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Affinity purification of immunoglobulins from chicken egg yolk using a new synthetic ligand

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Abstract

Due to the peculiar composition of the egg yolk and the lack of specific affinity ligands, Y immunoglobulins are normally purified using complex and time consuming procedures involving a combination of precipitation and chromatographic steps first to extract and capture and then to polish IgY. In this study, we have examined the applicability for IgY affinity purification of TG19318, a synthetic ligand for immunoglobulin, obtained from the screening of combinatorial libraries, and already characterized for its capability to purify immunoglobulins of class G, M, E and A. Soluble proteins were separated from the lipidic fraction of egg yolk by the water dilution method and loaded on to TG19318 affinity columns prepared by immobilizing the ligand on the commercially available support Emphaze™. In a single chromatographic step TG19318 affinity columns led to an efficient capture of IgY directly from crude samples, and with a purity degree higher than 90%, as determined by densitometric scanning of SDS–PAGE analysis of bound fractions, and with full recovery of antibody activity, as determined by ELISA assay. Higher recovery and purity of IgY was obtained by using loading buffers at pH close to 6.5. Column capacity, determined by applying 4× excess IgY to 1 ml bed volume column, and eluting the retained immunoglobulins, was close to 65 mg of IgY per ml of resin. Chemical and chromatographic stability of TG19318/Emphaze was tested before and after various treatments. The derivatized matrix was found to be very stable, in terms of ligand leakage and maintenance of IgY binding capacity, under conditions of normal column usage, cleaning and storage.

Keywords: Immunoglobulin

1. Introduction

The growing diffusion of monoclonal and polyclonal antibodies in immunotherapy and immunodiagnostics, and the strict standards for animal-care, has led to a renewed interest in the use of chickens for antibody production, as opposed to mammals. Egg yolk represents an economical source of polyclonal antibodies [1,2], since the amount of immunoglobulins (IgY) is similar or higher than in the serum of chickens or rabbits or humans reaching levels ranging from 15 to 25 mg IgY per ml of yolk in the case of hyperimmunized hen [3,4]. Production of immunoglobulins in chicken egg yolk offers several advantages over mammalian antibodies. The bleeding step, which causes remarkable distress to the animals involved, is eliminated and the number of animals used may be considerably reduced. For

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highly immunogenic antigens, one chicken may produce 120 rabbit equivalent of IgY over the period of one year, and at least 15 equivalent of antibodies even for less effective antigen [5]. Therapeutic and diagnostic potentialities of chicken antibodies are strictly related to the phylogenetic differences between mammals and birds, and are greater than between mammalian species. Immunoglobulins produced in chickens against mammalian antigens are, therefore, directed against more antigenic epitopes, especially for highly conserved proteins, such as hormones [6,7]. In addition, Y immunoglobulins don't cross-react with mammalian IgG and don't bind to rheumatoid factors and bacterial or mammalian Fc receptors, thus reducing non-specific binding and minimizing interference in immunological assays involving mammalian sera [8,9]. Despite the advantages related to the production and use of chicken antibodies, only less than 2% of the total number of polyclonal antibodies produced worldwide and commercially available are raised in chickens. This low diffusion is strictly related to the difficulties in isolating IgY from egg yolk, which is essentially composed of a considerable lipidic fraction dispersed in a water soluble fraction containing the immunoglobulins, to the failure of Protein-A or Protein-G, which are the most widely used affinity ligands for the purification of IgG from biological fluids, to bind to IgY [10], and to the restricted availability of secondary reagents for IgY detection. Many methods have been described to extract and purify IgY from egg yolk, based on a combination of several steps, including salt precipitation, alcohol precipitation, water dilution, ultrafiltration, gel filtration, ion-exchange chromatography and dialysis [11–17]. All of these protocols are time-consuming, labor intensive, may alter Ig functionality and can not easily be scaled up for industrial applications.

