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Alterations in nanoparticle protein corona by biological surfactants: Impact of bile salts on β-lactoglobulin-coated gold nanoparticles

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ABSTRACT

The impact of biological surfactants (bile salts) on the protein (β-lactoglobulin) corona surrounding gold nanoparticles (200 nm) was studied using a variety of analytical techniques at pH 7: dynamic light scattering (DLS); particle electrophoresis (ζ-potential); UV–visible (UV) spectroscopy; transmission electron microscopy (TEM); and surface-enhanced Raman scattering (SERS). The bile salts adsorbed to the protein-coated nanoparticle surfaces and altered their interfacial composition, charge, and structure. SERS spectra of protein-coated nanoparticles after bile salt addition contained bands from both protein and bile salts, indicating that the protein was not fully displaced by the bile salts. UV, DLS and TEM techniques also indicated that the protein coating was not fully displaced from the nanoparticle surfaces. The impact of bile salts could be described by an orogenic mechanism: mixed interfaces were formed that consisted of islands of aggregated proteins surrounded by a sea of bile salts. This knowledge is useful for understanding the interactions of bile salts with protein-coated colloidal particles, which may be important for controlling the fate of colloidal delivery systems in the human gastrointestinal tract, or the gastrointestinal fate of ingested inorganic nanoparticles.

1. Introduction

Proteins are widely used in the food and pharmaceutical industry as emulsifiers and stabilizing agents. The adsorption of globular proteins to particle surfaces is important in a number of physicochemical and biological phenomena relevant to the development of food and pharmaceutical products. Protein-coated lipid droplets are often used as delivery systems for lipophilic bioactive agents, such as nutraceuticals and drugs [1–4]. Proteins may adsorb to the surfaces of inorganic nanoparticles (such as gold, silver, or titanium dioxide) [5,6], which may alter their gastrointestinal fate (*e.g.,* absorption by epithelium cells or interactions with gut microbiota). Proteins may also adsorb to nanoparticles within the circulatory system of the human body due to a process known as opsonization, which again alters their potential biological fate [7,8]. Consequently, there has been much interest in understanding the way in which protein structure and function are altered after adsorption to particle surfaces. Many food and pharmaceutical products contain a mixture of different types of surface active agents, such as proteins, polysaccharides, phospholipids and surfactants. Consequently, the interfacial properties of a protein-coated particle may change in a product over time due to competitive adsorption, co-adsorption, or multilayer formation processes. In addition, a protein-coated particle may encounter a compositionally complex environment within the gastrointestinal tract, since the intestinal fluids contain a mixture of different surface active agents, such as bile salts, phospholipids, and proteins. There has therefore been substantial interest in understanding the interaction between protein-coated interfaces and surfactants [9–11].

One of the most important classes of surface active agents present within the human gastrointestinal tract are bile salts (BS) [12]. Bile salts are surface active mixtures consisting mainly of the sodium salts of taurocholates. They have previously been shown to be able to displace adsorbed materials from air–water and oil–water interfaces [12–14]. Atomic force microscopy (AFM) has been

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used to characterize the interfacial structures formed by surfactants and proteins at interfaces during competitive adsorption processes, which had led to the proposal of the "orogenic model" for displacement of adsorbed proteins by surfactants [12,15–18]. In this model, the protein molecules initially have a relatively homogeneous distribution at the interface. When the surfactant molecules are introduced, they adsorb to the interfaces and lead to two-dimensional phase separation: protein-rich regions surrounded by surfactant-rich regions. As the surfactant concentration is increased, the proteins further congregate and form thick islands, which are completely displaced from the interfaces at sufficiently high surfactant concentrations.

In this study, protein-coated nanoparticles were prepared using β-lactoglobulin (βLg) as a representative globular protein and gold nanoparticles (GNPs) as representative colloidal particles. β-Lactoglobulin is the major globular protein in bovine milk and is widely used in the food industry as an emulsifier [2]. Its structure, properties, and biological role are well established [19,20]. This protein usually exists as a dimer at neutral pH with a molecular weight of nearly 36 kDa, but dissociates into monomers below pH 3 [21]. Its three-dimensional structure consists of an eight-stranded antiparallel β-hydrophobic barrel unit (54%) with an α-helix (17%) [21,22]. GNPs are attractive for many biological and medical applications due to their chemical stability, biocompatibility, and unique optical properties [23,24]. β-Lactoglobulin-coated GNPs (200 nm) were used as model systems for protein-coated lipid droplets that are found in many food and pharmaceutical delivery systems [2]. An advantage of using GNPs rather than lipid droplets is that their interfacial properties can be measured *in situ* using surface enhanced Raman scattering (SERS). Knowledge of the influence of bile salts on protein-coated GNPs may also be important for understanding the intestinal fate of certain types of ingested inorganic nanoparticle.

