

The membrane bound bacterial lipocalin Blc is a functional dimer with binding preference for lysophospholipids

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Abstract

Lipocalins, a widespread multifunctional family of small proteins (15–25 kDa) have been first described in eukaryotes and more recently in Gram-negative bacteria. Bacterial lipocalins belonging to class I are outer membrane lipoproteins, among which Blc from *E. coli* is the better studied. Blc is expressed under conditions of starvation and high osmolarity, conditions known to exert stress on the cell envelope. The structure of Blc that we have previously solved (V. Campanacci, D. Nurizzo, S. Spinelli, C. Valencia, M. Tegoni, C. Cambillau, *FEBS Lett.* 562 (2004) 183–188.) suggested its possible role in binding fatty acids or phospholipids. Both physiological and structural data on Blc, therefore, point to a role in storage or transport of lipids necessary for membrane maintenance. In order to further document this hypothesis for Blc function, we have performed binding studies using fluorescence quenching experiments. Our results indicate that dimeric Blc binds fatty acids and phospholipids in a micromolar K_d range. The crystal structure of Blc with vaccenic acid, an unsaturated C18 fatty acid, reveals that the binding site spans across the Blc dimer, opposite to its membrane anchored face. An exposed unfilled pocket seemingly suited to bind a polar group attached to the fatty acid prompted us to investigate lyso-phospholipids, which were found to bind in a nanomolar K_d range. We discuss these findings in terms of a potential role for Blc in the metabolism of lysophospholipids generated in the bacterial outer membrane.

Keywords

Lipocalins; Blc; Crystal structure; Lysophospholipids; Fatty acids

1. Introduction

The lipocalins are a widespread family of proteins identified initially in eukaryotes [1] and more recently in Gram-negative bacteria [2,3]. Lipocalins are carriers of lipophilic

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molecules whose functions, however, are often elusive and very diverse [4] (see also papers in *Biochem. Biophys. Acta* Vol. 1482). Retinol binding protein (RBP), which possess a well documented function and transport mechanism, has been the first lipocalin whose 3D structure has been solved [5]. Other lipocalins whose structures are known [6] include carriers of pheromones [7] and odorant molecules [8,9].

The lipocalin fold comprises an 8 stranded β -barrel followed by an α -helix at the C-terminus [6]. The number of disulfide bridges can vary from none to three in mammalian lipocalins. The amino acid sequences of lipocalins are very poorly conserved, with their most general sequence signature being the GXW motif at the N-terminus [1,6]. Sequence comparisons and evolutionary analyses of lipocalins have led to a classification in which ~14 clades have been identified [10]. Bacterial lipocalins belong to Clade 1 (the root clade) while the majority of eukaryotic lipocalins ranging from plants to invertebrates and mammals are found in the higher clades (2–14).

Many bacterial lipocalins are lipoproteins which contain a type 2 signal peptide allowing for export into the periplasm and anchoring in the inner leaflet of the outer membrane [2,3]. In the larger of the two identified groups, one or two cysteines are observed and no disulfide bridge is found. The first cysteine, located immediately after the signal peptide, is attached to the membrane anchor lipid forming the *N*-acyl-*Ssn*-1,2-diacylglycerylcysteine moiety at the N-terminus [2,3]. A smaller subgroup of bacterial lipocalins contains a putative disulfide bridge and its members have diverse predicted localizations in the cytoplasm, periplasm and the inner and outer membranes [2,3].

The paradigm for bacterial lipocalins is provided by Blc (Fig. 1) from *Escherichia coli*, the first bacterial lipocalin that has been identified [2] and whose 3D structure has been solved [11]. The structure of Blc at 1.8Å resolution revealed a fold similar to that of the moth bilin binding protein (BBP) [12] and the presence of an elongated and open cavity with the proper size to accommodate fatty acids or phospholipids. Blc is an outer-membrane bound protein, facing the periplasmic space. It is expressed during the stationary growth phase and under conditions of high osmolarity, during which the cell envelope suffers stress and requires maintenance [2,3].

