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Type II Domains of BSP-A1/-A2 Proteins: Binding Properties, Lipid Efflux, and Sperm Capacitation Potential

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Bovine seminal plasma contains a family of major proteins (collectively called [1]BSP proteins) that potentiate sperm capacitation by binding to capacitation factors such as heparin and by stimulating sperm membrane cholesterol efflux. Here, we investigated the structure-function relationship of type II domains of BSP proteins. We isolated from a tryptic digest of citraconylated BSP-A1/-A2 proteins the intact second type II domain (domain b or Db). Similar to native protein, Db bound to heparin-Sepharose, p-aminophenylphosphorylcholine-Agarose and liposomes containing phosphatidylcholine. When assessed for biological function, Db did not stimulate cholesterol efflux from human fibroblasts, a cell model for lipid efflux studies, and from bovine spermatozoa, or potentiate bovine sperm capacitation induced by heparin and high-density lipoproteins. Therefore, type II motifs of BSP proteins represent binding units for sperm membrane choline phospholipids and heparin but the second type II domain of BSP-A1/-A2 alone is not sufficient to stimulate lipid efflux nor is sufficient to potentiate bovine sperm capacitation. Thus, the presence of both type II domains in BSP proteins is essential for the expression of functional properties, namely lipid efflux and sperm capacitation.

Key Words: **phospholipid-binding proteins; heparin; type II structure; lipid efflux; sperm capacitation.**

A number of proteins are mosaics assembled from a variety of module-types defining structural elements [1, 2]. One type of module, designated type II structure, is characterized by a double cystine polypeptide loop pattern distinguished by disulfide bridges connecting Cys residue pairs 1-3 and 2-4. As shown in Table 1, they are found as a single unit, as two collinear domains or as three head-to-tail repeats in numerous proteins. The type II domains of fibronectin are collagen-binding sites [12]. In gelatinases A and B, the three tandem type II repeats contribute to form a single collagen-binding site [20]. Unfortunately, the functional role of this module in other proteins is unknown. The observed homology of amino acids and the gene organization of fibronectin, the two type II repeats are present on exons separate from the other portions of the gene, suggests that this domain has been incorporated into several proteins as a result of exon shuffling [2]. However, substitutions of amino acid side chains on the protein surface appear to lead to distinct binding specificity and different roles. Furthermore, the type II structures have been reported to be related to protease Kringle-modules [1, 21], the latter corresponding to autonomous structural/functional units abundantly present in blood clotting and fibrinolytic proteins [22, 23]. Kringle units are also found in lipoprotein (a), Lp(a) [24].

Among the type II motif containing proteins, BSP-A1/-A2 (also called PDC-109 [17]), BSP-A3 and BSP-30-kDa (collectively called BSP proteins) represent a family of closely related proteins found in bovine seminal fluid [25]. They are secretory products of the seminal vesicles and constitute the major protein fraction of bovine seminal plasma (>60%). With the exception of BSP-A3, all members of this family are glycoproteins. Each protein contains two similar type II domains arranged in tandem fashion [17–19] and a N-terminal portion that is variable between each protein. BSP homologous proteins have been isolated from stallion (HSP-1 and HSP-2; [14]) and boar (pB1; [15, 16]) seminal plasma.

The BSP proteins exhibit multiple binding and functional properties. Upon ejaculation, the BSP proteins

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Abbreviations: DMEM, Dulbecco's modified Eagle medium; mTALP, Tyrode's medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; BSP, bovine seminal plasma; BSA, bovine serum albumine; Db, the second type II domain of BSP-A1/-A2; HDL, high-density lipoprotein; lyso-PC, lysophosphatidylcholine; PPC, p-aminophenylphosphorylcholine; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis.

