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To the American Egg Board

FINAL REPORT

Ability of Hemin-binding Antibodies From Egg to Inhibit Lipid Oxidation in Muscle Foods

Eric W. Grunwald and Mark P. Richards

Department of Animal Sciences University of Wisconsin-Madison

EXECUTIVE SUMMARY

Lipid oxidation causes quality deterioration in muscle foods. The hen has been used previously as a bio-reactor to produce antibodies for various purposes. Our goal was to inject hemin bound to bovine gamma globin into laying hens so that antibodies specific for hemin would be deposited in the egg yolks. Those antibodies could then be harvested from the yolk and tested for their ability to inhibit hemin-mediated lipid oxidation in washed muscle tissue. A tight binding of hemin by the specific antibody has the potential to inactivate hemin as a catalyst of lipid oxidation. A previous report also showed that the hen can be used to produce antibodies specific for gamma-human hemoglobin. Therefore we also examined producing antibodies in the hen that could potentially inhibit fish hemoglobin-mediated lipid oxidation.

Antibodies endogenous to egg yolk from hens that received no antigen injection accelerated hemin-mediated lipid oxidation in washed muscle. This may be due to the ability of the native antibody proteins to weakly bind and subsequently deliver hemin into lipid phases of the washed muscle. After injecting hens with hemin-based antigens, it appeared that only a small fraction of the total antibodies obtained were specific for hemin. Also, the amount of hemin binding proteins was not elevated in antibodies from the hens injected with hemin-based antigens compared to control antigens lacking hemin. This may explain the inability of antibodies from hens receiving hemin-based antigens to decrease rates of hemin-mediated lipid oxidation in washed muscle. Alternatively, the hemin specific antibodies may also only weakly bind hemin and thus have a pro-oxidant effect.

When trout hemoglobin was used as the antigen, again a small fraction of the total antibodies obtained appeared to be specific for trout hemoglobin which can explain the inability of antibodies from hens injected with hemoglobin to affect rates of trout hemoglobin-mediated lipid oxidation in washed muscle. Future work could be to fractionate the total antibodies from egg yolks of hens injected with the antigens containing hemin (or hemoglobin) to determine if the desired antibodies can be obtained in sufficient purity and yield to inhibit hemin or hemoglobin-mediated lipid oxidation. However, this would likely not be cost-effective industrially.

Since the antibody preparations from egg yolk were not capable of inhibiting hemin or hemoglobin-mediated lipid oxidation, we investigated the presence of hemin-binding proteins in the underutilized egg shell. The major hemin-binding protein from egg shell was extracted, purified, sequenced, and identified as ovocleidin-116.

INTRODUCTION

Lipid oxidation causes many problems related to quality of muscle foods; odor, flavor, color, texture, and nutritive value are negatively affected (Kanner, 1994). It is likely that the heme proteins, hemoglobin and myoglobin, in muscle foods promote lipid oxidation. Recent studies indicate that the hemin porphyrin group within the globin is the moiety responsible for myoglobin-mediated lipid oxidation in a washed muscle fiber model system (Grunwald and Richards, 2006). The laying hen has been used in the past as a bio-reactor to produce antibodies for various purposes. The antibodies are deposited in the egg yolk after the hen is injected with a specific antigen. For example, injecting hens with phospholipase conjugated to bovine gamma globulin (BGG) caused the hen to produce specific antibodies that were harvested from the yolks and used to inhibit the phospholipase in the digestive tract of fed animals which boosted growth rates without compromising feed efficiency (Cook, 2004). The primary goal of our work was to inject hemin conjugated to BGG into laying hens so that antibodies specific for hemin would be deposited in the egg yolks. Those antibodies could then be harvested from the yolk and tested for their ability to inhibit hemin-mediated lipid oxidation in washed muscle tissue. A tight binding of hemin by the specific antibody has the potential to inactivate hemin as a catalyst of lipid oxidation. A previous report also showed that the hen can be used to produce antibodies specific for gamma-human hemoglobin (Jintaridth et al., 2006). Therefore we also examined producing antibodies in hen that could potentially inhibit fish hemoglobin-mediated lipid oxidation.

MATERIALS AND METHODS

Conjugation and emulsification of Hemin and BgG using Glutaraldehyde

The first antigen that was investigated was hemin. It was conjugated to bovine gamma globulin (BgG) using glutaraldehyde. Twelve mg of BgG are dissolved in 1.8ml of 0.1M sodium acetate, pH7.5. Then 0.3ml of a 40mg/ml solution of hemin dissolved in 0.1M NaOH was mixed into the BgG solution. Finally, 1.56ml of 0.02M glutaraldehyde was mixed into the hemin-BgG solution and the conjugation mixture was mixed on an orbital shaker for 3hr. To stop the reaction, 60mg glycine was mixed into the reaction for 60min. The reaction is dialyzed in the dark for 12-16hr against PBS at 4ºC using a 6-8kDa MWCO dialysis membrane. A carrier control reaction containing 0.3ml of 0.1M NaOH in place of the hemin was also performed. After dialysis, the reactions were centrifuged (3800xg for 10 min at 4ºC) to remove precipitates, concentrated to 4.5ml, and used in the preparation of emulsions for immunization of hens. Emulsions were prepared by combining 1ml conjugation reaction or control (approximately 2.8mg conjugated antigen or control on protein basis) with 1ml PBS and 2ml Freund's adjuvant in a 10ml glass syringe connected to

a second 10ml glass syringe via a 20 gauge emulsion needle and pumping the contents of the syringe between the needles until the pumping became very difficult. A milliliter (approximately 700µg antigen or control on a protein basis) from the 4ml emulsion is transferred to two separate 3ml injection syringes fitted with 18 gauge needles.

