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Inhibition of Amyloid Fibrillation of Lysozyme by Organic Solvents Investigated by Thioflavin T Assay and Atomic Force Microscopy

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ABSTRACT

Amyloid fibrillation is relevant to a variety of serious human disease. This study aims to understand the modulation effect of some common organic solvents such as ethanol and acetonitrile on amyloid fibrillation using lysozyme as a model protein. Thioflavin T fluorescence assay and atomic force microscopy studies clearly indicated that these two organic molecules have profound inhibition effects on the amyloid fibrillation of lysozyme. These preliminary results suggest that a molecule abundant with hydroxyl and cyano groups may be able to serve as potential amyloid inhibitors.

Keywords: *amyloid, fibrillation, lysozyme, ethanol, acetonitrile*

INTRODUCTION

Amyloid fibrillation is a protein aggregation process through which proteins binds to each other in a non-covalent way to form a special type of fibrous aggregate termed as amyloid fibril. [1-3] Accumulation of these fibril-like protein aggregates in tissue and organs in human body will lead to some serious human diseases including the disorders such as Alzheimer's disease, Parkinson's disease, type II diabetes, as well as transmissible spongiform encephalopathies. [4] In addition to its clinical significance, amyloid fibrillation is hypothesized to be a general property of all proteins. In other words, any protein has the potential to form amyloid fibril. Therefore, understanding amyloid fibrillation and its modulation factors is thus not only beneficial to the treatment of amyloid-related diseases, but also fundamentally important in life science.

In this work, we would like to explore the modulation effects of two commonly used organic solvent, ethanol and acetonitrile, on the amyloid fibrillation of lysozyme. Two detection techniques are used. One is thioflavin T (ThT) assay and the other is the atomic force microscopy (AFM). The former can be used to detect the decrease or increase of the amount of amyloid fibril and the latter can detect the morphological change of protein aggregates. Lysozyme is used here as a model system and it is a widely used model system in amyloid research. [5-9]

MATERIALS AND METHOD

Materials

Hen egg white lysozyme was obtained from Amresco. ThT was purchased from Acros. Ethanol (99%, analytical grade), acetonitrile (99%, HPLC grade), and hydrogen chloride (36%, analytical grade) were from local vendors. The chemicals were used without further treatment. Distilled water with 18.2 MΩ· cm resistivity was from a Millipore water purification system.

Sample Preparations

To prepare amyloid fibril of lysozyme, the incubation solution contains 140 mM NaCl and 10 mg/mL lysozyme. The incubation conditions were pH=2 and 65 C. Before incubation, the solution needed to pass a 0.22 um filter to filter out some particles. To test the effects of organic solvent, the aqueous incubation solution will contain ethanol or acetonitrile. The tested ethanol concentrations were 88 mM, 176 mM, and 264 mM; the test acetonitrile concentrations were 96 mM,193 mM, and 289 mM.

ThT Assay

ThT assay was performed with a Hitachi F-7000 fluorescence spectrophotometer using the following parameters: excitation wavelength, 450 nm, excitation slit width, 5nm, emission wavelength, 486 nm, emission slit width, 10nm, voltage of photo mulitiplier tube, 700 V. The concentration of ThT solution is 10 μM. The buffer is 20 mM phosphate buffer at pH=7.4. During incubation, aliquots of incubation solutions were taken out of the incubation vial at selected time points and were subjected to the assay. For each measurement, 10 μl of incubation solution was added into 1ml of ThT solution in a 1.0 cm quartz cuvette.

AFM Measurement

The AFM measurements were performed on mica surface using NT-MDT AFM system. A 100μm×100μm scanner was used throughout the AFM experiment. The silicon cantilevers were also purchased from NT-MDT with a resonance frequency of 100 kHz and a nominal force constant of 3 N/m. The samples for AFM measurement were prepared using the following protocol. A drop of 10 μL of incubation solution was first diluted by 1000 times. 100 μL of the diluted solution was then dropped onto a freshly cleaved mica surface. After 15 min resting time, the surface was rinsed with plenty of water. The mica surface was finally air dried and stored for AFM imaging.

