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A novel clade of cysteinyl leukotriene scavengers in soft ticks

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Abstract

Inflammation is an important vertebrate defense mechanism against ecto-parasites for which ticks have evolved numerous mechanisms of modulation. AM-33 and TSGP4, related lipocalins from the soft ticks *Argas monolakensis* and *Ornithodoros savignyi* binds cysteinyl leukotrienes with high affinity as measured by isothermal titration calorimetry. This was confirmed in a smooth muscle bioassay that measured contraction of guinea pig ileum induced by leukotriene C₄ where both proteins inhibited contraction effectively. Conservation of this function in two diverse soft tick genera suggest that scavenging of cysteinyl leukotrienes evolved in the ancestral soft tick lineage. In addition soft ticks also evolved mechanisms that target other mediators of inflammation that includes scavenging of histamine, serotonin, leukotriene B₄, thromboxane A₂, ATP and inhibition of the complement cascade. Inhibitors of blood-coagulation and platelet aggregation were also present in the ancestral soft tick lineage. Because histamine and cysteinyl leukotrienes are mainly produced by mast cells and basophils, and these cells are important in the mediation of tick rejection reactions, these findings indicate an ancient antagonistic relationship between ticks and the immune system. As such, modulation of the hemostatic system as well as inflammation was important adaptive responses in the evolution of a blood-feeding lifestyle in soft ticks.

Keywords

Argas monolakensis; *Ornithodoros savignyi*; TSGP4; AM-33; vasodilator; leukotrienes; mast cell; basophil

1. Introduction

During feeding ticks cut into the host's skin using their hypostome and chelicerae in order to create a feeding site (hematoma), where blood pools and from which the tick can engorge. This probing causes tearing of tissues and rupturing of capillary vessels, with the subsequent release of mediators that activate the host's defense mechanisms. As such, ticks need to modulate their vertebrate host's immune and hemostatic systems (Ribeiro, 1989). The hemostatic system is composed of blood clotting, platelet aggregation and vasoconstriction. The immune response is composed of innate (inflammation, the complement cascade and pain) and adaptive (B and T cell mediated) responses. These systems interact to form complex and redundant networks. In response, ticks evolved a reciprocal complex salivary gland repertoire of molecules that is

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secreted into the feeding site that target multiple key components of the host's defense systems (Ribeiro and Francischetti, 2003; Mans and Neitz, 2004a).

Edema is one of the characteristics of inflamed tissue and is caused by an increase in vascular permeability that leads to accumulation of fluids, swelling and infiltration of leukocytes in the extra-vascular bed. This leads to occlusion of blood vessels and decreased blood flow. Edematous inflammatory reactions at the tick feeding site are associated with tick rejection and ticks evolved various mechanisms to counteract inflammation (Ribeiro, 1989). Mediators of inflammation, such as histamine or serotonin are for example scavenged by lipocalins in both soft and hard ticks (Paesen et al. 1999; Mans et al. 2008a). In soft ticks, lipocalins that scavenge leukotriene B₄ (LTB₄), a potent inflammatory mediator, has also been identified (Mans and Ribeiro, 2008). Targeting of another class of inflammatory mediators, the cysteinyl leukotrienes, by ticks has not yet been characterized. The current study describes soft tick lipocalins that bind cysteinyl leukotrienes with high affinity.

Soft tick lipocalins (TSGP1 through 4) were previously identified as the most abundant proteins in the salivary glands of the soft tick *Ornithodoros savignyi* (Mans et al. 2001; Mans et al. 2003). TSGP2 and TSGP4 were implicated in sand tampan toxicoses (Mans et al. 2002). Recently, it was shown that TSGP1 act as a scavenger of histamine and serotonin (Mans et al. 2008a). TSGP3 has been shown to inhibit collagen-induced platelet aggregation and vasoconstriction by scavenging of thromboxane A₂ (TXA₂) (Mans and Ribeiro, 2008). Both TSGP2 and TSGP3 also inhibit edema by scavenging LTB₄ and interact with complement C5 to inhibit the complement cascade (Mans and Ribeiro, 2008). No function has yet been elucidated for TSGP4. In the current study we show that TSGP4 and AM-33, a related lipocalin from the tick *Argas monolakensis*, can bind the cysteinyl leukotrienes, leukotriene C₄, D₄ and E₄ (LTC₄, LTD₄, LTE₄) with high affinity. This finding is novel and indicates that scavenging of mediators of inflammation evolved in the ancestral lineage to soft ticks.