Covalent Conjugation and Emulsification of Hemin and BgG

A hemin derivative was prepared by combining 130mg hemin, 50mg Woodward's Reagent K, 10ml acetonitrile, and 300µl triethylamine in a glass reaction tube and mixing for 60 min on a clinical orbital shaker in the dark (Pikuleva and Turko, 1990). Unreacted hemin remains insoluble after the reaction and was pelleted (5000xg for 10 min at 4ºC) using Teflon centrifuge tubes. The activated hemin solution was bubbled with nitrogen to evaporate solvent. Dried activated hemin was stored at 4ºC in the dark until the conjugation reaction. The conjugation reaction was performed by mixing a 3ml volume of 55mg/ml BgG dissolved in 20mM potassium phosphate, 0.1M imidazole, pH 7.2 with the activated hemin that had been dissolved in 500µl methanol and shaking the conjugation reaction 60 min at 350rpm in a culture shaker. Removal of unbound hemin was accomplished by precipitating conjugate with 20ml acid-acetone (200µl 12N HCL in 100ml acetone), and washing twice with acid-acetone and once with water. The precipitated conjugate was suspended in 1ml PBS for a total volume of approximately 2ml and emulsified with 2ml Freund's adjuvant using the method described above. Each 1ml volume in injection syringe contained approximately 40mg of antigen on a protein basis. A control was prepared by combining an identical aliquot of 55mg/ml BgG solution with 20ml acid-acetone and following the same subsequent steps as for the BgG-hemin conjugate.

Hemoglobin (Hb) Preparation and Emulsification

Trout Hb was prepared as described previously (Richards et al., 2002). On day zero Hb, diluted to 1mg/ml with PBS, was emulsified with Freund's complete adjuvant and injected at two points in the *pectoralis* muscle in such a fashion that a total of 500µg of Hb was injected per hen. Boost injections are performed on days 14 and 28 using the same method, except that Freund's incomplete adjuvant is used for emulsification. Eggs were collected on days 23 through 63.

Immunization Schedule

Unless otherwise noted above, the immunization and collection schedule was: On day 0 of a trial of the antigen, hens were given a total of four 250µl injections of the antigen emulsified in Freund's complete adjuvant. Injections were made in each pectoralis and gracilis muscle, and each hen receives 500-1000µg of emulsified antigen. Control hens were injected with an emulsion of a control solution in the same adjuvant. Eggs were collected from all hens beginning on day 23 after the first injection. Boost injections of ≥ 500µg antigen/hen were given to test and control hens on days 14 and 28, and every three months from day 0, using antigen or control solution, respectively, emulsified in Freund's incomplete adjuvant.

IgY Preparation

IgY was purifed from a single egg yolk as described previously (Polson et. al., 1980): The egg yolk was separated from the white using a kitchen separator and volume was determined. Yolk was dissolved by mixing with twice the yolk volume of phosphate buffered saline (PBS). PEG 6000 (3.5% w/v) was dissolved into the mixture by inversion. The mixture was then centrifuged (10 min at 14000xg at 4ºC) to obtain IgY in the supernatant. PEG (8.5%

w/v) was dissolved into supernatant and the mixture was centrifuged again. A pellet containing mostly IgY was obtained and dissolved in a volume of PBS equal to the original yolk volume. IgY in the dissolved pellets was then precipitated with 12% w/v of PEG and centrifuged three consecutive times to sufficiently concentrate them. The pelleted IgY was then dissolved in a volume of PBS equal to one half of the original yolk volume.

Washed Cod Experiments

To determine the effect of IgY on the rate of hemin or Hb induced lipid oxidation, a washed cod muscle model was used. Washed cod was prepared as described previously (Richards et al., 2002). A plug of frozen muscle was thawed, mixed, and the pH adjusted to 5.7. In a typical experiment, eight grams of muscle were weighed into four amber reaction vials. Two IgY-hemin test binding reactions each containing 2.36ml of a 48mg/ml solution of concentrated PEG purified IgY in PBS and 90µl of a 5mM solution of hemin in 0.1M NaOH were prepared and incubated in tubes on ice for at least 1hr. Prior to addition of these solutions to the muscle, water was added so that final moisture content is 90% and streptomycin sulfate was added so final concentration is 200ppm. The components were mixed into the muscle with a plastic spatula for 2 min. In these two test reactions, the final IgY concentration was 60µmol/kg tissue and the final hemin concentration was 40µmol/kg tissue. In two control reactions, 2.36ml PBS and 90µl 5mM hemin in 0.1M NaOH were substituted for the test solutions such that final hemin concentration was 40µmol/kg tissue and IgY was absent. Experiments using Hb in place of hemin were assembled such that the final Hb concentration was 40µmol/kg tissue on a hemin basis. Zero time samples for TBARS were taken immediately upon combining all components of all reactions, pH of the reactions was measured, and reaction vials were stored on ice for the duration of the experiment. Subsequent sampling was preformed daily through day 9. Samples were stored at -80ºC until analysis. The content of TBARS produced in the reactions is determined as previously described (Buege and Aust, 1978).

