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Structure of a Premicellar Complex of Alkyl Sulfates with the Interfacial Binding Surfaces of 4 Subunits of Phospholipase A2[☆]

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Abstract

The properties of three discrete premicellar complexes ($E_1^{\#}$, $E_2^{\#}$, $E_3^{\#}$) of pig pancreatic group-IB secreted phospholipase A2 (sPLA₂) with monodisperse alkyl sulfates has been characterized [Berg, O. G., et al., *Biochemistry* 43, 7999–8013, 2004]. Here we have solved the 2.7 Å crystal structure of group-IB sPLA₂ complexed with 12 molecules of octyl sulfate (C₈S) in a form consistent with a tetrameric oligomeric that exists during the $E_1^{\#}$ phase of premicellar complexes. The alkyl tails of the C₈S molecules are centered in the middle of the tetrameric cluster of sPLA₂ subunits. Three of the four sPLA₂ subunits also contain a C₈S molecule in the active site pocket. The sulfate oxygen of a C₈S ligand is complexed to the active site calcium in 3 of the 4 protein active sites. The interactions of the alkyl sulfate head group with Arg-6 and Lys-10, as well as the backbone amide of Met-20, are analogous to those observed in the previously solved sPLA₂ crystal structures with bound phosphate and sulfate anions. The cluster of three anions found in the present structure is postulated to be the site for nucleating the binding of anionic amphiphiles to the interfacial surface of the protein, and therefore this binding interaction has implications for interfacial activation of the enzyme.

Keywords

sPLA₂; Alkyl sulfate; premicellar complex; interfacial enzyme; membrane associated protein

1. Introduction

Among the wide array of proteins in nature, roughly half are either embedded in a membrane bilayer, or associated with the surface of an aggregated amphiphilic substrate, such as on the surface of a lipid bilayer. Proteins that function on the surface and access their substrates from the aggregated substrates are referred to as interfacial enzymes. The proximity of these enzymes to their substrates or regulating ligands is a major factor in their design and evolved optimization. However, additional allosteric feature of these catalysts are often required by a combination of protein-interface structural changes and desolvation. For these systems, this process has been termed interfacial activation.

The 14-kDa family of secreted phospholipase A2 enzymes (sPLA₂), which catalyze the hydrolysis of the sn-2-acyl substituent of glycerophospholipids, have been thoroughly

[☆]The atomic coordinate files for the tetramer and monomer forms of sPLA₂ have been deposited in the Protein Data Bank (PDB) with the accession codes: 3fvi and 3fvj, respectively.

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characterized as functioning as an interfacial enzyme [1]. Several of the sPLA₂ family members have an additional interfacial preference for anionic interfacial interfaces. For these proteins the functional increase of activity is partly realized by an increase of the interfacial binding affinity for the enzyme with an anionic interface. Additionally for these cases, anionic allosteric activation plays an important role. Specifically, pancreatic group-IB sPLA₂ requires anionic charges at the interface in order to attain optimal activity [1,2]. The physiological significance of this activation is consistent with the environment of the natural substrate for the pancreatic sPLA₂ that is co-dispersed with anionic bile salts.

Since group-IB sPLA₂ preferably binds to an anionic interface, it is reasonable to expect that monodisperse anionic amphiphiles bind to the interfacial binding surface (i-face) of sPLA₂. Features of these interactions are relevant to understand the structural and functional consequences of interfacial activation. This paradigm is supported by a variety of recent observations. In the crystal structure of the anion-assisted dimer of group-IB sPLA₂ the anions were bound along well-defined sites on the i-face [3]. Additionally, monodisperse alkyl sulfate molecules below their CMC concentration bind to group-IB sPLA₂ to form a premicellar complex [4,5]. These results have provided insight into the specific short-range interactions of the anionic head group with the i-face of sPLA₂, and they suggest how such interactions with alkyl sulfates could promote the hydrophobic effect for the subunit oligomerization and premicellar formation of E_i[#] complexes. Figure 1 illustrates how an enzyme such as sPLA₂ can bind to an aggregated substrate above its CMC (E*) and to premicellar complexes (E_i[#]) below the CMC of the amphiphilic ligand. A comparison of the structural and functional differences of the E* interfacially activated form and the premicellar forms (E_i[#]) may give insight into the process of interfacial activation.

