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Separation and Reactivity of Avian Immunoglobulin Y

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Abstract

Immunoglobulin Y (IgY) is the mayor protein present in the avian egg yolk. This antibody fulfils important functions in the protection of the embryo against several challenging stimuli. Separation of IgY from the egg yolk of several birds was carried out by the Polson method. Their capacity to react with immunoglobulin-binding bacterial protein: protein A, L or LA was investigated. The cross-reactivity of an anti-chicken-IgY-HRP conjugate with different avian IgY was tested by ELISA. The results showed that protein L reacts with bantam hem IgY; and proteins A and LA react with ostrich, bantam hen or duck IgYs. These findings are important for the development of methods of detection and purification of avian IgY proteins.

Keywords: Protein A; Protein L; Protein LA; Immunoglobulin Y; Avian egg yolk antibodies

Introduction

Immunoglobulin Y (IgY) is the mayor protein present in the avian egg yolk. This antibody fulfils important functions in the protection of the embryo against several challenging stimuli [1]. Egg yolk antibodies are important therapeutically [2,3], but also they have been used as immunological tool in many immunodiagnostic assays [4-7].

Staphylococcal protein A, SpA [8], Peptostreptococcal protein L, SpL [9] and chimeric protein LA, pLA [10] are immunoglobulin-binding bacterial proteins (IBBPs). They have the ability of binding to the Fc-fragment of several immunoglobulins (Igs) in a non-specific manner [11]. This interaction is useful in serological diagnosis of infectious diseases, where enzyme-labelled-IBBPs are used as a marker in ELISAs and other procedures for the determination of antibodies, and in the Ig-purification. The binding of IBBPs to Igs of mammalian and avian species because is so important has been extensively documented, specially the interactions between SpA to mammalian Igs [8]. The binding of pLA to animal Igs has not been extensively documented but it is predicted positive if SpA, which is an integral component of pLA, binds to Igs. However the interaction of SpL to avian immunoglobulins is not well-known and deserves further studies and documentation since this IBBP could also be successfully used for IgY isolation [12], and serological studies.

This study reports on the separation of IgY from the egg yolk of several avian species using the Polson Method [13] and the IgY interaction with IBBPs, aims to establish binding affinities between them that are unknown or not documented yet, this is important for using IBBP in the purification of avian immunoglobulins.

Materials and Methods

Materials

Peroxidase labelled protein A, L or LA, rabbit anti-IgG, protein A antibody purification kit, ELISA microtitre plates were obtained from Sigma-Aldrich Co, St. Louis Missouri, USA. All other chemical were of reagent grade, obtained from the same company. Eggs from different avian species were collected in Westmorland, Jamaica.

Immunoglobulin Y Isolation and Determination by Direct ELISA

The IgY fraction was isolated from the egg yolks of a variety of birds including chicken, bantam hen, guinea hen, quail, goose, duck, pigeon, parakeet, cattle egret, pheasant, and ostrich. The IgY fraction was isolated by the chloroform-polyethylene glycol (PEG) method [13]. The eggs were washed with warm water and the egg yolk was separated from the egg white. The membrane was broken and the egg yolk collected and diluted 1:3 in phosphate buffered saline (PBS), pH 7.4. To 1/3 of the egg yolk mixture an equal volume of chloroform was added, the mixture was then shaken and centrifuged for 30 min (1000×g, RT). The supernatant was decanted and mixed with PEG 6000 (12%, w/v), stirred and incubated for 30 min (RT). The mixture was then centrifuged as previously described. The precipitate containing IgY was dissolved in PBS (pH 7.4) at a volume equivalent to 1/6 of the original volume of the egg yolk and dialyzed against 1L of PBS (pH: 7.4 for 24 h at 4°C). The IgY was removed from the dialysis tubing. IgY concentration was determined by the Bradford method. IgY samples were stored at –20°C.

