Protein Folding & Stability I

1138-Pos Board B48

Effects of Potassium and Sodium Ions on the Stability of Poly-L-Glutamate

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It has been recently reported through classical molecular dynamics simulations, that potassium ions have lower binding affinity for glutamate residues than water, leading to destabilization of the helical conformations of the peptide. In contrast, sodium ions have much stronger affinity for glutamate groups than for water, strongly stabilizing the helical conformations of the peptide. On the other hand, recent CD and UVRR experiments found that both ions: sodium and potassium, have the same effect, inducing just a very small stabilization on the helical conformations of the polypeptide for concentrations greater than 1M. In this work, we investigate the controversy presented above by performing classical molecular dynamics simulations of the poly-l-glutamate immersed in pure water, sodium chloride and potassium chloride. We present alpha helical contents in each solvent and give a quantitative estimation of how the barrier between alpha helix and unfolded states is affected by the presence of the ions.

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Missense Mutations in N-Terminal Actin Binding Domain of Dystrophin that Trigger Muscular Dystrophy Decrease Protein Stability and Lead to Cross-β Aggregates

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A deficiency of functional dystrophin protein in muscle cells causes muscular dystrophy (MD). More than 50% of missense mutations that trigger the disease occur in the N-terminal actin binding domain (N-ABD or ABD1). We examined the effect of four disease-causing mutations - L54R, A168D, A171P, and Y231N - on the structural and biophysical properties of isolated N-ABD. Our results indicate that N-ABD is a monomeric, well-folded α-helical protein in solution, as is evident from its α-helical circular dichroism spectrum, blue shift of the native state tryptophan fluorescence, well-dispersed amide cross-peaks in 2D NMR ^{15}N-^{1}H HSQC fingerprint region, and its rotational correlation time calculated from NMR longitudinal (T_1) and transverse (T_2) relaxation experiments. Compared to WT, three mutants - L54R, A168D, and A171P - show a decreased α-helicity and do not show a cooperative sigmoidal melt with temperature, indicating that these mutations exist in a wide range of conformations or in a 'molten globule' state. In contrast, Y231N has an α-helical content similar to WT and shows a cooperative sigmoidal temperature melt but with a decreased stability. All four mutants experience serious misfolding and aggregation. FT-IR, circular dichroism, increase in thioflavin T fluorescence, and congo red absorption spectral shift and birefringence show that these aggregates contain intermolecular cross-β structure similar to that found in amyloid diseases. These results indicate that disease-causing mutants affect N-ABD structure by decreasing its thermodynamic stability and increasing its misfolding, thereby decreasing the net functional dystrophin concentration.

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A comprehensive Analysis of Bovine α-Lactalbumin Molten Globule in Presence of Surfactants: Biophysical Correlates

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The role of different types of interactions and their contribution in the stabilization of bovine α-lactalbumin (α-LA) molten globule in presence of cationic surfactant, hexadecyl trimethyl ammonium bromide (HTAB) and anionic surfactant, sodium dodecyl sulphate (SDS) have been examined using a combination of spectroscopic, light scattering and calorimetric techniques. At lower concentration of the surfactants, the thermodynamic parameters obtained from UV-Visible spectroscopy suggested an increased exposure of non-polar groups in HTAB while a possible restructuring of non-polar groups were indicated in SDS. The fluorescence and circular dichroism spectroscopy showed the formation of an intermediate state at various concentrations in presence of HTAB and SDS while the lifetime measurements supported the assumption of protein-surfactant complex stability in HTAB as compared to SDS. The hydrodynamic diameter and ζ-potential were analyzed by dynamic light scattering (DLS) which also implicated the combined influence of electrostatic and hydrophobic interactions in α-LA unfolding in HTAB and only hydrophobic interactions in SDS. The binding parameters for ANS obtained from isothermal titration calorimetric (ITC) measurements suggested a high stability of α-LA molten globule and the role of enthalpic and entropic contribution in the binding of ANS in HTAB. It also indicated the fragility of α-LA molten globule in SDS. The possible binding sites as well as the interactions of ANS with the molten globule were also investigated from the thermodynamic parameters obtained from ITC. We, thus propose that the molten globule state obtained in HTAB and SDS are very different from one another as well as the conventional molten globule state of α-LA obtained in presence of chemical denaturants.

1141-Pos Board B51

Stereochemistry and Protein Folding: Spectroscopic and Molecular Dynamics Studies of Homopolypeptides in DMSO

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In addressing stereochemistry for likely relevance in protein folding, poly-L and alternating-L,D diastereomers of suitable homopolypeptides have been evaluated for effects of solvent with spectroscopy and statistical-mechanical modeling. Previous studies have shown that the models are contrasted in effect of water and methanol as the solvents.[1-3] A lysine-solubilized nonapeptide now studied for conformation as a function of stereochemistry, has been found based on NMR, to be unfolded in DMSO irrespective of stereochemistry. Molecular-dynamics studies concur with the results, showing that indeed the diastereomers are populated in beta-basins of phi, psi space and are unfolded in conformation. The contrasts of water, methanol, and DMSO as the solvents for the diastereomeric structures of the model peptides are discussed in relation to the proposed protein-folding model according to which poly-L stereochemistry is the fulcrum placing hydrogen bonds and electrostatics of polypeptide dipoles in mutual conflict, and thus conformation under dielectric control. The solvent role in protein folding will be discussed for its physical basis.

References

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1142-Pos Board B52

Exploring by Enhanced Sampling Techniques: The Protein's Conformational Space Beyond the PDB

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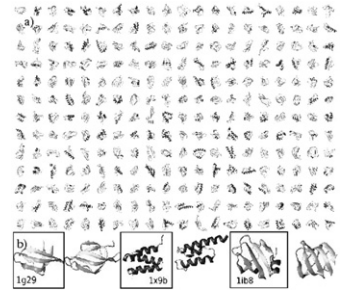
It is believed that the atlas of existing protein structures is faithfully represented in the PDB. However, whether this atlas covers the full universe of possible protein structures is still a debated issue. By using a sophisticated numerical approach we performed an exhaustive exploration of the conformational space of a 60a.a. polypeptide chain described with an accurate all-atom potential. We generated ~30,000 compact folds with at least 30% of secondary structure corresponding to local energy minima. This ensemble plausibly represents the universe of protein folds of similar length: indeed, all the known folds are represented with good accuracy. However, we discover that the known folds form a rather small subset, which cannot be reproduced by choosing random structures in the database. Rather, natural and possible folds differ by the contact order, on average significantly smaller in the former. This suggests the presence of an evolutionary bias, possibly related to kinetic accessibility, towards structures with shorter loops between contacting residues. The new structures open a range of practical applications such as the development of accurate structure prediction strategies, the optimization of force fields, and the design of novel folds.



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The Holding and Folding Chaperone Properties of Two Small Heat-Shock Pair Proteins IbpA and IbPB of Escherichia Coli

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IbpA and IbpB (inclusion body binding proteins A and B) are two small heat shock proteins in E. coli. Earlier studies report that IbpB has holdase property by which it binds denatured, aggregate-prone proteins forming stable complexes, but has no foldase property by which it can refold denatured, unfolded proteins. This study is conducted to investigate whether IbpA also has the holdase property, how the holdase activity of IbpB is modulated by the presence of IbpA, and if IbpA and IbpB, individually or in combination, have any foldase property on denatured proteins after prolonged incubation. Our results, based on the techniques of spectrophotometry, spectrofluorimetry, HPLC, gel electrophoresis and DLS show that a) IbpA possesses holding chaperone activity,