Functionally, the formation of the premicellar E_i[#] complexes have been monitored by Trp-3 fluorescence changes using monodisperse solutions of alkyl sulfates below their CMC concentration [4]. This analysis has provided a window of conditions for the structural characterization of a specific E_i[#] complex with an alkyl sulfate formed below its CMC. In this paper we report the X-ray crystal structure of an E_i[#] complex of sPLA₂ with C₈S amphiphiles. The structure, which we believe represents a premicellar complex of sPLA₂, is composed of four subunits of sPLA₂ complexed with 12 molecules of C₈S. The present structural results are discussed in context to recent functional characterization [4,5] and more generally as they relate to events linked with the interfacial activation of the enzyme [6,7].

2. Materials and methods

2.1. Crystallization and X-ray diffraction data collection

Porcine pancreatic group-IB sPLA₂ was expressed from *E. coli* as previously described [8], and was given to us as a kind gift from Dr. Mahendra Jain. The group-IB sPLA₂ enzyme was screened for protein crystallization in the presence of alkyl sulfates (Sigma-Aldrich) using protein at a concentration of 15 mg/ml in 10 mM CaCl₂, and a maximum concentration of alkyl sulfate of 4 mM. Crystals were obtained with C₈S using the hanging drop vapor diffusion method at 25 °C. Protein crystal screening using commercially available screens (Hampton Research) resulted in two new crystal forms of group-IB sPLA₂. Crystal form-1 was obtained from hanging drops made with an equal volume of the crystallization well solution containing 0.1 M sodium acetate pH 4.6 and 8% (w/v) PEG 8000. Crystal form-2 was obtained using the crystallization and well solution of 0.1 M sodium citrate pH 5.6, 20% (v/v) isopropanol and 20% (w/v) PEG 4000. For each crystal form, hanging drops were prepared using the optimized conditions by mixing a 1 µl pre-equilibrated aqueous solution containing 15 mg/ml protein, 10 mM CaCl₂ and 4 mM C₈S with a 1 µl aliquot of the crystallization solution described above. Crystals of form-1 appeared in an orthorhombic shape with dimensions of 50 × 50 × 300 microns. Crystals in form-2 appeared in an almost cubic shape with an edge dimension of 200

microns. For each crystal form, X-ray diffraction data sets were collected from a single crystal on a Rigaku-RU300 rotating anode generator with a RAXIS IV image plate area detector. The crystal were initially flash cooled at -180°C following a quick rinse through the crystallization solution that had been partially saturated with 30% xylitol or 30% glycerol solution as cryoprotectant for crystals form-1 and -2, respectively. The programs DENZO and SCALEPAK [9] were used for data processing and scaling. Crystal form-1 belonged to space group $P2_12_12_1$ with four protein subunits in the asymmetric unit. Crystal form-2 belonged to space group $P3_121$ with one subunit in the asymmetric unit.

