Isolation and purification of wheat germ agglutinin and analysis of its properties

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Abstract. In this paper, the wheat germ agglutinin was isolated and purified by affinity chromatography of chicken ovomucoid as ligand. The physicochemical properties were analyzed. The chicken ovomucoid was isolated from egg white and conjugated to affinity chromatography column agarose gel to prepare affinity adsorbent. The crude extract of wheat germ was freeze-dried by affinity chromatography. The physicochemical properties were analyzed by SDS-polyacrylamide gel electrophoresis and isoelectric focusing electrophoresis. And the relative molecular mass and isoelectric point of wheat germ agglutinin were obtained, and the high efficiency of purification of wheat germ agglutinin was proved by affinity chromatography.

1. Introduction

Wheat Germ Agglutinin (WGA) is a protein, which is specifically, reversibly binding to carbohydrates [1-3]. It can be used as a potent agent for the separation, purification and identification of biomolecules, which can be stably combined with other compounds present in or in the cell and can be easily dissociated by sugar [4-6]. Wheat germ agglutinin can react with adipocyte, which has the role of insulin. It can activate glucose oxidase and reduce blood sugar levels in the human body. Under appropriate conditions, WGA can significantly promote human blood B lymphocytes in vitro synthesis and secretion of immune globulin, which plays an important role in human production and life [7-10].

Affinity chromatography has been widely used in the separation and purification of biomolecules [11], such as binding proteins, enzymes, inhibitors, antigens, antibodies, hormones, hormone receptors, glycoproteins, nucleic acids and polysaccharides; It also could be used in separation of cells, organelles, viruses and so on. In recent decades, affinity chromatography technology has developed rapidly. For those biomolecules with long separation process, low concentration, impurity and difficult to separate by conventional methods, affinity chromatography shows its unique superiority. Affinity chromatography is the best way in the efficiency of separation and purification [12].

Affinity chromatography requires the selection of a durable, non-specific, less adsorbable, water-insoluble carrier and is covalently coupled to the ligand under mild conditions without affecting the original biological properties of the ligand [5, 11]. The most commonly used carrier is an agarose gel (Sepharose-4B). It has the advantages of high mechanical strength, good permeability and non-specific adsorption.

2. Experiment

2.1 The Isolation and Purification of WGA

2.1.1 Preparation of Affinity Adsorbents-Isolation and Purification of Ovomucin [13-15]

(1) Preparation of Crude Ovomucin

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Take 50ml chicken egg white, added with an equal volume of 10%, pH1.15 trichloroacetic acid solution, then it appears a large number of white precipitate, after stirring evenly, we measured pH value, and it is adjusted to pH 3.5 ± 0.2 using 5mol/L NaOH or 5mol/L HCl. After adjusting the pH value, let it stand for more than 4h at room temperature. After the protein was completely precipitated, it was centrifuged at 3000r/min for 10min. The supernatant was filtered off with a filter paper to remove the lipid and insoluble matter from the supernatant. The filtrate was collected and the pH of the filtrate was checked and adjusted to a pH of 3.5 ± 0.2 with 5 mol/L NaOH or 5 mol/L HCl. Placed in an ice bath for a moment, slowly add 3 times the volume of pre-cooled acetone, stir with a glass rod, and covered with plastic film to prevent acetone evaporation, at 4 ℃ for more than 4h (overnight). After the chicken ovomucin was completely precipitated, carefully discard some of the supernatant, the remaining part of the precipitation solution was all transferred to 50ml centrifugal cup, cover or plastic film sealed to 3000r/min centrifugal 15min. The supernatant was discarded and the bottom pellet of the centrifugal cup was evacuated in a vacuum drier to remove residual acetone and then dissolved in 20 ml of distilled water. If the dissolved solution is cloudy, insoluble matter can be used to remove the filter paper. The collected filtrate was desalted by Sephadex G-25 gel column.

(2) The Desalting of Sephadex G-25 Gel Chromatography

The resulting filtrate was added to a Sephadex G-25 gel column (20 mm x 400 mm) and the first elution peak was collected. The column information is shown in Table 1.

(3) DEAE-cellulose Ion Exchange Chromatography

The DEAE-cellulose ion exchange column (21 mm x 300 mm) was added to the solution obtained above and the second elution peak was collected in sections. The molish method was used to determine the sample to be left after the determination. The column information is shown in Table 1.

