Immunoglobulins from Egg Yolk: Isolation and Purification

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ABSTRACT

Simple water dilution was employed for the separation of water-soluble plasma proteins from egg yolk granules. An optimum recovery of immunoglobulin Y (IgY, 93–96%) in water-soluble fraction was obtained by sixfold water dilution at pH between 5.0–5.2 with incubation time of 6 hr at 4°C. Among the factors studied, pH was found to be the most important factor affecting IgY recovery. Active IgY of high purity with good recovery was obtained by a combination of several techniques including salt precipitation, alcohol precipitation, ultrafiltration, gel filtration and ion exchange chromatography. Salt precipitation, ultrafiltration, and get filtration is the recommended sequence. Over 100 mg of electrophoretically pure IgY was routinely obtained from one egg.

Key Words: egg, yolk, immunoglobulin, isolation, purification

INTRODUCTION

IT HAS BEEN ESTIMATED that five to ten million people in the developing countries die each year from diarrhea (Fedorak and Field, 1987). This diarrhea is mainly either of viral or bacterial origin. With diarrhea occurring in travelers to less developed countries, the main organism is enterotoxigenic *Escherichia coli* accounting for 30 - 70% of reported cases (Tacket et al., 1988). Although antimicrobial agents could be used to prevent illness, constant use of these drugs, some with several side effects (DuPont et al., 1986; Sack, 1986), would not be advisable. An acceptable microbial agent, if to be used on a prophylactic basis, should have few or no side effects.

Enhanced immunity due to oral administration of immunoglobulins has been observed by a number of researchers. Feeding vaccinated cow's colostrum prevented diarrhea in infants due to infection of *E. coli* (Hilpert et al., 1977; Brussow et al., 1987) or rotavirus (Ebina et al., 1985) and traveler's diarrhea (Tacket et al., 1988). Oral administration of immunoglobulins from chicken egg had been used successfully by Bartz et al. (1980) for the prevention of murine rotavirus infection in mice. No side effect was observed by Tacket et al. (1988) on using milk immunoglobulin concentrate as an effective prophylactic against traveler's diarrhea. Fortification of food products, especially infant formulas with immunoglobulins, since infants are the most vulnerable group, would therefore go a long way to help alleviate or solve this problem.

Egg yolk is recognized as a very good source of specific antibodies. The advantages it offers over conventional antibody production are well documented. These include the potential of producing more specific antibodies against mammalian antigens in birds compared to mammals, because of the phylogenetic distance between birds and mammals (Jensenius et al., 1981) lower cost and convenience (Polson et al., 1980) and, what is becoming more important, compatibility with modern animal protection regulations (Gottstein and Hemmeler, 1985). It has also been reported that production and maintenance of higher levels of specific antibodies is relatively easy (Orlans, 1967; Rose et al., 1974).

Many methods have been used for isolation and purification of immunoglobulins from egg yolk (Martin et al., 1957; Martin and Cook, 1958; Jensenius et al., 1981; Bade and Stegemann, 1984; Polson et al., 1985; Hassl and Aspock, 1988). However, most of those methods do not lend themselves to food applications. The procedure adopted for preparation of immunoglobulins should not only involve chemicals permitted for food use but should also be simple, easily scaled up and cost-effective.

Hatta et al. (1988) used food grade sodium alginate for the recovery of IgY in the water-soluble fraction (WSF) from egg yolk. Metal chelate interaction chromatography (McCannel and Nakai, 1989) and DEAE-ion exchange chromatography (McCannel and Nakai, 1990) have been used for partial purification of IgY from alginate-treated egg yolk. Kwan et al. (1991) used water dilution to fractionate water-soluble from water-insoluble components of egg yolk. They reported recovery of 60–90% of immunological activity of yolk in the water-soluble fraction.

Furthermore, egg yolk contains other important functional and biologically active components with diverse applications in the food and pharmaceutical industries. Work has been done in our laboratory to develop conditions for the isolation and purification of IgY from egg yolk with the above objectives in mind. Our purpose was to employ a water dilution procedure, and to optimize it for the isolation of IgY. The method is simple, rapid and efficient; and would allow separation and purification of IgY with high activity as well as other important components (e.g., phospholipids) from egg yolk.

MATERIALS & METHODS

Materials

Eggs from White Leghorn hens were obtained from the University of British Columbia Poultry Unit and stored at 4°C until used.

