Spectroscopic studies on the effect of temperature on pH-induced folded states of human serum albumin

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Abstract

Human serum albumin (HSA) is a very important multi-domain transporter protein in the circulatory system responsible for carriage of various kinds of ligands within the physiological system. HSA is also known to undergo conformational transformation at different pH(s) and temperatures. In this report we have studied the binding interactions of a photosensitizing drug, protoporphyrin IX (PPIX) with various conformers of HSA at different temperatures using picosecond time-resolved fluorescence spectroscopy. Also, using dynamic light scattering (DLS) and circular dichroism (CD) spectroscopy we have followed the structural transition of various conformers of HSA at different temperatures. Ensuring the intact binding of PPIX to various conformers of HSA at different temperatures as revealed through time-resolved fluorescence anisotropy decay and significant spectral overlap of emission of Trp214 residue (donor) in domain-IIA and absorption of PPIX (acceptor) bound to domain-IB of HSA, we have applied Förster's resonance energy transfer (FRET) technique to determine the interdomain separation under various environmental conditions. The alkali-induced conformer of HSA shows almost no change in donor–acceptor distance in contrast to the native and acid-induced conformers of HSA, which show a decrease in distance with increase in temperature. Through this study the non-covalently bound PPIX is shown to be an efficient FRET probe in reporting the different temperature-induced folded states of HSA in buffer solutions of widely differing pH values.
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Keywords: Human serum albumin (HSA); FRET; Fluorescence anisotropy; PPIX–HSA complex; Thermal unfolding; Inter-domain separation

1. Introduction

Serum albumins are the most abundant proteins present in the circulatory system [1]. They act as carrier for many endogenous substances like fatty acids, bilirubins, hormones and numerous small ligands [2–5]. Human serum albumin (HSA) (molecular weight 66,479 Da) comprising of 585 amino acid residues is a heart-shaped tridomain protein with each domain subdivided into two identical subdomains A and B [6]. Its amino acid sequence comprises of 17 disulfide bridges distributed over all domains, one free thiol (Cys34) in domain-I and a tryptophan residue (Trp214) in domain-IIA. HSA binds a wide variety of ligands with principal binding site being present in domains-IIA and IIIA [4]. HSA acts as an endogenous carrier of photosensitizing drugs known as porphyrins [7]. Porphyrins belong to a class of tetrapyrroles having extensive applications as photosensitizers in medicine [8,9]. Porphyrins are spectroscopically well characterized [10–12] and are known to serve as models for artificial solar energy capture as in photosynthesis [13]. The interaction of porphyrins with serum albumins has been a subject of extensive research till date [14–18]. Crystallographic study [19] reveals that protoporphyrin IX (PPIX), a member of porphyrin family, binds to domain-IB of HSA. Studies on binding of various drugs/ligands to different folded states of HSA are important as the conformation of carrier proteins depends on its immediate physiological environment. Moreover, clinical

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applications of HSA require good knowledge of thermal behavior of different folded states. Recently, advancement of time-resolved fluorescence and Fourier transform infra-red spectroscopy for other proteins open a scope for direct observation of protein folding pathways [20–22]. Also, recent advancement in the field of nanosciences requires the preparation of bioactive nanoparticles under different temperature and pH conditions using protein molecules as templates [23–25]. Hence, search for a protein molecule, which can retain its overall structure under conditions widely differing from the physiological condition is highly demanding. It is known that HSA undergoes reversible conformational transformation with change in pH of the solution containing the protein [26,27]. At normal pH 7, HSA assumes the normal form (N) which abruptly changes to highly charged fast migrating form (F) at pH values less than 4.3, as this form moves "fast" upon gel electrophoresis [1]. Upon further reduction in pH to less than 2.7 the F-form changes to the fully extended form (E). On the basic side of the normal pH above pH 8, the N-form changes to basic form (B) and above pH 10, the structure changes to another aged form (A) [28]. Serum albumin undergoes an ageing process when stored at low ionic strength at alkaline pH. The ageing process is catalyzed by the free sulfhydryl group and involves sulfhydryl-disulfide interchange that results in the conservation of the sulfhydryl at its origin position. This A-form is stable and does not undergo N–F transition [28,29]. Several studies on thermal denaturation of serum albumins have been carried out using differential scanning calorimetric [30–34], electronic and vibrational circular dichroism [35] and fluorescence techniques [36]. Previous studies involving resonance energy transfer on pH-induced folded states of HSA has been done using steady-state fluorescence spectroscopy [37,38] and chemically denatured HSA using time-resolved fluorescence spectroscopy [36]. To our understanding till date there exist no energy transfer studies on the thermally induced conformers of HSA at different pH(s). In this report we have studied the conformational transformation of three pH-induced conformers of HSA at three different temperatures – 25, 60 and 75 °C using circular dichroism (CD) and dynamic light scattering (DLS) techniques. We have also measured the inter-domain separation between domain-I and domain-II applying Förster's resonance energy transfer (FRET) between Trp214 (donor) in domain-IIA and PPIX (acceptor) in domain-IB using picosecond time-resolved fluorescence techniques.