Gram-negative bacterial outer membranes are built from diverse lipids and proteins. Lipid biosynthesis is restricted primarily to the inner (cytoplasmic) membrane. Many enzymes of lipid metabolism are integral membrane proteins, and among them only two structures are known. These are OMPLA [13] and PagP [14], both β -barrel proteins from the outer membrane of *E. coli*. In membrane metabolism, lipid trafficking events such as lateral diffusion and translocation between opposite leaflets are also essential processes, since the organization of lipids in the outer membrane is highly asymmetric; lipopolysaccharide (LPS) is located exclusively in the extracellular outer leaflet whereas phospholipids are normally restricted to the periplasmic inner leaflet. Although some trafficking events may occur spontaneously following chemical gradients or signals, some others may require a dedicated transporter, and Blc might be one of those. Two close eukaryotic neighbors of Blc, namely Lazarillo [15] and ApoD [16], are also believed to interact with membrane lipids [17].

In order to investigate the hypothesis that Blc might be a fatty acids/phospholipids/lysophospholipids carrier, we have performed binding experiments using tryptophan fluorescence quenching. We have also determined the crystal structure of Blc complexed to a fatty acid, vaccenic acid, and identified its dimeric functional organization. A likely role for Blc in the metabolism of lysophospholipids (LPLs) is indicated by the nanomolar affinity that Blc displays towards these compounds.

2. Materials and methods

2.1. Protein expression and purification

Sub-cloning and expression strategies used for our *E. coli* targets, including the *blc* gene, have already been described elsewhere [11,18]. Briefly, residues 1–22 in the native Blc have been replaced by the sequence MSYYHHHHHLESTSLYKKAGS, coming from the Gateway pDEST17 vector [11], thus removing the N-terminal cysteine.

2.2. Tryptophan fluorescence quenching studies

Fatty acids and Phospholipids, PtdGro, PtdEtn and extracts, were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Pools of 100% methanolic solutions of the ligands were freshly prepared. Fluorescence quenching was measured using a Cary Eclipse (Varian) using a right angle configuration, at 20 °C by using 2.5-nm excitation and 10-nm emission bandwidths. The excitation wavelength was 280 nm and the emission spectra were measured between 290 and 540 nm. In all experiments the final methanol concentration in the cuvette was kept below 1%. Binding samples contained 1 μM protein in 10 mM Tris buffer, 25 mM NaCl, pH 8.0; ligands were used at concentrations between 0.02 and 22.5 μM. For the experiments with LPLs, the final K_d values were obtained with a protein concentration of 0.1 μM.

In order to estimate the affinity of the compounds for Blc, the fluorescence intensities at 348 nm, the maximum of emission, at increasing concentrations of quencher were plotted versus the quencher concentration. The K_d values and the standard errors were estimated by non-linear regression using Prism 3.02 (GraphPad software, Inc) following procedures already described [19].

2.3. Static light scattering study

Static light scattering experiments were performed with a Malvern Zetasizer nano S instrument (Malvern, UK). The protein concentration was varied between 1 and 20 mg/mL, and data were collected at 25 °C against a toluene standard.

2.4. Crystal structure determination

Crystals of the protein were grown at 20 °C using sitting drops and the nano-drops technology [20] followed by optimization. The Blc/vaccenic acid complex was prepared by adding vaccenic acid (molar ratio 5:1) dissolved in methanol to Blc at 6–8 mg/mL in HEPES 5 mM, NaCl 150 mM, pH 7.5. A volume of 100 nL of the protein complex was mixed with 100 nL of precipitant solution consisting of sodium citrate 800–900 mM, sodium borate 50 mM, pH 7.0–7.5. Crystals grew within 1–3 days and proved to belong to the

orthorhombic space group $P2_12_12_1$ and are isostructural with the native Blc [11] ($a = 57.9.0\text{\AA}$, $b = 81.3\text{\AA}$, $c = 89.0\text{\AA}$) with two molecules per asymmetric unit ($V_m = 2.6\text{\AA}^3/\text{Da}$; 59 % solvent).