Recently, through the synthesis and screening of a multimeric combinatorial peptide library, our group has identified a new synthetic ligand, denoted PAM (Protein A Mimetic, TG19318), which binds specifically and selectively to the constant portion of immunoglobulins [18]. This ligand has been used in affinity chromatography applications [18–23], and results indicated a broader selectivity of this molecule than protein A, since mono- and polyclonal IgG from different sources, as well as IgA, IgE and IgM [19–23] may be purified by columns prepared immobilizing this molecule on solid supports. Due to the remarkable advantages related to the use of chicken immunoglobulins and the lack of specific ligands to purify IgY, in this study we have investigated the applicability of TG19318 in IgY purification, evaluating its ability to capture IgY directly from the soluble extract obtained from chicken egg yolk, determining the optimal parameters to achieve IgY high recovery, purity and functional activity, and examining ligand stability on sanitizing conditions and over repeated use.

2. Materials and methods

2.1. Peptide synthesis

TG19318 peptide was produced by solid-phase peptide synthesis following the Fmoc methodology on a fully automated peptide synthesizer 431A (Perkin-Elmer) as described previously [18]. After resin cleavage, the peptide was purified by Reverse-Phase HPLC and its identity was confirmed by amino acid analysis and time of flight matrix assisted laser desorption ionization (TOF–MALDI) mass spectrometry, which provided a molecular weight identical to the expected value (2141 a.m.u.).

2.2. Sample preparation

Egg yolks were separated from the egg white, washed with distilled water to remove as much albumen as possible and rolled on paper towels to remove adhering egg white. Yolks were pooled, mixed and 10 ml were processed according to the different protocols. For the water dilution method (WD), yolk was diluted 1:9 with distilled water, acidified with 0.1 *M* HCl to reach pH 5.0 and incubated for at least 6 h at 4°C. For polyethylene glycol (PEG) precipitation, after yolk dilution 1:4 with PBS, PEG 6000 was added to give a final concentration of 3.5% and the sample was incubated for 20 min at R.T. For dextran sulfate precipitation, the yolk was diluted 1:4 with TBS buffer, 6 ml of 10% dextran sulfate and 15 ml of 1 *M* calcium chloride were added and the mixture was incubated for 20 min at R.T. For the chloroform (CL) pro-

cedure, the sample was diluted 1:1 with chloroform and incubated for 20 min at R.T. After incubation, the samples were centrifuged at 10 000 *g* for 20 min, the supernatants were separated from the pellets, filtered on 0.45 μm Nalgene filters and characterized in terms of antibody purity, and IgY and total protein recovery.

2.3. Column preparation

Affinity columns were prepared by incubating 15 mg of peptide dissolved in 6 ml of 0.1 *M* $NaHCO_3$, 0.5 *M* NaCl, pH 8.5, with 130 mg of Emphaze (polyacrylamide-activated gel PIERCE), corresponding to 1 ml of matrix. The suspension was incubated for several h at room temperature under gentle agitation, by monitoring the extent of peptide incorporation by RP-HPLC analysis at different times. The coupling yield was close to 80–90%. After washing with 0.1 *M* Tris, pH 8.5, to deactivate residual active groups the matrix was then packed into a 100×10 mm I.D. glass column.

2.4. IgY affinity purification

The yolk extract obtained by the water dilution method was dialyzed or diluted 1:1 v/v with the starting buffer, and loaded on to the column equilibrated at a flow rate of 60 cm/h with the selected buffer. After elution of unbound material, the eluent was changed to 0.1 *M* acetic acid, pH 3.0, to elute bound material. The eluted IgY was immediately neutralized with a few drops of 1 *M* Tris, pH 9.5, and characterized by SDS–PAGE, gel-permeation, and Radial Immunodiffusion analysis in order to determine IgY recovery and purity, and by enzyme-linked immunosorbent assay (ELISA), to evaluate the immunoreactivity after purification.

2.5. SDS–PAGE

The purity of the bound fractions from the affinity columns was checked by SDS–PAGE analysis, performed under reducing and non-reducing conditions, using a 0.75 mm separating gel in a final concentration of 12 or 15% of acrylamide–bisacrylamide. The electrophoresis was performed using the Mini-PROTEAN II apparatus (Bio-Rad), following the manufacturer's instructions. Detection of the proteic bands was performed with the Brilliant Blue Coomassie R-250 (Merck) staining method.