Table 1. Purification Chromatography of Ovomucin

Type	Sephadex G-25	DEAE-cellulose Ion Exchange
Model (mm×mm)	20×400	21×300
Length (mm)	292	105
Buffer	0.02mol/L, pH6.5 Phosphate buffer	0.02mol/L, pH6.5 Phosphate buffer
Eluant	0.02mol/L, pH6.5 Phosphate buffer	0.02mol/L, pH6.5 Phosphate buffer with 0.3mol/L NaCl

2.1.2 Crude Purification of WGA 50g of wheat germ was added to 250ml water, 40 ℃ water bath, stirring constantly. After 4 h leaching, the mixture was filtered with 4 layers of gauze and the filtrate was centrifuged at 4000 r/min for 20 min. Discard the precipitate, the supernatant filter was adjusting the pH value to 5.0 ~ 5.5, 65 ℃ water bath 3 ~ 5min, which appeared a white flocculent precipitate. 3500r/min centrifugal 15min, discard the precipitation, and then adjust the supernatant pH to 7.5, the crude WGA was prepared. A small amount of it was measured coagulation activity.

2.1.3 Isolation and Purification of WGA by Affinity Chromatography The crude extracted WGA was chromatographed on an affinity chromatography column (10 mm*150 mm) using 0.5 mol/L KCl-0.05 mol/L LC-2 mol/L Tris-HCl buffer (pH 7.8) and 0.5 mol/L KCl (Flow rate is 0.2 ~ 0.4ml/min), with agglutination reaction detection, collecting active elution peak, the collection solution was dialyzed to be unable to be detected with Cl-, and then it concentrated, freeze-dried. The chromatography used Ø12 × 200mm chromatography column, plastic length is 112mm.

2.2 The Measurement of Coagulation Activity of WGA

2 ml of fresh red blood cells of type A was washed several times with 0.02 mol/L phosphate buffer (pH 6.5)-0.15 mol/L NaCl solution, and diluted to 100 ml to prepare 2% erythrocyte suspension. And then diluted to the original suspension with 1, 21, 22……29 times. Take each take 200μl of crude WGA and purified WGA (protein concentration is 2mg/ml), respectively, with different concentrations of equal volume of red blood cell suspension mixture, a little oscillation, standing 10min to find out the results.

2.3 The Determination of Molecular Weight of WGA

The molecular weight of WGA was determined by SDS-PAGE vertical plate type discontinuous system gel electrophoresis. The concentration of the separated gel was 12% and the concentration of the concentrate was 3%.

Take the low molecular weight standard protein as a reference marker, the sample and the amount of sample see Table 2.

Table 2. The Loading Amount Form of SDS-PAGE

Sample	WGA			Empty	Marker	Empty	ovalbumin	ovomucin	
Track	1	2	3	4	5	6	7	8	9
Loading amount/μl	20	15	10	0	12	0	10	20	20

2.4 Determination of WGA Isoelectric Point

The WGA isoelectric point was determined by polyacrylamide gel plate isoelectric focusing. The thickness of the sheet is 0.3mm, the gel concentration is 7.5%, and the 7.5% carrier ampholyte (pH 3.5 ~ 10) is contained. Take the amphoteric electrolyte as a reference marker, the sample and the amount of sample are shown in Table 3.

Table 3. The results of loading of isoelectric focusing electrophoresis

Sample	WGA			Marker	ovomucin
Track	1	2	3	4	5	6
Loading amount/mm	5	10	10	5	5	5

3. Results and Analysis

The results of the three chromatograms are shown in Figure 1. Fig. 1(a) is the results of Sephadex G-25 gel chromatography, Fig. 1(b) is DEAE-cellulose ion exchange chromatography, Fig. 1(c) is the result of affinity chromatography.

