Food Chemistry 158 (2014) 186–191

Contents lists available at ScienceDirect

Food Chemistry

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

Preparation of high purity egg phosvitin using anion exchange chromatography

Jiandong Ren, Jianping Wu *

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, T6G 2P5 Alberta, Canada

ARTICLE INFO

Article history:
Received 23 September 2013
Received in revised form 1 February 2014
Accepted 19 February 2014
Available online 28 February 2014

Keywords:
Phosvitin
Egg yolk
Phosphoprotein
Anion exchange chromatography
Functional food

ABSTRACT

Egg yolk phosvitin serves as a warehouse to provide metal ions for embryo development. It is significant for mineral metabolism study and also of great potential to be developed into functional foods with mineral absorption promoting ability. In this study, phosvitin was first extracted from yolk granules using 10% NaCl, dialysed and then adjusted to various pHs to remove impurities. The purity of phosvitin extracts was increased from 54.5% to 63.7% at decreasing pH from 8.0 to 5.5, and started to decrease afterwards. Both the yield and recovery were significantly decreased at decreasing pHs, especially at pHs close to its pI (pH 4.0), indicating the occurrence of co-precipitation of phosvitin with HDL. The extract prepared at pH 8.0 showed the highest recovery of 82.7%; its purity was increased from 54.5% to 97.1% by anion exchange chromatography, with a recovery of 42.0%. This simple method could be scaled up for industrial production.

1. Introduction

Phosvitin (PV) is the principal phosphoprotein in chicken egg yolk, and the most highly phosphorylated protein in nature (Mecham & Olcott, 1949). It represents about 11% of yolk proteins or 5.3% of yolk solids (Burley & Cook, 1961; Lei & Wu, 2012). Phosvitin contains 10% phosphorus which is mono-esterified to the 123 serine residues out of its total 217 amino acids (Byrne et al., 1984; Clark, 1985; Mecham & Olcott, 1949). Phosvitin shows great potential for food or functional food uses, as it has excellent emulsifying properties, antioxidant activities, antibacterial activities and metal chelating properties (Albright, Gordon, & Cotterill, 1984; Chung & Ferrier, 1992; Khan et al., 2000; Nakamura, Ogawa, Nakai, Kato, & Kitts, 1998).

Phosvitin was first purified by Mecham and Olcott (1949) using a lengthy protocol including precipitation by 0.08 M $MgSO_4$, and extraction subsequently with 0.4 M $(NH_4)_2SO_4$, ethyl ether and 0.25 M NaCl. Their recovery was 60–70%. Sundararajan, Kumar, and Sarma (1960) simplified the protocol without significant loss of phosvitin purity and yield compared with that of Mecham and Olcott (1949). This method started with water dilution and centrifugation of egg yolk, followed by extraction with 10% NaCl, butanol and ether, and precipitation at pH 1.8 in the presence of 0.4 M $MgSO_4$. Most of the subsequent extraction methods were developed based on these two protocols, employing organic solvent to remove lipids, and high ionic strength or magnesium ions to separate phosvitin from defatted egg yolk (Joubert & Cook, 1958; Ko, Nam, Jo, Lee, & Ahn, 2011; Losso & Nakai, 1994; Tsutsui & Obara, 1984). Phosvitin presents as lipovitellin–phosvitin complex. Lipovitellin (also known as a high-density lipoprotein (HDL) in egg yolk) is consisted of α-lipovitellin (α-HDL) and β-lipovitellin (β-HDL) which precipitate at pH 7.5–7.8 and pH 6.5–7.0, respectively (Sugano, 1958). At pHs lower than 5.0, both HDLs are precipitated (Castellani, Martinet, David-Briand, Guerin-Dubiard, & Anton, 2003). On the other hand, phosvitin shows extremely good solubility in water due to its negatively charged phosphoserine residues (Taborsky, 1991). Only 6.9% phosvitin was precipitated when pH was decreased from 7.0 to 5.0 (Castellani et al., 2003). Therefore, it is possible to improve the purity of phosvitin extract by decreasing pH to around 5.0 where HDL is supposed to precipitate. The objective of the study was to study the effect of pHs on phosvitin extraction with the aim to develop a method of phosvitin extraction without using organic solvent.

2. Materials and methods

2.1. Chemicals

Sodium chloride, sodium hydroxide, and hydrochloric acid were purchased from Fisher Scientific (Nepean, ON, Canada). Glycine, Precision Plus Protein Standard, sodium dodecyl sulphate (SDS),

* Corresponding author. Tel.: +1 780 492 6885; fax: +1 780 492 4265.
E-mail address: jwu3@ualberta.ca (J. Wu).

http://dx.doi.org/10.1016/j.foodchem.2014.02.120
0308-8146/

and precast gels (10–20% Tris–HCl) were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Phosvitin standard (P1253, from chicken egg yolk) was purchased from Sigma–Aldrich, Ltd. (Oakville, ON, Canada). The deionized distilled water (DD water) used for in the study was produced by a Barnstead water purification system (Thermo Scientific, Asheville, NC, USA).

2.2. Granules preparation

Fresh eggs were purchased from a local supermarket and egg yolk was manually separated from egg white. Granules were prepared according to the protocol of McBee and Cotterill (1979) with slight modifications. Briefly yolk was two times diluted with deionized water instead of 0.17 M NaCl, stirred for one hour at 4 °C, and centrifuged at 10,000*g* for 45 min at 4 °C to obtain the precipitate which was called granules.

