Colloids and Surfaces A: Physicochem. Eng. Aspects 522 (2017) 494–502

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Cationic gemini surfactant (16-4-16) interact electrostatically with anionic plant lectin and facilitates amyloid fibril formation at neutral pH

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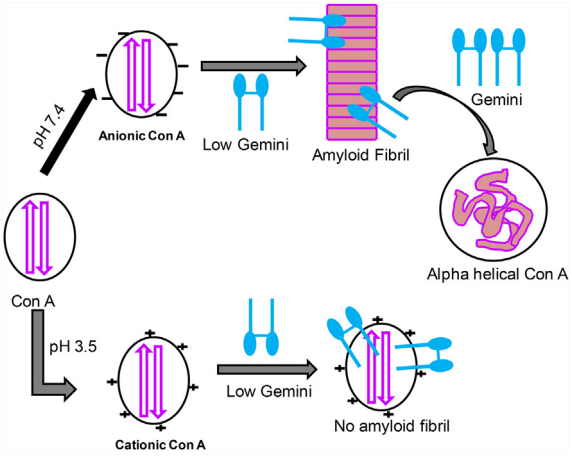
HIGHLIGHTS

- Low concentrations of gemini surfactant induces amyloid fibril in Con A protein.
- Higher concentrations of Gemini surfactant induces alpha helix in beta sheet Con A protein.
- Gemini surfactant interacts electrostaticaly and hydrophobicaly with anionic Con A protein.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 January 2017
Received in revised form 21 March 2017
Accepted 21 March 2017
Available online 23 March 2017

Keywords:
Amyloid fibril
Concanavalin A
Gemini surfactant and pH

ABSTRACT

Amyloid are fibrous clumps or aggregates of protein which usually deposited in various organs and tissues which direct their degenration. In neurodenrative disease, these proteincious aggregates leads degeneration of neuronal tissues/organs. In order to develop drug condidate which can dissolve the amyloid fibrils and turned protein functional, it is urgent need to elucidate the mechanism of amyloid fibril formation under different conditions. In this study, we have taken a step to find the mechanism of amyloid fibril formation in concanavalin A (Con A) protein via cationic gemini surfactant (16-4-16) at two different pHs (7.4 and 3.5). We used several biophysical techniques such as Rayleigh light scattering, turbidity, ThT dye binding, intrinsic fluorescence, extrinsic fluorescence, far-UV CD and transmission electron microscopy to characterize the amyloid fibril formation of Con A by cationic gemini surfactant. The results suggest that the Con A form amyloid-like aggregates in the presence of very low gemini concentrations (2.5–125 μM) at pH 7.4 while in the presence of higher concentrations (125–1000 μM),

Abbreviations: ThT, Thioflovin-T; Con A, Concanavalin A; AFM, Atomic force microscopy.

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http://dx.doi.org/10.1016/j.colsurfa.2017.03.048

Con A remained soluble. The Con A was not forming any aggregates or amyloid in the presence of same gemini concentrations at pH 3.5. The possible cause of gemini surfactant-induced amyloid fibril formation of Con A is electrostatic as well as hydrophobic interaction at pH 7.4 and strong electrostatic repulsion at pH 3.5. The far-UV CD spectra of Con A transformed into a cross β-sheet structure when incubated with low gemini surfactants while at higher concentrations the β-sheet structures of Con A transformed into α-helix.

1. Introduction

Amyloid fibrils have a direct connection with almost 30 types of human diseases, and the large number of cases are incurable at some stages. The known amyloid fibril linked diseases are Amyloid Lateral Sclerosis, Huntington's diseases, Perkinson, Diabetes and Alzheimer [1,2]. Amyloid fibrils are fiber like aggregate, unbranched, nano- to micrometer in length and encompass cross-β like structure [3]. For the treatment of amyloid diseases, it is crucial to understand the molecular mechanism of amyloid fibril formation. The amyloid fibril formation mechanism is broadly classified in two ways, i.e. nucleation-polymerization and isodesmic polymerization [4]. Nucleation dependent polymerization begins with the fibril nuclei formation, which is the rate limiting step. However, in isodesmic method, protein directly transformed into mature fibrils without going through fibril nuclei formation [5]. Recently, functional amyloid is also discovered in bacteria, fungi, and human [6]. The functional amyloid fibrils provide extra stability due to tight packing of many fibrils. The inter- and intramolecular interactions are involved in the tight packing of the fibrils. The amyloid fibril formation can be promoted by external factors, i.e. interaction with particular ligand and interaction with the environment [7]. In order to understand the trigger and mechanisms of fibril formation, it is indispensable to study the amyloid fibril formation under *in vitro* conditions. Various *in-vitro* factors, particularly temperature, pH, pressure, salts, interaction with lipid and surfactants are used to induce amyloid fibril in both human and plant proteins [8–10]. In the current work, we have tried to understand the mechanisms of gemini surfactant-induced amyloid fibril formation in Con A lectin.