Hemin Agarose Chromatography

Hemin agarose was prepared as previously described (Tsutsui, 1986). In a typical experiment, the percentage of hemin-binding protein in a preparation of IgY was determined as follows. Approximately 5ml of hemin-agarose resin was packed in a BioRad 10DG column and equilibrated with 20mM sodium phosphate, 0.5M NaCl, pH 7.5 (buffer A). A 2ml volume of a 5mg/ml IgY solution was applied to the column. After sample soaked into resin, volumes of buffer A were applied to column as 5min fractions were collected. After 50ml of buffer A were added, mobile phase was changed to 0.1M acetic acid and 5min fractions collected. OD 280 measurements and fraction volumes were then used to calculate the percentage of the protein preparation that demonstrated hemin-binding capability.

Separation of proteins in antibody preparations

IgY was separated using a MonoQ FPLC anion exchange column. Around 35 mg of protein was loaded for each separation. A linear gradient was used at a flow rate of 0.5 ml/min. Buffer A was 20 mM Tris, pH 8.0 and Buffer B was 20 mM Tris, pH 8.0, 350 mM NaCl.

Ovocleidin-116 Preparation

Previous methods were adapted to purify the hemin-binding matrix proteins from the shells of normal eggs (Mann, 1999, Mann and Siedler, 1999). Approximately 100g of eggshells were rinsed with water and 5% EDTA and pulverized. Shell powder was mixed with 10%

acetic acid overnight at 4ºC. Solution was adjusted to pH 7.50, filtered through cheesecloth, and swirled with hemin-agarose beads overnight. Next day, beads were collected and packed in a BioRad 10DG column. Column was washed with 10mM sodium phosphate, 0.5M NaCl pH 7.5 and hemin binding proteins were eluted with 0.2M citrate, buffer exchanged into PBS, and frozen at -20ºC.

RESULTS

Yield of total antibodies in different IgY preparations. Total antibodies (IgY) were prepared from egg yolks using polyethylene glycol reagent. Around 71 mg of IgY per egg was harvested from control eggs (i.e. those eggs that were not injected with any antigen) (Table 1). Around 80 mg of IgY/egg was obtained when bovine gamma globulin (BGG) was used as the antigen. Cross-linking hemin to BGG with glutaraldehyde [BGG-(glutaraldehyde)-hemin] increased the IgY yield to around 95 mg/egg compared to BGG antigen. This indicated there was a trend ($p=0.12$) that total antibody production was higher from BGG-(glutaraldehyde)-hemin antigen compared to BGG antigen (Table 1). Covalent cross-linking of hemin to BGG was also done using Woodward's K reagent [BGG-(covalent)-hemin]. This covalent BGG-hemin complex resulted in an IgY yield of around 107 mg/egg while the BGG antigen alone resulted in an IgY yield of around 97mg/egg (Table 1). Trout hemoglobin as an antigen was also examined. This was done with the intent of producing antibodies in the yolk that could potentially inhibit trout hemoglobin-mediated lipid oxidation. There was a trend ($p=0.12$) that the total IgY produced was greater when trout hemoglobin-antigen (160 mg/egg) was compared to an injection of the buffer (123 mg/egg) used to deliver the trout hemoglobin (Table 1).

Table 1. Yields of IgY Purified by Polyethylene Glycol (PEG) Method From Eggs of Hens Immunized With Specified Antigens as Determined by Biuret Method

Antigen	Conjugation Method	Quantity of IgY on a mg/egg basis*
none	Uninjected Normal Hen Egg	71.44 ± 22.22 (n=5)
BgG-Hemin	Glutaraldehyde	95.13 ± 14.32 (n=5)
BgG Control		79.45 ± 13.92 (n=5)
BgG-Hemin	Woodward's Reagent K (45-112µg hemin)	106.46 ± 4.33 (n=2)
BgG Control		96.98 ± 21.92 (n=2)
Hemoglobin	None	159.96 ± 35.78 (n=10)
PBS Control		122.91 ± 21.62 (n=3)

* replicates refer to individual IgY preparations of 1-5 eggs

Percent hemin-binding proteins in different IgY preparations. An enzyme-linked immunosorbent assay (ELISA) was not effective as a means to measure the amount of hemin-binding antibodies (e.g. proteins) present in the different IgY preparation. As a replacement method, hemin-agarose resin chromatography was used. In eggs from hens that were not injected with an antigen, around 35% of the total IgY were hemin binding proteins (Table 2). The amount of hemin-binding proteins was not enhanced when BGG-(glutaraldehyde)-hemin antigen was compared to BGG antigen (Table 2). Around 36% of the total IgY were hemin-binding proteins whether the antigen was BGG or BGG-(glutaraldehyde)-hemin (Table 2). The amount of hemin-binding proteins was also not enhanced when BGG-(covalent)-hemin antigen was compared to BGG antigen (Table 2). Around 40% of the total IgY were hemin-binding proteins whether the antigen was BGG or BGG-(covalent)-hemin (Table 2). The percentage of hemin-binding protein (around 28% of total IgY) was not enhanced when comparing trout hemoglobin antigen to an injection of saline (Table 2). Only around 15% of commercially purchased bovine serum albumin exhibited hemin binding properties under our experimental conditions.

