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The role of metal ions in emulsion characteristics and flocculation behaviour of phosvitin-stabilised emulsions

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Abstract

Phosvitin (Pvt) is an egg yolk and a natural metal-chelating protein presenting excellent emulsion-stabilising properties. During emulsification process, proteins are able to adsorb at the oil droplet interface decreasing the interfacial tension and, at the same time, stabilise the colloidal system by the formation of an interfacial film. Even if Pvt is a good stabilising agent, it could be susceptible to the presence of metal ions usually found in food systems.

In this work, we have measured the emulsifying capacity of Pvt in the presence of Mg and Fe ions. The influence of the latter was evaluated before and after the emulsion preparation. Pvt-stabilised emulsions, which naturally hold Mg, were highly flocculated at pH 6 and 7, as confirmed by rheology measurements. Flocculation was partially reversed by subsequent EDTA addition, and was totally reversed when EDTA was added before emulsion preparation. The addition of Fe ions to Pvt emulsions flocculates the system at pH values between 4 and 7, and especially at pH 6 and 7. EDTA (4.8 mM) was able to partially de-flocculate the emulsion. Similarly, SDS alone was not adequate to completely disaggregate oil droplet flocs, and only the presence of SDS and EDTA at the same time successfully de-flocculated the system. When Fe was added before emulsion formation, the Fe–Pvt complexes presented less emulsifying capacity than the crude preparation, and they exhibited a reduced flocculation compared with emulsions where Fe ions were added after the emulsion formation.

Keywords: Egg yolk proteins; Phosvitin; Emulsification; Flocculation; Fe; Mg; EDTA

1. Introduction

Egg yolk phosvitin (Pvt) has a particular amino acid composition, showing a high proportion of serine moieties (about 50%) (Losso & Nakai, 1994; Sundararajan, Sampath Kumar, & Sarma, 1960). As all of them are phosphorylated (Clark, 1985), Pvt can be represented as a natural polyelectrolyte. Moreover, the phosphoserines are arranged in a singular way, forming blocks that can carry up to 15 consecutive residues (Mabuchi et al., 1996). This unique primary structure makes this protein one of the strongest metal-chelating agents (Hegenauer, Saltman, & Nace, 1979). Grizzuti and Perlmann (1973) have described the interaction with Mg^{2+} and Ca^{2+}, showing that at pH 6.5 Pvt binds up to 103 and 127 ions/molecule, respectively, although at pH 4.5 it can only bind 40 and 32 ions of Mg^{2+} and Ca^{2+}, respectively.

Iron (Fe) is an important metal in food and health sciences, and Fe fortification is present in many processed and non-processed food products (Gaucheron, 2000). Moreover, its high Fe-binding capacity gives interesting characteristics to Pvt, for example, bactericidal properties (Sattar Khan et al., 2000) or antioxidant activity (Lee,

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Han, & Decker, 2002). When mixing Pvt and ferrous Fe solutions, Fe is oxidised into ferric Fe by autoxidation, forming Pvt– $Fe3+$ complexes (Grogan & Taborsky, 1986). This kind of structures can be formed by 60 ions/molecule of protein in optimal conditions (pH 6.5 and low ionic strength). They are very stable and resistant to pH modification or high-pressure treatments (Castellani, Guérin-Dubiard, David-Briand, & Anton, 2004).

Pvt can be represented as a di-block polymer consisting of a very large hydrophilic moiety with two small hydrophobic blocks at the N and C terminal portions (Dickinson, Pinfield, & Horne, 1997). Consequently it shows moderate interfacial adsorption; but at the same time, excellent emulsion stabilisation properties due to its high charge (Castellani, Belhomme, David-Briand, Guérin-Dubiard, & Anton, 2006; Dickinson, Hunt, & Dalgleish, 1991). Nonetheless, in view of its high phosphate content, Pvt-stabilised emulsions are sensitive to the presence of calcium ions. These ions affect mainly the flocculation state of the system (Dickinson, Hunt, & Horne, 1992). So, we would expect its emulsifying characteristics to be sensitive to other multi-charged metal cations.

For this reason, in this study, we have decided to investigate the characteristics of Pvt emulsions as influenced by Mg (already present in the sample) and Fe (added to the sample) and the reversibility of these processes.

