

Structural and Ligand Binding Analysis of Recombinant Blo t 13 Allergen from *Blomia tropicalis* Mite, a Fatty Acid Binding Protein

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Key Words

Fatty acid binding protein · Cytosolic lipid binding protein · *Blomia tropicalis* · Allergen · Recombinant allergen · Structural model · Blo t 13

Abstract

Background: We have previously described a cDNA (clone Bt6) encoding a novel allergen from *Blomia tropicalis*, which showed sequence similarities to the FABP/P2/cellular retinoic acid binding protein/cellular retinol binding protein, a family of cytosolic lipid transport proteins (cLTPs). This work was planned to better characterize this allergen to which the official name Blo t 13 had been assigned. **Methods:** Fluorescence-based lipid ligand binding assays and secondary structure analysis by circular dichroism were carried out using recombinant Blo t 13 (rBlo t 13) protein. Structural predictions and molecular modelling were performed based on the amino acid sequence inferred from the open reading frame of Bt6 cDNA sequence. **Results:** rBlo t 13 binds the natural fluorescent fatty acid *cis*-parinaric acid and oleic acid by competition, but not retinol, retinoic acid, cholesterol, dansylated or anthroxylated fatty acids such as dansyl-*DL*-aminocaproic acid and 12-(9-anthroxyl)-stearate. Circular dichroism analysis indicated that rBlo t 13 comprises 45% β -sheet and 13% α -helix. The amino acid sequence of Blo t 13 modelled

well to known crystal structures of cLTPs providing a tertiary structural model comprising ten β -strands organized into two β -sheets, and two short α -helices. **Conclusion:** Blo t 13 is a fatty acid-specific member of the β -rich cLTP family of proteins.

Blomia tropicalis is a domestic mite commonly found in tropical and subtropical regions causing a high frequency of sensitization among asthmatic patients [1, 2]. Some allergic individuals have a strong IgE reaction directed mainly to the Blo t 13 allergen, to which 11% of the mite-allergic population are sensitized [3]. Several allergens from the mite *B. tropicalis* have been cloned and sequenced, providing an important step for their functional and molecular characterization. We reported previously on the isolation of a cDNA encoding an allergen from *B. tropicalis* [3]. The inferred amino acid sequence of this allergen shows similarities to the β -rich family of cytosolic lipid transport proteins (cLTPs), but such is the sequence diversity of these proteins and the range of ligands they bind that direct biochemical analysis is required to formally establish its ligand binding propensities. Structural analysis may also shed light on how Blo t 13 is allergenic, and provide the basis for genetic modification of the protein for the examination of IgE binding sites. cLTPs are low molecular weight proteins that have a role in intracellular transport and trafficking of retinoids or fatty acids and acyl derivatives [4]. The family includes intestinal fatty acid binding protein (FABP), muscle FABP, adipocyte FABP, myelin P2 protein, cellular retinol binding

The coordinates of the molecular model of the Blo t 13 allergen can be obtained from malcolm.kennedy@bio.gla.ac.uk.

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protein (CRBP) I and II, and cellular retinoic acid binding protein (CRABP). Here, we report that Blo t 13 is indeed a functional FABP. There are no previous records of a member of the cytosolic lipid transporter protein family with allergen activity and this is the first functional analysis of an allergen from *B. tropicalis*.

Recombinant protein was expressed in *Escherichia coli* as a fusion protein with glutathione S-transferase and purified by glutathione Sepharose affinity chromatography following cleavage with thrombin [3]. The ligand binding activity of recombinant Blo t 13 (rBlo t 13) was examined as previously described [5], using *cis*-parinaric acid (cPnA), *trans*-parinaric acid, and the fluorescent fatty acid analogs 11-((5-dimethylaminonaphthalene-1-sulfonyl) amino) undecanoic acid (DAUDA) and 12-(9-anthroyloxy)-stearate (12AS), and all *trans*-retinol, cholesterol, dehydroergosterol, oleic acid and dansyl-*DL*-aminocaprylic acid (DACA). For estimation of the dissociation constant, additions of cPnA were made to a 2-ml sample of rBlo t 13. For competitive titration with oleic acid, 5 μ l of dilutions in PBS of a solution of oleic acid in ethanol were added to a sample of rBlo t 13 and cPnA. Data were fitted by standard nonlinear regression techniques (using Microcal ORIGIN software) to a single noncompetitive binding model, to give estimates of the dissociation constant (K_d) and maximal fluorescence intensity. Circular dichroism spectra were recorded at 20°C in a JASCO J-600 spectropolarimeter. Analysis of the secondary structure of the protein was performed using the CONTIN procedure [6] on data recorded over the range of 240–200 nm. Secondary structure predictions were performed using the Profile Network Prediction Heidelberg (PHD) program [7]. The Blo t 13 structure was modelled by letting the SwissModel [8] select five structures which its automatic alignment program found to be nearest to the sequence of Blo t 13. These are (with PDB accession codes). *Schistocerca gregaria* muscle FABP (1FTP), human adipocyte lipid binding protein (1ADL), murine adipocyte lipid binding protein (1ABO), a mutant mouse adipocyte lipid binding protein (1ACD), human muscle FABP (1HMR) and produce a model based on a composite. The model was checked for geometry by PROCHECK [9].

