prevention of migraines. In physiological conditions, CGRP is intrinsically disordered and, therefore, the binding to its receptor (or to drugs) will depend strongly on the structural and dynamical properties of the disordered unbound state. Such properties can be affected in vivo by changes in salt concentration and pH. However, while some information is available on CGRP's sampling of local secondary structural elements, very little is known about its long-range (''tertiary'') structural and dynamical properties. Detecting such properties is challenging because CGRP has a low molecular weight and samples many different conformations on very fast time scales. We use a nanosecond laser-pump spectroscopy technique, based on tryptophan triplet quenching, which allows probing the end-to-end distance and the rate of end-to-end contact formation in IDPs. This provides similar information to FRET, but without the use of prosthetic dyes. Our data show that CGRP populates compact states in buffer, which are extremely sensitive to pH and salt concentration. We find that a change from pH 8 to pH 3 can induce a significant expansion of conformations due to the modulation of charge interactions, with a dramatic change of the corresponding salt screening effects. This suggests a key role of specific charged residues in CGRP. In addition, we find a ''denaturant expansion'' effect that depends on the nature of the denaturant. The observations can be rationalized in terms of polymer models where the polyelectrolyte/polyampholyte nature of the peptide is taken into account.

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Gelation of Highly Cationic Alanine Based Peptide in Water in Absence of Charge Screening Anions

Jodi Kraus, Reinhard Schweitzer-Stenner.
Chemistry, Drexel University, Philadelphia, PA, USA.

Peptide aggregation and self-assembly is of interest due to the possible link to many neurodegenerative diseases, as well as possible biotechnological applications. Surprisingly, the alanine-based peptide, (AAKA)4, (''AK-16'') has been previously shown to aggregate and form a hydrogel in the presence of a sufficiently high concentration of anions, although it may seem intuitive that the positive lysine residues would prevent aggregation. Here, we report the delayed self-assembly of pre-aggregated AK-16 into a hydrogel in the absence of neutralizing anions. Self assembly kinetics was probed by both IR and vibrational circular dichroism. At low concentrations (<15mg/ml), the peptide initially forms β-sheet rich structures, which decays over a 5 day period, as evidenced by a decrease in the intensity of the amide I' band at 1616cm-1. This decay likely indicates the formation of more disordered structures or amorphous aggregates. At higher concentrations, the β-sheet content remains stable over a much longer time scale, and a stable hydrogel eventually forms. The intrinsic intensity of the amide I' band significantly increases upon gelation, revealing it as a spectroscopic marker for gelation. We attribute this to an increasing hydration of the peptide backbone. Moreover, the structural decay into amorphous aggregates as well as hydrogelation lead to rather substantial spectroscopic changes in the 1350 −1500 cm-1 region of the IR spectrum. AFM images reveal a complex nanoweb structure of the gelated peptide which is typical of noncovalently crosslinked fibrils. This cationic system lacks complimentary charges, a common feature of peptide hydrogels, and offers promise in regards to biotechnological applications.

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Randomizing Intrinsic Conformational Biases by Nearest Neighbor Interactions between Unlike Residues

Siobhan Toal[1], Christian Richter[2], Nina Kubatova[2], Harald Schwalbe[2], Reinhard Schweitzer-Stenner[1].
[1]Chemistry, Drexel University, Philadelphia, PA, USA, [2]Institute of Organic Chemistry and Chemical Biology, Johann Wolfgang Goethe University, Frankfurt, Germany.

To explore the influence of nearest neighbors on conformational biases in unfolded model peptides, we combined vibrational (IR, Vibrational Circular Dichroism, Raman), and 2D-NMR spectroscopy to study selected ''GxyG'' host-guest peptides in aqueous solution: GDyG, GSyG, GxLG, GxVG, where x/y={A,K,LV}. To obtain the conformational ensemble of each x/y residue, we utilized an excitonic coupling theory based formalism to simulate experimental amide I' profiles with conformational distributions composed of 2D-Gaussian distribution in Ramachandran space representing pPII-, β-strand-, helical-, and turn-like conformations. Experimental J coupling constants were similarly reproduced using these conformational distributions along with appropriate Karplus equations. Our data reveal large changes in conformational distributions due to neighbor interactions, contrary to the isolated pair hypothesis. Interestingly, residues that have large intrinsic biases towards specific subpopulations tend to loose these preferences upon interaction with a dissimilar neighbors, indicating a degree of conformational randomization. For instance, residues that prefer turn-like conformations (namely aspartic acid and serine) lose this turn preference in favor of increased pPII populations, which ultimately increases the total extended state sampling. In addition, we observe a decreased pPII content for alanine upon insertion of non-alanine neighbors, which generally increases with the bulkiness of the neighbors' side chain. Strong effects induced by residues with bulky aliphatic side chains suggests that the underlying mechanism occurs through disruption of the hydration shell. Thermodynamic analysis of 3J(HNHα) (T) data for each x,y residue reveals that modest changes in the conformational ensemble masks larger changes of enthalpy and entropy governing the pPII/β equilibrium suggesting a significant residue dependent temperature dependence of the peptides' conformational ensembles.

