JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation and Characterization of Immunoglobulin in Yolk (IgY) Specific against Hen Egg White Lysozyme by Immunoaffinity Chromatography

CHAO-CHENG CHEN,† YANN-YING TU,† TZY-LI CHEN,‡ AND HUNG-MIN CHANG*,‡

Department of Food Health, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan, and Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 106-17, Taiwan

Six hens were intramuscularly (im) immunized once a week for 3 weeks using chicken egg white lysozyme (LS) as antigen. Antibody (immunoglobulin in yolk, IgY) ELISA values of 10^3-fold diluted yolk were almost as high as 1.879 in the sixth week and maintained a value of 0.756 in the eighth week after the initial immunization treatment. The purification efficiency (specific activity of purified IgY against LS/specific activity of antibody in yolk against LS) of IgY specific against LS isolated by laboratory-prepared LS-bound (IgY-) Sepharose 4 Fast Flow immunoaffinity column was ~3380. By applying various amounts (0−22 mg) of the thusly obtained IgY specific against LS to the immunoaffinity column, the binding capacity (q_m) and dissociation constant (K_d, M^{-1}) of such immunoaffinity gel for IgY against LS were found to be 0.68 mg of IgY/mL of wet gel (0.54 mg of IgY/mg of LS) and 7.13×10^{-6} M, respectively, as determined by Langmuir-type adsorption isotherms.

KEYWORDS: Lysozyme; immunoglobulin in yolk; immunoaffinity chromatography; binding capacity; dissociation constant

INTRODUCTION

Lysozyme (LS) (mucopeptide *N*-acetylmuramoylhydrolase, EC 3.2.1.17) is a unique substance as it is able to lyse certain bacteria by hydrolyzing the β-linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine of mucopolysaccharides in the bacterial cell wall. Breakage of the β-(1−4)-glycosidic linkage leads to the decrease in turbidity of *Micrococcus lysodeikticus* suspension and to the methodology of the lysozyme activity assay. All of the properties including molecular weight, isoelectric point, and antibacterial functions of LS have long been known, and LS has been considered to have a high potential as a future food preservative.

The conventional method of preparing commercial LS is direct crystallization from the white of a hen's egg in the presence of 5% NaCl at pH 9.5. However, this is a lengthy process and usually requires a week until the enzyme is efficiently precipitated. Other methods such as ion-exchange chromatography (*1*, *2*), gel permeation chromatography (*3*), deacetylated chitin affinity chromatography, ascorbic acid and thermal treatment method (*4*), and anionic polysaccharide method (*5*) as well as membrane separation have been investigated and reported. However, lysozyme products thus obtained usually need subsequent purification to obtain products for medical use. Although these methods have proved to be useful, they have limitations such as low purity and/or low recovery of LS production. Thus, a simple, efficient, and rapid methodology for the production and purification of LS from egg white is needed.

Immunoaffinity chromatography is based on the specific affinity between antigen and the corresponding antibody. It has long been used to separate and/or isolate antigen or antibody in liquid samples by immobilizing the corresponding antibody or antigen on the gel matrices such as agarose and Sepharose (*6*, *7*). For the preparation of antibody for this purpose, use of monoclonal antibody specific against certain antigen is costly, lengthy, and, most importantly, difficult to achieve (*8*). Therefore, its industrial use appears to be impractical, whereas improving the use of polyclonal antibody is more useful and convincing. Recently, Tu et al. (*7*) prepared lactoferrin (LF) using anti-LF antibody (IgY) bound immunoaffinity chromatography and indicated that the purification efficiency of lactoferrin in milk by such immunoaffinity column was ~2420. However, affinity between antigen and the corresponding antibody is dependent on the chemical properties of antigen used to produce antibody. Therefore, more basic knowledge about such polyclonal antibody bound immunoaffinity chromatography was desired before such chromatography become practical.

The advantages of immunoglobulin in yolk (IgY) over serum immunoglobulin G (IgG) including its high immunoglobulin

* Author to whom correspondence should be addressed (telephone +886-2-2363-0231, ext 2776; fax +886-2-2362-0849; e-mail Changhm@ccms.ntu.edu.tw).
† Chia-Nan University of Pharmacy and Science.
‡ National Taiwan University.

