American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 1 (2): 106-120, 2011
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2011.106.120

Compositional Analysis α-Lactalbumin

[1]V. Velusamy and [2]L. Palaniappan
[1]Department of Physics, Manonmaniam Sundaranar University, Tirunelveli, TN, India
[2]Department of Physics, (DDE), Annamalai University-608 002, TN, India

Corresponding Author: L. Palaniappan, Department of Physics, (DDE), Annamalai University-608 002, TN, India

ABSTRACT

This review is about the α-Lactalbumin (α-LA), the major whey protein found in the milk of all mammals. It is a simple Ca^{2+} binding milk protein, homologous in sequence to the lysozyme family and has significant role in biosynthesis of lactose in the lactating mammary gland. The structural and functional analyses so far obtained about this protein were outlined here. As regards the structure, it displays the best-characterized molten globule state which has been studied by various techniques such as X-ray scattering, NMR, thermodynamic study, ultrasonic study etc. The functional aspect shows many remarkable features of its calcium binding sites and is found to be a valuable constituent to enrich the infant formula. In addition, α-LA can alter its biological function depending on the conformational state and actively interact with lipid membranes, which leads to its antimicrobial and antitumor activity that has a vital role to induce apoptosis in tumor cells. Recently it was found that fabrication of α-LA nanoparticles to use in drug and food delivery system in nanomedicine is also possible.

Key words: α-Lactalbumin, molten globule state, metal ion binding sites, membranes, infant formulas, apoptosis activity, biological nanoparticle

INTRODUCTION

Milk and its components: Man has been drinking the milk of cows, goats, sheep, buffalo, horses and other mammals for a long time. All mammals produce milk with different composition of its constituents. Of course the composition of milk varies from cow to cow, for the various breeds, the animal's feed, the stage of lactation etc.

Milk is an opaque white liquid produced by the mammary glands of mammals which is almost a complete natural food (Bowen and Lawrence, 2005). It is an emulsion or colloid of buffer fat globules within a water-based fluid. The major components of milk food are water, proteins, lactose, fat, minerals, vitamins and others (Velusamy *et al.*, 2007). Of all the macromolecules present, protein seems to be more significant owing to their selective soluble nature in acidic and alkaline mediums and its high absorption or low association properties (Velusamy and Palaniappan, 2004). Thus, the present study is centered about milk protein, in especially only one particular type.

Milk proteins: The total protein component of milk is composed of numerous specific proteins. The primary groups of milk proteins are the caseins. There are 3 or 4 caseins in the milk of most species; the different caseins are distinct molecules but are similar in structure. All other proteins found in milk are grouped together under the name of whey proteins. The major whey proteins in cow milk are β-lactoglobulin and α-LA (Hurley, 2009; Azman, 2009).

All these major milk proteins are synthesized in the mammary epithelial cells and are only produced by the mammary gland. The immunoglobulin and serum albumin in milk are not synthesized by the epithelial cells. Instead, they are absorbed from the blood (both serum albumin and the immunoglobulin).

Whey proteins: There are many whey proteins in milk and the specific set of whey proteins found in mammary secretions varies with the species, the stage of lactation, the presence of an intra-mammary infection and other factors.

α- LA and its importance: α-LA is the most abundant whey protein in human milk (Heine *et al.*, 1991). It is a small Ca^{2+} binding protein, of molecular weight 14,200 Dalton present in mammalian milk and function as a specificity modifier of an enzyme, galactosyltransferase (Hill and Brew, 1975; Hiraoka *et al.*, 1980; Mckenzie and White, 1991; Ashikin and Razak, 2009). α-LA plays an essential role in milk formation in all species (Brew and Grobler, 1992). During lactation, the mammary gland expresses both the enzyme β-1,4-galactosyltransferase (GT) and α-LA. These two proteins form an enzyme complex, lactose synthase that is present in the Golgi Vesicles during lactogenesis.

$$\text{GT/ } \alpha\text{-LA}$$

$$\text{UDP} - \text{GT} + \text{Glucose} \rightarrow \text{Lactose} + \text{UDP}$$

The important role of α-LA in lactose formation has been shown in experiments involving transgenic animals (Stacey *et al.*, 1995). In general, the metal cations such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Na^{+} etc. and/or the mineralogical compositions of heavier elements present in the individual specimen are specifically reflected in their physical compactness (Kannappan *et al.*, 1999). This special feature found in the case of α-LA aids to study about protein-membrane interaction and protein-organic compound interaction etc. (Kronman, 1989). In nutritional role, α-LA is rich in tryptophan which is typically the limiting amino acid in infant formulas and as a result α-LA-enriched infant formula has been developed. Feeding α-LA as a source of tryptophan has shown neurobehavioral benefits in adults (Markus *et al.*, 2000, 2002). Conformation is again a special phenomenon in cyclic molecules that offer two or more structural forms. The stable conformational variant of such molecules including proteins expose distinct response in biological activity (Sundaram and Palaniappan, 2005). An α-LA has different functions depending on its conformational or folding state. For example some forms of α-LA can induce apoptosis in tumor cells (Svensson *et al.*, 1999; Creighton *et al.*, 1996; Hakansson *et al.*, 1995). In recent years, the production of biological nanoparticles from α-LA is being achieved for drug delivery and food science applications (Mehravar *et al.*, 2009). In view of these vital importances of this milk protein, this review article is devoted to the detailed study of the compositional analysis of α-LA that includes both the structural aspects and functional aspects.

STRUCTURAL ANALYSIS OF α-LA

Structure of milk protein: The primary structure of proteins consists of a polypeptide chain of amino acid residues joined together by peptide linkages (Velusamy and Palaniappan, 2004) which may also be cross-linked by disulphide bridges. The three-dimensional organization of proteins, or

conformation also involves secondary, tertiary and quaternary structures (Sundaram and Palaniappan, 2005). The secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. The alpha-helix and β-pleated sheet are examples of secondary structures arising from regular and periodic steric relationships.

The tertiary structure refers to the spatial arrangement of amino acid residues that are far apart in the linear sequence, giving rise to further coiling and folding. If the protein is tightly coiled and folded into somewhat spherical shape, it is called a globular protein. Quaternary structure occurs when proteins with two or more polypeptide chain subunits are associated (http://www.foodsci.org). The structure of α-LA is different in its native and non-native states.

Native state of α-LA: α-LA is genetically and structurally homologous to C-type lysozyme and its high-resolution X-ray crystallographic structure has been reported (Acharya *et al.*, 1989, 1991; Harata and Muraki, 1992; Ren *et al.*, 1993). The structural properties of α-LA and its interrelationships with lysozyme were reported (Kronman, 1989; Mckenzie and White, 1991). However, lysozyme and α-LA also differ markedly in their metal-ion-binding properties (Kronman, 1989). Native α-LA consists of two domains and a β-sheet domain (Kuwajima, 1996). There are four α-helices-A[residues 5-11], B[23-24], C[86-99] and D[105-109] and three 3_{10}-helices[12-16, 101-104 and 115-119] in the α-helical domain and an anti-parallel β-sheet[40-50] and a 3_{10}-helix[76-82] in the β-sheet domain. The native state of α-LA is shown in Fig. 1.