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TABLE 1

Proteins Containing Type II Structures

Protein	Number of type II domains	Function	References
Factor XII	1	?	[3]
IGF-IIR/CIMan6PR	1	?	[4–6]
PLA_2 receptor	1	?	[7]
ManR	1	?	[8]
HGFA	1	?	[9]
DEC-205R	1	?	[10]
Fibronectin	2	Collagen binding	[11–13]
HSP-1, HSP-2	2	?	[14]
pB1/LAR	2	?	[15, 16]
BSP-A1/-A2	2	Choline binding	[17]
BSP-A3	2	Choline binding	[17]
BSP-30-kDa	2	Choline binding	[19]
Gelatinase A	3	Collagen binding	[20]
Gelatinase B	3	Collagen binding	[20]

Note. Factor XII, human coagulation factor XII; IGF-II, insulin-like growth factor II; CIMan6PR, cation-independent mannose-6-phosphate receptor; PLA_2, phospholipase A_2; ManR, mannose receptor; HGFA, hepatocyte growth factor activator; DEC-205R, dendritic cell receptor, HSP, horse seminal plasma; pB1, porcine heparin-binding protein; LAR, leukocyte adhesion regulator; BSP, bovine seminal plasma.

bind to choline phospholipids of the sperm surface [26, 27]. By equilibrium dialysis, we showed that BSP-A1/-A2 proteins contain two choline binding sites per mole of protein [28]. Our studies also indicate a structure-dependent hydrophobic interaction of the ligand with BSP proteins [28]. In addition to displaying choline-binding activity, BSP proteins bind to sperm capacitation factors namely, heparin [29] and high-density lipoproteins (HDL) [30]. Furthermore, BSP proteins potentiate bovine epididymal sperm capacitation induced by heparin [31] and HDL [32]. The mechanism how whereby BSP proteins capacitate sperm is unknown but they appear to increase binding of heparin to the sperm surface and also participate in removal of cholesterol from the sperm membrane [33], one of the earliest steps believed to occur in capacitation [34]. Our recent results show that the BSP proteins stimulate cholesterol and phospholipid efflux from fibroblasts [35]. This lipid efflux was inhibited by choline suggesting the involvement of choline-binding sites in this process. Although the BSP proteins modulate capacitation induced by heparin and participate in lipid efflux during sperm capacitation, the structural elements and molecular mechanisms involved are unknown.

In this study, we prepared the second type II domain (Db) of BSP-A1/-A2 using limited proteolysis and have studied its binding properties and biological function. The results indicated that type II domains of BSP proteins are binding units for choline phospholipids and heparin. However, the domain b (Db) alone is not sufficient to stimulate lipid efflux from human fibroblasts and bovine epididymal spermatozoa, or potentiate heparin/HDL-mediated bovine sperm capacitation.

EXPERIMENTAL PROCEDURES

Materials. Dulbecco's modified Eagle medium (DMEM), Fetal bovine serum (FBS), penicillin-streptomycin solution (10,000 units/ml and 10,000 μg/ml respectively), L-glutamine, trypsin (porcine), phosphate-buffered saline (PBS) and plastic culture dishes (100 mm diameter, Nunc) were from Gibco. Twelve-well culture plates were from Fisher. [1,2-^{3}H]-cholesterol (50 Ci/mmole) were from Dupont NEN. Fatty acid-poor bovine serum albumin (BSA), heparin, choline chloride, lysophosphatidylcholine (lyso-PC), citraconic acid and cholesterol were from Sigma (St. Louis, MO). Phosphatidylcholine was from Serdary Research Laboratories (NJ). p-Aminophenylphosphorylcholine immobilized on Agarose (PPC-Agarose) was from Pierce Chemical Company (Rockford, IL). Heparin-Sepharose and Sephadex G-50 were from Pharmacia Biotech. The liquid scintillation mixture (UniverSol) was obtained from ICN Biomedicals. All other chemicals used were of analytical grade and obtained from commercial suppliers.

Bovine testes were obtained from Abattoir Les Cèdres (St-Lazare, PQ) and semen was obtained from the Centre d'insémination artificiel du Québec (CIAQ, St-Hyacinthe, PQ). BSP-A1/-A2 proteins were isolated as described earlier [25]. High-density lipoproteins (HDL) were isolated from human plasma as described earlier [32].