2.6. Radial immunodiffusion

The IgY concentration was determined by Radial Immunodiffusion (RID) analysis carried out using a plate containing 3% rabbit anti-chicken IgG antisera (Sigma), 1% Agarose and 0.02% sodium azide in PBS. Five μl of appropriately diluted samples and standard IgY (Sigma) in the range of 0.1–1.25 mg/ml of immunoglobulins were added to 3 mm diameter wells. After 24 h of incubation, a standard curve was obtained by plotting the diameter of the antigen/antibody precipitation rings against concentration of standard immunoglobulins. The IgY concentration of unknown samples was determined by reference to this curve.

2.7. Enzyme linked immunosorbent assay (ELISA)

The immunoreactivity of IgY from crude yolk extract affinity purification was checked by enzyme linked immunosorbent assay (ELISA) performed on microtiter plates 96 wells as solid support, and using formaldehyde killed whole *E. coli* cells as antigen, as described by Akita et al. [13]. Wells were coated with 100 μl of *E. coli* sonicated whole cell suspension (2×10^7 cell per well) in PBS and incubated overnight at R.T. Plates were washed five times with PBS containing 0.05% Tween (PBS-T), followed by a blocking step using 200 μl of 3% milk in PBS for 2 h at 37°C. After incubation, the plates were washed five times with PBS-T and filled with standard IgY in the range of 0.01–50 μg/ml and crude, unbound and bound materials appropriately diluted with PBS containing 0.05% of milk (PBS-M), and incubated for 1.5 h at 37°C. Plates were then washed five times with PBS-T and 100 μl of rabbit anti-chicken IgG-peroxidase, diluted 1:1000 in PBS-M, were added to each well. After 1 h of incubation at 37°C, plates were then washed with PBS-T five times, and developed with a chromogenic substrate solution consisting of 0.2 mg/ml ABTS in 0.1 *M* sodium citrate buffer, pH 5.0 containing 5 m*M* hydrogen peroxide. The absorbance at 405 nm of each sample

was measured with a Model 2250 EIA Reader (Biorad).

2.8. Gel filtration analysis

Gel permeation analysis was performed using a Bio-Sil SEC 125 GF column, 300×7.8 mm (BIO-RAD) equilibrated at a flow rate of 1.0 ml/min with PBS, 0.01 *M* NaN_3, pH 6.8, monitoring the effluent at 280 nm. Crude material and Y immunoglobulins, purified on the affinity column, were 0.22 μm filtered, and applied to the column.

3. Results

3.1. IgY extraction from egg yolk

Due to the large amount of lipoprotein and phosvitin granules, a general outline for the isolation of Y immunoglobulins from egg yolk involves a preliminary step where water-soluble proteins are separated from the lipidic fraction. The most common procedures to extract IgY from egg yolk involve precipitation of non-aqueous material with polyethylene glycol (PEG) [16], dextran sulfate (DS) [2], and chloroform (CD) [15], or aggregation of proteic granules by a simple water dilution of the egg yolk under weakly acidic conditions [4,13]. Preliminary experiments were carried out to compare these four methods in terms of yield, purity and compatibility with an affinity purification step. Pooled egg yolks (10 ml) were processed to extract plasma proteins according to the protocols previously described. Recovery and purity of Y immunoglobulins were determined respectively by Radial Immunodiffusion assay and densitometric scanning of SDS–PAGE analysis of crude extracts, whereas the total protein amount was determined by the Biuret method using BSA as standard. In strict agreement with bibliographic data, the water dilution method gave the highest recovery and purity of IgY in comparison with the other protocols. With the WD method, 10.4 mg of antibodies per ml of egg yolk were obtained with a purity close to 34%, compared with 7.2, 7.3 and 8.9 mg/ml from the PEG, CD and DS methods, but with purity lower than 20% (Table 1).