10.1021/jf011567h CCC: $22.00
Published on Web 08/15/2002

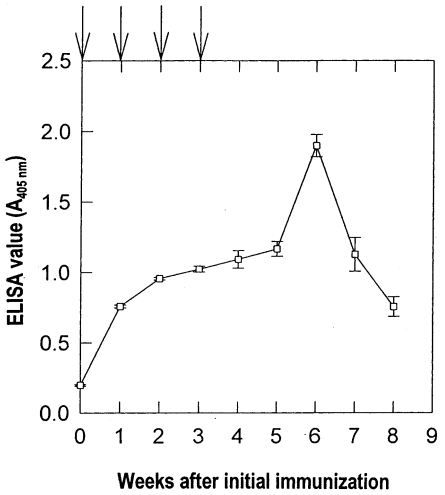


Figure 1. Changes in ELISA value of hen yolk during the immunization period. Six 160-day-old hens were intramuscularly immunized with lysozyme once per week for 3 weeks after initial immunization. Egg yolks from immunized hens were 10^3-fold diluted prior to ELISA assay. Each value is the average of three determinations. Arrows indicate the immunization schedule.

content (~15 mg/mL) in the egg yolk (*4*), the large quantities of chicken eggs available, and, most importantly, the ease of IgY collection without eventual sacrifice of the animals make IgY a potential source for use in immunochemical assays (*6*, *7*). Therefore, a trial on the use of IgY, instead of IgG, for affinity chromatography was done in the present study. First, to separate and recover LS from the white of a hen's egg, polyclonal antibodies specific against LS were prepared, isolated, and then immobilized on the matrix for the immunoaffinity chromatography. The procedure undertaken involved two phases. Second, the specific activities (ELISA value = 405 nm/mg protein) and purification efficiency of anti-LS antibody from the yolk purified by LS-Sepharose 4 Fast Flow immunoaffinity chromatography were determined. Finally, binding parameters such as dissociation constant and binding capacity of LS-Sepharose 4 Fast Flow immunoaffinity gel for IgY specific against LS were determined.

MATERIALS AND METHODS

Immunization of Hens. Antigens were prepared according to the method described by Tsai and Cousin (*9*) and Buchta (*10*). One milliliter of the hen egg white LS (L-6876, Sigma Chemical Co., St. Louis, MO) (52000 units/mg of protein) (500 μg of protein/mL of sterilized 0.02 M phosphate buffer saline, PBS, pH 7.0) was mixed well in a syringe with 3 mL of Freund's complete adjuvant to form an antigen emulsion for the initial immunization.

Antigens for the second and further immunizations were prepared in the same way as the initial immunization, the only difference being that Freund's incomplete adjuvant was used. Six 160-day-old hens were intramuscularly (im) injected on four spots in each leg (0.5 mL of antigen emulsion/leg). After the initial immunization, immunizations were repeated once a week for 3 weeks (as indicated in **Figure 1**). Eggs were sampled before each immunization for the determination of changes in binding activities of IgY to LS using the enzyme-linked immunosorbent assay (ELISA) analysis described below. Yolks from eggs collected over a 3 and 8 week period after the initial immunization were well mixed and kept frozen (−20 °C) prior to the antibody purification procedure as described below.

Partial Purification of IgY. Partial purification of IgY was conducted according to the method described by Chang et al. (*4*) using pectin (Sigma) with a degree of esterification of 64%. The collected supernatants (crude IgY) from the yolk−pectin mixture were filtered to obtain antibody recovery and purity rates of up to 73% (*4*). The crude IgY (5 mL) was subsequently applied to a Sepharose 4 Fast Flow immunoaffinity column, as described below, for the isolation of IgY specific against LS.

Lysozyme (LS)-Bound Sepharose 4 Fast Flow Immunoaffinity Chromatography. The CNBr-activated Sepharose 4 Fast Flow gel (4 g) (Pharmacia, Uppsala, Sweden) was washed five times with 1 mM HCl (200 mL/g gel) and then mixed well with 20 mg of LS in 30 mL of coupling buffer solution (0.5 M NaCl/0.1 M $NaHCO_3$, pH 8.3) (*6*, *7*, *11*). After an incubation period of 1 h at room temperature with slight stirring, the Sepharose 4 Fast Flow gel was washed with f gel volumes of coupling buffer and was resuspended in a 0.1 M Tris-HCl buffer (pH 8.0) for 2 h to block the residual active groups in the gel. The gel thus obtained was washed three times with 5 gel volumes of 0.5 M NaCl/0.1 M acetate buffer (pH 4.0) and of 0.5 M NaCl/0.1 M Tris-HCl (pH 8.0). Finally, LS-Sepharose 4 Fast Flow gel was packed in a column (20.0 cm in length × 1.0 cm in diameter) and was equilibrated with at least 5 column volumes of 0.25 M NaCl/0.02 M phosphate−citrate buffer (pH 7.2) in a chilled room (~5 °C). The LS bound to gel was determined to be 19.7 mg by subtracting the quantity of LS, determined by protein quantification as described below, in the coupling buffer from the original 20 mg of LS in the coupling buffer.