2.1. Structural refinement

Structures of the two crystal forms were solved by molecular replacement method using the program AMoRe [10]. The structure of porcine sPLA₂ (PDB code 1fxj) was used as the search model for molecular replacement. The X-ray data and refinement statistics are listed in Table 1. Molecular replacement solutions for both rotational and translational searches for both crystal forms-1 and -2 were unambiguous. The refinement was carried out using the program CNS [11]. A standard refinement strategy was used for each crystal form solved. Positional refinement was first carried out at the low resolution. As the refined model improved, data of higher resolution was added to the refinement. Simulated annealing and individual B-factor refinements were carried out. Models were manually adjusted on an interactive graphic station using the programs CHAIN [12] and O [13]. Crystal form-1 had four protein subunits built into the asymmetric unit. Subunit A and B are related by a non-crystallographic 2-fold axis and subunit C and D are related by a 158° rotation around another non-crystallographic symmetry axis. In the structure refinement we chose not to implement non-crystallographic symmetry averaging, so as not to bias differences that may appear between the 4 subunits of the asymmetric unit for the structure of crystal form-1. In the initial stages of refinement, residues in the 60s-loop (residues 59–75) were deleted from subunit C and D since the conformation of these two regions varied from the original molecular replacement search model. After two rounds of refinement consisting of positional, B-factor and simulated annealing refinement, the values of R_{free} and R_{working} were 0.350 and 0.274, respectively. After the 60s-loop residues were built into the model and one round of refinement, the R_{free} and R_{working} were 0.331 and 0.253, respectively. At this point, the difference electron density maps showed clear density for the sulfate head groups. Nine C₈S molecules were built into the model. After one round of refinement, the R_{free} and R_{working} values were 0.308 and 0.235, respectively. Overall a total of 12 detergent molecules were built into the model along with four calcium atoms, one sodium, two chloride and 71 water molecules. The final values of R_{free} and R_{working} are 0.275 and 0.206, respectively with reasonable protein geometry (Table 1).

Compared to crystal form-1, the refinement for crystal form-2 was more straightforward. One round of refinement at $2.5\text{ \AA}$ resulted in values of R_{free} and R_{working} of 0.310 and 0.259, respectively. The 60s-loop was manually rebuilt and two calcium atoms and 24 waters were added to the model, which improved the values of R_{free} and R_{working} to 0.290 and 0.237, respectively. Next, the refinement used data with a resolution extended to $2.3\text{ \AA}$. After several rounds of refinement involving manual intervention, a total of 79 waters, two calcium atoms and one chloride ion were added to the model. The final values of R_{free} and R_{working} are 0.249 and 0.201, respectively with good geometry (Table 1). Unlike crystal form-1, no C₈S molecules could be modeled into the crystal form-2 crystal structure, despite the presence of C₈S at 4 mM in the crystallization solution.

3. Results and discussion

3.1. Crystal structure of $E_1^{\#}$ complex

Crystallization conditions for sPLA₂ with C₈S were set for the premicellar $E_1^{\#}$ complex, which has been biophysically characterized previously [4,5]. Crystal form-1 has well ordered C₈S molecules bound in the core of an oligomeric state of sPLA₂. In contrast, crystal form-2 does not form a tetrameric oligomeric state, and the refined structure does not have any ordered C₈S molecules bound. The main difference of crystallization conditions between the two crystal forms was the presence of 20% (v/v) isopropanol in crystal form-2. These results are consistent with biophysical characterization of the $E_1^{\#}$ complex [4], and a conclusion that the binding of alkyl sulfate in the $E_1^{\#}$ complexes is dominated by the hydrophobic effect for the chain-chain interaction between the amphiphiles cooperatively bound to the i-face of sPLA₂. In contrast, crystal form-1 was obtained in the window of conditions under which the $E_1^{\#}$ complex was previously shown to be formed by Trp fluorescent spectroscopy [4,5]. The crystallographic asymmetric unit in this crystal form is a homotetramer containing 12 C₈S molecules as shown in Figure 2. Each subunit has the canonical phospholipase fold with subtle structural differences in the i-face surface 60s-loop (residues 59–75).

The four protein subunits associate by forming a spherical shaped oligomeric structure. The interfacial surfaces are buried in the core of the sphere and the eight long helices of sPLA₂ are exposed to the outside. Ten of the twelve C₈S molecules are clustered in the core and distributed among the four protein subunits as shown in Figure 2 and contacts described in Table 2. Two C₈S molecules bind to the exterior of subunits C and D. Subunits A and B only have contacts to the C₈S molecule through the i-face of each sPLA₂ subunit. When subunit A is positioned “horizontally” as the interface faces downward (as depicted in Figure 2), it can be seen as supported by a “tripod” made of the other 3 subunits, each subunit makes intermolecular contacts with subunit-A via residues located on the “edge” of the molecule. The same arrangement can be said for subunit B. At the core region of the tetramer structure very few water molecules were modeled into the crystal structure, which is consistent with the medium resolution of this structure (2.7 Å).