3.2. IgY affinity purification

3.2.1. Running buffer and sample treatment

In order to investigate the applicability of TG19318 for IgY purification from egg yolk, a full set of chromatographic experiments were carried out with the ligand immobilized on Emphaze™, a pre-activated commercially available support for affinity chromatography. Preliminary experiments, to define the best loading buffer and sample treatment, were performed with different buffers at the same pH and ionic strength. Samples, corresponding to the crude yolk extract deriving directly from the water dilution protocol, were diluted with the starting buffer to reach a final IgY concentration of 0.5 mg/ml and applied directly to the columns equilibrated with 25 m*M* sodium phosphate, or Tris or Bis–Tris buffers, pH 7.0, at a linear flow rate of 60 cm/h. After

Table 1
Comparison of water dilution (WD), polyethylene glycol (PEG), dextran sulphate (DS), and chloroform (CD) methods for the extraction of Y immunoglobulins from egg yolk

Sample	IgY		[b]Protein (mg)	[c]Purity (%)
	[a]Yield (mg)	Recovery (%)		
Egg yolk	141.2	100.0	N.D.[d]	7.0
WD-SN	128.7	91.1	286	33.6
PEG-SN	90.3	64.0	348	15.8
DS-SN	111.8	79.2	N.D.[d]	16.7
CD-SN	91.9	65.1	275	14.0

[a] IgY determination was done by RID.
[b] Protein determination was done by the Biuret method.
[c] IgY purity was evaluated by SDS–PAGE.
[d] N.D.: not determinable, due to interference (lipids or salts).

elution of unretained material, the buffer was changed to 0.1 *M* acetic acid, to elute the adsorbed antibodies. Bound fractions were characterized in terms of IgY recovery and purity respectively by SDS–PAGE and RID. As summarized in Table 2A, all samples showed a similar antibody purity (>95%), whereas the highest recovery (25.1%) was obtained by using the Bis–Tris buffer. All experiments were repeated using the crude extract dialyzed against each of the buffers used and then diluted to obtain a 0.5 mg/ml starting IgY concentration with similar results.

3.2.2. Effect of buffer composition on IgY purification

The effect of buffer composition on TG19318 recognition for IgY was examined by affinity chromatography experiments on 1 ml bed volume columns prepared immobilizing the ligand on Emphaze matrix. As described previously, 5 ml of crude yolk extract were diluted with different Bis–Tris buffers obtained by varying the pH from 5.0 to 8.0 and the ionic strength from 10 to 200 m*M*, and loaded onto the column equilibrated with the same diluting buffer, at a linear flow rate of 60 cm/h. Adsorbed material was eluted by lowering the pH with 0.1 *M* acetic acid, and all bound fractions, immediately neutralized with a few drops of 1.0 *M* Tris, pH 9.0, were characterized in terms of IgY recovery and purity by RID and SDS–PAGE. As shown in Table 2-B and -C, the buffer composition and pH affected IgY recovery significantly, with higher binding capacity at low ionic strength and slightly acidic pH, while all bound fractions showed a very high purity, ranging from 90 to 95% (Fig. 1). This indicated that the ability of the TG19318/Emphaze affinity column to selectively purify Y immunoglobulins was only weakly dependent on the buffer composition and pH. Fig. 2 shows a chromatographic profile related to the affinity purification of crude yolk extract with 25 m*M* Bis–Tris, pH 6.5, while the SDS–PAGE analysis of the corresponding bound and unbound fractions is showed in Fig. 3. Column-bound fraction (peak 2), only shows the protein bands at the expected molecular mass corresponding to the IgY in non-reduced (≈190 kDa) or reduced (≈70 and ≈30

Table 2
Dependence of TG19318 binding ability for yolk immunoglobulins on buffer, ionic strength and pH.

Molarity (m*M*)	Buffer	pH	[a]IgY Recovery		[b] IgY Purity (%)
			(mg)	(%)	
A					
25	Phosphate	7.0	0.65	11.1	>95
25	Tris	7.0	1.00	17.1	>95
25	Bis–Tris	7.0	1.47	25.1	>95
B					
25	Bis–Tris	5.0	–	–	–
25	Bis–Tris	5.5	5.40	92.3	>90
25	Bis–Tris	6.0	5.42	92.6	>90
25	Bis–Tris	6.5	5.76	98.5	>90
25	Bis–Tris	7.0	1.47	25.1	>95
25	Bis–Tris	8.0	0.15	2.6	>95
C					
10	Bis–Tris	6.5	3.54	60.5	>90
25	Bis–Tris	6.5	5.76	98.5	>90
50	Bis–Tris	6.5	4.08	69.7	>90
100	Bis–Tris	6.5	3.25	55.5	>95
200	Bis–Tris	6.5	0.30	5.1	>95

[a] IgY determination was done by RID.
[b] IgY purity was evaluated by SDS–PAGE.