The PHD program predicts Blo t 13 to be a β -rich structure with 10.0% α -helix, 56.9% β -sheet and 31% loops/remainder based on multiple alignments. PHD also predicted Blo t 13 to be structurally similar to cLTPs and for which crystal structures are known, in the order of similarity: 1ACD, 1FTP, human muscle FABP (1HMT), rat liver FABP (1LF0), human cellular retinoic acid binding protein II (1CBS), bovine myelin P2 (1PMP), and bovine, mouse and rat cellular retinoic acid binding proteins I (1CBR). In addition, PHD also predicted structural similarity of Blo t

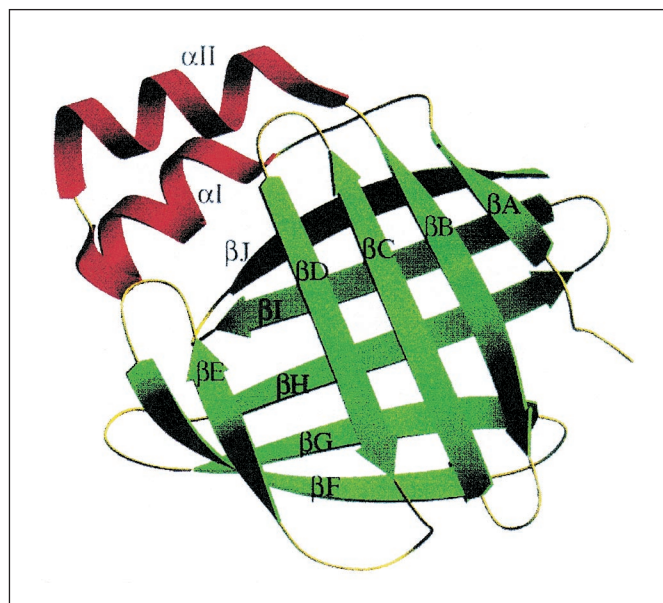


Fig. 1. Structural model of Blo t 13. The model was created using the SwissModel refined with GROMOS96, and the illustration made with SETOR.

13 to a theoretical model of *Ascaris suum* FABP homolog precursor (1AS1). The best model had 90.5% of residues in the most favored regions of a Ramachandran plot, 8.6% in the additionally allowed regions, none in generously allowed regions, and 0.9% in the disallowed regions. The single residue falling in a disallowed region (Asp 120) appears on the model in a loop, and so is unlikely to affect the overall fidelity of the predicted structure (fig. 1). The circular dichroism spectrum of rBlo t 13 exhibited a minimum at 217 nm and a maximum at 198 nm. Analysis of data provided an estimate of 13% of α -helix, and 45% β -sheet, values which are similar to those predicted by the PHD program and to the known secondary structure of the FABP/P2/CRBP/CRABP family [4]. Addition of rBlo t 13 to cPnA was accompanied by a substantial increase of the fluorescence emission intensity of this fatty acid (fig. 2). Similar data were obtained in which the *trans* form of parinaric acid was used (data not shown). rBlo t 13 also binds oleic acid by competitive binding with cPnA (fig. 2). No binding was observed with DAUDA, DACA, 12AS, dehydroergosterol, retinol or cholesterol (data not shown). Titration of rBlo t 13 with cPnA yields an apparent K_d of 1.31×10^{-6} M (fig. 3a). Analysis of the oleic acid titration curve yields an apparent K_d of 4.8×10^{-8} M (fig. 3b), which is of a similar order of magnitude to other fatty acid binding proteins of the family [10–12].