Data were collected on a single flash-cooled crystal (25% glycerol) on beamline ID14-EH4 at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France) using an ADSC Quantum 4 CCD detector. A total of 180 images have been collected using a 1° oscillation range per image at \AA wavelength. The data set were processed with MOSFLM and scaled using SCALA from the CCP4 [21] suite (Table 1). The isostructural crystals enabled the use of the native Blc model for initial phasing. Refinement was made with REFMAC [22] and model rebuilding with Turbo-Frodo [23]. Statistics for data collection and refinement are given in Table 1. Figures have been prepared with PyMol [24]. The coordinates have been deposited in the Protein Data Bank at RCSB (<http://www.rcsb.org/pdb/>) as entry 2ACO.

3. Results

3.1. Ligand binding studies using tryptophan fluorescence quenching

Fluorescence reporter molecules such as amino anthracene (AMA), NPS or 1-*N*-phenyl-naphthylamine (NPN) have proved to be very useful with some lipocalins, such as odorant binding proteins (OBPs) [7,25–27]. Such reporters display a strong increase of fluorescence when accommodated in the hydrophobic ligand-binding environment of lipocalins known as the calyx [7,25]. Chasing these fluorescence reporters with ligands provide a fast and easy tool to estimate their true affinity for the host protein [19,25]. The three reporters tested with Blc, namely AMA, NPS or NPN, however, failed to display any fluorescence increase in the presence of the protein, indicating that they were unable to bind into its hydrophobic pocket. Visual inspection of the Blc structure indicated that among the four tryptophans present in the protein, two belong to the wall of the pocket (Trp139 and Trp43), while the other two (Trp86 and Trp171) are located at the external surface. Based on this observation, we thought that the two former tryptophans might be excellent binding reporters as their fluorescence would probably be quenched when adding a hydrophobic ligand. As expected, quenching of the Blc tryptophan fluorescence (max emission at 348 nm) occurred with most of the lipidic ligands (Table 2). We also assayed, as non-specific references, two “outlier” bulkier molecules, ampicillin and retinol. They both displayed K_d values higher than FAs or PLs, 5 and 20 μM , respectively.

Since most, if not all, compounds were poorly soluble and had to be dissolved in methanol, we verified that the presence of small amounts of methanol did not significantly influence the binding. Among all ligands under study, fatty acids, ranging in acyl-chain length from C14 to C20, display good affinity for Blc. Binding of fatty acids, however, does not indicate a strong specificity as all the K_d values are in the narrow range of 3.3 μM (C14) and 1.8 μM (C20). A loose linear correlation is observed between the alkyl chain length and the K_d values, where the longer fatty acids yield the best affinity. The binding of three phospholipids was also studied. Pure phosphatidylglycerol (PtdGro) displayed a peculiar two range behavior: in the first range, quenching occurs as with fatty acids, and yields a K_d of 4.7 μM . However, upon increasing the concentration of PtdGro between 15 and 100 equiv.

(with respect to the protein), another increase in fluorescence occurred at 316 nm, which reaches saturation at 50 equiv. We attributed this latter effect to surface tryptophans probably becoming involved in large PtdGro aggregates. Deconvolution of the signals indicates that the new band has its maximum at 308 nm. Total *E. coli* phospholipid extracts were also assayed with two extracts commercially available (Section 2). The total extract is composed of phosphatidylethanolamine (PtdEtn) (57.5%), PtdGro (15.1%), cardiolipin (9.8%) and a mix of non-documented compounds (17.6%). The “polar extract” contains 67%, 23.2% and 9.8% of the first three above mentioned products, respectively, and no undocumented compounds. Both extracts bind with constants identical within the experimental errors, 2.5 and 2.9 μM , respectively (Table 2), values comparable to those obtained with fatty acids.