2. Materials and Methods

2.1 Bio-informatical analysis of the TSGP4 clade

The sequence of TSGP4 (Genbank accession: 25991438) were used to identify possible orthologs from the non-redundant Genbank protein sequence database by BLASTP analysis (Altschul et al. 1990). Sequences with E-values less than 10⁻¹⁰ were aligned using ClustalX and the edges trimmed manually to provide a conserved core of sequence alignment (161 informative positions) (Jeanmougin et al. 1998). Using this alignment, neighbor-joining analysis was performed using the Mega2 software (Kumar et al. 2001). Gaps were treated as pairwise deletion, amino acid distance were calculated using Poisson correction and branch support were estimated using bootstrap analysis (10 000 bootstraps). For molecular models the sequences for TSGP4 and AM-33 (Genbank accession: 114152974) were submitted to the PHYRE server (Bennett-Lovsey et al. 2008). Models obtained based on the structure of OMCI (Roversi et al. 2007), were visualized using the Pymol package (DeLano, 2002).

2.2 Recombinant protein expression

A gene for TSGP4 were synthesized (GeneArt) and for AM-33 were obtained from a cDNA library previously synthesized (Mans et al. 2008b). Genes were cloned and recombinantly expressed and analyzed for quality as previously described (Calvo et al. 2006; Mans and Ribeiro, 2008).

2.3 Isothermal titration calorimetry

Isothermal titration calorimetry was performed using a VP-ITC calorimeter (Microcal, Northhampton, MA) as described (Mans and Ribeiro, 2008). Lipid-derived ligands included

arachidonic acid (AA), LTB₄, LTC₄, LTD₄, LTE₄, U46619, carbocyclic thromboxane A₂ (cTXA₂), prostaglandin E₂ and platelet activating factor (PAF) (Cayman Chemical, MI, USA). All proteins were tested at 2 µM protein and 20 µM ligand unless otherwise indicated.

2.4 Smooth muscle bioassay

Contraction of guinea pig ileum by LTC₄ was measured isotonicity with a load of 1.5 g. A modified Tyrode solution (with 10 mM HEPES buffer) that was oxygenated by continuous bubbling of air was used in the assays (Webster and Prado, 1970). Ileum preparations were pre-incubated with 300 nM TSGP4 or AM-33. LTC₄ were added in 100 nM increments until maximum contraction was reached.

3. Results

3.1 The major functional lipocalin clades found within soft ticks

Previously, four major lipocalins (TSGP1-4) were described from the salivary glands of the soft tick, *O. savignyi* (Mans et al. 2003). Recent sialome projects on the salivary gland proteins from *A. monolakensis* and *O. parkeri* has indicated that most soft tick lipocalins falls roughly into three major classes as defined by the TSGPs (Francischetti et al. 2008; Mans et al. 2008b). TSGP1 and related proteins has been shown to be scavengers of histamine and serotonin (Mans et al. 2008a). TSGP2, TSGP3 and related moubatin-like proteins targets complement C5 and leukotriene B₄, while TSGP3 and moubatin can also scavenge thromboxane A₂ (Mans and Ribeiro, 2008). Only TSGP4 has no defined function yet and we thus set out to discover possible functions for TSGP4 and its homologs.

BLASTP analysis of the non-redundant Genbank protein database using the sequence of TSGP4 retrieved 8 soft tick lipocalins with E-values below 10⁻¹⁰. These included lipocalins from the sialomes of *A. monolakensis* and *O. parkeri* (Francischetti et al., 2008; Mans et al. 2008). Phylogenetic analysis indicated that the proteins grouped into two major clades, one for the genera *Ornithodoros* and the other for *Argas*, that each contained a number of genus specific proteins (Fig. 1). This suggested that the clades form an orthologous group (i.e. monophyletic) and that gene duplication events after divergence of the two genera led to a number of paralogous proteins. It should be noted that two of the proteins analyzed were previously annotated as “moubatin-like” (Francischetti et al. 2008). BLASTP analysis of the non-redundant database retrieves TSGP4 as best “other species” hit for both species. As such, their functional relationship to moubatin seems dubious and we infer a closer relationship to TSGP4. Similar annotations are addressed in regard to biogenic amine-binding proteins and their relationship to moubatin as well (Mans and Ribeiro, 2008).

