Interaction of bovine seminal plasma proteins with model membranes and sperm plasma membranes

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PDC-109 is the major protein of the bovine seminal plasma, which binds to the plasma membrane of spermatozoa upon ejaculation and plays a critical role in priming the sperm cells for fertilization to take place. Experimental studies from the last 15 years have shown that the cell-surface receptor for PDC-109 is a choline phospholipid and shed light on the molecular details of the interaction of this protein with model membranes containing phosphatidylcholine. PDC-109 contains two fibronectin type 2 domains and each domain binds one ligand molecule. Single crystal X-ray diffraction studies have shown that the specific binding of the soluble head group moiety of the choline phospholipids, phosphorylcholine, with PDC-109 is mediated by a cation–π interaction of the quaternary ammonium group with the indole side chain of a core tryptophan residue. The binding is further stabilized by hydrogen bonds of the phosphate group with the side chains of different amino acid residues or with the main chain amide groups of several residues. Spin-label ESR studies have shown that although PDC-109 does not bind to membranes lacking a choline-containing lipid, it does exhibit some, albeit weaker, interaction with other phospholipids such as phosphatidylglycerol and phosphatidylserine as well as cholesterol. Surface plasmon resonance studies on the mechanism of interaction with phosphatidylcholine membranes has shown that the tight binding of PDC-109 is characterized by a very slow dissociation process. Analysis of the activation parameters obtained from the SPR studies indicated that binding of PDC-109 to phosphatidylcholine membranes is favoured by a strong entropic contribution, whereas negative entropic contribution is primarily responsible for the rather weak interaction of this protein with phosphatidic acid and phosphatidylglycerol. These observations are discussed in the light of the role of PDC-109 in cholesterol efflux from sperm plasma membranes, which is a necessary event before capacitation, and subsequently fertilization, can take place.

IN mammals fertilization takes place by the interaction of spermatozoa from the male with the egg in the female uterus. The spermatozoa do not possess fertilizing capacity at the time of ejaculation, but acquire it during their transit through the female genital tract by a process known as capacitation. Studies on different mammalian species suggest that seminal plasma contains specific proteinaceous factors that influence the fertilizing ability of spermatozoa[1,2]. Considerable amount of work has been done during the last two decades on a group of acidic proteins present in the bovine seminal plasma which bind to sperm plasma membranes and appear to play a crucial role in the capacitation process. Collectively, these proteins have been termed 'bovine seminal plasma proteins', or in short, BSP proteins[3,4]. In order to understand the role of these proteins in the capacitation process better, it is necessary to study their interaction with model membranes and sperm plasma membranes. Our laboratory has been investigating the interaction of the major BSP protein, PDC-109 with phospholipid membranes by physical methods[5–7]. In this article, our studies on the interaction of PDC-109 with model membranes have been reviewed, together with the relevant studies from other groups on the interaction of the BSP proteins with model membranes and sperm plasma membranes.

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BSP proteins

Purification and primary structure determination of PDC-109 have been reported two decades ago[8]. It is a polypeptide of 109 amino acids and contains two tandemly repeating fibronectin type-II (Fn2) domains, preceded by a 23-residue *N*-terminal domain (Figure 1). This protein is present at about 15–25 mg/ml concentration in the seminal plasma[9]. Manjunath and coworkers[3,4] reported the purification and biochemical characterization of four gelatin-binding proteins and designated them as BSP-A1, BSP-A2, BSP-A3 and BSP30-kDa. BSP-A1 and BSP-A2 are identical in amino acid composition and differ only in glycosylation. BSP-A1 is a glycoprotein with a single *O*-linked oligosaccharide [Neu*N*Acα(2-3)Galβ(1-3)Gal*N*Ac-] attached to Thr-11, whereas BSP-A2 is not glycosylated[10,11]. Further, the amino acid composition of these two proteins is identical to that of PDC-109 and based on

this observation it was concluded that BSP-A1 and -A2 are identical to PDC-109. Therefore, a mixture of BSP-A1 and BSP-A2 is also referred to as PDC-109. BSP-A3 is homologous to PDC-109, with ca. 70% sequence identity and also contains two tandem Fn2 domains[12].

Modification of the lysine side chain amino groups of PDC-109 by citraconic anhydride, followed by tryptic digestion under controlled conditions yielded the intact second fibronectin type 2 domain, which was referred to as PDC-109 domain b (PDC-109/b) (ref. 13). The structure of this domain has been determined in solution by ^{1}H-NMR spectroscopy[14,15]. Binding of a series of putative ligands was investigated by monitoring changes induced in the chemical shifts of different resonances. Although the receptor for the molecule on the sperm surface was not known at that time, it is interesting to note that 3,3-dimethylbutylamine induced significant changes in the ^{1}H chemical shifts of several resonances, including Tyr-75 (Tyr-7 of the domain B), which was later shown by single-crystal X-ray diffraction studies to be involved in a H-bond with phosphorylcholine[15,16].