3.2. Tetramer interface

In the tetramer complex, the total surface area is 24,917 Å², of which 1,590 Å² are buried at the interface. The buried surface area was calculated by the method of Lee and Richardson as implemented in the CCP4 suite [14]. A 1.4 Å probe was used, and only protein residues were included in the calculation. More than 50% of the buried residues are sPLA₂ i-face residues as previously described [15]. In sPLA₂s the 20s-loop and 60s-loop refer to the two flexible surface loops that are often found at the subunit-subunit interface or crystallographic contact regions. They contain residues 13–25 and 59–75, respectively. These two surface loops along with the c-terminal residues display the largest conformational variations among solved sPLA₂ structures. In the C₈S structure, these regions that are buried at the tetramer interface are either close to the C-terminal region or belong to the 20s (residues 13–25) or 60s loop (residues 59–75) of sPLA₂. Each of these regions are known to be involved in i-face interactions [15]. Greater than 70% of the solvent buried residues are polar or charged residues. Subunits A and B each have close contacts with residues from subunit C, and to a smaller extent with subunit D. Subunit C has a nearly equal amount of contact with subunits A, B and D at the buried interfaces. And finally subunit D has the least close contact with subunits A, B or C at the buried interfaces. Judging from the percentage of accessible surface buried, the association of the tetramer is not extensive, and therefore would not be expected to be a highly stabilized species in solution without detergent bound.

3.3. Alkyl sulfate binding

Twelve ordered C₈S molecules were found in the tetramer structure. Figure 2 shows the distribution of C₈S molecules relative to the tetramer. The 12 C₈S molecules can be grouped into four subgroups according to their interactions with the protein or with themselves. Table 2 lists close contacts of protein ligands and the C₈S alkyl sulfates. Group-I contains four C₈S molecules: C₈S-2, C₈S-4, C₈S-7 and C₈S-11. The sulfate portions of C₈S-2, C₈S-4 and C₈S-7 each associate solely with subunit B, D and C, respectively. The sulfate head groups of the present structure occupies the same relative position as three of the anion binding sites as describe in the anion-assisted dimer crystal structure [3]. In the previously solved crystal structure of the anion-assisted dimer, phosphate or sulfate ions were modeled in this anionic binding pocket made up of R6, K10 and M20 of sPLA₂ [3]. In the sPLA₂-C₈S structure, the head groups of molecules C₈S-2, C₈S-4 and C₈S-7 interact with the positively charged R6 and residues from the 20s-loop (L19 and M20). The molecule C₈S-11 on the other hand, interacts with both subunits A and B, and therefore occupies a different position as the anion binding site. As a result the sulfate head group does not interact with the 20s-loop residues. A second group of detergent molecules (group-II: C₈S-3, C₈S-5 and C₈S-8), each associate with subunit B, D and C, respectively. This group of C₈S molecules is located inside the substrate binding pocket. The sulfate head group occupies a similar position as the sn-2 phosphate in the active site directed inhibitor MJ33 seen in previous crystal structures [3]. It interacts with the calcium ion through weak coordination with a binding distance of 2.97 – 3.27 Å. It also forms a hydrogen bond with the hydroxyl of Y69. In addition, it forms a hydrogen bond with the main chain nitrogen and carbonyl oxygen of the calcium binding loop residues Y28 and G30. This alkyl sulfate binding site was not observed in subunit A. Instead a Cl⁻ ion was modeled and refined in the place of the sulfate head group. The third group of C₈S molecules (C₈S-10 and C₈S-12) forms strong hydrogen bonds with the main chain carbonyl oxygen of L31. This anion binding site was not observed in the anion-assisted dimer crystal structure [3]. The C₈S-10 and C₈S-12 molecules were found associated with two subunits simultaneously. Similarly, the fourth group has C₈S molecules (C₈S-1, C₈S-6 and C₈S-9) that simultaneously interact with the 20s and 60s loops of two different subunits. Similarly, this site was also not observed in the anion-assisted crystal structure [3].

