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Polyclonal Antibody against Muscle Actin of *Ascaris suum*: Cross-reactivity against Actin from Various Sources

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Abstract

Rabbits were immunized with purified actin from obliquely striated muscle of body wall of *Ascaris*. The rabbits produced antibodies specific for the antigen. Polyclonal IgG specific for the antigen was obtained using affinity chromatography. This antibody demonstrated cross-reactivity with the actin of other species of parasitic helminths and various types of muscle actin of vertebrates. From among the IgG that recognized the *Ascaris* actin molecules, approximately 20% cross-reacted with actin of skeletal muscle and cardiac muscle, while approximately 10% cross-reacted with actin of smooth muscle.

Key words: *Ascaris*, Actin, Actin antibody, Nematode, Muscle contraction

Introduction

Actin is an essential contractile protein of eukaryotic cells (Pollard and Weihing, 1974). Its primary structure consists of sequences that are similar even between organisms that come from separate evolutionary ladders. The immunogenicity of actin is extremely low. The preparation of polyclonal antibodies against actin has been attempted in the past (Lazarides and Weber, 1974). Actin chemically modified or coupled to other protein molecules has been immunized into laboratory animals. However, either the antiserum had a remarkably low antibody activity or it contained antibodies to other proteins (Benyamin *et al.*, 1983 and Polzar *et al.*, 1989). Well-developed obliquely striated muscle exists in the body wall of the parasitic nematode *Ascaris*. The molecular structure of the actin purified from this obliquely striated muscle is in close agreement with various types of muscle actin of higher animals that serve as hosts (Nakamura *et al.*, 1979). However, mammals immunized with native *Ascaris* muscle actin produced antibody having high reactivity (Nishioka *et al.*, 1982, 1983). Anti-*Ascaris* actin antibody obtained from rabbits had a broad range of cross-reactivity covering various types of actin, from that of higher animals to that of parasitic protozoa (Endo *et al.*, 1988; Yasuda *et al.*, 1988). This paper describes quantitative analysis regarding the reactivity of skeletal, cardiac, and smooth muscle actin for antibody against actin from the muscle layer of *Ascaris suum*.

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Materials and Methods

Actin preparation: Purified actin was obtained from acetone-treated powder (Nakamura *et al.*, 1979) of the obliquely striated muscle of *Ascaris suum*, chicken skeletal muscle, rabbit skeletal muscle, canine cardiac muscle, and chicken gizzard smooth muscle using the method of Pordee and Spudich (1982). Crude extracted actin was prepared by extracting with 0.2 mM ATP containing 0.2 mM $CaCl_2$, 1 mM dithio-

threitol (DTT), and 2 mM Tris-HCl (pH 8.0) from acetone-treated powder of whole worms of *Dirofilaria immitis, Toxocara canis, Spirometra erinacei, Dipylidium caninum,* and *Fasciola hepatica.* The protein concentrations were determined using a slightly modified regent (Yamaguchi and Sekine, 1966) of the biuret reaction (Weichselbaum, 1946).

Antibody preparation: Purified *Ascaris* actin (1 mg) was emulsified in Freund's complete adjuvant and injected three times at two-day intervals into axillary lymphnodes and the gluteal region of rabbits. Three to five weeks after the initial immunization, 1 mg of protein was injected in the same manner as a booster. Each rabbit's entire volume of blood was drawn one week later. Specific antibody was then purified using affinity chromatography from the antisera.

Affinity chromatography: Sepharose-NH $(CH_2)_5$ COOH (CH-Sepharose) was prepared coupling 6-amino-n-caproic acid to cyanogen bromide-activated Sepharose 4B (Cuatrecasas, 1970; Cuatrecasas and Anfinsen, 1971). Activated CH-Sepharose was then prepared by reacting the above with *N,N'*-dicyclohexylcalbodiimide and *N*-hydroxysuccinimide (Cuatrecasas and Parikh, 1972). The purified F-actin from each of the muscle samples was coupled to the activated CH-Sepharose in 0.1 M borate buffer containing 0.5 M NaCl (pH 8.3, coupling buffer). Four to five mg of protein was coupled to 1 g of Sepharose gel. Anti-*Ascaris* actin serum (10 ml) containing 10 mM EDTA or crude globulin fraction of the antisera (100–200 mg) obtained by 50% ammonium sulfate saturation, was then applied to the *Ascaris* actin-CH-Sepharose column (0.9 × 10 cm) equilibrated with the coupling buffer. The column was thoroughly washed with the coupling buffer containing 1% Nonidet P40 (BDH Ltd., England), and finally the detergent was removed. The protein that coupled to Sepharose-actin matrix was eluted with 0.1 M glycine-HCl buffer (pH 2.3). Antibody that had been adjusted to a pH of 8 with 1 M Tris was further applied to a protein-A-Sepharose CL-4B (Pharmacia Co., Sweden) column. After washing with the coupling buffer, *Ascaris* actin specific IgG antibody (anti-*Ascaris* actin IgG) was eluted with 0.1 M glycine-HCl buffer (pH 3.0). The IgG fraction was dialyzed against 0.15 M NaCl containing 10 mM phosphate buffer (pH 7.4, PBS), and then stored at −80°C until the time of use. The binding of anti-*Ascaris* actin IgG to skeletal, cardiac, or smooth muscle actin was measured by affinity chromatography (0.9 × 5 cm column). A protein value of $E_{280}^{1\%} = 13.8$ was used for the calculation of IgG (Schultze and Heremans, 1966).

