RESEARCH ARTICLE | AUGUST 21 2023

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AIP Advances 13, 085023 (2023)
https://doi.org/10.1063/5.0154129

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Cite as: AIP Advances 13, 085023 (2023); doi: 10.1063/5.0154129
Submitted: 26 April 2023 • Accepted: 16 June 2023 •
Published Online: 21 August 2023

View Online Export Citation CrossMark

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ABSTRACT

Biological systems are often exposed to mechanical perturbations, which may modulate many biochemical processes. Ligand binding involves a wide range of structural changes in the receptor protein, from hinge movement of entire domains to minor sidechain rearrangements in the binding pocket residues. Hydrophobic ligand binding to protein alters the system's vibrational free energy, allowing different conformational states of allosteric proteins. Excess hydrophobicity in protein–ligand binding generates mechanical force along the peptide backbone through the hydrophobic effect. We describe mechanically strained peptide structures involved in protein aggregation to determine the transition between the initial condensation of hydrophobic polypeptide chains into ordered fibrillar structures. This transition is due to the excess attractive hydrophobic force by ligand binding within proteins into fibrillar assemblies. The process of fibrillar formation has a mechanosensitive nature, which significantly influences the pathogenesis of several neurodegenerative diseases.

The physicochemical properties depend on the conformation changes in inbound and unbound forms of proteins to regulate physiological functions.[1] The binding of a ligand to a protein can expose hydrophobic residues on the protein's surface if the ligand has hydrophobic properties. In protein–ligand binding, a ligand produces a signal by binding to a site on a target protein initiating a downstream signaling cascade, where the signal is amplified through a series of protein–protein interactions. Signaling by the ligand binding provides chemical to mechanical energy transduction by generating molecular forces inside them.[2] However, the mechanical energy alters the protein's conformation and increases hydrophobicity for the protein to which it binds. Hydrophobicity affects a receptor protein's three-dimensional shape and orientation, which ultimately impact the protein's functional state.

In a peptide bond, the likely conformations for a polypeptide chain are quite restricted due to the limitation of rotational freedom at φ (C_α–N) and ψ (C_α–C) angles by steric hindrance between peptide backbone and sidechain, reducing correspondingly the available conformational space. The rotations around two other bonds linked to the C_α atom can rotate freely. Hence, each amino acid residue in the polypeptide chain has many possible confirmations. The polypeptide conformations do unfold at high temperatures, and thus, the hydrophobic amino acid sidechains are exposed to the surrounding aqueous environment (Fig. 1). Due to the mechanical nature of the unfolded state (more flexible),[3] the total conformational entropy of the unfolded state is much greater than that of the folded state (more rigid). However, conformational entropy[4] on protein unfolding[5] involves hydrophobicity,[6] as the hydrophobic sidechains are free to explore a large range of conformations in the aqueous environment.

When a polypeptide chain is subjected to stretching force and cross-linking,[7] the conformation of the chain can be decreased. The stretching force can pull a polypeptide chain into an extended conformation (Fig. 1), where it has fewer degrees of freedom than when it is in a more compact state. Similarly, cross-linking can physically restrict the range of motion of the polypeptide chain, reducing the number of possible conformations.

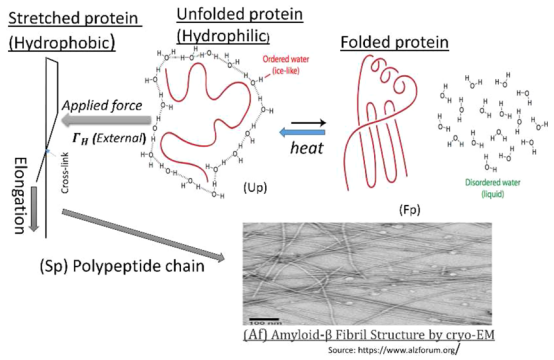

FIG. 1. Stretching of the unfolded protein.

Assuming that Γ_H is the maximum force applied to the α-helix of which elongation is Δd_e (Fig. 1).

This force Γ_H always considers higher than the mechanical stiffness[12] of an α-helix hydrogen bond.

The stretching force on protein unfolding is described by the following equation:

$$\Gamma_H = \frac{\left(-T\Delta S^{conf}\right)}{\Delta d_e} = \frac{k_B T \ln W}{\Delta d_e}.$$

The conformational energy[8] $T\Delta S^{conf}$, which is the energy associated with changes in the conformation of a protein, is dissipated in the polypeptide thermal vibrations. Thermal vibration energy in a receptor protein can be stored as mechanical deformations in all microstates W.

