

Cloning and characterization of tropomyosin from the mite *Chortoglyphus arcuatus*

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ABSTRACT

Tropomyosin is a pan-allergen that shares a high homology among species. It is involved in cross-reactivity among mites, crustaceans, mollusks and insects. The objectives were to express and purify recombinant tropomyosin from the storage mite *Chortoglyphus arcuatus*, and to investigate the homology and cross-reactivity with tropomyosin from other invertebrates.

Recombinant *C. arcuatus* tropomyosin (r-Cho a 10) was selected from a library by screening with a pool of patient sera. r-Cho a 10 (UniProt: H2DFL1) was sequenced, expressed in *Escherichia coli* and purified by ion exchange and affinity chromatography. Polyclonal anti-tropomyosin antibodies were produced in mice. IgE recognition of r-Cho a 10 was checked by immunoblot. Immunoblot inhibition assays were used to identify the native tropomyosin in the complete extract from *C. arcuatus* and study cross-reactivity between r-Cho a 10 and Der p 10. Identification of tropomyosin in other allergenic sources was performed by immunoblot.

r-Cho a 10 showed a high homology (54–96%) with other tropomyosins from different allergenic sources. IgE recognition was observed using a pool of sera from sensitized individuals. Tropomyosins from different extracts were identified not only in the whole *C. arcuatus* extract but also in *Dermatophagoides pteronyssinus*, shrimp, mussel, cockroach and *Anisakis* extracts with polyclonal α -Cho a 10. r-Cho a 10 completely inhibited the recognition of Der p 10.

Recombinant *C. arcuatus* tropomyosin maintained its capacity to recognize IgE. r-Cho a 10 was used to prove cross-reactivity among tropomyosins from other invertebrate species, including mites.

This is the first *C. arcuatus* allergen included in the WHO/IUIS (World Health Organization/International Union of Immunological Societies) Allergen Nomenclature database.

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1. Introduction

Chortoglyphus arcuatus is a storage mite which belongs to the family Chortoglyphidae. Its presence is common in barns, stored foods and on farms, but it is also frequently found in house dust samples from different regions worldwide (Boquete et al., 2006a,b; Marx et al., 1993; Morales et al., 2015; Puerta et al., 1993). Differ-

ent studies have demonstrated that sensitization to storage mites is more common than expected and not solely restricted to occupationally exposed individuals (Morales et al., 2015; Puerta et al., 1993; Tee, 1994; van Hage-Hamsten and Johansson, 1998; Vidal et al., 2004). *C. arcuatus* is abundant in the domestic environment of rural areas in the northwest of Spain (Galicia) (Boquete et al., 2006b). Sensitization to this mite in different areas (Chile and Galicia, Spain) ranged from 54 to 58% in patients with rhinoconjunctivitis and/or asthma (Armentia et al., 2006; Boquete et al., 2006a). The allergenicity of this mite has been confirmed by *in vivo* and *in vitro* studies (Boquete et al., 2006a) and the enzymatic activity of its allergenic particles has been associated with its high allergenicity (Carnés et al., 2008; Morales et al., 2013). Prelimi-

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Fig. 1. *C. arcuatus* tropomyosin sequence. Vector sequences are shown in grey. Primer sequences are underlined.

et al., 2014). Tropomyosin is a protein found in muscle and other non-contractile cells. In striated muscle, it is associated with the actin filaments that regulate contraction (Brown and Cohen, 2005). It is probably the most important primary allergen responsible for seafood allergies and has been associated with cross-reactivity between invertebrates, including mites, cockroaches, mollusks and crustaceans (Ayuso et al., 2002; DeWitt et al., 2004; Purohit et al., 2007). Recently, the importance of other allergens (ubiquitin, α -actinin and arginine kinase) has been suggested (Gamez et al., 2014). Sequence identity between tropomyosins from mites and other arthropods is 75–80% (Gamez et al., 2011; Reese et al., 1999). Additionally, seven IgE-binding epitopes from tropomyosin have been characterized, and they are highly conserved in shrimp and other tropomyosins from invertebrates, including mites (Ayuso et al., 2002). In mite sensitized individuals, the sensitization to