3.4. Alkyl sulfate self-association

As shown in Figure 2, C₈S molecules tend to aggregate together through alkyl chain association. The average inter-chain distance is around 4–5 Å. Two distinctive patches can be found: patch I contains ligands C₈S-1, C₈S-2, C₈S-3, C₈S-4 and C₈S-5; patch II contains ligands C₈S-7, C₈S-8, C₈S-9 and C₈S-10. These two groups of alkyl sulfates interact with subunit B, C and D, but not subunit A. The C₈S molecules that interact with subunit A (C₈S-6 and C₈S-11) do not show inter alkyl chain association with other C₈S ligands. The tendency for hydrophobic aggregation of the short alkyl chain might be the driving force for the tetramer formation. This differential pattern of C₈S aggregation in the tetramer furthermore explains why the subunits do not exhibit symmetrical structures.

3.5. Calcium coordination and waters

Due to medium resolution (2.7 Å) of the C₈S crystal structure, the presence or absence of waters should not be over interpreted. No waters could be modeled in coordination with calcium or around the catalytic center. The calcium ions in subunits B, C and D have 6–7 coordination due to binding of one C₈S ligand. The sulfate-calcium coordination is found to be bidentate in subunit B and monodentate in subunits C and D. In subunit A the calcium has a coordination of 5 since no C₈S ligand was found in this subunit's active site.

3.6. Comparison of the C₈S sPLA₂ structures in two crystal forms

The crystal structure of sPLA₂ was solved in the presence of 4 mM C₈S in two crystal forms. The tetrameric crystal form-1 with 12 bound C₈S molecules provided us with a structural insight of a premicellar aggregate of sPLA₂. In contrast, the structure of crystal form-2 has a single subunit of sPLA₂ and does not contain ordered C₈S molecules. The two crystal forms do offer a comparison of potential structural differences that may occur due to C₈S ligand binding. Figure 3 shows a backbone superposition of the two structures. The 60s loop (residues 60–70) is the only region that differs between the two structures. However, when one compares the sPLA₂ backbone structure of crystal form-2 with the previously solved sPLA₂-cholate bound structure [16], the 60s loops are nearly identical (not shown). These structures were each solved from crystals of the same symmetry and space group P3₁21. Also, in each of the P3₁21 crystal form structures, a second calcium was found to be involved in the crystal contact. The calcium is coordinated by six ligands from the carboxylates of E71 and E92 and the main chain carbonyl oxygen of S72 and their symmetry related counterparts. Again, this implies that the conformation for the 60s loop is space group dependent rather than physical chemically determined.

4. Conclusions

This study is motivated by the paradigm that if sPLA₂ binds along its i-face to the anionic interface, then monodisperse anionic amphiphiles are also likely to bind to this same i-face. Previously a variety of biophysical and kinetic approaches [4,7] have shown that at least three distinct premicellar complexes of sPLA₂ are sequentially formed with alkyl sulfates. Results in this paper provide structural insights into the first of these premicellar complexes. The tetrameric sPLA₂ structure from crystal form-1 may be related to the E₁[#] complex. The 2.7 Å crystal structure solved is composed of four subunits of sPLA₂ complexed with 12 molecules of C₈S in an oligomeric state indicative of a stable species. Figure 4 shows one of the four subunits of sPLA₂ and all of the C₈S molecules. The notable features of this structure, which are each consistent with the premicellar E₁[#] form with alkyl sulfate [4] are:

