Higher incorporation of heterologous chicken immunoglobulin Y compared with homologous quail immunoglobulin Y into egg yolks of Japanese quail (Coturnix japonica)

H.-D. Bae, K. Kitaguchi, F. Horio, and A. Murai

Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, 464-8601 Nagoya, Japan

ABSTRACT In avian species, blood IgY is selectively incorporated into the yolks of maturing oocytes, although the precise mechanism is poorly understood. Our previous study showed that 22% of i.v.-injected heterologous chicken IgY (cIgY) was incorporated into egg yolks of Japanese quail (Coturnix japonica). However, it is not known whether homologous quail IgY (qIgY) can be more efficiently incorporated into quail egg yolks than cIgY. Therefore, we compared the uptakes of qIgY and cIgY i.v. administered into quail egg yolks and further characterized the uptakes of these 2 antibodies into quail ovarian follicles. Quail IgY and cIgY purified from the blood of the respective bird were labeled with digoxigenin, and their uptakes into quail egg yolks were determined by ELISA. Unexpectedly, total incorporation of the injected qIgY was only one-third of that of cIgY, although much more qIgY was left in blood compared with cIgY, suggesting that qIgY is the less preferable antibody as a transport ligand into quail egg yolks. On the other hand, deposition of the qIgY into heart, lung, liver, spleen, kidney, and ovarian follicular membrane was markedly higher than that of cIgY. Amino acid sequence analysis of 3 peptides derived from the trypsin-digested qIgY heavy chain revealed low homology between qIgY and cIgY. In conclusion, our results show that heterologous cIgY is more efficiently incorporated into quail egg yolks than homologous qIgY, possibly due to a distinctive antibody transport system existing in oocytes. The present results also may provide a new strategy for delivering useful proteinaceous substances into egg yolks in an attempt to produce designer eggs.

Key words: quail, chicken, immunoglobulin Y, homologous, heterologous

INTRODUCTION

A recently developed genetic engineering technique has enabled us to utilize poultry eggs for the production of recombinant Ig (Kamihira et al., 2005; Zhu et al., 2005). Either the albumen fraction or the yolk fraction is a feasible target for the deposition and encapsulation of newly synthesized Ig in poultry eggs. Because a mammalian IgG-like molecule, referred to as IgY, is selectively incorporated into egg yolks, delivery of synthesized Ig-like molecules into egg yolks is a possible means of retrieving them from the body of the hen. In general, the major constituents of egg yolks are transported from blood to the ovarian follicles by receptor-mediated endocytosis at the oocyte plasma membrane (Shen et al., 1993). Immunoglobulin Y is also believed to be incorporated into the yolks by receptor-mediated endocytosis (Loeken and Roth, 1983; Morrison et al., 2001), although the relevant receptor involved in IgY transport is still unidentified. Therefore, investigation of the structural requirements for efficient Ig transport into ovarian follicles may offer a new perspective on the utilization of eggs as harvesters or designer foods by fortifying the transport efficiency of the newly synthesized Ig and Ig-like molecules.

In our previous report, the Ig structure required for effective transport into ovarian follicles was investigated by using Japanese quail (Coturnix japonica; Kitaguchi et al., 2008b). The quail has a small body size and excellent egg productivity, and these characteristics are advantageous for monitoring the transport of exogenously injected Ig into ovarian follicles. Our study has shown that 22% of i.v.-injected heterologous chicken IgY (cIgY) was incorporated into the egg yolks of quail and intact IgY or its Fc fragments were incorporated into yolks more effectively than Fab and F(ab')2 fragments. These results suggest that an IgY-Fc carrier protein or specific IgY-Fc receptor exists on the oocyte membrane, and the Fc region is required for its
effective transport into egg yolks. In addition to clgY, mammalian IgG and IgA are also incorporated into egg yolks when i.v. administered to laying chickens and quails (Morrison et al., 2001; Kitaguchi et al., 2008b). However, the uptakes of human IgG and human IgA into quail egg yolks were markedly lower than clgY uptake, probably due to interspecies differences in Ig structure and amino acid sequences. Conceivably then, homologous Ig, namely quail IgY (qIgY), is the most promising candidate to be effectively incorporated into egg yolks of quail. It has been reported that qIgY and clgY are similar in several respects, including sedimentation coefficients, molecular weights, and the yielding pattern of their heavy and light chains under reducing conditions (Hersh et al., 1969; Leslie and Benedict, 1969). However, information on the genome or amino acid sequences of qIgY is very limited, which is disadvantageous for investigation of the structural requirements for efficient Ig transport into egg yolks. Indeed, uptake of exogenously injected qIgY into egg yolks has not previously been investigated in any avian species.