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunoblotting: Electrophoresis was performed using a slab-type gel electrophoresis system (Nihon Eido Co.), based on the method of Fairbanks *et al.* (1971) and Weber and Osborn (1969) with slight modification (Nakamura *et al.*, 1975). The binding of the electrophoresed protein and antibody was observed using a variation of the methods of Towbin *et al.* (1979) and Howe and Hershey (1981). More specifically, transfer of protein to nitrocellulose membrane was performed in a 25 mM Tris-glycine buffer (pH 8.3) containing 20% CH_3OH, 0.1% SDS, and 1 mM EDTA. The residual binding sites of the membrane were blocked by saturation with 3% gelatin solution (pH 7.4) containing 0.15 M NaCl. The membrane was incubated with antibody (primary antibody) diluted with the same solution. The membrane after being adequately washed with 0.15 M NaCl (pH 7.4) containing 0.1% SDS, 0.5% Nonidet P40, and 0.5% gelatin, was then reacted with horse radish peroxidase (HRPO)-conjugated goat antibody (IgG) against rabbit IgG (Bio-Rad Inc., USA) as the second antibody. After being washed in the same manner as the primary antibody, the coupled HRPO was colored in the presence of 3,3'-diaminobenzidine-4HCl and H_2O_2.

Enzyme-linked immunosorbent assay (ELISA): Diluted primary antibody (less than 5 μg/ml of IgG, 0.1 ml) was allowed to react on a micro-immunoplate (Nunc No. 4-39454, Denmark) sensitized with a fixed amount (100 ng/ml, 0.1 ml) of actin from various sources. After washing and subsequent incubation of the HRPO-conjugated second antibody, the plate was colored using *o*-phenylenediamine-H_2O_2 as

the substrate. The reaction was terminated by the addition of 3 N H_2SO_4. The antibody reactivity to the antigen was determined by measuring the maximum absorbance at 490 nm during coupling of antibody to a fixed amount of antigen using the Langmuir plot (Stanley *et al.*, 1983).

Results

An average of 1.1 (±0.7) mg of anti-*Ascaris* actin IgG was obtained from 1 ml of antiserum using 8 rabbits (data not shown).

Crude actin was extracted from acetone-treated powder of parasitic helminth adults using a low concentration aqueous solution of ATP. The results of SDS-polyacrylamide gel electrophoresis of the crude extract are shown in Fig. 1A. Although a large number of protein bands can be observed for *D. immitis, T. canis, S. erinacei, D. caninum,* and *F. hepatica,* respectively, these existed in common with the protein having a molecular weight of 42k, which agrees with the purified *Ascaris* actin. By immunoblot methods, anti-*Ascaris* actin IgG showed strong cross-reactivity only to those 42k proteins of various parasite species (Fig. 1B).

The reaction between the muscle actin of higher animals and the specific antibody is indicated in Fig. 2. The antibody reacted with the actins of skeletal, cardiac, and smooth muscle, respectively. It has been previously reported (Nishioka *et al.*, 1982; 1983), in studies using immunoblot methods, that actin from rabbit skeletal muscle was recognized by rabbit auto-antibody. ELISA was performed to compare the reactivity of the antibody to each of the types of actin (Table 1). The antibody had equally strong cross-reactivity for the actin of chicken and rabbit skeletal muscle, as well as that of canine cardiac muscle. The antibody had weak reactivity for

