Food Hydrocolloids 25 (2011) 809–816

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Investigating the effect of surfactants on lipase interfacial behaviour in the presence of bile salts

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ARTICLE INFO

Article history:
Received 31 March 2010
Accepted 9 September 2010

Keywords:
Pluronic F68
Phospholipids
Lipase
Bile salts
Interfacial tension
Interfacial rheology

ABSTRACT

It is known that non-ionic surfactants and phospholipids provide large protection in emulsions against lipase-induced destabilization as compared to proteins, even in the presence of bile salts. In relation to this, the aim of this study is to probe the ability of two surfactants of industrial interest, poloxamer Pluronic F68 (non-ionic) and Epikuron 145V (phospholipid), to modify the adsorption of lipases at an oil–water interface under the physiological conditions existing in the duodenum. We have designed an experimental procedure by means of a pendant drop film balance equipped with a subphase exchange technique, which allows sequential adsorption of the compounds. This allows the investigation of the interfacial behaviour of lipase in the presence and absence of surfactant. According to this experimental approach, the lipase is added directly into the subphase only after the surfactant has been adsorbed onto the oil–water interface. We have used interfacial dilatational and shear rheology techniques to characterise the interfacial layers. The results suggest that Pluronic F68 reduces the interfacial activity of lipase more efficiently than Epikuron 145V. Furthermore, it seems that Pluronic F68 affects the accessibility of the lipase to the oil–water interface, even in the presence of the bile salts. These results may have applications in the development of novel strategies to rationally control lipid digestion in the diet.

1. Introduction

The design of food emulsions that allow controlled digestion of lipids has been a subject of interest in recent years (Dickinson, 2008; Golding & Wooster, 2010; Singh, Ye, & Horne, 2009). In some cases, such as obesity or cardiovascular diseases, it may be important to decrease the bioavailability of these lipids. During the digestion process, most lipids ingested, that are non-homogenized, are converted into oil-in-water (O/W) emulsions due to the mechanical stresses they experience and the role of different stabilizing agents. In addition, there are normally appreciable changes in the interfacial properties that may influence bioavailability of the fats (Golding & Wooster, 2010). On the one hand, there may be changes in the total area of the oil–water interface in the system, which may promote either adsorption or desorption of surface-active substances. On the other hand, many different types of surfactants, such as emulsifiers originally located at the surfaces of the lipid droplets in the food and various other surface-active substances arising from the food or the human body (free fatty acids, phospholipids, proteins…), compete for the available area and the nature of the resulting interfacial structure may influence bioavailability of the fats. Around 70–90% of fat digestion occurs in the small intestine (Fave, Coste, & Armand, 2004; Mun, Decker, & McClements, 2007). In this process, enzyme lipase has to adsorb onto the droplet surface, with the help of its cofactor co-lipase and in the presence of bile salts, before it can hydrolyse the lipids into a form that can be adsorbed by the human body (lipolysis) (Bauer, Jakob, & Monsenthin, 2005; Mun et al., 2007; Wickham, Wilde, & Fillery-Travis, 2002). Therefore, interfacial properties, like the composition of the interfacial layer surrounding the lipid droplets, will affect the rate and extent of lipid digestion (Fillery-Travis, Foster, & Robins, 1995; Mun, Decker, Park, Weiss, & McClements, 2006). Due to the apolar nature of oils and fats, the oil–water interface in emulsified fats is where eventually digestion takes place. Therefore, bioavailability of fat could be ultimately controlled by the interfacial layer that stabilises the emulsion.

The use of fundamental interfacial measurements such as interfacial tension and interfacial rheology in combination with other techniques in O/W emulsions are necessary to investigate the molecular changes at interfaces under digestion conditions, giving

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doi:10.1016/j.foodhyd.2010.09.007

additional information to this subject. In this sense, several interfacial tension studies were developed by Verger and Carrière group (de La Fournière, Ivanova, Blond, Carrière, & Verger, 1994; Gargouri, Julien, Bois, Verger, & Sarda, 1983; Labourdenne, Brass, Ivanova, Cagna, & Verger, 1997; Tiss, Carrière, & Verger, 2001), as well as by Reis et al. (Reis, Holmberg, et al., 2008; Reis, Holmberg, Miller, et al., 2009; Reis, Miller, et al., 2008; Reis, Raab, et al., 2008) to evaluate the measurement of fatty acid formation. Regarding interfacial rheology of adsorbed layers, it also contains vital information regarding the mechanical properties of the interfacial films (Krägel et al., 2003; Maldonado-Valderrama et al., 2008). In addition, neutron and X-ray scattering techniques have been used to establish the role of specific micelles (bile and surfactant micelles) on the activation of the lipase–co-lipase complex (Hermoso et al., 1997; van Tilbeurgh et al., 1993). The use of an inhibitor that irreversibly binds to the active site of the lipase in its lid-open conformation is also known (Borgström, 1988). Furthermore, atomic force microscopy has been used in the study of interfacial structuring (Chu et al., 2010; Maldonado-Valderrama et al., 2008), as well as electron microscopy has been used in the study of the inactivation by lipolytic products (Pafumi et al., 2002) and zeta-potential measurements to evaluate the lipolytic activity (Mun et al., 2006). It has been recently demonstrated that non-ionic surfactants and phospholipids provide larger protection in emulsions against lipase-induced destabilization in the presence of bile salts if compared to proteins (Mun et al., 2007). The goal of this study was to explore this protective functionality in more detail. For that reason, we investigated the ability of two surfactants of industrial interest, poloxamer Pluronic F68 (non-ionic) and Epikuron 145V (phospholipid), to affect the adsorption of lipases at an oil–water interface under the physiological conditions of the duodenum. Another objective of the present study was the design of a novel experimental procedure that allows the simulation of the digestion process based on a pendant drop film balance equipped with a subphase exchange technique (Cabrerizo-Vílchez, Wege, Holgado-Terriza, & Neumann, 1999).

