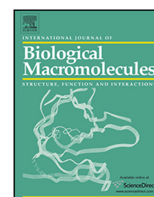
International Journal of Biological Macromolecules 92 (2016) 573–580

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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



Amyloid-like aggregates formation by bovine apo-carbonic anhydrase in various alcohols: A comparative study

Ali Es-haghi [a,*], Azadeh Ebrahim-Habibi [b], Marjan Sabbaghian [c], Mohsen Nemat-Gorgani [d]

[a] *Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran*
[b] *Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Andrology at Reproductive Biomedicine Research Center Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran*
[d] *Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA*

ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form 11 July 2016
Accepted 24 July 2016
Available online 25 July 2016

Keywords:
Carbonic anhydrase
Metalloprotein
Pre-molten globule
Alcohol
HFIP

ABSTRACT

Peptides and proteins convert from their native states to amyloid fibrillar aggregates in a number of pathological conditions. Characterizing these species could provide useful information on their pathogenicity and the key factors involved in their generation.

In this study, we have observed the ability of the model protein apo-bovine carbonic anhydrase (apo-BCA) to form amyloid-like aggregates in the presence of halogenated and non-halogenated alcohols. Far-UV circular dichroism, ThT fluorescence, atomic force microscopy and dynamic light scattering were used to characterize these structures. The concentration required for effective protein aggregation varied between the solvents, with non-halogenated alcohols acting in a wider range. These aggregates show amyloid-like structures as determined by specific techniques used for characterizing amyloid structures. Oligomers were obtained with various size distributions, but fibrillar structures were not observed. Use of halogenated alcohols resulted into smaller hydrodynamic radii, and most stable oligomers were formed in hexafluoropropan-2-ol (HFIP). At optimal concentrations used to generate these structures, the non-halogenated alcohols showed higher hydrophobicity, which may be related to the lower stability of the generated oligomers. These oligomers have the potential to be used as models in the search for effective treatments in proteinopathies.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Alcohols affect proteins and peptides native conformations, may induce helices, dissolve peptide aggregates, or be used as co-solvents to induce fibrillar structures [1]. Amyloid fibrils occupy an important place in the pathophysiology of several diseases such as Alzheimer's disease, dialysis-related amyloidosis, and type II diabetes mellitus [2]. In addition, a number of proteins and peptides have been observed to form similar fibrillar deposits *in vitro*, even though not related to any pathology [3]. Numerous studies have been conducted on these biologically important protein forms, but the fine details of fibril formation mechanism are still not elucidated [4]. For Most proteins, fibril formation will initiate under denaturing conditions [5], provided by a variety of conditions, including halogenated alcohols [6].

We have used carbonic anhydrase as a model protein to address the lack of a comprehensive study on various alcohols in a similar setting. Carbonic anhydrase II (CA, EC 4.2.1.1) is a globular protein of 259 amino acids with a molecular weight of 30 kD. Found in red blood cells, this enzyme is normally present as a monomer with a zinc atom in the active site. The bovine enzyme is composed of 10 β-strands and a small amount of α-helices [7,8]. At low pH, the apo form of bovine carbonic anhydrase (apo-BCA) unfolds via an apparent four-state process, comprising molten and pre-molten globule species [9]. Apo-BCA molten globule tends to form amorphous aggregates when incubated at high temperature, while pre-molten globule is able to convert to amyloid fibrils. Surface hydrophobicity and conformation of the starting structures are likely to play important roles in the obtained aggregates characteristics [10].

Here, we have compared the amyloid-like structures formed by apo-BCA in the presence of various concentrations of halogenated (TFE, HFIP) and non-halogenated (methanol, ethanol, propanol)

Abbreviations: BCA II, bovine carbonic anhydrase II; PMG, pre molten globule; MG, molten globule; ThT, thioflavin T; CD, circular dichroism; AFM, atomic force microscopy; ANS, 1-anilino-naphthalene 8-sulfonate; DLS, dynamic light scattering; TFE, 2,2,2-trifluoroethanol; HFIP, 1,1,1,3,3,3-hexafluoroisopropanol.

* Corresponding author at: Faculty of science, Department of biology, Islamic Azad university, Mashhad Branch, Mashhad, Iran.
E-mail address: ashaghi@gmail.com (A. Es-haghi).

http://dx.doi.org/10.1016/j.ijbiomac.2016.07.083
0141-8130/© 2016 Elsevier B.V. All rights reserved.

alcohols, using CD spectroscopy, ThT fluorescence and atomic force microscopy (AFM). In the absence of alcohol and at low pH, apo-BCA takes the pre-molten globule form [11]. Addition of alcohols to the protein causes its aggregation, and these aggregates are capable to bind specific amyloid-reporting molecules. To binding to these compounds (ThT and Congo red), extensive β-sheet structures should be present in the protein, and these are characteristic of amyloid structures [12]. These species may be related with the protein and peptide states involved in the “protein deposition diseases” and could be useful for cell toxicity assays and for modeling pathogenic states of the actual disease-linked proteins.

2. Results and discussion

2.1. Detection of structural changes by ThT fluorescence and CD spectra

Effects of buffer type, ionic strength, solution pH, and different concentrations of the protein have been previously investigated on amyloid or amyloid-like formation [10]. Only when the protein is incubated at pH 2.4, and takes a pre-molten globule state, is it able to move toward amyloid formation. At pH 3.5, in which the protein has a molten globule structure [13], amyloid formation is not observed. This indicates the importance of both structure flexibility and hydrophobicity in the polymerization process [14].