A direct ELISA was used to determine the presence of avian egg yolk IgYs as follows: 96 well microtitre plates were coated overnight at 4°C with 100 µg of duplicates of each IgY sample in carbonate-bicarbonate buffer pH 9.6. Plates were washed 4X with 150 µl PBS-Teen 20 buffer. Then 50µl of a commercial anti-chicken IgG-HRP diluted 1:30,000 (according to manufacturer's instructions) in PBS-non-fat milk, was added to each well and incubated for 1h at RT. The plates were washed 4X with PBS-Tween. 50 µl of 4 mg/ml o-phenylenediamine solution (OPD) was added and the plates were incubated 15 minutes at RT. The

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reaction was stopped with 25µl of 3M H_2SO_4 solution. The plates were read in a microplate reader at 492 nm.

Enzyme linked immunosorbent assay for investigating the immunoglobulin-binding bacterial protein (IBBPs) bacterial Ig-receptors (proteins A, L and LA) binding to avian immunoglobulins

An ELISA was used to study the interaction of proteins A, L and LA with different avian IgY preparations. The 96 well microtitre plates were coated overnight at 4°C with 1 µl/mg per well of unlabelled SpL, SpA or pLA in carbonate-bicarbonate buffer pH 9.6. Then plates were treated with bovine serum albumin solution and washed 4X with PBS-Tween. 50 µl of IgY (1 mg/ml) solution was added and incubated for 1h at room temperature and washed. Then 50 µl of rabbit anti-chicken IgG-HRP conjugate diluted 1:1000 in PBS-non-fat milk, was added to each well and incubated for 1h at RT. The plates were washed 4X with PBS-Tween. 50 µl of 4 mg/ml o-phenylenediamine solution (OPD) was added and the plates were incubated 15 minutes at RT. The reaction was stopped with 50 µl of 3M H_2SO_4 solution. The plates were visually assessed for the development of colour (indicating a positive result) and were also read in a microplate reader at 492 nm. Colour development and optical density readings were used to assessed the Igs-IBBP interactions. Blanks (buffer) and positive and negative controls (Human IgG and turtle Igs respectively) were included in each test.

Results

IgY from the egg yolk of several species of birds including guinea hen, pheasant, duck, bantam hen, chicken, ostrich, pigeon, cattle egret, parakeet, goose and quail were isolated by the Polson method also known chloroform-PEG isolation technique (Figure 1). This is a standard procedure, which yields IgY of a high purity that is well-documented [13].

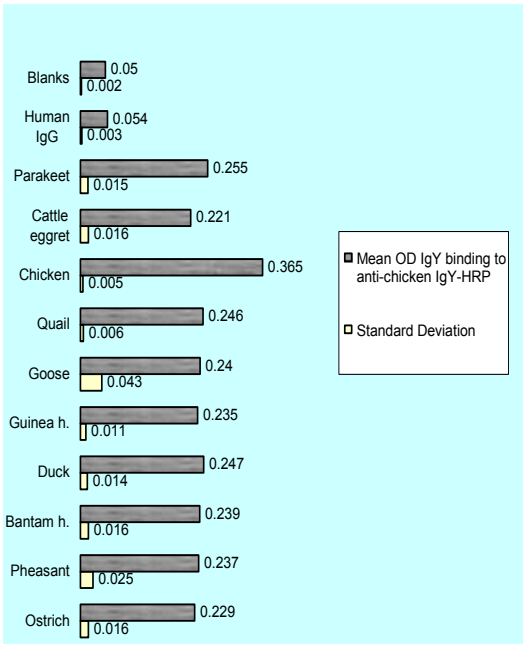

Figure 1: Direct ELISA for the detection of avian IgY extracted from the egg yolk of several birds. Triplicate samples of avian IgY were detected using a rabbit anti-chicken IgG antibody conjugated with horseradish peroxidase (Sigma). Human IgG was used as a negative control. Cut-off point=0.185.

IgY	Colour development	XOD (SD)
Bantam	+	0.22 (0.014)
Duck	-	0.9 (0.003)
Pigeon	-	0.09(0.01)
Chicken	-	0.09 (0.006)
Quail	-	0.08(0.001)
Guinea hen	-	0.07 (0.04)
Pheasant	-	0.08 (0.008)
Cattle egret	-	0.06 (0.005)
Parakeet	-	0.06 (0.01)
Goose	-	0.06 (0.02)
Ostrich	+	0.28 (0.011)

Cut off point=0.16
Eight replicates of each sample were tested

Table 1: The binding of peptostreptococcal protein L to avian IgY by direct enzyme linked immunosorbent assay*.