AM-33 was the first *Argas* derived protein retrieved from the non-redundant protein database by TSGP4 and reciprocal BLASTP analysis against the non-redundant protein database gave as best hit TSGP4. AM-33 is a highly abundant lipocalin found in the salivary glands of *A. monolakensis* (Mans et al. 2008b). Given the basal position in the phylogenetic tree we considered it possible that it would be the ancestral ortholog of TSGP4. As such, a comparative analysis of AM-33 and TSGP4 were performed in order to derive information that will give an indication as to their function.

3.2 Alignment of TSGP4 and AM-33

TSGP4 and AM-33 show 23% sequence identity and 50% sequence similarity based on the PAM250 matrix (DN, EQ, ST, KR, FYW, LIVM) (Fig. 2). Conserved residues occur in discrete clusters along the sequence and correspond with regions predicted to be involved in secondary structure formation. Each possess six cysteine residues that are conserved and correspond to the previously proposed unique disulphide bond pattern for TSGP4 (Mans et al. 2003). This

cysteine arrangement is found in all members of the TSGP4/AM-33 clade (results not shown) and differs from that of the other soft tick lipocalin clades, notably the histamine and serotonin-binding and the moubatin clades (Mans et al. 2003; Mans et al. 2008a; Mans and Ribeiro, 2008).

3.3 Molecular modeling of TSGP4 and AM33

TSGP4 and AM-33 were submitted to the PHYRE server (Bennett-Lovsey et al. 2008), and for both models were obtained based on the structures of the histamine-binding protein (FS-HBP2) from *Rhipicephalus appendiculatus* (E-14, E-15) and OMCI, the complement inhibitor from *O. moubata* (E-6, E-7), respectively (Paesen et al. 1999; Roversi et al. 2007). The estimated precision for each model were 100% and confirmed that TSGP4 and AM-33 belongs to the lipocalin family. However, given the low sequence identity between TSGP4, AM-33, FS-HBP2 and OMCI (<20% sequence identity, <30% sequence similarity), the models can only approximate the true structures of TSGP4 and AM-33. However, it should be possible to investigate general features such as topology and overall hydrophobicity within the lipocalin binding cavity.

Residues that lined the binding cavity of the lipocalin beta-barrel were selected and space fill models of their van der Waals radii were constructed for models based on the structure of OMCI (Roversi et al. 2007). Models for TSGP4 and AM-33 indicated that the binding cavity is hydrophobic in character, with polar residues occurring at the periphery of the binding pocket (Fig. 3). This is in contrast to for example, the structure of the histamine and serotonin binding lipocalins from *R. appendiculatus* and *A. monolakensis* that possess several polar residues within the binding pocket (Paesen et al. 1999; Mans et al. 2008). Similar results were obtained using models based on the structure of FS-HBP2 (results not shown).

OMCI binds ricinuleic acid and possesses polar residues in the binding pocket that allows interaction with the hydroxyl groups of ricinuleic acid (Roversi et al. 2007). In addition, the OMCI-related proteins moubatin, TSGP2 and TSGP3 was shown to be able to bind LTB₄ and TXA₂ (Mans and Ribeiro, 2008). The overly hydrophobic character of the structure models for TSGP4 and AM-33 thus suggested that they will bind hydrophobic ligands that do not possess any polar groups in the part that binds within the hydrophobic pocket of the beta-barrel. Potential bioactive ligands that fall within this classification will be lipid-derived ligands such as platelet-activating factor, TXA₂ or various leukotrienes (Fig. 4). As other soft tick lipocalins were recently found to be able to bind lipid-derived ligands (Mans and Ribeiro, 2008), we investigated the ability of AM-33 and TSGP4 to bind similar ligands.

3.4 Binding of hydrophobic bioactive ligands by TSGP4

Binding to hydrophobic ligands by recombinant expressed AM-33 and TSGP4 were tested using micro-calorimetry. No significant binding were observed for the TXA₂ analog, U44619 or PAF at the protein concentration (2 μM) tested (results not shown). Arachidonic acid and LTB₄ were bound at low affinities by both proteins. However, both bound the cysteinyl peptide leukotrienes LTC₄, LTD₄ and LTE₄ with high affinities, in the low nanomolar range (Table 1; Fig. 5 and Fig. 6).

3.5 Bioassay for TSGP4 and AM-33

LTC₄ and LTD₄ act as potent inflammatory mediators released by mast cells and basophils during hypersensitivity reactions (Drazen et al. 1980; Austen, 2008). Before molecular characterization of the cysteinyl leukotrienes, they were known as the slow-reacting substance (SRS) of anaphylaxis, because supernatants of cells undergoing anaphylaxis produced a slow contraction of guinea pig ileum treated with anti-histamines (Austen, 2005). TSGP4 and

AM-33 were both able to inhibit contraction of guinea pig ileum by LTC₄ in a dose dependent manner (Fig. 7).

