STRUCTURAL CHARACTERISTICS OF THE ASCARIS ALLERGEN, ABA-1

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Summary:
The structure of the Ascaris allergen, ABA-1 was characterized at several levels. Purified allergen monomers eluted from reducing PAGE were found to reassociate into dimers in phosphate buffered saline containing 0.9 mM Ca2+. This association may involve the formation of disulfide bonds between monomers. The primary amino acid sequence was used to predict secondary structure and compare the allergen to other known proteins sequences. ABA-1 appears to be highly helical protein of two domains. Sequence analysis reveals short regions (25 amino acids) of high homology (76%) between ABA-1 and the major body wall myosin of Onchocerca volvulus. In addition, ABA-1 has sequence similarity to a family of EF-hand containing calcium binding proteins called S100 proteins. The dimerization and two-domain structure of ABA-1 is consistent with the possibility that ABA-1 is a member of the S100 family of calcium binding proteins.

KEY WORDS: Ascaris, allergen, nematode, S100 proteins, ABA-1.

INTRODUCTION

Ascaris lumbricoides and Ascaris suum are important intestinal roundworm parasites of man and pigs. An estimated 22% of the world population, mostly young children, are infected with A. lumbricoides (Crompton, 1988) and up to 20,000 deaths annually result directly from this infection. A. suum results in significant economic loss to the pork industry due to a decreased weight gain/feed ratio during swine rearing. Ascaris, like other nematodes, is well known for its ability to strongly stimulate allergic responses in the host. These responses are characterized by mast cell hyperplasia, eosinophilia, and increased levels of circulating IgE (Urban, Gamble and Katona, 1989). These immune responses contribute significantly to the pathology of nematode infections and, although previously thought to be integral to resistance to infection, recently have been suggested to be advantageous to parasite survival by activating T helper cell mechanisms which result in a decrease in protective inflammatory responses (Pritchard, 1993).

In an effort to understand the stimulation of these characteristic responses, there have been many efforts to identify allergenic molecules from Ascaris and several have been partially purified from worm homogenates (reviewed in Greenspon, 1986).

Recently, we described (McGibbon, Christie, Kennedy and Lee, 1990) the identification of the major Ascaris allergen, ABA-1, purified to homogeneity from Ascaris body fluid (ABF) with high performance liquid chromatography (HPLC). The allergenicity of this protein was established by the induction of histamine release from rat peritoneal mast cells which were sensitized in vitro by A. suum infection. ABA-1 is referred to as the major allergen because it is responsible for greater than 80% of the specific reaginic, IgE-dependent, mast cell degranulation seen by incubation with ABF and mast cells sensitized by infection (McGibbon, Christie, Kennedy and Lee, 1990). Although IgE is directed against ABA-1, it is unclear if this protein is responsible for stimulating both the specific and non-specific IgE responses seen during Ascaris infection. Our recent work has identified a role for the allergen in the prolongation, but not the potentiation, of IgE responses to third party antigens (Lee and McGibbon, 1993).
Clearly ABA-1 is important in the immune response to *Ascaris*, but the function of this molecule within the parasite is not known. ABA-1 comprises > 60% of the total protein content of ABF. ABA-1 has been identified in all larval stages of *Ascaris* (Kennedy and Qureshi, 1986). In addition, homologues to ABA-1 have been identified in a number of different parasitic nematodes (Kennedy, Tiemey, McMonagle, McIntosh and McLaughlin, 1988) using anti-ABA-1 monospecific polyclonal antiserum and in the free-living nematode, *Caenorhabditis elegans* (McGibbon, unpublished observations). The conservation of ABA-1 between nematode genera is suggestive of an important function in development or metabolism. Despite the many studies using *Ascaris* and its products, however, the biological functions of this protein have not been determined.

In an effort to understand the functions of the protein ABA-1 both as a major allergen and as an important constituent of *Ascaris* body fluid, an understanding of its structure and comparisons to other known proteins are critical. The present study has examined the structural characteristics of ABA-1 at several different levels. The cloning of fragments of the cDNA for ABA-1 (Spence, Moore, Brass and Kennedy, 1993) has provided a complete amino acid sequence which we have used to predict secondary structure with computer analysis programs. The proposed dimeric quaternary structure of ABA-1 has been investigated using polyacrylamide gel electrophoresis. In addition, analysis of the complete amino acid sequence has revealed that ABA-1 may be related to the S100 family of calcium-binding proteins, thereby providing the first suggestion of the allergen's potential function in the worm and the first structural/functional link between ABA-1 and another major allergen, Allergen M of cod.

