Colloids and Surfaces B: Biointerfaces xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Formation and functionality of self-assembled whey protein microgels

Taco Nicolai

LUNAM Université du Maine, IMMM UMR-CNRS, Polymers, Colloids, Interfaces, 72085 Le Mans Cedex 9, France

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form 8 May 2015
Accepted 13 May 2015
Available online xxx

Keywords:
Protein
Whey
Microgel
Aggregation
Emulsion
Gel

ABSTRACT

Whey proteins spontaneously form spherical particles when heated in aqueous solutions at conditions where their net charge density is below a critical value. The particles are microgels consisting of a hydrated crosslinked network of proteins with a diameter between 100 nm and 1 μm. Stable suspensions of these microgels can be formed in a narrow range of conditions when the protein charge density is low enough to induce their formation, but high enough to inhibit further association into larger clusters or macroscopic gels. The formation of microgels and their application to stabilize emulsions and foams; form core–shell particles; form gels; or modify the texture of polysaccharide solutions and gels are reviewed.

1. Introduction

The proteins in milk may be divided into two groups: caseins and whey proteins [1]. Caseins are present in milk in the form of spherical complexes with a radius of about 100 nm that are called casein micelles. For cheese production casein micelles are destabilized, which causes them to agglomerate. The liquid that is exuded during this process is called whey and contains different types of globular proteins that are, contrary to caseins, rigid, dense and highly structured. The main whey proteins are β-lactoglobulin (β-lg) and α-lactalbumin (α-lac) that have a net negative charge at neutral pH and an isoelectric point close to pH 5. Purified whey protein isolate (WPI) contains about 80% β-lg and 15% α-lac and is produced on an industrial scale [2].

When WPI is heated above about 60 °C in aqueous solution, the peptide chains become mobile, which allows them to interact with other whey proteins. This may cause the formation of bonds between different proteins leading to aggregation. For recent detailed reviews of the aggregation of whey proteins see Refs. [3,4] and for protein aggregation more in general see Refs. [5,6]. Below 85 °C, the rate limiting step of WPI aggregation is denaturation of the native proteins, which is characterized by a high-activation energy. There is no lower critical temperature for aggregation to occur, but it is in practice too slow to be observed below 60 °C. The morphology of the aggregates depends on the pH, see Fig. 1. Curved strands with a diameter of a few nm are formed when the effective charge density of the proteins is high, while spherical particles with a radius of roughly 100 nm are formed when it is low. The spherical protein particles consist of a hydrated network of covalently crosslinked proteins and may therefore be considered as microgels [7]. At very low pH (1.5–2.5) and low ionic strength, long rigid fibrils are formed when whey proteins are heated extensively. However, at this low pH the proteins hydrolyze and the fibrils are formed by a fraction of the resulting peptides. At higher protein concentrations the strands or the microgels randomly associate into larger self-similar aggregates and above a critical concentration gels are formed.

Whey protein microgels are an example of protein micro- and nanoparticles that have attracted increased attention recently for their potential application in food and pharmaceutics [9]. The present review focuses on the formation and the properties of stable solutions of protein microgels that form spontaneously when WPI or pure β-lg is heated in aqueous solution.

2. Formation and characterization of stable microgel suspensions

2.1. Effect of the pH

A first indication that stable solutions of whey protein microparticles form spontaneously during heating was reported by Britten [10]. He found that when WPI solutions were heated in a narrow pH range around pH 6.0, they became highly turbid, but remained stable as long as the protein concentration was kept below a critical value. This finding remained largely unnoticed until

E-mail address: Taco.Nicolai@univ-lemans.fr

http://dx.doi.org/10.1016/j.colsurfb.2015.05.055
0927-7765/

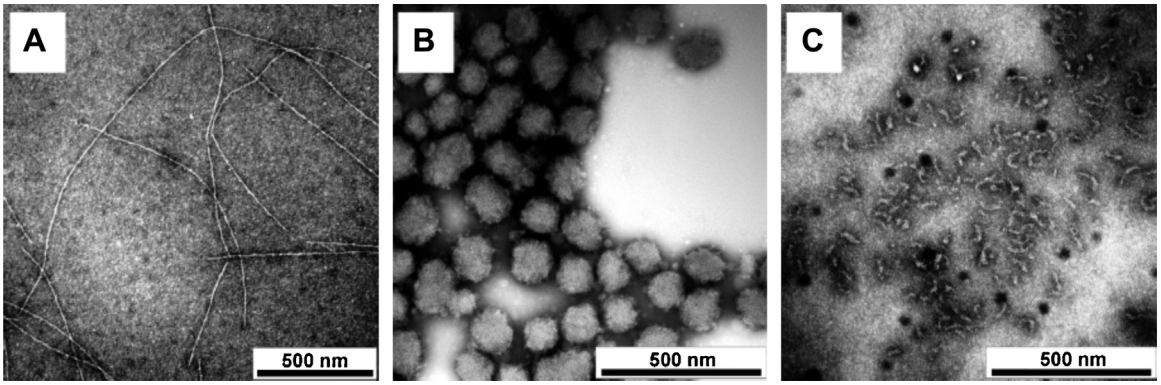

Fig. 1. Negative-staining TEM images of aggregates formed during heating 10 g/L β-lg in aqueous solution at pH 2.0 (A), pH 5.8 (B) and pH 7.0 (C). Scale bars represent 500 nm. Reproduced from Jung et al. [8].