Molten globule state of α-LA (non-native state): A composed denatured state is obtained (Shortle, 1996; Dill and Shortle, 1995) under a mild denaturation condition for various proteins. In this order, α-LA is the typical example for molten globule state which is also known as non-native state. In non-native state, two types of conformation are observed. One is a highly denatured or fully unfolded state and the other is the compact intermediates which have been observed under both transient and equilibrium conditions. The potential energy barrier will not so high enough between the conformers so that transitions from one form to other are quite possible (Palaniappan, 2001a). Thus the molten globule is a rigorously defined structural state with: (1) Substantial secondary structures, (2) absence of native-like tertiary structure, (3) compact (Dolgikh *et al.*, 1981) and globular and (4) without cooperative thermal denaturation (Ptitsyn, 1987, 1992, 1995).

X-ray scattering study: The non-native conformation of α-LA is equally important as the determination of the high-resolution 3-dimensional structure of the native state of α-LA. X-ray scattering technique, in particular the solution X-Ray scattering, has been successfully utilized to describe the characteristics of various non-native conformations (Damaschun *et al.*, 1991, 1993; Kataoka *et al.*, 1993, 1995; Kataoka and Goto, 1996; Konno *et al.*, 1995). In the X-ray scattering study, it is observed that the radius of gyration (Rg) for the molten globule is 20-30% larger than that for the native state. Radius of gyration of a molecule determines the scattering process. Velusamy *et al.* (2007) have reported that size of the molecules is important in explaining the cohesion effects whereas the mass is important regarding the inertial effects. The reports of Ptitsyn, (1987, 1992, 1995) are in line with this observation as α-LA is a heavier molecule with larger size, in which the Rg of the molten globule of α-LA was almost identical to the value for the native state. Further the structural characterization of the molten globule of α-LA studied by small-angle X-ray scattering which revealed that the molten globule is not as compact as native state. Arai *et al.*

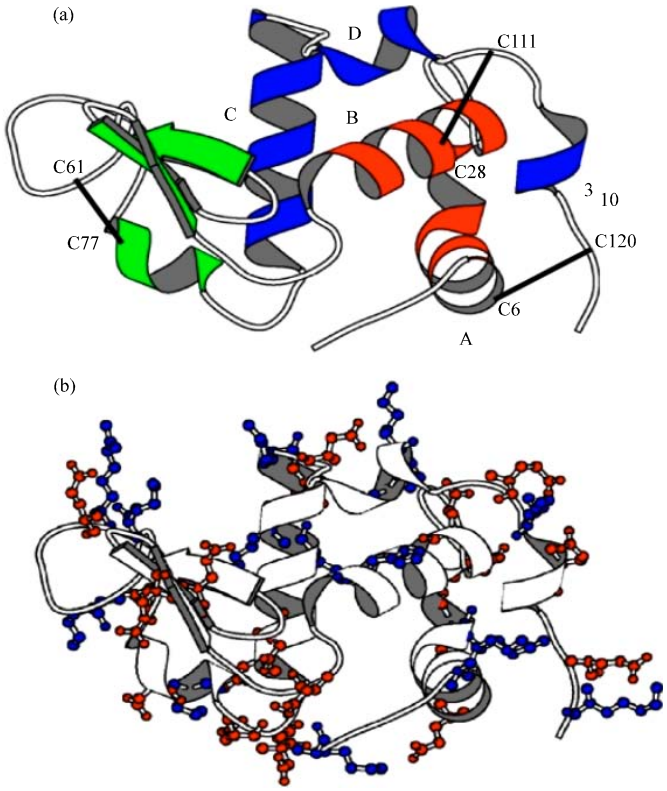

Fig. 1: Schematic representation of the native structure of human α-LA. (a) The α-domain is shown in red (residues 1-39) and blue (residues 82-123) and the β-domain (residues 40-81) is shown in green. The disulfide bonds present in 4SS and [28-111] α-LA are indicated and the α-domain helices are labeled. (b) Acidic (red) and basic (blue) residues are shown in a ball-and-stick representation. The diagrams were generated using MOLSCRIPT and the X-ray coordinates for the native protein (Rösner and Redfield, 2009)

(2002) observed the fast compaction of α-LA during folding, studied by stopped-flow X-ray scattering. Water being an aprotic solvent that can interact both in acidic and alkaline environment it was suggested that the folding intermediate is more hydrated than the native state and that the hydrated water molecules are dehydrated when specific side-chain packing is formed during the change from the molten globule to the native state (Velusamy and Palaniappan, 2004; Samuel Ebinezer *et al*., 2004).

NMR study: Based on NMR study of α-LA, the molten globule is a bipartite structure with an α-helical domain containing substantial secondary structure and native tertiary fold and with a disordered β-sheet domain (Baum *et al*., 1989; Alexandrescu *et al*., 1993; Chyan *et al*., 1993; Peng and Kim, 1994; Schulman *et al*., 1995). Its compactness suggested that the disordered β-sheet domain would be collapsed. The conceptual model was combined with the bipartite structural model. This model suggested a more detailed concept in which (1) the tertiary fold formed in the α-helical domain is stabilized by two disulfide bonds as well as by hydrophobic interaction (Thiyagarajan and Palaniappan, 2007b), (2) the disordered β-sheet domain is collapsed by one disulphide bond, (3) the two domains are also connected by another disulfide bond to maintain molecular compactness and (4) the disordered part cannot fluctuate and extend so randomly.

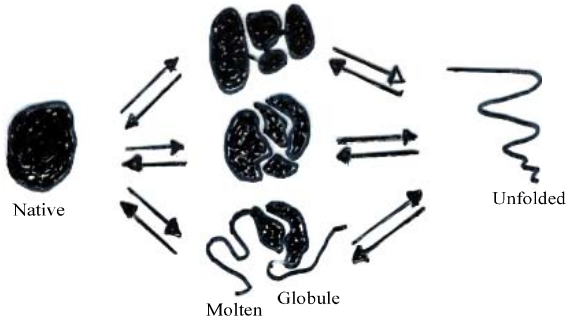

Fig. 2: Schematic description of various types of molten globule derived from the results of solution X-ray scattering (Kataoka *et al.*, 1997)

The various types of molten globule derived from the results of solution X-ray scattering is shown in Fig. 2. Proteins folding of α-LA studied by Temperature –jump NMR indicate a formation of non-native hydrophobic cluster in the unfolded state (Masaru and Kazuyuki, 1999). Further Redfield *et al.* (1999) reported that α-LA forms a compact molten globule in the absence of disulfide bonds using NMR spectroscopy.