Table 2. Percentage of Hemin-binding Protein (HBP) in Polyethylene Glycol (PEG) Yolk Protein Preparations of Immunized Hen Eggs Determined by Hemin-Agarose Chromatography

Antigen	Conjugation Method	% HBP in Preparation
Uninjected Normal Hen Egg	none	35.36 ± 15.50%
BgG-Hemin	Glutaraldehyde	38.70 ± 0.57%
BgG Control		34.50 ± 7.36%
BgG-Hemin	Woodward's K Reagent (45-112ug hemin)	40.37 ± 8.20%
BgG Control		40.95 ± 2.78%
Hemoglobin	none	26.59 ± 10.85%
Buffer Control		30.87 ± 7.45%
BSA[a]	none	14.78 ± 6.75%

* Percentage of HBP was based on OD280 of HBP fractions divided by total OD280 of PEG preparation.

Ability of egg yolk antibodies from hens not injected with antigen to affect hemin-mediated lipid oxidation. Since egg yolk antibodies (IgY) from hens not injected with antigen contained a substantial amount of hemin binding proteins (Table 2), the ability of these antibodies to affect hemin-mediated lipid oxidation was examined. Bovine serum albumin (BSA) was also examined considering it is known to have weak affinity for hemin (Bunn and Jandl, 1968). After 1 day of storage at 2°C, BSA and IgY both enhanced the ability of hemin to promote lipid oxidation in washed cod muscle ($p<0.05$)(Figure 1).

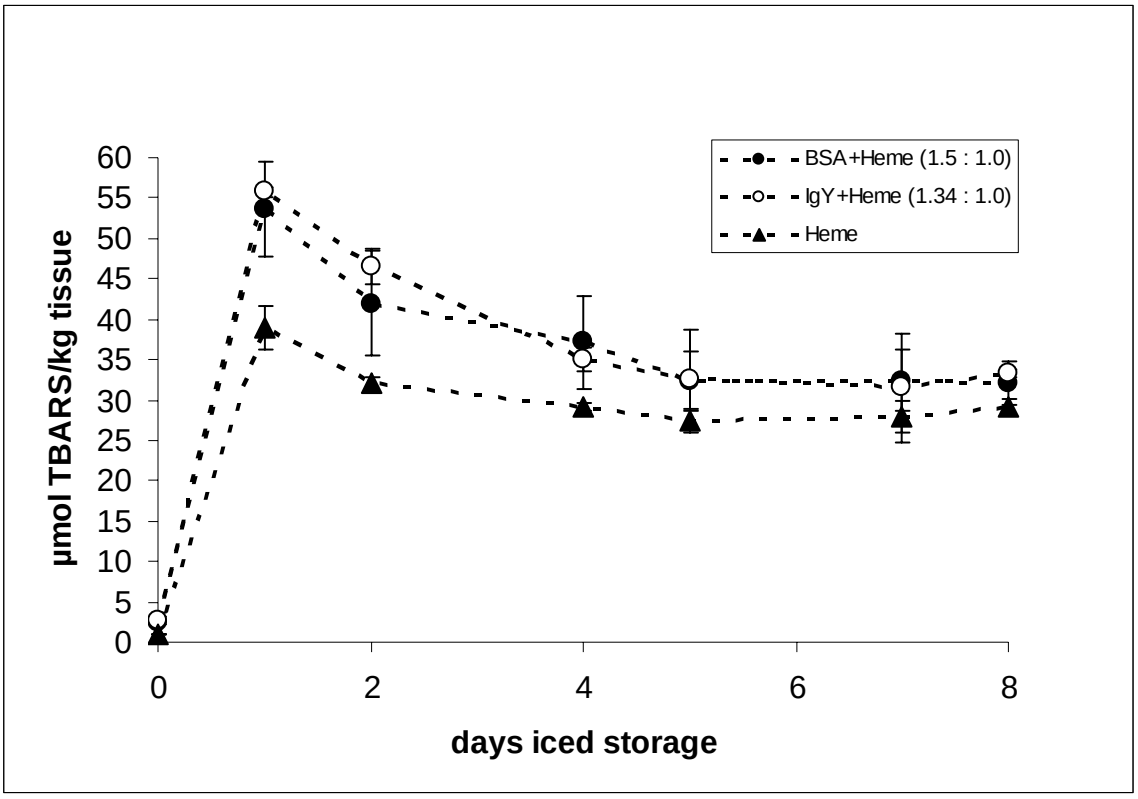

Figure 1. Thiobarbituric acid reactive substances (TBARS) values resulting from hemin-mediated lipid oxidation in washed cod muscle as affected by bovine serum albumin (BSA) and IgY from hens not injected with antigen. pH was 5.8. hemin concentration was 40µmol/kg tissue. BSA and IgY concentration was 54 µmol/kg tissue.

Ability of BGG and BGG-hemin (glutaraldehyde conjugation) to affect hemin-mediated lipid oxidation. Antibodies from BGG and BGG-(glutaraldehyde)-hemin injected hens enhanced the ability of hemin-mediated lipid oxidation ($p<0.05$) (Figure 2). There was no difference between the antibodies from BGG compared to BGG-(glutaraldehyde)-hemin injected hens. Our attempts to add higher levels of antibodies to the washed muscle were limited by the amount of solutes that could be added to the model system.