Domain b (Db) preparation. The preparation of the second type II domain of BSP-A1/-A2 proteins was accomplished following the procedure described by Banyai et al [13]. Briefly, lyophilized BSP-A1/-A2 proteins (50 mg) were citraconylated and digested with 1 mg of trypsin for 15 min at 25 °C. The reaction was stopped with 10% acetic acid and the solution was incubated for 24 h at 37 °C. The decitraconylated protein fragments were purified by gel filtration on a Sephadex G-50 column (2.5 × 92 cm) equilibrated in acetic acid (10%) and the purity was verified by SDS-electrophoresis on a 15% gel.

Affinity chromatography. Chromatographic analysis was performed at room temperature using an FPLC System (Pharmacia Fine Chemicals, Dorval, Québec). Native BSP-A1/-A2 proteins and tryptic fragments corresponding to the second type II domain of BSP-A1/-A2 proteins were solubilized in a Tris-buffered saline (TBS: 10 mM Tris, 150 mM NaCl, pH 8.0) and applied to a PPC-Agarose column (1 × 4 cm) which were previously equilibrated in the same buffer. Bound proteins were eluted with a linear gradient of choline chloride. The Heparin-Sepharose column (1 × 4 cm) was equilibrated in 50 mM phosphate buffer, pH 7.4 and the solubilized BSP-A1/-A2 proteins and Db fragment, in the same buffer, were applied to the column. The elution was performed with a linear gradient of NaCl.

Efflux studies with fibroblasts. The efflux studies with the fibroblasts were performed following the procedure described previously [35]. Human fibroblasts were seeded in 12-well plates for 36 h and incubated with [1,2-^{3}H]cholesterol in 1 ml DMEM (20 μCi/well) containing 5% FBS for 48 hours. The radioactivity released into the incubation medium in the presence of BSP proteins and Db was measured by using a standard liquid scintillation LKB counter. For each well, the radioactivity remaining in cellular extract was measured. The efflux of lipids was expressed as the percentage of the total radioactivity released into the medium.

Cholesterol efflux from spermatozoa. The cholesterol efflux studies with spermatozoa were performed as described previously [33]. Briefly, washed epididymal spermatozoa (500 × 10^6 cells) were radiolabeled for 1 h with [^{3}H]cholesterol (45.5 μCi) in 2.5 ml of Tyrode's medium (mTALP) as described by Langlais et al [34]. Labeled spermatozoa (50 × 10^6 cells) were resuspended in 1 ml mTALP and incubated at 37 °C for 6 h in the presence of BSP-A1/-A2 proteins,

Db fragment or in mTALP (control). The cholesterol efflux was expressed as the percentage of the total radioactivity released into the medium.

Capacitation of spermatozoa. Capacitation of cauda epididymal sperm was evaluated as described previously [31]. Briefly, washed epididymal sperm (25×10^6 cells) were preincubated at 37 °C for 20 min with BSP-A1/-A2 proteins and Db fragment in mTALP. After preincubation, the cells were washed twice ($300 \times$ g, 10 min), resuspended in mTALP containing 12 μg/ml of heparin or 160 μg/ml of HDL, and incubated for 8 h in 11×75-mm culture tubes at 37 °C. The acrosome reaction was induced by adding 100 μg/ml of lyso-PC for 15 min. This concentration of lyso-PC was previously shown to induce the acrosome reaction in capacitated sperm while having no effect on noncapacitated sperm [32]. The lyso-PC-induced acrosome reaction is a well-defined method that has been correlated with the in vitro fertilization rate and validated by electron micrograph studies. The percentage of sperm that were acrosome-reacted was determined on air-dried sperm smears with a naphthol yellow-erythrosin B staining procedure [36].