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Conformational Effects on Alanine Induced Induced by Various Alcohol Cosolvents

Bridget Milorey, Stefanie Farrell, Siobhan E. Toal, Reinhard Schweitzer-Stenner.
Department of Chemistry, Drexel University, Philadelphia, PA, USA.

The conformational ensemble of alanine, in the model peptide GAG, in aqueous solution, is known to exhibit a largely two-state equilibrium between polyproline II (pPII) and β-strand conformations with a high pPII population. If solvation by water is indeed pivotal for pPII stabilization as suggested in the literature, the addition of alcohol co-solvents such as ethanol and propanol could be expected to de-stabilize pPII in favor of β-strand conformations. Through the use of HNMR and UV circular dichroism (CD) spectroscopy, the conformationally sensitive 3J(HNHα) coupling constant and the dichroism at 215nm (Δε215, a pPII indicator) were obtained as a function of temperature. A two-state (pPII-β) thermodynamic analysis did not reproduce the temperature dependence of the J-coupling constant, indicating that alanine samples an increased turn-like population upon addition of co-solvent. The obtained Δε215 values were found to depend nonlinearly on the co-solvent concentration. This observation is likely to reflect different phases of the non-ideal mixture of water and primary alcohols. In order to further explore the relationship between peptide conformation and solvent mixture, we performed vibrational analyses and simulated the amide I' band in IR, VCD and polarized Raman using an excitonic coupling algorithm and variable 2D conformational distributions reflecting sub-populations (pPII, β, various turns). Preliminary results of this analysis, which has not yet been completed, suggest that the addition of alcohol co-solvents affect both the relative population of sub-distributions assignable to pPII, β-strand and turn-like conformations as well as their specific locality of these distributions in the Ramachandran space.

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Does the Golgi Reassembly and Stacking Protein (GRASP) Behave as a Well-Structured Protein in Solution?

Luis F. Santos Mendes[1], Assuero F. Garcia[1], Marcio L. Rodrigues[2], **Antonio J. Costa-Filho**[1].
[1]Department of Physics, University of São Paulo, Ribeirão Preto, Brazil, [2]Microbiology Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Among all proteins localized in the Golgi apparatus, a two-PDZ domain protein plays an important role in the assembly of the cisternaes and is also responsible for many other functions. This class of proteins, known as Golgi Reassembly and stacking Proteins (GRASP), has puzzled many researchers due to its large array of functions. In this work, the GRASP homologue in the fungus Cryptococcus neoformans (CnGRASP) was studied. This protein is associated with the unconventional secretion mechanisms required for the export of the most important virulence factor in that fungus. Biophysical techniques were used to assess structural aspects of CnGRASP in solution. We were able to detect a relevant secondary structural content, but with a large amount of disordered regions. The overall structure is less compacted compared to those values expected for a globular protein, which also leads to a high structural flexibility and water accessibility of the hydrophobic core. All the results together indicate an unusual behavior of CnGRASP in solution that closely resembles the behavior previously observed for molten globule proteins. To the best of our knowledge this is the first direct observation of the molten globule-like behavior of a GRASP protein in physiological conditions. We also speculate the possible implications due to this unusual behavior and how it can explain the multiple facets associated with this intriguing class of proteins. Financial support: FAPESP, CNPq, CAPES.

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Assembly and Dynamics of Liquid Phase Protein Droplets Comprised of the DEAD-Box RNA Helicase, LAF-1

Shana Elbaum[1], Younghoon Kim[2], Sua Myong[2], Clifford Brangwynne[1].
[1]Princeton University, Princeton, NJ, USA, [2]University of Illinois, Urbana-Champaign, Champaign, IL, USA.

Liquid-liquid phase separation has emerged as a key process underlying intracellular organization, including the regulation of RNA/protein assemblies, and