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Oil-in-water emulsion properties and interfacial characteristics of hen egg yolk phosvitin

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Abstract

Phosvitin is an egg yolk protein, constituted by 50% of serines, which are all phosphorylated. This singularity makes of phosvitin one of the most safety natural iron binding molecules. When hen egg yolk is used as emulsifying agent, the adsorption of phosvitin could alter this iron binding property. Consequently, this work was performed to better understand emulsifying and interfacial properties of phosvitin in optimal metal binding conditions. We have studied sunflower oil-in-water emulsions of phosvitin at pH 5 and 6, and ionic strengths of 0.05 and 0.15 M. The oil droplet size, the stability against coalescence and flocculation, the composition of the interfacial protein film and the interfacial activity at model interfaces were analysed. Finally, the capacity of phosvitin to bind iron when it is anchored at the emulsion interface was investigated.

Phosvitin showed satisfactory emulsifying capacity in conditions favouring iron fixation. In these conditions, emulsifying activity is sensitive to pH whereas flocculation is influenced by ionic strength. Coalescence destabilisation is not extended and the interfacial protein film has better characteristics at pH 5. Phosvitin was not efficient to reduce the oil-in-water interfacial tension, although at 0.1 mg/ml the interfacial tension was reduced from 31 mN/m to 15 mN/m. The high iron binding capacity of phosvitin in solution is kept by interfacial adsorbed phosvitin. Finally, to explain the poor adsorption efficiency coupled with the suitable emulsifying properties of phosvitin and the preservation of the iron binding capacity, it is likely that phosvitin is anchored at the interface only by a tiny terminal portion, presenting the rest of the molecule solvated on the aqueous phase.

Keywords: Phosphoprotein; Phosvitin; Emulsion; Interfacial-protein film

1. Introduction

Phosvitin (PVT) is a phosphoprotein that constitutes, in combination with high-density lipoproteins (HDL), the granules of hen egg yolk. It represents 25% of granules yolk proteins, and one of its singularities is that it presents a high proportion of serines which are almost all phosphorylated (Clark, 1985). Some phosvitin polypeptides have been sequenced, and, from the amino acid sequences, phosvitin can be represented like an elongated core of negatively charged phosphoserines, with a C-terminal part of about 15 residues relatively rich in hydrophobic amino acids (Byrne et al., 1984; Mabuchi et al., 1996). Phosvitin has important features as metal chelator, and is one of the molecules with the highest iron and calcium binding capacity (Grizzuti & Perlman, 1975; Hegenauer, Saltman, & Nace, 1979). The metal-chelator antioxidant activity of phosvitin in solution is well established (Lu & Baker, 1987; Yamamoto, Sogo, Iwao, & Miyamoto, 1990).

Hen egg yolk is commonly used in emulsion industry to form and stabilise emulsions, and it is a key ingredient in products such as mayonnaise or salad dressing. During the past 15 years, emulsifying behaviour of egg yolk has begun to be studied intensively (Anton & Gandemer, 1997; Kiosseoglou, 1989; Martinet, Beaumal, Dalgalarrondo, & Anton, 2002; Martinet, Saulnier, Beaumal, Couthaudon,

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& Anton, 2003; Mine, 1998a,b). The main studies have been focused on the lipoproteins of egg yolk and it has been specified that, among egg yolk constituents, low-density lipoproteins bring a great contribution to the emulsifying properties of yolk.

Concerning phosvitin, the principal investigations have been essentially focused on iron fixation in solution (Castellani, Guérin-Dubiard, David-Briand, & Anton, 2004; Lee, Han, & Decker, 2002), and, on conditions affecting the emulsifying and adsorption properties (Chung & Ferrier, 1991, 1992; Damodaran & Xu, 1996), However, conditions and mechanisms allowing simultaneously good iron fixation and suitable emulsifying properties have not been studied yet. When employing egg yolk in emulsion formation, phosvitin migrates to the interface and its iron affinity could result modified. If the iron affinity of phosvitin decreases, it could release iron to the solution, and therefore, increase the oxidant capacity of solution.

Consequently, our objectives in this study were to characterise phosvitin emulsions and interfacial activity of phosvitin in conditions where it can bind iron (pH 5–6). To increase the knowledge of interfacial properties of this protein, we have determined the oil–water interfacial protein composition (qualitatively and quantitatively), and both the structural modifications followed by phosvitin and its iron binding capacity when it is placed at the interface.

2. Materials and methods

2.1. Materials

Isabrown eggs (three or four days old) were obtained from local wholesale distributor. All chemicals (analytical grade) were purchased from Sigma (Saint Quentin-Fallavier, France), excepted Hydrochloric acid 37% (pro-analysis) which was purchased from Carlo Erba Reagenti (Val de Reuil, France).

2.2. Methods

2.2.1. Phosvitin 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. After that, 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 $10{,}000\times g$ 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% wt/vol %). 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 $10{,}000\times g$ 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 ($10{,}000\times g$ for 30 min), a precipitate of phosvitin was collected at the bottom of the tubes and it was dialysed against distilled water and freeze-dried (PVT).

2.2.2. Protein determination

The protein concentration was determined by means of phosphorus content considering 9 wt% of this mineral in PVT (Castellani, Martinet, David-Briand, Guérin-Dubiard, & Anton, 2003). Phosphorus was determined by triplicate, using the colorimetric method described by Bartlett (1959), with hydrazine sulphate and sodium molybdate as reagents.

2.2.3. Emulsion preparation

Dispersions of 5 mg/ml of lyophilised PVT were made at pH 6 (0.05 M 2-(*N*-porpholino) ethanesulfonic acid (MES) buffer) and at pH 5 (0.05 M acetic acid/sodium acetate buffer). NaCl was added to obtain either 0.05 or 0.15 M values of ionic strength. They were gently stirred for 1 h, then solutions were centrifuged 30 min at $3000\times g$, and the supernatant was recovered. PVT final concentration of all supernatants were measured and fixed at 3.3 mg/ml. Oil-in-water emulsions were prepared with 3 ml of sunflower oil and 27 ml of the respective PVT solution (oil volume fraction 0.1). The system was premixed for 1 min at 20,000 rpm using a 12 mm diameter head attached to a polytron PT 3000 homogeniser (Kinematica, Switzerland). Then, homogenisation of the emulsion premix was achieved with a high-pressure valve homogeniser at 70 bars (Stansted Fluid Power Ltd. model AO 812 W, Stansted, Essex, UK). Recirculation of each emulsion was made during 5 min at a flow rate of 80 ml/min.

2.2.4. Particle size distribution

The volume-surface area average diameter distribution (d_{32}) and the volume frequency distribution (d_{43}) of the emulsion droplets were determined by laser light diffraction using a Saturn DigiSizer™ 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. After homogenisation, a 1/12.5 emulsion dilution in 0.05 M Tris–HCl buffer (pH 8) containing 1 wt/vol.% of sodium dodecyl sulphate (SDS) was made to deflocculate the oil droplets.

2.2.5. Flocculation

Emulsions were kept at ambient temperature (18 °C) for 4 h, and then the volume frequency distribution was measured as indicated previously. To detect the flocculation process, emulsions were diluted in buffers with and without SDS. The flocculation index was evaluated as: $F_i = (d_{43}/d_{43SDS} - 1)\times 100$, where d_{43} is the volume frequency