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Effect of Different Crowding Agents (Dextran, PEG and Ficoll) on the Chaperone Ability of β-Casein

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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 different molecular crowding agents (dextran, ficoll and PEG) on chaperoning effect of β-casein in preventing of aggregation of α-lactalbumin. Our results show that dextran and PEG increase the rate of aggregation of α-lactalbumin while ficoll decrease the rate of this aggregation. β-Casein, as a molecular chaperone, prevented aggregation of α-lactalbumin which increased in the presence of ficoll, while its protection activity decreased in the presence of dextran and PEG. The decrease in protection activity of β-casein in the presence of dextran and PEG, is maybe because of enhance in nonspecific interaction between β-casein and α-lactalbumin and environment, or because of the effect of dextran and PEG on the rate of amyloid fibril formation of α-lactalbumin. On the other hand, the increase in chaperone activity of β-casein in the presence of ficoll could be due to the effect of ficoll on the aggregation of target protein and/or reducing the nonspecific interaction between protein and environment. In summary, our data suggest that crowding agents have different effect on the aggregation of α-lactalbumin as well as chaperone activity of β-casein.

Keywords: Crowding agents, β-Casein, Amyloid, α-Lactalbumin

INTRODUCTION

In vitro protein folding procedure was used widely as an efficient tool for finding the folding pathway in the cell. During folding pathway, a series of partially folded intermediate of protein exist with exposed hydrophobic area which result to abnormal interaction between protein and environment and cause to aggregation [1,2]. The intracellular aggregation forms amyloid fibrils which have been seen in many diseases like Alzheimer and type 2 Diabetes [3,4]. In the cell environment, most of protein must fold into their three dimensional structure to gain active form, but new synthesized poly peptide is at high risk of abnormal folding and produce the toxic species and so aggregate. Then to avoid aggregation of protein, cells contrive a complex of network of molecular chaperoning to prevent misfolding and aggregation an also lead to perfect folding [5,6].

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Chaperone molecules are a group of proteins which participate in cell homeostasis by two functions, first they assist in protein folding and second they play role in protein degradation [7]. Some chaperones can participate in the recovery from stressful condition of cell by modification or degrading damaged proteins and by then restoring homeostasis and promoting cell survival [8,9]. The new synthesized protein and the old protein are dispose 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 [10,11].

β-Casein with 23-24 KDa is a milk protein includes 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 [12].

The bovine α-lactalbumin (14 KDa) is a globular calcium methaloprotein which is stable with 4 disulfide bounds without free thiol so it considers as thermal stable protein [13]. This protein is a proper model for aggregation studies because it produces the stable molten globule states under a range of conditions such as acidic pH, high temperature and in the presence of reducing agent [13,14].

In vitro experiments usually conduct in a simple buffer system with low concentration of protein to avoid the aggregation of protein during refolding. The big disparity between the ideal test tube *in vitro* and cell environment is that cell environment are overcrowded because of high concentration of soluble and insoluble macromolecular in cytoplasm, so molecular crowded have large effect on molecular interaction [15]. The molecular crowding has a multifaceted impact on proteins both qualitatively and quantetivility [16]. It has both thermodynamic and kinetic effect on the properties of macromolecules resulting in large quantitative effects on both the rate and the equilibria of interaction involving macromolecules [17]. Study by Minton have been shown that crowding has two opposing effects on the rate of protein aggregation. It decrease the diffusion coefficient of the reactants, which decrease the reaction rate. Nevertheless, crowding agent increase the equilibrium constant and this thermodynamic effect would increase the reaction rate [18-19].

The chaperone activity of β-casein in preventing the aggregation of different target protein has been reported. It is also has been found that β-casein not only prevent aggregation of the target proteins, but also solubilize the protein aggregates already formed reported [20-22].

Chaperone action of β-casein in preventing aggregation of protein has, until now, been investigated in the absence of crowding agent. Thus to mimic the chaperone action of β-casein in *vivo*, this study explored the effect of crowding agent on aggregation of target protein and β-casein's ability to suppress it.

Dextran, ficoll and PEG widely accepted as an appropriate crowding agent *in vitro* [23-25]. In our previous study the effect of dextran on the amyloid fibril formation of α-lactalbumin and chaperone action of α-casein has been investigated [3]. Different crowding agents, however, have different effect on the thermodynamics and kinetics of protein folding, aggregation and other biological effects [23, 26].

So here we report the effect of three crowding agent on the aggregation of α-lactalbumin as well as chaperone action of β-casein. Our result indicates ficoll reduced the rate of amyloid formation of α-lactalbumin but dextran and PEG enhanced it. Of particular note, the results from this study also show that chaperon action of β-casein increased in the presence of ficoll against amyloid fibril formation of α-lactalbumin. The chaperone action of β-casein, on the other hand was not as good in the presence as it was in the absence of dextran and PEG. Thus, our results suggest that crowding agents with different size have different effect on the stability and chaperone action of β-casein.