Two LPLs have been chosen to complete the panel of putative ligands: oleoyl-*sn*-2-lyso-PtdEtn (LOP) and palmitoyl-*sn*-2-lyso-PtdEtn (LPP). Contrary to the “flat” range of K_d constants obtained with FAs or PLs, LPLs exhibited much higher affinities. LPP has 70 times higher affinity than oleic acid, while LPP has 245 times higher affinity than palmitic acid. Interestingly, while the longer acyl-chain and double bond in oleic acid gave a slightly favourable effect on the affinity compared to palmitic acid (ratio 1.35), LOP binds 5 times less well than LPP. This may reveal a much more constrained binding for LPLs than for fatty acids.

3.2. Crystal structure with vaccenic acid

We have co-crystallized Blc in the presence of vaccenic acid, and collected data at 1.8 \AA resolution. All attempts to crystallize Blc-LPLs complexes were unsuccessful, and vaccenic acid has provided the best diffracting crystals. The structure is isostructural with that of the native Blc. Two monomers of Blc are contained in the asymmetric unit. Blc monomer has a typical lipocalin fold consisting of a β -barrel with eight anti-parallel strands and an α -helix at the C-terminus. Inside the cavity of monomer B, a well defined elongated electron density could easily accommodate a vaccenic acid molecule, while monomer A was empty. The molecule is bent at the position of the *cis*-double bond (Z11–12). Careful examination of monomers A and B relationship revealed that they interact tightly together: the interaction covers 786 \AA^2 and 825 \AA^2 of water accessible surface (WAS) area, on monomer A and B, respectively, upon binding (Fig. 2). The total WAS area of each monomer being 7800 \AA^2 , the buried WAS area represents ~10% of the total surface, a value indicating that dimerisation is not a crystallization artefact [28]. For comparison, these values of interacting surface area are comparable to those observed in immunoglobulin fragments/protein complexes (Table 3A) [29]. The interaction between the two monomers was present and essentially identical in the original native structure, but it escaped our observation and is not described in [11].

The oligomerization state of Blc, alone or in complex with palmitoleic acid, was investigated using static light scattering. In both cases, Blc appears as a dimer of 43.1 ± 2.3 kDa and 38 ± 3 kDa, respectively. This confirms the crystallographic data that Blc dimerisation is an intrinsic property of the protein. It is interesting to note that such an asymmetric binding pocket (as shown in Fig. 2A and B) in a homodimer is maintained even without a ligand present. Furthermore, binding of two ligands to the Blc dimer is impossible due to steric clashes between them.

The comparison of subunits A and B indicate significant conformational differences that we think are necessary for dimerisation (Fig. 2C). In order to fit within the other monomer, strands 6 and 7, and particularly the loops between them, move up to 5 Å in one monomer relative to the other (Fig. 2C). The dimer interface involves in large part these loops, and other residues among which the aromatics Tyr113, Tyr137, and several surface-exposed phenylalanines including Phe53 A and B, Phe108 A and B, Phe109 A and B and Phe112 A and B together form an inter-dimer hydrophobic core (Fig. 2D, Table 3A). The presence of many exposed phenylalanines is an uncommon feature at the surface of a monomeric globular protein, and has to be regarded as a hallmark of protein-protein or protein-lipid interactions. Both subunits are involved in the binding site of Blc, accounting for the stoichiometry of one vaccenic acid molecule per dimer (Fig. 2A and B). The vaccenic acid interacts with both subunits and covers 89 Å² and 171 Å² of the WAS area of monomers A and B, respectively (Table 3B).