2. Materials and methods

2.1. Materials

Isabrown eggs were obtained from local wholesale distributor. They were 3 days old. All chemicals (analytical grade) were purchased from Sigma (Saint Quentin-Fallavier, France).

2.2. Methods

2.2.1. Pvt isolation

Hen eggs were manually broken, and yolks were carefully freed of adhering white and chalazae by rolling on a filter paper (Whatman). The vitellin membrane was punctured with a lancet and the content was collected in a beaker cooled in iced water. Temperature was maintained at 4 °C all through the process. Granules were extracted from yolk according to the method of McBee and Cotterill (1979). Yolk was diluted with an equal mass of a 0.17 M NaCl solution and mixed with a magnetic stirrer. After 1 h, the solution was centrifuged at 10,000*g* for 45 min in a Jouan centrifuge (model GR 2022, St. Herblain, France) and the pellet (granules) was collected and dissolved in a 1.74 M NaCl solution (10 w/v%). The mixture was stirred to complete dissolution keeping the pH adjusted to 7.25. The solution was then dialysed against several changes of distilled water for 24 h and centrifuged at 10,000*g* for 30 min. The high-density lipoproteins precipitated, and the supernatant was diluted with 0.9 M $MgSO_4$ solution to obtain a 0.2 M final concentration of this salt. After centrifugation (10,000*g* for 30 min), a precipitate (Pvt) was collected at the bottom of the tubes and it was freeze-dried. This sample contained 65% of protein and about 37 Mg atoms per molecule of protein as already described (Castellani, Martinet, David-Briand, Guérin-Dubiard, & Anton, 2003).

2.2.2. Emulsion preparation

Dispersions of 5 mg/ml of lyophilised Pvt were made at pH 4 and 5 (0.05 M sodium acetate/acetic acid buffer) and at pH 6 and 7 (0.05 M 2-(*N*-porpholino) ethanesulfonic acid (MES) buffer). NaCl was added to obtain 0.05 M values of ionic strength when necessary. They were gently stirred for 1 h and after that, solutions were centrifuged 20 min at 2000*g* and 15 °C. Pvt final concentration of all supernatants were measured by phosphorus content (Bartlett, 1959), considering 9 w/v% of phosphorus in Pvt (Castellani, Martinet, David-Briand, Guérin-Dubiard, & Anton, 2003). Then Pvt concentration was fixed at 3.3 mg/ml. Oil-in-water emulsions were prepared with 36 ml of the corresponding Pvt solution and 4 ml of sunflower oil. The system was premixed 1 min at 20,000 rpm using a polytron PT 3000 homogeniser (Kinematica, Switzerland) equipped with a 12 mm diameter head. Then, homogenisation of emulsion premix was achieved with a high-pressure valve homogeniser at 75 bar (Stansted Fluid Power Ltd. model AO 812 W, Stansted, Essex, UK). Each emulsion (40 ml) was left recirculating in the homogeniser for 5 min at a flow rate of 80 ml/min.

Fe addition: a variable volume of a 100 mM $FeSO_4$ solution was added, before or after emulsion formation, to obtain the final equivalent of 25%, 50%, 75% and 100% Fe saturation of Pvt. The system was left for 1 h, and then particle size distribution was determined. As described by Taborsky (1991), the 100% of Fe saturation corresponds to an Fe/P ratio of 0.5, and Pvt preparation presents 9 w/v% of organic phosphorus. So, for the 25%, 50%, 75%, and 100% of saturation, the final Fe concentration in solution were 1.2, 2.4, 3.6 and 4.8 mM, respectively.

Ethylenediaminetetraacetic acid (*EDTA*) *addition*: EDTA solution (50 mM in the corresponding buffer) was added to Pvt solutions or to Pvt-stabilised emulsions after 1 h of emulsion preparation or Fe interaction. The final concentration of EDTA was 5 mM. The corresponding solutions were gently mixed by 1 h and then particle size distribution of the systems was determined.

2.2.3. Particle size distribution

Volume frequency distribution (%) of the emulsion droplets was determined by laser light diffraction using a Saturn DigiSizer^{TM} 5200 (Micromeritics Instrument Corporation, USA). The refractive index of the oil was 1.475 and the imaginary part of refractive index (due to absorption) was fixed at 0.01. Before measurements, emulsions were diluted 12.5 times in a 0.05 M Tris–HCl buffer (pH 8), in the presence or in the absence of 1 w/v%