MATERIAL AND METHOD

Bovin α-lactalbumin (14 kDa), β-casein (24 kDa), dextran70, ficoll70, polyethylene glycol, 1,4-dithiotherithol (DTT), NaN_3, Na_2HpO_4, thioflavin T (ThT), 1-anilino-8-naphthalene sulfonic acid (ANS), all obtained from sigma-Aldrich.

UV-VIS SPECTROSCOPY

The aggregation of α-lactalbumin (2 mg ml^{-1}) was investigated in 50 mM phosphate buffer, 100 mM Nacl, pH 7.4, at the presence of crowding agents (at the final concentration of 10% w/v) and β-casein (1:1 molar ratio). The effect of macromolecular agents of dextran, ficoll and PEG on chaperoning 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^{-1}) was investigate in presence and absence of dextran, ficoll and PEG (10% w/v), β-casein (1:1 molar ratio). All sample incubated in 50mM sodium phosphate, pH, 7.4 in an incubator (A-Q, Germany) at 37 °C. 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. ThT

fluorescence intensity was monitored using a Cary Eclipse spectro-fluorimeter (Varian, USA). The wavelength of excitation and emission were respectively 446 nm and 450-600 nm with 5 and 5 nm slit widths.

INNER FILTER EFFECTBNAND MEASURMENT OF SYNCHRONOUS FLUORESCENCE

Since, there is an inner filter effect due absorption by β-casein in the region of excitation or emission wavelength and thus, reduce the fluorescence intensity (FI) the following equation was used to correct the inner filter effect [27]:

$$Fcor = Fobs\ e^{(Aex+Aem)/2}$$

Where, Fcor and Fobs are the corrected and observed fluorescence respectively. Aex and Aem represent sum of LNF absorbance at the excitation and emission wavelengths respectively.

INTRINSIC FLUORESCENCE SPECTROSCOPY

The intrinsic fluorescence intensity of α-lactalbumin (10 μM), β-casein (1:1 molar ratio), and 20 mM DTT in 50 mM sodium phosphate, 0.1% NaN_3 and pH 7.4 in presence and absence of dextran, ficoll and PEG 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 wavelengths of tryptophan residue were 295 nm and 300-400 nm with 2.5 nm and 5nm slit widths, respectively.

ANS BINDING ASSAY

The ANS binding assays were done to investigate the hydrophobicity changes of α-lactalbumin (10 μM) in presence and absence of crowding agents (dextran (10% w/v), ficoll (10% w/v) and polyethylene glycol (10% w/v) and β-casein (1:1 molar ratio). Experiment was done in 50 mM Na_2HPO_4, pH 7.4 and 0.1% NaN_3. The ANS fluorescence of samples were done in a Varian Cary eclips fluorescence spectroflurimetre. Samples were titer by 1 μM of a 10 mM ANS stock solution in 50 mM phosphate buffer, pH 7.4 and 0.05% (w/v) NaN_3 with 1 min of stirring after each addition, until the fluorescence intensity reached a constant value. The excitation wavelength was set on 400 nm with 2.5 slit width and the emission wave length was set on 405-600 nm and 5 slit width.

CIRCULAR DICHROISM SPECTROSCOPY

The overall secondary and tertiary structure of α-lactalbumin in the presence and absence of dextran, ficoll and PEG and β-casein were examined using Far UV (190-250 nm) and Near UV (250-350 nm). The samples were incubated 3 h, 10 mM phosphate buffer, 20 mM DTT and pH 7.4. Spectra were recorded using a circular dichroism spectrometer Aviv model-215 in a 1-cm path length cuvette.

RESULTS

In order to investigate the effect of macromolecular crowding (dextran, polyethylene glycol and ficoll) 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 as can be seen in light scattering result (Fig. 1) [28]. In the presence of dextran and PEG the rate of aggregation of a-lactalbumin increased and the lag phase decreased while ficoll 70 decreased the rate of aggregation and increased the lag phase of the reaction (Fig. 1).

This is also evident from the first rate constant so that in the presence of dextran and PEG, the rate constant of aggregation of α-lactalbumin increased from 0.2775 ± 0.0001 to 1.0401 ± 0.0001 and 3.5919 ± 0.0001, respectively. In the presence of ficoll, however, the rate constant decreased to 0.0154 ± 0.0001.

Adding β-casein to α-lactalbumin decreased the rate constant effectively. In the presence of dextran and PEG, however, β-casein was less effective chaperone in protecting aggregation of α-lactalbumin compare to its activity in the absence. The chaperone activity of β-casein, although, increased in presence of ficoll (Table 1).