Separation and purification of immunoglobulins

Egg yolk was separated from the white, washed with distilled water to remove as much albumen as possible and rolled on paper towels to remove adhering egg white. The membrane was punctured and the yolk allowed to flow into a graduated cyinder without the membrane. Egg yolk was diluted with distilled water (acidified with 0.1N HCL, predetermined to give the pH desired after dilution), and held for at least 2 hr before centrifugation (10,000 × g for 1 hr at 4°C) or filtration through Whatman No. 1 filter paper in the cold. The resulting immunoglobulin-containing filtrate or WSF was further purified by salt precipitation, alcohol precipitation, ultrafiltration (UF), gel filtration and anion exchange chromatography. Purity and recovery of immunoglobulin were monitored at various stages by SDS-PAGE and radial immunodiffusion (RID). The activity of IgY was evaluated by enzyme-linked immunosorbent assay (ELISA).

SDS-polyacrylamide gel electrophoresis

SDS-PAGE was done under nonreducing conditions on Pharmacia Phast System using a 10 - 15% gradient PhastGel and Coomassie brilliant blue staining according to the manufacturer's recommendations (Pharmacia-LKB, Uppsala, Sweden). Protein samples (1mg – 3mg/mL) were adjusted to around pH 8.0 and 10% SDS was added to give a final SDS concentration of 2%. Quantification and molecular weight determination of proteins on PhastGels were done with a Pharmacia Phast Image Gel analyzer. SDS-PAGE was also done under the same non-reducing conditions using the Mini-PROTEAN II cell (Bio-Rad, Richmond, CA).

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Radial immunodiffusion

Radial immunodiffusion was done essentially by the method described by McCannel and Nakai (1990). Rabbit anti-chicken IgG antisera (0.35 mL, Sigma Chemical Co., St. Louis, MO) mixed with 1.65 mL barbital buffer (ionic strength 0.075, pH 8.6) were incubated in a 56°C water bath. Agarose A (70 mg, Pharmacia-LKB, Uppsala, Sweden) was dissolved in 5.0 mL of barbital buffer containing 0.02% sodium azide, in boiling water, then cooled to 56°C, mixed with the anti-chicken IgG (Sigma Chemical Co., St. Louis, MO) solution and poured into RID plates. Six μL of appropriately diluted samples and standards in the range of 0.1 mg IgY/mL to 1.0 mg IgY/mL were added to 3-mm-diameter wells. A standard curve was obtained by plotting the diameter square (d^2) of the precipitation rings after 18 hr against $\log_{10}$ concentration. IgY concentration of unknown samples was determined by reference to this curve.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay was performed as described by Shimizu et al. (1988), with modifications, using formaldehyde-treated whole *E. coli* cells as the antigen. Immulon II microtiter plate (96 wells, Dynatech Laboratories Inc., Chantilly, VA) was used as the solid support. Wells were coated with 100 μL of *E. coli* sonicated whole cell suspension (10^7 cells per well) in potassium phosphate buffer saline (PBS, 10 mM phosphate buffer, pH 7.0, 0.14M NaCl), and incubated for 1 hr at 37°C or overnight at 4°C. Plates were washed three times with PBS-Tween (0.05% Tween 20), followed by a blocking step using 100 μL of 2% bovine serum albumin for 30 min at 37°C. Plates were incubated with the appropriate dilutions of IgY for 1 hr at 37°C. The plates were then washed again three times with PBS-Tween and 100 μL of rabbit anti-chicken IgG coupled to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO, 1:500 in PBS-Tween) was added to each well. After 1 hr incubation at 37°C, the plates were washed again followed by addition of 50 μL freshly prepared substrate solution (0.1% p-nitrophenyl phosphate disodium in diethanolamine buffer, pH 9.8). The reaction was stopped by addition of 50 μL 2.5 N NaOH. For each plate, controls for nonspecific binding of IgY antibodies and enzyme-labeled antibodies were prepared. Absorbance was read at 405 nm using a ER-400 ELISA reader (SLT Labinstruments, Salzburg, Austria).

Ultrafiltration

Ultrafiltration was done using a Harp™ hollow fiber ultrafiltration membrane cartridge (Supelco Inc., Bellefonte, PA) with a molecular weight cut-off of 100 kilodaltons (kD). The protein sample was initially concentrated 5 times followed by diafiltration with PBS. Elution of protein in filtrate was monitored by absorbance at 280 nm. Diafiltration was stopped when a negligible amount of protein was eluted in the filtrate, i.e., after 7 concentrate-volumes of PBS had been passed through the system. Experiments were done at room temperature.