Antibodies specific against LS were prepared according to the method described by Tu et al. (*6*). Five milliliters of crude IgY was applied to the above-mentioned LS-Sepharose 4 Fast Flow column. The column was eluted with 0.5 M NaCl/0.1 M phosphate−citrate buffer (pH 2.8) (elution buffer) after being washed with ~2 column volumes of 0.25 M NaCl/0.02 M phosphate−citrate (pH 7.2) (until the absorbance of effluent at 280 nm was nearly zero). Absorbance at 280 nm was monitored, and the effluent was collected (2.0 mL/fraction) in test tubes to which had been previously added 120 μL of 1 M Tris-base (pH 9.0) to neutralize the pH to ~7. Fractions corresponding to antibody were pooled as determined by the ELISA analysis. Concentration and desalting of the pooled antibody fractions were simultaneously conducted with a membrane (Millipore ultra-free 15, Bio-Rad, Hercules, CA) by centrifugation (5000*g*, 4 °C, 30 min), followed by lyophilization. Antibody samples were kept at −20 °C for the binding capacity and dissociation constant determinations of LS-Sepharose 4 Fast Flow immunoaffinity gel.

Determination of Protein. Protein content was determined according to the method described by Bradford (*12*). The microtiter plate protocol of the Bio-Rad protein assay was conducted using chicken serum IgG (Sigma) as the standard solution (50−500 μg/mL) for the standard curve (*13*). Three samples were each tested in duplicate.

Determination of Antibody. ELISA and antibody titer (*10*) were performed to determine the changes in binding activity of IgY to LS during the immunization period. LS solution (50 μg/mL carbonate buffer, pH 9.6) at 100 mL/well was used to coat an ELISA plate and incubated it at 4 ± 1 °C overnight. After four washings with PBS−0.05% Tween-20 (PBS-Tween), 100 μL of 10^3-fold diluted yolk with 0.5% gelatin−PBS was added to each well and incubated at 37 ± 1 °C for 90 min. Wells were washed again six times with PBS-Tween. Then 100 μL of 2000-fold diluted alkali phosphatase-conjugated rabbit anti-chicken IgG (Sigma) (0.5 μL/mL) for IgY was added to each well. Incubation occurred at 37 ± 1 °C for 90 min. Each well was again washed with PBS-Tween six times; then to each of them was added 100 μL of freshly prepared substrate solution (0.5 mg of *p*- nitrophenyl phosphate disodium salt/ mL, in 0.1 M diethanolamine buffer, pH 9.8). The plates were allowed to develop for 1 h at 37 ± 1 °C. The color developed was read at 405 nm with an ELISA reader (Multiskan Ex. Labsystem Co., Helsinki, Finland). Absorbance of sample was at least 3 times higher than that of control. Reverse value of the maximal dilution fold of yolk was used as antibody titer (*10*). Three samples were tested, each in duplicate. Yolks and crude IgY solution obtained from eggs laid by hens before the initial immunization were used as controls. The specific activity and purification efficiency were computed using the following equations (*6*, *7*, *10*). Specific activity = ELISA value (absorbance at 405 nm)/mg of protein; purification efficiency = specific activity of anti-LS antibody/specific activity of yolk against LS.

