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Research paper

Short chain polyethylene glycols unusually assist thermal unfolding of human serum albumin

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ABSTRACT

In the present study we have investigated the thermal stability of the globular transport protein human serum albumin (HSA), in the presence of two small chain polyethylene glycols (namely PEG 200 and PEG 400). Both near- and far-UV circular dichroism (CD) study reveal that addition of PEG moderately increases the α-helical content of the protein without abruptly changing its tertiary structure. The hydration structure at the protein surface experiences a notable change at 30% PEG (v/v) concentration as evidenced from compressibility and dynamic light scattering (DLS) measurements. Thermal denaturation of HSA in the presence of PEG has been studied by CD and fluorescence spectroscopy using the intrinsic fluorophore tryptophan and it has been found that addition of PEG makes the protein more prone towards unfolding, which is in contrary to what has been observed in case of larger molecular weight polymers. The energetics of the thermal unfolding process has been obtained using differential scanning calorimetry (DSC) measurements. Our study concludes that both the indirect excluded volume principle as well as interaction of the polymer at the protein surface is responsible for the observed change of the unfolding process.

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1. Introduction

Cellular environments are crowded with several macromolecules like lipids, sugars, nucleic acids and proteins, along with macromolecular arrays (e.g. cytoskeleton fibre) and the large volume occupied by such molecules can have profuse effect on a number of processes of biological importance [1]. Such non-specific influence of this high macromolecular concentration affects the structure and dynamics of proteins as they experience volume restriction owing to the surrounded macromolecules as well as restricting the allowed protein conformations [2]. The phenomenon of macromolecular crowding has generally been explained in terms of the 'exclusion volume' model which predicts a relative stabilization of the native state compared to the fully unfolded states [1,3] as has also been experimentally realized in many earlier studies [4–9] including those in which weak attractive protein-crowder interaction has been envisaged [2,10–18]. In all these studies increase in native → unfolded transition temperature has been explained on the basis of the relative stabilization of the folded state of the protein in comparison to the destabilization of the unfolded state in the potential energy landscape by making the unfolded state more compact and thereby energetically less favourable [1,6,19]. This model is based on hardcore repulsion which arises because the crowders decrease the space available to the proteins. Such an effect is mostly entropy driven as it involves only the arrangement of molecules and not the interaction among themselves [15,20]. However, a point of concern that emerges is whether there exist any 'true' crowding agent that does not cause any additional effect apart from the volume exclusion [2], also direct effect of the crowding agent on the folded as well as unfolded states of proteins could not be ruled out [3] as there have been reports available in which crowding agents are found to ease the unfolding process [21,22]. This could make a-priori prediction on the effect of macromolecular

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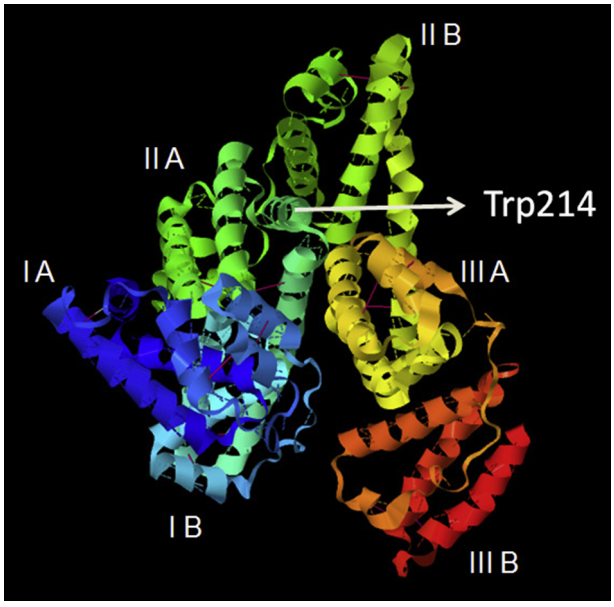
crowding on protein stability counterintuitive and a proper experimental as well as theoretical drive on rationalizing the effect on the type and content of the crowding agent is still on demand. In the present investigation we report an unusual stabilization of the unfolded state of a transport protein human serum albumin (HSA) in the presence of polyethylene glycols (PEG) of small molecular weights.