In the present study, we examined the uptake of homologous qIgY and heterologous clgY into quail egg yolks after i.v. administration and further characterized the uptakes of qIgY and clgY into quail ovarian follicles. Serum-derived qIgY and clgY were purified from blood serum of each bird using a combination of ammonium sulfate precipitation and ion-exchange chromatography. Briefly, blood samples were collected from laying quail and chickens. After centrifugation of blood samples, the collected serum was mixed with an equal volume of 40% ammonium sulfate. After incubation for 4 h, the mixed solution was centrifuged at 12,000 × g for 30 min, and the precipitate was dissolved in PBS. To remove lipids, sodium dextran sulfate (Sigma-Aldrich Inc., St. Louis, MO) was added to the sample PBS solution (Agne et al., 1983), and then the solution was centrifuged at 3,000 × g for 30 min at 4°C. The obtained supernatant was dialyzed against 20 mM phosphate buffer. The final solution was filtered through a sterile 0.45-μm filter (Millipore Corp., Cork, Ireland), and IgY in the clear supernatant were further purified by anion-exchange chromatography with a column (13 × 12 cm i.d.) packed with diethylenoethyl cellulose DE-52 (Whatman Corp., Kent, UK) and equilibrated with 0.15 M Tris-HCl (pH 8.0) buffer including 1 M urea at a flow rate of 0.8 mL/min. The sample load was between 0.5 and 5 mg of protein. The trapped IgY in the column was eluted by the same buffer with a concentration gradient of 0 to 1 M NaCl over 20 min. The purity of eluted samples was analyzed by 5% SDS-PAGE under reducing and nonreducing conditions. The gels were stained with 0.5% Coomassie Brilliant Blue R-250. The purified serum qIgY and clgY were labeled with digoxigenin (DIG) using a DIG protein labeling kit (Roche Diagnostics Corp., Mannheim, Germany) according to the recommendations of the manufacturer. The protein concentrations of the DIG-labeled qIgY and clgY were determined by the Lowry method (Lowry et al., 1951).

Intravenous Injection and Isolation of the DIG-Labeled qIgY and clgY in Quail Egg Yolks

The DIG-labeled qIgY and clgY were dissolved in PBS (100 μg/mL), and 20 μg of each IgY was injected into the wing vein of regularly laying quail (5 birds each). All laid eggs were collected for 6 d after the injection and were stored at 4°C until analysis. The isolation of the DIG-labeled IgY from yolk was carried out by the method of Akita and Nakai (1993) with minor modifications (Kitaguchi et al., 2008a). Briefly, egg yolk was separated from egg white. The yolk membrane was punctured and the whole yolk was allowed to drain into a glass dish and was mixed. One gram of the well-mixed yolk was transferred into a 50-mL polypropylene tube. The yolk sample was diluted with 9 volumes of distilled water acidified to pH 5.1 with 0.1 N HCl and stored at 4°C overnight, then centrifuged at 10,000 × g for 25 min at 4°C. The supernatant was collected and an equal volume of 40% saturated ammonium sulfate was gently added. After incubation for 4 h at room temperature, the sample was centrifuged at 12,000 × g for 30 min at 4°C, and then the supernatant was removed. The obtained pellet was dissolved in 1 mL of PBS and

**MATERIALS AND METHODS**

**Experimental Birds and Management**

Female Japanese quail and female Single Comb White Leghorn-type commercial chickens (Dekalb) were purchased from a local hatchery (Cyubu-Kagaku-Shizai Co., Nagoya, Japan) and maintained individually with free access to water and a commercial diet (Uzura-mash for quail; Toyohashi Feed Mills Corp., Toyohashi, Japan; S-seven for chicken; Nosan Corp., Yokohama, Japan). The photoperiod was set at 16L:8D during the experiment. Room temperature was controlled at 23 ± 2°C. Egg production was recorded daily, and continuously laying 7-wk-old quail and 40-wk-old chickens were used for the animal experiments. Animal care was in full compliance with the guidelines of the Nagoya University Policy on Animal Care and Use.