The solvent accessibility and side-chain dynamics of aromatic residues in the molten globule of α-LA was investigated by the use of selective isotope labeling and (19) F-NMR and comparison was made for properties of both the native and unfolded protein, which indicates that the α-LA molten globule is highly heterogeneous; each residue has its unique solvent accessibility and motional environment. From this study, it was suggested that hydrophobic (Palaniappan and Thiyagarajan, 2008; Kannappan and Palaniappan, 1998) and van der Waals interactions (Palaniappan, 2001b) mediated by the inaccessible surface area could be sufficient to account for all the stability of the α-LA molten globule, which is approximately 50% of the value for the native protein (Bai *et al.*, 2000).

The 15N-1H HSQC NMR spectrum of the human α-LA molten globule at pH 2 and 20°C was characterized by broad lines and found that an increase in temperature to 50°C leads to a dramatic sharpening of peaks in the 15N-1H HSQC spectrum of human α-LA at pH 2 (Ramboarina and Redfield, 2003). SOFAST real-time 2D NMR technique used by Schanda *et al.* (2007) to monitor the conformational transition of α-LA from a molten globular to the native state for a large number of amide sites along the polypeptide chain revealed that the kinetic behavior was observed for the disappearance of the molten globule and the appearance of the native state was mono exponential and uniform along the polypeptide chain. This observation confirmed previous findings that a single transition state ensemble controls folding of α-LA from the molten globule to the native state. Further the effect of higher temperatures on fast time-scale backbone dynamics of molten globules was investigated and found that the disulfide bonds have a significant effect on backbone dynamics within the β-domain of the molten globule; within the α-domain, dynamics are not significantly influenced by these bonds (Ramboarina and Redfield, 2008).

Rösner and Redfield (2009) reported that the pH-dependent changes in stability observed for the molten globule must arise from differences in the number and distribution of charged amino acids at the two pH (2 and 7) values. On the other hand, the differences in pH-dependent changes in stability were observed between the three α-LA protein variants and all-Ala α-LA must arise from the way that disulfide bonds influence the relative positions of charged groups. Global Protein

folding State mapping by multivariate NMR(GPS NMR) study had identified a number of known thermodynamically stable partially folded α-LA states and the transitions between them (Malmendal *et al*., 2010; Creighton *et al*., 1996).

Thermodynamic study: Thermodynamic and thermoacoustic studies form another important tool for defining the structure of any component, in particular about the molten globule state. The intermolecular interactions revealed from such studies have been widely used to interpret the structure and functional aspects between the various species (Kannappan and Palaniappan, 1997; Palaniappan, 2002; Thairiyaraja *et al*., 2003). Yutani *et al*. (1992) have reported that the heat capacity of the molten globule state is similar to that of the unfolded state in the absence of salt. These studies suggested that factors other than hydrophobic interaction would also contribute to the formation of α-LA molten globule. The thermal unfolding of the molten globule of α-LA were shown by some thermodynamic studies which reflects the heat capacity changes upon thermal unfolding (Xie *et al*., 1991, 1993; Griko *et al*., 1994; Relkin and Mulvihill, 1996). The thermally denatured states have been studied to elucidate the energetic residual structure and its contributions to the stability of the native conformation.

Griko (1999, 2002) observed that a degree of residual structure in the denatured state must be taken into account to yield a more accurate description of protein structural energetic. The value of the heat capacity increment of α-LA denaturation correlates closely with the amount of residual secondary structure in the denatured protein, therefore reflecting the degree of its disordering and accessibility to solvent. Thermodynamic parameters such as the enthalpy and entropy of the denaturation of α-LA to compact denatured state are always greater than the enthalpy and entropy of its unfolding. This difference represents the unfolding of the molten globule state. Calorimetric measurements of the heat effect associated with the unfolding of the molten globule state reveal that it is negative in sign over the temperature range of molten globule stability. It was concluded that at physiological temperatures the entropy of dehydration is the dominant factor providing stability for the compact intermediate state on the folding pathway, while for the stability of the native state, the conformational enthalpy is the dominant factor.

The stability of different α-LA forms toward urea and thermal denaturation was studied at low and physiological salt and in the absence as well as presence of 1 mM or 10 mM Ca^{2+}. Denaturation was studied by Differential Scanning Calorimetry (DSC). Three lines of evidence indicate that human α-LA made lethal to tumor cells (HAMLET) is a kinetic trap: (1) It has lower stability than α-LA, although it is a complex of α-LA and oleic acid; (2) its denaturation is irreversible and HAMLET is lost after denaturation; (3) formation of HAMLET requires a specific conversion protocol (Fast *et al*., 2005).

The thermodynamic folding barriers of two relatively large proteins of the same size and topology: bovine α-LA (BLA) and hen-egg-white lysozyme (HEWL) were investigated by Halskau *et al*. (2008) and Dolgikh *et al*. (1985). From the analysis of DSC experiments with the variable-barrier model, a high barrier for HEWL and a marginal folding barrier for BLA were obtained. Peculiarly, it was found that the tuning of folding barriers *in vitro* by the engineering of electrostatic interactions is quite possible. In recent years, computational procedures to design surface charge distributions toward enhancement of protein stability have been developed and tested (Loladze *et al*., 1999; Ibarra-Molero *et al*., 1999; Pace, 2000; Perl *et al*., 2000; Sanchez-Ruiz and Makhatadze, 2001; Strickler *et al*., 2006; Pey *et al*., 2008).

Ultrasonic study: The ultrasonic characterization technique finds wide application in bio-liquids (Thiyagarajan and Palaniappan, 2007a, b). The magnitude of density as well as the velocity of sound in human body fluid or its constituents are of vital importance for carrying out acoustical analysis of human system or organs as a sudden excess or reduction of sound velocity indicates abnormalities (Nithyanandham and Lakshmanan, 2010; Velusamy, 2003). Specific partial volume, partial compressibility and sound absorption changes induced by the native-to-molten globule state (acid) transition of the human α-LA were measured by means of densitometry and ultrasonic techniques (Samuel Ebinezer and Palaniappan, 2007) and interpreted in terms of the protein molecule phase transition and inter phase water transfer. It was found that the molten globule is a highly hydrated state containing about 270 water molecules inside. The compressibility measurement (Palaniappan, 2008; Thiyagarajan and Palaniappan, 2008) indicated that water inside the molten globule interior occupies less volume and is less compressible than in solvent phase (Kharakoz and Bychkova, 1997). Recently, ultrasonic effect on physiochemical and functional properties of α-LA were analyzed by Jambrak *et al.* (2010). The result showed that pH did not change significantly upon the passage of ultrasound, however conductivities increased significantly after 20 kHz sonication. Electrical conductivity decreased significantly for ultrasound treatments in baths at 40 and 500 kHz for all samples. Solubility increased significantly for all samples at 20 kHz. Foam capacities and foam stabilities were improved after ultrasound treatments for both 20 and 40 kHz treatments. Foaming properties were not improved for protein model suspensions for 500 kHz treatments. The molecular weight of the protein decreased significantly after ultrasound treatments both using a 20 kHz probe and 40 kHz bath. The flow behavior of α-LA was observed to be shear-thickening after all treatments.

