

Characterization of Partially Folded States of Proteins by Isothermal Titration Calorimetry and Energetics of Its Binding with Fluorescent Probe ANS

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The enhanced fluorescent intensity upon binding of 8-anilino-1-naphthalene sulphonate (ANS) to the proteins has been routinely used as one of the criterion to characterize their partially folded states. We have demonstrated that isothermal titration calorimetry can also been used to characterize the partially folded states both qualitatively and quantitatively from the titration of ANS with the native, partially folded and denatured states of the protein. The interaction of 2,2,2-trifluoroethanol with concanavalin A has been investigated by using a combination of differential scanning calorimetry, isothermal titration calorimetry, circular dichroism and fluorescence spectroscopy. In the presence of 4 mol kg⁻¹ TFE at pH 2.5, concanavalin A is observed to be in the partially folded state with significant loss of native tertiary structure. The loss of specific side chain interactions in the transition from native to TFE induced partially folded state is demonstrated by the loss of cooperative thermal transition and reduction of the CD bands in the aromatic region. Acrylamide quenching, ANS binding and energy transfer also suggest that in the presence of 4 mol kg⁻¹ TFE at pH 2.5, concanavalin A is in the molten globule state. Isothermal titration calorimetry has been used for the first time to characterize the energetics of ANS binding to the molten globule state. ITC results indicate that binding of ANS to molten globule state and acid induced state at pH 2.5 displays heterogeneity with two classes of non-interacting binding sites. The presentation will also include characterization of partially folded states of some other proteins using ITC.