Results and Discussion

The following are the results of the inhibition effect of ethanol on lysozyme amyloid fibrillation investigated by ThT assay. ThT assay quantifies the amount of amyloid fibril formed during incubation. The higher the ThT intensity is, the more the generated amyloid fibrils are. When a compound is added to the incubation solution, the lowered ThT intensity would indicate the inhibited amyloid fibrillation. Figure 1 shows the effect of ethanol on the amyloid fibrillation of lysozyme. Three concentrations, 88 mM, 176 mM, 264 mM, were tested and all of them induced obvious ThT intensity decrease, suggesting the inhibition effect of ethanol on amyloid fibrillation. Figure 2 shows the effect of acetonitrile on the amyloid fibrillation of lysozyme. Three concentrations, 96 mM, 193 mM, 289 mM, were tested and all of them induced obvious ThT intensity decrease, suggesting the inhibition effect of acetonitrile on amyloid fibrillation. Furthermore, these effects were concentration dependent. With increasing concentrations of organic solvent like ethanol and acetonitrile, the inhibitory effects are more pronounced.

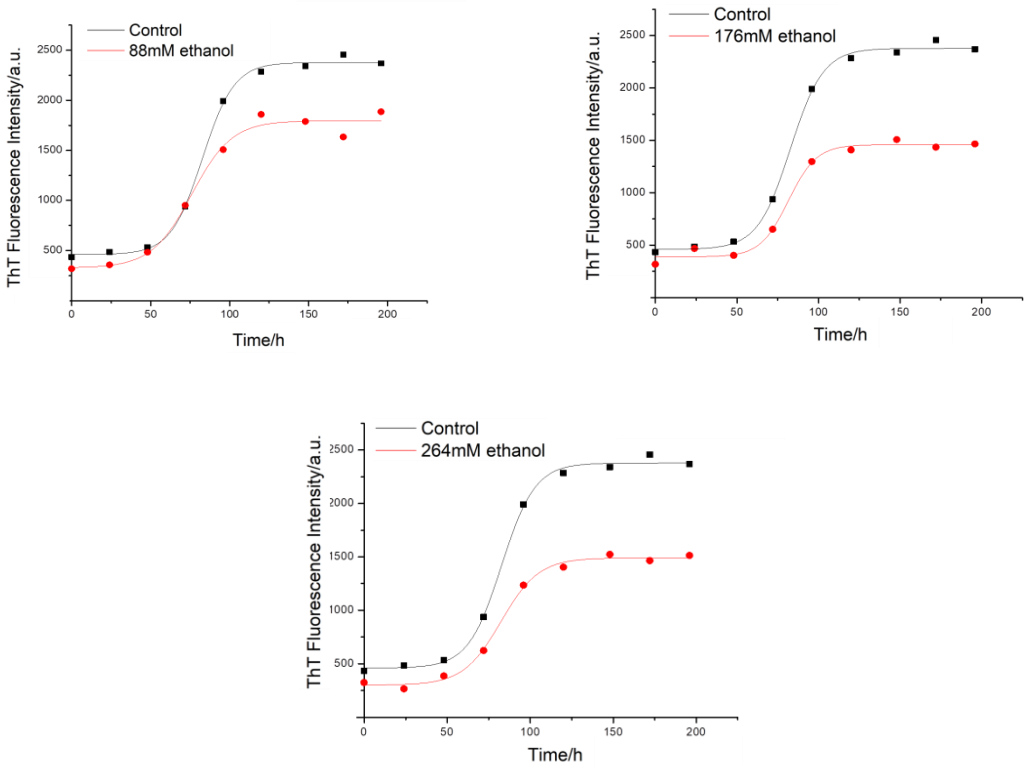

Figure1. *Effects of ethanol at three different concentrations on the amyloid fibrillation of lysozyme by ThT assay.*