In general as regarding the structural aspects it is clear that the term molten globule is used in α-LA to describe a wide range of non-native conformations. The α-LA displays the best-characterized molten globule state and is the best model protein in protein folding studies when compared with the AZ-68 of CSF protein (Velusamy and Palaniappan, 2004).

FUNCTIONAL ANALYSIS OF α-LA

Significance of Calcium binding sites: α-LA is metalloprotein with calcium as its natural ligand, is the regulatory component of the lactose synthase (Hill and Brew, 1975; Hiraoka *et al.*, 1980). α-LA has a number of binding sites for metal-ions including Ca^{2+}, Mn^{2+}, Zn^{2+} (Kronman, 1989). Cat ion binding to the strong calcium site increases the stability of α-LA. The second derivative FTIR study of heat-induced and pressure-assisted cold-induced changes in the secondary structure of bovine α-LA was carried out for native holoprotein and calcium ion depleted apoprotein. The secondary structure and compactness of α-LA were examined in a temperature range from 20 to -15°C during the pressure-assisted cold treatments (Dzwolak *et al.*, 2001). From this study, it is observed that the protein's compactness and secondary structure were both considerably stabilized against an increase and decrease in temperature by the presence of calcium ion. The binding of metal cations also increases the stability of α-LA against the cations denaturing agents such as urea or guanidine hydrochloride (Permyakov *et al.*, 1985). Further it is observed that from the above study, any denaturation transition in α-LA (pressure, temperature, denaturation concentration) depends upon metal ion concentration.

Interaction with membranes: The interaction α-LA also extend with lipid membranes (Cawthern *et al.*, 1996; Grishchenko *et al.*, 1996; Montich and Marsh, 1995; Banuelos and Muga,

1996; Permyakov *et al.*, 1993; Pelligrini *et al.*, 1999). The interaction of α-LA with the liposome affects the phase transition from gel to liquid-crystalline state in liposome. From Calorimetric and spectroscopic studies (Palaniappan, 1991, 1997), it was suggested that formation of flexible structural intermediate of α-LA in solution is a preparation for its association with membranes. Aggregation of the unfolded α-LA molecules and burial of hydrophobic surface upon formation of ordered tertiary structure significantly reduce their membrane perturbing activity (Thiyagarajan *et al.*, 2004).

The overall exchange behavior of the membrane-bound state is molten globule-like, suggesting an overall destabilization of the polypeptide. Nevertheless, the backbone amide protons of residues R10, L12, C77, K98, V99 and W104 show significant protection against solvent exchange in the membrane-bound protein. The membrane-bound conformation of α-LA includes initial protonation of acidic side-chains at the membrane interface and formation of an interacting site with the membrane which involves helixes A and C. Further, these helices would slide away from each other, adopting a parallel orientation to the membrane and would rotate to maximize the interaction between their hydrophobic residues and the lipid bilayer (Halskau *et al.*, 2002).

The effect of lipid structure on α-LA-membrane interaction and bi-layer integrity may throw new light on the membranes disrupting mechanism of a conformer of human α-LA (HAMLET) that induces death of tumor cells but not of normal cells (Rodland *et al.*, 2005). Surprisingly, it was found that under certain conditions α-LA may interact reversibly with a plasma membrane (Halskau *et al.*, 2009). Recent studies show that HAMLET interacts with lipid membranes and perturbs their structure and integrity (Mossberg *et al.*, 2010).

Apoptosis-inducing activity: α-LA can alter its biological function depending on the conformational state (Jaafar *et al.*, 2007). As α-LA is a second example of a protein that can acquire different functions depending on its folding state (Svensson *et al.*, 1999) an α-LA complex from acid-precipitated human milk casein was shown to induce apoptosis in tumor cells and immature cells, but not in mature, differential cells (Hakansson *et al.*, 1995). HAMLET is a tumoricidal complex of apo α-LA and oleic acid, formed in casein after low pH treatment of human milk (Svensson *et al.*, 2000; Pettersson *et al.*, 2006, 2010).

Role in infant formulas: α-LA has an vital role in enriching the infant formula because α-LA contains a relatively high concentration of tryptophan and other essential amino acids compared with whole cow milk (Heine *et al.*, 1991; Lien, 2003). Only infants receiving the formula with a high concentration of α-LA had serum tryptophan concentrations as high as those of breastfed infants, as shown by Heine *et al.* (1996). Feeding infants α-LA-rich formulas results in higher plasma tryptophan concentrations than did feeding infants by standard formulas. Tryptophan is the precursor of the neurotransmitter serotonin and the neuro-secretory hormone melatonin. Serotonin and melatonin regulate many neurobehavioral effects such as appetite, satiation, mood, pain perception and the sleep-wake rhythm (Yogman *et al.*, 1983; Heine *et al.*, 1991). Thus, feeding α-LA has shown neuro behavioral benefits in adults.

Possibilities of biological α-LA nanoparticles: In recent years, ferrofluids (Samuel Ebinezer *et al.*, 2003, 2005; Saraswathi *et al.*, 2005; Thiyagarajan *et al.*, 2006), a special system of Nanoparticles have been emerged as versatile systems for the specific delivery of

drugs to organs and tissues (Langer *et al.*, 2008). Especially polymeric nanoparticles have attractive physiochemical properties such as size, surface potential, hydrophilic-hydrophobic balance etc. (Jaafar *et al.*, 2007) and for this reason they have been recognized as potential drug carrier for bioactive ingredient such as anticancer drugs, vaccines, oligonucleotides, peptides etc. (Jahanshahi *et al.*, 2008b).

Nanomaterial derived from proteins, especially protein nanoparticles are biodegradable, non-antigenic, metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. In view of the defined primary structure of proteins, the protein-based nanoparticles may suggest various possibilities for surface alteration and covalent drug attachment (Weber *et al.*, 2000).