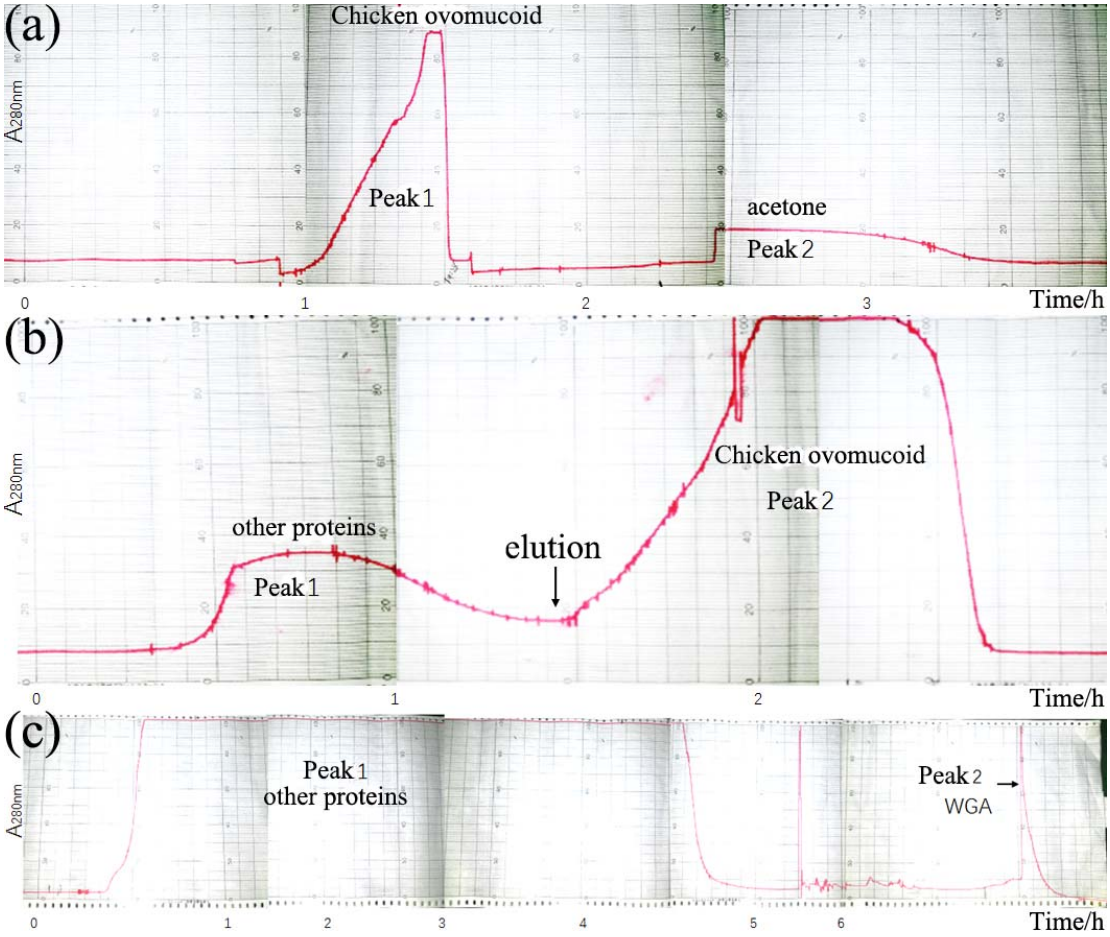


Figure 1. Three chromatographic results of chicken ovomucin. (a) Sephadex G-25 gel column chromatography (b) DEAE-cellulose ion exchange (c) the separation map of affinity chromatography column

As can be seen from Fig. 1(a), the maximum value of the peak 1 exceeds the machine limit, and the peak 1 changes the detector sensitivity, so the size of the peak is not used as a reference. It can be seen from Figure 1(b) that the maximum value of the peak 2 exceeds the machine limit, so the size of the peak is not a reference. The peak 2 is detected by molish with a purple ring. For Figure 1(c), the maximum value of the peak 1 exceeds the machine limit, so the size of the peak is not a reference.

The standard bovine serum albumin concentration as the abscissa, with its 280nm UV spectrophotometry for the vertical plot. The results of the three-stage sample recovery analysis are shown in Fig. 2.

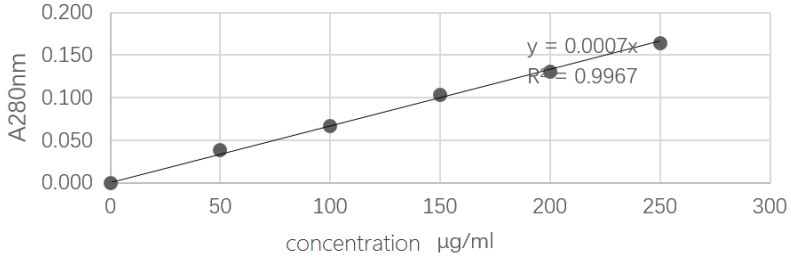


Figure 2. Bovine serum albumin-A280nm standard curve

The results of the recovery analysis are shown in Table 4.