**Purification of Serum IgY and Labeling**

Quail IgY and clgY were purified from blood serum of each bird using a combination of ammonium sulfate precipitation and ion-exchange chromatography. Briefly, blood samples were collected from laying quail and chickens. After centrifugation of blood samples, the collected serum was mixed with an equal volume of 40% ammonium sulfate. After incubation for 4 h, the mixed solution was centrifuged at 12,000 × g for 30 min, and the precipitate was dissolved in PBS. To remove lipids, sodium dextran sulfate (Sigma-Aldrich Inc., St. Louis, MO) was added to the sample PBS solution (Agne et al., 1983), and then the solution was centrifuged at 3,000 × g for 30 min at 4°C. The obtained supernatant was dialyzed against 20 mM phosphate buffer. The final solution was filtered through a sterile 0.45-μm filter (Millipore Corp., Cork, Ireland), and IgY in the clear supernatant were further purified by anion-exchange chromatography with a column (13 × 12 cm i.d.) packed with diethylenoethyl cellulose DE-52 (Whatman Corp., Kent, UK) and equilibrated with 0.15 M Tris-HCl (pH 8.0) buffer including 1 M urea at a flow rate of 0.8 mL/min. The sample load was between 0.5 and 5 mg of protein. The trapped IgY in the column was eluted by the same buffer with a concentration gradient of 0 to 1 M NaCl over 20 min. The purity of eluted samples was analyzed by 5% SDS-PAGE under reducing and nonreducing conditions. The gels were stained with 0.5% Coomassie Brilliant Blue R-250. The purified serum qIgY and clgY were labeled with digoxigenin (DIG) using a DIG protein labeling kit (Roche Diagnostics Corp., Mannheim, Germany) according to the recommendations of the manufacturer. The protein concentrations of the DIG-labeled qIgY and clgY were determined by the Lowry method (Lowry et al., 1951).
dialyzed against 20 mM phosphate buffer at 4°C overnight. The dialyzed sample was used for determination of the DIG-labeled IgY concentration by ELISA.

**Measurement of Isolated DIG-Labeled qIgY and cIgY Concentration in Egg Yolks by ELISA**

Microtiter plates with 96 wells were coated with 100 µL/well of sheep anti-DIG (1:500; Roche Diagnostics Corp.) in 50 mM sodium carbonate (pH 9.6) for 60 min at room temperature. After washing 3 times with washing buffer [0.14 M NaCl, 50 mM Tris-HCl at pH 8.0 with 0.05% (vol/vol) Tween 20], the plates were blocked by adding 200 µL/well of blocking solution [0.14 M NaCl, 50 mM Tris-HCl at pH 8.0 with 1% (vol/vol) BSA] and incubated for 30 min, then washed an additional 3 times. The plates were then incubated with standards (DIG-labeled qIgY and cIgY) and samples at 100 µL/well for 60 min. The standards were serially diluted within the range of 1 to 100 ng/mL, and the samples were diluted 50-fold. The plates were then washed 5 times and incubated for 1 h with 100 µL/well of horseradish peroxidase-conjugated sheep anti-DIG IgG Fab fragment (1:3,000; Roche Diagnostics Corp.). The plates were washed in an additional 5 times, and a color reaction was initiated by adding 100 µL of o-phenylenediamine solution [50 mM citric acid and 50 mM Na2HPO4 at pH 5.0 containing an o-phenylenediamine tablet (Sigma-Aldrich Inc.) with 0.01% (vol/vol) H2O2] for 5 min. The reaction mixture was terminated by adding 100 µL of 3 M H2SO4, and absorbance was measured at a wavelength of 490 nm with a microtiter plate reader.

