The Effect of Dextran on Chaproning Action on Beta - Casein

Seyed Hosseini B*, and Gahghaie A

Abstract - Amyloid aggregation is produced from deposition of intermediately folded protein states. Chaperones are assistant molecules that prevent aggregation of protein approximately. In this study we evaluated the effect of dextran on chaproning effect of β-casein in preventing of aggregation of α-lactalbumin using light scattering spectroscopy, binding assay, intrinsic fluorescence study we evaluated the effect of dextran on chaproning effect of β-casein as a molecular chaperone prevented aggregation of α-lactalbumin. The protection activity decreased in presence of dextran. The decrease of protection activity of β-casein is occurred because of enhancing in non-specific interaction between β-casein and α-lactalbumin and environment in the presence of dextran, or because of the effect of dextran in enhancing the amyloid aggregation of α-lactalbumin.

Index Terms- Crowding agents, β-casein, Amyloid Fibril, α-Lactalbumin

I. INTRODUCTION

In vitro protein folding process was used widely as an efficient tool for finding the folding process in the cell. 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]. The intra cellular aggregation forms amyloid fibril which has been seen in Alzheimer disease and type 2 Diabte [3]. Common feature in most protein aggregation disorders during amyloid formation is disappearing alpha helix domain and producing beta sheet structure [4].

The new synthesized protein and the old protein are disposing to misfolding and consequently aggregation. The sediment and accumulation of damage protein can disturb the homeostasis of the cell, and provoke aging and even death at the end but the cell contrive a powerful molecular system to counteract protein misfolding aggregation [5],[6].

Some of chaperones are stress or heat shock protein because cells need them in stress conditions. These stress condition can be unfold or misfold proteins. There are two main chaperon classes: hsp70 and chaperonin cylindrical complexes which prevent polypeptide misfolding with ATP dependent function in cytosol, chloroplast and mitochondria of prokaryotes and eukaryotes [7]. The common feature of chaperone is a hydrophilic head(to increase solubility) and hydrophobic head(to bind to substrate) [8].

Chaperone molecules increase the correct folding of proteins by separate new polypeptides from their environment [9]. β-casein with 23-24 KDa is a milk protein contain 209 amino acids. It contains a hydrophilic region (N-terminal domain) and a hydrophobic region (C-terminal domain) and so able to produce oligomeric micelles. According to previous study, β-casein is one of the most hydrophobic caseins because of wide extent hydrophobic region and act like chaperone molecule [10]. The bovine α-lactalbumin (14 KDa) is a globular calcium methalo protein which is stable with 4 disulfide bounds and also doesn't have a free thiol so it consider as thermal stable protein [11] because α-lactalbumin form classic molten globule in acidic pH, high temperature conditions and apo HLA structure, so it make α-lactalbumin a proper model for molten globule studies [11],[12].

The aim of this study was to investigate the effect of macromolecular crowding agents contain dextran on the functional and structural features of β-casein action as a molecule chaperon in preventing the amyloid formation and amorphous aggregation of α-lactalbumin.

II. MATERIAL AND METHODS

Bovin α-lactalbumin (14 kD), 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.

UV - visible light Spectroscopy

The aggregation of α-la (2mg/ml) was investigated in 50 mM phosphate buffer, 100mM Nacl, pH7.4, at the presence of crowding agent (at the final concentration of 10% w/v) and β-casein (1:1 molar ratio). The effect of macromolecular agents of dextran on chaproning function of β-casein was assayed by light scattering spectroscopy assay at the wave length of 340 nm using a Biotek Elisa plate reader spectrophotometer with temperature control.

Fibril formation

Fibril formation of α –lactalbumin (2/5 mg/ml) was investigate in presence and absence of dextran (10% w/v), β-casein ( 1:1 molar ratio). All sampe incubated in 50mM sodium phosphate, pH:7/4 in an incubator(A-Q, Germany) at 37°. DTT was added to a final concentration of 20 mM 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 440nm and 450-600nm with 5nm-5nm slit width.