3.6 Biological significance of TSGP4 and AM-33

TSGP4 and AM-33 both occurs at 5% and 3.3% of the total salivary gland proteins (Mans et al. 2001; Mans et al. 2008b). If it assumed that only 50% of all salivary gland proteins are secreted during feeding and that the feeding site is between 10–50µl total volume, then TSGP4 (3–18µM) and AM-33 (0.4–2µM) can attain concentrations at the feeding site that is well above the physiological ranges of the cysteinyl peptide leukotrienes (Drazen et al. 1980). Given the high affinity for the cysteinyl peptide leukotrienes and the fact that LTC₄ and LTD₄ are bio-active at similar concentrations (Drazen et al. 1980), as well as the results from the bioassay, we conclude that both TSGP4 and AM-33 would indeed be important scavengers of the cysteinyl peptide leukotrienes during feeding.

4. Discussion

Cysteinyl peptide leukotrienes derive from the conjugation of glutathione and leukotriene A₄ (Austen, 2007). Synthesis occurs in neutrophils, macrophages, basophils and mast cells in response to external stimuli. Neutrophil derived leukotriene A₄ are also efficiently converted to LTC₄ by platelets (Maclouf and Murphy, 1988). LTC₄ are in turn further metabolized to yield LTD₄ and LTE₄. Collectively these leukotrienes were first characterized as slow reacting substance of anaphylaxis (SRS-A) due to their potent ability as vasoconstrictors of the bronchial and other smooth muscle systems (Austen, 2007; Austen 2008). Although LTC₄ is a skin vasodilator and as such should enhance tick blood feeding, it's profound action in increasing endothelial permeability leads to edema that result in occlusion of blood vessels and decreased blood availability. Upon skin injection of LTC₄, erythema and wheal formation occurs within 10 minutes, peaks at 1 hour and persist for more than 2 hours (Soter et al. 1983). At the tick feeding site, this would lead to accumulation of a serous-rich and red cell depleted fluid in the feeding cavity and hence a less efficient blood meal. The time frame observed for erythema and wheal formation falls within that normally described for the feeding of soft ticks that ranges from 15 minutes to a few hours (Hoogstraal, 1985). As such, targeting of cysteinyl leukotrienes would be an important strategy during soft tick feeding.

The present study shows that two related lipocalins, TSGP4 and AM-33 binds cysteinyl leukotrienes with high affinities. Non-cysteinyl leukotrienes (LTB₄) and TXA₂ analogs (U46619) were not bound with high affinity, indicating specificity towards the cysteinyl leukotrienes. It should be noted that LTB₄ and U46619 possess hydroxyl groups at the C12 and C15 carbons, respectively, and that this was previously shown to have been determinants of specificity in the moubatin-clade of lipocalins (Mans and Ribeiro, 2008). This could explain the low binding affinities of TSGP4 and AM-33 for LTB₄. The cysteinyl leukotrienes, however, lack hydroxyl groups within the saturated hydrocarbon chain and would bind within a predominantly hydrophobic cavity.

The relatively low affinities for arachidonic acid, that is also predominantly hydrophobic, suggest that the peptidyl part of the cysteinyl leukotrienes play an important part in the interaction with the lipocalins. In this regard, there seem to be a direct correlation between the presence of peptidyl region and binding affinity, when binding of LTC₄ (5-(S)-hydroxy-6-R-S-glutathionyl) and LTD₄ (6-S-cysteinylglycyl) is compared to binding of LTE₄ (6-S-cysteinyl), LTB₄ and arachidonic acid. This would imply that while the lipid part of the cysteinyl peptide leukotrienes bind within the hydrophobic cavity of the lipocalin fold, that the peptidyl part (especially the terminal cysteinylglycyl) play an important role in the affinity of the lipocalins for the leukotrienes. This correlate with the relatively high negative enthalpy measured by microcalorimetry, that suggests that hydrogen bonds contribute significantly to

cysteinyl leukotriene binding. On the other hand, the relative positive entropy contribution, suggests that hydrophobic interactions within the lipocalin barrel also play an important role in eicosanoid binding.

