ELUCIDATING THE MECHANISM OF PROTEIN AGGREGATION AND CONFORMATIONAL STABILITY OF SOME PROTEINS

ABSTRACT

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JAVED MASOOD KHAN

INTERDISCIPLINARY BIOTECHNOLOGY UNIT
ALIGARH MUSLIM UNIVERSITY
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ABSTRACT
Protein folding process is an extensively studied topic. Folding of proteins is vital for performing various biological functions. Therefore, understanding the mechanisms by which protein fold is an important issue in current proteomic era. These folded proteins are stabilized by various covalent and non-covalent interactions and can be denatured by denaturant, surfactant, change in pH and temperature. In vivo misfolding of proteins occurs because of change in environmental conditions or due to mutations that lead to the exposure of hydrophobic residue and aggregation. These aggregates are deposited at various parts of the central nervous system and other organs hence causing development of human diseases including Alzheimer, Parkinson, Prion, Huntington and type II diabetes etc. In view of above in the first part of review we have focused on protein folding mechanisms, factors that affect the protein folding and stability under in-vitro and in-vivo conditions. The second part is devoted to protein aggregation mechanisms and detection of the aggregates and its morphology by using different dyes. The pros and cons of dyes are also discussed. Additionally, we have uncovered the mechanisms by which negatively charged surfactants and lipids induce amyloid fibril formation.

Chapter 1

Sodium dodecyl sulphate (SDS), an anionic surfactant that mimics some characteristics of biological membrane has also been found to induce aggregation in proteins. The present study was carried out on 25 diverse proteins using circular dichroism, fluorescence spectroscopy, dye binding assay and electron microscopy. It was found that an appropriate molar ratio of protein to SDS readily induced amyloid formation in all proteins at a pH below two units of their respective isoelectric points (pI) while no aggregation was observed at a pH above two units of pI. We also observed that electrostatic interactions play a leading role in the induction of amyloid. This study can be used to design or hypothesize a molecule or drug, which may counteract the factor responsible for amyloid formation.

Chapter 2

Different proteins have different amino acid sequences as well as conformations, and therefore different propensity to aggregate. Electrostatic interactions have an important role in the aggregation of proteins as revealed by our previous report (Khan et al. PLoS One 2012, 7, e29694). In this study, we designed and executed
experiments to know the role of charge variations on protein during the events of protein aggregation with lysozyme as a model protein. To impart positive and negative charge on protein we incubated lysozyme at different pH values, which were below and above the pl of lysozyme (~11). Negatively charged SDS were used to antagonize positive charges on the lysozyme at the respective pH values. We examined the effect of pH variations on SDS-induced amyloid fibril formation of lysozyme using methods such as far-UV CD, Rayleigh scattering, turbidity measurements, dye binding assays and dynamic light scattering. We found that submicellar concentrations of SDS (0.1 to 0.6 mM) induced amyloid fibril formation of lysozyme in the pH range 10.0-1.0 and the maximum aggregation was found at pH 1.0. The morphology of aggregates was fibrillar in structure, as visualized by transmission electron microscopy. Isothermal titration calorimetry studies demonstrated that fibril formation is an exothermic reaction. To our current understanding on the mechanism of aggregation this study demonstrates the crucial role of electrostatic interactions during amyloid fibril formation. Conclusively, we propose the mechanistic model of protein aggregation through this study. The model proposed here will help in designing molecules that can prevent or reverse the amyloid fibril formation or the aggregation.

