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Properties of oil-in-water emulsions stabilized by β-lactoglobulin in simulated gastric fluid as influenced by ionic strength and presence of mucin

Anwesha Sarkar [a,b], Kelvin K.T. Goh [b], Harjinder Singh [a,*]

[a] Riddet Institute, Massey University, Private Bag 11 222, Palmerston North 4442, New Zealand
[b] Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11 222, Palmerston North 4442, New Zealand

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ABSTRACT

The effects of ionic strength (0–150 mM NaCl) and the presence of mucin (0.1 wt%) on the properties of oil-in-water emulsions [20.0 wt% soy oil, stabilized by 1.0 wt% β-lactoglobulin (β-lg)] under simulated gastric conditions (with/without 0.32 wt% pepsin at 37 °C, with continuous shaking at approximately 95 rev/min for 2 h) were investigated. Changes in *Z*-average diameter, ζ-potential and microstructure were determined as a function of incubation time. The emulsions mixed with simulated gastric fluid (SGF) (without added pepsin) were stable at low ionic strength (≤50 mM NaCl) but showed some aggregation at high ionic strength (≥150 mM NaCl). Extensive droplet flocculation with some degree of coalescence was observed in emulsions with 0.32 wt% added pepsin, the flocculation being potentially accelerated in the presence of NaCl. The addition of 0.1 wt% mucin resulted in a greater extent of flocculation, possibly because of non-specific binding of mucin to the positively charged β-lg emulsion droplets. Ionic strength and the presence of mucin had a significant influence on the rate of hydrolysis of β-lg by pepsin. The behaviour of the emulsion in SGF was predominantly driven by electrostatic interactions, which varied as a function of digestion time, ionic strength and the presence of pepsin and mucin.

1. Introduction

In recent years, there has been increasing interest in designing food matrices with various interfacial compositions to achieve either desired sensorial attributes or specific site-dependent controlled release of bioactive molecules (Dickinson, 2008; Lundin, Golding, & Wooster, 2008; McClements, Decker, & Park, 2009; McClements, Decker, Park, & Weiss, 2008; Parada & Aguilera, 2007; Singh, Ye, & Horne, 2009). Currently, there is little understanding of the interactions of emulsions under physiologically active conditions *in vivo*. In an attempt to understand the physico-chemical changes that occur during the consumption of food, researchers have devised less expensive and time-efficient *in vitro* methodologies (as widely used in drug delivery studies) to simulate the physiological conditions at different stages of the gastrointestinal tract. Such systems provide better understanding of how the surface characteristics and microstructures of colloidal systems influence the digestibility and bioavailability of nutrients.

A few *in vitro* studies have focused on the interactions of food emulsions in the oral environment (Sarkar, Goh, & Singh, 2009a; Silletti, Vingerhoeds, Norde, & van Aken, 2007a, 2007b; Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005). These authors have shown different degrees and mechanisms of flocculation, which are influenced by the droplet charge and the presence of salts and biopolymers in unstimulated human saliva or in an environment that simulates the composition of saliva.

The behaviours of emulsions under gastrointestinal conditions have also been studied (Beysseriat, Decker, & McClements, 2006; Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009; Mun, Decker, & McClements, 2007; Sarkar, Horne, & Singh, 2010) but not extensively. When an emulsion enters the upper gastric lumen, it is destabilized, mostly because of the acidic environment (pH 1–3). Furthermore, the emulsion is exposed to different enzymes (pepsin, gastric lipase), biopolymers (mucin) and electrolytes (Na^+, Cl^-, Ca^{2+}, etc.) under the moderate shearing conditions that result from peristalsis (Ekmekcioglu, 2002; Kalantzi et al., 2006; Lindahl, Ungell, Knutson, & Lennernäs, 1997; Pal, Brasseur, & Abrahamsson, 2007; Sarkar, Goh, Singh, & Singh, 2009b; Weisbrodt, 2001). More in-depth research in this area to establish the role of physiological conditions in emulsion stability is required.

Recent work in our laboratory showed an almost instantaneous change in the electrostatic charge of β-lactoglobulin (β-lg) emulsion droplets (from negative charge to positive charge) in a simulated gastric fluid (SGF) environment (Sarkar et al., 2009b). It was

* Corresponding author. Tel.: +64 6 356 4401; fax: +64 6 350 5655.
E-mail address: H.Singh@massey.ac.nz (H. Singh).

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also reported that β-lg, which is usually resistant to pepsin attack in its native state, became susceptible to proteolysis when present as the interfacial layer in emulsions, which is in agreement with another recent study (Macierzanka et al., 2009). A change in the conformation of the β-lg molecules upon adsorption at the oil–water interface exposes the peptic cleavage sites for proteolysis, leading to emulsion instability and some degree of coalescence. The above study used SGF with a constant ionic strength (34 mM NaCl).

It is recognized that the ionic strength varies significantly in real physiological circumstances (Kalantzi et al., 2006). Furthermore, the role of mucin, which is a high molecular weight glycosylated protein (molecular weight about 2.0–4.0 $\times$ 10^6 Da) and forms a self-associated networked structure under gastric conditions (low pH and at high mucin concentrations) (Bansil & Turner, 2006; Lee, Muller, Rezwan, & Spencer, 2005; Nordman et al., 2002), has not been explored. Mucin has been shown to play an important role in the flocculation of emulsions in the oral environment because of its negative charge at neutral pH (Sarkar et al., 2009a; Silletti et al., 2007a, 2007b; Vingerhoeds et al., 2005). However, the influence of mucin at acidic pH and at higher ionic strengths on the stability of emulsions, particularly in the presence of a proteolytic enzyme, has not been reported.

