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Source: Journal of Wildlife Diseases, 32(4) : 687-690

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-32.4.687

Journal of Wildlife Diseases, 32(4), 1996, pp. 687–690

Brief Characterization of Muskrat (*Ondatra zibethicus*) Immunoglobulin G (IgG) Separated from Serum on Protein A

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ABSTRACT: Muskrat (*Ondatra zibethicus*) immunoglobulin fraction was separated from whole serum by Protein A Sepharose chromatography. In serum electrophoresis, this fraction had a gamma motility; when electrophoresed on a polyacrylamide gel with sodium dodecyl sulfate it resolved into two protein bands of approximately 52 and 25 kilodaltons, respectively. These bands were consistent with molecular weights of known heavy and light chains of immunoglobulin G (IgG) in other closely related species. Furthermore, the putative muskrat immunoglobulins had a strong cross-reactivity with mouse IgG_1, IgG_3, and kappa chain in an enzyme-linked immunosorbent assay. We propose, that the proteins bound to the Protein A Sepharose represent muskrat immunoglobulins of the IgG class.

Key words: Muskrat, *Ondatra zibethicus*, IgG separation.

The muskrat (*Ondatra zibethicus*) is a semiaquatic microtine rodent common in North America and Europe (Boutin and Birkenholz, 1987). Although it has been used as a research animal (Doyle, 1988) and some physiological data are available (Nagel and Kemble, 1974), little is known about it's immune system. To our knowledge, this is the first report on the separation and characterization of a muskrat immunoglobulin of the immunoglobulin G (IgG) class.

Mature muskrats have been live-trapped into modified box-diver type traps (30 × 30 × 1000 cm) from 1990 to 1993 in Wayne County, Pennsylvania (USA) (41°30′N, 75°30′W), and Storrs, Connecticut (USA) (41°48′N, 72°15′W). These animals were part of a muskrat breeding colony established at the University of Connecticut, Storrs. The colony was housed in two rooms with automatically controlled artificial light cycles (16 hr light; 8 hr dark) and a temperature of 12 C. Animals were kept in 1.5 × 2 m cages with concrete floors and 100 × 100 × 20 cm water basins. Wooden boxes with hay as nesting material were provided for each breeding pair. A commercial diet (Laboratory Rodent Diet 5001, PMI Feeds Inc., St. Louis, Missouri, USA) was fed ad libitum and water was changed daily.

Blood was collected from jugular veins of muskrats anesthetized with 1–2 mg/kg of xylazine hydrochloride (Rompun, Miles Inc., Shawnee Mission, Kansas, USA) followed by 10 mg/kg of ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Inc. Fort Dodge, Iowa, USA), both given into the semitendinosus muscle. The skin above the jugular vein was shaved and 2cc of blood were taken into a 5 ml syringe using a 22 gauge, 25 mm, needle. The blood was allowed to clot at room temperature for 30 min and then was centrifuged at 900 × *G* for 10 min to separate serum. Serum was stored at −70 C until used.

The binding of immunoglobulin through the Fc region to Protein A has been well documented in several species (Kronvall et al., 1970). Based on this, we isolated immunoglobulin from muskrat serum using Protein A Sepharose chromatography (Kronvall et al., 1970). The Protein A Sepharose column was equilibrated with borate buffer, pH 9.0 (0.6 M boric acid, 0.1 M sodium borate and 0.37 M NaCl). Muskrat serum was diluted 1:3 in the same buffer and loaded into the column. The column was allowed to run at 15 ml/hr and the absorbency (280 nm) of the run-through fraction was monitored with an ultraviolet detector (Bio Rad, Richmond, California, USA) until it reached baseline values. At this point, the

bound protein was eluted from the column with a glycine-HCl buffer, pH 3.0 (0.56 M glycine, 0.2 M NaCl and 0.1 N HCl). The eluted fraction was buffered with 1M Tris-Cl, dialyzed several times against fresh changes of phosphate buffered saline (PBS), (Sigma Chemical Company, St. Louis, Missouri, USA) and stored at −70 C.

As an initial characterization step, both whole muskrat serum and the protein A eluate were electrophoresed in 1% noble agar (FMC Bio Products, Rockland, Maine, USA). The electrophoresis profile obtained for the eluate had a gamma motility consistent with IgG (data not shown). To analyze further, the Protein A Sepharose column eluate and whole muskrat serum were electrophoresed over a 12% polyacrylamide gel with sodium dodecyl sulfate (PAGE-SDS) as described by Maizles et al. (1991). Briefly, a minigel apparatus, SE 250 Mighty Small (Hoefer Scientific Instruments, San Francisco, California), was used according to the manufacturers directions. Prior to loading onto the gel, the blood and serum samples were boiled with two volumes of sample buffer containing 0.625M Tris-HCl, pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate, 5% beta mercaptoethanol and 0.042% bromophenol blue in distilled water, for 3 min and then centrifuged at 12,000 × *G* at 4 C for 10 min. Ten μl of the supernatant were loaded into wells and electrophoresed until the front dye reached the bottom of the gel. The gels were stained with Coomassie Blue (Maizels et al., 1991). Molecular weight color markers (6,500 to 205,000 molecular weight markers, Sigma Chemical Company) were used to determine the molecular weights of the separated proteins.