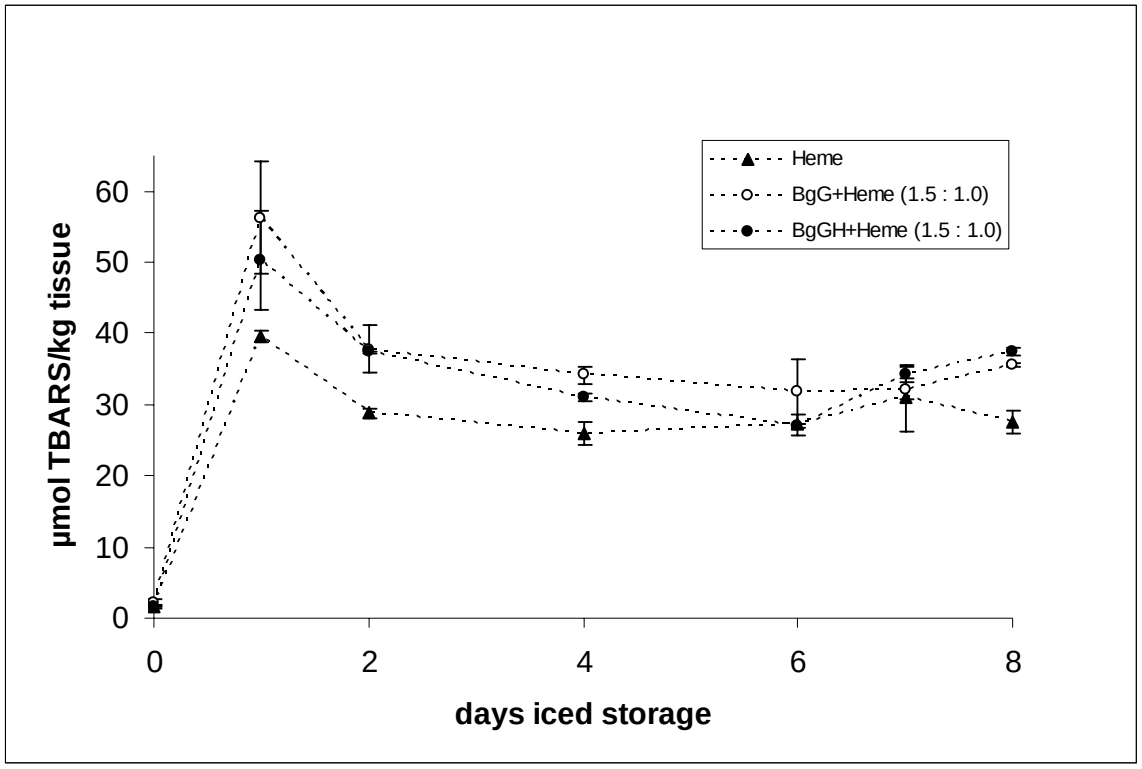

Figure 2. Thiobarbituric acid reactive substances (TBARS) values resulting from hemin-mediated lipid oxidation in washed cod muscle as affected by IgY from BGG injected hens and IgY from hens injected with BGG-hemin crosslinked with glutaraldehyde. pH was 5.8. hemin concentration was 40µmol/kg tissue. IgY concentration was 60 µmol/kg tissue.

Ability of BGG-hemin (covalent conjugation) to inhibit hemin-mediated lipid oxidation. After 1 day of storage at 2°C, there was a trend that BGG and BGG-(covalent)-hemin both enhanced the ability of hemin to promote lipid oxidation in washed cod muscle ($p=0.10$ to 0.16) (Figure 3). There was no difference in TBARS values when comparing the antibodies from BGG injected hens to BGG-(covalent)-hemin injected hens.