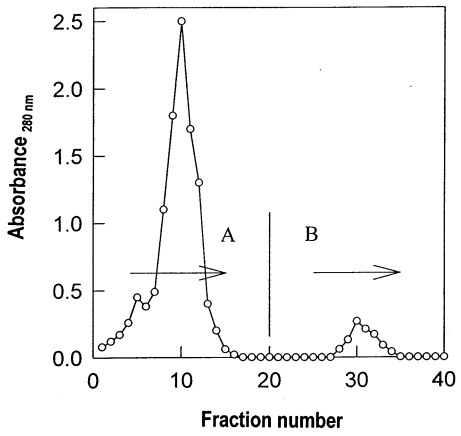
Binding Capacity (q_m) and Dissociation Constant (K_d) of LS-Sepharose 4 Immunoaffinity Gel for LS. Binding capacity and dissociation constants of adsorbent were determined by carrying out LS-Sepharose 4 Fast Flow immunoaffinity chromatography. Different amounts (0−22 mg of IgY) of IgY purified by LS-Sepharose 4 Fast Flow immunoaffinity chromatography were added to the column, which was then washed with 0.25 M NaCl/0.02 M phosphate−citrate buffer (pH 7.2) and eluted with an elution buffer (0.5 M NaCl/0.1 M phosphate−citrate buffer, pH 2.8). Fractions corresponding to IgY fractions were pooled, and the total protein content was determined to calculate the level (milligrams per milliliter of gel) of antibody bound to the immunoaffinity gel. This process was repeated to develop an adsorption isotherm line, which would represent the equilibrium amount of bound antibody at increasing concentrations of free antibody in solution. Binding parameters (q_m and K_d) of the adsorbent were then calculated using the isotherm line. The apparent adsorption isotherm of anti-LS antibody against amounts of antibody bound to the immunoaffinity gel and the Lineweaver−Burk line of anti-LY antibody were plotted according to the procedure of Chen and Wang (*14*).

Using the equation described by Chen and Wang (*14*), q_m and K_d were computed as determined by a Langmuir-type adsorption isotherm (*6*, *15*): $q^* = (q_m p^*)/(p^* + K_d)$, where K_d is the dissociation constant, p is the concentration of free adsorbate (IgY specific against LS) in solution (mg/mL), q_m is the maximum concentration of adsorbate, and q is the concentration of adsorbed adsorbate (mg/mL adsorbent); the asterisk (*) denotes equilibrium values (q^* is the concentration of adsorbed adsorbate at equilibrium and p^* is the concentration of free adsorbate at equilibrium). Both q and q_m are expressed in terms of mg/mL of wet gel.

RESULTS AND DISCUSSION

Productivity of IgY against LS. When immunization was conducted through the im route once a week for 3 weeks, the level of antibody against LS in 10^3-fold diluted yolk began to climb, reaching a peak (1.879) approximately in the sixth week. Subsequently, the level declined sharply to 0.756 in the eighth week (**Figure 1**) after initial immunization. A gradual increase in the antibody level was observed in the present study, whereas a sharp increase in the level of antibody in yolk specific against *Streptococcus mutans* serotype c was reported by Chang et al. (*16*). Compared with the changes in antibody titer for serum IgG from rabbits and IgY from eggs laid by hens immunized with casein (*17*), hens immunized with LS (**Figure 1**) showed almost the same changes in antibody titers. The choices of molecular weight, properties or source of antigen, the immunization routes, and experimental animals are all influential on the productivity of antibody against the specific antigen (*18*, *19*). IgY is derived from immunoglobulin G (IgG) in chicken serum and, therefore, its level is relatively lower than that of IgG (*16*, *20*). The increase in antibody titer could be achieved by boosting treatment to maintain a high level of antibody specific against certain antigen (*16*, *21*). The long period of time for a high antibody titer in yolk appears to be beneficial for IgY to be used as a potential source for immunological food supplement or for uses in immunoaffinity (*16*).

Purification of IgY Specific against LS. Five milliliters of crude IgY, prepared with the pectin method, was applied to the LS-Sepharose 4 Fast Flow column, and the elution profile for crude IgY is shown in **Figure 2**. Fractions corresponding to IgY (fractions 28−34), eluted with 0.5 M NaCl/0.1 M phosphate−citrate buffer (pH 2.8), were pooled (*6*), and the specific activity of purified IgY against LS was determined after pH neutralization. The specific activity of the yolk mixture collected over a 3−8 week period after the initial immunization was only 0.02 (**Table 1**). However, through the use of LS-Sepharose 4 Fast Flow immunoaffinity chromatography, the specific activity and the purification efficiency of IgY were raised to about 68 and 3380, respectively (**Table 1**), revealing the superiority of the LS-Sepharose 4 immunoaffinity column. The purification efficiencies of rabbit serum IgG and IgY purified by a lactoferrin (LF)-bound Sepharose 4B immunoaffinity column (0.5 mg of LF/mL of wet gel) were only 2420 and 2310 (*6*), respectively, inferior to the result in the present study. Hitrap Protein G (group G streptococci) shows a stronger affinity, than protein A, to the Fc fragment of mammalian IgG (*22*) and has been used to isolate total IgG in samples such as serum and milk. However, the antibodies isolate by such a protein G affinity column are nonspecific to certain antigen and, therefore, low purification efficiency was determined (*6*). Li-Chan et al. (*23*) determined the percentage (~11%) of IgY specific against LF in total IgY and isolated lactoferrin by immunoaffinity chromatography using yolk antibodies; however, no data regarding the purification efficiency of antibody or target protein (*7*) were reported. In addition, no determination of the recovery of antibody specific against certain antigen has been reported thus far due to the complex preparation of such an antibody.



