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Transition of transferrin from native to fibrillar state: An implication for amyloid-linked diseases

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ABSTRACT

Human transferrin (hTF), an α/β protein, transforms from its native soluble form to proto-fibrils and amyloid fibrils at 20% TFE after prolonged incubation. This type of amyloid fibrils is observed in a number of pathological disorders. Existence of dry molten-globule state, at 5% TFE, was characterized by native-like secondary structure, Trp fluorescence and negligible ANS binding, indicating its dry interior. At 15% TFE, decrease in Trp and increase in ANS fluorescence was observed with native-like secondary structure, indicating exposure to water molecule and hence, this was referred to as Wet MG state. AFM revealed protofibrils as smaller in size howbeit amyloid fibrils were long and stiffer in morphology. Amyloid fibrils were found to possess cross-linked β-sheet, lack of tertiary contacts, as revealed by CD and ATR-FTIR, enhanced Thioflavin T fluorescence and shift in Congo red absorbance. These results showed that formation of amyloid fibrils becomes favorable when protein is destabilized in suitable conditions and non-covalent interactions, particularly intermolecular hydrogen bonding becomes prominent. Protofibrils were genotoxic in nature albeit amyloid fibrils lack this effect.

1. Introduction

Every polypeptide has a natural tendency to transform from native state to toxic aggregates in the presence of destabilizing conditions [1]. Misfolded proteins gets deposited in different tissues as amyloid or plaques that are the origin of a number of degenerative diseases such as Alzheimer's and Parkinson's diseases, type II diabetes mellitus, spongiform encephalopathy and a wide range of amyloidosis [2]. Protein aggregates, in the form of amyloid plaques, neuro-fibrillary tangles, and/or intra-cytoplasmic or intra-nuclear inclusions, are thought to cause damage to cell membrane and disruption of ion homeostasis, induction of apoptosis and finally, cell death [3]. Stefani and others argued, whether mature amyloid fibrils (AF) or early formed aggregates are responsible for cell toxicity [4]. But recently, Stefani proposed that small soluble oligomeric amyloid intermediates are the main for cell toxicity [5]. Although a number of models have been suggested for the formation of protofibrils (PF), but the most popular accepted model depicts that either the refolding of polypeptide into parallel, left-handed β-helix or the 3 dimension domain swapping [6] is responsible for PF formation. A deeper understanding of the comprehensive mechanism of protein aggregation and the resulting cellular toxicity should lead to rational drug design for the above mentioned diseases. Thus characterization of these partially folded states is the chief purpose in protein folding study. Biophysical examination of aggregation processes normally requires the use of chemical cosolvents to reduce experimental inconsistency, imitate cellular environment, or provoke the formation of atypical aggregate structures. 2,2,2-Trifluoroethanol (TFE) is commonly used as a co-solvent to alter the conformations of proteins and peptides because of its ability to disrupt tertiary interaction by lowering solvent polarity and they favor α-helical structure by promoting intra-molecular hydrogen bonding [7]. TFE also impersonate the role of membranes in inducing structural alterations and aggregation of protein, which make this type of study commendable for analyzing protein conformation. Transferrin family is a collection of proteins that function in the transport of iron around the blood stream after forming an iron-protein complex and they also acts as bacteriostatic agents in a variety of biological fluids [8]. Human transferrin (hTF) is a glycoprotein consisting of 679 amino acid residues with a molecular weight of 79570 Da. It has a combination of α-helices and β-sheet

Abbreviations: AFM, atomic force microscopy; ATR-FTIR, attenuated total reflection Fourier transformed infra red spectroscopy; AF, amyloid fibrils; ANS, 8-Anilino-1-naphthalene-sulphonic acid; CD, circular dichroism; CR, Congo red; DMG, dry molten globule; PF, proto-fibrils; SCGE, single cell gel electrophoresis; ThT, thioflavin T; TFE, trifluoroethanol; WMG, wet molten globule.

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which comprises a bi-lobal protein having N- and C-terminal each of which reversibly binds to a ferric (Fe^{3+}) ion. A concomitant binding of an anion, mainly carbonate anion, is essential for iron binding [9]. Fe^{3+} is bound octahedrally to the side chains of Tyr^{95}, Tyr^{188}, Asp^{63}, His^{249}, and two oxygens from carbonate. hTF, when bound to two Fe^{3+}, becomes more compact and highly resistant to denaturation in comparison to the unbound state. Native hTF is folded in a way so as to create a cleft having favorable environment for Fe^{3+} binding. This environment is favorable not only for iron but also for other metal ions. Approximately half of the total hTF present in an adult body is located in blood plasma while the remaining are distributed among different body fluids including lymph, tears, cerebrospinal fluid, bile, amniotic fluid, milk, saliva, aqueous humor and seminal fluid. About 4% of the plasma protein content is plasma transferrin (2.5 g/l) and it is the fourth most abundant plasma protein. hTF also plays an important role in proliferation and differentiation along with antioxidant activity [10]. Besides this, it can be employed as cell culture media supplement and can also serve as a conjugation (fusion) partner to stabilize and improve the pharmacokinetics of certain therapeutically-important biomolecules in the drug delivery process. Whenever there is deficiency of hTF, as in rheumatoid arthritis, osteoarthritis, kidney failure or certain forms of cancer, iron is retained in the storage pool. This hampers red blood cells production owing to the lack of iron in bone marrow. Reduction in red blood cell production causes anemia of chronic disease. A number of diseases like haemophilia, haemolytic anaemia, diabetes mellitus etc occur when there is heme loss in the body. Therefore, TFE can be implied in discovering remedial ways for such diseases.