**Measurement of DIG-Labeled qIgY and cIgY Half-Life in Blood Circulation**

To determine the half-life of DIG-labeled IgY, birds were injected i.v. with 20 µg of either DIG-labeled cIgY or qIgY (6 birds each). Blood samples were collected at 1, 6, 12, and 24 h postinjection. The concentration of DIG-labeled IgY in serum was determined by ELISA. The half-life of the DIG-labeled IgY in blood circulation was calculated by fitting with the 1-compartment model.

**Measurement of DIG-Labeled qIgY and cIgY Deposition in Body Tissues by Western Blotting Analysis**

To determine the tissue distribution of DIG-labeled qIgY and cIgY, birds (4 birds each) were injected i.v. with 300 µg of qIgY or cIgY dissolved in 1 mL of PBS. At 6 h after the injection, the birds were killed by decapitation, and the heart, lung, spleen, liver, kidney, and ovarian follicles were collected. The ovarian follicles were separated into follicular membranes and yolk fractions. All tissue samples were quickly frozen and stored at −80°C until analysis. The yolks were diluted with 9 volumes of distilled water for isolation of the DIG-labeled IgY. The homogenates were centrifuged to remove debris (12,000 × g for 30 min). After the centrifugation, the protein concentration in the supernatant was determined by the Lowry method and the tissue homogenates were frozen at −80°C until Western blotting analysis.

**Peptide Sequencing of the Trypsin-Digested qIgY Heavy Chain**

To isolate the qIgY heavy chain consisting of variable region and constant region domains, the purified qIgY derived from blood was separated by SDS-PAGE under nonreducing conditions. The proteins separated by electrophoresis were transferred onto polyvinylidene difluoride membranes, and the blotting membranes were blocked with 5% skimmed milk to prevent nonspecific binding. The membranes were incubated with horseradish peroxidase-conjugated sheep anti-DIG IgG Fab fragment (1:1,000) at 4°C overnight. The DIG-labeled IgY on the membrane were visualized by the chemiluminescence detection method (SuperSignal West Dura Extended Duration Substrate; Pierce Biotechnology Inc., Rockford, IL) with a charge-coupled device camera (AE6960/C; Atto, Tokyo, Japan). The visualized data were analyzed by densitometric image analysis software (Atto).

**Statistical Analysis**

The means were compared by Student’s t-test. All error bars were expressed as the SEM, and differences between means were considered to be significant at *P*
RESULTS

Purification of IgY and Quantification of DIG-Labeled IgY

Before the administration of purified qIgY and cIgY, the purities of both molecules were verified by SDS-PAGE under nonreducing and reducing conditions (Figure 1). Under nonreducing conditions (Figure 1, panel A), qIgY was detected as a single band of 170 kDa, whereas cIgY was detected as a band of over 180 kDa. Similar results were obtained when qIgY and cIgY were electrophoresed on urea-formic acid starch gels (Leslie and Benedict, 1969). Two clear bands corresponding to the heavy (68 kDa) and light chain (25 kDa) were also detected under reducing conditions (Figure 1, panel B) in both qIgY and cIgY. Densitometry analysis demonstrated that the isolated qIgY and cIgY were of >95% purity. These results suggest that highly purified qIgY and cIgY were successfully prepared by the combination of ammonium sulfate precipitation and HPLC.

The purified qIgY and cIgY were labeled with DIG to discriminate them from the endogenous qIgY in body fluids after i.v. injection. Serial dilutions of DIG-labeled IgY ranging from 1 to 100 ng/mL produced dose-dependent antibody titration curves in ELISA (Figure 2). The half maximal absorbance of the DIG-labeled qIgY standard curve and that of the DIG-labeled cIgY standard curve were reached at 9.4 and 8.7 ng/mL, respectively. The endogenous qIgY included in the yolk extract and serum sample was not detected in this assay, showing that DIG-labeled IgY can be clearly distinguished from endogenous IgY and can be quantified by ELISA.