Schmitt et al. [11] reported that in the absence of added salt stable suspensions of spherical particles with a radius of about 60 nm formed in solutions of 10 g/L WPI during heating at pH 6.0, whereas at pH 6.6 and pH 7.0 small curved strands were formed. The ζ-potential of the two types of aggregates was similar, but the surface hydrophobicity was lower for the spherical particles. Larger aggregates could also be formed at higher pH by adding NaCl before heating. However, these aggregates consisted of randomly associated strands and were much less dense due to their fractal structure.

Mehalebi et al. [12] characterized β-lg aggregates that were formed by heating aqueous solutions between pH 5.8 and 8.0, as a function of the protein concentration. They found that at low protein concentrations the average hydrodynamic radius (R_h) of the aggregates was less than 20 nm between pH 6.0 and 8.0, whereas at pH 5.8 it was around 100 nm. The size of the aggregates formed at pH 5.8 was found to be sensitive to very small changes in the pH. At higher protein concentrations, random association of these primary aggregates led to the formation of larger self-similar aggregates or a gel. Jung et al. [8] also found that β-lg aggregates formed by heating at pH 5.8 (C = 10 g/L) where much larger than those formed at pH 7.0. Transmission electron microscopy (TEM) images showed that the aggregates formed at pH 5.8 were roughly spherical with a radius of about 100 nm, whereas those formed at pH 7.0 were curved strands with a diameter of about 10 nm and a length of about 50 nm, see Fig. 1. Scattering experiments showed that the spherical aggregates had well defined boundaries and that their structure was homogeneous down to a length scale of 20 nm. The authors suggested that the structure of the spherical aggregates corresponded to that of microgels.

Schmitt et al. [13] investigated in more detail the formation of microgels by β-lg as a function of the pH at a fixed protein concentration of C = 10 g/L. They showed that stable solutions of microgels were formed in a narrow pH-range both above (6.1–5.8) and below (4.3–4.6) the iso-electric point. In the intermediate pH-range the proteins precipitated, while above pH 6.1 or below pH 4.3 strands were observed. The hydrodynamic radius of the microgels was found to be slightly larger at pH 4.6 (R_h = 110 nm) than at pH 5.8 (R_h = 80 nm). The ζ-potential, the hydrophobicity and the accessibility of thiol groups depended on the pH, but was not correlated with the change in the aggregate morphology from strands to microgels.

The effect of heating time and temperature on the formation of microgels by β-lg at pH 5.7 or 5.9 was investigated by Donato et al. [14] for C = 10 g/L. TEM images showed that solutions heated at 70 °C for 1 h contained spherical particles with a diameter of 50–70 nm and that after heating for 24 h they had grown to up to 200 nm. The larger microgels were observed already after 1 h when the solutions were heated at 85 °C. Microgels formed at pH 5.7 were larger than those formed at pH 5.9, but at the lower pH a fraction of the proteins precipitated. The increase of the size with increasing heating time was corroborated by measurements of the hydrodynamic radius. The microgels sedimented after centrifugation for 30 min at 16.870 g at 20 °C, but the supernatant still contained smaller aggregates and residual native proteins. The fraction of native protein determined by precipitation of the aggregates at pH 4.6 decreased with heating time and more rapidly at 85 °C than at 70 °C. The fraction of microgels increased with heating time and was systematically larger at pH 5.7 than at pH 5.9. When the proteins were heated at pH 7.0 no sedimentation of proteins was observed. Steady state was reached after a few hours at 85 °C, but not yet after 24 h at 70 °C. Analysis by size exclusion chromatography (SEC) of the supernatant at pH 5.7 and pH 5.9 showed that it contained besides native β-lg, small oligomers (trimers and tetramers) and aggregates. The latter were much smaller than the microgels and similar to the strands that were formed at pH 7.0. The supernatant probably also contained denatured monomers and dimers, but it was not possible to clearly distinguish them from native β-lg with SEC. An important observation was that the pH increased during heating from 5.7 to 6.3 or from 5.9 to 6.5, whereas when the initial pH was set at 7.0 heating led a small decrease of the pH to pH 6.8.

The origin and consequence of the pH increase during microgel formation was discussed by Phan et al. [15] who studied the formation of β-lg aggregates over a range of concentrations (10–90 g/L) and pH (5.6–7.0). Stable microgel suspensions were observed between pH 5.75 and pH 6.2. At lower pH, protein precipitated and at higher pH only strands were formed. The rate of microgel formation was determined at pH 5.8, 5.9 and 6.0 for different temperatures between 72 °C and 85 °C. It was found to be independent of the pH and could be well-described by an activation energy of about 300 kJ/mol, indicating that denaturation was the rate limiting step. Steady state was reached when the solution no longer contained native proteins.

TEM images showed that the heated solutions contained both microgels and small strands, see Fig. 2. Centrifugation at different speeds of the solutions at steady state showed that all microgels could be sedimented, while maintaining most of the strands in the supernatant. By characterizing the supernatant at different centrifugation speeds the size and molar mass (M) distribution of the microgels could be determined. The average R_h of the microgels was found to increase with decreasing pH from about 70 nm at pH 6.0 to about 130 nm at pH 5.75, but their protein density calculated as $\rho = M/[(4/3)\pi R_h^3 \cdot N_a]$ did not depend significantly on the pH and was found to be 0.15 ± 0.5 g/mL. In this pH-range the size of the microgels did not depend on the protein concentration between 10 and 50 g/L, A similar increase of R_h with decreasing pH was reported

Please cite this article in press as: T. Nicolai, Formation and functionality of self-assembled whey protein microgels, Colloids Surf. B: Biointerfaces (2015), http://dx.doi.org/10.1016/j.colsurfb.2015.05.055