Alcohol precipitation

Ethanol precipitation of IgY was done by the method described by Polson et al. (1985). Ethanol (50%) precooled to −20°C was added to an equal volume of 1.0–1.5% protein at 4°C to give a final ethanol concentration of 25%. The suspension was held at between −5 to −10°C for 30 min and centrifuged at −5°C. Unless otherwise stated, centrifugation as done at 10,000 × *g for 20 min.*

DEAE-Sephacel anion exchange

The method described by Shimizu et al. (1988) was used. Anion exchange chromatography was done at ambient temperature at a flow rate of 1.0 mL/min. Protein sample was applied to DEAE-Sephacel columns (2.0 × 4 cm or 2.5 × 50 cm) equilibrated with 0.025M phosphate buffer (PB), pH 8.0.

Choice of column was dependent on the amount of IgY to be purified, up to 100 mg for the small and 1–2g of IgY for large columns. Column was washed extensively (5 bed-volumes) with equilibration buffer. IgY was eluted with 0.250M PB, pH 8.0. Strongly bound protein impurities on the column were removed by eluting with 0.01M PB, pH 7.0 containing 1.5M NaCl. Elution of the protein was monitored by absorbance at 280 nm. Sodium azide (0.02%) was included in the buffers to control microbial growth. Fractions from the IgY peak were pooled, dialyzed extensively against distilled water or the appropriate buffer in the cold and freeze-dried.

Gel filtration

Gel filtration was performed on columns (1.0 × 80 cm or 2.5 × 90 cm) packed with Sephacryl S-200 Superfine gel (Pharmacia-LKB, Uppsala, Sweden). After equilibration with 0.01M phosphate buffer pH 7.0 containing 1.5M NaCl, protein solution (10–40 mg/mL) in the same buffer was applied. A sample volume of 1–2% of the bed volume was applied and gel filtration was done at a flow rate of 1.0 mL/min. Elution of protein was monitored by absorbance at 280 nm.

Protein determinations

Protein content was determined by the micro-Kjeldahl method of Concon and Soltess (1973). After digestion, the nitrogen content was determined on a Technicon Auto Analyzer II system. Protein content was calculated from nitrogen content using the conversion factor of 6.25. Concentration of protein was routinely determined by the biuret method using Sigma's Total Protein Reagent (Sigma Chemical Co., St. Louis, MO), with slightly modified manufacturer's instructions in order to use microtiter plates. Briefly, 200 μL of the Total Protein Reagent was added to 50 μL of protein sample or protein standard solution (1 – 16 mg/mL). Absorbance was read at 500 nm after incubation for 15 min at room temperature.

Lipid determination

Lipids were extracted with a chloroform:methanol (3:1) solvent system. Total lipids were determined gravimetrically by taking aliquots from the chloroform layer and evaporating the solvent under nitrogen at 50°C.

RESULTS & DISCUSSION

EGG YOLK is essentially composed of granules (lipoproteins and phosvitin, Burley and Cook, 1961) dispersed in a soluble fraction (livetins and low-density lipoproteins, McCully et al., 1962). We used simple dilution of egg yolk with water to separate the plasma proteins from the granular proteins, since the egg yolk granules were found to aggregate with dilution. The livetins in the supernatant could then be recovered either by centrifugation or filtration.

The general outline for the separation and purification of IgY from egg yolk is shown in Fig. 1. Since the protein of interest was γ-livetin, which was believed to be IgY (Polson et al., 1980), a number of factors were investigated with the aim of increasing its yeld in the supernatant with simultaneous decrease of the other fractions. These include extent of dilution, incubation time, pH of diluted egg yolk and addition of sodium chloride.

Effect of dilution

Egg yolk was diluted 4, 6, 8, 10, 12, 14, 16, 18, 20 and 40 times with distilled water. The pH of the diluted egg yolk was found to be between 6.3 – 6.6, with the pH value increasing with age. After overnight incubation, samples diluted 10 times and above gave relatively clear supernatants with only slight lipid contamination. The supernatant-containing lipids appeared yellowish, possibly due to lipid-soluble pigments. Subsequently, further experiments were done using 10x dilution.