**MATERIALS AND METHODS**

ABF and ABA-1 were collected and prepared as described by McGibbon (McGibbon, Christie, Kennedy and Lee, 1990). Briefly, HPLC was used to purify ABA-1 from *Ascaris* body fluid using a TSK 3000SW size exclusion column on a Beckman System Gold HPLC (Beckman Instruments, Mississauga, Ont.). Molecular weight estimations were determined. The supernatant was decanted from the gel fragments and run through Centricon 10 microconcentrators (Amicon, Oakville, Ont.) at 5,000xg until the volume was reduced two fold. The concentration of eluted ABA-1 was then determined with a Bio-Rad protein assay kit using bovine serum albumin as a standard. PAGE were transferred electrophoretically to nitrocellulose paper for western blotting. The polyclonal anti-ABA-1 antiserum was prepared, in rabbits, as described in Knudsen (1985) and was used at 1:50 in 2% skim milk as a blocking solution. The detection system used horseradish peroxidase conjugated goat anti-mouse IgG as a secondary antibody and BioRad HRP Color Development Reagent for visualization. Amido black was used to stain a nitrocellulose strip of transferred PAGE containing molecular weight markers purchased from BioRad (Mississauga, Ont.) and containing the proteins phosphorylase B (97,400 Da), bovine serum albumin (66,200 Da), ovalbumin (45,000 Da), carbonic anhydrase (31,000 Da), trypsin inhibitor (21,500 Da) and lysozyme (14,400 Da).

The amino acid sequence of ABA-1 was analyzed with the computer program GCG, Version 5 (University of Missouri). The programs run included PeptideStructure and PlotStructure (Jameson and Wolf, 1988). Sequence searches for homology were performed using BLAST network service at the National Centre for Biotechnology Information and included searches of SWISS-PROT 24.0 and updates PIR 35.0, Gen Bank Release 75.0, Kabat Sequences of Proteins of Immunological Interest Release 5.0, and others. The amino acid sequence was also analyzed with Blocks Database Version 6.0 (Fred Hutchinson Cancer Research Centre, Seattle, Wa.; Henikoff and Henikoff, 1991).
RESULTS

SECONDARY STRUCTURE OF ABA-1

The N-terminal forty-two amino acids of the purified allergen were amino acid sequenced (McGibbon, Christie, Kennedy and Lee, 1990), and ABA-1 has now been cloned (Spence, Moore, Brass and Kennedy, 1993). The complete amino acid sequence (133 amino acids) of ABA-1 has been analysed for secondary structure with several computer analysis programs. GCG Version 5 was used to predict the secondary structure of ABA-1. Both Chou-Fassman (CF) and Garnier-Osguthorpe-Robson (GOR) algorithm predictions are in strong agreement and predict >90% alpha helix from the ABA-1 primary sequence. PeptideStructure indicates two long alpha helical structures (amino acids 1-61 and 71-111 by CF prediction) separated and ended by beta sheet. There is a random coil structure from amino acid 121-127 by GOR prediction. Jameson-Wolf antigenic index is maximum at amino acids 61-63, coinciding with a predicted beta turn (Jameson and Wolf, 1988). GOR algorithms are used to predict a two dimensional secondary structure of ABA-1 diagrammed in Figure 1. The two cysteines in ABA-1 are at amino acids 64 and 120, both in beta sheet structures. Although these predictions are useful in visualization of the protein secondary structure, it is important to emphasize that two dimensional representation does not predict folding and intra or intermolecular interactions.

STRUCTURE OF NATIVE ABA-1

With gel filtration HPLC, the molecular weight of native ABA-1 is in the range of 22-30 kDa, however this protein migrates below the 14kDa marker (lysozyme) on our reducing SDS-PAGE system. To explain this discrepancy, we have proposed (McGibbon, Christie, Kennedy and Lee, 1990) that ABA-1 exists as a dimer whereby the reduced monomers are visible on SDS-PAGE and the native form is seen with HPLC. To investigate this possibility and to determine the association of the monomers, the ABA-1 band, at approximately 10 kDa, was eluted (EABA-1) from SDS-PAGE in PBS and then run on HPLC (Fig. 2). The eluted monomer reassociates into its native form in PBS as indicated by the appearance of a single peak at 25-27kDa when compared to gel filtration standards.

To investigate the nature of the association between the monomers of ABA-1, the SDS-PAGE eluted ABA-1 monomer was re-associated in PBS and then re-run on SDS-PAGE with or without 2-mercaptoethanol in the sample buffer. Both samples were boiled for five minutes in the presence of 1 % SDS. On SDS-PAGE, the eluted protein ran at the usual 10-12 kDa under reducing conditions (Fig. 3), however, when the
Fig. 2. – Gel permeation HPLC of ABA-1 eluted from reducing SDS-PAGE in PBS. The single peak has a retention time identical to that of ABA-1 in *Ascaris* body fluid run on this column and corresponds to a molecular weight of approximately 25-27 kDa.