It should be stressed that Blc is anchored to membranes by a covalently attached lipid modification at the amino terminus of the protein (Fig. 1). The manner in which the lipid-anchor of each monomer is arranged relative to the other is a critical issue. Our Blc construct has the signal peptide and the first four residues (CSSP) removed and replaced by the Gateway ATTB1 sequence (Fig. 1) [11,30]. We have modelled into our dimeric structure the four original residues of Blc, as well as the major part of the anchoring lipid, *S-sn*-1,2-diacylglycerylcysteine, that we included at each *N*-terminal cysteine. This model reveals that the lipid-anchors of each of the 2 monomers are close to each other in the dimer, and are located on the same face in a geometry compatible with the insertion of the dimer into the membrane (Fig. 2E). The binding site for vaccenic acid is located opposite to the membrane insertion site where it is expected to face the periplasmic space (Fig. 2E).

4. Discussion

Using intrinsic fluorescence quenching, Blc has been shown to bind several FAs and PLs with μM affinities. In contrast, Blc binds weakly to retinol and does not bind fluorescent reporters such as AMA, amino-naphthalene sulfonate (ANS) or NPN. Ampicillin was found to bind Blc, but with slightly lower affinity than FAs. Blc exhibits, therefore, some preference for FAs and PLs over other compounds, the K_d differences being less than a factor 2 (Table 2). LPLs, in contrast, bind Blc two orders of magnitude better than the corresponding FAs or PLs (Table 2). Interestingly, while the K_d s ratio oleoyl-LPL/oleic acid is 51, the K_d s ratio for palmitoyl-LPL/ palmitic acid is 5 times larger. It seems that binding of the LPL polar headgroup is modulated by the FA aliphatic chain length.

The crystal structure of Blc in complex with vaccenic acid reveals a stoichiometry of one ligand per dimer. Blc dimerisation seems to be biologically relevant, since the WAS area buried in the dimer is larger than the accepted threshold, comparable to those observed in immunoglobulins/proteins complexes. Dimerisation of another lipocalin, bovine OBP (bOBP), by domain swapping was observed in the crystal structure. In this case, however, a ligand is bound by monomer [25,31]. The exchange of the α-helix in bOBP was made possible by the absence of a disulfide bridge and resulted in that case only in the stabilisation of the protein. The different arrangement of the Blc dimer, driven by the unusual number of

hydrophobic Phe groups exposed to the surface and possibly by a few movements of surface loops, results in two major advantages: it creates a larger binding site bridging two subunits, and it orients the polarity of the protein so as to promote the insertion into the membrane on one side and the binding function on the opposite side.

The trafficking of lipids between the inner and outer membranes of the Gram-negative bacterial cell envelope is a subject of intensive current interest. Significant recent progress has been made in the study of lipid export and import across both the inner and outer membranes. The ATP-binding cassette transporter MsbA accelerates the export of both phospholipids and LPS across the inner membrane [32,33], while the outer membrane protein Imp is needed for the display of LPS on the extracellular surface of the outer membrane [34]. Additionally, mechanisms for the uptake of fatty acids across both the outer and inner membranes are now well established [35]. However, the mechanisms for transperiplasmic movement of lipids between the inner and outer membrane systems remain largely unknown.

Pathways for the rapid exchange of glycerophospholipids between the two membranes and for the unidirectional export of LPS in Gram-negative bacteria were first revealed by the ground-breaking studies of Jones and Osborn [36,37]. In eukaryotic cells, lipid trafficking has been documented to proceed by lipid transfer proteins or by vesiculation mechanisms. In Gram-negative bacteria, vesiculation models seem less likely given the structural barrier provided by the peptidoglycan exoskeleton, which is sandwiched between the two membranes [38]. Bacterial lipoproteins clearly depend on a specific transfer protein during outer membrane biogenesis, suggesting that other lipids are transported by similar mechanisms [39]. Lipocalins represent an important class of lipid transfer proteins, and Blc, while a bacterial lipoprotein anchored in the inner leaflet of the outer membrane, is a lipocalin of unknown function.