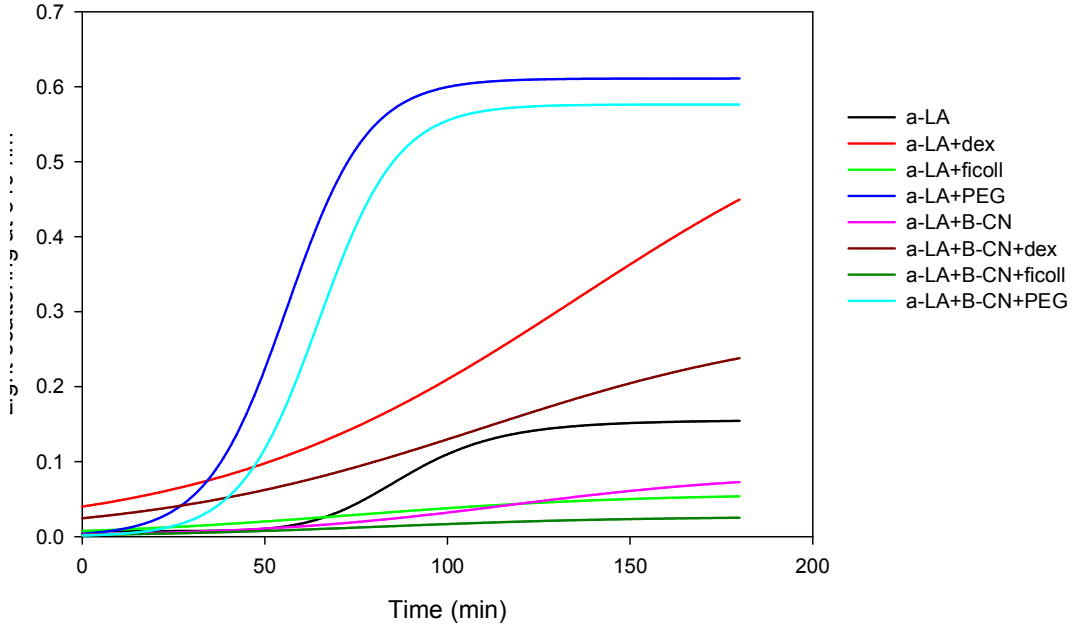

Fig. 1. Visible absorption profiles at 340 nm of α-lactalbumin (2 mg ml^{-1}) in presence and absence of β-casein (1:1 molar ratio) and dextran, ficoll and PEG (10% w/v), in phosphate buffer 50 mM , 0.05% NaN_3 and pH 7.4 at 37 °C.

Table 1. Summary of Rate Constant of α-Lactalbumin in Visible Absorption Assay. The Rate Constants were Calculated by Ftting Exponential Function to Visible Absorption Data Using Sigma Plot Software

Sample components	Rate constant (min^{-1})
α-Lactalbumin	0.2775 ± 0.0001
α-Lactalbumin + dextran	1.0401 ± 0.0001
α-Lactalbumin + ficoll	0.0154 ± 0.0001
α-Lactalbumin + PEG	3.5919 ± 0.0001
α-Lactalbumin + β-casein	0.0332 ± 0.0001
α-Lactalbumin + β-casein + dextran	0.3056 ± 0.0001
α-Lactalbumin + β-casein + ficoll	0.0038 ± 0.0001
α-Lactalbumin + β-casein + PEG	3.3843 ± 0.0001

AMYLOID FIBRIL FORMATION OF α-LACTALBUMINBY ThT BINDING ASSAY

Reduced α-lactalbumin at natural pH 7-7.4 adopts a molten globule conformation which makes it prone to amyloid fibril formation [29].

ThT binding assay showed that the fluorescence intensity of reduced α-lactalbumin increased at pH 7.4 [30].