The Protein A Sepharose eluate from muskrat serum resolved on PAGE-SDS gels into two distinct bands with molecular weights of approximately 52 and 25 kDa, respectively (Fig. 1). These molecular weights are consistent with heavy and light chains of IgG (Rose et al., 1986). Electrophoresed whole muskrat serum had multiple bands including the IgG heavy and light chains (Fig. 1), confirming the specific separation of IgG over Protein A Sepharose.

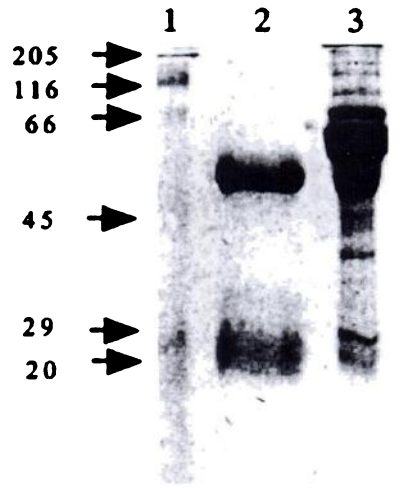

FIGURE 1. Analysis of muskrat IgG isolated on Protein A Sepharose by polyacrylamide gel with sodium dodecyl sulfate (PAGE-SDS). Lane 1: molecular weight markers; lane 2: muskrat IgG; lane 3: whole muskrat serum.

The putative IgG separated from muskrat serum was finally analyzed by means of a standard enzyme-linked immunosorbent assay (ELISA) using a mouse-specific isotyping kit according to the manufacturers recommendation (SBA Clonotyping System III, Fisher Biotech, Pittsburgh, Pennsylvania; USA). Mouse-specific reagents were used in the attempts to isotype muskrat IgG, because muskrats and mice both are in the microtine rodent family. Heavy and light chain, peroxidase-conjugated goat anti-mouse antisera including anti IgM, IgG_1, IgG_3, IgG_{2a}, IgG_{2b} and anti kappa and lambda light chains, were used. Briefly, a standard flat-bottomed, 96-well polystyrene microtiter plate was loaded with 100 μl per well of the immunoglobulin from muskrats mixed with carbonate/bicarbonate buffer pH 9.6 (13 mM Na_2CO_3 and 35 mM $NaHCO_3$). After incubation at 37 C for 1 hr and 4 C for 12 hr, the plate was washed five times with PBS, pH 7.3 containing 0.05% Tween 20

TABLE 1. Characterization of muskrat immunoglobulin G (IgG) using a mouse-specific isotyping ELISA kit.

	Goat anti-mouse antisera							
Antisera	Blank	IgM	IgG1	IgG3	IgG2a	IgG2b	Kappa	Lambda
1.[a]	0.000[b]	0.068	0.480	0.505	0.019	0.101	1.183	0.083
2.[a]	0.000	0.085	0.514	0.588	0.010	0.089	1.079	0.074

[a] Muskrat IgG in solid phase as antigen; samples run in duplicate.
[b] OD, Optical density.

(Sigma Chemical Company). The nonspecific sites were blocked by adding 200 μl per well of 1% normal goat serum in PBS-Tween 20. The plate was incubated for 60 to 120 min at 37 C followed by three washes in PBS-Tween 20. The goat antisera were diluted 1:300 and 100 μl per well were added to each well; all samples were run in duplicate. The plate was incubated for 45 min at 37 C followed by four washes in PBS-Tween 20. The presence of bound conjugate was determined by the addition of 100 μl per well of substrate (0.6 μg/ml H_2O_2 and 0.4 mg/ml of 0-phenylenediamine dihydrochloride in phosphate-citrate buffer, pH 5.0, (Sigma Chemical Company). The mixture was allowed to react at 18 C in the dark for 15 min, after which optical densities were measured at 450 nm by using the Titertek Multiscan Spectrophotometer (Flow Laboratories, McLean, Virginia, USA). The isotyping ELISA had strong responses with the muskrat IgG with anti-mouse IgG_1, IgG_3 and kappa light chain-specific antibodies (Table 1). Based on these results, muskrat IgG isolated on protein A Sepharose has cross-reactivity with mouse IgG_1 and IgG_3 isotypes, and it has a kappa light chain.

We concluded that the protein fraction separated from muskrat serum in a Protein A Sepharose column represents immunoglobulins of one or possibly more IgG classes. The muskrat IgG was subsequently used in our laboratory to obtain an anti-muskrat IgG rabbit antiserum (Borucinska, 1995). We used this antiserum successfully in Western blots (Borucinska, 1995) recognizing immune responses in muskrats infected experimentally with *Capillaria hepatica* (Nematoda) (data not shown).

The research was supported by the Northeastern Research Center for Wildlife Disease and the University of Connecticut Research Foundation, Storrs, Connecticut. We thank the Department of Environmental Protection in Connecticut, Bureau of Wildlife Management in Pennsylvania, and the New York State Department of Environmental Conservation for giving us live trapping and holding permits for muskrats. We thank all the trappers that submitted muskrats for this study, particularly Fred Becker from Connecticut and Alan Keister from Pennsylvania. We also thank Roger Zemek, Don Bessette, and Edan Tulman for technical assistance.

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Received for publication 21 December 1995.