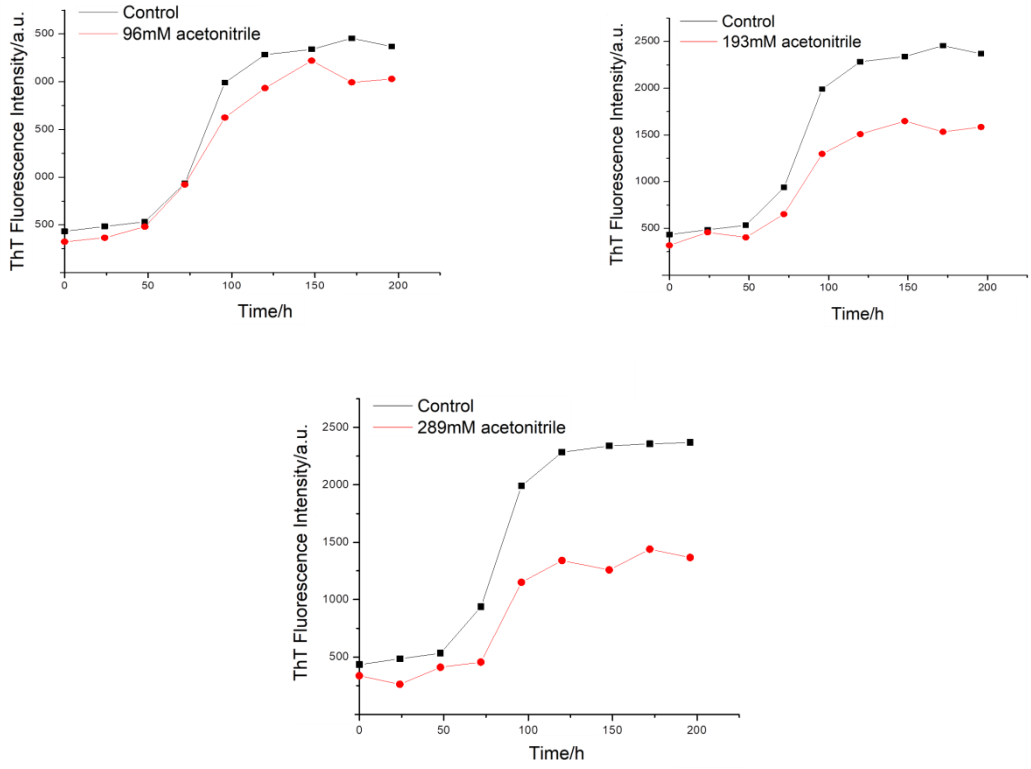

Figure2. *Effect of acetonitrile at three different concentrations on the amyloid fibrillation of lysozyme by ThT assay.*

The ThT assay clearly showed the inhibition effects of ethanol and acetonitrile on lysozyme fibrillation. We now used AFM to tackle the nature of the inhibition effect. As shown in Figure 3A, the control system, i.e., the incubation solution of lysozyme displays fibrillation aggregates. Yet, after the addition of ethanol (Figure 3B) or acetonitrile (Figure 3C) only amorphous aggregates were obtained. The AFM results thus indicated that the organic solvent like ethanol and acetonitrile could inhibition amyloid fibrillation of lysozyme through the induced formation of some amorphous aggregates.

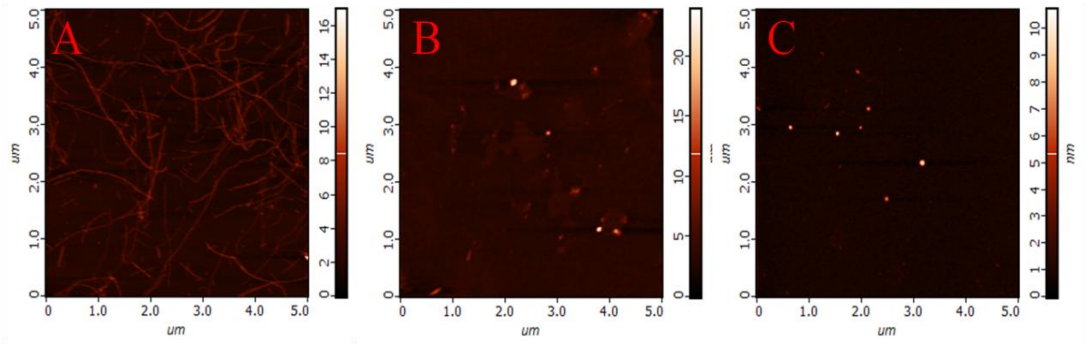

Figure3. *AFM images of lysozyme incubation solution. A: control; B: with ethanol; C: with acetonitrile.*

CONCLUSION

The ThT assay and AFM study on the amyloid fibrillation of lysozyme under the influence of ethanol and acetonitrile were conducted. The results showed that both of the two organic solvents could inhibit amyloid fibrillation of lysozyme. Our work further implicates that a chemical abundant with hydroxyl or cyano groups may be a potential candidate as amyloid inhibitor.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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