Incorporation of DIG-Labeled qIgY and cIgY in Sequentially Laid Eggs

To compare the qIgY and cIgY uptakes, regularly laying quail were injected i.v. with 20 µg of either DIG-labeled qIgY or DIG-labeled cIgY, and the presence of these molecules in the egg yolks was measured by ELISA. On the next day, the injected DIG-labeled qIgY and cIgY were detected in the egg yolks (Figure 3). Both incorporations reached their maximal level at 2 d after the injection, then gradually decreased until...
reaching the basal level at 6 d after the injection. Unexpectedly, the incorporation of DIG-labeled qIgY was significantly lower than that of DIG-labeled cIgY at 2 to 4 d after the injection (P ≤ 0.05), and the total uptake of qIgY was approximately one-third that of cIgY (2.11 ± 0.40 µg for qIgY vs. 6.70 ± 0.62 µg for cIgY). Namely, approximately 11% of the injected qIgY was recovered from egg yolks, whereas 34% of the injected cIgY was recovered from egg yolks.

### Blood Clearance Kinetics of DIG-Labeled qIgY and cIgY

To investigate why qIgY uptake was lower than cIgY uptake in the egg yolks of quail, the blood clearance kinetics of the injected IgY was studied by fitting with a 1-compartment model. The injected DIG-labeled qIgY and cIgY concentrations in blood decreased time-dependent (after 1 to 24 h of the injection) and approached the basal level, but the qIgY concentrations were always higher than the cIgY concentrations at all time points (Figure 4). A 1-compartment model was applied to each set of time-clearance curves to determine the initial concentration (C₀; µg/mL), volume of distribution (mL/g), and half-life (h). The area under the curve (µg·h/mL) was also calculated by using C₀. The half-life was 1.3-fold longer for the DIG-labeled qIgY (5.98 ± 0.55 h) than for the DIG-labeled cIgY (4.63 ± 0.1 h), and the area under the curve was 1.5-fold higher for the DIG-labeled qIgY than for the cIgY (Table 1). The C₀ and volume of distribution values were not significantly different between DIG-labeled qIgY and cIgY, suggesting that both DIG-labeled IgY were distributed to body tissues to a similar extent after the injection.

### Tissue Distribution of DIG-Labeled qIgY and cIgY

The blood clearance kinetics revealed that the i.v.-injected qIgY and cIgY were almost equally distributed to body tissues, although the total uptake of qIgY in egg yolks was approximately one-third that of cIgY. These results raised the possibility that the injected DIG-labeled qIgY might be more preferentially distributed to normal body tissues rather than to the inside of ovarian follicles (i.e., egg yolks). Accordingly, the i.v.-injected DIG-labeled IgY were detected by Western blotting analysis, and then depositions of the injected IgY in various body tissues were compared between qIgY and cIgY. At 6 h after the injection, both intact

### Table 1. Blood clearance kinetics of i.v.-injected digoxigenin (DIG)-labeled quail IgY (qIgY) and DIG-labeled chicken IgY (cIgY) in laying quail

<table>
<thead>
<tr>
<th>DIG-labeled IgY</th>
<th>t₁/₂ (h)</th>
<th>C₀ (µg/mL)</th>
<th>Vd (mL/g)</th>
<th>AUC (µg·h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qIgY</td>
<td>5.98 ± 0.55ᵃ</td>
<td>1.34 ± 0.06</td>
<td>0.11 ± 0.01</td>
<td>11.58 ± 1.26ᵇ</td>
</tr>
<tr>
<td>cIgY</td>
<td>4.63 ± 0.10ᵇ</td>
<td>1.18 ± 0.07</td>
<td>0.09 ± 0.01</td>
<td>7.91 ± 0.55ᵇ</td>
</tr>
</tbody>
</table>

ᵃᵇValue is mean ± SEM of 6 birds. Mean values that have different letters within a column are different at P ≤ 0.05.

ᵇThe kinetic parameters of IgY in serum circulation were calculated by fitting a 1-compartment model. t₁/₂ = half-life; C₀ = initial concentration in serum; Vd = volume of distribution; AUC = area under the curve.
qIgY and intact cIgY molecules were detected in the heart, lung, liver, spleen, kidney, and ovarian follicular membranes (Figure 5, panel A). Standard curves of the DIG-labeled cIgY and qIgY concentration versus luminescence intensity showed that both could be quantitated to low nanogram concentrations. In all tissues analyzed here, the deposition of DIG-labeled qIgY was significantly higher than that of DIG-labeled cIgY, as expected (Figure 5, panel B). Even in the ovarian follicular membranes, the deposition of the DIG-labeled qIgY was surprisingly higher than that of DIG-labeled cIgY ($P \leq 0.01$ for F1 and $P \leq 0.05$ for F2). Nevertheless, the reverse was true for incorporation of DIG-labeled IgY into egg yolks; the deposition of DIG-labeled qIgY in yolks was one-fifth of the DIG-labeled cIgY ($P \leq 0.01$).

