INTERACTION BETWEEN WHEY PROTEIN NANOPARTICLES AND FATTY ACIDS

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Abstract

The cytotoxicity of formulated nanoparticle complexes of different fatty acids (oleic, eliedic, Cis-vaccenic, Trans-vaccenic, and linolenic acids) in the presence or absence of whey protein isolate (WPI) was investigated in this study. Nanoparticle complexes formed with WPI was examined for surface tension, circular dichroism (CD), turbidity, isothermal titration calorimetry (ITC) and Cytotoxic activity. Surface tension values were decreased with adding fatty acid to WPI.

Cis-fatty acids such as oleic, cis-vaccenic and linolenic caused higher decrease in the surface tension of WPI nanoparticles than that of trans-fatty acids (eledic and trans-vaccenic acids). The tertiary structure of WPI was lost and changed from fold to unfold after binding with fatty acids. The changes in WPI structure would be correlated to exhibit a cytotoxic activity to tumor cells. The turbidity values for nanocomplexes of WPI/fatty acids were lower confirming higher ability in binding fatty acids.

All nano complexes formed of WPI/fatty acids exhibited a cytotoxic ability as a lysis in erythrocytes. Nanocomplexes can be formed from WPI with good cytotoxic effect to tumer cells using cis-vaccenic and linolenic fatty acids comparable to oleic acid. It was a new interesting observation being that the nanocomplexes formed of WPI with fatty acids has a comparable cytotoxcisty to that of α-LA and β-lg and can be used in tumor therapy.

Keywords: Nanoparticles; Whey protein isolate; Fatty acids; Surface tension; Circular dichroism; Turbidity; Cytotoxicity.

Introduction

Whey proteins which are valuable by-products from cheese industry are widely used in a variety of foods for their superior nutritional and functional properties. Molecular interactions between protein and lipid or lipid-like molecules are central to one of the most important functions of protein, namely the adsorption at biological interfaces, and the structure of bio-membranes ***(Adamson 1990)***. In the last few years, a remarkable apoptotic-like activity towards cultured cancer cells of a complex between the calcium-free form of human α-LA and oleic acid (OA) has been described ***(Mok et al., 2007)***. The complex could either be isolated from human milk ***(Hakansson et al., 1995)*** or formed on a diethylaminoethyl (DEAE) trisacry column equilibrated with OA ***(Svensson et al., 2000)***.

The complex was named HAMLET (human alpha-lactalbumin made lethal to tumor cells) and defined as a complex between partially unfold α-lactalbumin and oleic acid. When tested against several different cell types, HAMLET showed strongest activity against tumor cells, whereas mature differentiated cells were not affected ***(Svanborg et al., 2003)***. In contrast to human milk, no similar naturally occurring activity was found in bovine milk. However, it has been possible to make a complex between bovine α-LA and OA called BAMLET (bovine alpha-lactalbumin made lethal to tumor cells), capable of inducing cell death in trans-formed cells in vitro. Meanwhile, the OA complex retains its activity upon replacement of human α-LA with closely related amino acid sequences. ***(Pettersson et al., 2006)***.

Therefore, the aims of the present study were to the ability of bovine whey protein to formulate a nanocomplex with different unsaturated fatty acids that have a cytotoxic activity was investigated. Thus, the study was planned to formation and properties of nanocomplexes from whole whey protein (WPI) preparation and different fatty acids.

Materials

Whey Protien Isolate (WPI) was purchased from Arla Foods Ingredients, Denmark. All of fatty acids used in this study were purchased from Sigma-Aldrich Co., St Louis, USA.

Methods

Preparation of WPI solutions

In this study (at Denmark Laboratories) whey protein isolate (WPI) was dissolved in double distilled water at 4°C. The WPI concentration was determined using Spectrophotometer at 280nm. Samples of WPI solution for suspended fatty acids and heat treatment were diluted to a final concentration of 1 mg/mL in glycine buffer at pH 9.

Preparation of stock fatty acid solutions

Oleic acid was dissolved in Na2CO3 0.1 M while eliedic, cis-vaccenic, trans-vaccenic and linolenic fatty acids were dissolved in ethanol at concentration of 5mg/ml and stirred until complete solublization.