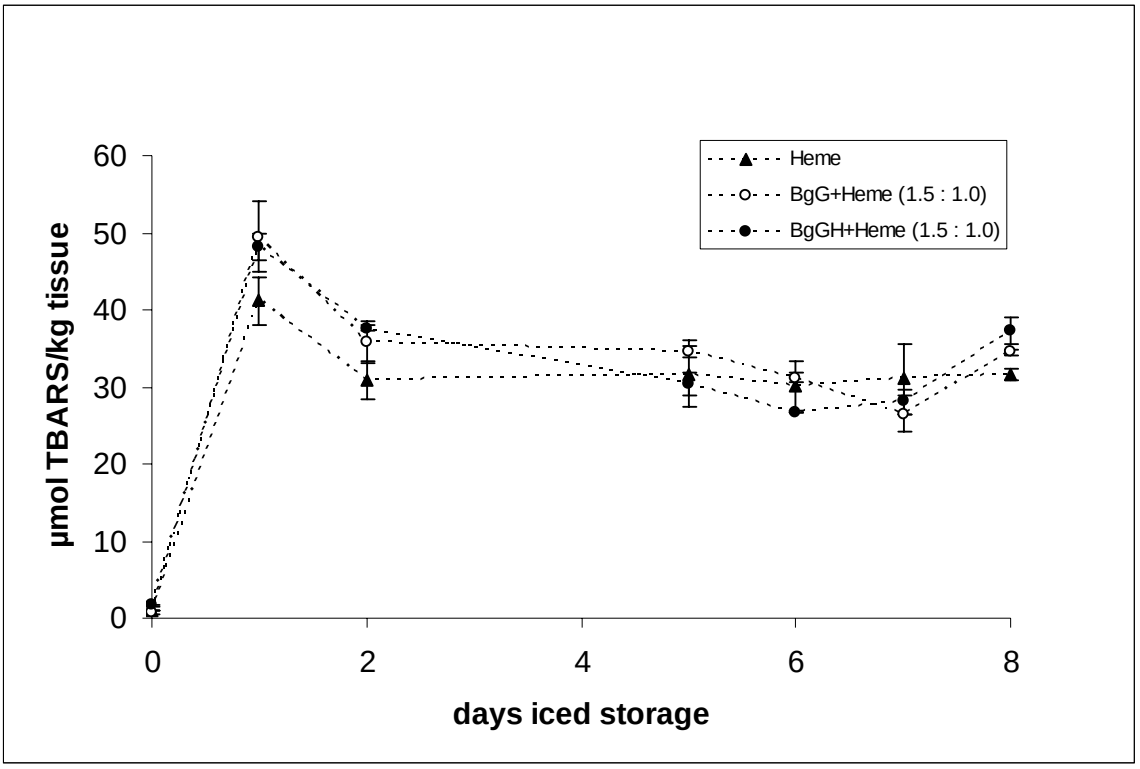

Figure 3. Thiobarbituric acid reactive substances (TBARS) values resulting from hemin-mediated lipid oxidation in washed cod muscle as affected by IgY from BGG injected hens and IgY from hens injected with BGG-hemin covalently crosslinked with Woodward's K reagent. pH was 5.9. hemin concentration was 40µmol/kg tissue. IgY concentration was 60 µmol/kg tissue.

Ability of trout hemoglobin (Hb) antigen to stimulate antibodies that inhibit Hb-mediated lipid oxidation. Antibodies were prepared from hens that were either injected with trout hemoglobin as an antigen or injected with saline as a control. Neither of the antibody preparations affected trout Hb-mediated lipid oxidation in washed muscle (Figure 4). Antibodies were also prepared from seven additional hens that were injected with trout hemoglobin as an antigen (0.5 or 1 mg antigen). Although one of the preparations showed a tendency to inhibit lipid oxidation, the other 6 preparations did not appear to inhibit Hb-mediated lipid oxidation (data not shown).

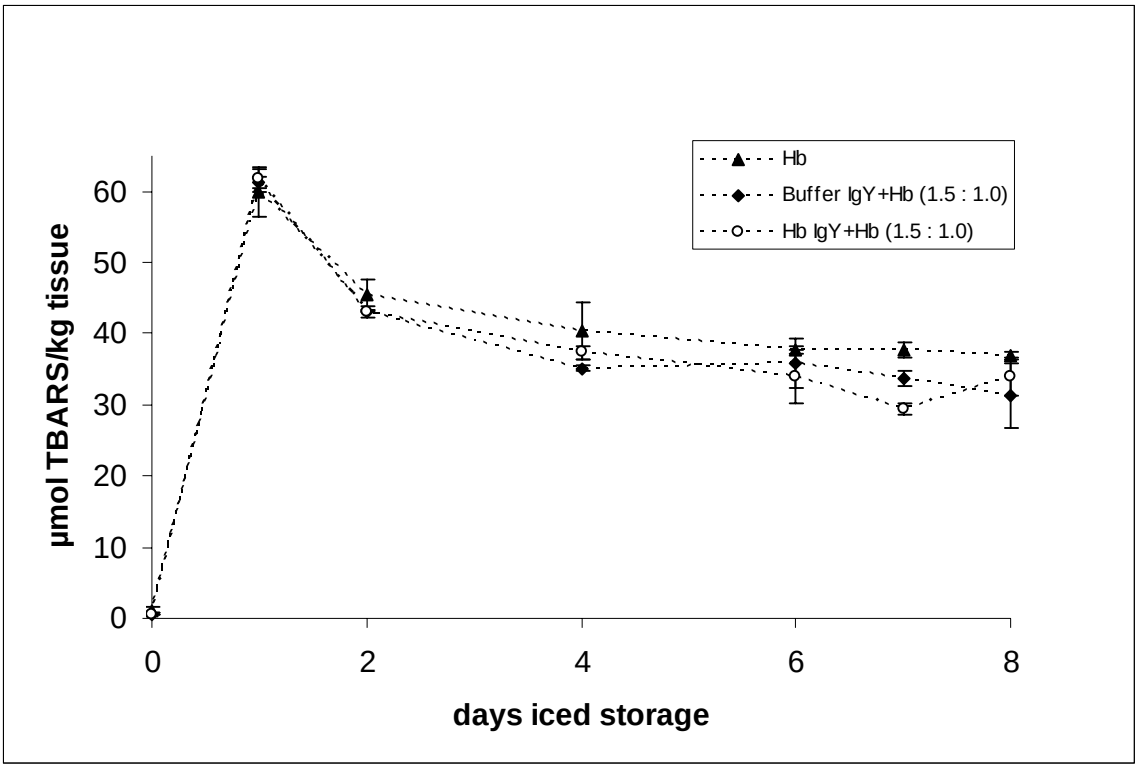

Figure 4. Thiobarbituric acid reactive substances (TBARS) values resulting from trout hemoglobin-mediated lipid oxidation in washed cod muscle as affected by IgY from trout hemoglobin injected hens and IgY from hens injected with saline. pH was 5.8. hemin concentration was 40µmol hemin/kg tissue. IgY concentration was 60 µmol/kg tissue.