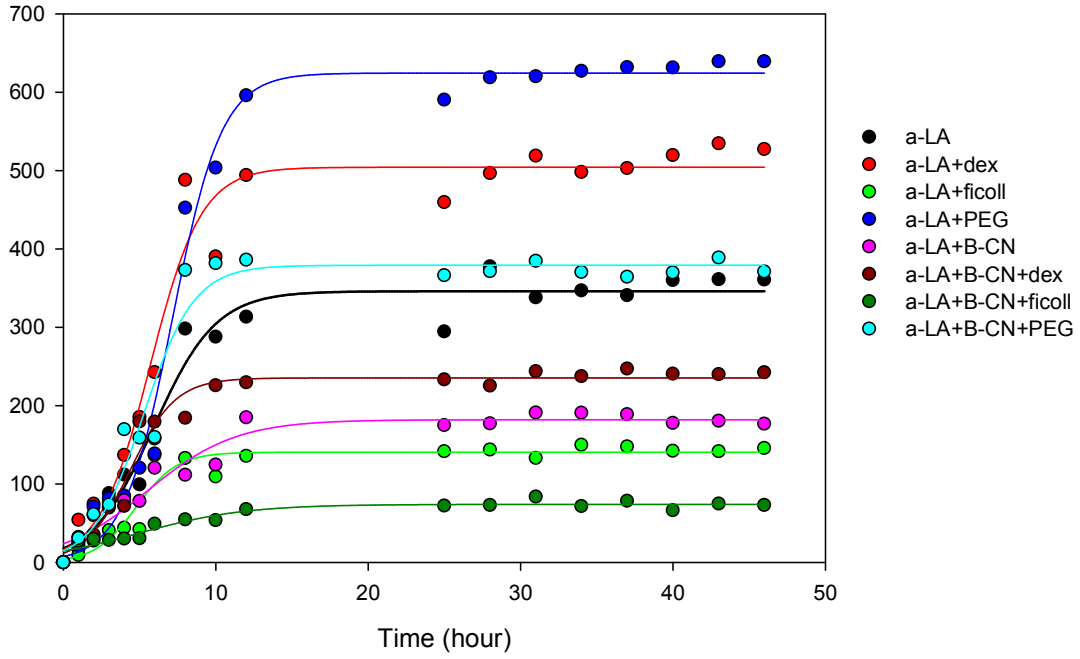

Fig. 2. Amyloid formation of α-lactalbumin (2.5 mg ml^{-1}) in the presence and absence of β-casein (1:1 molar ratio) and macromolecular crowding agent (dextran (10% w/v), ficoll (10% w/v) and PEG (10% w/v)). Protein was in 50 mM phosphate buffer and 0.05% NaN_3, pH 7.4 and the samples was incubated at 37 °C. The experiment was done 3 times and fitted using sigma plot software.

Table 2. Summery of Rate Constant for α-Lactalbumin in Thioflavin T-binding Assay in Presence and Absence of Crowding Agents (10% w/v) and β-Casein. The Rate Constants were Calculated by Ftting Exponential Function to Thioflavin T Binding Data Using Sigmaplot Software

Sample components	Rate constant × 10^{-1} (min^{-1})
α-Lactalbumin	0.596 ± 0.02
α-Lactalbumin + dextran	0.621 ± 0.01
α-Lactalbumin + PEG	0.743 ± 0.03
α-Lactalbumin + ficoll	0.490 ± 0.02
α-Lactalbumin + β-casein	0.343 ± 0.04
α-Lactalbumin + β-casein + dextran	0.604 ± 0.02
α-Lactalbumin + β-casein + PEG	0.674 ± 0.02
α-Lactalbumin + β-casein + ficoll	0.285 ± 0.06

After adding β-casein to α-lactalbumin, ThT intensity was decreased which could be concluded that β-casein prevented amyloid formation of α-lactalbumin compare with that observed when α-lactalbumin was incubated alone. According to Fig. 2 and Table 2, the rate constant of amyloid formation of α-lactalbumin was 0.596 ± 0.02 while

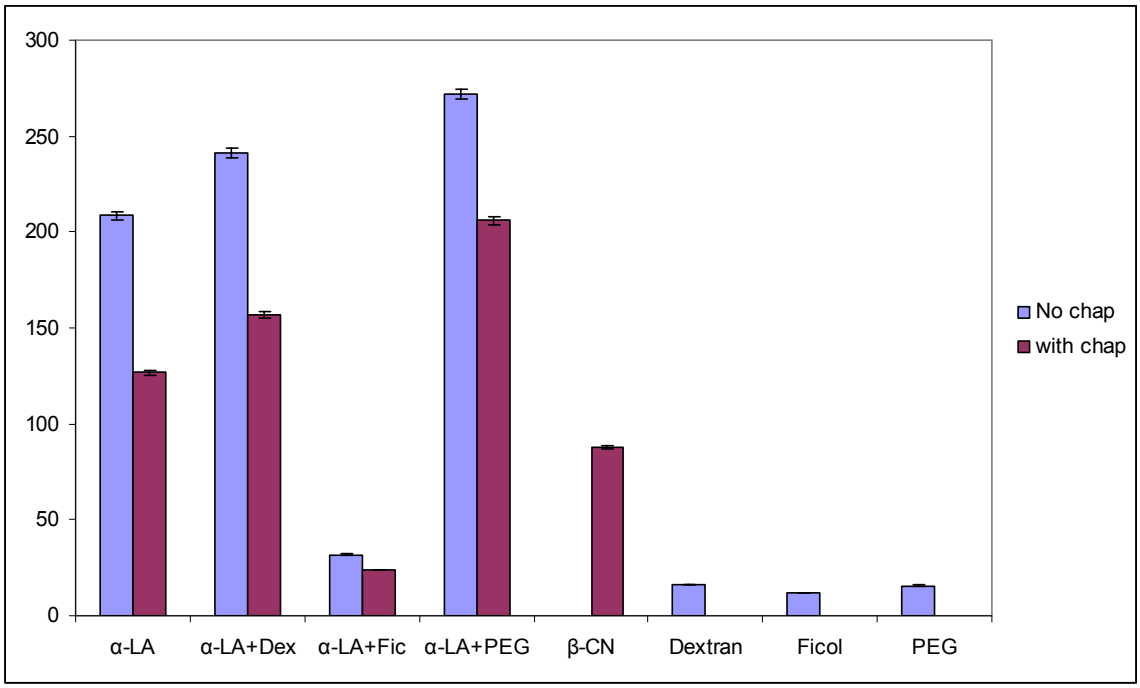

Fig. 3. The maximum fluorescence intensity of α-lactalbumin (10 μM) in presence and absence of dextran, ficoll, PEG (10% w/v) and β-casein (1:1 molar ratio). All samples were incubated 3 h in 50 mM phosphate buffer, 0.005% NaN_3, pH7.4 and at 37 °C.