In this way, α-LA has significant properties such as nutritional, rich in tryptophan, bacterial or antitumor activity etc so that it finds important role in the preparation of nanoparticles. Basically three different preparations of such protein nanoparticles have been described based on emulsion formation, desolvation or coacervation (Jahanshahi *et al.*, 2008a). The nanotubular structures was formed by partial hydrolysis of α-LA by a protease from *Bacillus licheniformis* under appropriate conditions (Graveland- Bikker *et al.*, 2006), as similarly reported by Yahaya (2009) and Palaniappan *et al.* (2008). The growth of α-LA nanotubes and their dimensions were analyzed using transmission electron microscopy, static light scattering and small-angle X-ray scattering. The scattering data were fitted using a model describing the growth of the tubes and using the form factor of a hollow tube. The cylinder diameter was calculated to be 19.9 (2) nm and the cavity diameter 8.7 (7) nm. Further the elongation rate of the nanotubes was about 10 nm min^{-1} under the experimental conditions. Recently, Mehravar *et al.* (2009) prepared nanoparticles (102 nm and 454 nm size) from α-LA by the two step desolvation process. Many researches are being carried out to explore the possibilities and the characteristics of α-LA nanoparticles.

CONCLUSION

Surprisingly, there is no exhaustion to find the biological and biochemical application from α-LA. Even a small part of whey protein found in milk, α-LA, has numerous applications such as lactose synthesis, model protein for protein folding study, enrich infant formula, therapy for tumor cells and amyloidoses, suitable carrier for drug and delivery system etc. The review made here shows that wide interdisciplinary researches still required among all science and technology branches especially in Nanomedicine to explore many subtler details of the precise structure and more proper functional analysis of this whey protein, α-LA.

REFERENCES

Acharya, K.R., D.I. Stuart, N.P.C. Walker, M. Lewis and D.C. Phillips, 1989. Refined structure of baboon α-Lactalbumin at 1.7 A° resolution. Comparison with C-type zysozyme. J. Mol. Biol., 208: 99-127.

Acharya, K.R., J. Ren, D.I. Stuart, D.C. Phillips and R.E. Fenna, 1991. Crystal structure of α-Lactalbumin at 1.7 A° resolution. J. Mol. Bio., 221: 571-581.

Alexandrescu, A.T., P.A. Evans, M. Pitkeathly, J. Baum and C.M. Dobson, 1993. Structure and dynamics of the acid-denatured molten globule state of α-Lactalbumin: A two dimensional NMR study. Biochemistry, 32: 1707-1718.

Arai, M., K. Ito, T. Inobe, M. Nakao and K. Maki *et al.*, 2002. Fast compaction of α-lactalbumin during folding studied by stopped-flow X-ray scattering. J. Mol. Biol., 321: 121-132.

Ashikin, N.N. and B.N.A. Razak, 2009. Effect of lactobacillus acidophilus towards ultrasonic velocity of cow milk. MS. Thesis, School of Physics, University of Sains, Malaysia.

Azman, N.Z.B.N., 2009. Radiation studies of lactose content in polluted cow milk samples. Masters Thesis, School of Physics, Universiti Sains Malaysia.

Bai, P., L. Luo and Z. Peng, 2000. Side chain accessibility and dynamics in the molten globule state of alpha-lactalbumin: A (19) F-NMR study. Biochemistry, 39: 372-380.

Banuelos, S. and A. Muga, 1996. Structural requirements for the association of native and partially folded conformations of alpha-lactalbumin with model membranes. Biochemistry, 35: 3892-3898.

Baum, J., C.M. Dobson, P.A. Evans and C. Hanley, 1989. Characterisation of partly folded protein by NMR methods: Studies on the molten globule state of guinea pig α-Lactalbumin. Biochemistry, 28: 7-13.

Bowen, H. and A. Lawrence, 2005. Comparison of the cariogenicity of cola, honey, cattle milk, human milk and sucrose. Pediatrics, 116: 921-926.

Brew, K. and J.A. Grobler, 1992. Alpha-Lactalbumin. In: Proteins: Advanced Diary Chemistry, Fox, P.F. (Ed.). Elsevier Applied Sciences, London, pp: 191-229.

Cawthern, K.M., E.A. Permyakov and L.J. Berliner, 1996. Interactions of α-lactalbumin with fatty acids and spin label analogs. Protein. Sci., 5: 1394-1405.

Chyan, C.L., C. Wormald, C.M. Dobson, P.A. Evans and J. Baum, 1993. Structure and stability of the molten globule state of guinea-pig α-lactalbumin: A hydrogen exchange study. Biochemistry, 32: 5681-5691.

Creighton, T.E., N. Darby and J. Kemmink, 1996. The roles of partly folded intermediates in protein folding. FASEB J., 10: 110-118.

Damaschun, G., H. Damaschun, K. Gast, C. Gernat and D. Zirwer, 1991. Acid denaturated apo-cytochrome C is a random coil: Evidence from small-angle X-ray scattering and dynamic light scattering. Biochim. Biophys. Acta., 1078: 289-295.

Damaschun, G., H. Damaschun, K. Gast, R. Misselwitz, J.J. Miller, W. Pfeil and D. Zirwer, 1993. Cold denaturation-induced conformational changes in phosphoglycerate kinase from yeast. Biochemistry, 32: 7739-7746.

Dill, K.A. and D. Shortle, 1995. Denaturated states of proteins. Ann. Rev. Biochem., 60: 795-825.

Dolgikh, D.A., R.I. Gilmanshin, E.V. Brazhnikov, V.E. Bychkova, G.V. Semisotnov, S.Y. Venyaminov and O.B. Ptitsyn, 1981. Alpha-lactalbumin: Compact state with fluctuating tertiary structure. FEBS Lett., 136: 311-315.

Dolgikh, D.A., L.V. Abaturov, I.A. Bolotina, E.V. Brazhnikov and V.E. Bychkova *et al.*, 1985. Compact state of a protein molecule with pronounced small-scale mobility: Bovine α-Lactalbumin.Compact state of a protein molecule with pronounced small-scale mobility: Bovine α-Lactalbumin. Eur. Biophys. J., 13: 109-121.

Dzwolak, W., M. Kato, A.Shimizu and Y. Taniguchi, 2001. FTIR study on heat-induced changes in structure of bovine α-Lactalbumin: Stabilizing role of calcium ion. Biospectro., 62: 29-39.

Fast, J., A.K. Mossberg, C. Svanborg and S. Linse, 2005. Stability of HAMLET: A kinetically trapped α-lactalbumin oleic acid complex. Prot. Sci., 14: 329-340.

Graveland- Bikker, J.F., G. Fritz, O. Glatter and C.G. de Kruif, 2006. Growth and structure of α-lactalbumin nanotubes. J. Appl. Cryst., 39: 180-184.

Griko, Y.V., E. Freire and P.L. Privalov, 1994. Energetics of the α-lactalbumin states: A calorimetric and statistical thermodynamic study. Biochemistry, 33: 1889-1899.

Griko, Y.V, 1999. Denaturation versus unfolding: Energetic aspects of residual structure in denaturated alpha-lactalbumin. J. Prot. Chem., 18: 361-369.

Griko, Y.V., 2002. Energetic basis of structural stability in the molten globule state: Alpha-lactalbumin. J. Mol. Biol., 297: 1259-1268.