Preparation of WPI/fatty acid mixtures

Mixtures of WPI solutions with different fatty acids were prepared as described by ***Kamijima et al., (2008).*** Different fatty acids were directly suspended into the (WPI) protein solutions of different concentration as a molar ratio. These mixtures were heated at 60°C for 15 min. to facilitate dispersal of the fatty acids and structural change of the protein.

After incubation for 15 min, the mixtures were cooled to room temperature (20°C).

Surface tension measurements

Surface tension for all samples were measured at room temperature (20°C) using the Pendent drop (KSV Instruments', CAM 101).

Circular Dichroism (CD) spectra

Spectra of CD was measured at 37°C with a Jasco J-810 spectropolarimeter (Jasco Spectroscopic Co. Ltd., Hachioji City, Japan) equipped with Jasco PTC-348WI temperature control unit. Eight scans were recorded and averaged for each spectrum. The WPI was 38.62 µM and 3.862 µM for near UV and Far UV, respectively.

Turbidimetric analysis

The turbidity of protein fatty acid mixtures were measured at 400 nm using Spectrophotometer.

Cutotoxic activity by using erythrocytes

The interaction effect of different protein/fatty acid complexes on erythrocytes as indication of cutotoxic activity was examined as described by ***Dobrovolskaia et al., (2008).***

Statistical analysis

Data of the three experimental were analyzed using the Statistical Analysis System ***SAS (1998).***

Results and Discussions

Surface tension measurements

The surface tension values of solution from WPI nanoparticles with different fatty acid in complex are plotted in Fig. (1), as a function of fatty acid in the presence or absence of WPI after heating at 60°C for 15 min. The effect of additing fatty acids to the WPI nanoparticles (1mg/ml) on surface tension was depended on fatty acid concentration. In general, addition of WPI nanoparticles led to much faster decrease of surface tension as compared to the solution without WPI nanoparticles (the cis fatty acids have more ability to bind with WPI nanoparticles than the trans fatty acids).

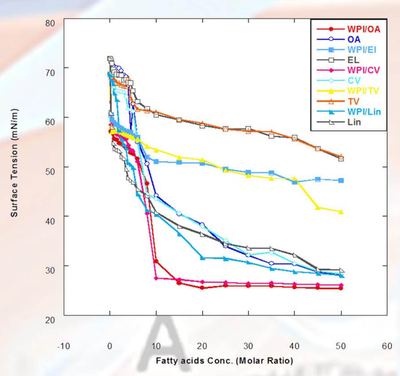

Fig. (1): Surface tension (mN/m) of different fatty acid solutions in glycine buffer (pH 9.0) either alone or as a complex with whey protein isolate (WPI 1mg/ml) by heating at 60°C/15 min.

The reduction in surface tension value was always higher with WPI nanoparticle complex than that without WPI. Therefore, the WPI/fatty acid complexes have higher ability to kill cancer cell more than fatty acids only.

Fatty acids in the cis conformation are U-shaped around the double bond, with both carbon chains projecting in one direction. Tran's fatty acids are rod shaped around the double bond due to the carbon chains on opposite sides of the double bond. This may explain the ability of cis fatty acids that have more ability of binding with WPI nanoparticles more than trans fatty acids. The results thus indicate that the cis conformation allows fatty acids a close stereo-specific fit, and the additional critical feature of the fatty acid is the carbon chain length. Fernandez et al., (2007) found a significant decrease in surface tension when films prepared from whey protein isolate (WPI) together with were saturated and unsaturated fatty acids.

Circular dichroism (CD) Spectra

Spectra of heated WPI solution (1mg/ml in glycine buffer, pH 9.0) with different fatty acids (oleic, eledic, cis--vaccenic, trans-vaccenic and linolenic) in nano complex form circular dichroism (CD) was measured to confirm the changes in structure characteristics and the spectra is presented in ***Figs. (2 & 3)***. The ability of WPI/oleic acid nanoparticle complexes to kill cancer cell has been shown to increase after losing the tertiary structure. The lower signal intensities of heat treated WPI compared to native WPI is commonly indicating the loss of tertiary structure. The spectrum of heat treated complex showed an increase in helicity form from that of native WPI.