Usaally, surfactants are used as mimic membrane model in protein-membrane interaction study. These mimic model membrane are very commonly used in pharmaceutical industry like, drug delivery, cosmetics and other biotechnological studies [11]. It is reported that both (cationic and anionic) surfactant intracted with lysozyme at different sites [12,13]. It is also found that these surfactant play significant role in induction of protein fibril along with simulteniously destabilizing proteins and or transforming unstructured into structured conformation [14,15]. Several categories of surfactants i.e. cationic, anionic and hydrophobic are known to induce the amyloid fibril in proteins [16]. The micromolar concentrations of sodium dodecyl sulfate accelerate amyloid fibril formation in recombinant α-synuclein proteins [17]. Cationic surfactants such as cetyltrimethylammonium bromide (CTAB) and dodecyl trimethylammonium bromide (DTAB) are reported to facilitate amyloid fibril formation in many proteins, namely hen egg white lysozyme and beta-amyloid [18,19]. Single chain cationic surfactants (CTAB and DTAB) are extensively used to check the aggregation propensity in various proteins. Only a few reports are available about gemini surfactant-induced amyloid fibril formation in proteins [20]. Gemini surfactant is a dimeric surfactant, consist of two positively charged heads, two sixteen carbon tails and one four carbon spacer groups shown in Fig. 1A. Gemini surfactants are better than available conventional cationic surfactants because of low CMC, unusual viscosity, low Krafft temperature, specific aggregation and high affinity towards oppositely charged surfactants. gemini surfactant effectively interacted with the bovine serum albumin proteins compared to conventional single-chain cationic surfactant (CTAB and DTAB) [21].

The aim of this work to probed the mode of gemini surfactant interaction with plant lectin i.e. Con A at neutral as well as acidic pH. Con A is carbohydrate binding proteins made up of four subunits. Con A is rich in β-sheet structures and possessing one carbohydrate and two metal (Ca^{2+} and Mn^{2+}) binding sites on each monomer of Con A [22]. Con A has a very important biological function i.e. agglutinates erythrocytes and kill the cancerous cells [23].

Con A and other lectin form aggregates at different unphysiological conditions like pH and temperature [24,25].

Understanding the mechanism of Con A aggregation with response to gemini surfactants is very important because of Con A has a similar resemblance to a human serum amyloid protein which is involved in amyloidosis diseases. This will be the first report about gemini surfactant induced amyloid fibril formation in Con A proteins. We used various types of spectroscopic and microscopic techniques to complete this study.

2. Materials and methods

2.1. Materials

Concanavalin A lectin (Con A), Tris–HCl, Thioflavin-T (ThT), and 8-anilino-1-naphthalene-sulfonate (ANS) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals were used of analytical grade. Milli-Q water was used throughout the study.

2.2. Protein concentration measurements

Con A stock was prepared in 20 mM Tris-HCl buffer, pH 7.4. The stock concentrations of Con A was calculated via Perkin Elmer (Lambda 25) spectrophotometer, with molar extinction coefficients, $E^{1\%}_{1/4}$ =11.4 at 280 nm.

2.3. pH measurements

pH of every solution was prepared by the use of Mettler Toledo pH meter (seven easy S 20-K) with the least count of 0.01 pH units, using an expert "Pro3 in 1" type electrode. The buffers were filtered through syringe filters (Millipore Milex-HV).

2.4. Rayleigh light scattering (RLS) measurements

The RLS study was executed on Hitachi F-4500 Fluorescence spectrofluorometer. The role of gemini surfactant into Con A aggregation was measured by the Rayleigh light scattering (RLS) methods. All the samples with and without gemini surfactant were excited at 350 nm and emission were taken at the same wavelength. The excitation and emission slit width were kept constant 5 nm for all samples. The 0.2 mg ml^{-1} of Con A was incubated with and without different concentrations of gemini surfactant (0–1000 μM) at