Table 3. Summary of Protection Percentage of β-casein in Spectroscopy Results in the Presence and Absence of Dextran, Ficol and Polyethylene Glycol (10% w/v)

Sample components	Protection (%)
α-LA + β-casein	63.36
α-LA + β-casein + dextran	52.53
α-LA + β-casein + ficoll	77.17
α-LA + β-casein + PEG	52

in presence of β-casein it decreased to 0.343 ± 0.04. Adding dextran and PEG increased the intensity of the ThT binding indicating that dextran and PEG increased the rate of amyloid fibril formation which is also apparent from the first order rate constant (Fig. 2, Table2).

In the presence of dextran and PEG, however, the effect of β-casein in reducing amyloid fibril formation of α-lactalbumin decreased compare to the absence of them. In contrary the ThT intensity of α-lactalbumin decreased in presence of ficoll which means ficoll could suppress amyloid formation of α-lactalbumin. The chaperone activity of β-casein was also increased in the presence of ficoll toward amyloid fibril formation of reduced α-lactalbumin. This result also evident from calculating the first order rate which are shown in Table 2.

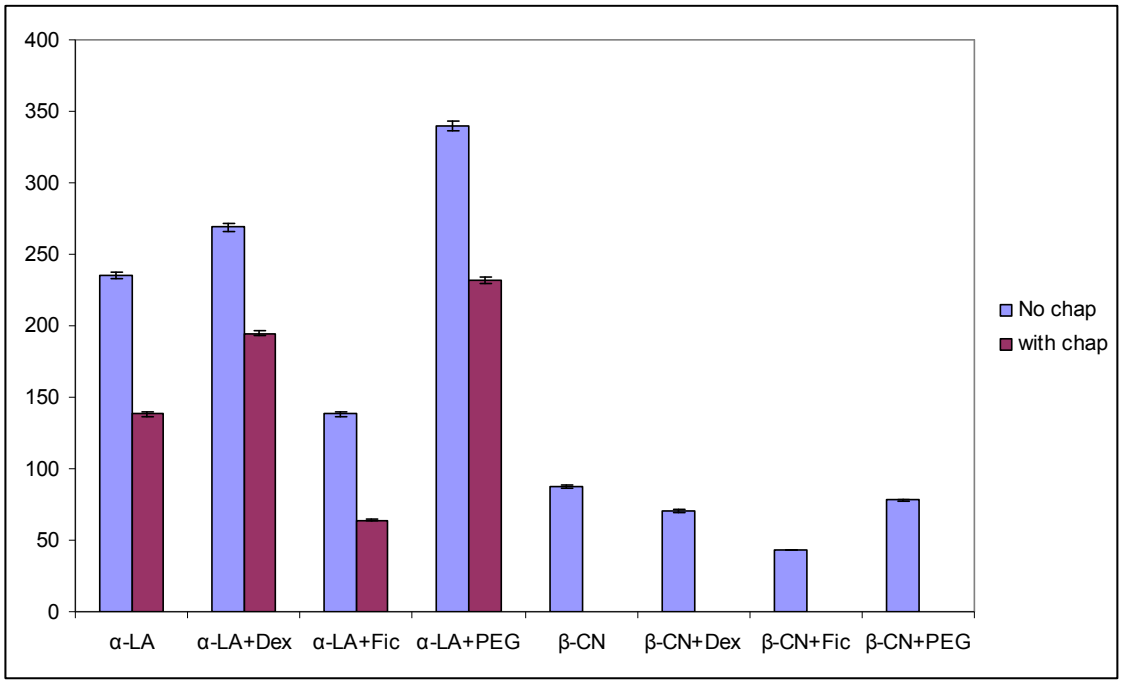

Fig. 4. Average maximum ANS fluorescence of α-lactalbumin in presence and absence of dextran, ficoll and polyethylene glycol (10% w/v) and β-casein (1:1 molar ratio). All experiment were done in 50 mM phosphate buffer, 0.05 NaN_3, and pH = 7.4.