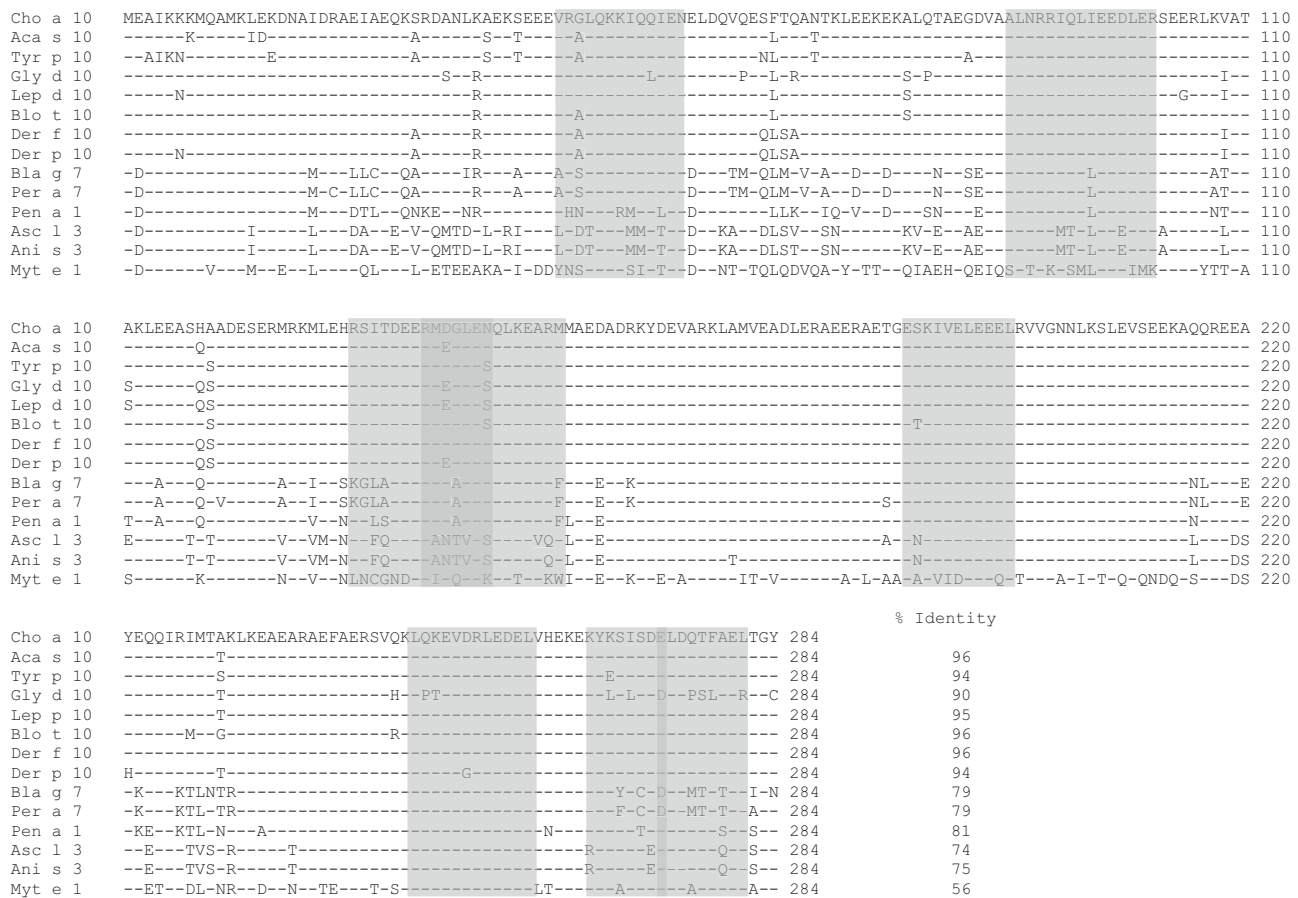


Fig. 2. Alignment of tropomyosin sequences and percentage of identity related to Cho a 10. Cross reactive epitopes previously described are in grey boxes (Ayuso et al., 2002).

tropomyosin is low (5–10%) (Armentia et al., 2006). A lack of cross-reactivity between *C. arcuatus* and shrimp has also been described (Boquete et al., 2011). However, the same authors explain this lack of cross-reactivity by the different tropomyosin concentrations in both extracts (Boquete et al., 2011). The availability of *C. arcuatus* tropomyosin could be a good tool to study the relationship between this allergen and other mites and seafood and would be useful in component-resolved diagnosis (CRD).

The objectives of the study were (1) to express and purify recombinant tropomyosin from the storage mite *C. arcuatus* (Cho a 10), and (2) to investigate the homology and cross-reactivity with other invertebrate allergenic sources.

2. Methods

2.1. Patient population and serum samples

Serum samples from 28 individuals from Galicia, Spain, were included in the study. They all had rhinitis and/or asthma and a positive skin prick test (SPT) to a battery of common inhaled mite allergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *C. arcuatus*. All patients were recruited in the Hospital Universitario Xeral-Calde (Lugo) for previously published studies (Boquete et al., 2006a; Boquete et al., 2011).

Specific IgE to *D. pteronyssinus* and *D. farinae* was determined by the ImmunoCAP system (Thermo Scientific, Uppsala, Sweden), following the manufacturer's instructions, and specific IgE to *C. arcuatus* was determined by direct ELISA. Briefly, microplates were coated with 20 µg/mL of *C. arcuatus* extract and incubated overnight. After washing, serum samples from patients were added

(dilution 1:1) and incubated for 2 h. Finally, after washing, an antihuman-IgE horse radish peroxidase (1:800) was added and the reaction was developed with TMB (3,3',5,5'-tetramethylbenzidine), stopped and absorbance was read at 450 nm. A pool of sera was prepared from 8 individuals with high levels of specific IgE to *C. arcuatus* (optical density [O.D.] greater than 1.8; range 1.8–3.4; mean 2.8 ± 0.5) who had low levels of specific IgE to Der p 1 and Der p 2 (at least one of them lower than 1 kUA/L) measured by ImmunoCAP; Der p 1 ranged from negative to 12 kUA/L, mean 1.8 ± 4.1 kUA/L; Der p 2 ranged negative to 8 kUA/L, mean 3 ± 3.5 kUA/L.

