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Hen egg white fractionation by ion-exchange chromatography

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Abstract

Major hen egg white proteins have been widely studied for their functional properties but these studies still are unable to explain, alone, all of the biological properties of hen egg white. Hence, it is still interesting to produce pure and non-altered proteins to improve our knowledge on the biological properties of hen egg white. Presently, identification and characterization of both bioactive peptides and minor proteins from hen egg white is essential work for progressing in the understanding of hen egg white biological properties. With this objective in mind, a new process for a complete "mucin free" hen egg white fractionation based on ion exchange chromatography is proposed. "Mucin free" egg white is fractionated into six different fractions. Four of them are high-recovery yield purified fractions of lysozyme, ovotransferrin, ovalbumin and flavoprotein. The two other fractions are enriched in recently detected minor proteins in hen egg white.

Keywords: Hen egg white fractionation; Ion exchange chromatography; Electrophoresis; Reverse-phase chromatography; LC–MS–MS; Ovalbumin; Ovotransferrin; Lysozyme; Flavoprotein; Ovalbumin gene X; Ovalbumin gene Y; Ovoglycoprotein; Ovomucoid

1. Introduction

Hen egg white represents an essential ingredient, which has been used for many years by the food industry because of its excellent technological properties. Moreover, hen egg white possesses many biologically active proteins that could offer a better valorisation for hen egg white: lysozyme as anti-microbial, antiviral, antiphlogistic and antalgic agent [1–4], ovotransferrin as an anti-microbial agent [4–6], avidin as a vitamin carrier and antimicrobial agent [7], flavoprotein as a vitamin stabilizer, ovokinin from ovalbumin as an anti-hypertensive agent [8,9] and ovomucin as a source of glycopeptides with antiviral activities, anti-tumor and immunomodulating effects [10–13].

Many procedures for purifying these proteins were developed to study, and in some case to use, the biological activity of hen egg white proteins. Purifications were mostly performed on liquid chromatography because of the absence of protein denaturation and its high selectivity. Presently, lysozyme (3.5% of egg white proteins) and avidin (0.05%) are the main egg white proteins extracted for commercial applications; lysozyme is extracted on an industrial scale by a combination of chromatography and salting out precipitation techniques [14,15] whereas avidin is purified by affinity chromatography [16]. With a pI of 4.5, ovalbumin (54% of egg white proteins) was mainly purified by anion exchange chromatography [17–23]. Ovotransferrin (13%) has been purified either by cation exchange chromatography [24,25] or anion exchange chromatography [26–30]. Ovomucin has been precipitated at low ionic strength and acidic pH. Its purity rate was increased through different water or salt washings [31,32]. Moreover, some authors proposed to hydrolyse ovomucin to increase its solubility [33–37]. Flavoprotein was isolated by different methods involving several separation steps such as salt precipitation, anion exchange chromatography and gel filtration chromatography [38–40].

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A single step procedure based on anion exchange chromatography was also proposed [41].

In spite of having a wide spectrum of action, these proteins are unable to explain, alone, all the biological activities of hen egg white. Identification and characterization of both bioactive peptides from main egg white proteins and minor egg white proteins is an important research axis to understand the biological properties of hen egg white. From a practical point of view, minor egg white protein identification needs the clarification of egg white from its quantitatively major egg white proteins, i.e. ovalbumin and ovotransferrin. Moreover, the characterization of their biological activity needs the extraction of significant quantities of unaltered proteins. The aim of this study was to develop an easy procedure for fractionating the whole egg white for further biological activity studies. Using three successive steps on ion exchange chromatography, egg white was fractionated into six fractions. Four of them are high-recovery yield purified fractions of the well-known egg white proteins: ovalbumin, ovotransferrin, lysozyme and flavoprotein. They represent well-characterized fractions for bioactive peptide identification from major hen egg white. The two other fractions are enriched out in recently detected minor proteins in hen egg white.

