

Alcohol-Salt Induced Partially Folded State of α -Lactalbumin and Energetics of ANS-Binding: Isothermal Titration Calorimetric and Spectroscopic Studies

R. Sharma^S and N. Kishore^C

*Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai, India
nandk@chem.iitb.ac.in*

The interaction of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and isopropanol in the presence of equimolar quantities of guanidine thiocyanate (GndSCN) with bovine α -lactalbumin has been investigated by using a combination of isothermal titration calorimetry, circular Dichroism, fluorescence and ultra-violet spectroscopy at pH 7.0. All the thermal unfolding transitions, in presence both the alcohol-salt mixtures were found to be irreversible. In the presence of 0.25 mol dm⁻³ HFIP GndSCN mixture, 0.6 and 0.85 mol dm⁻³ isopropanol GndSCN mixture α -lactalbumin was observed to be in the partially folded state with significant loss of native tertiary structure. Intrinsic fluorescence results, acrylamide and potassium iodide quenching, ANS binding and energy transfer also corroborate the presence of proposed partially folded states of α -lactalbumin. Apart from the generation of the partially folded states, it was also observed that destabilizing action of GndSCN is reduced in the presence of isopropanol compared to that in HFIP. Isothermal titration calorimetry has been used to characterize the energetics of ANS binding to the partially folded states of the protein. ITC results indicate that ANS binds to these partially folded states at pH 7.0 due to the presence of two sequentially binding sites on the protein under the solvent conditions employed, and the binding exhibits positive cooperativity in the presence 0.25 mol dm⁻³ HFIP GndSCN mixture and 0.6 mol dm⁻³ isopropanol GndSCN mixture, whereas a negative cooperativity is observed in the presence of 0.85 mol dm⁻³ isopropanol GndSCN mixture. The results also demonstrate that ITC can be used to characterize and differentiate between the partially folded states of the protein both qualitatively and quantitatively. In light of the fluorescence results, possible binding sites where ANS can bind to the protein have also been suggested.