2.2. Extracts preparation

All extracts used in the study were manufactured using standardized internal protocols (Laboratorios LETI, Madrid, Spain). Briefly, *C. arcuatus*, *D. pteronyssinus* and cockroach were extracted for 4 h at 4 °C in 0.01 M PBS pH 7.4. After centrifugation, a second extraction was performed for 8 h under the same conditions. Seafood was boiled in 0.01 M PBS pH 7.4 (1:4 w/v). The edible parts were homogenized in the same buffer and kept under continuous magnetic stirring for 16–18 h at 4 °C. *Anisakis simplex* was blended in 125 mM NH_4HCO_3 (1:50 w/v) and extracted for 4 h at 4 °C. The extraction process were performed under continuous stirring. After the extractions, all extracts were centrifuged, and the supernatants were collected, dialyzed, filtered and freeze-dried.

2.3. Construction of cDNA library

A *C. arcuatus* mite culture (Dr. Arlian, Wayne State University, Dayton, OH, USA) containing approximately 8 g of mites was

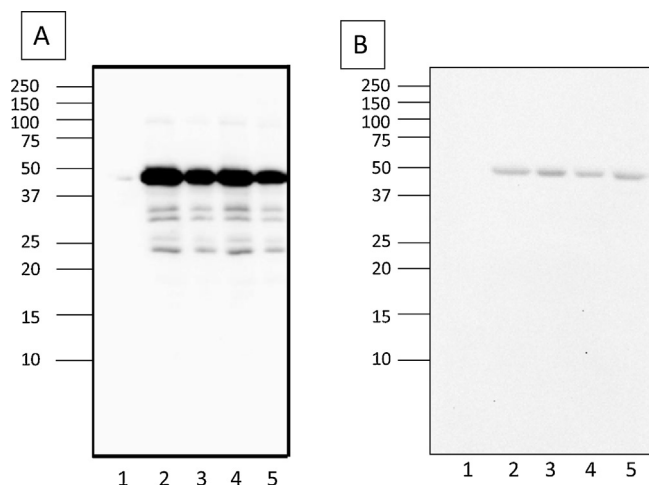


Fig. 3. Immunoblots of bacterial clones expressing: 1-Empty pRSET-B, 2-5- Four different clones containing the His-Cho a 10 construction cloned in pRSET-B. A- Anti-HisG-HRP antibody and B-A pool of sera from patients sensitized to *C. arcuatus* (IgE).

washed with bidistilled water in order to remove food particles or other contaminants, flash frozen in liquid nitrogen and shipped on dry ice. Isolation of total RNA and purification of polyA-RNA was performed by Clontech laboratories (Mountain View, CA, USA). The cDNA library was constructed and amplified in λ Triplex-2 vector (Clontech) according to the manufacturer's instructions.

2.4. Screening of the cDNA library with IgE antibodies

The pool of sera was diluted to 1:2 in SuperBlock Blocking Buffer (Thermo Scientific, Rockford, IL, USA). The library was diluted to produce 10^4 pfu/mL (titer of the amplified library was $\geq 10^9$ pfu/mL). In all, 10 μ L of the diluted library were added to 200 μ L of XL1-Blue cells (Stratagene, La Jolla, CA, USA). After the phage was allowed to adsorb to the XL1-Blue cells, 3 mL of top agar was added and plated onto LB/MgSO₄. Nitrocellulose filters presoaked with 10 mM IPTG (isopropyl- β -D-thiogalactopyranoside) were used to induce protein expression and transfer the plaques. Filters were washed, screened with the diluted serum pool and developed with goat anti-human IgE-AP-conjugated, followed by NBT/BCIP solution to identify positive plaques. More than 50 IgE-binding plaques were identified. Positive plaques were extracted, transferred to a tube containing SM buffer (0.1 M NaCl, 10 mM MgSO₄, 50 mM Tris-HCl, pH 7.5, 0.01% gelatin) and chloroform, and plated again.

Phages of positive plaques were eluted from isolated agar plugs, replated and rescreened to obtain single clones, which were subsequently converted to plasmids in *Escherichia coli* BM25.8, according to the manufacturer's recommendations.

2.5. Identification, cloning, expression and purification of r-Cho a 10

The isolated clones were sequenced using Applied Biosystems 3100 (Applied Biosystems, Foster City, CA, USA). Sequence comparison was done with Blastx (<http://blast.ncbi.nlm.nih.gov/>). Clones were selected containing sequences homologous to previously described tropomyosins.