Given the high affinity of Blc for LPL's, it seems likely that Blc may fulfil a role in cell envelope LPL transport. Although LPL's are key inner membrane intermediates of phospholipid metabolism, we do not know of any evidence to indicate that LPL's are exported to the outer membrane. However, Rock and colleagues have established that exogenously supplied LPL's can be taken up by deep-rough LPS mutants and converted by reacylation into glycerophospholipids using inner membrane-associated enzymes [40,41]. To date, no accessory factors needed for LPL uptake have been identified. In wild-type cells, at least two enzymes can generate LPL's in the outer membrane, namely, the phospholipase OMPLA [13] and the lipid A palmitoyltransferase PagP [14,42–45]. Both enzymes preferentially generate the *sn*-1 LPL regioisomers, but these are known to spontaneously rearrange into the more stable *sn*-2 LPL's [46] shown here to bind Blc with high affinity in our fluorescence quenching studies.

In eukaryotic cells, *sn*-2 LPL's are generated by phospholipases that mobilize arachidonic acid for eicosanoid-mediated signal transduction. Recently, the *sn*-2 LPL products have been shown to function as potent biological mediators in their own right [47]. Mammalian apolipoprotein D and *Drosophila* lazarrillo are the closest eukaryotic homologous of Blc and the only eukaryotic lipocalins that, like Blc, are anchored to lipid membranes. The ligand for

lazarillo is unknown, but apolipoprotein D binds a variety of ligands including arachidonic acid [48]. Recently, functional studies on ApoD and lazarrillo clearly pointed to their essential role in lipid metabolism [49,50]. Perhaps the high affinity of Blc for LPL's may help shed some light on the enigmatic functions of lazarrillo and apolipoprotein D.

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Abbreviations

AMA	amino anthracene
ANS	amino-naphthalene sulfonate
LPS	lipopolysaccharide
NPN	1- <i>N</i> -phenylnaphthylamine
FA	fatty acid
PL	phospholipid
LPL	lysophospholipid
PtdGro	phosphatidylglycerol
PtdEtn	phosphatidylethanolamine
LOP	oleoyl- <i>sn</i> -2-lyso-PtdEtn
LPP	palmitoyl- <i>sn</i> -2-lyso-PtdEtn
WAS	water accessible surface
SLS	static light scattering

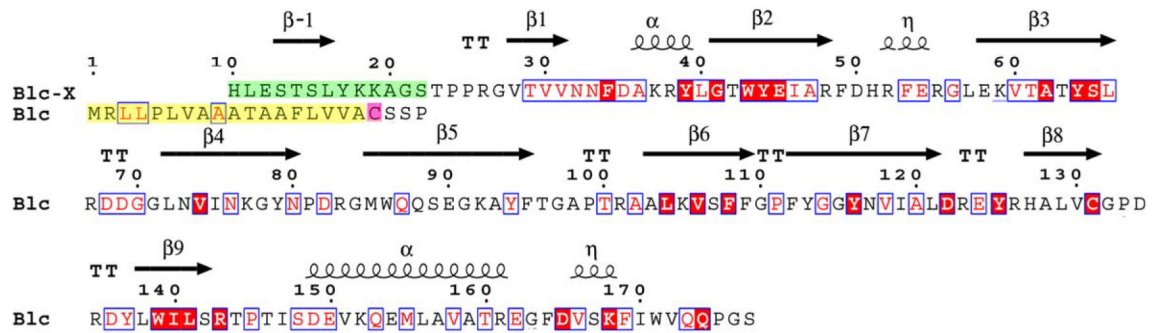
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**Fig. 1.**

Sequence of the native Blc (Blc) and of the Blc construct used in this study (Blc-X). The N-terminus of Blc-X (green) replaces the N-terminus sequence in native Blc (yellow). The red filled boxes indicate identity with ApoD and Lazarillo, while the white ones indicate similarity. The secondary structure is represented as arrows and spirals (strands and helices) above the sequence. The cysteine linked to a membrane anchor in native Blc is identified in pink.