Effect of pH

The effect of pH (2 –12) on the recovery of IgY in the water-soluble fraction (WSF) was studied next. In our initial experiments, egg yolk dilutions were adjusted to the desired pH by adding 0.1N HCl or 0.1N NaOH. However, to avoid possible disruptions of egg yolk granules due to localized high concentrations of acid or alkali, chilled acidified distilled water or

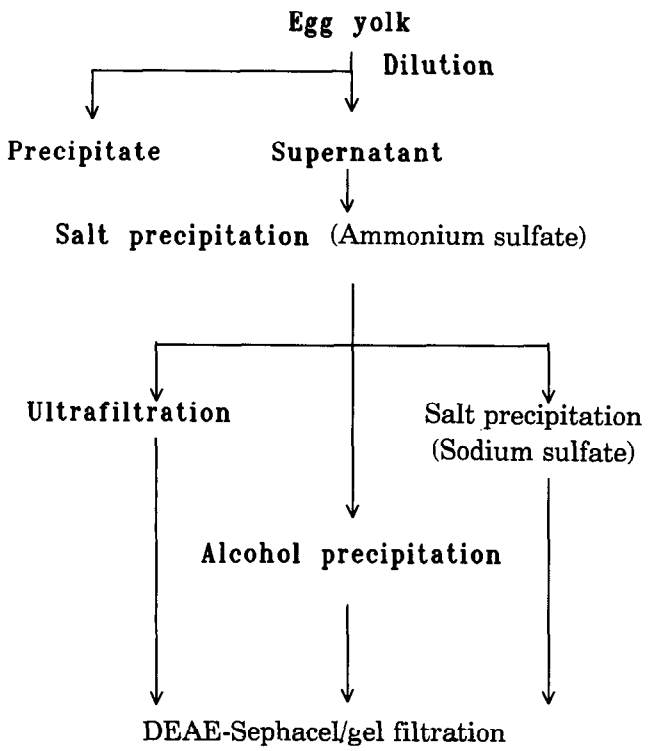

Fig. 1 – Flow diagram for the isolation and purification of IgY from egg yolk.

Table 1 – Effect of pH on the recovery of IgY and lipids from water-soluble fraction of egg yolk[a]

pH	IgY Yield[b]	IgY % Recovery	Lipids Yield[b]	Lipids % Recovery
4.3	9.98	75	253.20	88.5
4.6	10.28	77.3	4.20	1.5
4.8	10.40	78.2	3.90	1.4
5.0	12.33	92.7	3.50	1.2
5.2	12.53	94.2	5.40	1.9
5.5	9.59	72.1	20.40	7.1
6.0	8.69	65.2	54.90	19.2
6.6	7.24	54.5	59.10	20.6
Untreated egg yolk	13.30	–	286.20	–

[a] Averages of two determinations. Egg yolk was diluted 10×, incubated for 9 hr and centrifuged at 10,000× *g* for 1 hr at 4°C. IgY concentration was estimated by RID.
[b] mg/mL egg yolk.

dilute alkali, predetermined to give a desired pH, was used for the dilution.

Extremes of pH, below 4.2 and above 9.0, prevented settling of the egg yolk granules. The amount of lipids in the WSF was affected by the pH (Table 1). The WSF was almost devoid of lipids at mild acidic conditions (pH 4.6 to 5.2) with increasing levels of lipids at pH's below and above this range. The pH effect could be attributable to changes in the integrity of the egg yolk granules which may have increased the lipid binding ability of the granules. A possible indicator of pH effect on integrity of the granules was the nature of the precipitate formed.

After overnight incubation at pH values close to neutral, granules aggregated to give a compact sediment, about one-tenth of the total volume with WSF slightly yellowish in color indicating presence of lipids. Consequently, the WSF could easily be poured or siphoned off. However, at the lower pH values (e.g., 5.0), the egg yolk assumed a creamy color and the granules appeared fluffy, forming a loose aggregate and settling to more than 90% of the total volume giving a very clear colorless WSF. Consequently, at the lower pH values there was a need for filtration or centrifugation for efficient separation of the granules from the WSF. Lowering the pH