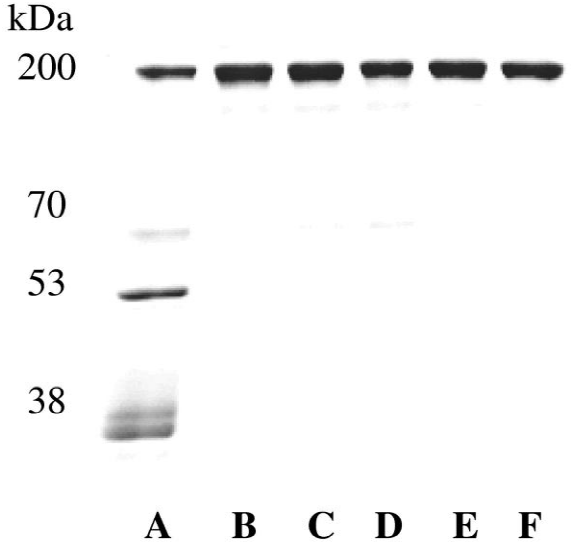

Fig. 1. SDS–PAGE analysis (12% slab-gel under non reducing conditions, comassie staining) of bound fractions obtained from the purification of chicken IgY from crude yolk extract by varying the ionic strength. About 7 μg of total proteins were loaded in each lane. Lane A: crude yolk extract, lane B: 10 m*M* bound fraction, lane C: 25 m*M* bound fraction, lane D: 50 m*M* bound fraction, lane E: 100 m*M* bound fraction, lane F: 200 m*M* bound fraction.

kDa) form, while the flow-through material (peak 1), shows all contaminants with no traces of antibodies. Gel filtration profile of the affinity purified IgY (Fig. 4) validated the SDS–PAGE data, since no traces of contaminants were detected in the bound fraction, thus confirming the capacity of TG19318/Emphaze column to capture and concentrate IgY from the soluble yolk extract in a single affinity step.

3.2.3. Column capacity

Capacity determination of TG19318/Emphaze affinity matrix was estimated by overloading 1 ml bed volume column with purified IgY under the experimental conditions showing the best results in terms of recovery and purity. 200 mg of purified immunoglobulins were dialyzed against 25 m*M* Bis–Tris, pH 6.5, and applied to the column, equilibrated with the same diluting buffer, with an IgY concentration of 1.85 mg/ml and a linear flow rate of 60 cm/h. After elution of the flow-through material, the buffer was changed to 0.1 *M* acetic acid, to elute the