Human serum albumin (HSA) is a principal extracellular globular protein abundantly present in blood plasma [23] consisting of three structurally similar domains (I, II, and III), each containing two subdomains (A and B) and stabilized by 17 disulfide bridges [24]. The choice of this protein lies on the fact that the crystal structure of the protein is well established, and the various steps involved in its thermal denaturation have now been well understood [25,26]. Furthermore, the protein has an intrinsic fluorophore in the form of a single tryptophan moiety in the IIA sub-domain which can selectively be used to extract domain specific information. Polyethylene glycol (PEG) is a highly water-soluble synthetic polymer which has extensively been used as a macromolecular crowing agent [27–29]. PEG is best modelled as a spherical particle and belongs to one of the 'modificator' groups which are essentially treated as protein structure stabilizers [30]. PEG is regarded as a flexible spherical coil in the dilute regime and in the semi dilute as well as in concentrated regime the polymers begin to penetrate one another forming a mesh-like entangled network [27]. Large molecular weight PEGs experience predominantly repulsive interaction with proteins and induce macromolecular association and compaction in accordance to the crowding theory [31]. However, a number of studies have shown that this interaction cannot exclusively be described in terms of excluded volume alone [32,33], rather an attractive interaction between PEG and nonpolar or hydrophobic side chains on the protein surface needs to be taken into consideration [34,35]. Such dual behaviour of PEG to act both as a crowding agent obeying the excluded volume theory as well as being covalently or non-covalently adsorbed at the protein surface seems intriguing. PEGs of higher molecular weights (e.g., M_w 8000, 10000) stabilize the native compact structure of HSA; the interaction between these polymers and HSA is thermodynamically unfavourable and becomes even more unfavourable for the denatured protein [22], PEG 3500 has been found to bind HSA non-specifically and induces its conformational change at high PEG concentration [36]. On the other hand, higher molecular weight PEGs (M_w 6000, 10,000) have been found to stabilize the unfolded state of lysozyme and consequently reduce the unfolding temperature of the protein [21] whereas they offer only marginal effect on the thermal denaturation of ribonuclease [37]. Most of these previous studies involve high molecular weight ($M_w > 1000$) PEGs and the role of PEG in modifying protein stabilization either in the form of an osmolyte or as a ligand has not explicitly been addressed. A systematic and thorough understanding of the way small chain PEG molecules interact with proteins is still demanded. Recently Knowles et al. [38] have studied the concentration dependent effect of PEGs with varying chain length (starting from the monomer) on intramolecular hairpin and intermolecular duplex DNA formation. The stability of the formation is found to be dependent on two factors, namely the excluded volume and the preferential interaction. The authors reported that for the large PEGs the later factor has a positive stabilizing effect whereas the former one offers a less destabilizing effect compared to the smaller PEGs. Interestingly the destabilization to stabilization transition shows a threshold for PEG 200 and 400. This prompted us to carry out the present investigation with these two small chain PEGs, namely PEG 200 and PEG 400 to understand their effect on the stability of HSA. The structure of the protein in the presence of PEG has been studied using far-UV circular dichroism (CD) spectroscopy. Hydration at the surface of the protein has been analyzed using high precision densimetry and sound velocity measurements. Temperature induced unfolding of the protein in the presence of the polymer has been studied using CD, fluorescence spectroscopic techniques and the corresponding energetics are measured using differential scanning calorimetry (DSC) technique.



Scheme 1. Structure of human serum albumin (HSA) [based on Sugio et al., Protein Eng. 12 (1999) 439–446].

2. Materials and methods

HSA (Human serum albumin) (M_w 66 kDa) (Scheme 1), and polyethylene glycols (PEG 400, PEG 200) were purchased from Sigma–Aldrich. All the chemicals were used without further purification. All aqueous solutions were prepared in sodium phosphate buffer (50 mM) at pH 7.0. The PEG concentration was varied from 10% to 50% (v/v). For far-UV CD measurements the protein concentration was fixed at 2 μM. For near-UV CD and volumetric study 30 μM protein was used, while for DSC and DLS measurements 20 μM protein solution was used. For fluorescence measurements it was fixed at 1 μM. Far- and near-UV circular dichroism (CD) measurements were performed on a JASCO J-815 spectrometer (Jasco International Co., Japan) with an attachment for the temperature dependent measurements (Peltier) using quartz cuvettes of 0.1 cm path length. The secondary structural analysis of the CD spectra was done using CDNN software (http//bioinformatik.biochemtech.uni-halle.de/cdnn). Volume and compressibility of HSA solution in the absence and in the presence of PEG were calculated using the density and sound velocity data obtained with a high precision density meter (model DSA5000) from Anton Parr (Austria) with an accuracy of 5×10^{-6} g cc^{-1} and 0.5 m s^{-1} in density and sound velocity measurements, respectively. Adiabatic compressibility (β_p) of the protein solution was determined by measuring the protein solution density (ρ_p) and sound velocity (u_p) and applying Laplace's equation, $\beta_p = 1/\rho_p u_p^2$. The apparent specific volume of the protein, ϕ_v is obtained by,

$$\phi_v = \frac{1}{\rho_0} + \frac{\rho_0 - \rho_p}{[P]\rho_0} \quad (1)$$

where $[P]$ is the concentration of the protein solution and ρ_0 and ρ_p are the densities of the solvent and protein solutions, respectively.

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