Chapter 3

Many proteins form amyloid fibril which is associated with several neurodegenerative diseases such as Alzheimer’s, type II diabetes and dialysis-related amyloidosis. In this study different serum albumins were used at pH 3.5 below two units of pl (pH 5.5) to examine the role of negative charge and hydrophobicity in amyloid fibril formation. In this context three anionic surfactants viz SDS, SDBS and AOT were taken, bearing same negative head but different hydrophobic tail. The propensity of SDS, SDBS and AOT to form amyloid fibril were investigated by using turbidity, Rayleigh light scattering measurements, ThT and Congo Red dye binding assays, dynamic light scattering as well as by far-UV CD methods. At submicellar concentrations (0.5-2.5 mM) of SDS and SDBS amyloid fibril were formed in all albumin taken in this study while at higher concentration amyloid fibril formation was completely inhibited. Interestingly AOT promotes amyloid fibril formation up to 11 mM without any inhibition as SDS and SDBS does. The amyloid induced by SDS, SDBS and AOT
have fibrilar morphology as confirmed by Congo Red, ThT and also visualized by transmission electron microscopy. Albumins surfactant interaction was exothermic as confirmed by isothermal titration calorimetry (ITC). At lower concentrations of SDS and SDBS electrostatic interaction play the major role while at higher concentration only hydrophobic interaction were found effective. In case of AOT electrostatic interaction persists even at higher concentration of detergents. From the turbidity, Rayleigh light scattering and dynamic light scattering it was concluded that the order of amyloid induction was more in AOT followed by SDBS and least in SDS. Similar studies were performed at pH 7.4 approximately above two units of pl and no amyloid fibril were noticed it may be due to electrostatic repulsion. From this study we have concluded that the negatively charged surfactant induced amyloid fibril formation in serum albumins with the help of electrostatic and hydrophobic interaction. Beside this study performed pH above two units of pl confirmed that hydrophobic interaction alone can not induce aggregation in serum albumins.

Chapter 4

The effect of sodium dodecyl sulphate (SDS) on human, bovine, porcine, rabbit and sheep serum albumins were investigated at pH 3.5 by using various spectroscopic techniques like circular dichroism (CD), intrinsic fluorescence and dynamic light scattering. In the presence 4.0 mM SDS the secondary structure of all the albumins were not affected as measured by CD but fluorescence spectra reveled 8.0 nm blue shift in emission maxima. We further checked the stability of albumins in the absence and presence of 4.0 mM SDS by urea induced unfolding at pH 3.5. In the absence of SDS, urea unfolds secondary as well as tertiary structural elements of the albumins at very low concentrations (~2.0 M) but in the presence of 4.0 mM SDS urea was unable to unfold even up to 9.0 M. Thermal stability of albumins were also checked under similar condition. The albumins were very less stable at pH 3.5 with decrease in Tm values 54 °C to (HSA), 57 °C (BSA, PSA), 59 °C (SSA) and 63 °C (RSA) but in the presence of 4.0 mM SDS, the Tm was increased to 74 °C (HSA), 75 °C (BSA), 76 °C (PSA, SSA) and 75 °C (RSA). From this study it was concluded that SDS is showing a protective effect against urea as well as thermal denaturation of albumins. This behavior may be due to electrostatic as well as the hydrophobic interaction of SDS with albumins. Further we have proposed the mechanism of action of urea. We have
proposed that urea interacted with proteins directly when proteins are in charged form. Indirect interaction may be taking place when the environment is more hydrophobic.

Chapter 5

Banana lectin (BL) is a homodimeric protein categorized among jacalin-related family of lectins. The effect of acidic pH was examined on conformational stability of BL by using circular dichroism, intrinsic fluorescence, 1-anilino-8-naphthalene sulfonate (ANS) binding, size exclusion chromatography (SEC) and dynamic light scattering (DLS). During acid denaturation of BL, the monomerization of native dimeric protein was found at pH 2.0. The elution profile from SEC showed two different peaks (59.65 ml & 87.98 ml) at pH 2.0 while single peak (61.45 ml) at pH 7.4. The hydrodynamic radii ($R_h$) of native BL was 2.9 nm while at pH 2.0 two species were found with $R_h$ of 1.7 and 3.7 nm. Furthermore at, pH 2.0 the secondary structures of BL remained unaltered while tertiary structure was significantly disrupted with the exposure of hydrophobic clusters confirming the existence of molten globule like state. The unfolding of BL with different subunit status was further evaluated by urea and temperature mediated denaturation to check their stability. As inferred from high $C_m$ and $\Delta G$ values, the monomeric form of BL offers more resistance towards chemical denaturation than the native dimeric form. Besides, dimeric BL exhibited a $T_m$ of 77 °C while no loss in secondary structures was observed in monomers even up to 95 °C. To the best of our knowledge, this is the first report on monomeric subunit of lectins showing more stability against denaturants than its native dimeric state.