Advance Journal of Food Science and Technology 6(4): 517-519, 2014
DOI:10.19026/ajfst.6.64
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: January 07, 2014 Accepted: January 24, 2014 Published: April 10, 2014

Research Article

Effective and Low-cost Purification of Lysozyme by Combination of Conventional Processes

[1]Yunfeng Ma, [2]Kuiling Xue, [1]Li Li, [1]Shibin He and [1]Fude Shang
[1]College of Life Science, Henan University,
[2]Hospital of Henan University, Kaifeng, 475004, P.R. China

Abstract: In this study, a simple method to purify lysozyme was introduced, based on combination of conventional processes, including thermal treatment is electric point precipitation and gel chromatography. The sample was purified well by compared with lysozyme standard and no other proteins appeared in Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Keywords: Gel chromatography, isoelectric point precipitation, lysozyme, purification, thermal treatment

INTRODUCTION

Lysozyme is a soluble enzyme consisting of a single polypeptide chain containing 129 amino acids with the ability to break down bacterial cell walls. It has a molecular weight of ~14.3 kDa and an isoelectric point value of 10.7. As one of the potential bio-preservatives, lysozyme has been received attention from researchers in the food industry. It also has many applications in the current biopharmaceutical industry, including medicinal use, antibacterial, hemostasis, the reduction of swelling and inflammation and an important soluble agent for cell-free extracts in the fermentation industry. Chicken egg white contains the highest amount of lysozyme, making it the best source of purified lysozyme (Chiu *et al*., 2012; Kim *et al*., 2012).

Besides the classical methods, new technologies have been adopted for lysozyme purification in recent years. Functionalized Fe_3O_4 nanoparticles conjugated with Polyethylene Glycol (PEG) and Carboxymethyl Chitosan (CM-CTS) were developed and used as a novel magnetic absorbing carrier for the separation and purification of lysozyme from the aqueous solution and chicken egg white respectively (Sun *et al*., 2011). Affinity chromatography strategies for lysozyme purification were reported (Li *et al*., 2009; Han *et al*., 2012). Ion-exchange nanofibrous membrane for direct purification of lysozyme from chicken egg white was reported (Chiu *et al*., 2012). Aqueous two-phase extraction of lysozyme was applied from crude hen egg white using response surface methodology (Lu *et al*., 2012). These technologies are generally complicated, or expensive for large-scale production.

Egg white is composed of 88.5% water, 10.5% protein, 0.5% carbohydrate and the remainder of other solutes and is relatively homogeneous, containing very little particulate material and most of the solutes are proteins, among which most components have neutral pI values and only lysozyme (*Mr* 14,300) and avid in (*Mr* 68,300) have alkaline pI values (Stevens, 1991). Isoelectric point precipitation and gel chromatography were used for purification of proteins and the procedure was worked out for large scale production because the operations involved are simple and cheap and a high purification could be obtained (Steindl *et al*., 1987).

Changes in physical and functional properties of lysozyme due to heat have been reported. Lysozyme was not extensively damaged by heating egg white several minutes at temperatures up to 62°C if the pH was less than 7.5 and heat resistance of lysozymein phosphate buffer was 50 times higher than in egg white (Cunningham and Lineweaver, 1965). Lysozyme recoveries increased with increasing ascorbic acid concentration (0-1.0%) in the egg white samples and the residual ascorbic acid in the lysozyme thus obtained should be removed to protect lysozyme from being denatured and so lysozyme heated with ascorbic acid was stable at 70°C for 1-10 min and (Chang *et al*., 2000). Then other proteins will be removed from the solution by heat treatment.

This study introduces a simple method to purify lysozyme based on a combination of conventional processes, such as thermal treatment, isoelectric point precipitation and gel chromatography.

MATERIALS AND METHODS

Chemicals and enzyme: Sephadex G-100 and blue dextran 2000 were purchased from Pharmacia Company, USA. Lysozyme standard (twice crystalline)

Corresponding Author: Fude Shang, College of Life Science, Henan University, Kaifeng, 475004, P.R. China, Tel.: 13839993933

was purchased from Sinopharm Chemical Reagent Co. Ltd., China. All the other chemicals are of analytical grade.

Egg white used in the following experiments was obtained from fresh shell eggs broken out in the laboratory.