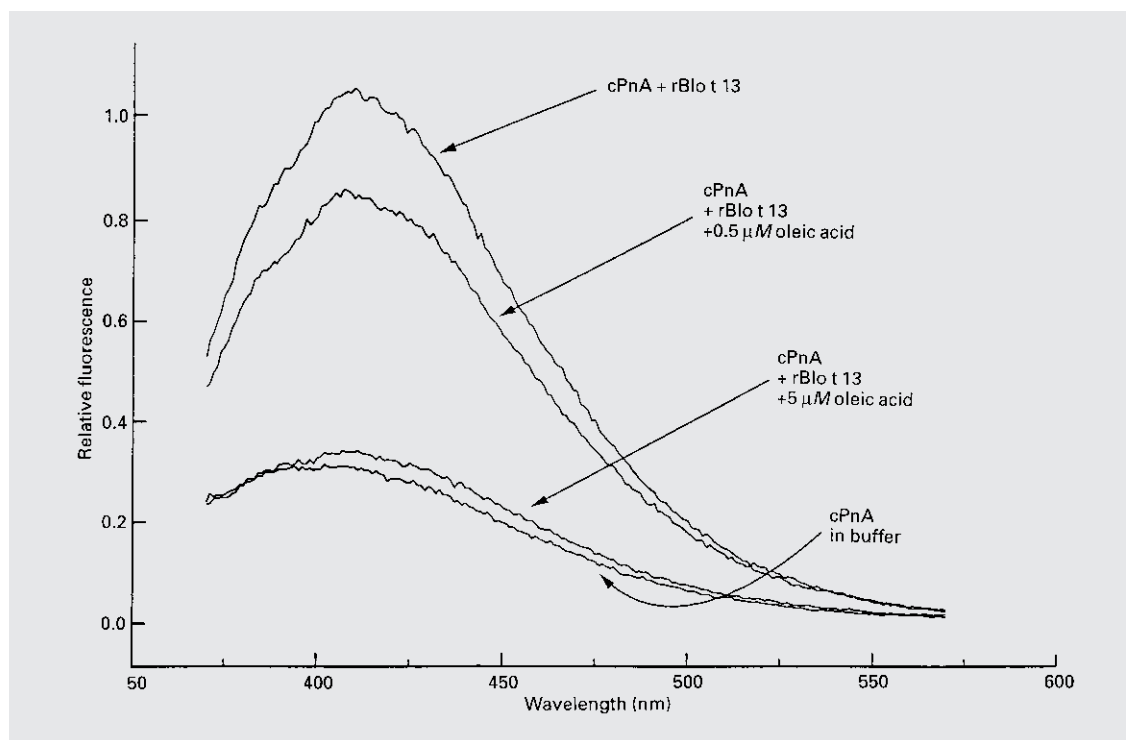


Fig. 2. Binding of cPnA to recombinant Blo t 13 protein. Fluorescence emission spectra of cPnA in the absence or presence of rBlo t 13. The fluorescence enhancement brought about by addition of rBlo t 13 to cPnA is reversed by the subsequent addition of oleic acid, showing competition for the rBlo t 13 binding site by oleic acid.

Fluorescence-based ligand binding analysis showed that rBlo t 13 binds to cPnA by competition with oleic acid, and the dissociation constants measured were similar to those for other cLTPs. However, like some other members of the cLBP family, rBlo t 13 failed to bind fluorophore-tagged fatty acids which have been successfully used in the analysis of other FABPs [4, 5, 11, 13]. This is presumably due to steric hindrance by the aromatic fluorophores. Other FABPs expressed in *E. coli* show the same binding specificity as their naturally produced counterparts [11, 13], so the specificity of rBlo t 13 is likely to be representative of mite protein. The proteinase treatment used in the production of rBlo t 13 is also unlikely to have modified its binding specificity because the uncleaved GST-rBlo t 13 fusion protein exhibited an identical pattern of fatty acid binding (data not shown). Moreover, other FABPs expressed as fusion protein with GST bind to cPnA, oleic acid and dansylated or anthroxylylated fatty acids with the same affinities as the native form [11].

The architecture (10 antiparallel β -strands and two short α -helices) of cytosolic lipid transfer proteins is conserved throughout the family. The β -strands are organized into two β -sheets surrounding an internal ligand binding cavity [4].