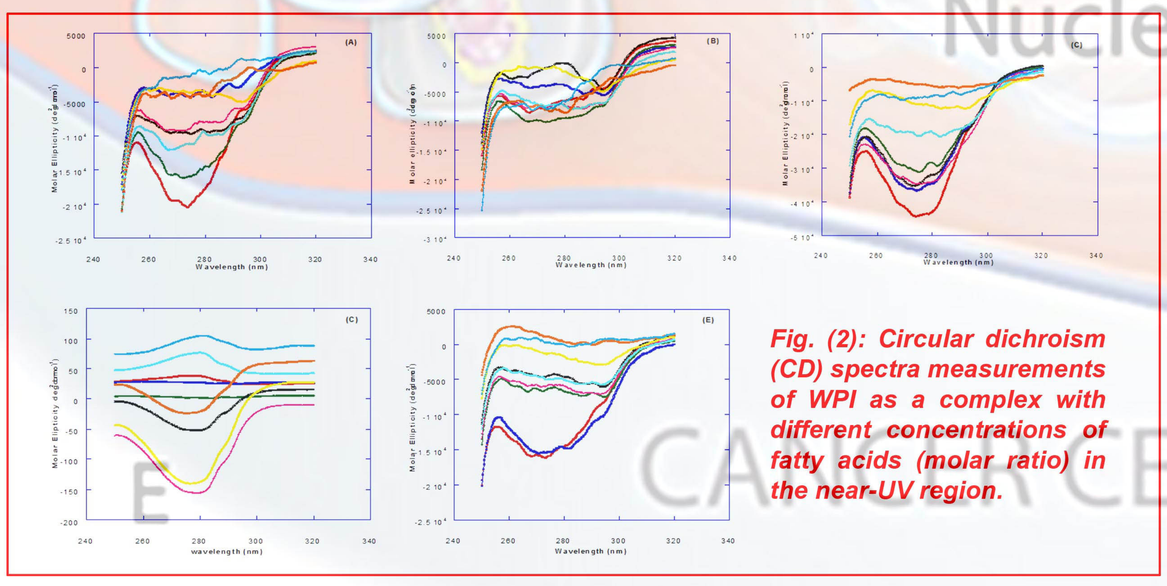

Fig. (2): Circular dichroism (CD) spectra measurements of WPI as a complex with different concentrations of fatty acids (molar ratio) in the near-UV region.