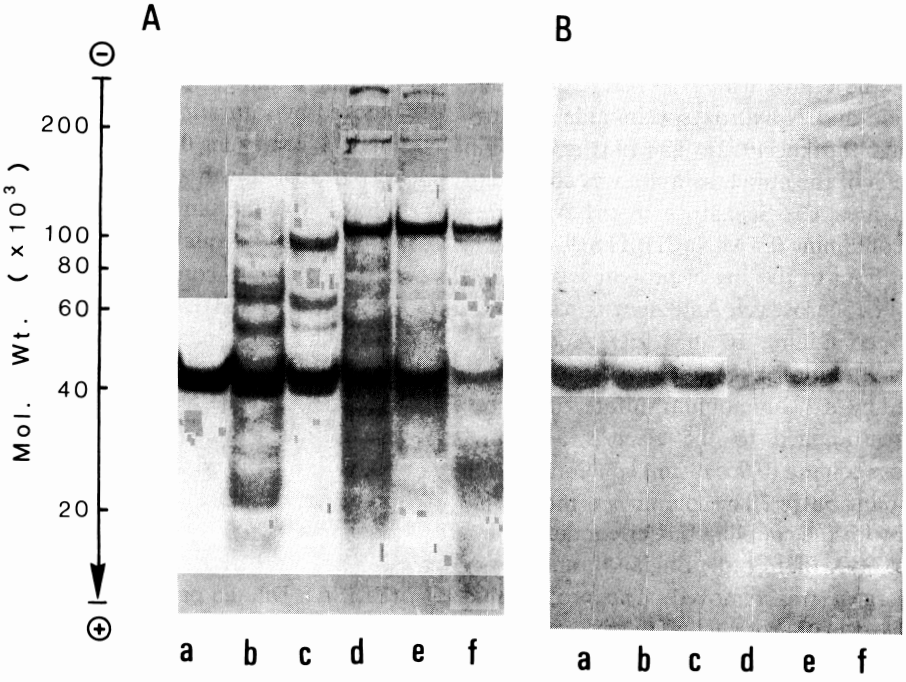

Fig. 1 SDS-polyacrylamide gel electrophoresis and immunoblotting of the crude-extracted actin from various parasites. A, protein stain by Coomassie brilliant blue G-250; B, immunoblotting with anti-*Ascaris* actin IgG on a nitrocellulose membrane. Extract from (a) *Ascaris* (purified actin), (b) *Dirofilaria immitis,* (c) *Toxocara canis,* (d) *Spirometra erinacei,* (e) *Dipylidium caninum,* and (f) *Fasciola hepatica.*

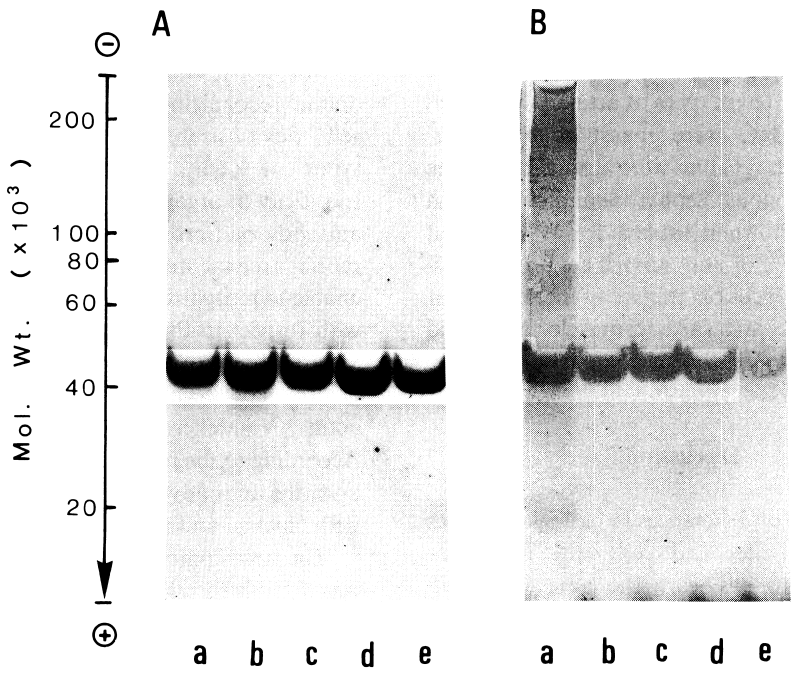

Fig. 2 SDS-Polyacrylamide gel electrophoresis and immunoblotting of purified actin from various muscles. Actin from (a) *Ascaris* obliquely striated, (b) chicken skeletal, (c) rabbit skeletal, (d) canine cardiac, and (e) chicken (gizzard) smooth muscle. A and B, as in Fig. 1.

