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In vitro disintegration of goat brain cystatin fibrils using conventional and gemini surfactants: Putative therapeutic intervention in amyloidoses

Waseem Feeroze Bhat[a], Imtiyaz Ahmad Bhat[b], Sheraz Ahmad Bhat[a], Bilqees Bano (Prof.)[a,*]

[a] *Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, UP, India*
[b] *Department of Chemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, UP, India*

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ABSTRACT

Many protein misfolding diseases in mammalian system are characterised by the accumulation of protein aggregates in amyloid fibrillar forms. Several therapeutic approaches include reduction in the production of the amyloidogenic form of proteins, increase in the clearance rate of misfolded or aggregated proteins, and direct inhibition of the self-assembly process have been explained. One of the possible remedial treatments for such disorders may be to identify molecules which are capable of either preventing formation of fibrils or disintegrating the formed fibrils. In this work, we have studied the effect of conventional surfactants; sodium dodecylsulphate (SDS), cetyl trimethylammonium bromide (CTAB) and dicationic gemini (16-4-16) surfactant on the disintegration of the goat brain cystatin (GBC) fibrils above their critical micelle concentrations (CMC) using ThT fluorescence, CD, TEM, Congo red and turbidity approaches. The results obtained are significant and showing the best disintegrating potency on GBC fibrils with gemini surfactant. The outcome from this work will aid in the development and/or design of potential inhibitory agents against amyloid deposits associated with amyloid diseases.

1. Introduction

Protein misfolding attributes to the failure of a protein to achieve its native conformation efficiently or its inability to maintain that conformation due to minimization in stability as a result of mutation or environmental change or interaction with other molecules. It has been accepted that failure of protein folding is a general phenomenon at elevated temperatures and under other stressful conditions such as local change in pH, heat and oxidative stress [1]. The most common consequences of misfolded proteins are degradation, aggregation and amyloid fibril formation. When a polypeptide emerges from the cell, it may fold to the native state, degrade by proteolysis, or form aggregates. Aggregation usually results in disordered species that can be degraded within the organism but it may also result in highly insoluble fibrils that accumulate in tissues. Amyloid fibrils are ordered protein aggregates that have comprehensive beta sheet structure due to intermolecular hydrogen bonds. The formation of the amyloid fibrils is the result of prolonged exposure to at least partially denatured conditions. There are about twenty known diseases resulting from the formation of amyloid material including Alzheimer's and Parkinson's diseases, the spongiform encephalopathies and late-onset diabetes, are known to be directly associated with the deposition of such aggregates in tissues [2].

Although this layout knowledge, the way by which protein aggregation results in pathological behaviour is not yet understood in detail. In the case of systemic disease, the insoluble protein may physically disrupt the functioning of specific organs [3]. However for neurodegenerative disorders, such as Alzheimer's disease (AD), it is known that the primary symptoms result from the destruction of cells such as neurons by a "minimization in the function of communication" that results from the fibril formation process [4]. It has recently been found that the early pre-fibrillar aggregates of proteins associated with neurological diseases can be highly damaging to cells. Also as with the advance of age it is likely that the activity of our chaperone response and degradatory mechanisms declines, similarly, the new agricultural practices, a changing diet and new medical procedures have been adopted [5,6]. These in turn

Abbreviations: GBC, goat brain cystatin; SDS, sodium dodecylsulphate; CTAB, cetyl trimethylammonium bromide.

* Corresponding author.
E-mail address: bilqeesbano99@gmail.com (B. Bano).

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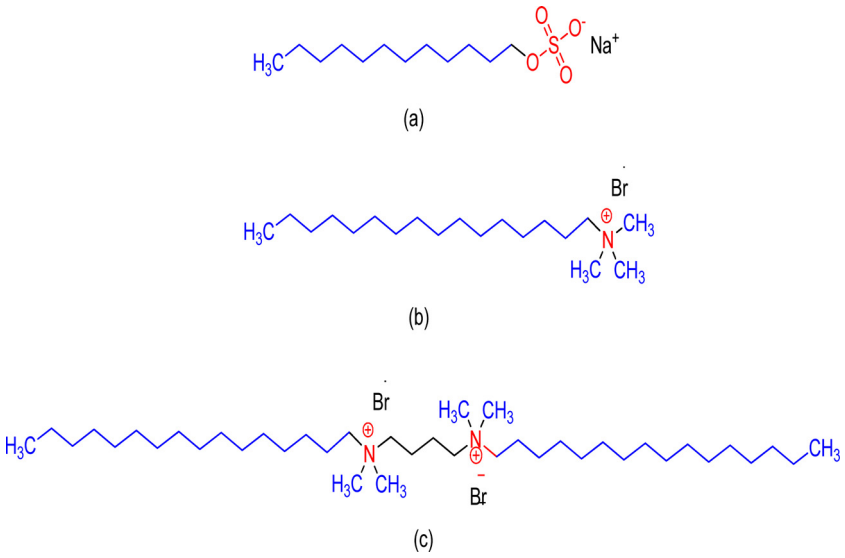