Table 4. The recovery analysis of samples in three types

	G-25	DEAE	Sepharose 4B
Loading Volume/ml	17.2	19	82
Dilution ratio	\	20	500
A_{280nm}	\	0.1027	0.0637
Collected Volume/ml	23	20	10
Dilution ratio	20	16	4
A_{280nm}	0.1027	0.0913	0.082

Note: A280nm in the table are the average of three repeated sets.

3.1 The Determination of Agglutination Activity of WGA

The results of WGA agglutination activity test are shown in Table 5.

Table 5 . The purified WGA agglutination activity

Reagent	Dilution ratio of erythrocyte									
	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
Crude WGA	+	+	+	+	+	+	+	+	+	-
Purified WGA	+	+	+	+	+	+	+	+	+	+

Note: +: agglutination -: no agglutination.

3.2 The Determination of Molecular Weight of WGA

The results of SDS-PAGE determination are shown in Fig. 3.

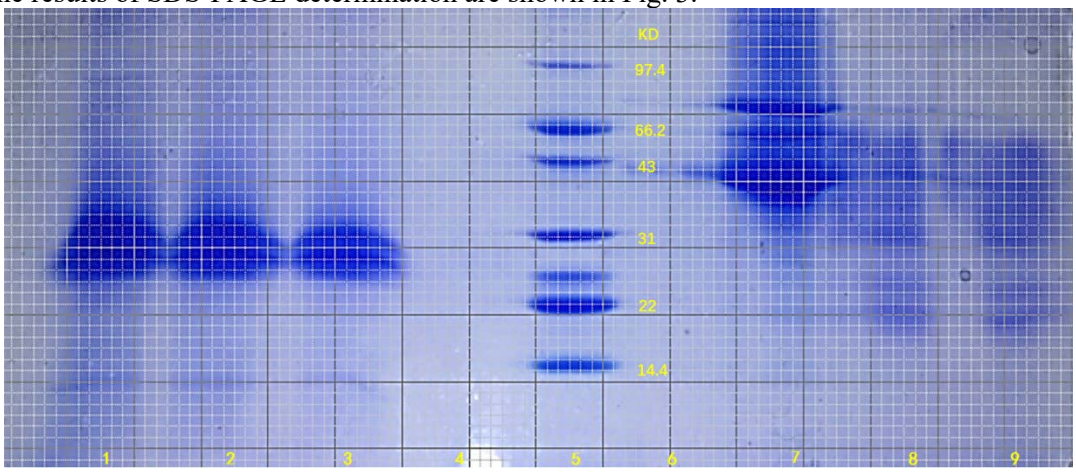


Figure 3 . The results of SDS-PAGE

Where, from left to right, there are 1 to 9 tracks, 4, 10 tracks are empty, 5 track is marker.

Table 6. Bandage mobility and molecular weight of each track

Item	**bandage**	**Distance (cm)**	**Relative mobility**	**Molecular weight (KD)**	**logarithm of molecular weight**
Marker	1	1.20	0.150	97.40	1.989
	2	2.36	0.295	66.20	1.821
	3	2.80	0.350	43.00	1.633
	4	4.14	0.518	31.00	1.491
	5	5.36	0.670	22.00	1.342
	6	6.20	0.775	14.40	1.158

Track 1-3	1	4.40	0.550	28.65	1.457
	2	4.60	0.575	26.62	1.425
	3	6.40	0.800	13.74	1.138
Track 7	1	2.00	0.250	69.20	1.840
	2	2.40	0.300	59.74	1.776
	3	3.40	0.425	41.37	1.617