The receptor for BSP proteins on sperm plasma membrane is a lipid

It has been shown by Manjunath and colleagues[17] in 1988 that the BSP proteins bind to the plasma membrane of spermatozoa. Around the same time Scheit and coworkers[18] also reported that the major protein of the bovine seminal plasma, PDC-109 binds to the surface of the spermatozoa. Freshly ejaculated and washed spermatozoa bind approximately 9.3×10^6 molecules of PDC-109 per cell[10]. The binding sites were found to be resistant to protease treatment and heat-stable, but could be extracted with organic solvents. The extracted material when coated on plastic microtitration plates bound radiolabeled BSP proteins, leading to the conclusion that the receptor for PDC-109 on spermatozoa is a lipid rather than a protein[19]. The specificity of binding was subsequently investigated by examining the binding of BSP proteins to a variety of lipids. These studies revealed that BSP-A1, -A2 and -A3 proteins specifically recognized phospholipids containing the phosphorylcholine group, such as phosphatidylcholine (PC), sphingomyelin (SM), lyso-PC, PC plasmalogen, platelet activating factor (PAF), and lyso-PAF. BSP-30-kDa protein, on the other hand, recognized phosphorylcholine containing lipids with greater affinity, but also interacted with phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol, phosphatidic acid and cardiolipin[19]. Consistent with this specificity, BSP proteins could be purified by affinity chromatography on *p*-aminophenyl phosphorylcholine coupled to sepharose, or quaternary methylamine coupled to silica particles, or DEAE-sephadex and eluted by the specific ligand, phosphorylcholine. Equilibrium dialysis studies performed at 4°C to investigate the binding of choline yielded a stoichiometry of 1.8 binding sites per PDC-109 monomer, and a binding constant of 0.95 mM[20].

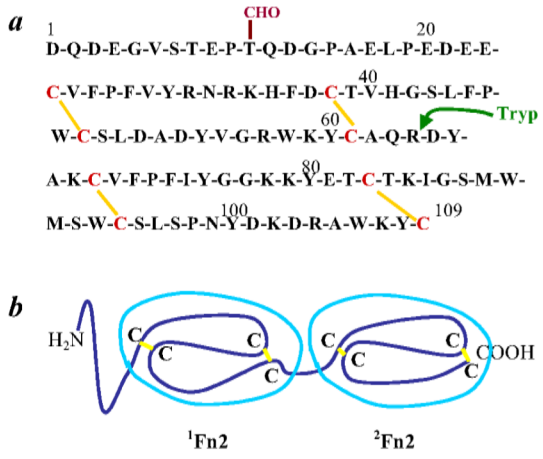

Figure 1. Primary structure and domain organization of PDC-109. ***a***, The primary structure of PDC-109 (ref. 8). The cysteine residues are shown in red and the disulphide bonds are indicated by yellow lines connecting them. CHO denotes the *O*-linked carbohydrate attached to Thr-11. As shown by the green arrow, when the lysine residues are modified by citraconylation, trypsin cleaves at the C-terminal side of Arg-64 to yield the ^{2}Fn2 domain (also referred to as PDC-109/b (ref. 13). The 3-dimensional structure of this domain was determined in solution by NMR spectroscopy[15] (see Figure 2 *d*). ***b***, The domain organization of PDC-109. The polypeptide chain is shown as a dark blue line and the disulphide bonds are shown as yellow lines connecting the cysteine residues. The fibronectin type-II (Fn2) domains are circled in cyan. The two Fn2 domains (^{1}Fn2 and ^{2}Fn2) are very similar, but not identical.

The above experiments suggested that binding of PDC-109 with phospholipid membranes takes place by the specific interaction of the protein with the phosphorylcholine head group of choline phospholipids and led to further studies aimed at investigating the effect of phosphorylcholine binding on the macromolecular properties of this protein[21]. Gel filtration experiments showed that PDC-109 exists as a polydisperse aggregate in solution, which upon binding of *O*-phosphorylcholine dissociates into dimers. Differential scanning calorimetric studies demonstrated that binding of phosphorylcholine increases the thermal unfolding temperature of the protein by ca. 13 degrees, suggesting that the protein structure is stabilized by ligand binding. Fourier-transform infrared spectral studies on native PDC-109 and on its complex with *O*-phosphorylcholine revealed that binding of the soluble phosphorylcholine molecule results in a small increase in the turn content, with a proportional decrease in the unordered structure, which is consistent with the above calorimetric studies[21].