Grishchenko, V.M., L.P. Kalinichenko, G.Y. Deikus, D.B. Veprintsev, K.M. Cawthern, L.J. Berliner and E.A. Permyakov, 1996. Interactions of α-lactalbumin with lipid vesicles studied by tryptophan fluorescence. Biochem. Mol. Biol. Int., 38: 453-466.

Hakansson, A., B. Zhivotovsky, S. Orrenius, H. Sabharcoal and C. Svanborg, 1995. Apoptosis induced by a human milk protein. Proc. Natl. Acad. Sci. USA., 92: 8064-8068.

Halskau, O., N.A. Froystein, A. Muga and A. Martinez, 2002. The membrane-bound conformation of α-lactalbumin studied by NMR-monitored 1H exchange. J. Mol. Biol., 321: 99-110.

Halskau, O., A. Muga and A. Martinez, 2009. Linking new paradigms in protein chemistry to reversible membrane-protein interactions. Curr. Prot. Pept. Sci., 10: 339-359.

Halskau, O., R. Perez-Jimenez, B. Ibarra-Molero, J. Underhaug, V. Munoz, A. Martinez and J.M. Sanchez-Ruiz, 2008. Large-scale modulation of thermodynamic protein folding barriers linked to electrostatics. PNAS., 105: 8625-8630.

Harata, K. and M. Muraki, 1992. X-ray structure evidence for a local helix-loop transition in α-lactalbumin. J. Biol. Chem., 267: 1419-1421.

Heine, W.E., P.D. Klein and P.J. Reeds, 1991. The importance of α-lactalbumin in infant nutrition. J. Nutr., 121: 277-283.

Heine, W.E., M. Radke, K.D. Wutzke, E. Peters and G. Kundt, 1996. Alpha-lactalbumin-enriched Loe-protein infant formulas: A comparison to breast milk feeding. Acta. Paed., 85: 1024-1028.

Hill, R.L. and K. Brew, 1975. Lactose synthetase. Adv. Enzymol. Relat. Areas Mol. Biol., 43: 411-490.

Hiraoka, Y., T. Segawa, K. Kuwajima, S. Sugai and N. Murai, 1980. Alpha-lactalbumin: A calcium metalloprotein. Biochem. Biophys. Res. Commun., 95: 1098-1104.

Hurley, W., 2009. Milk Composition and Synthesis Resource Library. University of Illinois, Urbana, Champaign.

Ibarra-Molero, B., V.V. Loladze, G.I. Makhatadze and J.M. Sanchez-Ruiz, 1999. Thermal versusMguanidine-induced unfolding of ubiquitin. An analysis in terms of the contributions from charge-charge interactions to protein stability. Biochem., 38: 8138-8149.

Jaafar, M.S., L. Palaniappan and R. Thiyagarajan, 2007. Ultrasonic study of the self association of aniline in ethanol-cyclohexane mixtures. J. Phy. Sci. Malaysia, 18: 81-88.

Jahanshahi, M., G.D. Najafpour and M. Rahimnejad, 2008a. Applying the taguchi method for optimized fabrication of Bovine Serum Albumin (BSA) nanoparticles as drug delivery vehicles. Afr. J. Biotechnol., 7: 362-367.

Jahanshahi, M., M.H. Sanati, S. Hajizadeh and Z. Babaei, 2008b. Gelatin nanoparticle fabrication and optimization of the particle size. Phys. Stat. Sol., 205: 2898-2902.

Jambrak, A.R., T.J. Mason, V. Lelas and G. Kresic, 2010. Ultrasonic effect on physicochemical and functional properties of α-lactalbumin. LWT-Food Sci. Technol., 43: 254-262.

Kannappan, A.N. and L. Palaniappan, 1997. Thermodynamic functions of binary liquid mixtures at various temperatures. J. Acoust. Soc. India, 25: 9.1-9.2.

Kannappan, A.N. and L. Palaniappan, 1998. Acoustical properties of water-dioxane mixtures. Proceedings of the National Conference on Advances in Condensed Matter Physics, Pondicherry University, Pondicherry, India.

Kannappan, A.N., L. Palaniappan and R. Mani, 1999. Ultrasonic investigations in selected rock specimens. Indian J. Pure Applied Physics, 37: 861-864.

Kataoka, M., Y. Hagihara, K. Mihara and Y. Goto, 1993. Molten globule of cytochrome c studied by the small angle X-ray scattering. J. Mol. Biol., 229: 591-596.

Kataoka, M., I. Nishii, T. Fujisawa, T. Veki, F. Tokunaga and Y. Goto, 1995. Structural characterization of the molten globule and native states of apomyoglobin by solution X-ray scattering. J. Mol. Biol., 259: 215-228.

Kataoka, M. and Y. Goto, 1996. X-ray scattering solution scattering studies of protein folding. Folding Design, 1: 107-114.

Kataoka, M., K. Kuwajima, F. Tokunaga and Y. Goto, 1997. Structural characterization of the molten globule of α-lactalbumin by solution X-ray scattering. Prot. Sci., 6: 422-430.

Kharakoz, D.P. and V.E. Bychkova, 1997. Molten globule of human α-Lactalbumin: Hydration density and compressibility of the interior. Biochemistry, 36: 1882-1890.

Konno, T., M. Kataoka, Y. Kamatari, K. Kanaori, A. Nosaka and K. Akasaka, 1995. Solution of X-ray scattering analysis of cold-heat and urea-denatured states in a protein streptomyces subtilsin inhibitor. J. Mol. Biol., 251: 95-103.

Kronman, M.J., 1989. Metal–ion binding and the molecular conformational properties of α-lactalbumin. CRC Crit. Rev. Biochem. Mol. Biol., 24: 565-667.

Kuwajima, K., 1996. The molten globule state of α-lactalbumin. FASEB J., 10: 102-109.

Langer, K., M.G. Anhorn, I. Steinhauser, S. Dreis and D. Celebi *et al*., 2008. Human serum albumin (HAS) nanoparticles: Reproducibility of preparation process and kinrtics of enzymatic degradation. Int. J. Pharmaceut., 347: 109-117.

Lien, E.L., 2003. Infant formulas with increased concentrations of α-lactalbumin. Am. J. Clin. Nutr., 77: 1555-1558.

Loladze, V.V., B. Ibarra-Molero, J.M. Sanchez-Ruiz and G.I. Makhatadze, 1999. Engineering a thermostable protein via optimization of charge-charge interactions on the protein surface. Biochemistry, 38: 16419-16423.

Malmendal, A., J. Underhaug, D.E. Otzen and N.C. Nielsen, 2010. Fast mapping of global protein folding states by multivariate NMR: A GPS for proteins. PLoS ONE, 5: 10262-10262.