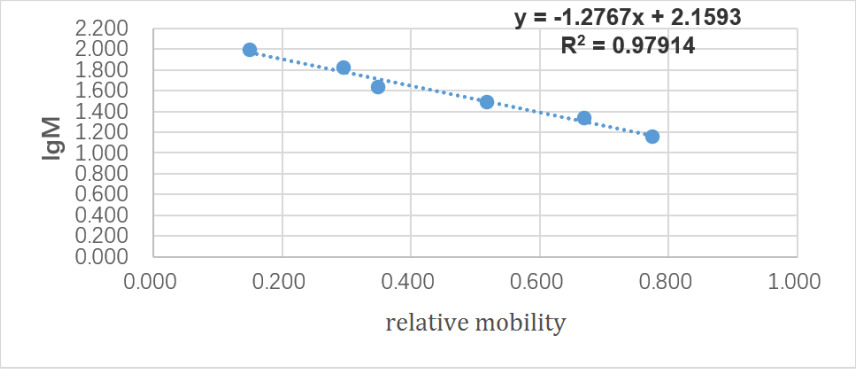


Figure 4. The logarithmic standard curve of relative mobility - molecular weight

3.3 The Determination of pI of WGA

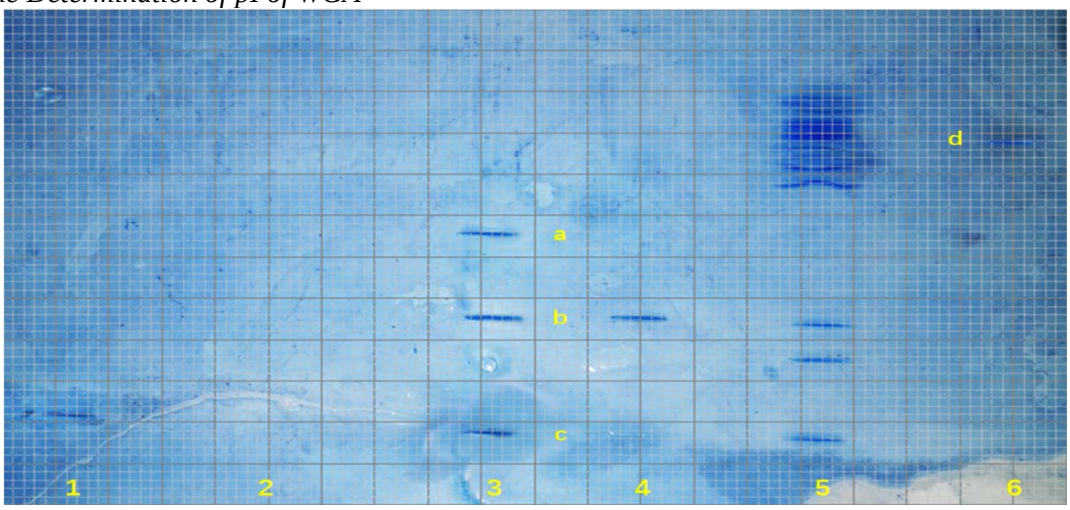


Figure 5. The results of isoelectric focusing electrophoresis

Where, tracks 1 to 4 are WGA, for the points of 1 and 2, the mirror paper is not completely soaked, the results do not make sense.

Compared with table 3, it can be concluded: the 5 track is the amphoteric electrolyte, that is, marker. According to the band, we can draw the pH gradient of the electrophoresis, as shown in Figure 6.

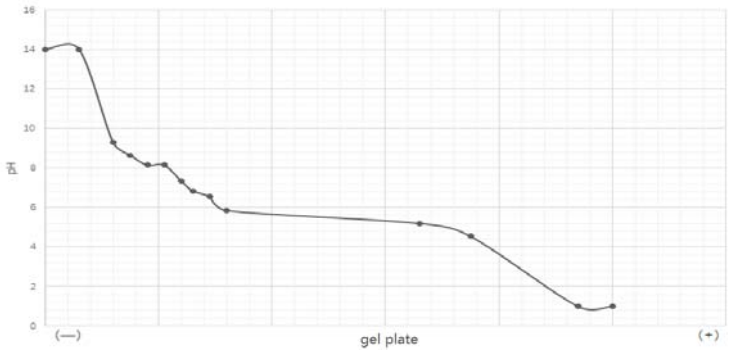


Figure 6. The graph of pH gradient

There are three bands in the track 3 of the WGA sample, ie a, b and c in the figure, and there is one band in track 4, ie b in the figure. Then according to Figure 5 and 6, where pIa = 5.60, pIb = 5.22, pIc = 1.30. There is one band in track 6, that is, the figure d. We can figure out, pId = 8.00.

4. Conclusion

In this paper, wheat germ agglutinin was isolated and purified by affinity chromatography with chicken ovomucin as ligand, and then purified by freeze drying to obtain WGA. The molecular weight and isoelectric point were determined by SDS-polyacrylamide gel electrophoresis and isoelectric focusing electrophoresis. However, both WGA bands had miscellaneous bands, and for the molecular weight and isoelectric point, there is a certain gap between the measured value and true value. This may be because the freeze-dried powder is old, which contains impurities or protein that has been degeneration. In this paper, it is proved that affinity chromatography has a unique superiority in the purification of wheat germ agglutinin.

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