The most direct evidence for the specificity of PDC-109 towards the phosphorylcholine moiety came from the single crystal X-ray diffraction studies[16]. These studies showed that each of the two Fn2 domains bind one phosphorylcholine molecule with both the binding sites located

on the same face of the molecule. Binding of phosphorylcholine to the Fn2 domains involves a cation–π interaction between the quaternary ammonium group of the ligand and a core tryptophan ring of the polypeptide (Figure 2 *a–c*). Additional stabilization comes from hydrogen bonding interaction of the hydroxyl groups of tyrosine side chains or main chain amide groups with the phosphate groups. The loop comprising residues 41–44 of the ^{1}Fn2 domain, which is extended away from the core of the protein in the unbound structure[15], packs closer to the binding site and interacts with the ligand (Figure 2 *d*). This conformational change most likely triggers the dissociation of the polydisperse aggregate form of PDC-109, resulting in the formation of dimers in which all the four choline-binding sites lie on the same face of the protein. Such an orientation in which the four choline-binding sites of the PDC-109 dimer are all on the same face of the protein is consistent with the binding and optimal interaction of this protein with choline lipid-rich membranes[5–7,22,23]. Since choline phospholipids comprise 60–70% of the total phospholipids of bull sperm plasma membrane[24,25], the specific interaction of PDC-109 with the phosphorylcholine moiety of these lipids seems to be an effective means of coating the sperm plasma membrane with this protein, which leads to the efflux of cholesterol and choline phospholipids.

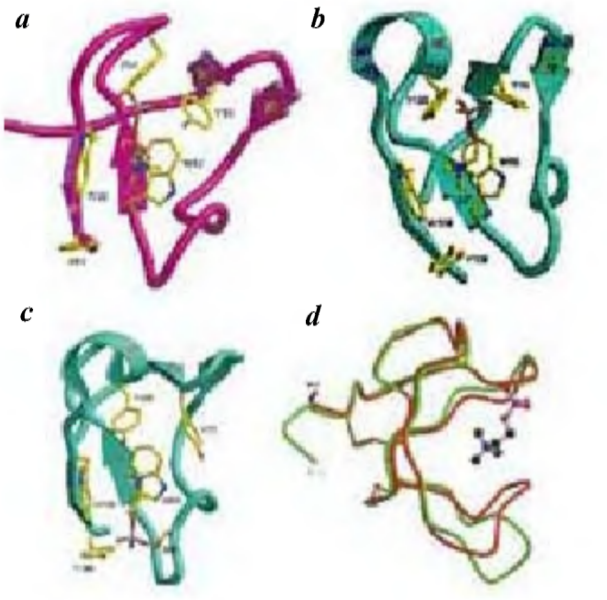

Figure 2. Binding of phosphorylcholine by the Fn2 domains of PDC-109. ***a***, Structure of ^{1}Fn2 domain with bound phosphorylcholine. The binding orientation is identical for both the PDC-109 monomers (***a*** and ***b***), present in the asymmetric unit. ***b***, Structure of the ^{2}Fn2 domain of monomer ***a*** with bound phosphorylcholine. ***c***, Structure of the ^{2}Fn2 domain of monomer ***b***. ***d***, Structure of the ^{1}Fn2 domain with bound phosphorylcholine (red) and without ligand (green). Note that in the phosphorylcholine-bound structure the loop at the lower right corner has moved closer to the ligand (shown as a ball-and-stick model). Parts ***a***, ***b***, and ***c*** were reproduced from Figure 3 of ref. 16 and part D was reproduced from Figure 4 *a* of ref. 16, with permission from Elsevier. Copyright (2002) by Elsevier Science Ltd.

The X-ray structure shows that although the four sites of the two PDC-109 molecules in the asymmetric unit bind phosphorylcholine, the binding orientation of all the four phosphorylcholine molecules is not identical[16]. In ^{1}Fn2 of monomers A and B of PDC-109 the quaternary ammonium cation interacts with the indole side chain of Trp-47 whilst the phosphate forms hydrogen bonds with Tyr-30 and Tyr-54 (Figure 2 *a*). The binding of phosphorylcholine to the ^{2}Fn2 domains of monomers A and B differs from one another, and also differs from its binding to ^{1}Fn2 domains. In the ^{2}Fn2 domain of monomer A, the trimethylammonium group interacts with the side chain of the outer Trp-106 residue (rather than with the core Trp-93 residue, as is the case in its binding to the ^{1}Fn2 domain), whereas the phosphate group forms hydrogen bonds with the side chains of Tyr-75 and Tyr-100 (Figure 2 *b*). Finally, in the binding of phosphorylcholine to the ^{2}Fn2 domain of monomer B, the positions of the quaternary ammonium group and phosphate are reversed as compared to its orientation in the binding pocket of the ^{2}Fn2 domain of monomer A. Due to this, in its binding to the ^{2}Fn2 domain of monomer B the quaternary ammonium group interacts with the core Trp-93, and the phosphate group forms a hydrogen bond with the side chain of Tyr-108, as well as with the main chain amides of Met-89, Trp-90, and Met-91 (Figure 2 *c*)[16].

Interaction of PDC-109 with phospholipid membranes

Although the BSP proteins have been implicated in sperm capacitation, ultrastructural and biochemical studies have suggested that spermatozoa undergoing capacitation exhibit a membrane remodeling process[17–19], the molecular events of this process were not well understood. In order to understand the molecular details of lipid binding by the BSP proteins and to investigate the role of their binding in sperm capacitation and fertilization process, biophysical and spectroscopic studies have been carried out on the interaction of PDC-109 with lipid membranes.