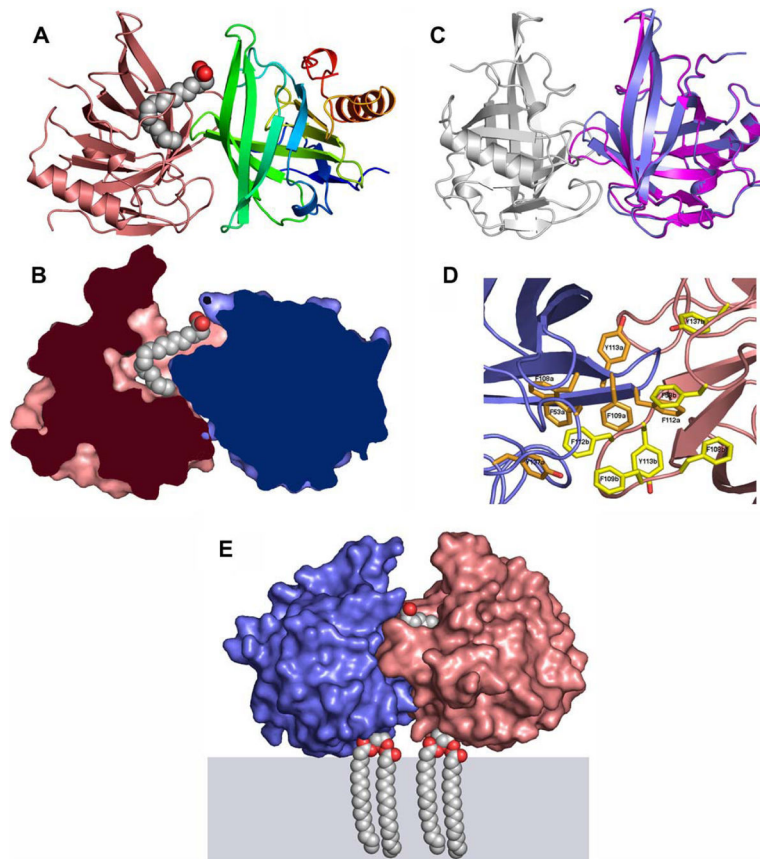


Fig. 2. X-ray structure of Blc in complex with vaccenic acid. (A) Electron density map (2fo–fc) contoured in the binding site of Blc with vaccenic acid (red sticks) inside. (B) Ribbon view of Blc with vaccenic acid inside (spheres). Monomer B (left) is pink; monomer A (right) is rainbow colored from N-(blue) to C-terminus (red). (C) Compact view of Blc sliced at the level of the inside cavity, illustrating shape complementarity of both monomers and of vaccenic acid. Same orientation as in (B); vaccenic acid in spheres; monomer B (left) is pink; monomer A (right) is blue. (D) View of Blc dimer after superposition of monomers A and B (blue and pink) showing the structural difference involving strands 6 and 7 and the loop in between. (E) Inter-monomers hydrophobic core. Monomer B (right) is pink with yellow side-chains; monomer A (left) is blue with orange side-chains. (F) Compact view of the Blc dimer attached to the membrane anchor lipid forming a *S*-sn-1,2-diacylglycerylcysteine moiety attached to the first cysteine (computer model). Monomer B (right) is pink and monomer A (left) is blue; vaccenic acid and lipidic anchors are represented as spheres.

