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Guanidine Hydrochloride-Induced Denaturation of Bovine Serum Albumin: A Comparative Study and Analysis using Different Probes

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ABSTRACT The denaturation of bovine serum albumin (BSA) by guanidine hydrochloride (GdnHCl) showed a single-step, two-state transition, when monitored by different probes such as intrinsic fluorescence at 338 and 333 nm after excitation at 280 and 295 nm respectively, UV difference spectral signal at 288 nm, 1-anilinonaphthalene-8-sulphonate (ANS) fluorescence at 470 nm after excitation at 380 nm, bromophenol blue (BPB)-induced difference spectral signal at 619 nm and λ_{max} of positive difference spectral signal of BPB-BSA complex. A comparison of the denaturation curves obtained with the above mentioned probes showed differences in the requirement of GdnHCl concentration for the transition to start and complete. The values for the mid-point of denaturation transition and free energy change associated with GdnHCl denaturation ($\Delta G_D^{H_2O}$) also varied from each other, using different probes.

(Bovine serum albumin, bromophenol blue, denaturation, fluorescence measurements, guanidine hydrochloride)

INTRODUCTION

Most of the proteins require specific interactions to fold into their globular conformation from a structure-less denatured state to their biologically-active native state. This makes the study of protein folding an interesting subject to get insight into the basic mechanism and producing large amount of refolded proteins from inclusion bodies [1, 2]. In order to understand the protein folding/denaturation phenomena, many biophysical techniques such as kinetics, fluorescence, ellipticity, ultraviolet (UV) difference spectroscopy and 1-anilino-naphthalene-8-sulphonate (ANS) fluorescence measurements [3, 4, 5, 6] have been used and conclusions have been drawn regarding conformational stability of proteins and pathways of denaturation/folding [7, 8, 9]. It is necessary to choose appropriate probe to monitor conformational changes in search of intermediate(s) formed, if any, during protein denaturation process [10, 11, 12]. Different probes like UV absorption difference at 287 nm [5], fluorescence at 340 nm upon excitation at 282 nm [13], tryptophan fluorescence upon excitation at 295 nm [5], ellipticity at 222 nm [6, 14], ANS fluorescence [6], differential calorimetry [15] and bromophenol blue (BPB) binding [16] have been successfully used by several groups in studying the denaturation of a single chain multidomain protein, serum albumin. Denaturation of serum albumin has been reported to follow a single-step [6, 13], two-step [6, 11, 12, 13] or multiple-step [17] transition depending upon the reaction conditions and probes used. For example, urea denaturation of serum albumin has been shown to follow a single-step, two-state transition when studied by UV difference spectroscopy at 293 nm [5] but became two-step, three-state transition involving one intermediate when monitored by fluorescence, UV difference spectroscopy at 288 nm and ellipticity measurements [5, 6, 14]. Similarly, denaturation of serum albumin with guanidine hydrochloride (GdnHCl) which has about the same effect as urea on protein conformation [18], has been reported to follow both single-step and two-step transitions [6, 12]. Both ANS and BPB binding probes have shown the urea/GdnHCl denaturation of serum albumin as a single-step transition [6, 16]. Although denaturation of serum

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