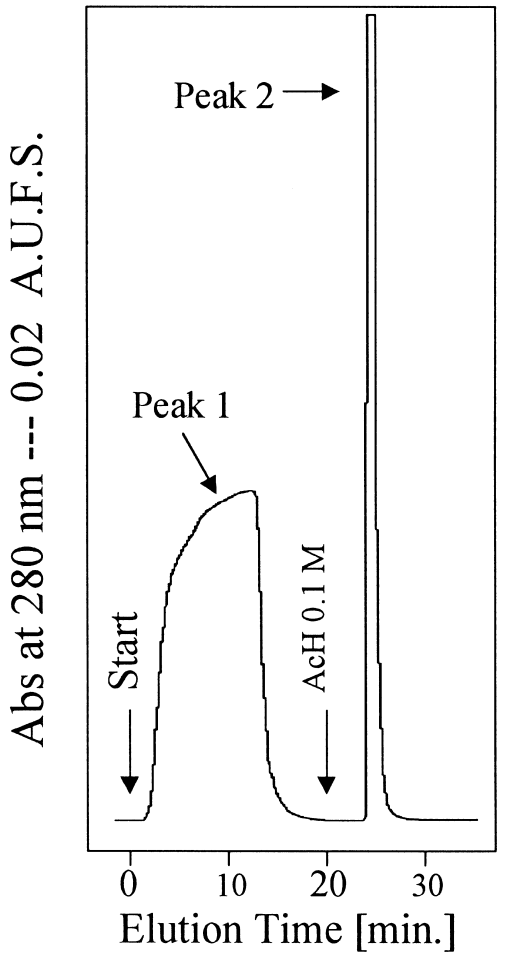

Fig. 2. Purification of crude yolk extract on TG19318/Emphaze affinity column. 5 ml of crude extract was applied to the column equilibrated with 25 m*M* Bis–Tris buffer, pH 6.5, at a flow rate of 60 cm/h. After elution of unbound material (peak 1), the adsorbed material was eluted with 0.1 *M* acetic acid (peak 2). Each fraction was collected for SDS–PAGE, RID and ELISA analysis.

adsorbed antibodies. Bound fractions were immediately neutralized and characterized in terms of IgY recovery by UV adsorption (ϵ=1.51 cm^{-1} mg^{-1} ml) and RID. The maximal binding capacity obtained was close to 64 mg of IgY per ml of matrix.

3.2.4. IgY activity

Since the eggs used for this work were laid by hens not immunized against a specific antigen, to study antibody activity we used the inherent activity

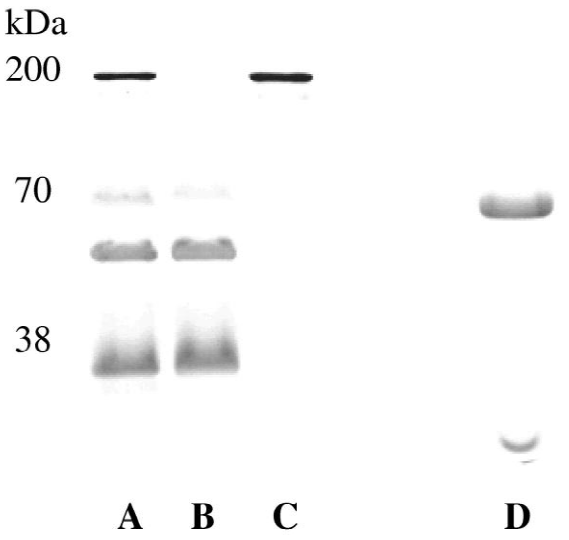

Fig. 3. SDS–PAGE analysis (15% polyacrylamide gel, comassie staining) of fractions obtained from the purification of chicken IgY from water dilution supernatant with 25 m*M* Bis–Tris buffer, pH 6.5. About 7 μg of total proteins were loaded in each lane. Lane A: crude extract, lane B: unbound fraction (peak 1), lane C: bound fraction (peak 2) non reduced, lane D: bound fraction reduced.

