Available online at www.sciencedirect.com
ScienceDirect
Archives of Biochemistry and Biophysics 457 (2007) 160–169
ABB
www.elsevier.com/locate/yabbi

Role of electrostatic interactions in 2,2,2-trifluoroethanol-induced structural changes and aggregation of α-chymotrypsin

Nasrollah Rezaei-Ghaleh [a], Azadeh Ebrahim-Habibi [a], Ali Akbar Moosavi-Movahedi [a], Mohsen Nemat-Gorgani [a,b,*]

[a] *Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran*
[b] *Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA*

Received 21 August 2006, and in revised form 23 October 2006
Available online 2 November 2006

Abstract

It has been recently demonstrated that α-chymotrypsin (CT) can be driven toward amyloid aggregation by addition of 2,2,2-trifluoroethanol (TFE), at intermediate concentrations. In the present article, the process of TFE-induced CT aggregation was investigated in more detailed kinetic terms where the effects of medium conditions, such as temperature, presence of kosmotropic and chaotropic salts, pH and chemical modification of lysine residues were examined. Various techniques, including light scattering, fluorescence and circular dichroism spectroscopy, were used to follow and characterize this process. The kinetics of aggregation was found to obey a second-order reaction with respect to protein concentration. The aggregation-prone A-state and aggregation-deficient TFE- or T-state of CT were found to be induced at lower TFE concentrations in the presence of salts. Use of acidic and alkaline conditions and lysine modification also promoted the formation of the T-state. Results presented suggest a role for electrostatic interactions in the aggregation process.

Keywords: Aggregation; α-Chymotrypsin; Trifluoroethanol; Amyloid; Electrostatic interaction; Hydrophobic interaction

The study of protein aggregation, the self-association of non-native polypeptide chains to form amorphous or ordered multimeric complexes, is one of the most exciting frontiers in protein science today [1]. Protein aggregation may occur in the form of inclusion bodies during protein expression in host cells or as a kinetically competing reaction during the recovery of active proteins, hence diminishing the yield of productive refolding in biotechnological processes [2,3]. Furthermore, it is well established that protein misfolding followed by aggregation into amyloid fibrils may lead to a group of pathologic states known as amyloid diseases [4,5]. Alzheimer's, Parkinson's and Huntington's diseases, transmissible spongiform encephalopathies and systemic amyloidoses are some examples of this important group of diseases [6]. Due to the clinical and biotechnological importance of protein aggregation, there is now a strong motivation to characterize the aggregation-prone state of polypeptide chains, elucidate the mechanism of protein aggregation, and seek novel methods for controlling this process. It is widely believed that the partially folded molten globule-like protein structures are especially susceptible to aggregation [7–9]. Diverse physical and chemical measures, e.g. temperature, pressure, pH, co-solvents and ligands, could be used to induce such partially unfolded structures [7,10], which may also be populated transiently during folding/refolding events [9].

The effects of alcohol co-solvents on proteins and peptides have been studied extensively over the last few decades [11,12]. Various alcohols, especially those substituted

* Corresponding author. Fax: +1 650 812 1975.
E-mail address: mohsenn@stanford.edu (M. Nemat-Gorgani).

0003-9861/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2006.10.031

with fluorine such as 2,2,2-trifluoroethanol (TFE)[1] and 3,3,3,3′,3′,3′-hexafluoro-2-propanol (HFIP) have been found very effective in causing protein denaturation and inducing the α-helical structure in peptides [12]. This effect can presumably be attributed to low dielectric constants of alcohols [13,14]. The drop in solvent polarity weakens solvophobic interactions that stabilize the compact native structure of proteins, and simultaneously strengthens electrostatic interactions, thereby stabilizing local secondary structures, particularly the α-helix [15]. The specially marked effect of TFE and HFIP on protein structure has been attributed to the formation of relatively large micelle-like clusters of alcohol molecules, resulting in a high local alcohol concentration [16]. The helix-inducing effect of TFE has been either explained by its direct stabilizing effect on the folded chain [17,18] or, alternatively, by its selective destabilization of the unfolded structure [11,19,20]. The detailed effects of TFE on protein conformation have been found to be diverse, depending on experimental conditions, as well as on the structures involved [21]. Some proteins may exhibit a distinct aggregation-prone state at low TFE concentrations before they reach the α-helical-rich state (called as TFE-state), while other proteins are directly transformed to TFE-state [22]. In one case, this aggregation-prone state of protein has been claimed to have molten globule-like features and resemble the kinetic intermediate appearing at the early stages of protein folding [22].