IgY	Colour development	XOD (SD)
Bantam	+	0.22 (0.012)
Duck	+	0.21 (0.014)
Pigeon	-	0.08 (0.011)
Chicken	-	0.08 (0.014)
Quail	-	0.08 (0.003)
Guinea hen	-	0.09 (0.018)
Pheasant	-	0.07 (0.004)
Cattle egret	-	0.08 (0.015)
Parakeet	-	0.07 (0.001)
Goose	-	0.09 (0.015)
Ostrich	+	0.23 (0.006)

Cut off point=0.16
Eight replicates of each sample were tested

Table 2: The binding of staphylococcal protein A to avian IgY by direct enzyme linked immunosorbent assay*.

IgY	Colour development	XOD (SD)
Bantam	+	0.25 (0.01)
Duck	+	0.24 (0.014)
Pigeon	-	0.1 (0.015)
Chicken	-	0.09 (0.007)
Quail	-	0.07 (0.009)
Guinea hen	-	0.08 (0.005)
Pheasant	-	0.09 (0.008)
Cattle egret	-	0.06 (0.006)
Parakeet	-	0.07 (0.008)
Goose	-	0.1 (0.006)
Ostrich	+	0.25 (0.008)

Cut off point=0.16
Eight replicates of each sample were tested

Table 3: The binding of recombinant protein LA to avian IgY by direct enzyme linked immunosorbent assay*.

The presence of the purified IgY from the egg yolk of different birds was assessed by an ELISA where a rabbit anti-chicken IgG-HRP conjugate was used for immunodetection. The interaction of IgY with IBBPs was proved by ELISA. Protein L reacted with bantam hen and ostrich IgYs (Table 1) and Proteins A or LA reacted with IgY of bantam hens, ostriches and ducks (Tables 2 and 3). IgYs from guinea hen, pheasant, chicken, cattle egret, parakeet, goose and quail did not react with IBBPs. These interactions involve the Fc region of IgY and the immunoglobulin binding region of IBBPs.

Discussion

Closely antigenic relationships among avian egg yolk IgY molecules

were shown by the capacity of an anti-chicken IgG-HRP conjugate to cross-react with the entire panel of IgYs in this study. It confirmed previous reports of the cross-reactivity of anti-turkey IgY antibodies with IgY of chickens, ducks and geese [14]. Probably the Fc region of avian IgY molecules is much conserved than that of the mammalian species. It may explain why antibodies produced against chicken IgY cross-reacts well with IgY from other birds. However, cross-reactivity among mammalian IgGs seems to be more restricted. Pretorius and collaborators [15] demonstrated the binding of labelled anti-rabbit IgG with species of the order Lagomorpha and anti-mouse IgG with most species of Rodentia; anti-human IgG did not react with sera from other species. This suggested a much higher variability in the amino-acidic sequences of the Fc fragment in mammalian in comparison with avian.

The binding of SpL to chicken IgY has been reported [16], however the results of this study did not confirm this report. The SpL binding to bantam IgY and the lack of SpL binding to chicken IgY could reflect the diversity in Ig molecules between these closely related avian species. The reactivity of SpA with some avian immunoglobulins such as ostrich IgY has been previously reported [17]. The purification of these immunoglobulins by affinity chromatography confirmed their ability to bind to SpA. Similarly protein A-affinity chromatography was used by Higgins to purify duck serum IgY [12], which binding to SpA was again demonstrated in our study.

The experiments described in this study suggest that the avian immunoglobulins are antigenically related. This property could be used in the preparation of anti-avian-IgY antibodies to be used as immunological tool in the detection of several avian imunoglobulins. The reactivity of proteins A, L or LA to IgY is lower than that of the mammals. The use of affinity chromatography using these bacterial proteins as anti-globulin could be exploited in the production of IgY purified fractions of some avian species [17]. On the other hand SpL could promissory be used in serological studies for the immunodetection of antibodies to bacteria, viruses and parasites in bantam hens and ostriches. The results of these experiments suggest that IBBP could be used for immunodetection of avian immunoglobulins that were successfully separated from the egg yolk.

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