**Fluorescence spectroscopy**

**Intrinsic fluorescence intensity**

The intrinsic fluorescence intensity of α-lactalbumin (10 μM), β-casein (1:1 molar ratio), and 20mM DTT in 50mM sodium phosphate, 0/1% NaNO2 and pH 7/4 in presence and absence of dextran was studied after 3 hours incubation at 37°C. Fluorescence intensity were obtained on a Varian Eclips fluorescence spectrofluorimetre equipped with temperature 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**

In order to investigate the effect of macromolecular crowding agent, dextran, on the aggregation of α-lactalbumin and chaperoning action of β-casein, amorphous aggregation was examined. α-Lactalbumin aggregate after the adding DTT due to reduction of its four disulfide bounds. Our investigations showed that dextran increase the rate of aggregation and decrease the lag phase (Table I). This is also evident from the first order rate constant i.e. in the absence of β-casein rate constant was (41.49 ± 0.0007) while in the presence of β-casein it decreased to (36.76 ± 0.0019) (Table I).

In the presence of dextran, however, β-casein was less effective in protecting aggregation of α-lactalbumin compared to its activity in the absence of them (table I). So that in the absence of β-casein, the rate constant was (98.03 ± 0.003) in presence of dextran.  

**Table I.** Summary of rate constant of α- lactalbumin in visible absorption assay in the presence and absence of β-casein and crowding agents

<table>
<thead>
<tr>
<th>Sample components</th>
<th>Rate constant x10^3(min^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-lactalbumin</td>
<td>41.49 ± 0.0007</td>
</tr>
<tr>
<td>α-lactalbumin+Dextran</td>
<td>98.03 ± 0.003</td>
</tr>
<tr>
<td>α-lactalbumin+β-casein</td>
<td>36.76 ± 0.0019</td>
</tr>
<tr>
<td>α-lactalbumin+β-casein+</td>
<td>41.32 ± 0.0004</td>
</tr>
</tbody>
</table>

To further investigate the effect of crowding agent on protein aggregation of α-lactalbumin and chaperone ability of B-casein, intrinsic fluorescence intensities was conducted.

According to figure I adding β-casein to α-lactalbumin compared with individual protein showed a decrease in the maximum fluorescence intensity about 52/40% which this could be means there was some complex production between α-lactalbumine and β-casein which it showed some chaperone activity that could cause changes around tryptophan. Adding dextran to α-lactalbumin showed an increase in the maximum fluorescence intensity and adding β-casein to α-lactalbumine in the presence of dextran decreased the maximum fluorescence intensity (Fig.I).

**IV. DISCUSSION**

The crowding theory suggest 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 crowding agents including dextran on β-casein action in preventing amyloid formation.

β-casein is one of molecular chaperones which can bind to the exposed hydrophobic region of protein and also could prevent the aggregation of protein in vitro and in vivo. β-casein molecule not only prevent the formation of aggregation but also can dissolve the previous accumulation. Some studies showed that β-casein function is related to size of molecules [15]. The experiment of light scattering show that dextran reduced the lag phase and increase the rate of amyloid formation of α-lactalbumin.

To investigated these results we used intrinsic fluorescence in presence of crowding agents. The intrinsic experiment showed that fluorescence intensity increase in presence of dextran. The action of β-casein decreased in presence of dextran that may come from acting of dextran on hydrophobicity of β-casein.

Some study show non-specific interactions, could lead to unstable protein formation, also small proteins unstable the proteins and big protein stable proteins [16],[17].

**V. CONCLUSION**

We have shown that crowding agent of dextran can affect on β-casein chaperoning activity and on hydrophobicity and polarity changes around amino acids of β-casein and α-lactalbumin. Thus, our results suggest that both the target protein and the structural change in β-casein in the presence of dextran play a critical role in decreasing the protective activity of β-casein.

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**REFERENCES**

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