Table 4. Secondary Structure Prediction from far CD for Native and Incubated α-Lactalbumin in the Absence and Presence of β-Casein and Crowding Agents

Sample	β-Sheet [%]	α-Helix [%]	Random coil [%]
α-LA (native)	93	5.4	2.5
α-LA + DTT	31.3	44.8	24
α-LA + dextran	14.6	43.2	42.3
α-LA + ficoll	33	30.5	36.3
α-LA + PEG	27.1	38.4	34.4
α-LA + β-CN	79.9	15.1	1.4
α-LA + β-CN+ dextran	50.8	48.6	0.2
α-LA + β-CN + ficoll	82	17.8	0.2
α-LA + β-CN + PEG	22.8	22.3	54.9

INTRINSIC FLUORESCENCE SPECTROSCOPY

To further investigate the effect of crowding agent on protein aggregation of α-lactalbumin and the chaperone ability of β-casein, intrinsic fluorescence was conducted. The intrinsic fluorescence of tryptophan shows environment changes of Trp residue during the folding/unfolding process

or during the interaction between two proteins like chaperone and target protein [31].

According to Fig. 3, the fluorescence intensity of α-lactalbumin was increased in presence of dextran and PEG, while it decreased in the presence of ficoll. Adding β-casein to α-lactalbumin decreased the maximum fluorescence intensity about 52.40% compare with fluorescence intensity of individual proteins. By adding β-casein, the fluorescence intensity of the mixture of α-lactalbumin and dextran and the mixture of α-lactalbumin PEG decrease to 49.91% and 42.96%, respectively.

On the other hand, adding β-casein to α-lactalbumin in the presence of ficoll decreased the maximum fluorescence intensity by 79.98%. So the intrinsic fluorescence α-lactalbumin and β-casein in the presence of ficoll was about 29% less than the sum of individual proteins. In summary, dextran and PEG had negative effect on both proteins, while ficoll had a positive effect on α-lactalbumin and β-casein action.

ANS BINDING ASSAY

ANS binding assay have been done to monitoring the changes in exposed hydrophobicity in α-lactalbumin upon incubation in the presence and absence of crowding agents and β-casein. As we showed in Fig. 4 the ANS fluorescence intensity in α-lactalbumin was increased by adding dextran and PEG compare with absence of them, which it could be means that dextran and PEG increase the exposed hydrophobicity in α-lactalbumin. On the other hand, the ANS fluorescence of α-lactalbumin decreased in the presence of ficoll which means less exposed hydrophobicity area of protein. The ANS fluorescence result also showed a decreased in ANS fluorescence value of α-lactalbumin about 63.36% in the presence of β-casein and implying a reduction in exposed hydrophobicity in α-lactalbumin and showed chaperoning effect of β-casein in prevent amyloid formation of α-lactalbumin.

By adding β-casein to the mixture of dextran and α-lactalbumin, as well as the mixture of PEG and α-lactalbumin the level of ANS fluorescence decreased about 52% and 53%, respectively. But by adding β-casein in the presence of ficoll the level of ANS fluorescence decreased about 77%, so it is concluded that in the presence of ficoll the chaperone activity of β-casein increased.

NEAR UV CD

Near UV CD assay was used to show the effect of crowding agents on changes in tertiary structure in α-lactalbumin. As shown in Fig. 5, the Near UV CD spectrum of native α-lactalbumin shows a minimum ellipticity at 270-280 and 290 nm which it refers to four tyrosine and 4 tryptophan residues respectively [32]. A spectrum recorded after adding DTT to α-lactalbumin showed an increase in the tryptophan signal at around 290 nm, which illustrate some changes around the environment of aromatic residue. By adding dextran and PEG to reduced α-lactalbumin, there was a shift of peak around 270 nm and increased in band intensity which shows some changes in the structure of α-lactalbumin. By adding ficoll to the α-lactalbumin, however, the change in the CD spectra around 290 nm was not significant with concomitant increase in overall intensity, implying little alteration in the tertiary structure.

CD spectra of reduced α-lactalbumin in the presence of β-casein revealed that there was a signal intensity changes in Near UV CD signal. In the presence of dextran and polyethylene glycol the effect of β-casein on tertiary structure of α-lactalbumin was reduced compare with absence of dextran and PEG. Adding ficoll to reduced α-lactalbumin bring to signal shift and negative peak to 290 nm and cause some shift in the tryptophan signal, indicating some changes in tryptophan residue environment.

FAR UV CD

The effect of macromolecular crowding agents on the secondary structure of α-lactalbumin in the presence and absence of β-casein has been investigated by Far UV CD spectra. The CD spectrum of native α-lactalbumin showed a minimum on 208 and 222 nm which it presence the α-helix structure in the protein [33]. By adding DTT there was disputable change in intensity of α-lactalbumin on 208 and 222 nm. The Far UV spectra of α-lactalbumin in the presence of dextran and PEG show a substantial loss in 222 and 208 nm respectively which show change in the secondary structure. In the presence of ficoll, however, the structure of α-lactalbumin is still similar to that obtained for