Monitoring the fluorescence properties of the protein, it has been shown that maximal binding of PDC-109 to phosphatidylcholine SUV occurred with a lipid/protein ratio of 10–11 PC molecules/PDC-109 molecule[22]. Incorporation of PE or PS into PC vesicles decreased binding, indicating that density of phosphorylcholine groups is an important factor for the binding to take place. Stopped-flow fluorescence studies have shown that binding of PDC-109 to PC vesicles (SUV) is a very rapid, biphasic process with half times of less than one second. In additional experiments these authors have incorporated 1-palmitoyl 2-(4-doxylpentanoyl)-PC and -PE into PC vesicles and obtained ESR spectra in the absence and in the presence of PDC-109. Spectra obtained at 4°C in the presence of the protein consisted of two components – a rigid

component and a fluid component – which could be resolved by spectral subtraction. On the other hand, spectra obtained from PC vesicles alone contained only the fluid component, demonstrating that binding of PDC-109 to phosphatidylcholine vesicles led to a rigidification of the membrane. FTIR studies on PDC-109 bound to phosphatidylcholine membranes[23] showed that the solvent exposed loop content increases by 5–6%.

Studies from our laboratory have shown that binding of PDC-109 to dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles resulted in a steep decrease in the sample turbidity, suggesting that binding led to a decrease in the size of the lipid assemblies[5]. Gel filtration on Sepharose CL6B yielded the apparent mass of particles obtained when PDC-109 was bound to DMPC multilamellar vesicles as ca. 1.3×10^6 Daltons. The lipid efflux particles obtained from human fibroblasts (a cell model to study the lipid efflux) upon binding of PDC-109 were characterized to be ~80 nm in diameter[26]. The mass of 1.3×10^6 Da, estimated for the lipid particles, is consistent with the particle size and suggests that the efflux of choline phospholipids and cholesterol from sperm plasma membrane by PDC-109 is mediated by the direct interaction of the protein with the membrane lipids[5].

When the DMPC vesicles contained 25 mol% cholesterol the decrease in turbidity was less steep, indicating that cholesterol provides a partial stabilization of the liposomes from the PDC-109-induced solubilization[5]. These results are qualitatively consistent with the earlier observations[23] that the leakage of internal contents from dioleoylphosphatidylcholine (DOPC) unilamellar vesicles, induced by PDC-109, is considerably reduced in the presence of cholesterol. However, it must be borne in mind that these two experiments are rather different in nature.