A: 0.25 M NaCl/0.02 M phosphate-citrate (pH 7.2)
B: 0.5 M NaCl/0.1 M phosphate-citrate (pH 2.8)

Figure 2. Elution profile of IgY from egg yolk of hens immunized with lysozyme by LS-Sepharose 4 Fast Flow immunoaffinity chromatography: column size, 20 cm in length × 1.0 cm in diameter; sample, crude IgY; elution buffer, 0.5 M NaCl/0.1 M phosphate–citrate buffer (pH 2.8); flow rate, 0.2 mL/min; fractionation, 2.0 mL/tube.

Table 1. Specific Activity[a] and Purification Efficiency[b] of Anti-lysozyme Antibody from Yolk Purified by LS-Sepharose 4 Fast Flow Immunoaffinity Chromatography

method	specific activity[a]	purification efficiency[b]
immunoaffinity chromatography	67.6 ± 0.1	3382.5 ± 3.8
control[c]	0.02	1.00[d]

[a] ELISA value (A_{405nm})/mg of protein. Average ± SD of triplicate analyses. [b] Specific activity of anti-LS antibody/specific activity of yolk against LS. Average ± SD of triplicate analyses. [c] Mixture of yolks from eggs collected over a 3–8 week period after the initial immunization. [d] Purification efficiency of control was treated as 1.00.

q_m and K_d. Figure 3 represents the apparent adsorption isotherms (**Figure 3A**) and Lineweaver−Burk line (**Figure 3B**) of LS-Sepharose 4 Fast Flow for anti-LS IgY. The absorption of IgY specific against LS increased with the increasing amount (0−22.0 mg) of IgY specific against LS to the immunoaffinity column (*6*, *15*). Therefore, it was apparent that q_m and K_d values of such an immunoaffinity column for IgY specific against LS were 0.68 mg of IgY/mL of wet gel (0.54 mg of IgY/mg of

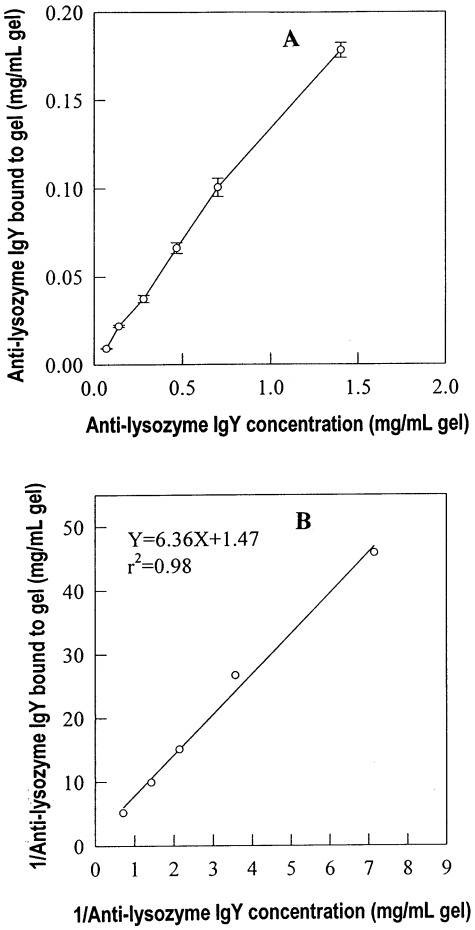


Figure 3. Apparent adsorption isotherm (A) and Lineweaver–Burk line (B) of LS-Sepharose 4 Fast Flow immunoaffinity gel for IgY against LY: column size, 20 cm in length × 1.0 cm in diameter (total volume = 15.7 mL gel); sample, 0–22 mg of IgY specific against LY; elution buffer, 0.5 M NaCl/0.1 M phosphate–citrate buffer (pH 2.8); flow rate, 0.2 mL/min; fractionation, 2.0 mL/tube.