If the conformational energy of a protein is higher than its enthalpy, then the protein's conformation is likely to be highly dynamic and flexible. In this case, ligand binding to the protein provides unfolding free energy,[9] $\Delta G_U = -k_B T \ln \kappa_a$ (T = Temperature and κ_a = Binding association constant), which contributes to the stabilization of the protein's structure. The unfolding free energy compensates for the protein's own hydrophobicity deficiencies by exposing hydrophobic sidechains to a hydrophobic ligand for creating a favorable interaction between the protein and the ligand. On the other hand, the ligand's excess hydrophobicity can be compensated by the protein's unfolding free energy by exposing hydrophilic residues to the aqueous environment, thereby minimizing unfavorable interactions with water. Therefore, the excess hydrophobic force[10,11] drives the ligand into a binding site in the protein (Fig. 2), where electrostatics and hydrogen bonding (e.g., salt bridges) govern specificity.

The excess hydrophobic force can induce a mechanical deformation of a polypeptide chain[13] by generating mechanical forces[14] (Fig. 2). When a mechanical force[15] is applied to a protein, it causes the protein to undergo mechanical unfolding, also known as the unfolding force or stretching force Γ_H, which involves the conversion of the native protein conformation to a misfolded conformation.[16] The misfolded protein initiates self-assembly and forms molten oligomers, which can be mechanically strained and stabilize charged residues and hydrophobic forces, leading to further self-assembly and the formation of larger aggregates. However, the mechanical force can work along the length of the unfolded domain[17] to cause peptide stretching and ultimately result in an elongated state. Elongation can lead to changes in secondary structure that can affect the hydrophobicity of the protein. This hydrophobicity can trigger further misleading and the accumulation of other proteins aggregates or oligomers.

The hydrophobicity released during protein–ligand binding at a specific distance,[18] $\Delta\Omega_b = 4 \times 10^{-10}$ m, tends to stretch and orient geometrically bend peptides in the loading direction. The fully stretched polypeptide chain is less flexible due to the increased attraction among hydrophobic sidechains, which are energetically favorable and can contribute to the stability of the stretched conformation.

The strong attraction of polypeptide sidechains in water is reflected in the large interfacial energy of the protein–water interface. The hydrophobic sidechains in water have high surface tension and contact angle values. Hence, the hydrophobicity of a protein in contact with a hydrophobic surface will be dominated by van der Waals, long-range forces, and adhesion energy.

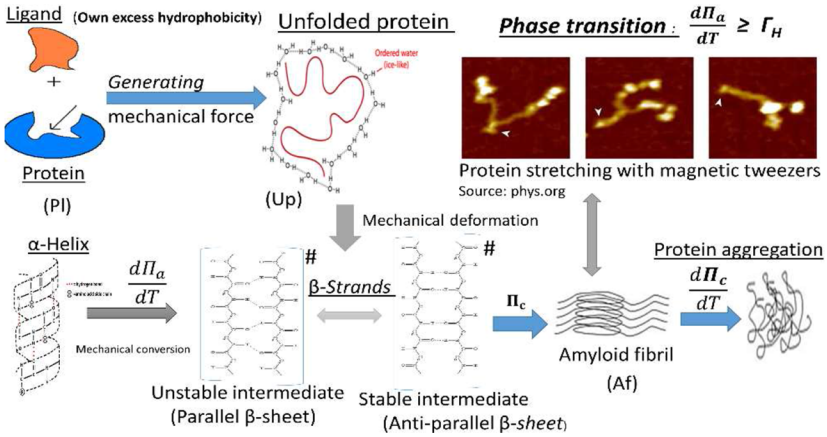

FIG. 2. Ligand binding hydrophobicity.

At a protein–water interface, the tendency of the hydrophobic protein–ligand complex is to minimize the contact area with water for maximizing hydrophobicity. The thermodynamics of protein self-assembly at a water interface can be described by the thermal aggregation parameter Π_a. This parameter is a measure of unfolding free energy associated with the aggregation of protein–ligand binding complexes at the interface. It is defined as

$$\Pi_a = \frac{\Xi_H \delta_e^2 \nabla \varepsilon_H}{\Delta\Omega_b k_B T \ln \kappa_a}.$$

Since the protein aggregation parameter, Π_a depends on the hydrophobicity of the peptide sequence[19] σ_i, i.e., the number of hydrophobic peptide monomers. Thus, the characterization of hydrophobicity Ξ_H of a peptide sequence takes into account the number of hydrophobic peptide monomers $И_H$ normalized concerning the total number of hydrophobic sidechains $И$,

$$\Xi_H(\sigma) = \frac{И_H}{И} = \frac{1}{И}\sum_{i=1}^{И} \sigma_i$$

The average hydrophobicity over a set of designing sequences of a given length $И$ can then be introduced as

$$\langle \Xi_H \rangle_И = \frac{1}{\mathcal{C}_И} \sum_{\sigma \in \mathcal{C}_И} \Xi_H(\sigma)$$