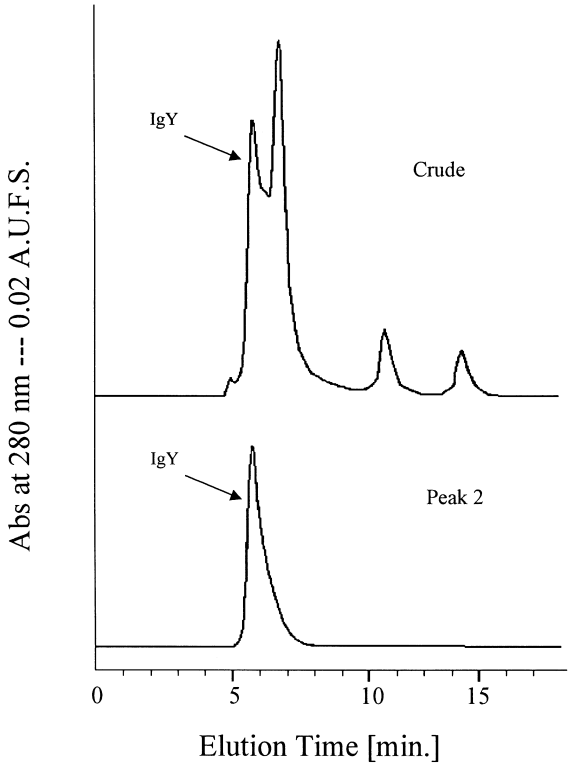

Fig. 4. Gel permeation profiles on Bio-Sil SEC 125 GF column (300×7.8 mm) of crude yolk extract and affinity purified IgY from the TG19318/Emphaze column equilibrated with with 25 m*M* Bis–Tris buffer, pH 6.5. Samples (150 μg) of crude extract (top) and purified IgY (bottom) were applied on the gel permeation column equilibrated with PBS at a flow rate of 1 ml/min, monitoring the effluent at 280 nm. The peaks were identified by comparison with the retention times of molecular mass standards.

that Y immunoglobulins show towards formaldehyde-treated E.coli, as described by Akita et al. [13]. The activity of IgY in the WD supernatant and in the fractions obtained from the affinity purification with 25 m*M* Bis–Tris, pH 6.5, were compared by direct ELISA in order to evaluate the effect of purification conditions on the maintenance of the antibody–antigen recognition. Results indicated that after the affinity fractionation step, antibodies were recovered in a fully active form, with the majority of the immunoreactivity, about 99%, recovered in the bound fraction, in strict agreement with the data of immunoglobulins recovery obtained from Radial Immunodiffusion analysis.

3.3. Matrix stability

The chemical and chromatographic stability of TG19318/Emphaze resin has been evaluated measuring matrix ligand leakage and retention of IgY binding capacity before and after various treatments. Studies have been performed in the batch and column mode.

3.3.1. Batch experiments

A small amount of resin was settled, washed with distilled water and incubated under gentle shaking, at room temperature, under various conditions. At chosen times, 2 ml of swollen gel, corresponding to 1 ml of resin, were centrifuged, the supernatant analyzed for ligand content by RP-HPLC and the resin packed and tested for retention of IgY binding capacity. Similar experiments were performed on 1 ml of autoclaved matrix (Table 3).

3.3.2. Column experiments

Two types of experiments were carried out, test runs without loading the sample and test runs loading samples containing IgY. Resin (1 ml) was packed into a 100×10 mm I.D. glass column, equilibrated

Table 3
Stability of TG19318 affinity matrix

Conditions	Incubation time	SN[a]	Run1[b]	Run3[b]	Run4[c]		Run5[c]		Regeneration[b]		Run 7[c]		Run 12[c]	
									pH 4	pH 8				
		L1[d]	L1[d]	L1[d]	L2[e]	B[f]	L2[e]	B[f]	L1[d]	L1[d]	L2[e]		L2[e]	B[f]
Untreated	0	0	1.9	0.63	0.11	0	0.07	0	1.6	0.35	0.02	1.0	0.02	0
Ethanol 20%	7 days	3.1	1.9	0.76	0.11	0	0.08	1.7	1.5	0.38	–	–	–	–
	6 months	27.2	2.3	0.82	0.13	0	0.11	0.9	1.6	0.36	0.05	1.7	0.04	0.8
Ethanol 70%	7 days	4.1	2.2	0.95	0.13	1.7	0.08	0.9	1.9	0.57	–	–	–	–
Acetic acid	15 h	6.0	1.3	0.54	0.35	1.7	0.38	5	4.8	0.88	–	–	–	–
0.1 *M*, pH 3.0	7 days	29.6	3.1	0.39	0.31	0	0.25	3	–	–	–	–	–	–
NaOH 0.1 *M*	1 h	482	22	2.5	5.2	34	–	33	–	–	–	–	–	–
Autoclaving		230	35	4.1	17	40	–	38	–	–	–	–	–	–

[a] Performed in batch without IgY.
[b] Performed in column without IgY.
[c] Performed in column with IgY.
[d] L1: TG19318 leakage determined without loading IgY containing samples [μg/ml of resin].
[e] L2:TG19318 leakage determined loading IgY containing samples [μg/mg of bound IgY].
[f] B: reduction in total binding capacity [%].