In this study, we investigate TFE-induced structural rearrangement and aggregation using hTF as a model system. Our analysis has shown that after 4 h incubation hTF at 5% and 15% v/v[1] TFE convert to its dry molten globule (DMG) and wet molten globule (WMG) state, respectively, and form aggregates at 20% TFE. Aggregates on further incubation for 24 and 48 h (in the similar environment) form PF and AF, respectively.

2. Materials and methods

2.1. Materials

Transferrin from human plasma was purchased from Sigma (St. Louis, MO, US). Purity of hTF was checked by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. 8-Anilino-1-naphthalene-sulphonic acid (ANS), thioflavin T (ThT) and Congo red (CR) were bought from Sigma (St. Louis, MO, USA). Sodium phosphate monobasic and dibasic was purchased from SRL (Mumbai, India) for making sodium phosphate buffer of pH 7.

Stock solution of 5 mg/ml hTF was prepared in 20 mM sodium phosphate buffer of pH 7 and it was then dialyzed in the same buffer. The concentration of native protein in 20 mM sodium phosphate buffer, pH 7, was determined from extinction co-efficient of 11.1 A/1%/1 cm by UV absorption at 280 nm on a Shimadzu UV-1700 spectrometer with the help of a cuvette having 1 cm path length.

2.2. Sample preparation

Aliquots of hTF were prepared with varying concentration of TFE i.e. 0% to 50% at pH 7. These were then incubated at 25 °C for 4 h followed by 24 and 48 h before carrying out spectroscopic methods. Three replicates for each set were analyzed for the results.

[1] Unless and otherwise indicated all the TFE concentration has been expressed in v/v

2.3. Intrinsic fluorescence measurements

The fluorescence spectra were recorded on a Shimadzu RF-5301 spectrofluorophotometer (Tokyo, Japan) in a 10 mm path length quartz cell. The excitation wavelength was 295 nm and the emission was recorded in the range of 300–400 nm [11]. Finally, 2.5 μM hTF was present in the aliquots.

2.4. Acrylamide quenching studies

In the acrylamide-quenching experiments, aliquots of 5 M acrylamide stock solution were added to a protein stock solution (15 μM) to achieve the desired acrylamide concentration. Aliquots were excited at 295 nm so as to excite only Trp fluorescence and the emission was recorded in the range of 300–400 nm. The decrease in fluorescence intensity at λ_{max} was analyzed according to the equation given by Stern–Volmer [12]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where F_0 and F are the fluorescence intensities at an appropriate wavelength in the absence and presence of acrylamide, respectively, K_{SV} is the Stern–Volmer constant for the collisional quenching process, and [Q] is acrylamide concentration

2.5. ANS fluorescence measurements

ANS binding was measured by fluorescence emission with excitation wavelength at 380 nm and emission spectra were recorded from 400 to 600 nm. Protein (2.5 μM) was incubated with 100 fold molar excess of ANS for 30 min.

2.6. Attenuated total reflection Fourier transformed infra red spectroscopy (ATR-FTIR)

ATR-FTIR spectra were recorded with an Interspec 2020 FTIR spectrometer in D_2O in the amide I region in the range of 1710 to 1550 cm^{-1}. 75 μM of hTF was taken for analysis. The scanning wave number was from 1000 to 4000 cm^{-1} [13].

2.7. Circular dichroism (CD) measurements

CD was measured with a JASCO J-810 spectropolarimeter equipped with a Jasco Peltier-type temperature controller (PTC-424S/15) and calibrated with ammonium D-10-camphorsulfonate. All the CD measurements were carried out at 25 °C with scan speed of 100 nm/min and response time of 1 s. hTF concentration for far and near-UV CD is 3.75 and 25 μM, respectively. Cells of path length 0.1 and 1 cm was used for scanning between 250–200 and 300–250 nm, respectively. For good signal to noise ratio, each spectrum was the average of 4 scans. Base lining and analysis were done using Jasco J-720 software. The results were expressed as MRE (Mean Residue Ellipticity) in deg/cm^2/dmol which is defined as:

$$\text{MRE} = \frac{\theta_{obs} \times \text{MRW}}{C \times l \times 10}$$

where θ_{obs} is the CD in milli-degree, MRW is the mean residue weight (~117 for hTF), l is the path length of the cell in cm and C is concentration in mg/ml. Helical content was calculated from value of the α-helical content was computed using DICHROWEB software [14] with the help of K_2D algorithm [15].

2.8. Size exclusion chromatography (SEC)

SEC experiments were carried out on a Sephadex G 200 (76 91.15) cm column. The column was pre-equilibrated with 20 mM