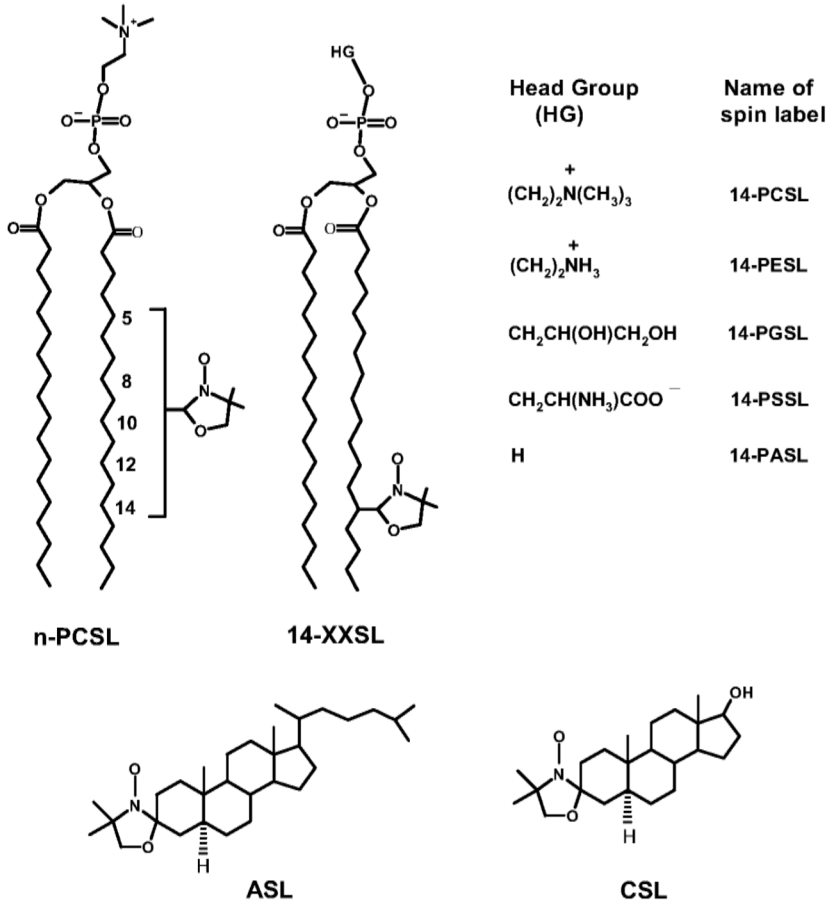

Figure 3. Structures of spin labels used in the ESR studies. In the phosphatidylcholine spin labels, *n*-PCSL, the nitroxide moiety is attached at C-5, C-8, C-10, C-12, or C-14 of the *sn*-2 acyl chain. All the phospholipid spin labels, 14-XXSL, have the nitroxide spin label attached to the C-14 of the *sn*-2 acyl chain, but have different head group structures. These are: 14-PCSL (phosphatidylcholine), 14-PESL (phosphatidylethanolamine), 14-PGSL (phosphatidylglycerol), 14-PSSL (phosphatidylserine) and 14-PASL (phosphatidic acid). The *sn*-2 acyl chain is stearoyl as indicated, but the *sn*-1 acyl chain (from egg lecithin) is predominantly palmitoyl and stearoyl. The sterol-based spin labels are: androstanol spin label (ASL) and cholestane spin label (CSL).

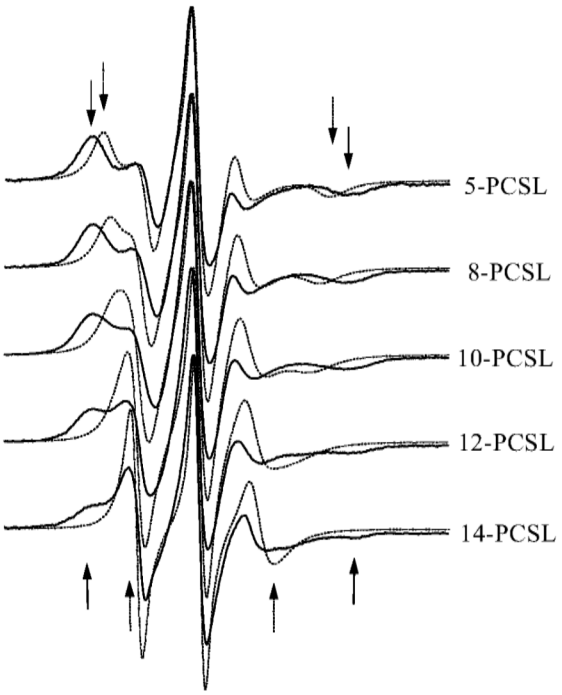

Figure 4. ESR spectra of phosphatidylcholine spin labels, *n*-PCSL, in DMPC membranes and in DMPC/PDC-109 recombinants. Dotted lines correspond to spectra recorded from DMPC membranes alone, and solid lines correspond to the spectra recorded from DMPC membranes in the presence of PDC-109 (lipid : protein ratio, 1 : 2 w/w). Arrows indicate the outer hyperfone splitting, $2A_{max}$. The two components in the spectrum of 14-PCSL obtained in the presence of PDC-109 could be resolved by spectral subtraction. The spectral width is 100 Gauss (Figure taken from ref. 5, Copyright (2001) by the Biophysical Society).

The experiments of Gasset *et al.*[23] involve permeability/leakage of contents from small, 90 nm diameter unilamellar vesicles of DOPC, whereas the binding experiments reported by us correspond to the fragmentation of large multilamellar vesicles of DMPC[5].

The interaction of PDC-109 with DMPC membranes has been investigated by us in considerable detail by spin-label ESR spectroscopy[5]. The spin-label probes employed in these studies include: (i) a series of spin-labeled phosphatidylcholines, *n*-PCSL, bearing the doxyl moiety at C-5, C-8, C-10, C-12 and C-14 of the *sn*-2 acyl chain, (ii) a series of phospholipid spin labels, 14-XXSL bearing the doxyl moiety on the C-14 of the *sn*-2 acyl chain but differing in the head group structure, and (iii) two sterol-based spin labels, androstanol spin label (ASL) and cholestane spin label (CSL) (see Figure 3). Other phospholipid spin labels used are 14-NAPESL, which has the doxyl moiety attached to the C-14 of the *N*-acyl chain of dipalmitoyl(*N*-stearoyl)phosphatidylethanolamine, and 14-SMSL, which is a sphingomyelin derivative with a 14-doxylstearoyl chain acylated to the nitrogen atom of the sphingoid base.

In our ESR studies, the spin labels were incorporated into DMPC membranes at ca. 1 mol% concentration and a 2 : 1 (w/w) protein/lipid ratio was used (which corresponds to ca. 9.4 lipids/PDC-109 monomer). The ESR spectra of all the phosphatidylcholine spin-labels were perturbed by PDC-109, and seemed to consist of two components (Figure 4), suggesting that the protein or a part of it inserts into membrane interior and interacts directly with the lipid acyl chains. Further, the two components in the spectra of 14-PCSL obtained from the PDC-109/DMPC recombinants could be resolved by spectral subtraction. One of the two resolved components resembled the spectrum of the spin label in DMPC membranes in the fluid phase. The second component was characterized by much larger values of the outer hyperfine splitting ($2A_{max}$) and is similar to the spectra of spin label that is directly in contact with integral membrane proteins[27]. Extent of quenching of the protein intrinsic fluorescence by acrylamide and iodide decreased when PDC-109 was bound to DMPC vesicles, which is consistent with a partial penetration of the protein into the hydrophobic interior of the lipid membrane, resulting in it being shielded from the quenchers present in the aqueous medium (V. Anbazhagan and M. J. Swamy, unpublished observations).

