Tata Institute of Fundamental Research
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad - 500107

Internal Seminar

Kinetic and Thermodynamic Studies on the Effect of Macromolecular Crowding Agents (Dextran and Ficoll) on Proteins

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Carbonmonoxycytochrome c refolds to a native-like compact state (NCO-state), where the non-native Fe^{2+}-CO interaction persists. Slow thermal-dissociation of CO transforms the NCO-state to native-state (N-state), where the native Fe^{2+}–M80 bond recovers. To determine the role of crowding agents on the structural-fluctuation of NCO and carbonmonoxymyoglobin (MbCO), the kinetic and thermodynamic parameters for CO-dissociation from NCO (NCO → N + CO) and MbCO were measured at varying concentrations of crowding agents (dextran 70, dextran 40, ficoll 70) and at the different [GdnHCl] or [Urea] in the presence of crowding agents (dextran 40, dextran 70 and ficoll 70) demonstrate that, (i) the rate coefficient or dynamics of NCO and MbCO depends on size, shape, and viscosity of the crowding agent (ii) at low denaturant concentrations, crowder presence enhances the denaturant-mediated restricted dynamics of NCO and MbCO, and (iii) at higher denaturant concentrations, large-scale unfolding fluctuations dominate the dynamics and inclusion of crowder counteracts the structural-fluctuations causing the unfolding of proteins. Thermodynamic analysis of thermal and urea-unfolding curves of cytochrome c (Cyt c) and myoglobin (Mb) measured at different [GdnHCl] in presence of crowding agents reveals that crowder presence counterbalances and strengthens the destabilizing action of GdnHCl on the stability of Cyt c and Mb, respectively.

Structural and molecular properties extracted from circular dichroism (CD), tryptophan fluorescence and 1- anilino-8-napthalene sulfonate (ANS) binding experiments suggest that the high concentration of synthetic crowding agents (dextran 40, dextran 70 and ficoll 70) stabilizes and refolds the base-denatured ferricytochrome c (Ferricyt c), apomyoglobin (apoMb) and lysozyme (Lyz) at pH 12.9 (±0.1) to molten globule (MG) states (CB-states).

Monday, Nov 5th 2018

2:30 PM (Tea/Coffee at 2:00 PM)

Seminar Hall, TIFR-H