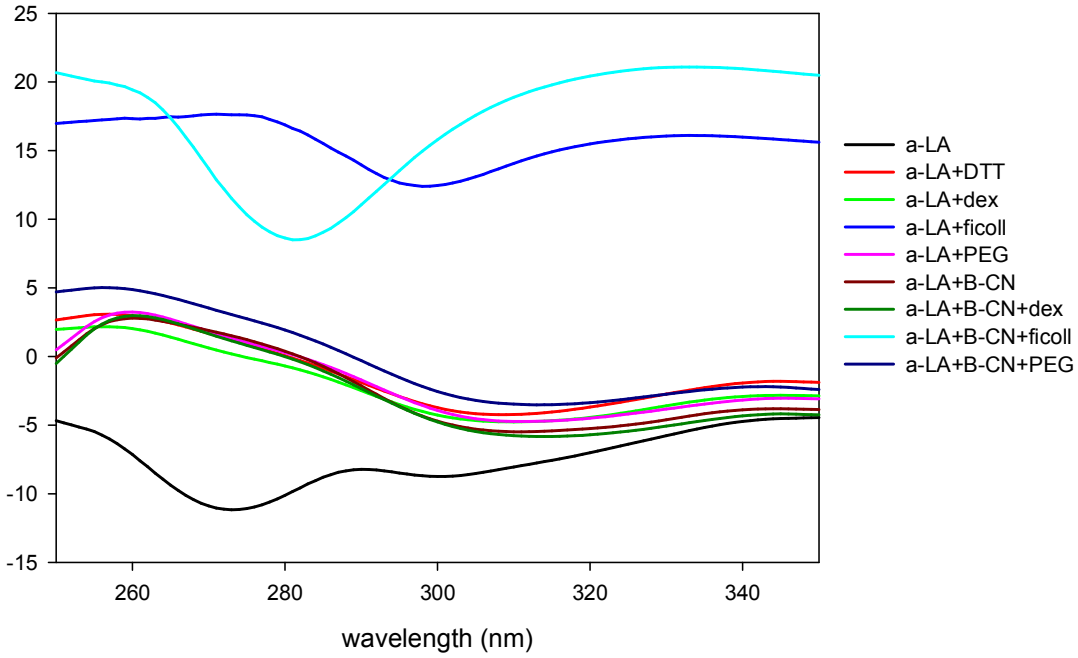

Fig. 5. Near UV spectra of α-lactalbumin (0.4 mg ml^{-1}) in presence and absence dextran, ficoll, PEG (10% w/v) and β-casein. All experiments were conducted in 10 mM phosphate buffer, pH 7.0 at 37 °C in a Aviv 215 spectropolarimetre with a 1-ch path length cell.

native protein.

Adding β-casein to the protein, however, showed the same signal as native α-lactalbumin with decrease in the size of the negative CD signal. In the presence of all crowding agent this deviated pattern is still similar with different size of intensity (Fig. 6).

The content of the secondary structure of native and reduced α-lactalbumin in the presence and absence of β-casein, dextran, ficoll and PEG are shown in Table 1.

DISCUSSIONS

The intra cellular environment is full of crowded because of the high concentration of soluble and insoluble macromolecule [34,35]. 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 results is decrease in molecular diffusion rate and this effect happen in all small and big molecules. Furthermore, crowded effects are complex in biochemical interaction, because albeit it decreases the diffusion of molecules, it makes an increase in thermodynamic interaction [15,36]. Obviously, the volume that occupied by a molecule is not available to other molecule since two molecules 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 alteration in the rate of the other chemical reaction, folding protein and interaction between macromolecules [37,38].

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 of protein [12]. The aim of this study is to find out the effect of crowding agents including dextran, ficoll and PEG on chaperone action of β-casein in preventing amyloid fibril formation of α-lactalbumin.

Light scattering data show that dextran and PEG had significant effect on the rate and time of the onset of