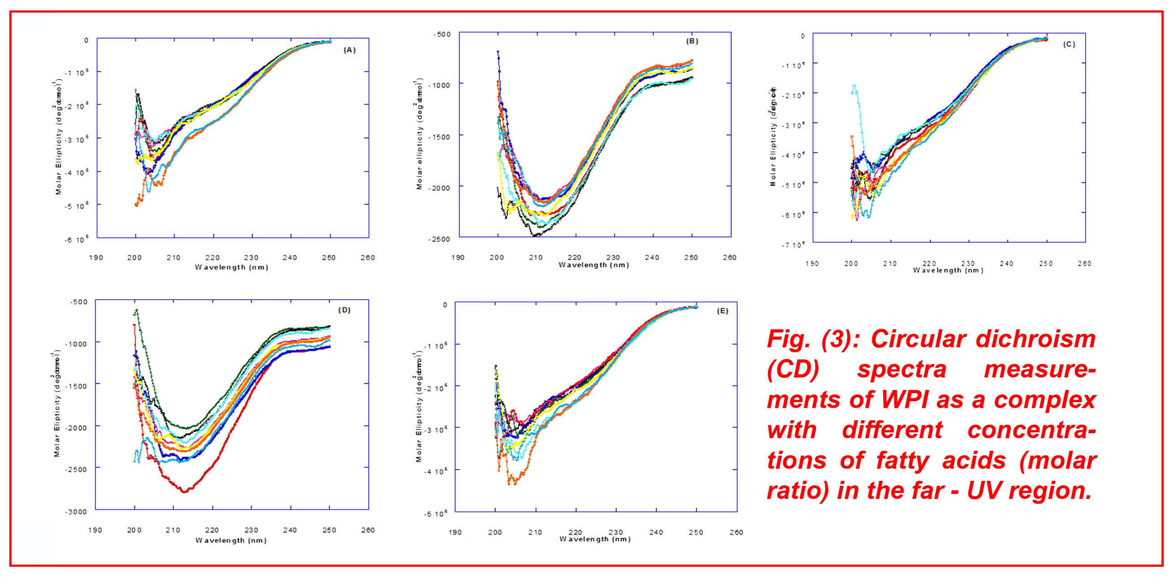

Fig. (3): Circular dichroism (CD) spectra measurements of WPI as a complex with different concentrations of fatty acids (molar ratio) in the far - UV region.

These results may indicate that structural features of heated WPI/oleic acid complexes are slightly different from that of HAMLET although their apoptotic activities resemble each other. The ability for complexes of WPI/oleic acid nanoparticles to kill cancer cell increased after losing tertiary structure and can be used to work like HAMLET or BAMLET. Interestingly, the peak intensities of the near-UV seemed to be correlated with cytotoxic activity. These results may indicate that the amount of the active components increases as WPI loses its conformation by increase the addition from oleic acid and heat treatment.

Generally, the signal of CD spectra increased with increasing the fatty acid concentrations. Increasing the signal refers to the changes of protein (WPI) from fold to unfold conformation. Clearly, all fatty acid used have changed the structure and even WPI alone at (0.0) fatty acid concentration exhibited a structure change since it was heat before used.

Our results are in agreement with previous research which clearly demonstrated that whey proteins form small disulfide linked aggregates when heated at low salt concentrations and neutral pH ***(Marangoni et al. 2000; Hoffmann and van Mil, 1997; Roef and de Kruif, 1994; Sawyer, 1968; Shimada and Cheftel, 1989).***

Changes in the collective secondary structures of WPI were assessed by CD spectroscopy. The binding of fatty acids with WPI have significant effect on the protein secondary structures of the WPI and the results of far-UV CD spectra indicated significant loss in protein secondary structural.

The far-UV CD spectra of pre-extruded and freeze-dried WPI approximate an appropriate mixture of α-LA and β-lg with double negative peaks at 205 nm and 222 nm, similar but less well defined (especially at 222 nm) than that of pure α-LA ***(Qi and Onwulata 2011).***

Turbidity measurements

The turbidity measurements of different fatty acids at different concentration ratios in glycine buffer solution (pH 9.0) either alone or in the presence of WPI (1mg/ml) as a complex by heating at 60°C/15 min are illustrated in Fig (4).

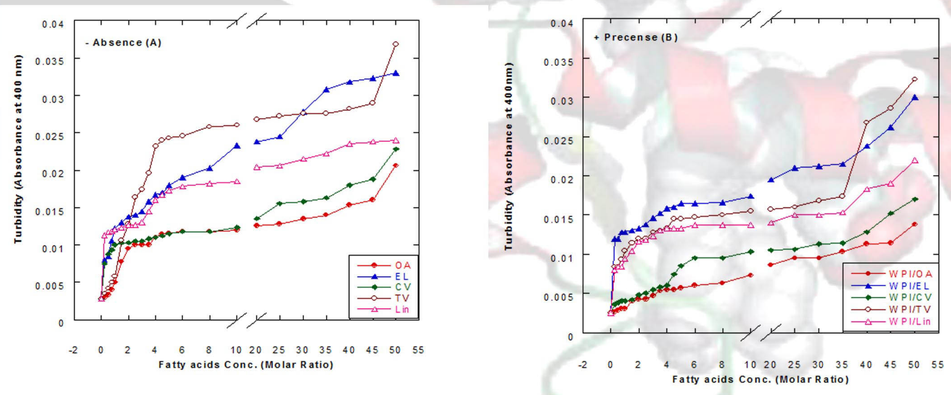

Fig. (4): Turbidity measurements of different fatty acid solutions in glycine buffer (pH 9.0) either alone (A) or as a complex (B) with whey protein isolate (WPI 1mg/ml) by heating at 60°C/15 min.

