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Molecular characterization and B cell membrane expression analysis of Fc fragment gene of goose IgY

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ABSTRACT

A novel goose immunoglobulin υ chain (Igυ) Fc fragment gene was cloned from splenic tissue mRNA using RT-PCR. Deduced amino acid sequence data from different vertebrates revealed high similarity to IgY-Fc fragments of duck (91%) and chicken (64%). Molecular characterization showed that the goose IgY-Fc fragment was consistent with the definition of immunoglobulin, and had the same antigenicity to natural IgY. Flow cytometry and laser scanning confocal microscopy showed that the polyclonal antibody against GoυFc reacted with the membrane surface of B lymphocytes in peripheral blood, which indicates that IgY was expressed on the surface of B cells. Analyses of the gene sequence of the goose IgY-Fc fragment and expression of B cell membrane may provide insight into the evolution of the Ig heavy chain gene family and benefit future studies on the avian immune system.

Immunoglobulin (Ig), an important effector molecule of humoral immunity, has an important function in immune regulation and represents the primary component of the adaptive immune system in all jawed vertebrates. Birds have three classes of antibodies, namely, IgM, IgY, and IgA, which are present in serum and secretions with a distinct tissue distribution (Davison et al., 2008). IgY is an avian version of IgG that is the major systemic antibody produced after IgM in the primary antibody response, and it is the main isotype produced in the secondary response (Davison et al., 2008). A small form of IgY that lacks an Fc region IgY (ΔFc) and a surface membrane-bound (sIg) receptor form with a hydrophobic membrane-spanning C-terminus are also found in duck and some species of turtles (Leslie and Clem, 1972; Magadan-Mompo et al., 2013; Magor et al., 1994). These forms are produced by the same υ gene that expresses the intact IgY form (CH1-4) using different transcriptional termination sites (Magor et al., 1992, 1994). The Fc fragment of υ chain is the main difference between IgY and IgY (ΔFc), which can be used to study the immune response of goose. After chicken, the birds with the most recognized IgY are ducks. Anseriform birds (ducks, goose, and their relatives) are the closest relatives of chickens, so generalizations about goose IgY can be made from chicken and duck. As of this writing, only some studies have been conducted on goose Ig (Guo et al., 2012, 2014a, 2014b; Wang et al., 2012), but none have focused on goose IgY.

The Igs of all heavy-chain isotypes can be produced either in secreted form or as an sIg receptor form (Schroeder and Cavacini, 2010). B cells are the major lymphocyte lineage involved in gene conversion for developing the Ig repertoire. Different B cells produce Ig molecules with different specificities, and each B cell can produce one Ig specificity (Sogn and Kindt, 1988). The antibody repertoire of B cells is generated by Ig gene rearrangement, which leads to sIg expression and continues throughout life (Schroeder and Cavacini, 2010). B cells develop as a function of Ig rearrangement, and sIg is a feature of B cells in most species (Bird et al., 1995). A sIg receptor form IgY that can be expressed on the surface of B lymphocytes is found in duck and chicken (Magor et al., 1994; Parham, 1995; Parvari et al., 1988), but no research has been conducted in goose.

First, RT-PCR was used to amplify the goose IgY-Fc fragment. A pair of specific primers (SY1: 5′-CAGAGCTGCAGCCCCATCCA-3′ and AY1: 5′-TGGGGTGGTGACGAATTCGG-3′) was designed according to the duck Igυ constant region (IgCυ) sequence (GenBank: X65219.1) and chicken Igυ (GenBank: X07174.1). The anchored primers (SY3-1: 5′-AGCACTTCAACAGCACCTTCACG-3′, SY3-2: 5′-GCTTCCAGCCTGAGAACGTG-3′, AY3-1: 5′-ATTAGCGGCCGCGATATC$_{EcoRV}$TTTTTTTTTTTTTTTTT-3′, and AY3-2: 5′-ATTAGCGGCCGCGATATC$_{EcoR\ V}$T-3′) were then designed to amplify the 3′ end of the Igυ gene. Total RNA was extracted from the splenic tissue of 18-month-old Northeast white goose using TRIzol reagent (Invitrogen Co., Beijing, China). Two

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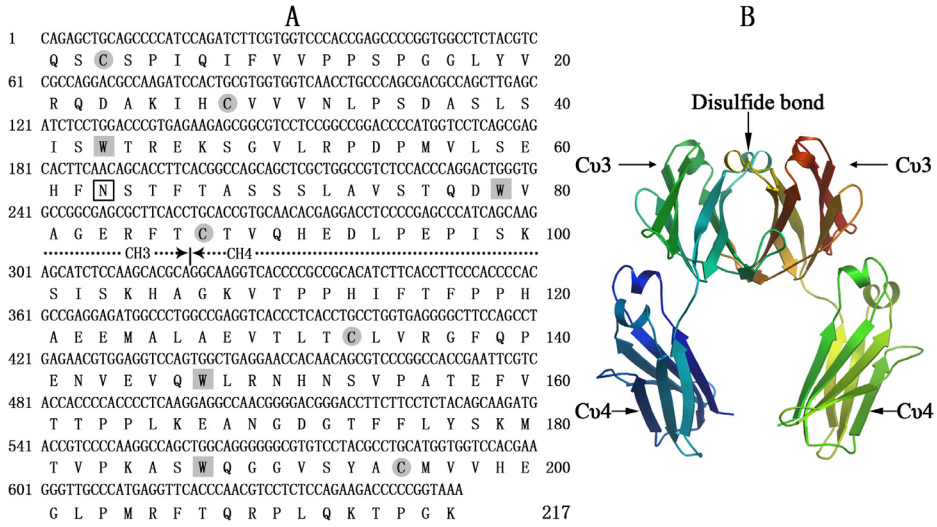