Peptide sequences that are symmetric under the reversal of hydrophobic sidechains only once in the statistics are known as palindromic sequences. Taking into account all $2^{И}$ peptide sequences of length $И$, where each position can be occupied by any of the 20 standard amino acids, would lead to a binomial distribution for $И_H$ sin ce there are exactly $\binom{И}{И_H}$ sequences with $И_H$ hydrophobic peptide monomers.

The hydrophobic force, thermodynamic in nature and relatively stronger than dehydrons,[20] is an average of a fluctuating force $-\nabla\varepsilon_H$, the average attractive force between hydrophobic groups being the negative gradient of free energy. The hydrophobic force, a force of mean-field normalized[21] to 1 for ordered stable oligomers, relates to the energy $\delta_e \nabla \varepsilon_H$ involved in the salvation of hydrophobic sidechains that become exposed in the unfolded state[22] (Fig. 1). However, the resultant attractive forces between water molecules tend to increase the area of the protein–water interface; the force is the negative gradient of the resultant free energy change $-\delta_e \nabla \varepsilon_H$. The transfer of a protein sidechain from water to nonpolar solvent at a distance of $\{\delta_e = 3.5 \times 10^{-10}\ \text{m}\}$[23] is thermodynamically favorable with a transfer-free energy change of −1.39 kJ/mol.

The thermal aggregation shows a linear relationship between Π_a and the inverse of absolute temperature T, assuming Ξ_H, δ^2, and Ω_b are independent of temperature. If Π_a is determined using $\Xi_H = 1$, $\delta_e = 3.5 \times 10^{-10}$ m, $\nabla\varepsilon_H = 1$, $\Delta\Omega_b = 4 \times 10^{-10}$ m, $\{\kappa_a = 104\}$,[24] $k_B = 1.380\,649 \times 10^{-23}$ m^2 kg s^{-2} K^{-1} at different temperatures, a linear plot of Π_a vs 1/T can be obtained (Fig. 3).