Analytical methods. Liposomes of cholesterol/PC (30:70%) were prepared as described previously [27]. After 30 min of incubation with proteins, the liposomes were sedimented at $100{,}000 \times g$ for 45 min and the proteins present in the supernatant were precipitated with trichloroacetric acid (TCA). The pellet was incubated with 200 mM choline (15 min) and centrifuged. The BSP proteins released into the supernatant by the addition of choline were precipitated with TCA and analysed by SDS-PAGE. The apparent molecular mass was determined by using the low-molecular weight calibration kit of Pharmacia Biotech (Dorval, PQ, Canada). Amino acid composition and polypeptide sequencing were performed as described by Basak et al [37].

Statistical analysis. Statistical differences were evaluated by the two-tailed Student's t-test. A level of 0.05 was considered as significant.

RESULTS

Isolation and Biochemical Characterization of the Domain b

BSP-A1/-A2 proteins contain two type II structures arranged in a tandem fashion (Fig. 1A). In order to determine the choline- and heparin-binding sites more precisely as well as to define the mechanism of lipid efflux and sperm capacitation mediated by BSP proteins, we prepared a tryptic fragment corresponding to the second type II domain of BSP-A1/-A2 proteins (Fig. 1B) following a reported procedure [13]. Citraconylated protected tryptic digestion yielded an intact fragment of 5 kDa whereas the first domain was cleaved at multiple sites. The amino acid composition and sequence analysis confirmed that the purified fragment corresponded to the second type II domain (domain b or Db) of BSP-A1/-A2 proteins (data not shown).

Ligand-Binding Properties of Domain b

We then compared the binding properties of Db and BSP-A1/-A2 proteins. The Db bound to p-aminophenylphosphorylcholine (PPC)-Agarose column (Fig. 2A). When compared to BSP-A1/-A2, the molar choline required to elute Db from the column was lower (90 mM vs 45 mM). As observed with BSP-A1/-A2 proteins, Db bound to phosphatidylcholine liposomes (data not shown). In a similar manner to BSP-A1/-A2, Db bound to the heparin-Sepharose column (Fig. 2C). The concentration of NaCl required to elute Db was lower than for BSP-A1/-A2 (0.5 M vs 0.25 M). The material that did not bind to either column represented degradation products as judged by SDS-PAGE analysis (data not shown). Furthermore, the Db fragment bound to a gelatin-Agarose column and elution was achieved either with a linear gradient of urea or choline (data not shown).

Cholesterol Efflux Potential of Domain b

Our previous studies indicated that BSP-mediated cholesterol efflux was inhibited by choline suggesting the involvement of BSP type II domains in their cholesterol efflux potential [35]. We thus investigated the functional properties of Db. At concentrations from 9 to 50 μg/ml, the Db fragment failed to stimulate the efflux of cholesterol from human fibroblasts after 3 h of incubation (Table 2). However, under similar conditions, 9 μg/ml of BSP-A1/-A2 proteins stimulated cholesterol efflux by 2.6-fold after 3 h of incubation as previously reported [35]. Longer period of incubation (6 h) in the presence of the Db fragment did not result in cholesterol efflux from fibroblasts (data not shown).

As observed with the fibroblasts, BSP proteins also stimulated cholesterol efflux from [^{3}H]cholesterol labeled epididymal spermatozoa (Table 3). In contrast, Db fragment, up to 120 μg/ml, failed to stimulate the cholesterol efflux from epididymal spermatozoa (Table 3).

Effect of Domain b on Sperm Capacitation

We have previously demonstrated that preincubation of bovine epididymal spermatozoa with BSP proteins potentiate subsequent heparin-mediated [31] and HDL-mediated [32] capacitation. However, incubation of bovine epididymal sperm with 50 μg/ml of Db fragment did not enhance heparin-mediated or HDL-mediated capacitation (Table 4). After the incubation, the percentage of acrosome-reacted spermatozoa preincubated with Db fragment was not significantly different from the control without BSP-A1/-A2 proteins.