- i. The protein to C₈S (4:12) stoichiometry is consistent with the Hill number of 2 to 2.5 for the E₁[#] premicellar complex [4].
- ii. The alkyl tails of the C₈S molecules are clustered in the middle of the four-subunit complex, and one molecule of C₈S occupies the hydrophobic pocket of 3 of the 4 sPLA₂ active sites observed in the asymmetric unit of the crystal structure.
- iii. The sulfates cluster into a group of three anions next to the R6 and K10 and are predicted to be the nucleating anion-binding positions of the E₁[#] form [4]. Interactions with R6 and K10, as well as the backbone amide of M20, are analogous to those observed in the anion-assisted dimer crystal structure [3].
- iv. The sulfate oxygen of one of the C₈S ligands in the active site is complexed to calcium; however, its environment resembles that of the inert solution form of sPLA₂.

The limiting resolution of 2.7 Å for the tetrameric sPLA₂-C₈S structure does not allow a more thorough analysis and prediction of changes to the active site from a crystal form that mimics the unactivated solution form of the enzyme. For example, the current model of what represents an allosterically activated sPLA₂ has been linked to the presence of two water molecules in line between H48 and the catalytic calcium [2]. At the current level of resolution, the identity and placement of water molecules in the active site is unreliable. The limit of resolution and asymmetry of the tetrameric sPLA₂ structure raises the possibility that in solution premicellar aggregates of sPLA₂ with alkyl sulfates may contain a distribution of several possible oligomeric states (i.e. 3–10). The crystallization of a tetramer in this case can be considered to have been a fortuitous event. In general, the use of detergents in the crystallization of membrane

proteins may also rely to some extent on either the prevention of such pre-micellar aggregates, or in some case the fortuitous crystallization of them, as was the case here.

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Abbreviations used

B-factor	temperature factor
E _i #	pre-micellar complex of enzyme
E*	interfacial bound form of enzyme
i-face	interface binding surface of enzyme
MJ33	1-hexadecyl-3-(trifluoroethyl)- <i>sn</i> -glycero-2-phosphomethanol
C ₈ S	octyl sulfate
PDB	Protein Data Bank
sPLA ₂	14 kDa secreted phospholipase A2
RMSD	root mean squared deviation
R _{free}	free R-factor
R _{working}	working R-factor

References

1. Berg OG, Gelb MH, Tsai MD, Jain MK. Interfacial enzymology: the secreted phospholipase A(2)-paradigm. *Chem Rev* 2001;101:2613–2654. [PubMed: 11749391]
2. Bahnson BJ. Structure, function and interfacial allostery in phospholipase A2: insight from the anion-assisted dimer. *Arch Biochem Biophys* 2005;433:96–106. [PubMed: 15581569]
3. Pan YH, Epstein TM, Jain MK, Bahnson BJ. Five coplanar anion binding sites on one face of phospholipase A2: relationship to interface binding. *Biochemistry* 2001;40:609–617. [PubMed: 11170377]
4. Berg OG, Yu BZ, Chang C, Koehler KA, Jain MK. Cooperative binding of monodisperse anionic amphiphiles to the i-face: phospholipase A2-paradigm for interfacial binding. *Biochemistry* 2004;43:7999–8013. [PubMed: 15209495]
5. Yu BZ, Apitz-Castro R, Tsai MD, Jain MK. Interaction of monodisperse anionic amphiphiles with the i-face of secreted phospholipase A2. *Biochemistry* 2003;42:6293–6301. [PubMed: 12755634]
6. Berg OG, Yu BZ, Jain MK. Thermodynamic Reciprocity of the Inhibitor Binding to the Active Site and the Interface Binding Region of IB Phospholipase A2. *Biochemistry* 2009;48:3209–3218. [PubMed: 19301847]
7. Yu BZ, Bai S, Berg OG, Jain MK. Allosteric Effect of Amphiphile Binding to Phospholipase A(2). *Biochemistry* 2009;48:3219–3229. [PubMed: 19301848]
8. Jain MK, Tao WJ, Rogers J, Arenson C, Eibl H, Yu BZ. Active-site-directed specific competitive inhibitors of phospholipase A2: novel transition-state analogues. *Biochemistry* 1991;30:10256–10268. [PubMed: 1931954]
9. Otwinowski Z, Minor W. Processing of x-ray diffraction data collected in oscillation mode. *Methods Enzymol* 1997;276:307–326.
10. Navaza J. Amore - an Automated Package for Molecular Replacement. *Acta Crystallographica Section A* 1994;50:157–163.