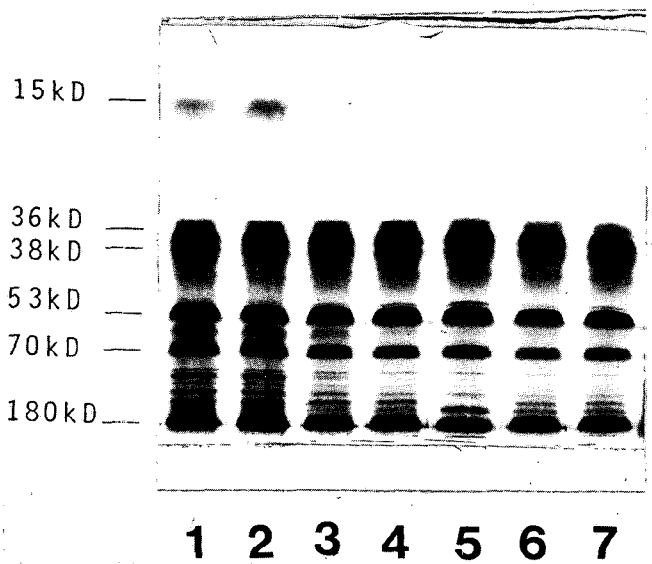

Fig. 2 – SDS-PAGE (nonreducing) on 10% gel (Mini-PROTEAN II cell) of effect of pH on the recovery of IgY in the water-soluble fraction of egg yolk: (1) 6.6, (2) 6.0, (3) 5.5, (4) 5.2, (5) 5.0, (6) 4.8, (7) 4.6.

led to the elimination of some of the contaminating proteins (Fig. 2). For example, the 15kD protein was almost eliminated at pH below 5.2. The highest yield of IgY was obtained between pH 5.0 - 5.2 (Table 1).

Decreasing pH did not only increase recovery of IgY but also led to a decrease of low-density lipoprotein in the supernatant. Similar results were obtained by Sugano and Watanabe (1961) who studied the solubility of lipoproteins as a function of pH at different ionic strengths. They found decreased lipoprotein solubility with lowering of pH up to pH 4.3, especially at low ionic strengths.

It was observed, that when fresh eggs were used without lowering the pH, there was more contamination with lipids and longer incubation time was needed to give a relatively clear supernatant. This is attributable to the fact that in fresh eggs the lipids are combined noncovalently with protein, particularly with lipoprotein and to a small extent in other lipid-protein structures such as yolk membranes and globules (Burley and Vadehra, 1989). Use of old eggs was found to alleviate this problem, requiring less incubation time and less contamination of the WSF with lipids. However, this created problems in the processing of the eggs since the yolk membranes are fragile and high losses were encountered in the separation of yolk from the white. Processing at lower pH provides an added advantage since this allows use of fresh eggs with minimum contamination of the WSF with lipids.

Effect of incubation time

The effect of time of incubation on 10x diluted egg yolk, pH 5.0, on separation of granular proteins from the plasma proteins was investigated over a period of 49 hr at 4°C (Fig. 3). A clear filtrate was obtained after 2 hr. The IgY concentration was found to remain almost constant after 5.5 hr. Samples were therefore routinely incubated for about 6 hr before filtration in the cold.

Effect of sodium chloride concentration

It has been reported that the action of certain substances (e.g., 0.1M NaCl), would cause the yolk granules to settle out slowly leaving a clear supernatant solution (Vadehra and Burley 1978). The effect of sodium chloride on the precipitation of the granules was therefore investigated. Two levels of sodium chloride, 0.16M and 1.5M, were compared to diluted

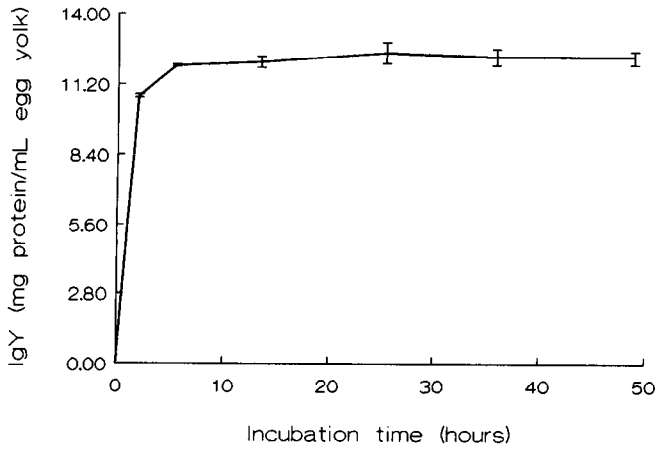

Fig. 3 — Effect of time of incubation on the recovery of IgY in the water-soluble fraction of egg yolk as estimated by RID. Egg yolk was diluted 10x at pH 5.0.