Hence, in this study, we attempted to elucidate the interactions of β-lg-stabilized emulsions in SGF media containing pepsin and mucin under different ionic strength conditions. The focus was on the changes in the physico-chemical properties and microstructures of the emulsions.

2. Materials and methods

2.1. Materials

The β-lg protein from bovine milk (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) contained approximately 90.0% β-lg. Pepsin (EC 3.4.23.1) from porcine gastric mucosa was purchased from Sigma–Aldrich Chemical Co., St. Louis, MO, USA. The pepsin had an enzymatic activity of 800–2500 units/mg protein, as stated by the manufacturer. Porcine stomach mucin Type II (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) contained 1.0% bound sialic acid. Refined bleached deodorized soy oil was obtained from Davis Trading Company, Palmerston North, New Zealand, and was used without any further purification. All other chemicals were purchased from BDH Chemicals (BDH Ltd, Poole, UK) unless otherwise stated. Analytical-grade reagents were used for the preparation of all solutions. Milli-Q water (water purified by a Milli-Q apparatus, Millipore Corp., Bedford, MA, USA) was used as a solvent in all experiments.

2.2. Emulsion preparation and mixing with SGF

The β-lg (1.0 wt%) solution was prepared in Milli-Q water by stirring for 2 h at 20 °C. Sodium azide (0.02 wt%) was added to prevent microbial growth during storage of the emulsions. The pH of the solution was adjusted in the range 6.8–7.0 using 1 M NaOH or 1 M HCl. An emulsion was prepared by mixing appropriate quantities of β-lg (1.0 wt%) solution and soy oil (20 wt%). The mixture of soy oil and protein solution was heated to 50 °C and pre-emulsified using a conventional rotor–stator type mixer (Heidolph DIAX 600, Schwabach, Germany) at a speed of 20 500 min^{-1}. The sample was homogenized by two passes in a two-stage valve homogenizer (APV 2000, Copenhagen, Denmark) operating at 25 MPa and 5 MPa for the first and second stages respectively. All emulsion samples were prepared in duplicate.

In vitro gastric model containing SGF (United States Pharmacopeial Convention, 2000) at pH 1.2 (with or without 0.32 wt% pepsin) was prepared according to the procedure described previously (Sarkar et al., 2009b). However, the ionic strength of the SGF was varied from 0 to 150 mM NaCl. To prevent the autolysis of pepsin, SGF solution was prepared on the day of the experiments and was stored at 4 °C until required. In some experiments, 0.1 wt% mucin was added to SGF (O'Gara, Maslin, Nevill, & Hill, 2008). Each stock emulsion was mixed with SGF (protein:pepsin ratio 3:1 w/w) at 37 °C with continuous agitation at 95 rev/min in a temperature-controlled water bath (Lab-Line shaker bath, Model LZ33070, Barnstead International, Dubuque, IA, USA) for 2 h. The final mixture contained 10.0 wt% dispersed phase. Samples of the emulsion–SGF mixture were characterized as a function of digestion time at regular intervals up to 2 h.

The pH of the emulsion–SGF mixture was maintained at 1.5 using 1 M HCl to simulate the harsh conditions when liquid foods enter the fasted human stomach. It must be recognized that typically the pH in fasted gastric conditions ranges from pH 1–3 (Kalantzi et al., 2006). However, on consumption of food, the gastric pH considerably increases to ~pH 6.0 before decreasing back to ~pH 2.0, depending upon the composition and buffering capacity of food consumed, meal residence time in the stomach and individual's physiology (Kalantzi et al., 2006; McClements et al., 2009). In this study, we envisage an extreme case of acidic pH (emulsion–SGF mixture pH 1.5), where pepsin has the optimum activity to focus predominantly on understanding the influence of ionic strength and mucin on gastric proteolysis of emulsions.

2.3. Measurement of droplet size and charge

The mean hydrodynamic diameter (denoted here as the *Z*-average diameter) and the ζ-potential value of the emulsion–SGF mixture samples were measured using a Zetasizer Nano ZS, Model ZEN 3600 (Malvern Instruments Ltd, Malvern, Worcestershire, UK).

Droplet sizing based on dynamic light scattering was carried out at 37 °C in a particle-sizing cell using backscattering technology at a detection angle of 173°. The autocorrelation function was transformed into the size distribution using cumulant analysis (Koppel, 1972) by the Zetasizer Nano ZS software package (Malvern Instruments Ltd, Malvern, Worcestershire, UK) and the intensity of scattered light from the emulsion droplets was converted to the mean hydrodynamic diameter (*Z*-average diameter), using Stokes–Einstein equation, assuming the emulsion droplets to be spherical. For each sample, the mean and the standard deviation were calculated from a series of five measurements.

For ζ-potential measurement, an emulsion–SGF mixture sample of approximately 0.005 wt% droplet concentration, diluted using the appropriate background buffer solution, was put in a white folded electrophoresis cell (Model DTS 1060C, Malvern Instruments Ltd, Malvern, Worcestershire, UK) and the electrophoretic mobilities were measured using a laser Doppler velocimetry and phase analysis light scattering (M3-PALS) technique. This electrophoresis cell has electrodes at either end, to which a potential is applied. Droplets within the dispersion move towards the oppositely charged electrode at a certain velocity, which is determined by measuring the frequency shift of the incident laser beam. This velocity (electrophoretic mobility) at which the droplets move in the applied electric field is converted to a ζ-potential by substituting the known variables, such as the dielectric constant of the medium, the viscosity, etc. in Henry's equation. The temperature of the electrophoresis cell was maintained at 25 °C using a water jacket that was temperature controlled by the Peltier system. For each sample, the mean and the standard deviation were calculated from a series of ten measurements.