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On the complexation of whey proteins

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ABSTRACT

Milk proteins have a rich diversity of physical chemistry and biodegradable properties which makes them appealing for different food and pharmaceutical applications. Theoretical coarse grained models and numerical simulations were employed here in order to gain novel insight into the understanding of the fundamental mechanisms of the process of milk proteins complexation in a diversity of environmental conditions. The interactions between α-lactalbumin, β-lactoglobulin and lactoferrin were investigated by means of Monte Carlo simulations. The comparison between the free energies associated with the complexation of α-lactalbumin–lactoferrin and β-lactoglobulin–lactoferrin at different pH and ionic strengths let us to explain why is experimentally observed the later complex and not the α-lactalbumin–lactoferrin complex.

1. Introduction

Since probably the domestication of cattle and other animals after the eighth millennium BC, milk has gained a vital role on men survival and development (Evershed et al., 2008). Milk uses have a great impact in our present daily lives and important applications in various areas (Bösze, 2008; McSweeney and Fox, 2013; Thompson, Boland, & Singh, 2009; Watson, Zibadi, & Preedy, 2010), including odontology (Tenovuo, 2002), medicine (Permyakov, Pershikova, Khokhlova, Uversky, & Permyakov, 2004; Steijns & van Hooijdonk, 2000; Tomita et al., 2009), biodefense (Clare, Catignani, & Swaisgood, 2003), food (Aly, Ros, & Frontela, 2013; Dalgleish, 1992; Dickinson, 2008; Dickinson, 2013; Donald, 2000; Kinsella & Whitehead, 1989; de Roos, Walstraa, & Geurts, 1998; Steijns & van Hooijdonk, 2000; Syrbe, Bauer, & Klostermeyer, 1998; Vardhanabhuti, Yucel, Coupland, & Foegedinga, 2009), cosmetics (Secchi, 2008; Steijns & van Hooijdonk, 2000), pharmaceuticals (Dickinson, 2008), and others industries (Audica, Chaufera, & Daufin, 2003). There also have been considerable increase on its importance specially due to the interest in developing biodegradable materials. Some of these applications are also essential to many academic areas.

In addition to water, lipids and sugar, milk contains several proteins with a large diversity of physical chemistry properties which let them be used in a quite wide number of applications ranging from nutritional to functional and biological functions (Thompson et al., 2009). From all milk constituents, these proteins are particularly interesting for industrial applications. For instance, they are highly effective emulsion stabilizing agents and gelling ingredients (Dalgleish, 1992; Dickinson, 2008, 2013; Havea, Singh, & Creamer, 2001; Kinsella & Whitehead, 1989; Ryan et al., 2012; Tcholakova, Denkov, Ivanov, & Campbell, 2006) responsible to texturing a variety of foods (Batt, Brady, & Sawyer, 1994; Donald, 2000; Girard & Schaffer-Lequart, 2007), used in sports drinks to retain clarity and improve nutritional functions in beverages (Ryan & Foegeding, 2015; Vardhanabhuti et al., 2009), effective micro-encapsulating agents in food (Egan, Jacquier, Rosenberg, & Rosenberg, 2013; Egan, O'Riordan, O'Sullivan, & Jacquier, 2014; Eratte, Wang, Dowling, Barrow, & Adhikari, 2014), used to the design of infant formula with similar functional effects than human milk (Aly et al., 2013), as models in medicine for *in-vitro* studies on human tear film deposits for the development of optical lens (Luensmann & Jones, 2008; Soltys-Robitaille, Ammon, Valint, & Grobe, 2001), and large-scale protein purification on biotechnology (Wang, Gao, & Dubin, 1996).

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Together with caseins, bovine whey (or milk serum) proteins [β-lactoglobulin (β-LG), α-Lactalbumin (α-LA), immunoglobulin (IgG), serum albumin (SA), lactoferrin (LF), lactoperoxidase (LP) and lysozyme (LSZ)] (Kinsella & Whitehead, 1989; Korhonen, 1998) are of considerable importance on dairy products (McSweeney and Fox, 2013; Smithers, 2008; Watson et al., 2010). The exploitation of the properties of the whey proteins may contribute to solve some technological shortcomings that makes difficult their widespread comercial applications (Smithers et al., 1996), despite the fact that there is a need to satisfy the market demand (Kinsella & Whitehead, 1989). In fact, the food marketplace requires whey proteins to be continuously manipulated in order to improve their functionality (Clare & Daubert, 2011). As already pointed out by other authors, the research on the improvement and manipulation of milk proteins, milk protein–polysaccharide and milk protein–-protein interactions may have an effect on economy and lead to the development of intelligent nanoscale encapsulation systems for food and pharmaceutical applications (Batt et al., 1994; Dickinson, 2008).

The major whey proteins are β-LG, α-LA, SA and LF. From these proteins, LF seems to be the most versatile one both in terms of its physical chemistry properties and applications. Just to name a few, LF might be involved in several physiological functions as such as inflammation, bacteriostasis and bactericidal effects, autoimmune diseases, growth, and absorption of iron from breast milk (El-Loly & Mahfouz, 2011; Lönnerdal & Iyer, 1995; Takayama, 2012; Wakabayashi, Oda, Yamauchi, & Abe, 2014). Particularly in the food technology, heteroprotein coacervates of β-LG and LF are of interest in new food products (Anema & Kruif, 2014; Yan et al., 2013). Perhaps one of the most exciting features of LF is its ability to easily form complexes, as noticed by Hekman already in 1971 (Hekman, 1971).