Table 2. Binding Parameter of Laboratory-Prepared Lysozyme-Sepharose 4 Fast Flow Immunoaffinity Column for IgY Specific against Lysozyme

binding parameter	IgY
q_m[a] (mg/mL wet gel)	0.68 ± 0.03
K_d[b] (× 10^{-6} mg/mL)	7.13 ± 0.32

[a] Maximal binding capacity. Average ± SD of triplicate analyses. [b] Dissociation constant. Average ± SD of triplicate analyses.

LS) and 7.13 × 10^{-6} mg/mL, respectively (**Table 2**). In comparison with the binding capacity (0.81 mg of IgY/mL of wet gel) (1.620 mg of IgY/mg of LF) and the dissociation constant (6.4 × 10-6 M) of LF-Sepharose 4 immunoaffinity gel for IgY specific against LF (*6*), the immunoaffinity column for IgY specific against LS in the present study was lower in q_m by 19% but higher in K_d by ~10%. Properties such as size, composition, and charge (isoelectric point) of bound antigen in the gel matrix strongly influence its affinity to the corresponding antibody and, therefore, are crucial to the binding parameters of the immunoaffinity chromatography (*24*). Ling and Mattison (*25*) have indicated that to get a calculated recovery of >90% target protein when at least 99% of other contaminants were washed out, the dissociation constant must be <10^{-6} M. In the present study, the dissociation constant of such an immunoaffinity column for IgY specific against LS was 7.13 × 10^{-6} M, and the binding capacity was much lower than that (17.8 mg/mL of wet gel) of protein G Sepharose for IgG (*14*). However, protein G Sepharose is not suitable for the isolation of IgG specific against a certain antigen (*22*).

Conclusion. Hens were immunized with LS, and the ELISA value of IgY against LS was determined. The trend of the change in antibody against specific antigen was different with the antigen used and the immunization route applied on the animals. Although lower in binding capacity and higher in dissociation constant, compared to those of the monoclonal antibody immunoaffinity column chromatography, the LS-Sepharose 4 immunoaffinity column was effective in isolating IgY specific against LS to achieve purification efficiency as high as ~3380 and was competent in separating IgY specific against LS from yolk. Therefore, for the sakes of cost and efficiency, the use of a polyclonal antibody such as IgY appeared to be practical in immunoaffinity chromatography to purify and/or isolate limited components with bioactivity in foodstuffs. Leakage of bound LS during use of the column was not precisely determined. However, a constant amount of isolated IgY was collected during the 4 months and 30 isolation operations during the reproducibility test.

LITERATURE CITED

(1) Li-Chan, E.; Nakai, S.; Sim, J.; Bragg, D. B.; Lo, K. Lysozyme separation from egg white by cation-exchange column chromatography. *J. Food Sci.* **1986**, *51*, 1032−1036.

(2) Jiang, C. M.; Wan, M. C.; Chang, W. H.; Chang, H. M. Isolation of lysozyme from hen albumen by alcohol-insoluble cross linked pea pod solid ion-exchange chromatography. *J Food Sci.* **2001**, *66*, 1089−1092.

(3) Palladio, D. K.; Ball, H. R.; Swaisgood, H. E. Separation of native and acylated egg white proteins with gel chromatography and DEAE-cellulose ion exchange. *J. Food Sci.* **1981**, *46*, 778−780.

(4) Chang, H. M.; Lu, T. C.; Chen, C. C.; Tu, Y. Y. Isolation of immunoglobulin from egg yolk by anionic polysaccharides. *J. Agric. Food Chem.* **2000**, *48*, 995−999.

(5) Yang, C. C.; Chen, C. C.; Chang, H. M. Separation of egg white lysozyme by anionic polysaccharides. *J. Food Sci.* **1998**, *63*, 962−965.

(6) Tu, Y. Y.; Chen, C. C.; Chang, H. M. Preparation of immunoglobulin in yolk (IgY) and rabbit serum immunoglobulin (IgG) specific against lactoferrin by immunoaffinity chromatography. *Food Res. Int.* **2001**, *34*, 783−789.