Fig. 3. – Eluted ABA-1 monomers cut from reducing SDS-PAGE are re-run on PAGE in the presence of the reducing agent, 2-mercaptoethanol (left lanes-R) or without 2-mercaptoethanol present (right lanes-NR). The centre lane contains molecular weight markers (M) with the molecular weight of each indicated in kDa. The protein marked by the arrow has an estimated molecular weight of 27 kDa indicating it is dimeric ABA-1.

Fig. 4. – Western blot of *Ascaris* body fluid at two concentrations run on non-reducing SDS-PAGE with anti-ABA-1 antiseraum showing the ladder of bands in ABF which may result from dimerization and aggregation of monomers. Markers are indicated to the left in kDa.
2-mercaptoethanol was omitted from the sample buffer, the protein ran as a ladder with the majority of the protein being visualized at 10-12 kDa but a significant band appearing at 28kDa and lighter bands appearing in multiples of these.

Although unlikely, the dimerization and aggregation of ABA-1 may be an artifact of the elution conditions. To ensure that this is not the case, western blotting was performed on Ascaris body fluid (ABF), a heterogeneous mixture of proteins and the source of ABA-1. With our monospecific polyclonal anti-ABA-1 antiserum, the results show the same ladder of bands seen on non-reducing PAGE (Fig. 4) indicating that in native conditions, ABA-1 may be found in dimers and aggregates.

**SEQUENCE COMPARISONS**

Searches for homologies with sequence banks indicate that ABA-1 has sequence homology to antigens of Brugia malayi and Dirofilaria immitis as described by Spence et al. (1993). We also find ABA-1 has short regions (25 amino acids) of high homology (75%) to Onchocerca volvulus major body wall myosin (Fig. 5). These types of comparisons may not be as useful as that provided by Blocks, a program designed to recognize highly conserved functional regions within families. Analysis with this program has identified homology between ABA-1 and a calcium binding protein isolated from rabbit lung tissue called calgizzardin (Watanabe, Ando, Todoroki, Minami and Hidaka, 1991). The initial recognition of similarity between portions of ABA-1 to functional calcium binding regions or EF hands of calgizzardin prompted further analysis. The primary sequence of ABA-1 was compared to other members of the S100 family of proteins to which calgizzardin belongs. Direct sequence comparison between the beta subunit of the human S100 protein and ABA-1 reveals 45% similarity (identical amino acids and conservative changes) with two gaps introduced for better alignment. Amino acids 40-80 of ABA-1 are > 50% identical to the region of S100b containing the N-terminal EF hand (Fig. 6a). In multiple sequence alignment (Fig. 6b), ABA-1 is compared to other members of the S100 family (Harder, Kube and Gerke, 1992).

**DISCUSSION**

There has been much interest in nematode models of allergic responses since they have been involved in the defining of T helper subset cytokine profiles and activities (Mossman, 1991 and Else and Grencis, 1991). To further our understanding of the mechanisms involved in this response, the allergens of nematodes are the subject of current research in many laboratories and the most studied of all nematode allergens is from Ascaris, now identified as ABA-1 (McGibbon, Christie, Kennedy and Lee, 1990). The function of this protein with respect to the host may be the stimulation of TH2 activities (as evidenced by its prolongation of IgE responses to irrelevant antigens; Lee and McGibbon, 1993) thereby protecting the worm from more effective host responses. This protein is present in abundance in all larval and adult stages of A. suum and A. lumbricoides suggesting an important function in the worm which has not yet been defined. The structural characteristics of ABA-1 have been investigated here and the secondary and quaternary structure of ABA-1 has revealed characteristics consistent with primary sequence homology to the S100 family of calcium-binding proteins.

There is now strong evidence that ABA-1 natively exists as a homodimer with the subunits varying by only a few amino acids if at all (Christie, Dunbar and Kennedy, 1993). ABA-1 amino acid sequence reveals a highly helical monomer by both Chou-Fassman and Garnier-Osguthorpe-Robson predictions and a two dimensional structure of the monomer (Fig. 1) shows the cysteines and a putative antigenic epitope in short beta sheet structures. Without more structural data (such as from X-ray crystallography), a discussion of the tertiary structure of this protein is speculative, however the separation of the two cysteines by a long alpha helix (45 amino acids in length) makes the formation of intramolecular disulphide bonds unlikely. The two cysteines, therefore, are probably involved in intermolecular associations between ABA-1 monomers.