A closer look at these proteins interactions often reveals some rather unexpected behavior. Despite the fact that electrostatic interaction is one of the most important driven force for protein complexation (Jönsson, Lund, & da Silva, 2007; Takahashi, 1997) and that both α-LA and β-LG acquire positive and negative charges at virtually the same pH, the interaction of these molecules with other proteins shows some peculiarities. Different reports have demonstrated that the complexation with β-LG occurs but not with α-LA (Puyol, Perez, Ena, & Calvo, 1991). Using gel filtration, Puyol and co-authors have analysed and compared the interaction of α-LA and β-LG with palmitic acid. They found that while β-LG binds to the fatty acid, α-LA does not (Puyol et al., 1991). Hekman has also raised some questions not conclusively solved about the ability of LF to easily forms complexes and what forces play a role in it (Hekman, 1971). In spite of the fact that LF does form hetero-complexes with β-LG, it is not clear if LF may associate with α-LA (Lampreave et al., 1990) which is an apparent contradiction with LF binding abilities. Recent studies show the complexation of β-LG and LF not only for the 1:1 stoichiometry but also observed the formation of clusters suggested as $\beta\text{-LG}_2\text{–LF–}\beta\text{-LG}_2$ (Kizilay et al., 2014; Yan et al., 2013). These reports also discussed the physical chemistry differences between the heteroprotein association and the self-aggregation processes.

Although whey proteins and whey protein complexes have been extensively reported by means of several experimental techniques (e.g. gel filtration (Puyol et al., 1991), calorimetry (Ju, Hettiarachchy, & Kilara, 1999; Raemy, Lambelet, & Rousset, 2005), spectophotometry (Morawetz & Hughes, 1952), transmission electron microscopy (Mudgal, Daubert, Clare, & Foegeding, 2011), scanning electron microscopy (Egan et al., 2013), fluorescence (Nigen, Tilly, Croguennec, Drouinkucma, & Bouhallab, 2009b), laser scanning microscopy (Kizilay et al., 2014), electrophoresis (Hattori, Bataldar, Kato, Bohidar, & Dubin, 2005; Havea et al., 2001; Mudgal et al., 2011; Seyrek, Hattori, & Dubin, 2004), rheology (Kizilay et al., 2014), light scattering (Li, Mattison, Dubin, Havel, & Edwards, 1996; Yan et al., 2013), small angle neutron scattering (Kizilay et al., 2014), turbidity and microscopic observations (Anema & Kruif, 2014; Nigen, Croguennec, & Bouhallab, 2009a; Ryan et al., 2012; Ryan & Foegeding, 2015; Yan et al., 2013), high performance liquid chromatography (Egan et al., 2014; Topuzogullari, Cimen, Mustafaeva, & Mustafaev, 2007), size exclusion chromatography (Ryan & Foegeding, 2015; Yan et al., 2013), ζ-potential (Anema & Kruif, 2014; Ryan et al., 2012; Ryan & Foegeding, 2015), atomic force microscopy (Touhami & Dutcher, 2009), potentiometric and turbidimetric titration and photon correlation spectroscopy (Laos, Brownsey, & Ring, 2007)), much less is known about milk protein–protein interactions at the molecular level, where is the origin of any functional characteristics of a protein. In fact, there is a well supported need for a better understanding of their molecular interactions (Ryan & Foegeding, 2015; Wijayanti, Bansal, & Deeth, 2014; de Wit, 2009; Zeiler & Bolhuis, 2015).

Theoretical coarse grained models and numerical simulations offer an opportunity to gain novel insight into the understanding of the fundamental mechanisms of the process of milk proteins complexation in a diversity of environmental conditions. As such, these techniques are a valuable tool for the nascent soft matter approach of the food science offering a rational approach to describe, explain and control complexation mechanisms that affect the functions. Moreover, due to the fact that these proteins have also important physiological functions, these tools are as well of relevance to the Biological sciences.

In this article, we illustrate the applicability of such molecular modeling approaches on food science and technology addressing the complexation issues of α-LA and β-LG interactions with itself and with LF. Doing that we demonstrate the physical reasons for the more favorable interactions of LF with β-LG but not so strong with α-LA (Lampreave et al., 1990). Ionic strength and pH effects are also discussed.

2. Model and methodology

Statistical mechanics provides an appealing theoretical framework in order to explore the main features of the complexation of two macromolecules in an electrolyte solution (Hill, 1986). Particularly interesting here is the fact that this approach provides free energy derivatives as a function of the macromolecules separation at different environmental conditions. Coarse grained models within this framework also offer the possibility to explore the main physical features of a system with a reduced number of parameters. With this in mind, several molecular models within the continuum solvent description have been devised to study protein–protein interactions (Kurut, Persson, Åkesson, Forsman, & Lund, 2012, 2015; Lund & Jönsson, 2003) and solved by Monte Carlo (MC) simulations (Frenkel & Smit, 1996; Metropolis, Rosenbluth, Rosenbluth, Teller, & Teller, 1953).

Monte Carlo methods are numerical integration tools that have become a common procedure to compute thermodynamic averages in theoretical biochemistry during the last 30 years. Applied to molecular systems, they provide a set of relevant configurations for a given physical model, that can be used to calculate detailed structural and thermodynamical properties. Compared with other popular approaches such as the Poisson-Boltzmann equation, MC simulations have the advantage to *exactly* (within statistical errors) solve the given model, providing a deeper understanding of the system at hand (Allen & Tildesley, 1989; Frenkel & Smit, 1996).

The model used here is based in previous works (Kurut et al., 2012; Lund & Jönsson, 2003; Persson, Lund, Forsman, Chatterton, & Åkesson, 2010). Two macromolecules build up by a collection