**Sequence Analysis of Trypsin-Digested qIgY Heavy Chain**

The N-terminal region of the 3 peptides derived from the trypsin-digested qIgY heavy chain was sequenced. One of the 3 peptides had a sequence with GFRPR (Table 2), which is 100% identical to the cIgY heavy chain at residues 427 to 431 within the CH4 domain (Parvari et al., 1988). In contrast, the amino acid sequences of the other 2 peptides (PSGEVLR and LRDGDKGEKC) had a low homology to that of the cIgY heavy chain; the alignment score of the 2 peptides was approximately 40% when analyzed by Clustal W (http://align.genome.jp/). By using the obtained amino acid sequences, we designed degenerate PCR primers to amplify the qIgY heavy chain gene. However, no target

---

**Figure 5.** Measurement of digoxigenin (DIG)-labeled quail IgY (qIgY) and chicken IgY (cIgY) tissue distribution. The birds were i.v. injected with 300 µg of either DIG-labeled qIgY or cIgY. Six hours after the injection, tissue samples were collected for Western blotting analysis. (A) The DIG-labeled IgY deposited in various tissues and standards (DIG-labeled qIgY and cIgY) were visualized. (B) The concentration of each sample was calculated by linear regression lines of standard (0.5, 0.3, and 0.1 ng/lane for qIgY; 0.5, 0.3, 0.1, and 0.05 ng/lane for cIgY). Each band was quantitated by a densitometric image analysis. Vertical bar indicates mean ± SEM of 4 birds. *$P \leq 0.05$; **$P \leq 0.01$. F1 = largest follicle; F2 = second largest follicle; M = membranes.
product was amplified by degenerate reverse transcription-PCR with quail spleen cDNA as a template (data not shown).

**DISCUSSION**

Contrary to our expectation, qIgY was less efficiently incorporated into quail egg yolks than cIgY when it was administered to Japanese quail, implying that heterologous cIgY is far preferable as a ligand for transport into the egg yolks of quail compared with homologous qIgY. This unanticipated result suggests the existence of a unique antibody transport system in avian ovarian follicles and also provides a clue for postulating key contributory factors involved in the efficiency transport of blood Ig into ovarian follicles.

Initially, we speculated that the rapid clearance of the qIgY from the blood circulation lowered the qIgY uptake in egg yolks, but our results showed that qIgY had a longer half-life than cIgY (6 h vs. 4.6 h; Table 1). This indicated that the lower qIgY uptake did not arise from the rapid and intense clearance of qIgY from blood circulation. Next, we determined the tissue distribution of i.v.-injected qIgY into the main body tissues. Quail IgY and cIgY showed contrasting distribution patterns in the egg yolks and the main body tissues; qIgY was deposited much more abundantly in the heart, lung, liver, spleen, kidney, and ovarian follicular membranes compared with cIgY, and the reverse was true in egg yolks (Figure 5). These results raise the possibility that the higher deposition of qIgY in main body tissues may cause a reduction in partitioning and lead to lower uptake of qIgY in ovarian follicles. In general, the rate and extent of antibody distribution will be dependent on the rates and extents of antibody extravasation within tissue, distribution within tissue, and elimination from tissue (Lobo et al., 2004). Extravasation of antibodies may occur by paracellular or transcellular movement of antibodies, which may proceed via convection (i.e., movement of antibody with fluid flow from blood to tissue), diffusion, or pinocytosis (e.g., receptor-mediated endocytosis, fluid phase endocytosis). Due to the large molecular size and high polarity of Ig, it is unlikely that significant extravasation occurs via transcellular diffusion. As such, receptor-mediated endocytosis may be an important contributor to the transport of Ig from blood to the interstitial fluid of tissues. It has been assumed that receptor-mediated endocytosis occurs via Fc receptors, such as Fcγ receptors, which are mainly expressed in immunocompetent cells, and FcRn, which are expressed in several organs and tissues, which may play a role in IgG transport. In avian species, evidence is accumulating that various types of Fc receptors, including CHIIR-A1 (Fcγ receptor; Viertlboeck et al., 2007), chFcR/L (homolog of the mammalian Fc receptor family; Taylor et al., 2007), and FcRY (avian-type FcRn; Ward, 2004), are expressed in various immunocompetent cells and body tissues. Although the potential relevance of these avian-type Fc receptors remains unknown, qIgY potentially retains a structure preferential for deposition in quail body tissues, whereas cIgY retains a structure preferential for uptake into quail egg yolks.