2.3. Phosvitin extraction

Ten times weight of 10% NaCl solution was added to dissolve granules under magnetically stirring at 4 °C overnight. The pH was adjusted to 7.25 by adding 3 M NaOH (Castellani et al., 2003). After 24 h dialysis with 4–5 water changes, the solution was centrifuged at 10,000×*g*, 4 °C for 25 min. The supernatant was collected and divided into 9 aliquots. The aliquots were adjusted to pH 8 (the "as is" pH, the pH of the extract without adjustment), 7, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0 and 3.5, respectively. The supernatants of aliquots were collected for further analysis after centrifugation at 10,000*g*, 4 °C for 25 min.

2.4. Anion exchange chromatography

Crude phosvitin extract was prepared at 15 mg/mL with buffer A (0.05 M Tris–HCl at pH 8.0) and filtered through 0.45 μm membrane (Millipore, Billerica, MA, USA) before loading to a HiPrep 16/10 Q FF anion exchange column (GE Healthcare, Piscataway, NJ, USA) coupled with an AKTA explorer 10S system (GE Healthcare, Piscataway, NJ, USA). The column was first equilibrated with buffer A for 5 column volumes (CVs) and then eluted by a gradient from 100% buffer A to 100% buffer B (1.0 M NaCl in buffer A) with 16 CV at a flow rate of 2 mL/min. The elution was monitored at 215 nm. Fractions were collected by a Frac-950 fraction collector (GE Healthcare, Piscataway, NJ, USA) in 16 × 125 mm glass tubes and lyophilized for analysis.

2.5. Gel filtration chromatography

Gel filtration chromatography was carried out using a Waters HPLC system (Waters, Milford, MA, USA) in a TSK-Gel G3000SW×L stainless column (0.78 × 30 cm, Tosoh Bioscience, Inc., South San Francisco, CA, USA) at room temperature which is constantly controlled at ~25 °C. The phosvitin extracts were 1:1 (v/v) diluted with running buffer and filtered with 0.45 μm PVDF filter (13 mm × 0.20 μm, MANDEL, Guelf, ON, Canada). The injection volume was 30 μL. The column was eluted by 0.1 M sodium phosphate buffer (containing 0.2 M NaCl, pH 7.0) at a flow rate of 0.5 mL/min. The elution was monitored at 215 nm. The experiment was controlled by Empower II Software (Waters, Milford, MA, USA). The content of phosvitin in the extracts was calculated from a standard curve prepared by the standard phosvitin at concentrations ranging from 0.1 to 5.0 mg/mL.

2.6. Calculation of phosvitin purity, recovery and yield

Phosvitin purity was calculated based on peak area integration in the HPLC chromatograms. Phosvitin recovery was calculated as percentage based on the weight ratio of amount of phosvitin in the extracts to the total phosvitin in the egg yolk, which was based on 5.3% of egg yolk solids according to Lei and Wu (2012). Phosvitin yield was calculated as the amount of phosvitin in extracts from 100 g yolk solids. The phosvitin content in extracts was calculated by gel filtration HPLC using Sigma's phosvitin standard.

2.7. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE analysis was carried out according to Shapiro, Vinuela, and Maizel (1967) using continuous system (10–20%) gels obtained from Mini-Protean Tetra Cell (Bio-Rad, Hercules, CA, USA). After electrophoresis, the gels were stained with 0.05% Coomassie brilliant blue R-250 in a solution of 0.1 M aluminum nitrate/25% isopropanol/10% acetic acid/1.0% Triton X-100, prepared according to the method of Hegenauer, Ripley, and Nace (1977), and destained in 7% acetic acid solution. Images of gels were taken in an Alphachem SP machine, and molecular weight (MW) was analysed by AlphaEase FC software version 6.0.0 (Alpha Innotech Corporation, Santa Clara, CA, USA) based on molecular weight markers.

2.8. Phosphorus determination

Phosphorus content was determined according to the protocol of the manufacture using malachite green phosphate assay kit provided by Bioassay Systems (Hayward, CA, USA).

2.9. Statistic analysis

Statistical analysis was carried out with using Statistical Analysis System Software, version 9.0 (SAS Institute, Cary, NC). Data were expressed as mean ± S.D. One way analysis of variance (ANOVA) was used to determine differences between means, and Tukey range post-hoc comparisons were used to determine the significant differences. It was considered statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Effects of pH on phosvitin purity and recovery

In this study, granules were obtained after two times water dilution of egg yolk and then mixed with 10% NaCl to disrupt HDL–phosvitin complex. After dialysis, HDL was partially precipitated from the granules solution, whereas phosvitin was presented in the supernatant. However, the purity of phosvitin in the supernatant was relatively low due to the incomplete precipitation of HDL. Effects of the pH on purity and recovery of phosvitin extracts (the supernatant of dialysate) were studied. The phosvitin extracts showed light orange colour at pH around 7.9–8.1. Decreasing pH of the extract did not result in any noticeable change until pH 6.5, where significant precipitate was occurred and the extract became transparent and colourless. Phosvitin extracts prepared at different pHs were analysed by gel filtration chromatography (GFC) in comparison with Sigma's phosvitin standard (Fig. 1a). Phosvitin was known to be heterogeneous (Abe, Itoh, & Adachi, 1982; Mcbee & Cotterill, 1979; Wallace & Morgan, 1986a, 1986b). Three major peaks were obvious in the phosvitin standard: the first fraction (B) with the largest molecular weight (MW) was usually considered as HDL, while the following two fractions were previously identified as β-phosvitin and α-phosvitin, with MW of 190 and 160 kDa, respectively (Abe et al., 1982). The purity of the standard phosvitin (fractions of β- and α-phosvitin) was estimated to be