The $2A_{max}$ values of 5-PCSL in DMPC membranes alone exhibited a sharp decrease at ca. 23°C, corresponding to the chain-melting phase transition of the lipid. On the other hand, in the presence of saturating concentrations of PDC-109 the $2A_{max}$ values of this spin label exhibited a gradual decrease with increase in temperature, suggesting that protein binding at saturation strongly perturbed the cooperativity of the lipid chain-melting phase transition. Further, the $2A_{max}$ values obtained in the presence of the protein at different temperatures were larger than those obtained with DMPC membranes alone, clearly demonstrating that protein interacts with the lipid molecules in both the gel and fluid phases and reduces the lipid mobility.

Müller and coworkers[28] investigated the binding of PDC-109 to PC membranes as well as to the plasma membranes of intact spermatozoa by ESR spectroscopy, employing spin-labeled phosphatidylcholines and phosphatidylethanolamines. It has been found that protein binding resulted in an immobilization of the lipids in both the gel and fluid phases. The immobilizing effect was larger in the fluid phase than in the gel phase. Additionally, the immobilization was also seen when PDC-109 was bound to the plasma membranes of bovine epididymal spermatozoa[28]. Larger immobilization was observed for phosphatidylcholine spin labels than phosphatidylethanolamine spin labels, consistent with the head group specificity of the protein[5,19,28].

Lipid selectivity of PDC-109 in membrane bilayers

The selectivity of PDC-109 towards different phospholipids and cholesterol was investigated by ESR spectro-

scopy, employing phospholipid and sterol spin labels[5]. When different phospholipids, spin labeled on C-14 of *sn*-2 acyl chain, or sterol probes, CSL and ASL (see Figure 3) were incorporated into DMPC host matrix, the relative fractions of the two spin label populations in the two-component spectra obtained when PDC-109 was bound would reflect the selectivity of the protein for the different lipids according to the expression[29]:

$$K_r/K_r^{PC} = (1/f_{PC} - 1)/(1/f - 1), \quad (1)$$

where K_r is the association constant for the spin-labeled lipid and K_r^{PC} is the association constant for DMPC (the host lipid), f_{PC} is the fraction of the motionally restricted 14-PCSL and f is the fraction of the motionally restricted spin-labeled lipid.

Based on the above analysis, the selectivity of PDC-109 was found to be in the following order: phosphatidic acid dianion (pH 8.5) > phosphatidylcholine ≈ sphingomyelin ≥ phosphatidic acid monoanion (pH 6.0) > phosphatidylglycerol ≈ phosphatidylserine ≈ androstanol > phosphatidylethanolamine ≥ *N*-acyl phosphatidylethanolamine ≫ cholestane. Although the highest selectivity is seen for phosphatidic acid dianion, the physiologically irrelevant pH of 8.5 where it is seen and the fact that phosphatidic acid is normally present in membranes at very low concentrations suggest that this is not the natural receptor for PDC-109. The selectivity of this protein is the highest for the phosphocholine-containing lipids, namely PC and SM among the remaining lipids. This is consistent with the selective binding of phosphorylcholine by PDC-109 as discussed above. Further, among the two sterol derivatives, PDC-109 exhibits a significant selectivity towards ASL, which is a sterol analog that contains the 17β-OH group attached to the steroid nucleus, but lacks the alkyl chain, which is replaced by the doxyl moiety. CSL, in which the doxyl moiety replaces the 3β-OH group of cholesterol is very poorly recognized by this protein, suggesting that the hydroxy group of cholesterol is critical for its recognition by PDC-109.

Effect of cholesterol on PDC-109/membrane interaction

Because binding of PDC-109 to the plasma membrane of spermatozoa and other cells such as fibroblasts leads to an efflux of choline phospholipids and cholesterol, it is of considerable interest to investigate the effect of cholesterol on the binding of this protein to lipid membranes in general and on the lipid selectivity pattern in particular. ESR studies employing different phospholipid and sterol spin label probes on the interaction of PDC-109 with membranes containing DMPC and cholesterol, performed essentially as described above, indicated that while the relative selectivity for different lipids was not significantly affected by cholesterol, its presence increased the association of different phospholipid and sterol probes with the protein[6]. In another study, it was shown that PDC-109 does not directly interact with cholesterol, but in the presence of phospholipids its mobility was considerably reduced. The fraction of immobilized cholesterol was shown to be highest in the presence of phosphatidylcholine, consistent with a preferential interaction of PDC-109 with phosphatidylcholine[30].