Markus, C.R., B. Olivier, G.E.M. Panhuysen, J. van der Gugten and M.S. Alles *et al*., 2000. The bovine protein α-lactalbumin Increases the plasma ratio of tryptophan to the other large neutral aminoacid and in Vulnerable subjects raises brain serotonin activity, reduces cortisol concentration and Improves mood under stress. Am. J. Clin. Nutr., 71: 1536-1544.

Markus, C.R., B. Olivier and E.H. De Haan, 2002. Whey protein rich in α-lactalbumin Increases the ratio of plasma a tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulneralbe subjects. Am. J. Clin. Nutr., 75: 1051-1056.

Masaru, K. and A. Kazuyuki, 1999. Protein folding studied by microwave temperature-jump NMR. NMR Toronkai Koen Yoshishu, Jpn., 38: 125-125.

Mckenzie, H.A. and F.H. Jr. White, 1991. Lysozyme and α-lactalbumin: Structure, function and interrelationships. Adv. Protein. Chem., 41: 173-315.

Mehravar, R., M. Jahanshahi and N. Saghatoleslami, 2009. Production of biological nano particles from α-lactalbumin for drug delivery and food science application. Afr. J. Biotechnol., 8: 6822-6827.

Montich, G.G. and D. Marsh, 1995. Interaction of alpha-lactalbumin with phosphatidylglycerol. Influence of protein binding on the lipid phase trasition and lipid acyl chain mobility. Biochemistry, 34: 13139-13145.

Mossberg, A., M. Puchades, O. Halskau, A. Baumann and I. Lanekoff *et al.*, 2010. HAMLET interacts with lipid membranes and perturbs their structure and integrity. PLos ONE, 5: 9384-9384.

Nithyanandham, S. and P. Lakshmanan, 2010. Ultrasonic study of adsorption in disaccharide (maltose) metabolism. Applied Acoustics, 71: 754-758.

Pace, C.N., 2000. Single surface stabilizer. Nat. Struct. Biol., 7: 345-346.

Palaniappan, L., 1991. Ultraviolet study of diabetes mellitus incidence in human beings. M.Phil Thesis, Annamalai University, India.

Palaniappan, L., 1997. Ultraviolet study of diabetes mellitus incidence in human beings. Proceedings of the National Conference on Spectrophysics, Feb. 10-12, Pachaiyappa's College Chennai, India, pp: 53-53.

Palaniappan, L., 2001a. ltrasonic study of 2-butanol in cyclohexane with toluene. Indian J. Phys., 75: 515-518.

Palaniappan, L., 2001b. Estimation of sound velocity in the ternary mixtures of iso-butanol in cyclohexane with toluene J. Acoust. Soc. India, 29: 149-153.

Palaniappan, L., 2002. Thermo acoustical studies of some iso-alcohols in cyclohexane with toluene. Indian J. Pure Applied Phys., 40: 828-830.

Palaniappan, L., 2008. Ultrasonic analysis in the ternary system of Pyridine + Benzene + 2-Butanol. Physica B: Condensed Matter, 403: 3887-3891.

Palaniappan, L. and R. Thiyagarajan, 2008. Molecular interaction study of two aliphatic alcohols with cyclohexane. Indian J. Pure Applied Phys., 46: 852-856.

Palaniappan, L., M.S. Jaafar and Velusamy, 2008. An *in vivo* study of colonic flora on the synthesis of lactase enzyme. UK-MEC Hatch End HA5 4TY, UK., USM03.

Pelligrini, A., U. Thomas, N. Bramaz, P. Hunziker and R. von Fellenberg, 1999. Isolation and Identification of three bacterial domains in the bovine α-lactalbumin molecule. Biochim. Biophys. Acta., 1426: 439-448.

Peng, Z.Y. and P.S. Kim, 1994. A protein dissection study of a molten globule. Biochemistry, 33: 2136-2141.

Perl, D., U. Mueller, U. Heinemann and F.X. Schmid, 2000. Two exposed amino acid residues confer thermostability on a cold shock protein. Nat. Struct. Mol. Biol., 7: 380-383.

Permyakov, E.A., L.A. Morozova and E.A. Burshtein, 1985. Cation binding effects on the pH, thermal and urea denaturation transitions in alpha-lactalbumin. Biophys. Chem., 21: 21-31.

Permyakov, E.A., I.L. Reyzer and L.J. Berlineer, 1993. Effects of Zn(II) on galactosyltransferase activity. J. Protein. Chem., 12: 633-638.

Pettersson, J., A.K. Mossberg and C. Svanborg, 2006. A-lactalbumin species variation, HAMLET formation and tumor cell death. Biochem. Biophy. Res. Comm., 345: 260-270.

Pettersson, J., A.K. Mossberg, M. Trulsson, Y.J. Yong, S. Min and Y. Lim, 2010. A-lactalbumin, engineered to be non-native and inactive kills tumor cells when in complex with oleic acid: A new biological function resulting from partial unfolding. J. Mol. Biol., 98: 629-629.

Pey, A.L., D. Rodriguez-Larrea, S. Bomke, S. Dammers, R. Godoy-Ruiz, M.M Garcia-Mira and J.M. Sanchez-Ruiz, 2008. Engineering proteins with tunable thermodynamic and kinetic stabilities. Proteins., 71: 165-174.

Ptitsyn, O.B., 1987. Protein folding: Hypotheses and experiments. J. Prot. Chem., 6: 273-293.

Ptitsyn, O.B., 1992. The Molten Globule State. In: Protein Folding, Creighton, E.E. (Ed.). W.H. Freeman and Company, New York, pp: 243-400.

Ptitsyn, O.B., 1995. The molten globule and protein folding. Adv. Protein. Chem., 47: 83-229.

Ramboarina, S. and C. Redfield, 2003. Structural characterisation of the human alpha-lactalbumin molten globule at high temperature. J. Mol. Biol., 330: 1177-1188.

Ramboarina, S. and C. Redfield, 2008. Probing the effect of temperature on the backbone dynamics of the human alpha-lactalbumin molten globule. J. Am. Chem. Soc., 130: 15318-15326.

Redfield, C., B.A. Schulman, M.A. Milhollen, P.S. Kim and C.M. Dobson, 1999. Alpha-lactalbumin forms a compact molten globule in the absence of disulfide bonds. Nat. Struct. Biol., 6: 948-952.

Relkin, P. and D.M. Mulvihill, 1996. Thermal unfolding of β-lactoglobulin, α-lactalbumin and bovine serum albumin. A thermodynamic approach. Crit. Rev. Food Sci. Nut., 36: 565-601.

Ren, J., D.I. Stuart and K.R. Acharya, 1993. Alpha-lactalbumin possesses a distince zinc binding site. J. Biol. Chem., 268: 19292-19298.