To produce the recombinant form of tropomyosin (r-Cho a 10), a clone containing the whole sequence of tropomyosin was amplified using the primers 5'-CGGATCCAATGGAGGCCATCAAGAAG-3' and 5'-CGGAATTCCTTAATAGCCAGTGAGTTCGG-3' (BamHI and EcoRI restriction sites underlined, first and stop tropomyosin codons in

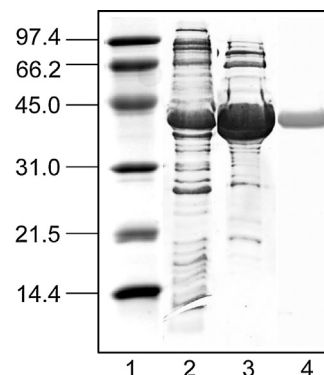


Fig. 4. SDS-PAGE with the different purification steps of r-Cho a 10. 1-Molecular weight standard (Bio-Rad), 2-*E. coli* crude extract, 3-rCho a 10 enrichment after IEX, 4-r-Cho a 10 purified after Ni²⁺ affinity chromatography.

bold), with iProof High-Fidelity DNA Polymerase (Bio-Rad, Hercules, CA, USA) and cloned between the BamHI and EcoRI sites of pRSET-B (Invitrogen, Carlsbad, CA, USA). r-Cho a 10 was produced as an N-terminal hexahistidine-tagged protein in *E. coli* Rosetta (Merck, Darmstadt, Germany). Besides the 6-His-tag, 23 amino acids that come from the vector were added to the N-terminal end (Fig. 1). Competent cells were transformed and induced with 0.4 mM IPTG. After 3 h at 30 °C, the cells were harvested and lysed by sonication. The recombinant protein was purified in a 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA). First, the lysate supernatant was passed through a HiTrap Q-FF column (GE Healthcare, Little Chalfont, UK) to obtain the fraction enriched in acidic proteins. r-Cho a 10 was eluted on an H₂PO₄⁻/HPO₄²⁻ 50 mM, pH 6.8, NaCl 1 M gradient. Fractions containing r-Cho a 10 were recovered and combined. In a second step, the pool was passed through a HisTrap FF affinity column (GE Healthcare), and was eluted on an H₂PO₄⁻/HPO₄²⁻ 20 mM, pH 6.8, NaCl 0.5 M, 500 mM imidazole gradient. Protein concentration was calculated using the Lowry-Biuret method.

2.6. IgE-binding to r-Cho a 10

Specific IgE to r-Cho a 10 was detected by immunoblot. The different bacterial clones expressing r-Cho a 10 were electrophoresed and transferred to an Immulon-P membrane (Millipore, Bedford, MA, USA) and hybridized with anti-HisG-HRP antibody (Invitrogen, Carlsbad, CA, USA) to confirm the presence of the fusion protein. Similarly, the same bacterial clones were incubated with *C. arcuatus* specific serum pool to check the recognition of the tropomyosin-specific IgE. After incubation with anti-human IgE-PO (Ingenasa), membranes were developed with luminol (Immun-Star HRP Chemiluminescent Kit; Bio-Rad Laboratories), and bands were detected using chemiluminescence.

2.7. Immunoblot inhibition studies

Inhibition studies were performed between *C. arcuatus* extract and r-Cho a 10 and between r-Cho a 10 and Der p 10 using the pool of sera or anti-Der p 10 monoclonal antibody MA-1A6 (provided by Indoor Biotechnologies, Charlottesville, VA, USA). Briefly, fifty micrograms of lyophilized *C. arcuatus* or *D. pteronyssinus* extract (Laboratorios LETI) were electrophoresed and electrotransferred to an Immulon-P membrane (Millipore). A total of 5 μ g of r-Cho a 10 was preincubated with the pool of sera (dilution 1:1) or with anti-Der p 10 monoclonal antibody (dilution 1:6000) for 2 h. Afterwards, the inhibition mixtures were incubated with the membrane for 2 h, washed and developed by chemiluminescence. Percentage

of recognition of each bands was calculated by densitometry with the software ImageQuant TL 8.1 (GE Healthcare, Uppsala, Sweden).

2.8. ImmunoCAP inhibition

An immunoCAP inhibition assay was performed in the ImmunoCAP 100E system (ThermoFisher Scientific) to demonstrate the IgE cross-reactivity between Der p 10 and Cho a 10. Different aliquots of the pool of sera (1/2 diluted) were previously incubated with amounts of r-Cho a 10 ranging from 0.02 to 100 ng. The same dilution of the pool of sera in 0.01 M PBS buffer without r-Cho a 10 was used as control. After 2 h of incubation, specific IgE binding to rDer p 10 (d205) was determined for the aliquots and for the control.

