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Stabilization mechanism of oil-in-water emulsions by β-lactoglobulin and gum arabic

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ABSTRACT

Natural biopolymer stabilized oil-in-water emulsions were formulated using β-lactoglobulin (β-lg), gum arabic (GA), and β-lg:GA solutions as an alternative to synthetic surfactants. Emulsions using these biopolymers and their complexes were formulated varying the biopolymer total concentration, the protein-to-polysaccharide ratio, and the emulsification protocol.

This work showed that whereas β-lg enabled the formulation of emulsions at concentration as low as 0.5 (w/w)%, GA allowed to obtain emulsions at concentrations equal to or higher than 2.5 (w/w)%. In order to improve emulsion stability, β-lg and GA were complexed through strong attractive electrostatic interactions. GA solution had to be added to previously prepared β-lg emulsions in order to obtain stable emulsions. Interfacial tension and interfacial rheological measurements allowed a better understanding of the possible stabilizing mechanism. β-lg and GA both induced a very effective decrease in interfacial tension and showed interfacial elastic behaviour. In the mixed system, β-lg adsorbed at the interface and GA electrostatically bound to it, leading to the formation of a bi-layer stabilized emulsion. However, emulsion stability was not improved compared to β-lg stabilized emulsion, probably due to depletion or bridging flocculation.

1. Introduction

Emulsions are widely used in the formulation of food, pharmaceutical, and cosmetic products. They are not thermodynamically stable (except microemulsions) and can phase separate. Addition of emulsifiers allows the formulation of stable emulsions thanks to the formation of structured interfacial films. The present study aimed at formulating oil-in-water emulsions for dermatologic use. Our objective was to replace synthetic surfactants that are potentially irritating for skin [1] by "natural" emulsifiers and stabilizers in order to answer the increasing demand of natural and biodegradable products [2,3]. This approach has already been applied in the food industry that currently uses proteins and polysaccharides as texturizing and stabilizing agents [4–7]. Proteins are less effective than synthetic emulsifiers in decreasing interfacial tension, but they lead to thermodynamically and kinetically more stable emulsions [8,9]. Indeed, they form a viscoelastic adsorbed layer onto the oil droplets and are able to generate repulsive steric and electrostatic interactions between these droplets [10]. Generally, proteins act as the main stabilizers and polysaccharides contribute to emulsion stability by their thickening and steric stabilizing behaviours [11]. Polysaccharides that do not adsorb at the globule interface would enhance the viscosity of the aqueous phase and thus slow down destabilizing mechanisms [12] such as creaming, flocculation, and coalescence. On the other hand, adsorbing ones would anchor at the interface by their hydrophobic residues, decrease the interfacial tension and form a steric barrier preventing coalescence [13].

Many studies have shown the interest of combining advantages of proteins and polysaccharides *via* the formation of protein:polysaccharide complexes to emulsify and stabilize emulsions [14–17]. These complexes can be formed through covalent bonding or electrostatic interactions. In the latter case, the efficiency of a polysaccharide to form an interfacial complex with a protein depends on the distribution of ionized groups onto the protein surface and the stability of the protein structure, but also on the flexibility, charge distribution, and density of the polysaccharide [14,18]. In this approach it is possible to formulate emulsions stabilized by multilayered interfacial membranes composed of alternatively oppositely charged biopolymers. Under appropriate formulation

Abbreviations: β-lg, β-lactoglobulin; GA, gum arabic; AG, arabinogalactan; GP, glycoprotein; AGP, arabinogalactan-protein; PEO, poly(ethylene oxide); ACE, affinity capillary electrophoresis; DSA, drop shape analysis; WPM, Wilhelmy plate method.

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conditions, this multiple layer deposition has been shown to confer better stability to environmental stresses than conventional oil-in-water emulsions stabilized by a single interfacial biopolymer layer [17,19–22].

In this work, two biopolymers have been chosen to stabilize oil-in-water emulsions: β-lactoglobulin (β-lg), the major whey protein, and gum arabic (GA), an exudate from *Acacia* trees.

β-lg is a compact globular protein (monomer molar mass = 18.3×10^3 g/mol) containing 162 amino acid residues with one thiol group and two disulphide bonds. It exists in several genetic variants of which A (Gln59, Asp64, Val118) and B (Gln59, Gly64, Ala118) are the most abundant ones [23]. The isoelectric point (p*I*) of β-lg is about 5.2. The protein displays a global positive charge below this value and a negative charge above it. Once adsorbed at the interface, there is a structural reorganization of the protein in order to partially unfold and expose hydrophobic residues toward the lipophilic phase [8,9].

GA is a hybrid polyelectrolyte containing both protein and polysaccharide subunits. It consists mainly of a mixture of arabinogalactan (AG) (80–90% of the total gum in weight), glycoprotein (GP) (2–4% of the total gum in weight), and arabinogalactan-protein (AGP) (10–20% of the total gum in weight) fractions [24]. Molar mass of the whole gum can vary from 3.0×10^5 to 5.8×10^5 g/mol depending on its origin and age [25]. GA carries a net negative charge for pH value above 2.0 conferred by its glucuronic acid residues. The AGP fraction has been considered to be responsible for the emulsifying properties of GA [26–29]. Indeed, the protein component of the gum would embed in the oil phase while the carbohydrate component would extend into water [28,30].