Rodland, I., O. Halskau, A. Martinez and H. Holmsen, 2005. Alpha-lactalbumin binding and membrane integrity-effect of charge and degree of unsaturation of glycerophospholipids. Biophys. Acta Biomemb., 1717: 11-20.

Rösner, I. and C. Redfield, 2009. The human α-lactalbumin molten globule: Comparison of structural preferences at pH 2 and pH 7. The human α-lactalbumin molten globule: Comparison of structural preferences at pH 2 and pH 7. J. Mol. Biol., 394: 351-362.

Sanchez-Ruiz, J.M. and G.I. Makhatadze, 2001. To charge or not to charge?. Trends Biotechnol., 19: 132-135.

Samuel Ebinezer, B., V. Velusamy and L. Palaniappan, 2003. Application of ferrofluids for the treatment of numbness. Proceedings of the International Workshop on Recent Advances in Nano Technology of Magnetic Fluids, Jan. 22-24, National Physical Laboratory, New Delhi, India, pp: 194-194.

Samuel Ebinezer, B., G. Arul and L. Palaniappan, 2004. Acoustical studies on surface water samples. Proceedings of the National Seminar on Crustal Evolution, (NSCE'04), Wadia Institute, Dehradun, India, pp: 75-75.

Samuel Ebinezer, B., G. Arul, S. Nithiyanandham and L. Palaniappan, 2005. Synthesis of a water based magnetic fluid with starch coating. Proceedings of the National Seminar on Coordination Compounds, (NSCC'05), Ghandhigram Institute, Dindigul, India, pp: 40-40.

Samuel Ebinezer, B. and L. Palaniappan, 2007. Effect of field strength in the velocity anisotropy of ferrofluids. J. Phys. Sci., 18: 1-13.

Saraswathi, K., V. Velusamy and L. Palaniappan, 2005. Preparation of n-propanol based magnetic fluid Proceedings of the National Conference on Recent Trends in Materials Science and Technology, Aug. 10-12, Vellore Institute of Technology, Vellore, India, pp: 5-5.

Schanda, P., V. Forge and B. Brutscher, 2007. Protein folding and unfolding studied at atomic resolution by fast two-dimensional NMR spectroscopy. Proc. Nat. Acad. Sci. USA., 104: 11257-11262.

Schulman, B.A., C. Redfield, Z.Y. Peng, C.M. Dobson and P.S. Kim, 1995. Different subdomains are most protected from hydrogen exchange in the molten globule and native states of human a-lactalbumin. J. Mol. Biol., 253: 651-657.

Shortle, D., 1996. The denatured state (the other half of the folding equation) and its role in protein stability. FASEB J., 10: 27-34.

Stacey, A., A. Schnieke, M. Kerr, A. Scott and C. Mckee *et al.*, 1995. Lactation is disrupted by α-lactalbumin deficiency and can be restored by human α-lactalbumin gene replacement in mice. Proc. Natl. Acad. Sci. USA., 92: 2835-2839.

Strickler, S.S., A.V. Gribenko, T.R. Keiffer, J. Tomlinson and T. Reihle *et al.*, 2006. Protein stability and surface electrostatics: A charged relationship. Biochem., 45: 2761-2766.

Sundaram, N. and L. Palaniappan, 2005. Molecular interaction studies in the ternary mixtures. Indian J. Phys., 79: 1173-1178.

Svensson, M., H. Sabharwal, A. Hakansson, A.K. Mossberg and P. Lipniunas *et al.*, 1999. Molecular charaterization of α-lactalbumin folding variants that induce apoptosis in tumor cells. J. Biol. Chem., 274: 6388-6396.

Svensson, M., A. Hakansson, A.K. Mossberg, S. Linse and C. Svanborg, 2000. Conversion of α-lactalbumin folding to a protein inducing apoptosis. Proc. Natl. Acad. Sci. USA, 97: 4221-4226.

Thairiyaraja, M., L. Palaniappan and D. Devadoss, 2003. Molecular interaction studies in the ternary mixtures of 1-hexanol+acetonitile+cyclohexane. Indian J. Phys., 77: 669-672.

Thiyagarajan, R., D.B. Anburaj, K. Ganesan and L. Palaniappan, 2004. FTIR studies of apatite formation of silica gel in a simulated body fluid. Proceedings of the National Seminar on Modern Trends in Applied Spectroscopy, March 25-26, Annamalai University, India, pp: 25-25.

Thiyagarajan, R., V. Velusamy and L. Palaniappan, 2006. Characterization of AU nanocrystal synthesis in organic layer. Proceedings of the National Conference on Recent Advances in Material Science, Feb. 16-17, Periyar University, Salem, India, pp: 99-99.

Thiyagarajan, R. and L. Palaniappan, 2007a. Ultrasonic investigation of molecular association in binary mixtures of aniline with aliphatic alcohols. Comptes Rendus Chimie, 10: 1157-1161.

Thiyagarajan, R. and L. Palaniappan, 2007b. Excess values studies in the mixtures of Aniline + 1-Propanol + Benzene. Main Group Chem., 6: 25-30.

Thiyagarajan, R. and L. Palaniappan, 2008. Effect of toluene in dipolar interaction ofsome aliphatic alcohols. Phys. Chem. Liquids, 46: 366-371.

Velusamy, V., 2003. Ultrasonic study of cerebro spinal fluid. M.Phil. Thesis, Annamalai University, India.

Velusamy, V. and L. Palaniappan, 2004. Ultrasonic study of human cerebro spinal fluid. Indian J. Pure Applied Phys., 42: 591-594.

Velusamy, V., L. Palaniappan and S. Nithiyanandham, 2007. Ultrasonic study of adsorption in polysaccharide metabolism. Main Group Chem., 6: 53-61.

Weber, C., C. Coester, J. Kreuter and K. Langer, 2000. Desolvation process and surface charaterisation of protein nanoparticles. Int. J. Pharmaceut., 194: 91-102.

Xie, D., V. Bhakuni and E. Freire, 1991. Calorimetric determination of the energeticsof the molten globule intermediate in protein folding: Apo-a-lactalbumin. Biochem., 30: 10673-10678.

Xie, D., V. Bhakuni and E. Freire, 1993. Are the molten globule and the unfolded states of apo-a-lactalbumin enthalpically equivalent. J. Mol. Biol., 232: 5-8.

Yahaya, N.Z., 2009. Spectrophotometric studies on the lactose content in cow milk samples. Masters Dissertation, School of Physics, University of Sains, Malaysia.

Yogman, M.W., S.H. Zeisel and C. Roberts, 1983. Assessing effects of serotonin precursors on newborn behavior. J. Psychiatr. Res., 17: 123-133.

Yutani, K., K. Ogasahara and K. Kuwajima, 1992. The absence of the thermal transition in apo-α-lactalbumin in the molten globule state: A study by differential scanning microcalorimetry. J. Mol. Bio., 228: 347-350.