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Design, properties, and applications of protein micro- and nanoparticles

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ABSTRACT

The design of protein particles with tailored properties has received an increased attention recently. Several approaches, from simple heat treatment in dilute systems to the combination of heat and mechanical treatments in concentrated protein solutions, have been used to obtain protein particles with varying functional properties. Control of particle size, morphology, surface- and internal properties is crucial for obtaining protein particles with the necessary properties for emerging applications. The latter include not only the use of protein particles in foods, where they can improve the stability of foods at high protein content, but also as food-grade particles for the delivery of bio-actives. By tuning the morphology and size of protein particles, protection or controlled release of various bio-active components may be obtained. We review the various methods that have been used to prepare protein particles and discuss the behavior of the particles in dispersed systems and their possible applications.

1. Introduction

The design of protein-based micro- and nanoparticles with controlled size, morphology and physico-chemical properties is a topic of growing interest in view of emerging applications in foods and pharmaceuticals, and products at the interface of foods and pharma. Food grade protein particles can be designed to meet the demands for different applications, in providing thermally stable foods with high levels of protein, or immobilization and controlled release for a range of hydrophobic to hydrophilic bioactives, and entire micro-organisms.

A first technique for preparing protein particles that was introduced early on by the food industry is the use of heat-induced protein aggregation, in combination with high shear to obtain small particles [1]. This approach is completely scalable, and is now widely used for preparing so-called micro-particulated protein, especially micro-particulated whey protein. Micro-particulated proteins having a range of functional properties can be obtained by tuning various process parameters [2–4]. In recent years there has been a drive to develop approaches that give better control over the relevant protein particle properties such as size, morphology and surface characteristics. With tight control over the solution and heating conditions, it has been possible to create well-defined spherical protein particles. For example, by heating dilute aqueous solutions of globular proteins in a narrow pH range around the protein iso-electric point [5–7••]. By way of another example, when heating protein solutions that have been emulsified in a continuous oil phase, good control over particle properties was also obtained. This process can be used to create protein particles with sizes of the order of microns [8•] and down to the order of 100 nm [9]. Instead of gelling small droplets of protein solutions dispersed in oil, segregative phase separation in mixed biopolymer systems, i.e. protein/polysaccharide, can also be used to create protein droplets, which subsequently can be gelled by a heating step [10]. Finally, not so much investigated yet, is the use of associative phase separation with an interacting biopolymer, which will give composite protein–biopolymer particles [11]. Hence the three main routes to protein particle formation are a) "simple" heat-induced protein aggregation, where size control at higher protein concentrations is possibly assisted by mechanical shear forces, b) heat-induced aggregation of dense protein droplets suspended in a protein-poor continuous matrix (either oil or a segregating biopolymer) and c) using associative phase separation that locally gives a high protein concentration (either a soluble complex or a dispersed droplet of a

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macroscopic coacervate phase), and which can be heated to give protein particles. A schematic of these basic routes and protein particles prepared through using one of these approaches is shown in Fig. 1.

A major potential application of nano- and micron-sized protein particles is as a food ingredient, in particular for tuning the heat stability of high protein foods. This application is highly relevant since high protein beverages are becoming more and more prevalent and their liquid character is rather heat sensitive, because high concentrations of protein (in most cases whey protein) readily gel upon heating. As a consequence there is only a limited concentration range that allows for the heat-treatments that are required in view of the microbiological safety, and which at the same time are stable. Usually, acidic conditions and lower protein concentrations are required to enable manufacturing and long term stability. As we will discuss, protein particles with controlled size, morphology, surface and internal properties can be used in designing heat-stable protein formulations at high concentration that have the appropriate textural properties.

The size of protein nano- and microparticles responds to the changes in the physical chemical conditions, such as pH and ionic strength [12,13]. Dispersions of these particles will therefore exhibit rheological properties that are highly dependent upon the environmental conditions. This behavior may be exploited for food structure design. Depending on their surface characteristics, protein nano- and microparticles may or may not adhere to a variety of interfaces (air–water, oil–water, water–water), and lead to Pickering stabilization of foams and emulsions. Finally, the responsiveness of food-grade protein nano- and microparticles, in combination with the fact that these particles bind a variety of bioactives, also makes them potentially interesting delivery vehicles for a range of (bioactives) molecules or small structures.