Finally, we investigated the general assumption that differences in ovarian uptake between the 2 IgY may be attributable to the dissimilarity of their primary structures. In other words, the qIgY domain required for ovarian transport has a different amino acid sequence than the corresponding cIgY domain, and thus, we attempted to clone the constant region of the qIgY heavy chain because the IgY-Fc region is required for efficient antibody transport into quail egg yolks (Kitaguchi et al., 2008b). Initially, we tried to amplify a portion of the qIgY heavy chain by reverse transcription-PCR using primers specific to the cIgY heavy chain (Parvari et al., 1988) and quail spleen cDNA as a template. However, we failed to amplify the qIgY heavy chain gene, probably due to the dissimilarity between the cIgY gene and the qIgY heavy chain genes. Hence, the amino acid sequences of trypsin-digested qIgY heavy chain fragments were directly analyzed. Among the 3 peptides (PSGEVLR, GFRPR, LRDGDKGEKFEC) analyzed here, only GFRPR was 100% identical to the cIgY gene and the qIgY heavy chain because the IgY-Fc region is required for efficient antibody transport into quail egg yolks. In general, the rate and extent of antibody distribution will be dependent on the rates and extents of antibody extravasation within tissue, distribution within tissue, and elimination from tissue (Lobo et al., 2004). Extravasation of antibodies may occur by paracellular or transcellular movement of antibodies, which may proceed via convection (i.e., movement of antibody with fluid flow from blood to tissue), diffusion, or pinocytosis (e.g., receptor-mediated endocytosis, fluid phase endocytosis). Due to the large molecular size and high polarity of Ig, it is unlikely that significant extravasation occurs via transcellular diffusion. As such, receptor-mediated endocytosis may be an important contributor to the transport of Ig from blood to the interstitial fluid of tissues. It has been assumed that receptor-mediated endocytosis occurs via Fc receptors, such as Fcγ receptors, which are mainly expressed in immunocompetent cells, and FcRn, which are expressed in several organs and tissues, which may play a role in IgG transport. In avian species, evidence is accumulating that various types of Fc receptors, including CHIIR-A1 (Fcγ receptor; Viertlboeck et al., 2007), chFcR/L (homolog of the mammalian Fc receptor family; Taylor et al., 2007), and FcRY (avian-type FcRn; Ward, 2004), are expressed in various immunocompetent cells and body tissues. Although the potential relevance of these avian-type Fc receptors remains unknown, qIgY potentially retains a structure preferential for deposition in quail body tissues, whereas cIgY retains a structure preferential for uptake into quail egg yolks.

Table 2. Amino acid sequence analysis of 3 peptides derived from trypsin-digested quail IgY (qIgY) heavy chain

<table>
<thead>
<tr>
<th>Item</th>
<th>(CH2) 167–172</th>
<th>(CH3) 366–377</th>
<th>(CH4) 427–431</th>
</tr>
</thead>
<tbody>
<tr>
<td>qIgY</td>
<td>PSGEVLR</td>
<td>LRDGDKGEKFEC</td>
<td>GFRPR</td>
</tr>
<tr>
<td>cIgY</td>
<td>PGGALLK</td>
<td>TDQDWLSGERETC</td>
<td>GFRPR</td>
</tr>
<tr>
<td>Homology</td>
<td>42%</td>
<td>41%</td>
<td>100%</td>
</tr>
</tbody>
</table>