2. Experiments

2.1. Preparation of mucin-free egg white solution

Hen eggs were purchased from a local market (10-day-old refrigerated eggs). Albumen from each egg was manually separated from the yolk and pooled. One hundred and ninety milliliters of egg white was diluted with 570 mL of distilled water and the mixture was adjusted to pH 6 with HCl 1 M. The solution was stirred overnight at 2 °C enabling ovomucin precipitation. The mixture developed a white, gelatinous precipitate, which was removed by 5 min centrifugation at $3000 \times g$ (4 °C). Prior to cation exchange chromatography, the mixture was adjusted to pH 8 with NaOH 1 M, and then centrifuged at $24{,}000 \times g$ for 30 min at 4 °C in order to remove insoluble material. The supernatant, called "mucin-free" egg white ("mucin-free" EW) was used in the following steps. "Mucin-free" EW protein concentration was 21 g L^{-1}.

2.2. Preparative ion exchange chromatography

Preparative chromatographies were performed with 100 mL (5 cm × 5 cm i.d.) of S Ceramic Hyper DF (cation exchanger from Biosepra, Cergy Saint-Christophe, France) and 250 mL (12.7 cm × 5 cm i.d.) of Q Sepharose Fast Flow (anion exchanger from Amersham Biosciences, Uppsala, Sweden). Resins were packed into two XK 50/20 columns Pharmacia Biotech AB (Saclay, France). The columns were connected successively to the same Biopilot TM system (Pharmacia Biotech AB) equipped with 280 nm UV, conductivity and pH detectors. Lysozyme and ovotransferrin were extracted in two successive steps by cation exchange chromatography on S Hyper DF. The co-product was then used as starting material for anion exchange chromatography. A fraction (F_A), ovalbumin fraction, B fraction (F_B) and flavoprotein fraction were extracted by single step anion exchange chromatography on Q Sepharose FF. The complete "mucin-free" EW fractionation procedure is described in Fig. 1. All egg white protein fractions were desalted by dialysis against deionised water, freeze-dried and lyophilised.

2.3. Nitrogen determination

Protein quantities were determined using the Kjeldahl method. The conversion coefficient was 6.35.

2.4. Analytical chromatography

Reverse-phase (RP) chromatography was performed on HPLC Spectra Physics (Series P200) on a Vydac C4 214 TP (5 cm × 0.21 cm i.d., particle size 5 μm) column (Touzart et Matignon, Vitry ^{s}/Seine, France). HPLC-grade acetonitrile (ACN) (Carlo Erba, Nanterre, France) containing 0.025% trifluoroacetic acid was used as the eluent under gradient elution conditions. The linear gradient elution increased from 7 to 70% ACN in 17 min, at a flow-rate of 0.8 mL min^{-1}, at room temperature. Detection was carried out at 280 nm with a UV–vis detector (Spectra Physics UV 100). The chromatograms were processed with Azur V2.0 software (Datalys, France).

2.5. Polyacrylamide gel electrophoresis

2.5.1. SDS–PAGE

SDS polyacrylamide gel electrophoresis was conducted using 12.5% acrylamide separating gel and 4% stacking gel containing 0.1% SDS, with a Biorad Mini Protean II system [42]. SDS-protein samples were heated at 95 °C for 3 min. Electrophoresis was carried out at 75 V in stacking gel and 150 V in separating gel for 1 h30 using an electrophoretic buffer of Tris-Glycine containing 0.1% SDS. The gel was stained with 0.05% Coomassie Blue R250, 49.95% water, 40% ethanol and 10% acetic acid for 1 h and subsequently destained with 50% water, 40% ethanol and 10% acetic acid.

2.5.2. Isoelectric focusing

Conventional isoelectric focusing (IEF) in ampholyte carrier buffers was performed using 7.5% acrylamide, 10% glycerol, and 3% ampholytes. Samples were diluted in 50% glycerol, 2% ampholyte and 2% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS). The cathode solution contained 20 mM lysine/20 mM arginine and the anode solution was 10 mM H_3PO_4. Migration was performed using a Biorad Mini Protean II system and running conditions were 1 h at 100 V, 1 h at 250 V and 30 min at 500 V.