The Effect of Ficol on α-lactalbumin Aggregation

Seyed Hosseini B', Gahghaie A

Abstract— During folding pathway surface of amino acids are available which result to abnormal interaction between protein and environment and cause to aggregation. The intra cellular aggregation forms amyloid fibrils which has been seen in Alzheimer disease, type 2 Diabte. Amyloid aggregation is produced from deposition of immediately folded protein states. In this study we evaluated the effect of molecular crowding agent, ficol, on α-lactalbumin amyloid using light scattering spectroscopy, Tht binding assay, intrinsic fluorescence intensity, ANS binding assay and CD spectroscopy. Our results showed ficol ficol70 decreased the rate of aggregation and increased the lag phase of the reaction. The decrease of α-lactalbumin amyloid aggregation is because of reduction in non specific interaction between ficol and α-lactalbumin and environment in the presence of ficol.

Keywords---Crowding agents, Amyloid aggregation, α-lactalbumin

I. INTRODUCTION

In vitro protein folding process was used widely as an efficient tool for finding the folding process in the cell. After protein synthesis, new synthesized poly peptide is unfold, so in the protein folding pathway, protein fold to its normal and active form. During folding pathway surface of amino acids are available which result to abnormal interaction between protein and environment and cause to aggregation [1], aggregations are insoluble which produce from unfold or semi-fold polypeptide and hold together by hydrophobic interaction. Aggregation is an inefficient end and abnormal in protein folding [2]. Common feature in most protein aggregation disorders during amyloid formation is disappearing alpha helix domain and producing beta sheet structure [3].

The bovine α-lactalbumin (14KDa) is a globular calcium methalzo protein which is stable with 4 disulfide bounds and also doesn't have a free thiol so it consider as thermal stable protein [4], because α-lactalbumin form classic molten globule in acidic pH, high tempreature conditions and apo HLA structure, so it make α-lactalbumin a proper model for molten globule studies [4], [5].

The phenomenon of macromolecular crowding is used for living systems describe that the total concentration of macromolecule is high enough to occupy amount of volume cell that is not accessible for other molecules. one of the crowded result is decrease in molecular diffusion rate and this effect happen in all small and big molecules, also crowded effect are complex in biochemical interaction, because albeit it decrease the diffusion of molecules, it make an increase in thermodynamic interaction [6], [7].

Obviously, the volume that occupied by a molecules is not available to other molecule since two molecule cannot be in the same place at the same time, and thermodynamically the result of this effect are affecting macromolecular equilibria such as protein-protein interaction and significant altertation in the rate of the other chemical reaction, folding protein and interaction between macromolecules [8], [9].

We used crowded to mimic the cell environment to investigate the effect of crowding on interaction between chaperon and the protein. Also we choose this subject because the study of three macromolecular crowding agents together on beta-casein was not done before. The aim of this study was to investigate the effect of macromolecular crowding agent ficol on the amyloid formation and amorphous aggregation of α-lactalbumin.

II. MATERIAL AND METHODS

Bovin α-lactalbumin (14kDa), β-casein (24kDa), dextran70, ficol70, polyethylene glycol, 1,4- dithiotherithol (DTT), NaN₃, Na₂HPO₄, thioflavin T (Tht), 1-anilino-8-naphthalene sulfonic acid (ANS), all obtained from sigma-Aldrich.

Fibril formation of α-lactalbumin (2/5 mg/ml) was investigate in presence and absence of dextran, ficol and PEG (10% w/v), β-casein (1:1 molar ratio). All sampe incubated in 50mM sodium phosphate, pH/4 in an incubator(A-Q, Germany) at 37°. DTT was added to a final concentration of 20mM to commence the unfolding and aggregation of α-lactalbumin. Samples were shaken at 210 rpm to accelerate amyloid fibril formation. To deliberate fibril formation and the effect of crowding agents on β-casein activity, Tht was added to the samples and amyloid formation was measured by Tht fluorescence on Varian spectrofluorimetre. The wave length of excitation and emission was respectively 446nm and 450-600nm with 5nm-5nm slit width.