Crystallographic and mutational analysis of fatty acid binding members of the cLTPs family has revealed three residues which are conserved and are involved in a hydrogen bonding network with the ligand's carboxylate [11, 13, 14]. The Blo t 13 model shows these residues (Arg 106, Arg 126 and Tyr 128) to be appropriately positioned within the model.

This structural model will facilitate the investigation of the immunology of Blo t 13 from several aspects. First, it will allow the mapping of IgE binding onto a three-dimensional structure. Second, it may reveal structural features of the protein which differ from those of human cLTPs which might pertain to the allergenicity of the protein. Third, the structure will allow the identification of amino acids which are likely to be crucial to the ligand binding function of the molecule. Allergens from mouse (Mus m 1), rat (Rat n 1), bovine (Bos d 2), milk β -lactoglobulin (Bos d 5), dog (Can f 1 and Can f 2), horse (Equ c 1 and Equ c 2) and cockroach (Bla g 4) have structural and sequence similarities to the ligand binding family of lipocalin proteins which function as carrier in the transport of hydrophobic ligands in the extracellular fluid [4, 15]. This is the first report of the function

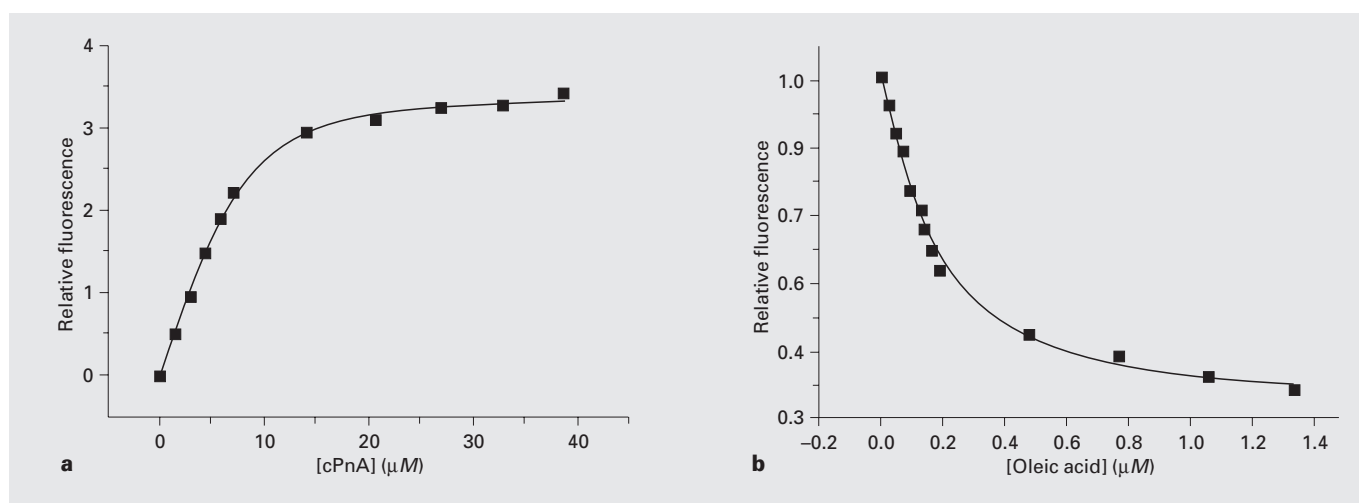


Fig. 3. **a** Titration curve for the binding of cPnA to rBlo t 13. Increase in relative fluorescence intensity on progressive addition of rBlo t 13 to cPnA in PBS. The solid line is a theoretical binding giving an apparent K_d of 1.31×10^{-6} M, and is consistent with 1:1 binding. **b** Stoichiometric decrease in relative fluorescence intensity due to the

displacement of cPnA from rBlo t 13 by progressive addition of oleic acid. The solid line is the theoretical curve for simple competitive binding of oleic acid with the cPnA binding site of rBlo t 13 with an apparent K_d of approximately 4.8×10^{-8} M.

of a mite allergen among the FABP/lipocalin superfamily of allergens. Given that cLTPs have a highly conserved structure and an important biological function, it is highly likely that other mite species have homologues to Blo t 13. Investigating whether Blo t 13 homologues in other mite species have allergenic activity could then assist in understanding the structural requirements for biological function and allergenicity of this and other lipid binding proteins.

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