Methods:

Chicken egg white treatment: According to salt in action of protein, lower concentration of salt solution promotes dissolution of proteins. Chicken egg white was added with 5-fold volume 1% NaCl solution. The mixture solution was heated at 70°C for 10 min with 1.0% ascorbic acid under pH 6.0. The composition of precipitate was removed by centrifugation and extraction filtration and the supernatant was concentrated by centrifugal ultra filtration.

Purification of lysozyme: The concentrated solution was added to 2-fold volume 0.1 mol/L Na_2CO_3-$NaHCO_3$ buffer (pH10.8). After centrifugation, the composition of the precipitate (crude product of lysozyme) was obtained.

Lysozyme standard and blue dextran 2000 were dissolved by 0.2 mol/L phosphate buffer solution (PBS, pH 7.2). The Lysozyme solution and blue dextran 2000 solution were separated through Sephadex G-100 gel filtration chromatography and the elution volumes were determined based on nucleic acid/protein UV analyzer. The crude product of lysozyme was dissolved by 0.2 mol/L PBS (pH 7.2) and the crude lysozyme solution was separated through gel filtration chromatography. The sample elution solution was collected according to lysozyme standard elution volume. The purification process was showed as Fig. 1.

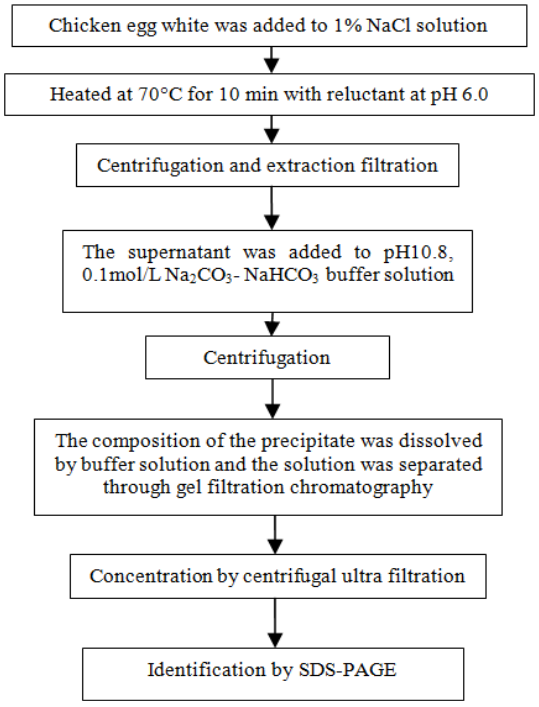

Fig. 1: Purification process of lysozyme

Identification and determination of lysozyme: The sample elution solution was concentrated by centrifugal ultra filtration. The concentration solution was identified with lysozyme standard by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

RESULTS AND DISCUSSION

The lysozyme standard and blue dextran 2000 solution were separated through Sephadex G-100 gel filtration chromatography and the elution volumes were determined as showed in Fig. 2. The crude lysozyme solution was separated through gel filtration chromatography as showed in Fig. 3.

The absorption peak of sample was wider than lysozyme standard, perhaps due to the larger sample

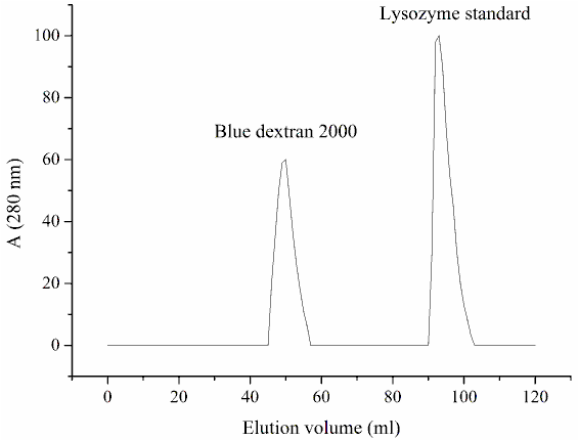

Fig. 2: The lysozyme standard and blue dextran 2000 solution were separated through sephadex G-100 gel filtration chromatography