Characterization of proteins in IgY from trout hemoglobin-injected hens and hens not injected with antigen. The protein elution profile of IgY from hens not injected with antigen is illustrated (Figure 5). There appears to be additional proteins in the IgY from trout hemoglobin injected hens (Figure 6). These additional peaks may be antibodies with specific reactivity to the injected hemoglobin. The chromatographic separation however was not adequate enough in resolution to evaluate the apparent additional protein bands.

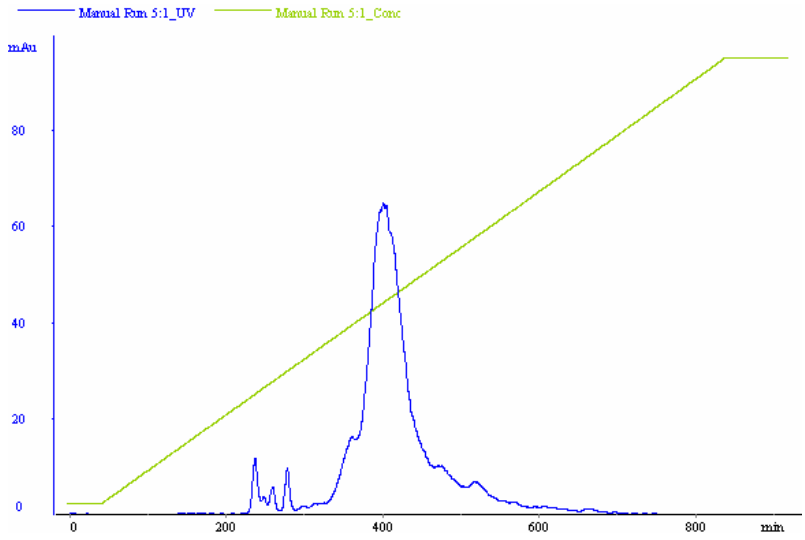

Figure 5. Chromatogram of IgY from hens not injected with antigen.

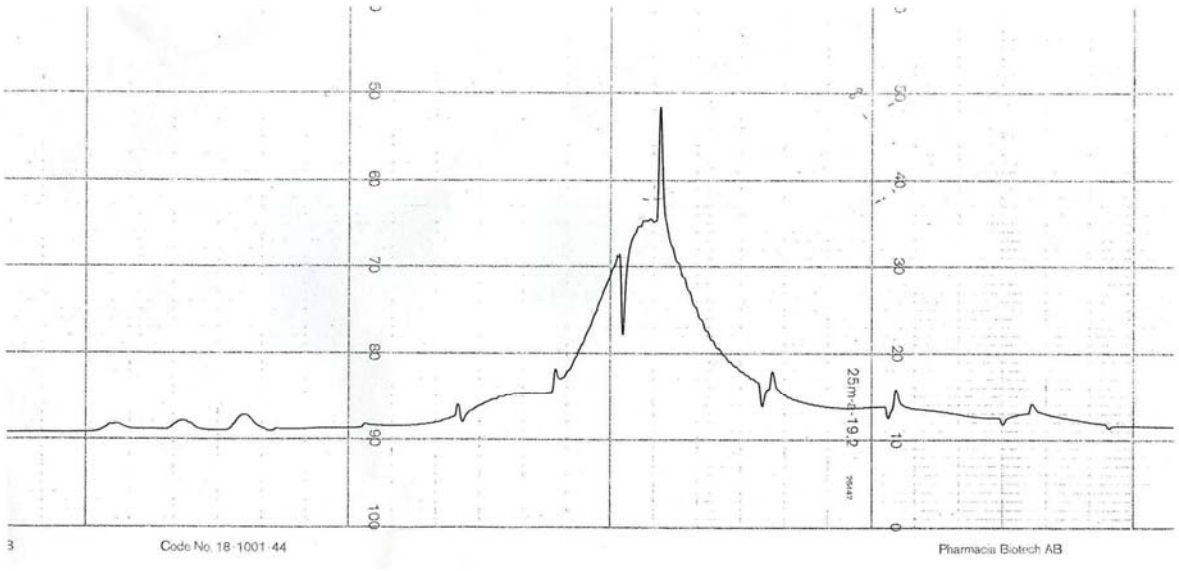

Figure 6. Chromatogram of IgY from hens injected with trout hemoglobin antigen.

Characterization of hemin binding proteins in egg shell. We also wanted to investigate the presence of hemin-binding proteins from egg shell. Hemopexin has been qualitatively reported in egg shell using proteomic analysis (Mann et al., 2006) . Hemopexin has the highest known affinity for hemin and thus may be an effective inhibitor of hemin-mediated lipid oxidation. Hemin agarose resin was used to prepare hemin-binding proteins from egg shell. The extract containing hemin-binding proteins was then exposed to gel electrophoresis.