Mechanism of membrane binding by PDC-109

The kinetics of PDC-109 binding to phosphatidylcholine small unilamellar vesicles was investigated by stopped-flow fluorescence spectroscopy at 30°C by monitoring the changes in the fluorescence intensity of PDC-109 (ref. 22). The stopped-flow trace could be fit to a biexponential process. However, as very limited measurements were carried out, the molecular origin of the two exponentials could not be identified.

Extensive kinetic studies have been performed on the binding of PDC-109 to various phospholipids by the surface plasmon resonance (SPR) technique[6]. When PDC-109 was allowed to bind to hybrid bilayers made up of a DMPC layer coated on the alkanethiol derivatized sensor chip, it was observed that the response showed a gradual decay after attaining a high value, suggesting that protein binding led to removal of the lipid from the sensor chip. Addition of cholesterol to DMPC at 20% (w/w) resulted in stable response curves, indicating that cholesterol stabilized the DMPC membrane from PDC-109-induced leaching from the surface of the sensor chip. Further experiments were therefore carried out with membranes of DMPC, dimyristoylphosphatidylglycerol (DMPG), dimyristoylphosphatidic acid (DMPA), and dipalmitoylphosphatidylethanolamine (DPPE), all containing 20% (w/w) cholesterol. Sensograms depicting the binding of PDC-109 to the hybrid bilayers made by coating these lipids on the alkanethiol derivatized sensor chip, are shown in Figure 5. It is seen that while binding of PDC-109 to DMPC yielded a large response, indicating good binding of the protein to DMPC membranes, binding of PDC-109 to membranes of DMPG and DMPA, is associated with considerably smaller changes in the instrument response, suggesting much weaker binding of these two lipids. Binding to DPPE gave a very low response and the data were not sufficiently reliable for determining the association and dissociation rate constants from the response curve.

The SPR data could be analysed satisfactorily by a single exponential fit, suggesting that both the association and dissociation processes take place by a single step mechanism for all the lipids studied. The association (k_1) and dissociation (k_{-1}) rate constants for the binding of PDC-109 to DMPC at 20°C were determined to be 5.7 ×

$10^5\ M^{-1}\ s^{-1}$ and $2.7 \times 10^{-2}\ s^{-1}$, respectively. From these values the K_a value was calculated to be $2.1 \times 10^5\ M^{-1}$. The k_1 values obtained at different temperatures for the binding of PDC-109 with DMPC membranes were higher than those for its interaction with DMPA and DMPG by about 3 orders of magnitude, while the dissociation rate constants (k_{-1}) obtained with the latter two lipids were 3–4 times larger than the k_{-1} values obtained with DMPC. Thus the higher affinity of PDC-109 for choline phospholipids is reflected in a faster association rate constant and a slower dissociation rate constant for DMPC as compared to the other phospholipids. The experiments with dimyristoylphosphatidylethanolamine (DMPE) and its higher homologue, dipalmitoylphosphatidylethanolamine (DPPE) yielded very low response, indicating that the interaction of phosphatidylethanolamine with PDC-109 is very weak. These results indicate that the association constants for the interaction PDC-109 with different phospholipids are in the following order: DMPC > DMPG > DMPA > DMPE/DPPE. Further, although all the phospholipids contained 20% (w/w) cholesterol, binding of PDC-109 to membranes made up of phospholipids other than PC was moderate or weak, suggesting that this protein does not interact directly with cholesterol. It is likely that the initial binding takes place by the interaction of PDC-109 with the phosphocholine moiety of PC, which leads to a conformational change in the protein, after which the protein aquires the ability to recognize cholesterol. Alternately, PDC-109 may not interact directly with cholesterol, but their interaction could be mediated by the binding of the protein to PC, which in turn interacts with cholesterol. The ESR results of Müller *et al.*[30] are in agreement with this model. Further, it has been shown that phosphatidylcholine and cholesterol associate in the membrane bilayer, most likely via a hydrogen bond between the 3β-hydroxyl of cholesterol and the sn-2 carbonyl of the phospholipid[31]. More recent studies show that cholesterol interacts with PC and other phospholipids in membranes and forms condensed complexes[32–34]. Thus, PDC-109 may influence the mobility of cholesterol through its interaction with PC.