The data presented cleared that the turbidity was increased in the solution of fatty acids either alone or in a complex with WPI by increasing the fatty acid concentration. All formed fatty acid complexes presented lower turbidity measurements ***(Fig. 4B)*** compared to the same fatty acid only ***(Fig. 4A).*** The minimum turbidity for the fatty acids alone without WPI was observed with oleic acid while the maximum was observed in trans-vaccenic acid. The fatty acids in cis-form (oleic, cis-vaccinic, linolenic) showed lower turbidity measurements compared to that in trans-form (eledic, trans-vaccinic).

A general observation could be noticed in our experiments that there were a lower turbidity measurements in all tested samples which could be explained to the higher pH value used (more than 8.0) in all solutions measured since it was adjusted to pH 9.0 using glycine buffer.

Cutotoxic activity of WPI/fatty acid complexes

The lysis of erythrocytes (O.D at 405 nm) by addition of either fatty acids only or WPI/fatty acids complexes after incubation for 3 h at 37°C is presented in ***Fig. (5).*** The obtained data indicated that the WPI/fatty acid complexes had more efficiency to lysis the erythrocytes than fatty acids alone. The erythrocytes lysis increased by increasing the fatty acid concentrations either in presence or absense of WPI. It could be also seen that at the low oleic acid concentration the complex of WPI/oleic acid showed slight lysis but still higher than that of WPI in buffer only. The fatty acids in cis conformation showed highest activity than that of fatty acids in trans conformation. Therefore, the lowest erythrocyte lysis was observed when eledic acid was used.

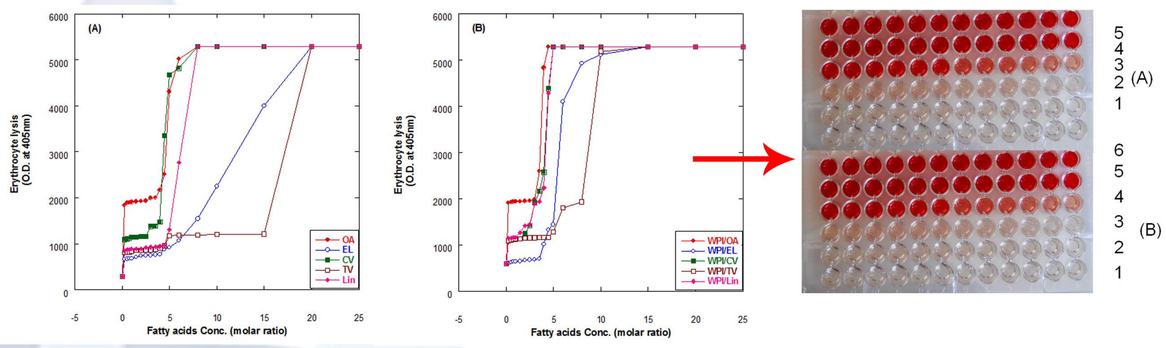

Fig. (5): Cutotoxic activity measurements as erythrocytes lysis happen after adding either fatty acid alone (A) or WPI/fatty acid complexes (B) at pH 9.0.

In general, all Complexes prepared of WPI/different fatty acids showed cytotoxic effect in different extent depending upon the type and concentration of fatty acid. The cytotoxicity of the complexes increased with higher numbers of fatty acids per WPI molecule. The fatty acid alone in buffer solutions showed also a cytotoxic effect to less extent as in complex with protein.

The cytotoxic function of the WPI/fatty acid complexes was similar to that of HAMLET and clearly formed apoptotic complexes where heat treated. However, heat treatment changed such a mixture to a cytotoxic component (Kamijima et al., 2008). There is no consensus as to whether the cytotoxicity can be ascribed to a synergistic effect of α-LA and OA or exerted per se by either of the two components (Brinkmann et al., 2011).

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