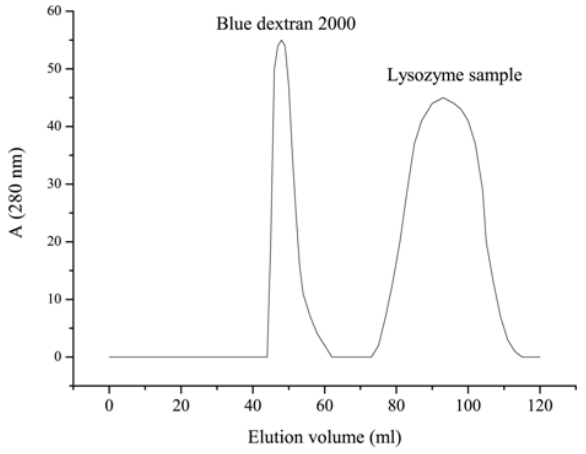

Fig. 3: The crude lysozyme solution was separated through gel filtration chromatography

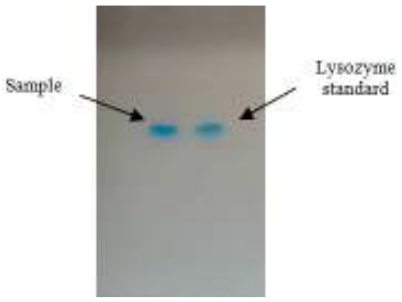

Fig. 4: The sample was identified by SDS-PAGE

volume or the effect of other proteins. In order to collect the target protein and eliminate interference of other proteins, the sample elution solution was collected according to lysozyme standard elution volume. The concentration solution was identified with lysozyme standard by SDS-PAGE as showed in Fig. 4. The sample was purified well by compared with lysozyme standard and no other proteins appeared in SDS-PAGE.

Heat treatment, isoelectric point precipitation and gel chromatography are all classical and low-cost methods. Therefore, the combination of heat treatment, isoelectric point precipitation and gel chromatography is probably the effective and low-cost purification.

ACKNOWLEDGMENT

The authors thank the Henan University Foundation (HDXJJG2013-131), China Postdoctoral Science Foundation (2013M5311670), Henan Provincial Postdoctoral Science Foundation (2011019), the Scientific Research Fund of Henan Provincial Education Department (13A416074). The authors also thank Li Nan, Qian Hui for the preparation wok of this study.

REFERENCES

Chang, H., C. Yang and Y. Chang, 2000. Rapid separation of lysozyme from chicken egg white by reductants and thermal treatment. J. Agr. Food Chem., 48: 161-164.

Chiu, H., J. Lin, T. Cheng, S. Chou and C. Huang, 2012. Direct purification of lysozyme from chicken egg white using weak acidic polyacrylonitrile nanofiber-based membranes. J. Appl. Polym. Sci., 125: 616-621.

Cunningham, F.E. and H. Lineweaver, 1965. Stabilization of egg-white proteins to pasteurizing temperatures above 60 °C. Food Technol., 19: 136-141.

Han, B., C. Zhao, J. Yin and H. Wang, 2012. High performance aptamer affinity chromatography for single-step selective extraction and screening of basic protein lysozyme. J. Chromatogr. B, 903: 112-117.

Kim, M., M. Park and Y. Jeong, 2012. Purification and characterization of lysozyme from filipino venus, *Ruditapes philippinarum*. Food Sci. Biotechnol., 21(5): 1463-1468.

Li, Q., Q. Cao, Z. Li, N. Li, K. Li and F. Liu, 2009. Highly efficient and low-cost purification of lysozyme: A novel tris (hydroxymethyl) aminomethane immobilized affinity column. J. Chromatogr. B, 877: 594-598.

Lu, Y., W. Lu, W. Wang, Q. Guo and Y. Yang, 2012. The optimization of aqueous two-phase extraction of lysozyme from crude hen egg white using response surface methodology. J. Chem. Technol. Biot., 88: 415-421.

Steindl, F., A. Jungbauer, E. Wenisch, G. Himmler and H. Katinger, 1987. Isoelectric precipitation and gel chromatography for purification of monoclonal IgM. Enzyme Microb. Tech., 9: 361-364.

Stevens, L., 1991. Egg white proteins. Comp. Biochem. Phys. B, 100(1): 1-9.

Sun, J., Y. Su, S. Rao and Y. Yang, 2011. Separation of lysozyme using superparamagnetic carboxymethyl chitosan nanoparticles. J. Chromatogr. B, 879: 2194-2200.