The combination of these two biopolymers is of interest to potentially induce a synergistic effect on emulsion stability [31]. Over the last decade, proteins and polysaccharides and their complexes have emerged in the pharmaceutical field mainly for microencapsulation [32–34]. However, to our knowledge, there is currently no marketed pharmaceutical emulsion stabilized by a combination of protein and polysaccharide. The aim of this study was thus to investigate the potentialities of β-lg, GA, and their mixtures to formulate sweet almond oil-in-water emulsions for dermatologic use. β-lg:GA complexation in solution was first studied by capillary electrophoresis. Affinity capillary electrophoresis (ACE) as a probe for studying molecular interactions has gained increasing popularity in the last few years [35]. The influence of biopolymer concentration, pH, protein-to-polysaccharide ratio, and emulsification process on emulsion stability was then thoroughly studied. In a last step, we have characterized the interactions of both biopolymers with the oil–water interface in order to better understand the emulsion stabilization mechanisms.

2. Materials and methods

2.1. Materials

β-lg powder (lot JE 002-8-992) was supplied by Davisco Foods International, Inc. (USA). Its composition was 89.8 (w/w)% protein, 8.8 (w/w)% moisture, and 1.4 (w/w)% ash [36]. GA (INSTANTGUM AA) was a gift from CNI Company (Rouen, France). Its composition contained 10 (w/w)% moisture and 4 (w/w)% ash with a molecular weight of about 350,000 g/mol as reported by the supplier. The protein content was 2.5 (w/w)% as determined by the Kjeldahl analysis ($N \times 6.66$). Sweet almond oil (lot 07120145 \ A) was provided by Cooper (Melun, France) and sodium azide (lot K305.2) by Roth (Lauterbourg, France). Sodium hydroxide, hydrochloric acid, and citric acid were analytical grades from VWR (Fontenay-sous-Bois, France). Poly(ethylene oxide) (PEO) with a molecular weight of 200,000 g/mol was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Thiourea was obtained from Sigma–Aldrich (Saint Louis, USA). Water used in all experiments was purified using a Millipore Synergy 185 apparatus coupled to a RiOs5™ with resistivity of 18.2 MΩ cm.

2.2. Preparation of biopolymers solutions

β-lg and GA solutions were prepared at total final concentrations of 0.5 and 2.5 (w/w)%. The powder was dissolved in MilliQ water under gentle stirring at 20 ± 1 °C for at least 2 h. Solutions were then allowed to hydrate overnight at 4 ± 1 °C. The pH of β-lg solutions was adjusted to 4.8 (lowest β-lg solubility pH [36]) using 0.1 or 1 N HCl solutions. β-lg solutions were then centrifuged at 10,000 g for 30 min at 20 ± 1 °C to remove insoluble particles and β-lg aggregates. This step induces a 10% loss of β-lg [36] that was taken into account when preparing the solutions. Sodium azide (0.05 (w/w)%) was added to β-lg and GA solutions as an antimicrobial agent. The final pH of both solutions was then adjusted to 4.2 using 0.1 or 1 N HCl solutions in order to allow attractive electrostatic interactions and thus the formation of complexes between β-lg and GA [36,37].

For interfacial measurements, β-lg solutions with concentrations ranging from 2.3×10^{-5} to 3 (w/w)% were prepared by dilution of a 3 (w/w)% β-lg stock solution, in order to measure protein adsorption over a 24-h period.

2.3. Oil-in-water emulsion preparation

Emulsions were prepared by homogenizing 30 (w/w)% sweet almond oil with 0.5 and 2.5 (w/w)% aqueous emulsifier solutions (β-lg, GA or β-lg:GA). When emulsions were stabilized by only one biopolymer, oil and emulsifier solutions were mixed by a rotor–stator homogenizer (Polytron PT-MR 3100, Kinematica AG, Bioblock Scientific) for 2 min at 15,000 rpm. Emulsions were then passed through a two-stage high-pressure valve homogenizer (APV Invensys) for 5 min and with two successive pressure steps, 500 and 50 bars.

To obtain emulsions simultaneously stabilized by β-lg and GA, three different protocols were set up to prepare the 0.5 (w/w)% biopolymer stabilized emulsions (Fig. 1).

The first protocol consisted in formulating an emulsion from a last minute mixed biopolymer solution. In the second protocol, emulsions were formulated using a GA solution that was subsequently diluted by adding a β-lg solution. Finally in protocol 3, emulsions were prepared with a β-lg solution, and then diluted by a GA solution. Note that this latter procedure can be used for

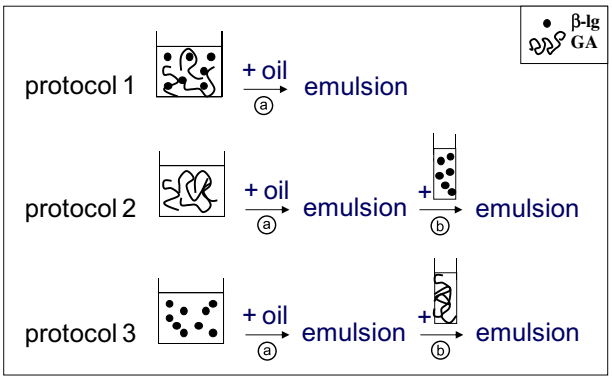

Fig. 1. Schematic depiction of the three protocols for emulsion preparation with β-lg and GA at 2:1 and 1:2 β-lg:GA ratios. (a) Rotor–stator and high pressure homogenizers. (b) Hand reversal shaking.