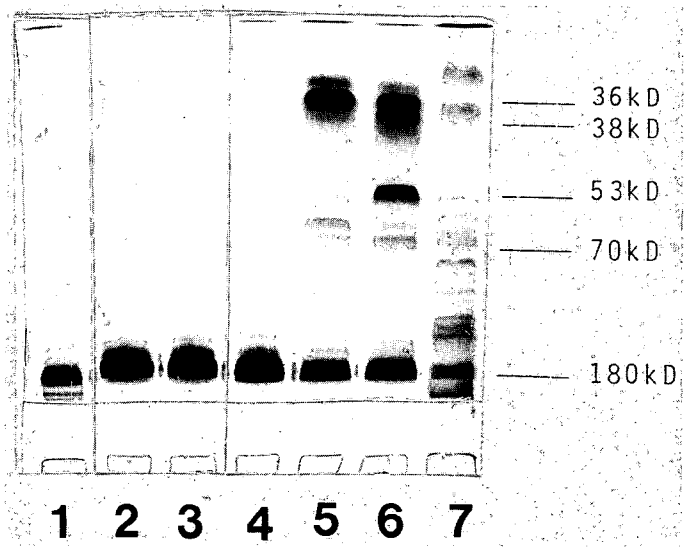

Fig. 4 — SDS-PAGE (nonreducing) on 7.5% gel (Mini-PROTEAN II cell) of IgY at various stages of purification. Lanes: (1) alcohol precipitation, (2) gel filtration, (3) DEAE-Sephacel, (4) ultrafiltration, (5) ammonium sulfate precipitation, (6) water-soluble fraction, pH 5.2, and (7) egg yolk. Egg yolk sample was defatted with chloroform:methanol mixture (3:1) to prevent streaking.

Table 2—Summary of recovery and purity of IgY by different purification techniques[a]

		IgY	
		% Recovery	% Purity
Stage 1	Water-soluble fraction	93–96	15–18
Stage 2	Salt precipitation[b]	89	30
Stage 3	Alcohol precipitation	>99	>93
Stage 3	Ultrafiltration	>98	>93
Stage 4	DEAE-Sephacel	95	>99
Stage 4	Gel filtration	98	>99

[a] Values are averages of two determinations. Recovery and purity of IgY was estimated by SDS-PAGE (Phast System). Total % recovery of IgY at a particular stage (Fig. 1) could be calculated from taking into consideration the reported % recoveries of the preceding steps.

[b] Ammonium sulfate (60% saturation at 0 °C).

egg yolk without sodium chloride. A concentration of 0.16M was used in this work since it is the isotonic concentration of the egg yolk granules. It was chosen with the hope that the granules would be more stable at that salt concentration, thereby reducing the possible contamination of WSF with granular proteins.

Sodium chloride (0.16M) was found to exert an inhibitory effect on the separation of egg yolk granules from the plasma proteins in 10x diluted egg yolk. This inhibitory effect increased with increasing sodium chloride (1.5M) concentration. The inhibitory effect of 0.16M NaCl in this work suggests the importance of low ionic strength for the efficient aggregation of egg yolk granules. The observation that aggregation of granules improved with higher dilution was further implication of the importance of low ionic strength in improving aggregation of granules.

Using pH 5.0 and incubation time of 6 hr, we investigated whether using less than 10x dilution would give similar IgY recovery. Sixfold dilution was found to be effective, giving an IgY recovery of 93–96%. Another approach which gave comparable results was using the WSF from an initial 20-fold diluted egg yolk, for the dilution of fresh egg yolk. This was repeated once more to give 6.6-fold (20x/3x mL egg yolk) final dilution of egg yolk.

Purification of IgY from WSF

After determining the optimum conditions for the separation of the egg granules from the livetins, the next stage of the purification scheme involved isolation of the IgY from the other water-soluble proteins. The major proteins present in the WSF include α– and β–livetins with molecular weight of 70 kD and 42 kD, respectively (Burley and Vadehra, 1979), and low-density lipoproteins.

