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Conformational Study of Human Serum Albumin in Pre-denaturation Temperatures by Differential Scanning Calorimetry, Circular Dichroism and UV Spectroscopy

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Thermal conformational changes of human serum albumin (HSA) in phosphate buffer, 10 mM at pH = 7 are investigated using differential scanning calorimetric (DSC), circular dichroism (CD) and UV spectroscopic methods. The results indicate that temperature increment from 25ºC to 55ºC induces reversible conformational changes in the structure of HSA. Conformational change of HSA are shown to be a three-step process. Interestingly, melting temperature of the last domain is equal to the maximum value of fever in pathological conditions, i.e. 42ºC. These conformational alterations are accompanied by a mild alteration of secondary structures. Study of HSA-SDS (sodium dodecyl sulphate) interaction at 45ºC and 35ºC reveals that SDS affects the HSA structure at least in three steps: the first two steps result in more stabilization and compactness of HSA structure, while the last one induces the unfolding of HSA. Since HSA has a more affinity for SDS at 45ºC compared to 35ºC, It is suggested that the net negative charge of HSA is decreased in fever, which results in the decrease of HSA-associated cations and plasma osmolarity, and consequently, heat removal via the increase in urine volume.

Keywords: Circular dichroism, Conformational changes, Differential scanning microcalorimetry, HSA, Sodium dodecyl sulphate

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Introduction

The mechanism by which proteins fold from a structure-free denatured state to their unique biologically active state is an intricate process. This process is even more complex in multi-domain proteins where each domain may be able to refold independently and interdomain interactions may affect the overall folding process (Wetlaufer, 1981; Privalov, 1982; Ptitsyn, 1994). The presence of stable intermediate conformations has helped in understanding of protein folding mechanisms (Redfield *et al*., 1994; Privalov, 1996). Elucidation of the mechanism of protein denaturation is important for understanding of protein stability (Ptitsyn, 1995). Proteins can be denatured in two ways, namely, chemical and thermal (Santoro and Bolen, 1988). Human serum albumin (HSA) has been used as a model protein for protein folding and ligand-binding studies over many decades (Kragh-Hansen, 1981; Dill *et al*., 1989). HSA is a single chain protein with 585 amino acids, with a molecular weight of ~67,000 Da. Serum albumin homologs with very similar properties are found in other mammals. The structure of this protein has been determined by X-ray crystallography of high resolution (Sugio *et al*., 1999); it includes three homologous domains (I-III) that assemble a heart-shaped molecule. Each domain is formed by two sub-domains that possess common structural motifs (Tanford, 1968; Carter and Ho, 1994). HSA has one cysteine residue at position 34 (in domain I) with a free sulfhydryl group (Farruggia and Picó, 1999; Sugio *et al*., 1999).

HSA plays a special role in transporting metabolites and drugs throughout the vascular system and also in maintaining the pH and osmotic pressure of plasma (Ikeguchi *et al*., 1992;