The kinetic mechanism of polypeptide chain aggregation has been frequently investigated [23–26]. Of the various models, formation of amyloid aggregates is proposed to obey a nucleation-dependent mechanism [27,28]. The effect of various solution conditions such as temperature, the presence of co-solvent and hydrostatic pressure, on the mechanism and the rates of protein aggregation have been examined to gain insight into transition states associated with protein aggregation [29]. However, the simple model described here, in addition to those reported in the literature, do not address all of the diverse issues related to this intricate process.

There exists an abundant amount of evidence suggesting that amyloid fibril formation is a generic property of polypeptide chains, being the most stable species of the polypeptide chain under specific conditions [30]. It has been recently demonstrated that α-chymotrypsin, a well-known serine protease with an all-beta fold, could be driven toward amyloid aggregation by addition of TFE at intermediate concentrations [31]. α-Chymotrypsin is a three-chain protein connected by five inter- and intra-chain disulfide bonds. This protein is folded into two anti-parallel β-barrel domains consisting of a Greek key motif followed by an anti-parallel hairpin motif [32]. The purpose of the present study was to investigate the process of TFE-induced α-chymotrypsin aggregation in more detailed kinetic terms and to examine the effects of medium conditions on the rate of aggregation. It is suggested that the resulting information may be useful in providing mechanistic insights into TFE-induced structural changes and aggregation of α-chymotrypsin and other protein structures.

Materials and methods

Materials

α-Chymotrypsin, 2,2,2-trifluoroethanol (TFE), thioflavin-T (ThT), Congo red, *N*-benzoyl-L-tyrosyl-ethyl ester (BTEE), citraconic anhydride, fluorescamine and sodium sulfate were purchased from Sigma (St. Louis, MO). 1-Anillino-8 naphthalene sulfonate (ANS), di-potassium hydrogen phosphate, ammonium sulfate, ammonium thiocyanate and sodium chloride were obtained from Merck (Darmstadt, Germany). Unless otherwise stated, all solutions were made in 50 mM K_2HPO_4 buffer (pH 7.0). The concentration of protein was determined by measuring 280-nm absorbance using an absorbance of 20.4 for 1 g/100 ml protein solution [33]. The reported results are averages of three separate experiments whenever the coefficients of variation were less than 5%.

Turbidity measurements

Turbidity measurements were made at specified wavelengths on a Cary-100 Bio VARIAN spectrophotometer. Temperatures were controlled to within ±0.1 °C by a Cary temperature controller. The path length of the sample cell used was 10 mm, so turbidity of samples (τ) in cm^{-1} unit was calculated by multiplying the measured optical density at 2.303. To determine the wavelength (λ) dependence of turbidity, the apparent absorbance of protein samples were obtained by scanning between 320 and 800 nm in 1 nm intervals, then $\ln\tau$ was plotted against $\ln\lambda$ and the slope of the best-fitted line was calculated as the turbidity wavelength exponent.

Centrifugation

Aliquots of the 100 μl sample were centrifuged at 13,000 rpm (9000*g*) for 20 min using the Beckman Avanti™ 30 centrifuge. The supernatant was removed and mixed with 900 μl buffer, while the pellet was re-suspended in 1000 μl buffer for each sample tested. Protein concentrations of the supernatant and re-suspended pellets were determined by measuring absorbance at 280 nm.

Density measurements

Density measurements were performed using a PAAR density meter (DMA58, Austria) at 25 °C, after calibration with deionized water and dry air at this temperature.

Quasielastic light scattering (QLS)

QLS experiments were conducted at room temperature with a SEMATECH correlator and a helium–neon laser (633 nm). The scattering angle was 90°. Protein concentration was 4 μM and TFE v/v percent 32%. Since the estimated size of TFE clusters at this concentration of TFE is well below the expected size of protein aggregates [22], there should be no interference with the scattering investigation of protein aggregation. To remove large dusts, samples were filtered twice by Whatman filter papers

[1] *Abbreviations used:* TFE, 2,2,2-trifluoroethanol; HFIP, 3,3,3,3′,3′,3′-hexafluoro-2-propanol; ThT, thioflavin-T; BTEE, *N*-benzoyl-L-tyrosyl-ethyl ester; ANS, 1-anillino-8 naphthalene sulfonate; QLS, quasielastic light scattering; CD, circular dichroism; DSC, differential scanning calorimetry.