11. Brunger AT, Adams PD, Clore GM, Delano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges N, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. Crystallography and NMR Systems (CNS): A New Software System for Macromolecular Structure Determination. *Acta Cryst* 1998;D54:905–921.
12. Sack JS, Quirocho FA. CHAIN: A crystallographic modeling program. *Methods Enzymol* 1997;277:158–173. [PubMed: 18488309]
13. Jones TA, Zou JY, Cowan SW, Kjeldgaard M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr Sect A* 1991;47:110–119. [PubMed: 2025413]
14. CCP4. The CCP4 Suite: Programs for Protein Crystallography. *Acta Crystallogr D Biol Crystallogr* 1994;50:760–763. [PubMed: 15299374]
15. Winget JM, Pan YH, Bahnson BJ. The interfacial binding surface of phospholipase A2s. *Biochim Biophys Acta* 2006;1761:1260–1269. [PubMed: 16962825]
16. Pan YH, Bahnson BJ. Structural basis for bile salt inhibition of pancreatic phospholipase A2. *Journal of Molecular Biology* 2007;369:439–450. [PubMed: 17434532]
17. Brunger AT. The free R value: A novel statistical quantity for assessing the accuracy of crystal structures. *Nature* 1992;355:472–474. [PubMed: 18481394]

Figure 1.

sPLA₂ forms an interfacial surface bound and activated form (E^*) following a desolvation and binding event to an aggregate substrate surface, such as a lipid bilayer. The E^* form is the functional form. Additionally, sPLA₂ forms micellar $E_i^\#$ complexes, which have a tendency to self-aggregate due to their amphiphilic properties.

Figure 2.

The 2.7 Å crystal structure of a tetrameric porcine group-IB sPLA₂ complexed with 12 C₈S molecules. **A** The C₈S alkyl sulfates are clustered in the middle of the tetrameric quaternary structure, shown as white balls and sticks. The residues K10, R6 and the active site calcium of subunit A are shown in blue, while the active site calcium of subunits B, C and D are rendered white. This figure was made using the program MOLSCRIPT. **B** Molecular model of the alkyl sulfate and sPLA₂ tetramer complex. The protein subunits A(green), B(cyan), C(yellow) and D(purple) are shown as a C_α trace. The interface residues shown in surface representations are A: L19, N24, L31, G32, V65, D66, N67 and T70; B: L2, H17, L19, M20, D21, F22, N23, N24, L31, D66, N67, T70, K113, K116, N117, and K121; C: W3, R6, K10, L19, M20, S60, K62,

F63, D66, S74, N89, E92 and A93; D: W3, R6, K10, L19, M20, N23, N24, L31, K62, F63 and V65. The c8s molecules are shown in sphere/mesh surface representation. Also shown are Ca^{2+} ions (burgundy sphere), one Cl^- ion (green sphere) and water molecules (blue sphere) that are within a 4 Å radius from the interface residues. This figure was made using the program PYMOL.

Figure 3.

The RMSD comparison of crystal form-1 and form-2. Overall RMSD were calculated in reference to form-2 and plotted for form-1 subunits A (red), B(green), C(blue) and D(violet) for each residue.

Figure 4.

The crystal structure of porcine group-IB sPLA₂ complexed with C₈S molecules. The B subunit of the four subunits is shown relative to 10 of the C₈S molecules of this structure for clarity. The residues K10, R6 and the active site calcium are shown in blue. The grey oval represents the interfacial binding region of the sPLA₂ enzyme. This figure was made using the program MOLSCRIPT.

