Journal of Photochemistry and Photobiology A: Chemistry 299 (2015) 73–79

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Effect of fibrillation on the excited state dynamics of tryptophan in serum protein – A time-resolved fluorescence study

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ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 2 November 2014
Accepted 13 November 2014
Available online 14 November 2014

Keywords:
Amyloid fibril
Time-resolved fluorescence
Solvation dynamics
Human serum albumin

ABSTRACT

The aggregation of proteins into the amyloid fibrils is mainly responsible for several neurological diseases. Knowledge of dynamics in amyloid fibril is very essential to understand its biological activity. Although, the effects of environment like pH, ionic strength, temperature, etc. on the fibril have been studied extensively, studies on the dynamics of amyloid fibril and the role of water in fibril are scarce. In this article, we have reported the results on the excited state dynamics of amyloid fibrils formed by a well-known blood plasma protein, human serum albumin (HSA), at neutral pH. The sole tryptophan residue, W214, has been used as the intrinsic fluorescent probe to monitor its excited state dynamics. Steady-state and time-resolved fluorescence data suggests that the W214 becomes more closer to the quencher amino acid residues in the fibrillar phase than in the native protein. From detailed time-resolved emission measurements, it is shown that despite having a more ordered structure, the water molecules around W214 in amyloid fibril is more labile than that in the native protein. Fluorescence depolarization studies also indicate that the W214 residue is located in a relatively more flexible region of the ordered amyloid fibril than that in the native protein.

1. Introduction

The aggregation of unfolded proteins and peptides into the filamentous form, known as amyloid fibrils, is responsible for several neurological disorders like Alzheimer's, Parkinson's, Huntington's and type II diabetes, etc. At present, twenty known diseases are related to the formation of amyloid fibrils in human body [1–8]. Most of the proteins form fibrils irrespective of their native structure indicating that fibrillation is a generic property of polypeptide chains [9–12]. The fibrillation occurs mainly under the condition that favors partially denatured or totally unfolded protein conformation [13,14]. The process follows through unfolding of protein, formation of oligomers, proto-fibrils, and finally to energetically stable matured fibrils [5]. However, it is also known that there are number of molecules that can de-aggregate or inhibit the fibril formation suggesting that the process of fibrillation is reversible in nature [15–17]. In some cases, de-aggregation of the fibrils is also seen to reduce the symptoms of the associated diseases [18,19]. Thus, it is very important to understand the structural and the dynamical behavior of the fibrils to develop new therapeutic strategies for amyloid based diseases. Although there are extensive biological and structural information on amyloid fibrils [20–23], limited information on the structural dynamics of amyloid fibrils is available in the literature.

Studies on the amyloid fibrils are mostly focused on the effect of protein conformation [13], amino acid sequence [1,24,25], and environmental conditions like pH, ionic strength etc. [26,27], to reveal the nature of protein–protein interactions. However, in all these studies water is treated as a spectator even though it is an important constituent in the biological systems [28]. In fact, the intermolecular interaction of proteins are highly dependent on the water molecules in their hydration shell [28]. Recently, Chong et al. have shown that the water molecules surrounding the protein mainly control its aggregation rather than the protein itself [29]. It is further shown that the electrostatic interaction between the protein and water molecules has substantial role in the protein aggregation process [30]. The importance of the water dynamics on the formation of amyloid fibril of ribonuclease A has been demonstrated by molecular dynamic simulation [31]. Simulation result shows that buried water molecules drive the structural dynamics of protein, whereas the surface water plays a vital role in protein aggregation. It is further shown that the surface water have very low residence time due to the dynamic exchange with the bulk water [32]. Thus, all these studies indicate that the structure and the dynamics of the water in and around the protein molecules play crucial role in the formation of amyloid fibril.

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http://dx.doi.org/10.1016/j.jphotochem.2014.11.012
1010-6030/

Recently, fibrillation of human serum albumin (HSA), an important blood plasma protein, has been investigated by several research groups [33–37]. Single tryptophan moiety (W214) in human serum HSA has been used as an internal fluorescent probe to report the local dynamics around it [38–41]. Native HSA protein has very low β-sheet content (~5%) and hence, the possibility of the presence of its pre-formed fibrils is negligible [42]. Formation of the partially unfolded form of the protein is the prerequisite for the formation of the amyloid fibril [43] and hence, heating at elevated temperature leads to the spontaneous formation of the HSA fibril [44]. The effect of pH, co-solvents, ionic strength, etc. on the fibrillation process of HSA has also been investigated in recent years [45–47]. Juarez et al. have shown that the electrostatic interaction between solvent and HSA affect the fibrillation kinetics, morphology and the extent of fibril formation [48]. Further, it is shown that the extent of hydrogen bonding between the solvent and the β-sheets can also change the morphology of HSA fibrils. Thus, it is evident that the interaction of water with proteins plays a very crucial role in HSA fibrillation. Although, it is well known that the interaction of water with protein controls its biological activity but very little is known about the interaction of water molecules with amyloid fibrils.