TSGP4 as well as another lipocalin (TSGP2) has been previously implicated in the toxicoses induced by the soft tick *O. savignyi* (Mans et al. 2002; Mans and Neitz, 2004a). It was shown that TSGP2 can bind arachidonic acid and LTB₄ (nanomolar affinities) and it was suggested that toxicity may be linked to eicosanoid metabolism within the tick (Mans and Ribeiro, 2008). The present study showed that TSGP4 can bind arachidonic acid (K_d~155 nM) as well as cysteinyl leukotrienes (nanomolar affinity). It is thus of interest that two proteins previously implicated in toxicity can both bind arachidonic acid derivatives that have been linked to anaphylactic shock, a recognized symptom in sand tampan toxicoses (Mans et al. 2002; Feuerstein and Hallenbeck, 1987).

In this regard it is of interest that ticks obtain large quantities of arachidonic acid during a blood-meal (Shipley et al. 1993). In soft ticks, adults feed more than once with extended non-feeding periods that may last for months or even years in *O. savignyi* (Mans and Neitz, 2004b). As such, salivary gland molecules are stored for prolonged periods within the secretory granules of the salivary glands between periods of feeding (Mans et al. 2004a). It is thus possible that arachidonic acid derivatives can partition to lipocalins amenable to high affinity binding. If this is the case for TSGP2 or TSGP4 it would suggest that they can act as carriers of host-derived or tick-transformed arachidonic acid derivatives. Whether the ability to act as transporters of eicosanoid derivatives are related to the pathogenesis of sand tampan toxicoses, remains to be determined. If this is, however, the case it would be a novel mechanism for toxicoses induced by ticks (Mans et al. 2004b).

To date, three major functional classes have been elucidated for the soft tick family of lipocalins that maps to well defined clades (Mans, 2005). This includes biogenic amine scavenging (Mans et al. 2008a), scavenging of TXA₂ and LTB₄ by the moubatin-clade (Mans and Ribeiro, 2008) and in the present study scavenging of cysteinyl leukotrienes. BLAST analysis and conserved disulphide bonds suggests that the biogenic amine-binding and moubatin-clades are evolutionary closer related than the TSGP4/AM-33 clade (Mans and Ribeiro, 2008). Similarity of soft tick lipocalins to hard tick biogenic amine-binding proteins also suggests that the latter clade could have been ancestral to the soft tick lipocalin family. If this is the case, scavenging of eicosanoids evolved on two independent occasions in the soft tick lipocalins, once in the ancestral soft tick lineage and once within the *Ornithodoros* genus, from the same biogenic amine-binding scaffold (Mans et al. 2008a; Mans and Ribeiro, 2008).

The present study adds to the emerging picture on how ticks modulate inflammatory responses by the host. Hard and soft ticks targets ATP, an agonist of neutrophils, via apyrase mediated hydrolysis (Ribeiro et al. 1985; Ribeiro et al. 1991; Mans et al. 1998). Histamine and serotonin are scavenged by lipocalins in both hard and soft ticks (Mans et al. 2008a). TXA₂, LTB₄ and complement C5 are targeted by the moubatin-related lipocalins from the genus *Ornithodoros* (Mans and Ribeiro, 2008). Kininases inactivate bradykinin in *Ixodes* ticks (Ribeiro and Mather, 1998). Soft ticks from both *Argas* and *Ornithodoros* genera scavenge LTC₄, LTD₄ and LTE₄ using the lipocalin family (this study). This essentially covers most of the known mediators of inflammation and suggests that regulation of the host's inflammatory response is an important survival strategy for soft ticks. The model of feeding that emerges includes the prevention of neutrophil migration to the feeding site by scavenging of ATP and LTB₄. This occurs most probably mainly to prevent neutrophil-platelet interactions that can lead to the formation of LTC₄ that will promote edema.

Conservation of function in the soft tick lineage is becoming more apparent. This includes cysteinyl leukotriene scavengers, biogenic amine-binding proteins (Mans et al. 2008a) as well as anti-thrombin and platelet aggregation inhibitors (Mans et al. 2008c). As such, inhibition of blood-coagulation, platelet or thrombocyte aggregation, vasoconstriction and inflammation emerges as some of the dominant targets in soft tick feeding. Leukotriene C4 synthase are conserved within most vertebrates, including bony fishes and amphibians and imply that edematous inflammatory responses were present at least 400 MYA (Bresell et al. 2005; Benton and Donoghue, 2007). This is well before any of the estimates on the origin of ticks and their adaptation to a blood-feeding environment (Mans et al. 2008c). As such, mast cells and basophils, or their equivalent leukocytes, must have been ancient defenses against tick feeding that persist until the modern day (Brown and Askenase, 1985). This would imply that hemostatic and inflammatory responses by the host were the primary hurdles that soft ticks had to overcome during their adaptation to a blood-feeding environment.