Experiments were carried out to determine the best concentration of ammonium sulfate for the optimum precipitation of IgY (i.e., maximum yield of IgY with minimum amount of impurities in the WSF). This was achieved by precipitating IgY at different concentrations (50 – 85% saturation of ammonium sulfate at 0°C). Yield and purity were evaluated by SDS-PAGE. Precipitation with 60% saturation gave optimum IgY recovery. Salt precipitation caused selective removal of some contaminating proteins, especially the 38 kD and 53kD fractions with reduction of the 70 kD protein (Fig. 4). A further purification scheme based on molecular weight was a next logical step.

A summary of the recovery and purity of IgY at the various stages of purification is shown in Table 2. It is evident that IgY of high purity with good recovery was obtained with the various purification techniques used in this work. Ultrafiltration of the salt-precipitated proteins gave IgY with high purity. A salt precipitation step preceding UF improved the efficiency, by allowing UF of high-concentration protein solution. The molecular weight cut-off of the UF membrane used was 100 kD. Selective removal of the 70 kD protein also improved the efficiency of UF, since the proteins with molecular weight close to the cut-off value of the membrane are less effectively removed. Direct UF of water-soluble egg yolk proteins under the same conditons as salt-precipitated proteins was found to be inefficient.

Precipitation of IgY with ethanol at sub-zero temperatures after salt precipitation also gave IgY of high purity (over 93%). The yield was almost 100%. However, direct precipitation of IgY in the WSF was not found to be efficient. In another experiment, ammonium sulfate precipitation was followed by precipitating twice with 14% sodium sulfate. This treatment also yielded IgY with high purity (over 96%).

Although the above protocols gave IgY of very high purity, an electrophoretically pure IgY was obtained by further purification using gel filtration (Fig. 5). Several workers (Hersh and Benedict, 1966; Kubo and Benedict, 1969) reported that chicken serum IgG forms polymers with a molecular weight of 560 kD and a sedimentation coefficient of about 14S in 1.5M NaCl. We also found a similar effect of high salt concentration of IgY (reported elsewhere).

Since gel filtration is based on differences in molecular weights of the proteins, high salt concentration was used to polymerize the IgY. This was done by dialyzing the IgY against the high

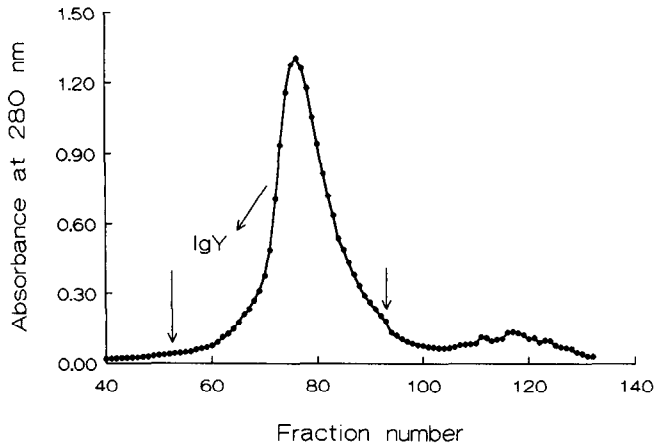

Fig. 5 — Purification of IgY on Sephacryl S-200 column. Column: 2.5 × 90 cm; flow rate, 1.0 mL/min; fractions, 2.5 mL; sample volume, 5 mL, sample concentration; 10 mg/mL. Column was equilibrated with 0.01M PB, pH 7.0 containing 1.5M NaCl. Fractions (indicated by arrows) of IgY peak were pooled and analyzed by SDS-PAGE (Fig. 4, lane 2).