DISCUSSION

Upon ejaculation, spermatozoa are mixed with secretions of the accessory sex glands and certain seminal plasma proteins bind to the sperm surface. Subsequently, sperm cells acquire fertilization potential in the female genital tract in a process called capacitation. This is a multistep process including interactions of sperm with capacitation factors of the female genital tract as well as a loss of sperm membrane cholesterol

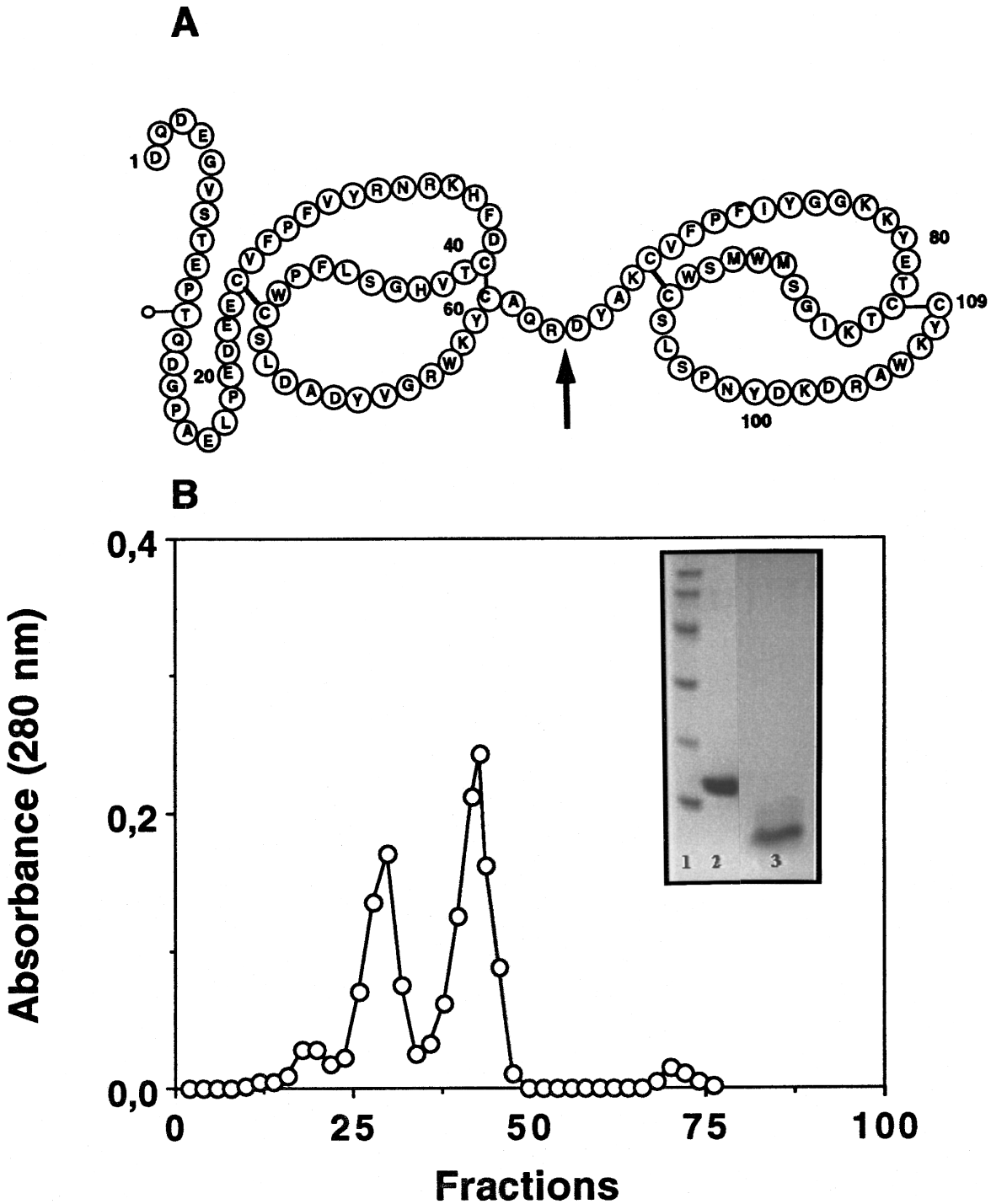

FIG. 1. Secondary structure of BSP-A1/-A2 proteins and purification of domain b fragment. A) Secondary structure of BSP-A1/-A2 proteins. The arrow indicates the tryptic cleavage site under limited proteolytic digestion as described under the Experimental Procedure section. The site of glycosylation of BSP-A1/-A2 proteins is indicated. B) The size-exclusion chromatographic profile of tryptic fragments of BSP-A1/-A2 proteins on a Sephadex G-50 column (fraction size of 4.1 ml with a wait of 380 min). Inset: reduced and denaturated BSP-A1/-A2 proteins (5 μg) and Db fragment (5 μg, pool from tubes 39 