Fig. 1. (A) Nucleotide sequence and inferred amino acid sequences of the cDNA encoding goose IgY-Fc fragment. The nucleotide and amino acid sequences are numbered on the left and right sides, respectively. The N-linked glycosylation sites are indicated by unshaded boxes, the conserved cysteines and tryptophans are indicated by shaded circles and gray-shaded boxes. (B) Three-dimensional model of goose IgY-Fc fragment. The Fc fragment has Cυ3 and Cυ4 domains, which is consistent with Ig molecular that is analyzed by crystal diffraction. Two monomers can be formed as dimer with the intra-domain disulfide bridges marked.

cDNAs were synthesized by RT-PCR and nested PCR, extending from the 5′ end of the Fc fragment to the poly(A) tail of the 3′ UTR. The total spliced sequence obtained was 783 bp, providing a 651 bp ORF that contained CH3 and CH4 (Fig. 1A), which were divided by comparison with known sequences of duck and chicken. The cloned sequence has been submitted to the GenBank database under the accession number JQ080260. The cDNA sequence showed 95% and 71% identities to the Fc fragment of duck and chicken, respectively. By contrast, the inferred amino acid sequence had 91% and 64% identities to the Fc fragment of duck and chicken, respectively.

The goose IgY-Fc fragment contains 217 amino acids, with a molecular mass of 23.871 kDa and theoretical isoelectric point of 7.03. The goose sequence cloned in this study was found to contain cysteines and tryptophans at the same positions as those in duck and chicken (Fig. 1A). These residues may have an important function in maintaining the secondary structure of goose Ig. One N-glycosylation site was predicted (Fig. 1A), which may be important in maintaining effector functions and raising the possibility for goose Igυ to form a glycoprotein. This site is a typical feature of Ig family members. Phylogenetic analysis suggested that the goose Igυ dendrogram was more similar to duck and chicken than other animals analyzed, indicating that the Igυ clusters of goose, duck, and chicken may have arisen during evolution by duplication of an ancestral cluster. Three-dimensional model analysis revealed that the goose IgY-Fc fragment was in agreement with the *Gallus gallus* IgY Fcυ3–4 template produced by X-ray diffraction (Guex and Peitsch, 1997; Taylor et al., 2009). This fragment was also consistent with the division of goose Igυ CH3 and CH4, and could be successfully formed as a dimer with Ig-like folds (Fig. 1B). The immunogenicity of the goose IgY-Fc fragment was analyzed by Western blot using the polyclonal antibody (PAb) prepared with the purified recombinant protein rGoυFc (Fig. 2A) containing the goose IgY-Fc fragment gene according to the sequence cloned in this study. PAb had a clear reaction with rGoυFc and goose Igυ from serum, whereas no reaction was observed between PAb and IgY lacking the Fc fragment (Fig. 2B). This result shows that rGoυFc had similar antigenicity to native goose IgY, confirming that the cloned sequence was the genuine gene of the goose IgY-Fc fragment.

Lymphocytes were isolated from peripheral blood samples of an 18-month-old Northeast white goose using a lymphocyte separation medium (TBD, Tianjin, China) according to the manufacturer's instruction. Flow cytometry was conducted to analyze the reactivity of PAb with B cells. Cells were fixed, permeabilized, incubated with PAb against GoυFc, and then stained with anti-rabbit-IgG-FITC antibody (ZSGB, Beijing, China). The results show that PAb against GoυFc could recognize 9% of the total B lymphocytes isolated from peripheral blood (Fig. 2C). Laser scanning confocal microscopy (LSCM) was performed to analyze sIgY expression on the surface of B cells with PAb against GoυFc. Cells were fixed, permeabilized, stained with PAb against GoυFc, and then stained with DAPI and anti-rabbit-IgG-FITC antibody. LSCM analysis indicated that the surface of B lymphocytes (size, 5 μm) in peripheral blood demonstrated green fluorescence, whereas the nucleolus exhibited blue fluorescence (Fig. 2D). This finding demonstrates that PAb against GoυFc could react with the surface of B lymphocytes in peripheral blood, and sIgY was expressed on the cell surface. This finding confirmed that goose IgY also had the sIg receptor form, which was in accordance with sIgY being the membrane surface marker of B cells. Moreover, 9% of blood lymphocytes were found to bear sIgY, which was consistent with the percentage of chicken sIgY blood lymphocytes (about 7–13%) (Hirai et al., 1981), but lower than the percentage of human sIgG blood lymphocytes (about 15%) (Nicod et al., 1973) and higher than the percentage of tortoise sIgY blood lymphocytes (about 5–8%) (Andreas and Ambrosius, 1989).

This paper presents the first evidence of an encoded cDNA for a goose IgY-Fc fragment and sIgY expression on the surface of B cells. These findings are novel and have not been reported. However, further research should be performed based on our preliminary results to investigate the structure and generation of antibody diversity for goose Ig. Ducks possess three forms of IgY: a secreted form, truncated form or IgY (ΔFc), and the receptor form that can be expressed on the surface of B lymphocytes (Davison et al., 2008; Magor et al., 1992, 1994; Parham, 1995). Chickens have no truncated form IgY (Parvari et al., 1988), whereas the IgY form of goose remains unknown. This research provided data to help identify the IgY form of goose.

This study is the first to report the cDNA encoding goose IgY-Fc fragment and sIg receptor form IgY that can be expressed on the surface of B lymphocytes. The results of this study may be used as a basis for further research on the genetic immunology of birds.