(7) Tu, Y. Y.; Chen, C. C.; Chang, H. M. Isolation of bovine milk lactoferrin (LF) by anti-LF antibody bound immunoaffinity chromatography. *J. Food Sci.* **2002**, in press.

(8) Kawakami, H.; Shinmoto, H.; Dosaka, S.; Sogo, Y. One-step isolation of lactoferrin using immobilized monoclonal antibodies. *J. Dairy Sci.* **1987**, *70*, 752−759.

(9) Tsai, G. J.; Cousin, M. A. Enzyme-Linked immunosorbent assay for detection of molds in cheese and yogurt. *J. Dairy Sci.* **1990**, *73*, 3366−3378.

(10) Buchta, R. Ovine lactoferrin: isolation from colostrum and characterization. *J. Dairy Res.* **1991**, *58*, 211−218.

(11) Carroll, S. B.; Stollar, B. D. Antibody to calf thymus RNA polymerase II from egg yolks of immunized hens. *J. Biol. Chem.* **1983**, *258*, 24−26.

(12) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(13) McCannel, A. A.; Nakai, S. Separation of egg yolk immunoglobulin into subpopulations using DEAE-ion exchange chromatography. *Can. Inst. Food Sci. Technol. J.* **1990**, *23*, 42−46.

(14) Chen, J. P.; Wang, C. H. Microfiltration affinity purification of lactoferrin and immunoglobulin G from cheese whey. *J. Food Sci.* **1991**, *56*, 701−706, 713.

(15) Cowan, G. H.; Gosling, I. S.; Laws, J. F.; Sweethenham, W. P. Physical and mathematical modeling to aid scale-up of liquid chromatography. *J. Chromatogr.* **1986**, *363*, 37−42.
(16) Chang, H. M.; Ou-Yang, R. F.; Chen, Y. T.; Chen, C. C. Productivity and some properties of immunoglobulin specific against *Streptococcus mutans* serotype c in chicken egg yolk (IgY). *J. Agric. Food Chem.* **1999**, *47*, 61−66.
(17) Otani, H.; Matsumoto, K.; Saeki, A.; Hosono, A. Comparative studies on properties of hen egg yolk IgY and rabbit serum IgG antibodies. *Lebensm. Wiss. Technol.* **1991**, *24*, 152−158.
(18) Kuby, J. *Immunology*, 2nd ed.; Freeman: New York, 1994; p 85.
(19) Hefle, L. S. Immunoassay fundamentals. *Food Technol.* **1995**, *49*, 102−107.
(20) Otake, S.; Nishihara, Y.; Makimura, M.; Hatta, H.; Kim, M.; Yamamoto, T.; Hirasawa, M. Protection of rats against dental caries by passive immunization with hen-egg-yolk antibody (IgY). *J. Dental Res.* **1991**, *70*, 62−166.
(21) Chang, H. M.; Lee, Y. C.; Chen, C. C.; Tu, Y. Y. Microencapsulation protects immunoglobulin in yolk (IgY) specific against *Helicobactor pylorie* urease. *J. Food Sci*. **2002**, *67*, 15−20.
(22) Akerstrom, B.; Brodin, T.; Reis, K.; Bjorck, L. Protein G: a powerful tool for binding and detection of monoclonal and polyclonal antibodies. *J. Immunol.* **1985**, *135*, 2589−2592.
(23) Li-Chan, E.; Ler, S. S.; Kummer, A.; Akita, E. M. Isolation of lactoferrin by immunoaffinity chromatography using yolk antibodies. *J. Food Biochem.* **1998**, *22*, 179−195.
(24) Loomans, E. E. M. G.; Gribnau, T. C. J.; Bloemers, H. P. J.; Schielen, W. J. G. The influence of binding capacity and affinity on the improved performance of N-terminally extended hCG peptides, determined by ELISA-based procedures. *J. Immunol. Methods* **1998**, *221*, 119−130.
(25) Ling, T. G.; Mattisson, B. Membrane filtration affinity purification (MFAP) of dehydrogenase using Cibacron Blue. *Biotechnol. Bioeng.* **1989**, *34*, 1321−1326.

Received for review November 28, 2001. Revised manuscript received May 30, 2002. Accepted June 19, 2002.

JF011567H