Surface-enhanced Raman scattering (SERS) is a derivative of Raman spectroscopy that can be used for sensitive molecular identification and structural characterization [25]. Enhanced Raman scattering occurs when an analyte is on or in close proximity of a SERS active surface, such as silver or gold nanoparticles. Raman signals can be enhanced tremendously by combining electromagnetic enhancement and chemical charge transfer mechanisms [26]. Detection limits of parts per billion (ppb) have been achieved, and even a single molecule can be detected using this method [27]. To study the conformation of biomolecules such as protein, Raman spectroscopy can provide information related to protein backbone conformation and the molecular environment of certain side chains, as well as transitions from ordered to disordered structure upon protein denaturation [28,29]. Raman scattering is also sensitive to changes in covalent bonds, such as disulfide linkages, and non-covalent bonds, such as electrostatic and hydrophobic interactions [28]. Raman spectroscopy also provides the capability to analyze compounds in aqueous solutions with minimal interference from water adsorption, which is a critical factor when considering biological applications [26]. Enhanced with nanotechniques, SERS is a sensitive and promising technique to observe the molecular conformation of biopolymers or substances on a nanosubstrate.

In this study, we used a variety of analytical techniques to better understanding the interaction between bile salts and protein-coated gold nanoparticles, including dynamic light scattering (DLS), particle electrophoresis (ζ-potential), localized surface plasmon resonance (LSPR), transmission electron microscopy (TEM), and surface-enhanced Raman scattering (SERS). Our intention was to unravel the mechanism of interaction between protein-coated GNPs and surface-active bile salts by primarily focusing on the physicochemical and compositional changes at the particle surfaces.

2. Materials and methods

2.1. Materials

Powdered βLg was obtained from Davisco Foods International (Lot JE 001-0-415, Le Sueur, MN). As stated by the manufacturer, the protein content was 97.4% (w/w dry basis), the purity was 95% (w/w) βLg and the nitrogen content was 15.6% (w/w). A suspension of 200 nm gold nanoparticles (7×10^8 particles/ml) was purchased from Ted Pella, Inc. (Redding, CA). Bile extract (porcine) was also purchased from the Sigma Chemical Company (St. Louis, MO). All other chemicals used were of analytical grade. Double distilled water was used throughout the experiments.

2.2. βLg Solution preparation

A βLg solution was prepared at ambient temperature (22 °C) by dissolving powdered β-Lg in 5 mM sodium phosphate buffer solution (pH 7). A βLg solution was prepared at concentration of 5000 μM. The βLg solutions were stirred gently for 3 h to ensure complete protein dissolution. If required the pH of these solutions was readjusted to pH 7.0 using either 1 N HCl and/or 1 N NaOH.

2.3. Gold nanoparticle (GNPs) interaction with βLg and bile salts

The interaction of the globular proteins with the gold nanoparticles (GNPs) was studied by adding 20 μL of βLg solution into a 1.5 ml microcentrifuge tube. Then, 1000 μL of GNP solution was added drop wise under vigorous mixing for 5 min. Thus, the original protein concentration was reduced by a factor of 20/1020, and the original GNP concentration was reduced by a factor of 1000/1020. The mixture was incubated overnight under consistent orbital rotation at ambient temperature (22 °C). The pH of the mixture was kept constant at pH 7.

After incubation, the bile salt solution was added to the GNP-βLg suspensions. The bile salt concentration was varied from 0 to 20 mg/ml to cover a range of potential physiological situations. The mixture was kept constant at pH 7.0 and incubated with continuous shaking at 100 rpm at 37 °C for up to 2 h. Each treatment was prepared in triplicate. The mixture of βLg-coated GNPs and βLg-coated GNPs with bile salts were denoted "GNPs-βLg" and "GNPs-βLg-BS", respectively.

2.4. Particle size and charge measurements

The particle size and charge of bare and coated GNPs were determined using a commercial dynamic light scattering/particle electrophoresis device (Nano-ZS, Malvern Instruments, Worcester-shire, UK). The particle size data were reported as the Z-average diameter, while the particle charge data were reported as the ζ-potential. Measurements were performed at ambient temperature (22 °C) and results were reported as the average of 6 measurements.

2.5. UV–visible spectrophotometer

The ultraviolet spectrum (250–800 nm) of the bare GNPs and coated GNPs was recorded with a UV/Vis scanning spectrophotometer (model UV-2101 PC, Shimadzu Scientific Instruments Inc., Columbia, MD) to determine the peak of protein and gold plasmon band.

To determine the change of the interfacial composition of the coated GNPs after bile salt addition for 2 h, the unabsorbed βLg (present in the aqueous phase) was examined by centrifuging mixtures for 20 min at 13,600 g (model 325C, Thermo Scientific,