In the present study, we have tried to elucidate and understand the local structural flexibility of protein and the nature of water in the fibrillar state by monitoring the time dependent fluorescence Stokes' shift (TDFSS) of the sole tryptophan moiety (W214) in HSA. To the best of our knowledge, there is no report on the water dynamics in amyloid fibril using intrinsic tryptophan as a local reporter.

2. Materials and methods

HSA, Tris (hydroxymethyl aminomethane) buffer were purchased from Sigma–Aldrich and used without further purification. Nanopure water (conductivity less than 0.1 $\mu S\,cm^{-1}$) from a Millipore Milli Q system was used for all sample preparations. All samples were freshly prepared just before the experiments. HSA fibrils were prepared by the method reported by Juarez et al. [42]. HSA (2 mg/ml) was dissolved in Tris–HCl buffer (pH ~7.4) and heated to 65 °C for 4 h. The formation of HSA fibril was confirmed by thioflavin-T fluorescence assay [49]. Further, the structural changes in the protein due to aggregation [50–53] or due to its interaction with small molecules [54–56] can also be monitored by measuring the circular dichroism (CD) spectra. Thus, CD measurements have been performed to confirm the formation of HSA amyloid fibril (Fig. S1-see Supporting information).

Steady-state fluorescence spectra were measured with Hitachi F-4500 spectrofluorometer. The emission spectra were corrected for the wavelength-dependent instrument responses using the standard spectrum of tryptophan in aqueous solution [57]. The measured wavelength domain spectra, $I(\lambda)$, were converted into the frequency domain spectra, $I(\nu)$, using the following equation [58]:

$$I(\nu) = \lambda^2 I(\lambda) \tag{1}$$

The fluorescence transients were collected over the entire emission spectrum of W214 at 10 nm intervals using time-correlated single-photon counting (TCSPC) instrument (IBH, UK). The samples were excited with 295 nm LED. The instrument response function (IRF) was determined by measuring the scattered light from the suspended TiO_2 in water and was found to be ~0.78 ns. All fluorescence decays were fitted with multi-exponential function using an iterative convolution method [59]. Time-resolved emission spectra (TRES) was constructed from the fitted parameters of the fluorescence decay traces following the method proposed by Maroncelli and Fleming [60]. The calculated data points for TRES were fitted with the following lognormal function [60].

$$I(\nu) = A\exp\left[-\ln(2)\left\{\frac{1}{B}\ln\left(1+\frac{2B(\nu-\nu_P)}{\Delta w}\right)\right\}^2\right] \quad if \frac{2B(\nu-\nu_P)}{\Delta w} > -1$$
$$= 0 \text{ if } \frac{2B(\nu-\nu_P)}{\Delta w} \le -1 \tag{2}$$

In the above equation, A is the amplitude, ν_P is the peak frequency, Δw is the spectral width parameter, and B is the asymmetry parameter. Full width at half maximum (FWHM) of TRES was calculated from Δw and B by the following equation [60].

$$FWHM = \Delta w\frac{\sinh(B)}{B} \tag{3}$$

Time-dependent fluorescence anisotropy was calculated from the following equation [58]

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \tag{4}$$

where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence decays with emission polarizations parallel and perpendicular to the vertically polarized excitation light. The factor G is introduced to consider the polarization dependent sensitivity of the detection system and is measured independently.

3. Results and discussions

3.1. Steady-state emission measurements

The steady-state emission spectra of W214 in native protein and in fibril, on excitation with 295 nm light, have been recorded and are shown in Fig. 1. The excitation wavelength is chosen in such a way that only tryptophan residue of the protein will be excited preferentially [58]. HSA, in its native form, shows emission maximum at 345 nm [61]. However, the emission maxima for amyloid fibril appears at 337 nm, which is 8 nm blue shifted as compared to its native form. Such blue shift in the emission spectra of W214 in HSA due to the fibrillation process is reported in the literature [46]. The observed blue shift indicates that the microenvironment of W214 changes substantially due to the formation of the amyloid fibril from the native protein [58]. Further, preferential quenching in the red side of tryptophan emission spectrum by charged amino acid residues can also cause the observed blue shift in the emission spectra in the fibrillar

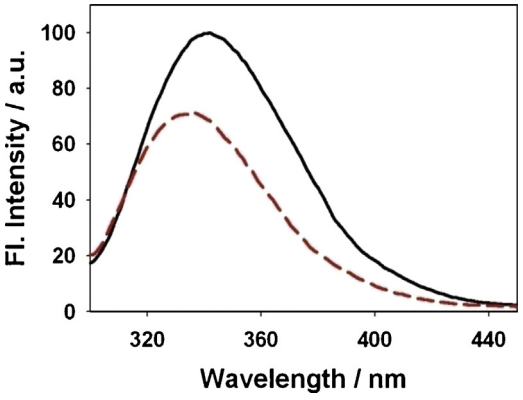

Fig. 1. Steady-state emission spectra of HSA (2 mg/ml in Tris–HCl buffer pH 7.4) native protein (—) and fibril (------) on excitation with 295 nm light.