimalistic Fgf-D2 Domain Interface

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Fibroblast growth factors (FGFs) are heparin binding proteins that help regulate key cellular processes such as wound healing and differentiation, cell proliferation, cell migration, morphogenesis, and angiogenesis. FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR; heparin is believed to play a major role in this interaction. The role of heparin on the FGF-D2 complex was determined by using sucrose octasulfate, an analogue of heparin, role in the FGF-D2 complex. In this study we have determined the three dimensional structure of the minimalistic FGF-D2 domain interface using a variety of biophysical techniques including multidimensional NMR spectroscopy. The SOS and FGF binding sites on D2 have been mapped using 1H-15N HSQC. The three dimensional structure of the minimalistic FGF signaling complex, has been validated by site-directed mutagenesis studies. The results of this study provide valuable information towards a rational design of therapeutic principles against FGF-induced pathogenesis.