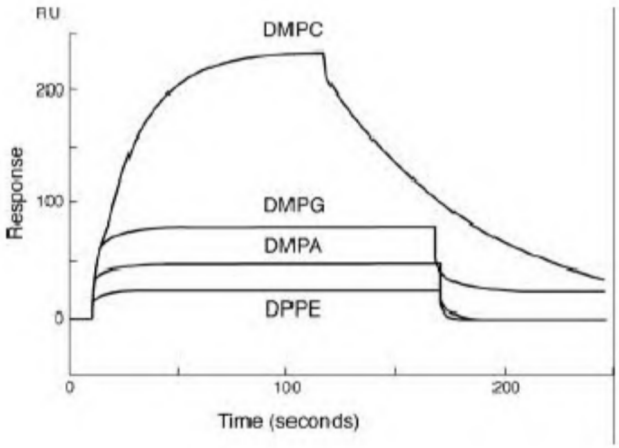

Figure 5. SPR sensograms depicting the binding of PDC-109 to different phospholipid membranes. The figure depicts the instrument response as a function of time when PDC-109 was passed over different lipid monolayers (DMPC, DMPG, DMPA, and DPPE), containing 20% (wt/wt) cholesterol at 20°C, coated on the alkanethiol-derivatized sensor chip. The concentrations of PDC-109 used were 0.05, 20, 100 and 150 μM, respectively, for DMPC, DMPG, DMPA, and DPPE. It can be seen that the response is quite large when the protein is passed over DMPC membranes, but significantly less when it is passed over other phospholipids, indicating the high specificity of PDC-109 for phosphatidylcholine (Figure taken from ref. 7, Copyright (2003) by the Biophysical Society).

A comparison of the activation parameters for the binding of PDC-109 to DMPC, DMPG and DMPA (Table 1) revealed the underlying thermodynamic factors that favour the strong binding of PDC-109 to DMPC as compared to DMPG and DMPA[7]. Although the association of PDC-109 to DMPG and DMPA was characterized by smaller values of activation enthalpy as compared to DMPC, the binding to the latter lipid was associated with a positive activation entropy, whereas the binding to the former two lipids was associated with relatively large negative values of activation entropy. This suggested that the interaction of PDC-109 with the different phospholipids was discriminated by disparate changes in the activation entropies – being favourable for PC and unfavourable for other phospholipids that lack the choline moiety in the head group structure[7]. It is likely that the positive change in entropy associated with the binding of PDC-109 to PC membranes may arise due to the disaggregation of the protein, which exists as a polydisperse aggregate in the absence of the ligand[21].

While the SPR studies indicate that the binding of PDC-109 to phospholipid membranes takes place by a single exponential process, the stopped flow fluorescence experiments could only be fit to a biexponential process. This discrepancy could be explained by the fact that in SPR

Table 1. Activation parameters and thermodynamic parameters obtained from them for the interaction of PDC-109 with phospholipid/cholesterol membranes. Units are: $\Delta H_a^{\ddagger}$, $\Delta H_d^{\ddagger}$, ΔH^o, $\Delta G_a^{\ddagger}$, $\Delta G_d^{\ddagger}$ and ΔG^o: kJ mol^{-1}; $\Delta S_a^{\ddagger}$, $\Delta S_d^{\ddagger}$ and ΔS^o : J mol^{-1} K^{-1}

	Lipid		
Parameter	DMPC	DMPA	DMPG
$\Delta H_a^{\ddagger}$	50.11	11.32	16.48
$\Delta H_d^{\ddagger}$	43.03	41.64	36.74
ΔH^o	7.08	–30.32	–20.26
$\Delta S_a^{\ddagger}$	36.36	–166.28	–136.39
$\Delta S_a^{\ddagger}$	–128.0	–121.06	–139.46
ΔS^o	164.36	–45.22	3.07
$\Delta G_a^{\ddagger}$	39.45	60.07	56.46
$\Delta G_d^{\ddagger}$	80.55	77.13	77.62
ΔG^o	–41.10	–17.06	–21.16

only the mass change is detected as a function of time, due to which subtle changes occurring in the protein conformation after the initial binding event (which most likely do not involve changes in the mass) may not be detected by this method. Consistent with this, FTIR studies have shown that PDC-109 undergoes a conformational change upon binding to PC membranes[23].

Future outlook

Although the studies reviewed here have shed much light on the interaction of PDC-109 with model membranes and with the plasma membrane of the spermatozoa, the mechanism by which cholesterol efflux takes place when this protein binds to the sperm plasma membrane is not yet clear. Further studies are therefore necessary to understand this process better. It is especially important to determine the structure of PDC-109 in the membrane bound state, which is likely to yield further insights into the molecular events leading to cholesterol efflux. Recent studies have shown that PDC-109 also binds L-fucose, D-fructose and D-mannose as well as the Le^a trisaccharide, which contains a terminal L-fucose moiety[35–37]. It has been further demonstrated that by its binding to fucose-containing glycoconjugates on the surface of oviductal epithelial cells PDC-109 enables spermatozoa to bind to oviductal epithelium and plays a major role in formation of the bovine oviductal sperm reservoir[38]. In the light of these observations, detailed biophysical and structural characterization of the binding of different saccharides, especially the Le^a trisaccharide, to PDC-109 will be necessary to understand the formation of oviductal sperm reservoir. Finally, additional studies are also required to understand the molecular events that are involved in the release of the spermatozoa attached to the oviductal mucosa, which is necessary before the sperm cell can fertilize the egg.

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