1Range of residues indicates the chicken IgY (cIgY) heavy chain domains.
2Quail IgY amino acid sequences are compared with cIgY heavy chain (Parvari et al., 1988).
3Sequence homology scores are analyzed by Clustal W multiple sequence alignment analysis. Underlined residues indicate sequence identities.
428 amino acids, and the sequence alignment by Clustal W revealed substantial homology between duck 7.8S IgY and clgY at CH1-CH4 domain, but its alignment score was 51%. This means that the homology of the IgY constant region is low within members of the same species. Considering these results together, it is plausible that the amino acid sequence of the clgY domain required for ovarian transport of quail is dissimilar to that of the corresponding IgY domain, which could have been the reason for the relatively lower uptake of qIgY compared with cIgY.

The fundamental question in this study was that of the mechanism by which qIgY and clgY were taken up into the inside of ovarian follicles; in other words, what determined the difference in ovarian uptakes between qIgY and clgY. Two plausible models have been proposed. The first model is that the lower deposition of clgY in main body tissues may cause an increase in partitioning and lead to higher uptake into quail egg yolks. If this is true, higher ovarian uptake of clgY may be secondary to the lower uptake in main body tissues. The second model is that clgY, rather than qIgY, may be more preferentially taken up into the inside of quail ovarian follicles. Although our results lack direct evidence to conclude which model is more probable, the observation that the blood clgY concentrations were always lower than the blood qIgY concentrations (Figure 4) permits us to speculate that clgY was much more preferentially taken up into the inside of ovarian follicles from blood circulation than qIgY. In this viewpoint, the most convincing model of IgY uptake is via the receptor-mediated endocytosis at the oocyte plasma membrane. However, the presence of an IgY-Fc receptor or some kind of IgY-Fc carrier proteins responsible for selective IgY transport remains unproven. West et al. (2004) have cloned FcRγY, which is expressed in the yolk sac membrane and several body tissues, including ovarian tissue, and demonstrated that FcRγY is involved in IgY transport from the yolk to embryo. A recent paper also revealed that FcRγY expressed in polarized mammalian epithelial cells functions in endocytosis, bi- directional transcytosis, and recycling of clgY and its Fc fragment, which represents the first cell biological evidence of functional equivalence between FcRγY and mammalian FcRn (Tesar et al., 2008). However, it is believed that a distinct receptor might be involved in IgY transport from maternal blood to egg yolks (Ward, 2004) because FcRγY shows no binding activity with human IgG (West et al., 2004), even though human IgG is transported into hen egg yolks (Mohammed et al., 1998; Morrison et al., 2001). At the same time, the present results suggest that IgY-Fc receptors involved in ovarian transport, if they exist, might specifically function in growing ovarian follicles because qIgY and clgY showed contrasting distribution patterns in egg yolks and in main body tissues (Figure 5). To prove this hypothesis, it is essential to identify the IgY-Fc receptor or some kinds of IgY-Fc carrier proteins expressed in ovarian follicles and to demonstrate that clgY has very high affinity for these receptors originating from quail.

In summary, the present study demonstrated that homologous qIgY is less efficiently incorporated into egg yolks of Japanese quail than heterologous clgY after exogenous injection; in other words, heterologous clgY is far preferable to homologous qIgY as a transport ligand to egg yolks. Sequence analysis of the qIgY heavy chain led us to speculate that sequence dissimilarity between qIgY and clgY produces a difference in ovarian uptakes and a difference in tissue distribution. The uptake patterns of homologous and heterologous IgY in egg yolks will provide valuable information for enhancing the incorporation of Ig and other proteinaceous substances. It might be possible to generate mutated antibodies or antibody fusion proteins fortifying ovarian-transportable activity using protein engineering techniques, which could provide a new strategy for delivering useful proteinaceous substances into egg yolks in an attempt to produce designer eggs.

ACKNOWLEDGMENTS

This research was supported in part by a Grant-in-Aid (No. 17780210 to A.M.) from the Japan Society for the Promotion of Science. H.B. would like to acknowledge the support of the NGK Insulators Ltd. through the NGK Foundation for International Students.

REFERENCES