Table 1X-ray data collection and refinement of sPLA₂- C₈S tetramer and monomer structures.

Crystal Data	Form-1	Form-2
Space group	P2 ₁ 2 ₁ 2 ₁	P3 ₁ 21
Cell parameters		
a (Å)	66.0	69.1
b (Å)	82.1	69.1
c (Å)	123.8	68.8
Subunits/asymmetric unit	4	1
X-ray Data		
Total reflections	153330	100652
Unique reflections	19134	8777
Resolution limit (Å)	2.70	2.30
Completeness (%)	99.1	98.7
R _{merge} ^a	11.1 (46.6)	6.3 (52.2)
I/Sigma(I)	8.5 (3.1)	16 (3.6)
Refinement		
PDB code	3fvi	3fvj
Resolution range (Å)	33.5-2.70	30.9-2.30
R _{working} ^b	0.206	0.201
R _{free}	0.275	0.249
RMSD observed ^c		
bond length (Å)	0.007	0.006
angle distance (°)	1.4	1.2
Ramachandran Plot		
% most favorable	84.9	86.5
% additional allowed	14.9	12.6
% generously allowed	0.2	0.0
% disallowed	0.0	0.0
B-value Wilson Plot (Å ²)	17.1	43.9
Mean B-value (Å ²)	26.9	44.9
Protein atoms	3884	971
C ₈ S molecules	12	0
Ca ²⁺ atoms	4	2
Na ⁺ atoms	1	1
Cl ⁻ atoms	2	1
Water molecules	71	76

^a $R_{merge} = \sum |I_o - I_a| / \sum (I_a)$, where I_o is the observed intensity and I_a is the average intensity, the sums being taken over all symmetry related reflections. Parentheses indicate values in the last resolution shell.

^b R-factor = $\sum |F_o - F_c| / \sum (F_o)$, where F_o is the observed amplitude and F_c is the calculated amplitude. R_{free} is the equivalent of R_{working}, except it is calculated for a subset of 5% of the unique reflections not used during refinement [17].

^cDeviations from ideal protein geometry are reported as the root mean squared deviation (RMSD).

Table 2

Pair wise contacts $< 5 \text{ \AA}$ of C₈S ligands with residues from the sPLA₂ tetramer structure.^a

C ₈ S	Subunit A	Subunit B	Subunit C	Subunit D
C ₈ S-1		L19, N23(OD1)	F63, L64, V65	W3, N23(OD1)
C ₈ S-2		R6(NH2), H17, P18, L19, M20	L64, T70	Y69
C ₈ S-3		L2, F5, R6, P18, L19, F22, Y28, C29, G30, C45, H48, D49, Y69		
C ₈ S-4		L19, N23(OD1), N67, Y69		R6(NH2), H17, P18, L19, M20
C ₈ S-5				L2, F5, R6, P18, L19, Y28(O), C29, G30(O), L31, C45, D48(O), D49, Y69
C ₈ S-6	L2, W3, L19, N23 (OD1)	H17		
C ₈ S-7	L2, L31, Y69, T70		R6, H17, P18, L19, M20	
C ₈ S-8			L2, F5, R6, P18, L19, Y28(O), C29, G30(O), H48(ND1), D49, Y69(OH)	L64
C ₈ S-9			L2, W3, L19, N23	F63, L64, V65
C ₈ S-10		K121	K56, N57(O), L58, D59, C61, K62	N24, C27, L31(O), G32, N117, L118, T120
C ₈ S-11	R6(NH2)	R6, K10(NZ)		
C ₈ S-12			N24, C27, G30, L31(O), G32, L118, T120	K56(O), N57, L58, D59, C61, K62

^aProtein residue atoms in parentheses have polar interactions $< 5 \text{ \AA}$ with the sulfate oxygens of C₈S. The other contacts are non-polar interactions between the C₈S alkyl chain and protein residue.