The primary protein band obtained was submitted for molecular weight determination and amino acid sequencing. The hemin binding protein was identified as Ovocleidin-116.

DISCUSSION

This work assessed the potential of utilizing laying hens to produce antibodies that could be used to inhibit hemin protein-mediated lipid oxidation in muscle-based foods. Three different antigens, used to stimulate antibody production, were tested. Those antigens were hemin bound to bovine gamma globulin (BGG) with glutaraldehyde as the cross-linking agent, hemin covalently linked to BGG through basic residues of BGG and the propionate groups of the hemin moiety, and trout hemoglobin as an antigen.

Bovine serum albumin (BSA) and IgY from non-injected hens enhanced hemin-mediated lipid oxidation (Figure 1). Considering the relatively low solubility of hemin in aqueous phases, it may be that non-specific proteins such as BSA or non-specific IgY weakly bind but facilitate the transport of hemin to the membrane phases in washed cod where the hemin breaks down pre-formed lipid hydroperoxides and thereby initiates lipid oxidation (Tappel, 1955). This could explain the pro-oxidant effect of IgY proteins from egg yolks of hens not injected with antigens and BSA which is known to have a weak affinity for hemin (Bunn and Jandl, 1968). It was hoped that injecting BGG-hemin complexes into laying hens would stimulate the production of specific antibodies that tightly bind and inactivate hemin. When IgY from BGG-hemin injected hens was added to washed cod containing added hemin, a pro-oxidant effect was observed whether the complex was formed with glutaraldehyde (Figure 2) or covalently (Figure 3). It can be seen that the total IgY produced was only modestly enhanced by injecting BGG-hemin compared to BGG injected controls (Table 1). Also, the amount of hemin binding proteins was not elevated in IgY from the hens injected with BGG-hemin (Table 2). It may be that an insufficient amount of hemin-specific antibodies were secreted to incur an inhibitory effect; rather the excess of non-specific IgY was driving hemin into the membrane. Future work could be to scale up the IgY preparations, chromatographically separate the various proteins in the total IgY preparation and test each sub-fraction for its ability to inhibit hemin-mediated lipid oxidation.

One explanation for a poor production of hemin-specific antibodies is that hemin may have bound to BGG in folds of the globin so that the immune response of the hen could not adequately detect the hemin portion of the foreign BGG-hemin complex. It is also possible

that an adequate amount of hemin-specific antibodies were produced but even these specific proteins may have only a weak affinity for hemin and therefore, like the non-specific IgY, facilitate transport of hemin to the lipid substrate.

Antibody preparations prepared from hens that were stimulated by trout hemoglobin antigen did not inhibit trout hemoglobin-mediated lipid oxidation in washed cod muscle (Figure 4). The total amount of antibody was only modestly enhanced by injecting the hemoglobin antigen compared to the saline control (Table 1). There may not be adequate amounts of hemoglobin-specific antibodies present to inhibit hemoglobin-mediated lipid oxidation. The protein chromatograms of IgY from hens not injected with antigen compared to those injected with Hb show somewhat different profiles (Figure 5 and 6). It would be of interest to examine individual proteins unique to the IgY from hemoglobin-injected hens. The possibility also exists that a specific antibody-hemoglobin complex was formed in washed muscle but was not chemically capable of preventing reactivity of the hemoglobin molecule or its subunits with the lipid substrate.

We did observe that the hemoglobin antigen solution rapidly turned brown when forming the adjuvant containing-emulsion that was to be injected into the hens. This indicates oxidation of the hemoglobin by some components in the adjuvant. Oxidized hemoglobin is more prone to denaturation than reduced hemoglobin (Hargrove and Olson, 1996). The oxidation facilitates denaturation of the globin which will likely decrease the ability of the immune system of the hen to produce antibodies specific to the trout hemoglobin. Thus, it is recommended to use fresh adjuvant when working with heme proteins as antigens. The monooleate in the adjuvant has been noted to be susceptible to peroxidation, which will promote oxidation of the hemoglobin (Nagy et al., 2005).

In conclusion, antibodies endogenous to egg yolk from hens that received no antigen injection accelerated hemin-mediated lipid oxidation in washed muscle. This may be due to the ability of the antibody proteins to weakly bind and transport hemin into lipid phases of the washed muscle. After injecting hens with hemin-based antigens, it appeared that only a small fraction of the total antibodies obtained were specific for the hemin-based antigens. Also, the amount of hemin binding proteins was not elevated in IgY from the hens injected with hemin-based antigens. This may explain the inability of antibodies from hens receiving hemin-based antigens to decrease rates of hemin-mediated lipid oxidation in washed muscle. Alternatively, the hemin specific antibodies may also only weakly bind hemin and thus have

a pro-oxidant effect. When trout hemoglobin was used as the antigen, again a small fraction of the total antibodies obtained appeared to be specific for trout hemoglobin which can explain the inability of antibodies from hens injected with hemoglobin to affect rates of trout hemoglobin-mediated lipid oxidation in washed muscle. Future work could be to fractionate the total IgY antibodies obtained from egg yolks of hens injected with the antigens containing hemin or hemoglobin to determine if the desired antibodies can be obtained in sufficient purity to inhibit hemin and hemoglobin-mediated lipid oxidation.

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