Here, purified protein 133 μM was incubated in a 50 mM Glycine buffer (pH 2.4), at room temperature, and in the presence of various concentrations of alcohols for 24 h. ThT assay was performed, which is a widely used technique to detect amyloid aggregates, and based on fluorescence emission at 485 nm. As shown in Fig. 1, sharp increases in emission intensity were observed in different ranges of alcohol concentrations. Accordingly, far UV-CD spectra of these samples showed conformational changes of the protein which indicates the presence of β-structure in the aggregates. CD spectrum of Apo-BCA at pH 2.4 has a minimum at 200 nm, which shows the presence of the pre-molten globule structure [11], and appearance of a negative peak at about 215 nm in far- CD spectrum is taken as the sign of beta-amyloid-type structure formation [15]. The term “amyloid-like” has been previously used for designing stable oligomeric structures which could be detected with amyloid-specific methods, but do not form the distinct fibrils that are characteristic of “amyloids”[16–18].

These results show suitable concentrations of the tested alcohols for amyloid-like aggregates formation ofapo-BCA. For non-halogenated-alcohols, as an increase occurs in carbon chain length, a higher concentration is required for amyloid-like aggregate formation. Optimal concentration for methanol to form amyloid-like aggregates was 35% (v/v), while in the case of ethanol a 20–60% (v/v) concentration range resulted in amyloid-like aggregates and the optimal concentration (i.e. concentration needed for maximum aggregation) was 45%. For 1 – propanol, aggregates were formed in the range of 40%–60% (v/v), and the optimal concentration was 60% (v/v). At higher concentration of propanol (above 65%), the protein was precipitated. Similar results were obtained for isopropanol, indicating that the position of the hydroxyl group has no effect on the aggregation process.

For halogenated alcohols, the process was different and only a small range of alcohol concentrations resulted in formation of amyloid-like structures. The optimal concentration to form amyloid-like aggregates was 12% and 5% for TFE and HFIP, respectively. The effect of HFIP is significantly different from other alcohols, because at high concentrations (greater than 50%–70%), it can induce alpha-helix in protein, which requires a large structural rearrangement. In the literature, this abnormal protein α-helix is described as an “open helical state” [19]. At high concentrations of HFIP, the hydrophobicity of the solution is high and may stabilize the monomeric state of α-helical proteins, inhibiting amyloid-like aggregate formation [20]. In a recent study, it has also been reported that at high concentrations of TFE, alpha helix structure**s** are detected in human carbonic anhydrase [21].

Electrostatic and hydrophobic interactions are both involved in the process. Low polarity of a solvent results in decreased strength of hydrophobic interactions (crucial in protein structural integrity) when at the same time, local hydrogen bonds are reinforced [22]. More hydrophobic alcohols with longer chain may thus exert their effects at higher concentration (e.g. propanol versus methanol).

On the other hand, dielectric constant decreases in organic solvents. Dielectric constant of TFE is 27, while that of water is 78; [23] consequently, enhanced electrostatic interactions, including polar interactions and charge–charge interaction, may occur in water-alcohol mixtures [24,25]. At low concentrations of HFIP, hydrophobic and electrostatic interactions are enhanced [26]. In HFIP and TFE, dynamics clusters are formed, to a higher extent compared with other alcohols [27,28]. Protein interaction with these dynamic clusters may increase the overall hydrophobicity, leading to aggregation [29]. This may explain why a particular concentration of HFIP and TFE are required to form amyloid-like structures.

Table 1
Height of oligomers formed in various alcohols. At least 20 oligomer samples (N) were selected and their height was measured by WSxM software. SD stands for standard deviation.

alcohol	Average height of oligomer (nm)	S.D.	N
HFIP	2.02	0.49	41
TFE	2.92	0.60	26
	7.23	1.01	26
	11.10	1.19	26
Methanol	8.59	1.09	22
	12.93	1.77	32
Ethanol	5.23	0.70	41
Propanol	7.46	0.66	38
	10.28	1.10	38

2.2. Aggregates morphology observed with atomic force microscope (AFM)

Structures formed under optimal concentrations of each alcohol were probed with the use of atomic force microscopy. Fig. 2 shows the images obtained for apo-BCA after 24 h of incubation in each optimal concentration of the alcohols. The observed aggregates possess regular structures since they bind to ThT and the CD spectra show that they contain large amounts of β- structure (Fig. 1), but mature fibrils were not formed. The height of each oligomer was analyzed by WSxM software, and results of statistical analysis shown in Table 1. Oligomers generated in HFIP and ethanol have uniform heights, while in other alcohols heterogeneity is observed. In HFIP, species with 2.02 ± 0.495 nm in height were generated while oligomers formed in 12% TFE had three different heights of 2.9 ± 0.600, 7.2 ± 1.01 nm, and 11.1 ± 1.195 nm. Two different heights of oligomers were also detected in 35% methanol as 8.5 ± 1.092 and 12.9 ± 1.77 nm.

In ethanol, only particles with heights of 5.23 ± 0.705, and in propanol two particles population with heights of 7.46 ± 0.664 and 10.28 ± 1.104 were formed. Maximal heights of oligomers formed in fluroalcohols are thus slightly less than oligomers produced in non- halogenated alcohols (Table 1).

In summary, based on AFM images, high amounts of large aggregates with various heights have been obtained in various alcohols with amyloid-like properties and high β-structure contents.

Similarly, stable oligomers have been observed for the Hypf-N protein in the presence of TFE and under alkaline conditions,

Download English Version:

https://daneshyari.com/en/article/8329725

Download Persian Version:

https://daneshyari.com/article/8329725

Daneshyari.com