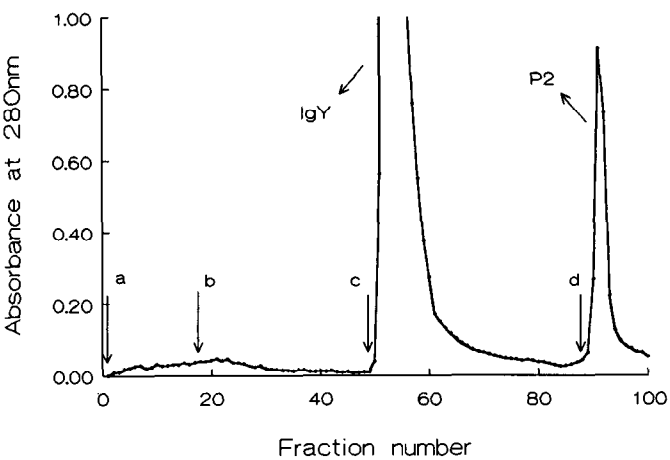

Fig. 6 — Purification of IgY by DEAE-Sephacel anion exchange. Column: 2.0 × 5 cm; flow rate, 1.0 mL/min; fractions, 2.9 mL. Protein solution (50 mL, 2 mg/mL) was applied (a) and washed (b) with 0.025M PB, pH 8.0, IgY was elute (c) with 0.25M PB, pH 8.0. Remaining bound proteins were (d) removed with 0.01M PB, pH 7.0 containing 1.5M NaCl. IgY peak was analyzed by SDS-PAGE (Fig. 4, lane 3).

salt buffer overnight in the cold. Selection of Sephacryl S-200 gel allowed elution of IgY in the void volume thereby improving the efficiency of the purification in terms of both speed and resolution. An additional advantage of gel filtration in high salt is that it eliminates the need to include agents for control of microbial growth.

Electrophoretically pure IgY was also obtained when DEAE-Sephacel anion exchange was used (Fig. 6). However, it was observed with one batch of egg yolk that DEAE-Sephacel anion exchange failed to give electrophoretically pure IgY. The advantage of using ion exchange is its high capacity compared to gel filtration.

Hassl et al. (1987) performed isoelectric focusing with IgY purified by three methods. They found several different bands located between pH 4.3 and 8.3. They concluded that fractionation of egg yolk proteins according to their isoelectric point was not likely to lead to development of a satisfactory purification step at yolk protein bands are very close and linked in isoelectric focusing. We found that direct application of WSF to DEAE-Sephacel anion exchange gave IgY with a lower purity (50 – 60%). Similar results were reported by McCannel and Nakai (1990). They purified water-soluble fraction of egg yolk after treatment with alginate on a DEAE-Sephacel column using two linear gradients of increasing phosphate concentrations. IgY purity of 40.1% was obtained for the first gradient and IgY purities of 59.8% and 18% respectively, for the first and second parts of the second gradient.

It is suggested that anion exchange chromatography should be preceded by other purification methods to remove some of the interfering proteins. This could explain why electrophoretically pure IgY was obtained in our work with DEAE-Sephacel anion exchange since the starting material was very pure (over 93%). Experiments are under way for the use of metal chelate interaction chromatography in the purification scheme of IgY because of its high capacity.

Activity of IgY

ELISA was used to study the activity of IgY produced by different methods. Since the eggs used for this work were laid by hens not immunized against a specific antigen, there was a need to find a suitable marker for antibody activity of IgY. We found that IgY had inherent activity toward formaldehyde-treated *E. coli* (0142:K86:H6), which was therefore used to study the antibody activity. Our results showed that IgY produced by the different purification schemes demonstrated similar antigen binding activities. This was not unexpected since the methods of purification were selected in part because they are mild. Although alcohol is known to denature proteins, under sub-zero conditions this is averted. Polson et al. (1985) also found that cryo-ethanol treatment had no adverse effect on the activity of IgY. However, when ethanol precipitation is done, care should be taken to remove the residual alcohol before drying to avoid possible denaturation of IgY.

CONCLUSIONS

ISOLATION of water-soluble plasma proteins from egg yolk granules was achieved by simple water dilution. Optimum recovery of IgY (93 – 96%) in the WSF was obtained by six-fold water dilution at pH 5.0 with incubation time of 6 hr at 4°C. Among the factors studied, pH was found to be the most important factor affecting IgY recovery. Our recommendation is that an efficient purification procedure should employ salt precipitaton, alcohol precipitation, ultrafiltration or a combination of these in the initial steps, whereas gel filtration or ion exchange should be used as the final steps. The purification scheme which involves "salt precipitation →UF→ Gel filtration" is the most preferred scheme, since it offers the most efficient method from a technical standpoint. Both concentration and buffer change could be done with UF allowing efficient freeze-drying. If further purification is needed, the retentate could be directly applied to gel filtration column. Advantages of the protocol include the following: The procedure is simple, rapid and may allow utilization of the rest of the egg yolk as a food product or further fractionation of other biologically active components. It produces high yields of active IgY with varying levels of purity. It can be easily scaled up for large-scale production of IgY.

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