This review focuses on recent progress in the design of protein nano- and microparticles with well-defined physical chemical properties. Specifically, in this review, we limit our attention to particles produced from hydrophilic proteins. First we give an overview of the various ways that have been used to produce these particles, basically following

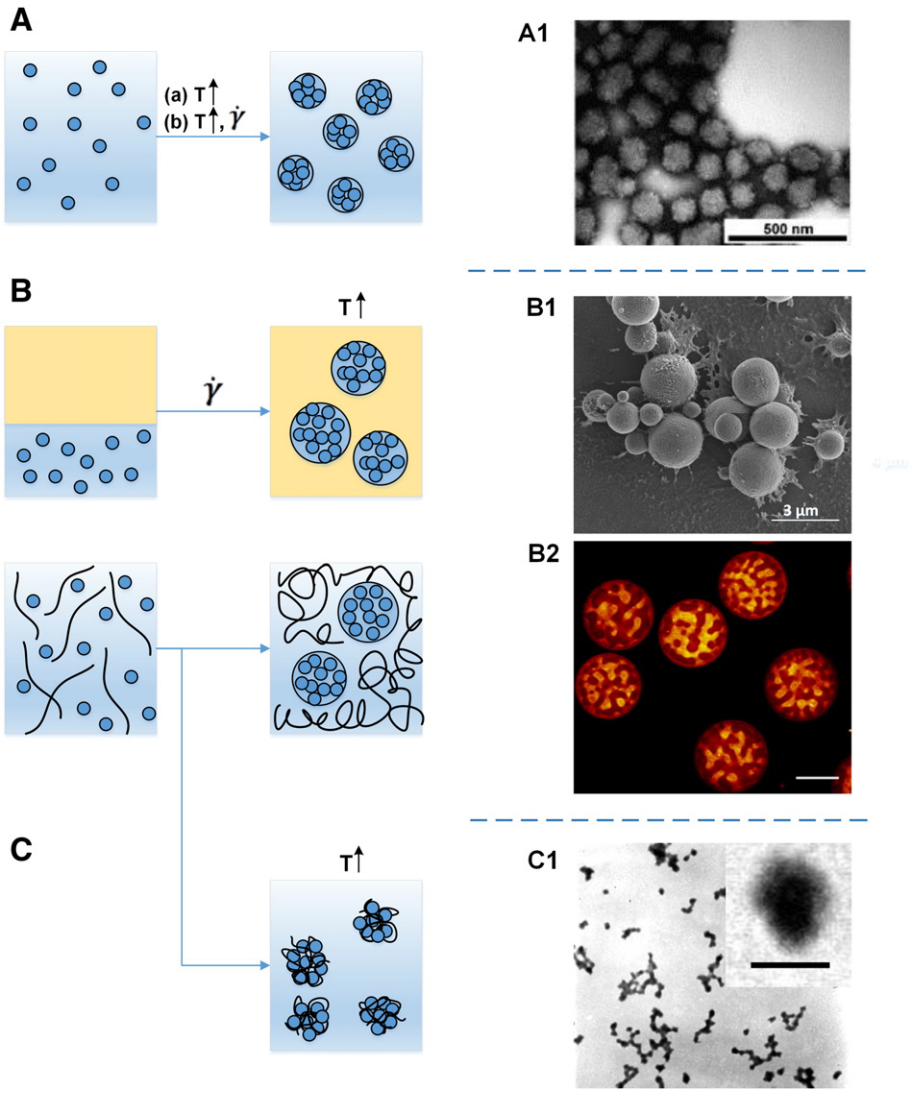

Fig. 1. Three main routes to protein particles. A) Protein aggregation in dilute solutions or aggregation at higher concentrations using mechanical force to keep aggregate size below a certain size, B) Emulsions (top) and segregative phase separation with a non-interacting biopolymer (bottom), C) Associative phase separation with an interacting biopolymer either aggregating proteins in soluble complexes or in dispersed droplets of coacervates. A1: TEM images of spherical protein aggregates prepared through heat induced aggregation of a 1%wt β-lg solution at pH 5.8. B1: SEM images of protein particles prepared through emulsification and heat induced gelation of WPI solution at pH 6.8. B2: Confocal scanning laser microscopy images of gelatine particles prepared through emulsification of phase separating and gelling biopolymer mixture. C1: TEM images of nanoparticles prepared through associative phase separation and heat treatment of ovalbumin/chitosan mixture. Image A1, reprinted with permission from Ref. [14], Biomacromolecules 9, 2477–2486 (2008), copyright 2008 American Chemical Society. Image B1, reprinted with permission from Ref. [15], Langmuir 28, 6551–6560 (2012), copyright 2012 American Chemical Society. Image B2, reprinted with permission from Ref. [16•], Food Hydrocolloids 28, 20–27 (2012), copyright 2012 Elsevier. Image C1, reprinted with permission from Ref. [17], Langmuir 22, 2754–2759 (2006), copyright 2006 American Chemical Society.