In this study we aim to focus on the fundamental processes underlying lipid digestion. A basic understanding of this complex phenomenon is crucial if we are to engineer food emulsions to control lipid digestion. This work provides a new insight into the interfacial processes affecting lipase activity at a fundamental level, being a useful tool in order to complement the existing techniques.

2. Materials and methods

2.1. Materials

As non-ionic surfactant we used the poloxamer Pluronic F68 from Sigma–Aldrich. It is a triblock copolymer based on poly (ethylene oxide)-block–poly(propylene oxide)-block–poly(ethylene oxide) structure which is also typically expressed as $PEO_{75}PPO_{30}$-PEO_{75} (8350 g/mol). The central block has an hydrophobic character and hence adsorbs onto the oil–water interface, whereas the two chains of poly(ethylene oxide) remain in the aqueous phase. As phospholipid we used Epikuron 145V (around 800 g/mol), a deoiled, wax-like phosphatidylcholine (PC) enriched soybean lecithin (min. 45% PC) from Cargill Ibérica S. L. Highly refined olive oil was purchased from Sigma–Aldrich, and purified with activated magnesium silicate (Florisil, Fluka) to eliminate free fatty acids and surface-active impurities. The oil was kept under mild agitation with the resins for 3 h and centrifuged at 12,000 rpm for 30 min in a bench centrifuge. It was then filtered and stored away from light.

For the duodenal juice preparation we used lipase from porcine pancreas L3126, Type II (100–400 units/mg protein, using olive oil – 30 min incubation), and bile extract porcine B8631, both of them purchased from Sigma–Aldrich, as this combination has been previously used for in vitro duodenal digestion models (Mun et al., 2007). The lipase sample hydrolyses tri-, di-, and monoglycerides (in decreasing order of rate) and contains amylase and protease activity as well. The composition of the bile extract has previously been analyzed: total bile salt content = 49 wt%; with 10–15% glycodeoxycholic acid, 3–9% taurodeoxycholic acid, 0.5–7% deoxycholic acid; PC 5 wt% (Zangenberg, Mullertz, Kristensen, & Hovgaard, 2001a). $CaCl_2 \cdot 2H_2O$ of analytical grade, manufactured by Sigma–Aldrich, was also used in a final concentration of 3 mM, which is in the range of concentration found in the fasted state of the duodenum (Hofmann & Mysels, 1992; Lindahl, Ungell, Knutson, & Lennernas, 1997).

All the samples were prepared in a 2 mM Trizma Maleate (Fluka) buffer with 150 mM NaCl (Scharlau Chemie S.A.). The pH was adjusted to 6.5 with 1 M NaOH (Panreac Quimica S.A.). All chemicals used were of analytical grade. Milli-Q purified water 0.054 μS was used for buffer preparation and all other purposes. All the glassware was cleaned with Micro-90 and ethanol, and repeatedly rinsed with distilled and ultrapure water. Only freshly prepared solutions were used for each experiment. The interfacial tension of the clean oil–water interface (γ_0) was measured before every experiment to ensure the absence of surface-active contaminants obtaining values of (26 ± 0.5) mN/m at 37 °C. All the experiments were performed at the physiological temperature of $T = 37$ °C and their reproducibility was verified through at least three replicate measurements.

2.2. Interfacial tension set-up and subphase exchange technique

The interfacial tension measurements have been performed in a pendant drop film balance fully assembled and developed at the University of Granada and is described in detail elsewhere (Cabrerizo-Vílchez et al., 1999). A solution droplet is formed at the tip of a coaxial double capillary, connected independently to a double microinjector. The computer program fits experimental drop profiles, extracted from digital drop micrographs, to the Young–Laplace equation of capillarity by using Axisymmetric Drop Shape Analysis (ADSA), and provides as outputs the drop volume V, the interfacial tension γ, and the interfacial area A. The adsorption process is recorded at constant interfacial area through a modulated fuzzy logic PID algorithm (proportional, integral, and derivative control) (Wege, Holgado-Terriza, & Cabrerizo-Vílchez, 2002). The drop is immersed in a glass cuvette (Hellma), which contains the oil phase and is kept in a thermostatized cell at 37 °C. The interfacial pressure values π are obtained from the relationship $\pi \equiv \gamma_0 - \gamma$, where γ_0 is the interfacial tension of pure oil–water interface, and γ is the interfacial tension of the solution.

The interfacial pressure is first recorded for the pure systems at the bare oil–water interface. Next, the subphase exchange accessory is used in order to basically simulate the transit through the duodenum of the previously covered interface. A coaxial double capillary enables to substitute the surfactant bulk solution once a stable layer has been formed at the oil–water interface at constant interfacial area. The subphase is exchanged by extracting the surfactant solution through the outer capillary, and injecting simultaneously through the inner one. Then, the interfacial behaviour of pancreatic lipase, in the absence and presence of bile salts, can be also monitored on a previously covered interface.

2.3. Interfacial dilatational rheology

The dilatational rheology of the surfactant layer and the sequentially adsorbed lipase and bile salts onto the pre-adsorbed surfactant layer was measured with the pendant drop technique