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Fig. 1.

Phylogenetic analysis of the TSGP4-related lipocalins. Lipocalins were retrieved from the non-redundant database by BLASTP analysis of TSGP4. Closely related sequences were selected based on an E-value cut-off less than 10^{-10} . Neighbor-joining analysis was performed, with a Poisson amino acid substitution model and gaps treated as pairwise deletion. Support values were obtained by bootstrap analysis (10000 replicates). Sequences are described by a species designation (AM: *A. monolakensis*; OP: *O. parkeri*, OS: *O. savignyi*), Genbank accession number in round brackets and annotation line in square brackets. Dots indicate proteins characterized in the present study. Squares indicate proteins for which the functional and evolutionary relationship with moubatin is uncertain.

Fig. 2.

Alignment of TSGP4 and AM-33. Residues shaded in black indicate similarity based on the PAM250 matrix. Disulphide bonds are indicated as predicted previously for TSGP4 (Mans et al. 2003). Secondary structures (a: α-helices; B: β-strands) were obtained from structure models obtained from the PHYRE server based on the structure of OMCI (Bennet-Lovley et al. 2008; Roversi et al. 2007).

Fig. 3.

Molecular modeling of TSGP4 and AM-33. Indicated are models for TSGP4 (top panels) and AM-33 (bottom panels). The first pair of pictures in each panel shows spacefill and cartoon models of the β -barrel viewed from the side. The second pair of pictures shows the same structures viewed from the top of the β -barrel looking down into the binding cavity. Space fill models indicate non-polar (dark gray) and polar (light gray) residues that lines the binding pocket.

Fig. 4.
Structures of cysteinyl leukotrienes and related eicosanoids.

Fig. 5. Binding of arachidonic acid, LTB₄, LTC₄, LTD₄ and LTE₄ by TSGP4. Protein (2μM) was titrated against 20 μM ligand. Indicated are thermodynamic parameters derived for each titration that includes the stoichiometry of binding (N), dissociation constant (K_D), change in enthalpy (ΔH) and change in entropy (TΔS) upon binding.

Fig. 6.
Binding of arachidonic acid, LTB₄, LTC₄, LTD₄ and LTE₄ by AM-33. Protein (2 μM) was titrated against 20 μM ligand. Indicated are thermodynamic parameters derived for each titration that includes the stoichiometry of binding (N), dissociation constant (K_D), change in enthalpy (ΔH) and change in entropy (TΔS) upon binding.

Fig. 7.

Inhibition of LTC₄-induced contraction of guinea-pig ileum by AM-33 and TSGP4. Ileum was pre-incubated with TSGP4 or AM-33 at a final concentration of 0.3 μ M. At set time points (arrows), LTC₄ (0.1 μ M final concentration) were added to give concentrations of 0.1, 0.2, 0.3 and 0.4 μ M.

Table 1
Thermodynamic parameters for binding of arachidonic acid, LTB₄, LTC₄, LTD₄ and LTE₄.

Protein	n ^a	ΔH ^b	TΔS ^b	Kd ^c	ΔG ^b
TSGP4					
Arachidonic acid	1.35	-5.3±0.2	4.1	155±43	-9.4
LTB ₄	0.9	-6.8±1.7	1.5	1000±476	-8.3
LTC ₄	1.1	-19.7±0.1		≤ 2	
LTD ₄	1.2	-21.1±0.2		≤ 2	
LTE ₄	1.3	-14.4±0.2	-3.3	10.8±2	-11.1
AM-33					
Arachidonic acid	0.7	-8.6±2	-0.02	613±198	-8.6
LTB ₄	0.9	-3.6±0.7	5.0	587±286	-8.6
LTC ₄	1.2	-21.6±0.2	-9.5	2.2 ± 0.4	-12.1
LTD ₄	1.3	-19.5±0.2		≤ 2	
LTE ₄	1.3	-11.5±0.2	-1.5	68.0±6.6	-10.0

^aValues indicate the stoichiometry of ligand binding.

^bValues are given as kcal/mol. S.E.M. represents the deviation of the experimental data from the fitted data.

^cValues are given as nanomolar. Values indicated as equal or smaller than two have c values not in the 1–1000 range that can be accurately measured by iso-thermal microcalorimetry (Wiseman et al. 1989). S.E.M. represents the deviation of the experimental data from the fitted data.