Table 1 Reactivity of anti-*Ascaris* actin IgG against various actins by ELISA

Antigen (Actin from)	$[A_{490\ nm}]$*
Ascaris obliquely striated muscle	1.91 (±0.04)
Chicken skeletal muscle	1.08 (±0.28)
Rabbit skeletal muscle	1.06 (±0.16)
Canine cardiac muscle	1.28 (±0.20)
Chicken (gizzard) smooth muscle	0.41 (±0.11)

*Data were obtained as an average of the calculated value from three rabbit antibodies.

the actin of chicken gizzard smooth muscle in comparison to the actin sources mentioned above. However, the actin of skeletal muscle, cardiac muscle, as well as smooth muscle, indicated considerably lower absorbance values in comparison to *Ascaris* actin. This suggests that

Table 2 Stoichiometry of cross-reacting antibody measured by affinity chromatography on various actin-coupled CH-Sepharose

Actin	cross-reacting IgG (%)
Chicken skeletal muscle	17.1 (±8.8)*
Canine cardiac muscle	18.0 (±7.4)*
Chicken (gizzard) smooth muscle	8.3 (±1.4)*
Ascaris obliquely striated muscle	90.8†

The percentage of cross-reacting IgG was calculated from the total volume of anti-*Ascaris* actin IgG applied to a column. *The average value of three rabbit antibodies, 4 weeks after initial immunization (one week after booster), were determined. † The value for positive control measured by rechromatography on *Ascaris* actin-CH-Sepharose column of pooled anti-*Ascaris* actin IgG.

the antibody is reacting to certain constituents of the different types of actin. Therefore, the amounts of antibody reacting with the respective molecules of different types of actin in the *Ascaris* actin-specific IgG were measured. The percentages of each IgG that were absorbed onto the muscle actin-coupled Sepharose and eluted at an acidic pH are shown in Table 2. It was found that roughly 10–30% of anti-*Ascaris* actin IgG cross-reacted with each of the actin molecules of skeletal muscle and cardiac muscle. Measured values that were 7–10% in comparison to these were obtained for smooth muscle actin.

Discussion

Irrespective of differences in biological species or between tissues and cells, the molecular structure of actin has nearly the same amino acid sequence (Pollard and Weihing, 1974; Vandekerckhove and Weber, 1984). Consequently, actin has been considered to be a protein extremely lacking in antigenicity. Laboratory animals most likely would have difficulty distinguishing native actin from other organisms as being "non-self." However, native actin purified from the muscle layer of *Ascaris* demonstrated high antigenicity against rabbits. When purification of the antibody was performed using affinity chromatography, 1 mg or more of *Ascaris* actin-specific IgG was present in 1 ml of rabbit antiserum.

Detailed research has been conducted on the free-living *Caenorhabditis elegans* with regard to the primary structure of nematode actin. Based on DNA sequences, at least four types of actin genes exist, and two similar types of primary structures have been determined (Files *et al.*, 1983). According to DNA sequences and electrofocusing, only slight differences can be observed between this nematode actin and the actin of vertebrates. There have been no reports thus far regarding major structural differences between *C. elegans* and *Ascaris* actin. However, at least in terms of the molecular structure of the actin of obliquely striated muscle of the body wall of *Ascaris* prepared here, it is believed that there are sufficient epitopes to allow immunological recognition as being "non-self" by a vertebrate.

In rabbits, antibodies that resulted from the animal recognizing *Ascaris* actin as being "non-self" demonstrated cross-reactivity with various types of actin, without possessing species specificity or organ specificity (Fig. 1 and 2). The antibody had cross-reactivity with the actin of rabbit skeletal muscles, with the rabbit being unable to recognize it as "non-self." Incidentally, with respect to the actin of skeletal muscle, all 375 residues of amino acid sequences are in common between rabbit, chicken, and bovine actin (Vandekerckhove and Weber, 1979). According to the results of ELISA described here also, the antibody demonstrated equal reactivity with chicken and rabbit skeletal actin (Table 1).

The stoichiometry of cross-reactivity antibody was examined using affinity chromatography (Table 2). Approximately 20% of IgG that recognized the *Ascaris* actin molecules bound with skeletal and cardiac muscle actin, while approximately 10% bound with the actin of smooth muscle. These quantitative results are in agreement with the ELISA-measured values (Table 1).

Experiments for determining the epitopes with respect to the various antibodies obtained in this research, in other words, the antibodies that react with skeletal muscle, cardiac muscle, and smooth muscle actin, as well as the *Ascaris* actin-specific IgG that does not react with the above muscle actin, are currently in progress.

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