2.9. Anti-r-Cho a 10 polyclonal antibody production

Five BALB/c mice were immunized on day 0 with 60 µg purified r-Cho a 10 in complete Freund's adjuvant, and with 30 µg r-Cho a 10 in incomplete Freund's adjuvant, as boosters on day 28 and 56. Sera were obtained after 70 days. The study was conducted at the Vivotecnia Research facilities (Madrid, Spain). All the procedures were approved by Vivotecnia Research's Institutional Review Board and complied with national regulations for animal experimentation. Sera were checked and titrated by direct ELISA with complete *C. arcuatus* extract and r-Cho a 10. Briefly, microtiter plates (Immulon 4; Dynex Technologies, Chantilly, VA) were coated with *C. arcuatus* extract (20 µg/mL) or r-Cho a 10 (10 µg/mL) overnight and blocked with 1% BSA in PBS-Tween. Serial dilutions (1:500 to 1:16000 v/v) were prepared in PBS 0.01 M with each mouse serum sample. After 2 h of incubation in the coated wells, the plates were washed and incubated for another 2 h with goat anti-mouse IgG-PO (dilution 1:2000) (Nordic-Cultek, Tilburg, The Netherlands). After 5 washes, the reaction was developed with TMB for 30 min, stopped with sulfuric acid 1N and read at 450 nm with a plate reader (Thermo Fisher Scientific). Preimmune sera were used as negative controls.

2.10. Recognition of tropomyosin in invertebrate extracts

Anti-r-Cho a 10 antibody was used in an immunoblot assay to identify the tropomyosin from other invertebrates: mite (*D. pteronyssinus*), seafood (shrimp: *Penaeus aztecus* and mussel: *Mytilus edulis*), insect (cockroach: *Blattella germanica*) and the nematode *A. simplex*. Extracts were prepared as explained in section 2.2. Fifty micrograms of each extract were electrophoresed and transferred to an Immulon-P membrane (Millipore) and hybridized with polyclonal anti-r-Cho a 10 antibody 1:2000 (v/v) diluted and detected with goat anti-mouse IgG-PO (dilution 1:2000). Developed and detection of the bands were performed as explain in section 2.6.

3. Results

3.1. cDNA and protein sequence analysis of r-Cho a 10

Six clones containing the tropomyosin sequence were isolated by 2 rounds of immunoscreening. All the clones had the same cDNA sequence of 1168 base pairs containing a sequence homologous to mite tropomyosins. The sequence corresponding to tropomyosin was amplified by PCR and cloned into pRSET-B. The sequence obtained has been deposited under GenBank accession number JN596422, UniProtKB H2DFL1 and named Cho a 10 by the WHO/IUIS Allergen Nomenclature Sub-Committee (<http://www.allergen.org/>) (Supplementary material, Fig. 1). r-Cho a 10 contains 284 amino acids (Fig. 1). Based on the ProtParam tool (<http://expasy.org/cgi-bin/protparam>), the putative protein had a molecular mass

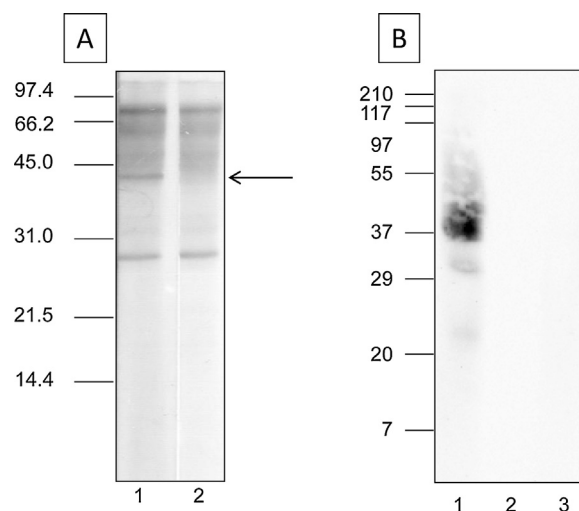


Fig. 5. A. Solid phase *C. arcuatus* extract, 50 µg/line. Immunoblot with 1-Pool of patients sera (dilution 1:1), 2-Pool of patient sera inhibited with 5 µg of rCho a 10. Position of tropomyosin is marked with an arrow. B. Solid phase *D. pteronyssinus* extract, 50 µg/line. Immunoblot with: 1-Anti-Der p 10 1:6000 2-Anti-Der p 10 inhibited with 500 µg of *D. pteronyssinus* extract, 3-Anti-Der p 10 inhibited with rCho a 10, 5 µg.

of 36.7 kDa (including the histidine tag at the N-terminus) and a theoretical pI of 4.88. *C. arcuatus* tropomyosin has 74–96% identity with allergenic tropomyosins from other mites (Der p 10, O18416; Der f 10, Q23939; Lep d 10, Q9NFZ4; Tyr p 10, AY623832; Aca s 10, B0KZJ4; Gly d 10, Q1M2L8; Blo t 10, A7XZ14), insects (Bla g 7, Q9NG56; Per a 7, Q9UB83), crustaceans (Pen a 1, Q3Y8M6), nematodes (Ani s 3, Q9NAS5; Asc l 3, COL3K2), and 56% identity with mollusks (Myt e 1, Q25457) (Fig. 2). All the sequences were obtained from UniProtKB (<http://www.uniprot.org/>) except Tyr p 10, which was obtained from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). The sequence of Cho a 10 contains all the tropomyosin IgE-binding epitopes previously described (Ayuso et al., 2002) (Fig. 2).