with running buffer (25 m*M* Bis–Tris, pH 6.5) at a flow rate of 60 cm/h, and eluted three times with 5–8 column volumes of elution buffer (acetic acid 0.1 *M*, pH 3.0). After the third cycle, the column was equilibrated with a starting buffer and tested for IgY binding capacity by loading 10 ml of soluble yolk extract diluted 1:1 with starting buffer. After two runs, the column was regenerated with 5 column volumes of acetic acid 0.1 *M*, NaCl 0.5 *M*, pH 4.0, followed by 5 bed volumes of Tris 0.1 *M*, NaCl 0.5 *M*, pH 8.0. In addition, 1 ml of resin was packed, washed with 5 column volumes of distilled water and circulated with 20 volumes of NaOH 0.5 *M*. After 30 min, the resin was washed with 20 volumes of water, equilibrated with running buffer and tested for ligand leakage and IgY binding capacity.

Table 3 summarizes all the data related to ligand leakage and retention of IgY binding capacity obtained by analyzing supernatants and eluates for ligand and IgY content. Results indicate that with all the buffers used to elute, clean, and store the matrix, stability was not significantly affected. On the other hand, ligand release from the matrix following exposure to NaOH 0.1 *M* for 1 h or after autoclaving was more significant (up to 5%) and as a consequence, binding capacity was reduced by approximately 30%.

4. Discussion

The production of immunoglobulins in egg yolk is convenient in terms of respect to animal care, high productivity, high immunogenicity against mammal proteins and high potentiality of Y immunoglobulins for the diagnosis and therapy of certain diseases, of which dental caries, cancer, salmonella enterica, Hepatitis B, botulism, gastroenteritis, represent only a small share [24–36]. While immunoglobulins from egg yolk are normally produced in large amount (about 1500 mg of IgY per month from each hen), their purification may constitute a problem that can be both attributed to the difficulties to separate plasma proteins from the egg yolk granules, and to the lack of specific and selective ligands for IgY (Staphilococcal protein A and Streptococcal protein G fail to bind IgY). Table 4 compares four common methods for IgY purification with TG19318/Em-

Table 4
Comparison of TG19318/Emphaze column with four conventional methods for the purification of IgY from egg yolk

Procedure	IgY		References
	Yield (mg/ml)	Purity (%)	
Dextran sulphate $_{prec.}$+2 Sodium sulphate $_{prec.}$	7.5	>87	[2], [4]
Xanthan$_{prec.}$+2 Sodium sulphate$_{prec.}$	7.3	>89	[14], [4]
2 PEG$_{prec.}$+Alcohol $_{prec.}$	9.8	>94	[16], [4]
Water dilution+Sodium sulphate$_{prec.}$+UltraFiltr.	4.9	>89	[4], [13]
Water dilution+TG19318/Emph. column	10.2	>90	[4], [13]

phaze affinity column, in terms of yield and purity of purified antibodies. Conventional procedures are essentially based on a combination of precipitation and chromatographic steps, such as polyethylene glycol, dextran sulphate, chloroform, natural gums, ultrafiltration, gel filtration, hydrophobic interaction and anion-exchange chromatography. These protocols are, however, quite expensive, time consuming, and can not be easily scaled up. The TG19318 ligand, a tetrameric tripeptide, can be obtained at a low cost by chemical solution phase or solid-phase synthesis in the multikilogram scale, does not contain biological contaminants such as viruses, pyrogens, DNA fragments as many recombinant or extractive biomolecules, and can withstand a large array of sanitizing agents with no denaturation problems. Immobilization on preactivated solid supports can be easily accomplished given the presence of four amino groups of which only a limited number are involved in the coupling to the solid-phase, leaving the others fully available for the interaction [37]. Immunoglobulin adsorption can be achieved under mild conditions, at a physiological pH, low ionic strength, and at room temperature, and elution of adsorbed antibody can be achieved under conditions not causing IgY denaturation or loss of antigen binding capacity, as detected by ELISA assays. The high column capacity, determined by saturating the resin with purified Y immunoglobulins and close to 65 mg of IgY per ml of resin, the availability of TG19318 as affinity ligand and the stability of the derivatized matrix in mild and harsh conditions, will make IgY purification much more convenient and affordable, opening up new avenues in the study and application of this important class of immunoglobulins.

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