The intrinsic fluorescence intensity of α-lactalbumin (10 μM), and 20mM DTT in 50mM sodium phosphate, 0/1% NaN₃ and pH 7/4 in presence and absence of ficol was studied after 3 hours incubation at 37°. Fluorescence intensity were obtained on a Varian Eclips fluorescence spectrofluorimetre equipped with tempreture control. The excitation and emission wave length of tryptophan residue were 295nm and 300-400nm with 2/5nm and 5nm slit width, respectively.

III. RESULTS

Reduced α-lactalbumin at natural pH 7-7.4 adopts a molten globule conformation which make it prone to amyloid fibril formation [10].
In order to investigate the effect of macromolecular crowding, ficoll, on the aggregation of α-lactalbumin. ThT binding assay showed that the fluorescence intensity of ThT of reduced α-lactalbumin increased at pH 7.4 (11). The ThT intensity of α-lactalbumin decreased in presence of ficoll which means ficoll could suppress amyloid formation of α-lactalbumin. According to table 1, the rate constant of amyloid formation of α-lactalbumin was 0.596± 0.02 while in presence of ficoll it decrease to 0.49 ± 0.02.

### Table I

<table>
<thead>
<tr>
<th>Sample components</th>
<th>Rate constant × 10⁻¹ (min⁻¹)</th>
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<tbody>
<tr>
<td>α-lactalbumin</td>
<td>0.596 ± 0.02</td>
</tr>
<tr>
<td>α-lactalbumin + Ficol</td>
<td>0.49 ± 0.02</td>
</tr>
</tbody>
</table>

Fluorescence spectroscopy: Intrinsic fluorescence

Intrinsic fluorescence intensity assay was used to show the effect of ficoll on structural changes of α-lactalbumin. The intrinsic fluorescence of tryptophan show environm Changes of Trp residue during the folding/unfolding process [12]. According to figure I, adding ficoll to α-lactalbumin showed an increased in the fluorescence intensity, So the intrinsic fluorescence of α –lactalbumin was about 70% less compare with sum of individual proteins.

![Figure I](image-url)

**Fig I.** The maximum fluorescence intensity of α-lactalbumin (10 μM) in presence and absence of ficoll (10% w/v). All samples were incubated 3 hours in 50 mM phosphate buffer, 0.005% Na₂SO₄, pH7.4 and at 37°C. 

### IV. DISCUSSION

The intra cellular environment is full of crowded because of the high concentration of soluble and insoluble macromolecule, so a large amount of intra cellular space isn't accessible to other molecules while these circumstance does not exist in in vitro environment. So the crowding theory suggests that the crowded of the cell can have effect on thermodynamic action of every molecule in the cell [13], [14]. The aim of this study was to find out the effect of macromolecular crowding agent, ficoll, on structural changes and amyloid formation of α-lactalbumin.

Ficoll70 is one of suitable model to create a cellular crowed enviroment, where the folding process done.

The thioflavin T binding assay showed that ficoll decreased the rate of amyloid aggregation of α –lactalbumin and increase the lag phase of amyloid formation. To investigated these results we used intrinsic fluorescence intensity in the presence of ficoll. The intrinsic experiment showed that the fluorescence intensity had a remarkable decrease in the presence of ficoll.

Some study show non-specific interactions, could lead to unstable protein formation, also small proteins unstable the proteins and big protein stable proteins [15],[16]. This study show there wasn't non- specific interaction between ficoll and α-lactalbumin. also Physiological heterogeneous crowding environment can lead to an environment which protein can be stable or unstable by total volume occupied and total non-specific interaction [1], [2].

### V. CONCLUSION

We have shown that ficoll as a crowding agent can affect the polarity changes around amino acids of α –lactalbumin. Also ficoll could decrease the rate of amyloid formation, so it can be conclude that ficoll can have positive effect on prevent the amyloid aggregation of α –lactalbumin.

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