3.2. Expression, IgE binding and purification of r-Cho a 10

A protein band of about 42–44 kDa was observed in immunoblot with anti-HisG-HRP for the clones that expressed r-Cho a 10 (Fig. 3A). IgE-binding of the same four clones was demonstrated by immunoblot with the pool of patient sera sensitized to *C. arcuatus* (Fig. 3B).

After the first purification step by IEX (ion-exchange chromatography), fractions with the highest r-Cho a 10 quantity were pooled (Fig. 4, line 3). In a second step, purified r-Cho a 10 was obtained by Ni²⁺ affinity chromatography. Purified r-Cho a 10 has a purity greater than 95% (Fig. 4, line 4) and a concentration of 0.6 mg/mL. The yield of purified protein was 1.85 mg/L of bacterial culture.

3.3. Identification of *C. arcuatus* tropomyosin

Tropomyosin was identified in the whole *C. arcuatus* extract by immunoblot inhibition. The band corresponding to tropomyosin was completely inhibited with r-Cho a 10 (Fig. 5A). Cho a 10 recognition was equivalent to 17.6% of the total obtained for the whole extract, measured by densitometric analysis.

3.4. Cross-reactivity between Cho a 10 and Der p 10

Immunoblot inhibition was performed to demonstrate the IgG cross-reactivity of mite tropomyosins. r-Cho a 10 completely inhibited the recognition of Der p 10 by a monoclonal anti-Der p 10

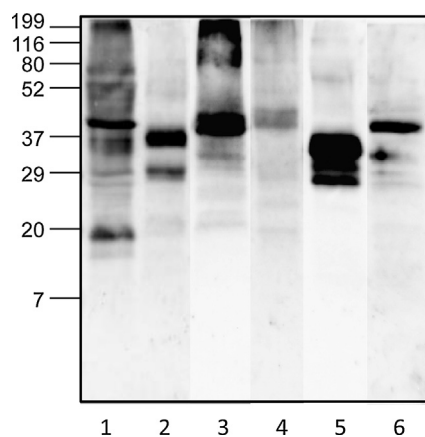


Fig. 6. Recognition of heterologous tropomyosins with polyclonal anti-rCho a 10 antibody. Lane 1-*C. arcuatus*, 2-*D. pteronyssinus*, 3-*P. aztecus*, 4-*M. edulis*, 5-*P. americana*, 6-*A. simplex*. Solid phase with 50 µg of each extract.

antibody (Fig. 5B). Self-inhibition with complete extract of Der p 10 was used as a positive control.

A 50% of inhibition of IgE binding to rDer p 10 was obtained with 0.22 ng of rCho a 10 (11 ng/mL). The maximum inhibition (76%) was obtained after incubating the pool of sera with 1.5 ng of rCho a 10 (75 ng/mL) (Supplementary material, Fig. 2).

3.5. Anti-r-Cho a 10 antibodies production and recognition of tropomyosin from other species

Polyclonal anti-r-Cho a 10 antibodies produced in mice recognized both r-Cho a 10 and the complete *C. arcuatus* extract in ELISA. Moreover, the antibody detected tropomyosin in all the invertebrate extracts tested (Fig. 6).

4. Discussion

Cross-reactivity between mites and seafood has been clearly demonstrated and the involvement of tropomyosin extensively studied (Ayuso et al., 2002; Boquete et al., 2011; DeWitt et al., 2004; Gamez et al., 2011, 2014; Lopata et al., 2010; Purohit et al., 2007; Reese et al., 1999). The characterization of tropomyosin in different species will cast light on invertebrate cross-reactivity and improve the selection of patients for immunotherapy.

In that sense, the objective of this study was to clone and purify tropomyosin from *C. arcuatus*. This is the first allergen from this mite to be identified and characterized. This allergen has been included in the WHO/IUIS Allergen Nomenclature database under the name Cho a 10.

r-Cho a 10 has been isolated from a cDNA library via screening with a pool of patient sera from Galicia. Patients were selected by their high IgE to *C. arcuatus*. In this area of Spain, *C. arcuatus* is very common (Boquete et al., 2006b). Additionally, shellfish consumption and allergies to this kind of food are high in Spain (Gonzalez-Fernandez et al., 2014).

The expression of the Cho a 10 cDNA produces a recombinant protein recognized by sera IgE from patients with *C. arcuatus* allergy, indicating that the recombinant protein is biologically active, containing IgE antibody binding determinants. Moreover, preincubation of the sera with r-Cho a 10 inhibited sera IgE reactivity to the band corresponding to native Cho a 10 in the *C. arcuatus* extract by immunoblotting, suggesting that r-Cho a 10 contains most or all the Cho a 10 IgE epitopes recognized by sera from patients with an allergy to this mite. The 8 IgE-binding epitopes described as cross-reactive from

shrimp, HDM and cockroach tropomyosins (Ayuso et al., 2002) only differ in 3 amino acids out of 88 (96.6% identical) between Cho a 10 and Der p 10, and 4 epitopes are completely identical. The difference between Cho a 10 and Pen a 1 is due to 12 amino acids (86.4% identical), and 2 epitopes are identical (Fig. 2).