SDS-PAGE was used to investigate the quaternary structure of ABA-1 and intramolecular disulphide linkage of monomers. Reduced ABA-1 monomers, eluted from SDS-PAGE, are able to reassociate into dimeric form in PBS (Fig. 1) giving a molecular weight estimation by HPLC identical to native ABA-1 in ABF. The reassociated dimers, when reapplied to SDS-PAGE in the presence of a reducing agent, appeared only as monomers (Fig. 2, lane R), however, when run on SDS-PAGE without a reducing agent, monomers, dimers and aggregates containing three monomer subunits were seen (Fig. 2, lane NR). The fact that dimers are present in nonreducing but not reducing conditions provides evidence of disulphide linkage of the dimers, but the presence of the majority of the ABA-1 protein as monomers in the nonreducing gel indicates that not all of the ABA-1 monomers readily re-associate covalently. Sequence analysis has indicated the relationship between
Fig. 5. – Sequence comparison between ABA-1 and *Onchocerca volvulus* major body wall myosin reveals a region of 25 amino acids showing 36% identity and 76% similarity. Identical amino acids are indicated by a dash, and similar amino acids by a dot.

Fig. 6. – **a.** Sequence comparison between ABA-1 and S100b in this region shows 33% identity and 77% similarity. Identical amino acids are marked with a dash and conserved amino acids with a dot. The position of a recognized Ca\textsuperscript{2+} binding site in S100b is indicated by the horizontal line. **b.** Alignment of the amino acids sequences of ABA-1 and members of the S100 family of calcium binding proteins. Gaps (−) have been introduced to allow for optimum alignment. Dots marked similar amino acids in ABA-1, Calgizarin (CALG), human S100 beta (S100b) and MRP8, also known as the CF antigen, P8 or calgranulin A. The asterix mark identical amino acids. The position of recognized Ca\textsuperscript{2+} binding EF hands in the S100 proteins have been marked by a horizontal line. The boxed region of ABA-1, coinciding with the N-terminal gap, is the area showing homology to *Onchocerca volvulus* myosin.
ABA-1 and the S100 family of proteins. These proteins also form dimers but dimerization is influenced by calcium concentration in that the binding of calcium to EF-hand structures allows conformational changes exposing portions of the protein involved in dimerization. For example, parvalbumin will exhibit dimer formation in 3 mM Ca\(^{2+}\), however the percentage of monomers associated as dimers increases when kept for one month at -20°C (Barger, Wolchok and Van Eldik, 1992). It is possible that ABA-1 dimer formation is dependent on factors such as calcium concentration and incomplete dimerization of ABA-1 monomers has occurred under the conditions used in these experiments.

Although the dimerization of ABA-1 appears to have characteristics of the S100 proteins, the formation of polymers or aggregates is not a feature of the family. cDNA cloning of the message for ABA-1 has suggested that ABA-1 exists as a proprotein which is post translationally cleaved into monomers (Spence, Moore, Brass and Kennedy, 1993). Although the ladder of bands in Figure 3 may represent different stages of the proprotein cleavage, the fact that the eluted monomers are capable of reassociating into the same ladder of bands (Fig. 2) renders this conclusion doubtful. The gene structure of several S100 proteins has been identified and do not support the proposed ABA-1 message structure translating a long proprotein. Commonly, the two EF hands (marked in Fig. 6b) are encoded on different exons and it is believed that they have arisen from a gene duplication event in a common ancestor. There are two domains in ABA-1 with 26% homology to each other which are also suggested to have arisen from gene duplication (Spence, Moore, Brass and Kennedy, 1993). It is not known if ABA-1 is also encoded on two exons.

The similarity (including identical amino acids and conservative changes) between ABA-1 and the beta subunit of human S100 is high, with the most conserved area being within the region of the N-terminal EF hand (calcium binding domain). Interestingly, amino acids 20-30, within the N-terminal gap, is the area that ABA-1 is most similar (up to 50%) to the invertebrate sarcoplasmic calcium binding proteins (Takagi and Konishi, 1986). In addition, exactly coinciding with this gap is the area of homology between Oeneococerca volvulus myosin and ABA-1 (Fig. 5; 76% similarity and 36% identity in 25 amino acids). ABA-1 may therefore represent a bridge point in the evolution of the calcium binding proteins.

The function of the S100 proteins are still not known, however S100 proteins are believed to exert their biologic activity by responding to Ca\(^{2+}\) levels and mediating physiological responses. The S100 proteins have been described with involvement in cell cycle progression, chemotaxis and cell differentiation (reviewed in Kligman and Hilt, 1988). Some S100 proteins, however, have EF hands which have evolved such that they are no longer functional calcium binding domains. It is not known if ABA-1 has functional EF hands which bind calcium, however by similarity, it is possible that ABA-1 acts within the worm in response to calcium. Interestingly, Parvalbumin pl 4.75 (or Allergen M), the major allergen of cod fish, is also a calcium binding protein related to S100 proteins (Elsayed, Apold, Holen, Vik, Florvaag and Dybendal, 1991). The similarity of potent allergens to this family of protein is suggestive of a link between structure/function and the potentiation of TH2 directed IgE responses; currently an area of intense research in numerous laboratories.

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