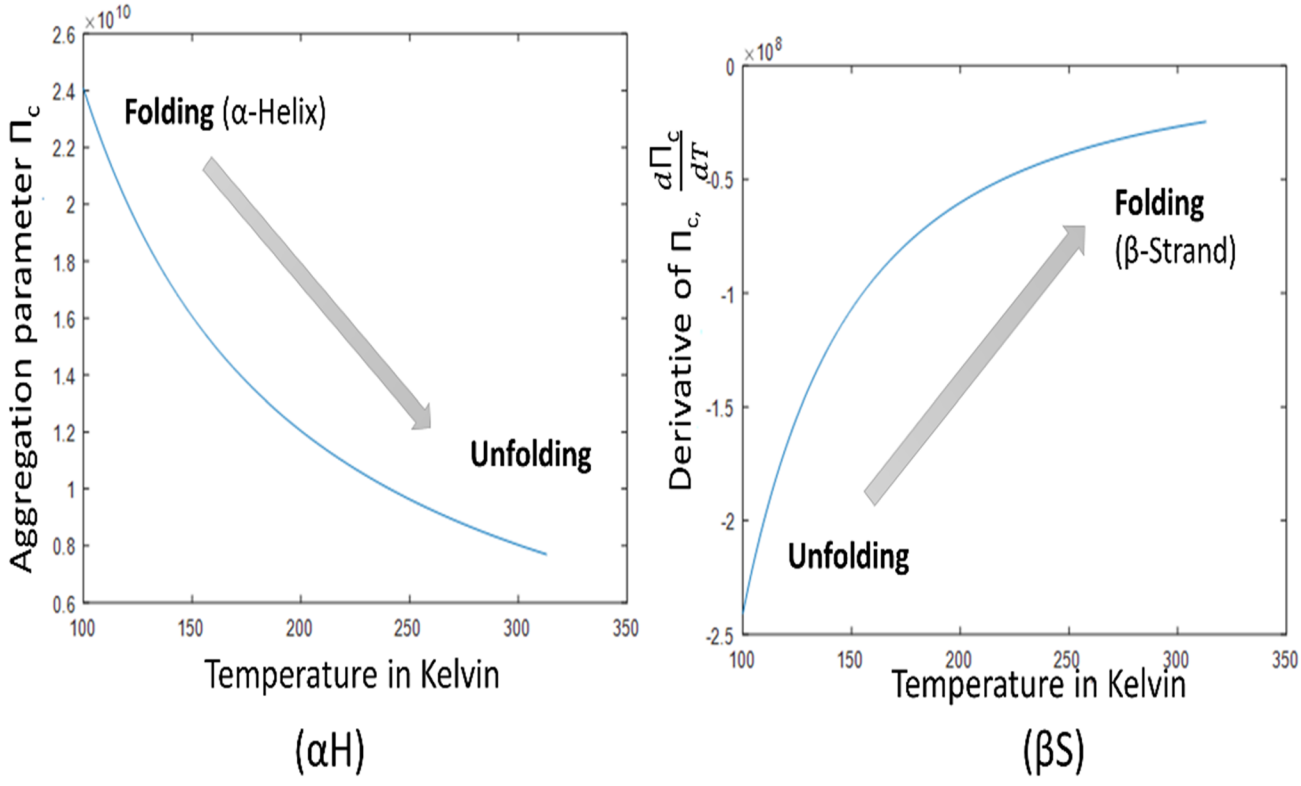

FIG. 3. Temperature dependence curves of aggregation parameter.

In Fig. 3, the curved function of temperature indicates the aggregation parameter changes with temperature, which is nonlinear and reaches an asymptotic value at high temperatures. The temperature increase enhances a critical α-helix to the random coil to β-sheet transitions, and thus, the rate of formation β-strand occurs through unfolding. In the α-helix to β-sheet transition, an increase in temperature ($T_c > T$) can disrupt the hydrogen bonding and van der Waals forces[25] that stabilize the α-helix conformation, causing it to unfold into a random coil. As the temperature continues to increase, the random coil can form into β-sheet structures through the formation of inter-strand hydrogen bonds {T (α-Helix) > T (β-Sheet)}.[25] The initial formation of a β-strand nucleus promotes the addition of more β-strands to form a stable β-sheet structure, which can lead to the formation of amyloid fibrils or other aggregates.

The derivative of Π_a concerning temperature T (Fig. 3) determines a switch from α-helical to a β-strand. A sharp increase (Fig. 4) in the derivative of Π_a with respect to temperature at a certain temperature indicates a transition from α-helical to β-strand conformations.

Varying the temperature T of the system while keeping all other variables in thermal aggregation Π_a fixed can lead to an unfolding-aggregation transition at critical temperature T_c. As the temperature increases toward T_c, the protein begins to unfold, exposing hydrophobic regions that can interact with other unfolded proteins to form aggregates. When T is near T_c, the thermal aggregation Π_c has a power law behavior,

$$\Pi_c = \frac{\Xi_H \delta_e^2 \nabla \varepsilon_H}{\Delta\Omega_b |T_c - T|^{\alpha} k_B \ln \kappa_a}.$$

In Fig. 4, the critical parameter Π_c of a polypeptide chain at $T_c = 311$ K has an aggregation-prone behavior near the unfolding-aggregation transition where the critical exponent $\alpha = 0.1$ indicates a weakly first-order phase transition, which is not fixed above and below the critical temperature. As the temperature approaches T_c from below, Π_c increases rapidly with decreasing temperature, consistent with a strong first-order phase transition. On the other hand, as the temperature approaches T_c from above, Π_c increases more slowly, consistent with a weak first-order phase transition. The critical temperature $T_c = 311$ K represents the highest temperature at which a protein can aggregate through ligand binding as shown in Fig. 4. This result ($T_c = 311$ K) provides important insights into the protein's thermal stability and aggregation behavior under these conditions. By comparing the critical temperatures from other techniques [Differential Titration Calorimetry (DSC), Isothermal Titration Calorimetry (ITC), Circular Dichroism (CD), etc.] with the value ($T_c = 311$ K) obtained from ligand binding, it may be possible to gain a complete understanding of the protein's thermal stability and aggregation behavior.

The analysis of sidechain flexibility can provide valuable insights into the behavior and interactions of amino acid sidechains in proteins. The development of an index of amino-acid sidechain