Pan-allergens, like tropomyosin, are markers of cross-reactivity (Valenta et al., 1999) and can explain sensitization to different sources, such as mites and seafood. Sensitization to pan-allergens may also predict the severity of allergic reactions to other allergen sources containing the homologous protein (Boquete et al., 2011). The use of *in vitro* CRD in clinical practice has improved patient selection for specific immunotherapy (IT) (Sastre et al., 2012; Stringari et al., 2014). In mite-sensitized patients, diagnosis with purified tropomyosin enables the initial selection of patients as candidates for IT. Tropomyosin positive patients are likely to be sensitized to other invertebrates (seafood, cockroaches or *Anisakis*) (Reese et al., 1999). To identify these patients, *in vitro* CRD is most commonly used.

We recently purified shrimp tropomyosin for SPT use (unpublished data). Mite-sensitized patients with a negative SPT to tropomyosin are most likely sensitized to major mite-specific allergens (from groups 1 and 2), in which case IT has a high probability of success.

Tropomyosins from mites (Der p 10) and shrimp (Pen a 1) have been used in CRD, but none from storage mites has been used until now. In this study, we demonstrated cross-reactivity between Der p 10 and Cho a 10. Cho a 10 is able to inhibit the binding of Der p 10 to its specific monoclonal antibody and a polyclonal anti-Cho a 10 antibody can recognize Der p 10. Allergic cross-reactivity was evaluated by showing inhibition of IgE reactivity to rDer p 10 by rCho a 10 using ImmunoCAP. A maximum inhibition of 76% indicated the presence of common, as well as species-specific epitopes between both allergens. Low cross-reactivity has been previously reported between *C. arcuatus* and *D. pteronyssinus*, a possible explanation could be that there are small amounts of tropomyosin in the extracts used to select the patients, as has been previously reported for 8 different extracts from different commercial manufacturers (Brunetto et al., 2010). CRD is highly useful for preventing the problem caused by the different allergen content in the extracts.

Anti-Cho a 10 antibodies also recognized tropomyosins from different invertebrate sources, with high specificity and sensitivity. These results suggest the presence of conserved regions between tropomyosin from storage mites and other invertebrates, but more studies related to cross-reactivity between tropomyosins from different sources are required. Such studies could help to improve the diagnosis of sensitization to tropomyosin. The use of purified tropomyosins may resolve the problem proposed in previous studies regarding the differences in allergen quantity between mite and crustacean extracts (Boquete et al., 2011).

In summary, we have cloned the cDNA sequence encoding the tropomyosin from *C. arcuatus* (Cho a 10) which has 56–96% homology with other tropomyosins. The recombinant protein maintains its IgE-binding capacity, and an antibody against Cho a 10 was able to recognize other tropomyosins. Cross-reactivity among Cho a 10 and Der p 10 was demonstrated. Cho a 10 is the first *C. arcuatus* described allergen and can be used in tropomyosin diagnostic assays.

Acknowledgements

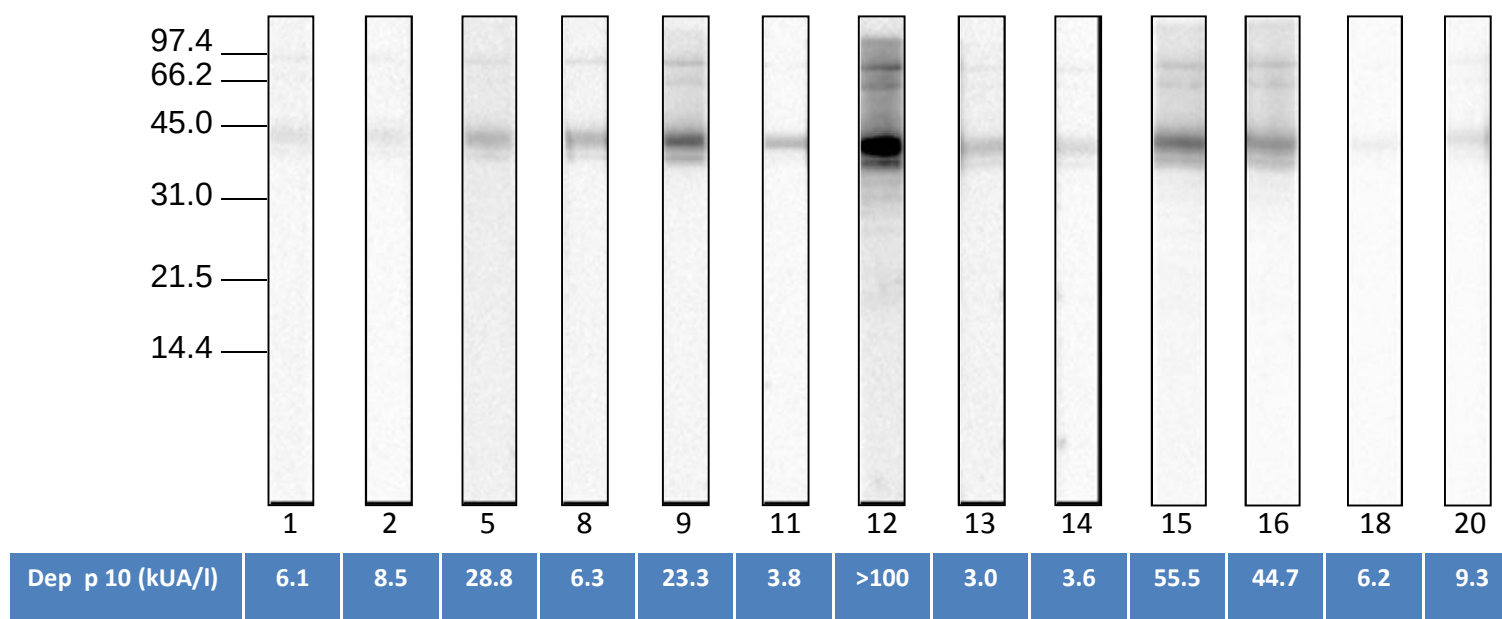
We would like to thank Ms. Olga Troubina for screening the cDNA library and identifying the rCho a 10 clones and Ms. Raquel Reyes for her technical work.