Fig. 1. Molecular structures of selected surfactants: (a) SDS, (b) CTAB and (c) $C_{16}C_4C_{16}Br_2$.

resulted in the suppression of the protective mechanisms. This phenomenon can be correlated to the lack of capacity of our bodies to deal with heavy metals such as mercury and cadmium, whose levels in the environment are increasing badly as a result of modern mining and industrial procedures; all the abundant elements in the earth with soluble compounds are essential to life but most of the rare elements or those with insoluble compounds are toxic [7]. There are distinct mechanisms for such toxicity, for example through the "doughnut" shaped aggregates that simulate to the toxins produces by bacteria that create pores in membranes and disturb the ion balance in cells [8]. It is also possible that disorganised early aggregates are toxic through a less specific mechanism; for example, the exposure of non-native hydrophobic surfaces may stimulate aberrant interactions with membranes or other cellular components [9]. Certainly, a host of exciting advents is being developed, representing interference at many of the steps in aggregation represented, and many show considerable promise [10].

Comprehensive efforts have been applied towards seeking or developing anti-amyloidogenic or anti-aggregating agents as potential approaches to overcome AD [11]. A variety of molecules/compounds have been reported to retard the formation of fibrillar or aggregated species of β-amyloid both *in vitro* and *in vivo*. Some drugs containing aromatic groups such as rifampicin and dopamine have been found to be able to disaggregate mature β-amyloid fibrils. Nicotine, nordihydroguaiaretic acid, tannic acid, curcumin, and aspirin, slowed or inhibited fibrillogenesis of β-amyloid peptides [12–16]. Besides, several synthesized compounds as well as surfactants [17] were found to delay or prevent fibril formation. Also it is reported that cationic alkylammonium surfactants favored fibril formation below the critical micelle concentration (CMC) and that the surfactants above their CMC can delay amyloid beta fibril formation [18].

In the present study the systematic examination of different surfactants (Fig. 1) especially, cationic single chain surfactant, cetyltrimethylammonium bromide (CTAB, $C_{16}H_{33})N(CH_3)_3Br$), anionic single chain surfactant sodium dodecyl sulfate (SDS) and gemini surfactant ($C_{16}C_4C_{16}Br_2$, where 16 and 4 respectively, represents the number of carbon atoms in tail and spacer), have been used for disintegration of the amyloid fibrils formed from goat brain cystatin (GBC). It has been investigated that lysosomal proteinase (cathepsins) and their endogenous inhibitors (cystatins) have been closely associated with senile plaques, cerebrovascular amyloid deposits and neurofibrillary tangles in Alzheimer disease (AD) and Parkinson's disease (PD) [19,20]. Geminis consist of two identical hydrophobic segments and two polar head groups covalently linked by a spacer. They are considered superior in having lower CMC's and a stronger self-aggregation ability than their single head/tail counterparts.

The three different surfactants chosen here have different effects, because their unique chemical structures thus conceded the effect of their chemical nature, hydrophobicity, charge and binding capacity on GBC amyloid fibrils. This study shows that the micelles formed from gemini surfactant ($C_{16}C_4C_{16}Br_2$) are the most powerful medium for disintegrating the amyloid fibrils of GBC. Comparisons with other two surfactants suggested that the disintegrating potency of gemini surfactant micelles to amyloid fibrils of GBC associated to their bicharged hydrophilic head and potent self-aggregation ability.

2. Materials and methods

2.1. Materials

Thioflavin T, Congo red, and ANS (8-anilino-1- naphthalenesulfonic acid) were purchased from Sigma, Aldrich USA. The solutions were prepared in 20 mM sodium phosphate buffer of pH 7.4. Salts were purchased from Merck (India). The protein concentration was determined spectrophotometrically. All other reagents were of analytical grade, and double distilled water was used throughout.

Gemini surfactant tetramethylene-1,4-bis (hexadecyldimethylammonium bromide) ($C_{16}C_4C_{16}Br_2$) was synthesized by method of Zana et al. [21]. Anionic surfactant sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB) were obtained from Aldrich. The structures of three concerned surfactants are shown in (Fig. 1).

2.2. Purification of goat brain cystatin (GBC)

GBC was isolated from goat brain by the method earlier reported by Bhat et al. [22]. The purified protein gave a single band under native conditions on 7.5% polyacrylamide gel electrophoresis (PAGE).