Table 1

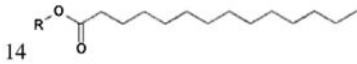
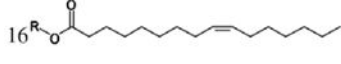
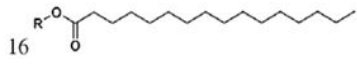

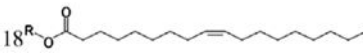
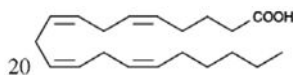
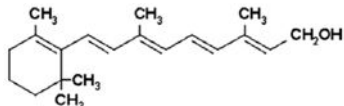
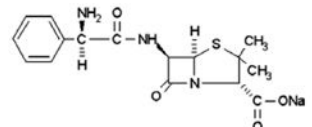
Data collection and refinement statistics

<i>Data collection</i>	
Resolution limits	1.8–60.0/1.8–1.85
% of data > 1 σ (overall/last shell) ^a	97.1 (89.1)
Overall I/ σ (I) (overall/last shell) ^a	13.7 (2.3)
R-merge (%) (overall/last shell) ^a	8.9 (32.7)
<i>Refinement</i>	
Protein/ligand/solvent atoms	2684/20/449
Resolution limits (Å)	1.8–60
R-/R-free value (%)	17.1/21.0
r.m.s.d. on bonds (Å) and angles (°)	0.011/1.3
Mean B factor (Å ²) protein	15.2
Vaccenic acid	27.2

^aLast shell: 1.8–1.85 Å.

Table 2

Binding results, obtained with tryptophan quenching, of fatty acids, phospholipids and other molecules with Blc

Fatty acids	Nr. carbons/chemical structure	K_d (μM)	Relative affinity
Myristic	14 	3.3 ± 0.3	0.8
Palmitoleic	16 	3.1 ± 0.2	0.9
Palmitic acid	16 	2.7 ± 0.2	1
<i>cis</i> -Vaccenic	18 	2.5 ± 0.3	1.1
Oleic	18 	2.0 ± 0.1	1.35
Arachidonic	20 	1.8 ± 0.1	1.5
Phospholipids			
PtdGro		4.7 ± 0.3	0.57
<i>E.coli</i> total mix		2.5 ± 0.3	1.1
<i>E.coli</i> polar mix		2.9 ± 0.4	0.9
Lysophospholipids	1-Acyl-2-Hydroxy- <i>sn</i> -Glycero-3-Phosphoethanolamine		
Oleoyl		0.039 ± 0.006	70
Palmitoyl		0.011 ± 0.001	245
Other molecules			
Retinol		20.2 ± 2	0.13
Ampicillin		5.0 ± 0.3	0.54

K_d values are given in μM .

Table 3

List of interactions within B1c dimer (A) and between B1c and vaccenic acid (B) as illustrated by the WAS (\AA^2) area buried in the interaction

Res	Nr	WAS	Res	Nr	WAS
A					
GLY	21	A	11	LYS	19
ARG	52	A	33	ALA	20
PHE	53	A	28	GLY	21
ARG	67	A	15	LEU	22
ASP	69	A	20	PRO	25
SER	89	A	4	HIS	51
GLU	90	A	2	ARG	52
LYS	92	A	53	PHE	53
TYR	94	A	42	VAL	106
LYS	105	A	64	PHE	108
SER	107	A	1	PHE	109
PHE	108	A	10	PRO	111
PHE	109	A	89	PHE	112
GLY	110	A	40	TYR	113
PRO	111	A	84	GLY	114
PHE	112	A	100	PRO	133
TYR	113	A	85	ASP	134
GLY	114	A	13	ASP	136
PRO	133	A	28	TYR	137
ASP	134	A	18	TRP	139
TYR	137	A	46	Total	823
Total			786		

B	
Nr	WAS
HIS	51
ARG	52
PHE	53

	Nr	WAS	
		B	A
GLU	54	B	4
ASN	76	B	13
SER	89	B	20
VAL	106	B	2
PHE	108	B	43
PHE	109	B	19
GLY	114	B	1
Total	B		171
ARG	52	A	40
PHE	53	A	2
PHE	109	A	16
PRO	111	A	10
PHE	112	A	21
Total	A		89

The columns A and B refer to the B1c monomer involved in the interaction B.