Appendix A. Supplementary data

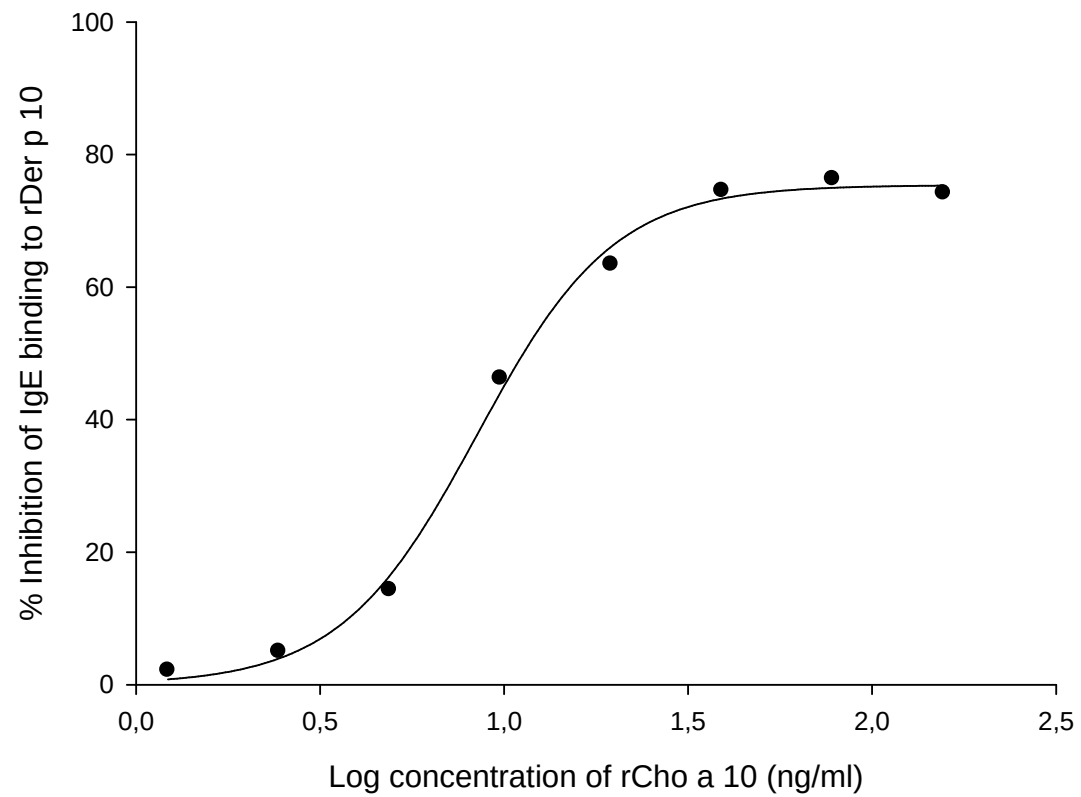
Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molimm.2015.10.004>.

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Supplementary material figure 1: Immunoblots with individual sera. Solid phase: 3 µg of purified rCho a 10 per lane. Values of sIgE to Der p 10 by CAP are shown under each blot assay. Figure submitted to the IUIS for the inclusion of Cho a 10 in the Allergen Nomenclature database (<http://www.allergen.org/>).



Supplementary material figure 2: Inhibition of IgE binding to rDer p 10 with rCho a 10 by ImmunoCAP.