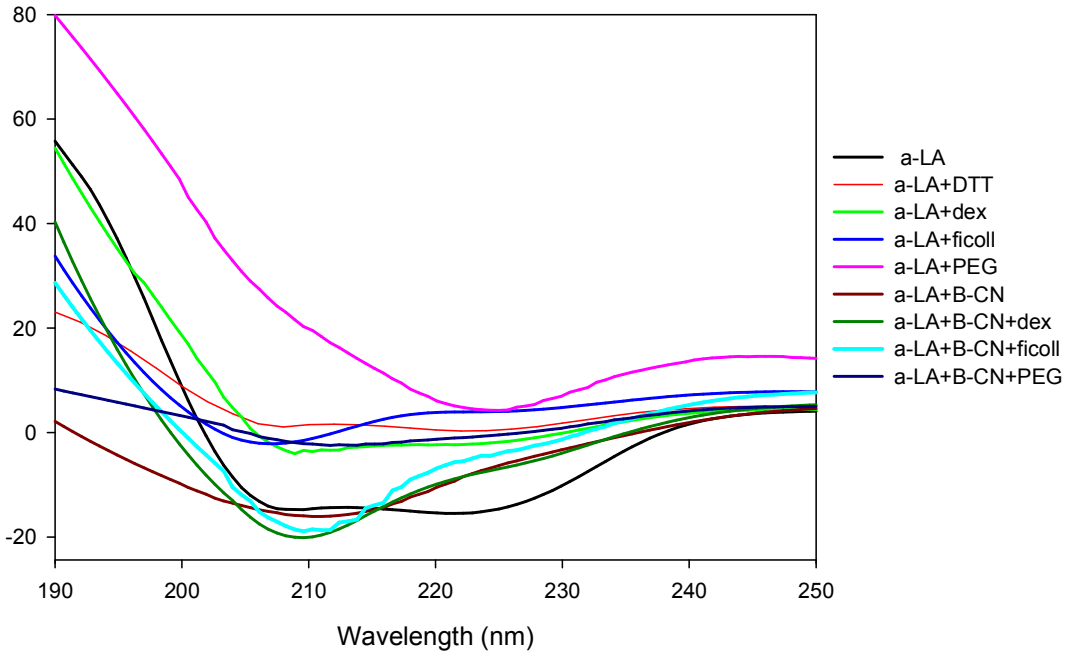

Fig. 6. Far UV spectra of α-lactalbumin (0.2 mg ml^{-1}) in presence and absence of dextran, ficoll, PEG (10% w/v) and β-casein. All experiments were conducted in 10 mM phosphate buffer, pH 7.0 at 37 °C in an Aviv 215 spectropolarimetre with a 1cm path length cell.

aggregation of α-lactalbumin. In fact, they increased the rate and decreased the time of onset of aggregation in α-lactalbumin. Ficoll, on the other hand, showed opposite results in which it decreased the rate of aggregation of α-lactalbumin. β-Casein effectively prevented aggregation of α-lactalbumin. It was found, however, to be less efficient in preventing this aggregation in the presence of dextran and PEG while its chaperone ability increased in the presence of ficoll.

Consistent with light scattering data, ThT binding assay also showed the same results. β-Casein decreased the rate and the extent of amyloid fibril formation. In the presence of dextran and PEG, however, β-casein was less effective in preventing amyloid fibril of α-lactalbumin. In contrast, ficoll decreased the rate of amyloid fibril formation and β-casein was more effective in preventing fibril formation in α-lactalbumin in the presence of ficoll [39-41,30].

The intrinsic experiment show fluorescence intensity increased in presence of dextran and PEG [30,3] and reduced in the presence of ficoll [40], In addition the result of intrinsic fluorescence intensity also showed that the chaperone activity of β-casein increased in the presence of ficoll while it decreased in the presence of dextran and PEG. Following intrinsic results, the ANS binding assay also show an increase in exposed hydrophobic area in α-lactalbumin in the presence of dextran and PEGS which confirm protein conformation changes whereas it decreased in the presence of ficoll [4,12]. The action of β-casein was decrease in presence of dextran and PEG which could be concludes that these two agents can have an effect both on hydrophobicity of α-lactalbumin and β-casein. β-Casein activity enhances in present ficoll which means ficoll could increase the hydrophobicity regions of β-casein.

CD results show that dextran and PEG could cause significant changes in the structure of α-lactalbumin while these change was less in presence of ficoll. β-Casein prevent structural changes in α-lactalbumin but not as well as in the presence of dextran and PEG [41,42].

As a result, dextran and PEG decrease the β-casein function which means dextran and PEG diminished the positive activity of β-casein on preventing amyloid aggregation in a-lactalbumin. The inhibitory effect of these

two agents on β-casein could be because of 3 reasons: a) decrease of interaction between β-casein and α-lactalbumin, b) increase rate of aggregation of α-lactalbumin, [43].

According to previous studies, the different crowding agents have different effect on the protein stabilization [39]. Macromolecular crowding is termed 'the excluded volume effect' because of the non-specific steric repulsion that occurs between the solute molecules. The amount of intracellular volume which is unavailable to other molecules depends on the number, size, shape and concentration of all molecules [44]. The volume-excluded effect of crowding agents can intensify by creating non-specific interaction between inactive form of protein and crowding agents 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].

In relation to point a maybe dextran and PEG slowing down diffusion and limiting chance encounters of α-lactalbumin with β-casein [45,46].

With regard to point b, dextran and PEG increased the rate of aggregation and amyloid fibril formation in α-lactalbumin (Figs. 1 and 2). In our previous study we also showed that dextran interfered with chaperone action of α-crystalline on preventing amyloid formation of α-lactalbumin [30].

In contrast with dextran and PEG, ficoll decreased the aggregation of α-lactalbumin and increased the chaperone ability of β-casein. In fact, ficoll increase the stability of α-lactalbumin which could be due to the structure of ficoll that affect its excluded volume effect and consequently non-specific interaction did not occur between ficoll and α-lactalbumin [47].

CONCLUSION

In conclusion the result from this study shows that crowding agents have different effect on the stability of target protein and chaperone action of β-casein. Crowding agents had effect on α-lactalbumin hydrophobicity and polarity changes of amino acids, as well as on the hydrophobic regions of β-casein as a chaperone. So we could conclude the effect of crowding agents depends greatly on size of molecules, volume occupied and non-specific interaction between molecules and type of crowding agents.

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