2. Materials and methods

Human serum albumin (HSA), protoporphyrin IX (PPIX), sodium acetate, sodium dihydrogen phosphate, disodium hydrogen phosphate were procured from sigma chemical (St. Louis, USA). Hydrochloric acid, sodium hydroxide and dimethyl formamide (DMF) were procured from Merck. Double distilled water was used for preparation of aqueous solutions. The molecular weight of HSA

has been checked by MALDI mass spectrometry which essentially reveals a peak at 66.8 kDa (data not shown) consistent with the literature value [39]. All the other samples were used as received without further purification. Alkaline pH solutions were prepared by adding NaOH to phosphate buffer, while acidic pH solutions were prepared by adding HCl to acetate buffer. A stock solution of HSA was prepared in 10 mM phosphate buffer solution. HSA was labeled with PPIX as follows. About 3.5 mg of PPIX was dissolved in 100 μl DMF and injected in 5 aliquots of 20 μl each to 2 ml of phosphate buffer containing 200 μM HSA at an interval of 15 min under vigorous stirring condition. In order to prepare HSA–PPIX complex, the mixture was allowed to vigorously stir for 1 h and then a mild dialysis was carried out against phosphate buffer for 4.5 h to remove the unreacted PPIX. The HSA–PPIX solution was added in equal amounts to measured volumes of acidic/alkaline pH solutions and allowed to stir vigorously for two hours in order to achieve various pH-induced conformers of HSA–PPIX complexes. These samples were then used for spectroscopic studies.

Steady-state absorption and emission were measured with Shimadzu UV-2450 spectrophotometer and Jobin Yvon Fluoromax-3 fluorimeter respectively. The circular dichroism study was done using Jasco 815 spectropolarimeter using a quartz cell of path-length 10 mm. The secondary structural data of the CD spectra were analyzed using CDNN deconvolution program. Dynamic light scattering (DLS) measurements were done with Nano-S Malvern instruments (UK), employing a 4 mW He–Ne laser ($\lambda = 632.8$ nm) and equipped with a thermostatted sample chamber. All measurements were taken at 173° scattering angle at 298 K.

All the fluorescence transients were recorded using pico-second-resolved time correlated single photon counting (TCSPC) setup from Edinburgh instruments (LifeSpec-ps), UK. PPIX and Trp214 residue of HSA were excited using a laser source of 409 nm (Instrument response function, IRF ~ 86 ps) and a LED source of 299 nm (IRF ~ 460 ps), respectively. The observed fluorescence transients were fitted by using a nonlinear least square fitting procedure [40], the experimental details including the construction of temporal fluorescence anisotropy decay, $r(t)$ are published elsewhere [41].

In order to estimate the fluorescence resonance energy transfer efficiency of the donor Trp214 and hence to determine distances of donor–acceptor pairs, we followed the methodology described in chap. 13 of Ref. [42]. The Förster's distance (R_0) is given by,

$$R_0 = 0.211[\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6} (\text{in Å}) \tag{1}$$

where κ^2 is a factor describing the relative orientation in space of the emission and absorption transition dipoles of the donor and acceptor, respectively. The value of the orientation factor κ^2 is calculated from the equation [42]

$$\kappa^2 = (\sin\theta_D \sin\theta_A \cos\phi - 2\cos\theta_D \cos\theta_A)^2 \tag{2}$$