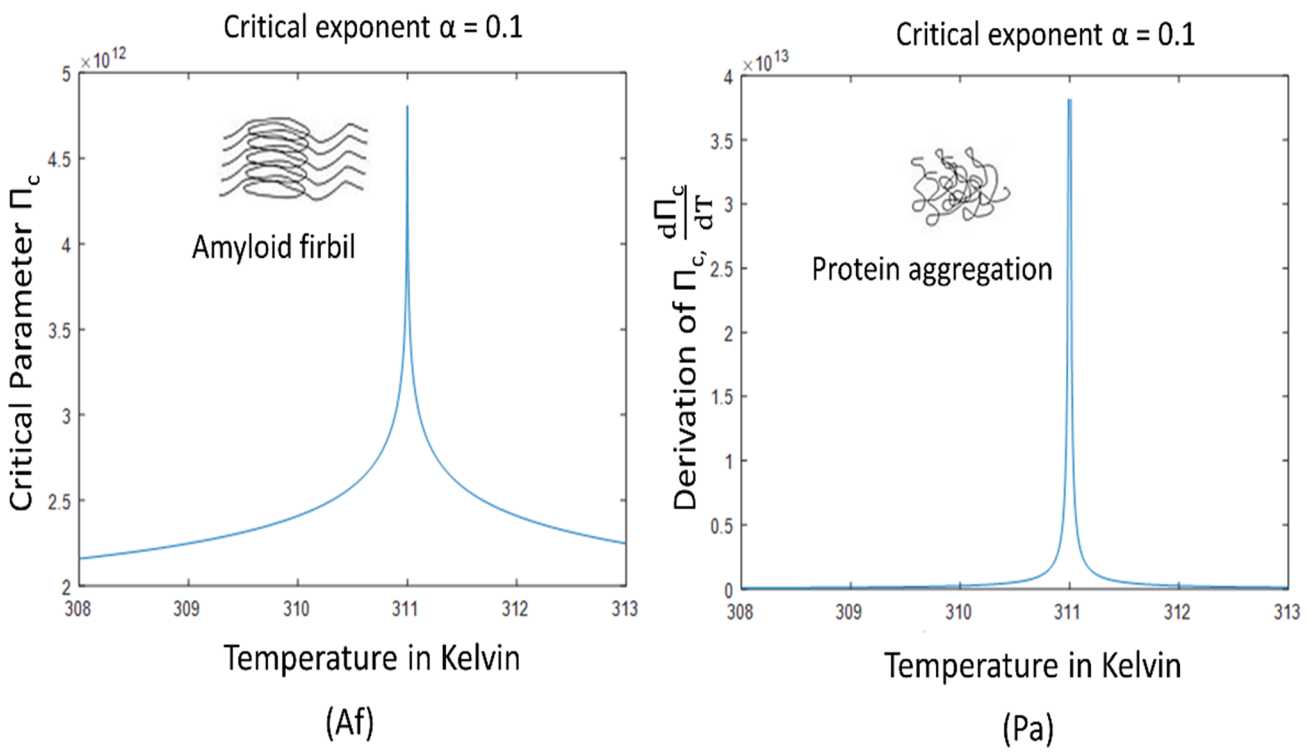

FIG. 4. Graphs for temperature range 308–313 K, α value on the top of each graph, T_c assumed to be 311 K. When ($T_c - T$) is negative, it becomes a complex number for some values of α. In these graphs, the real part of complex numbers is considered.

flexibility can be a powerful tool for predicting the behavior of proteins under different conditions, and for guiding the design of new proteins with desired properties. In this paper, we present molecular insights into the structure and the hydrophobicity of the β-sheet provides the aggregation process. However, protein aggregation is a complex phenomenon that is poorly understood at a molecular level. Hydrophobicity and apolarity are ubiquitous phenomena in protein aggregation. Quantifying the effect of the hydrophobic force on ligand-binding association has proven to be difficult because force is a vector magnitude and can act in different directions. This can result in the heterogeneous application of forces over uncontrolled reaction orientations, making it challenging to accurately quantify the contribution of hydrophobic forces to ligand binding. Minimization of the vector magnitude in ligand-protein association, the aggregation parameter is determined based on the hydrophobicity of dehydrated stretched polypeptides to sidechains. The aggregation parameter is measured by analyzing the physiochemical properties of the amino acids in the protein sequence. Protein aggregation results in the formation of oligomers from the mechanical conversion of unfolding proteins rich in β – sheets at the temperature T_c = 311 K. The temperature T_c = 311 K is significant because it is a physiological temperature close to the melting temperature of many proteins prone to aggregation. At this temperature, protein molecules are in a state of high energy and are more likely to undergo structural changes that can lead to aggregation. This provides a physical process of aggregation at a molecular level and suggests that the ligand-binding contacts control the observed amyloid fibril architectures. This is a valuable feature for understanding the structure and mechanical aspects of amyloid fibrils that might provide a solution to many therapies.

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Md. Mozzammel Haque: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review & editing (lead). **Muhammad Abdul Kadir**: Software (lead). **Richard Bayford**: Investigation (equal); Validation (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the findings of this study are available within the article.

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