to 53) were submitted to electrophoresis on a 15% polyacrylamide gel. Low molecular weight standards (lane 1), BSP-A1/-A2 (lane 2) and Db (lane 3) were stained with Coomassie Brilliant.

that render these cells responsive to inducers of the acrosome reaction [33, 34]. Many studies suggest that factors present in the seminal fluid are important for the acquisition of fertility [31–33, 38]. In bovine, a family of proteins secreted by the seminal vesicles represent the major protein fraction (20–40 mg/ml) of semi-

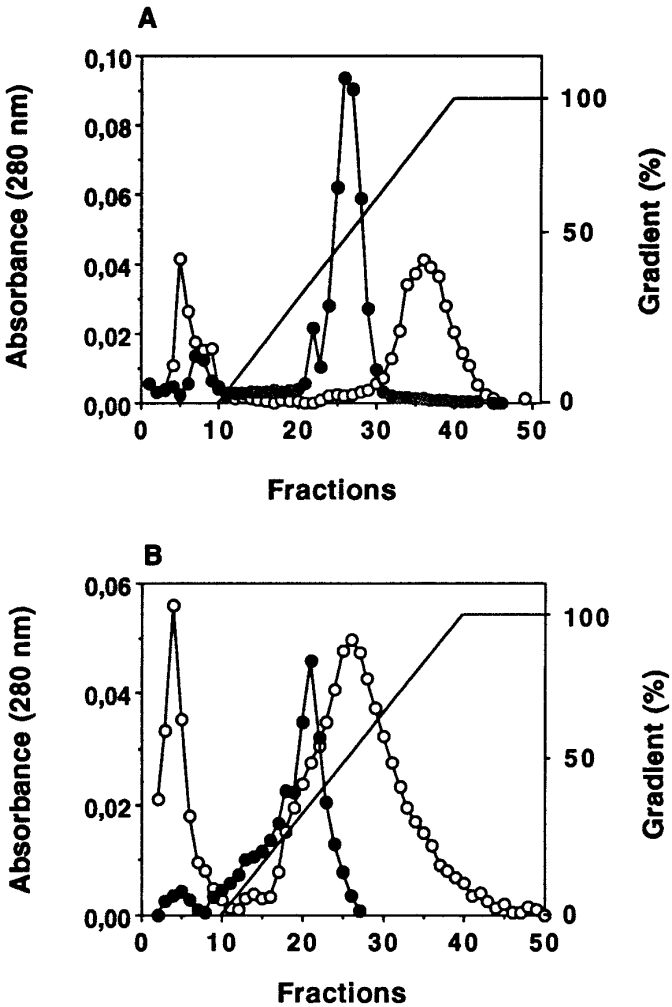

FIG. 2. Choline- and heparin-binding of Db fragment. A) BSP-A1/-A2 proteins (○) and Db fragment (●) (200 μg) solubilized in TBS were applied to a PPC-Agarose column. Fractions of 1 ml were collected and elution of proteins was assessed with a linear gradient of choline chloride (0–100 mM). B) BSP-A1/-A2 proteins (○) and Db fragment (●) (200 μg) solubilized in 50 mM phosphate buffer were applied to heparin-Sepharose column. Fractions of 1 ml were collected and elution of proteins was assessed with a linear gradient of NaCl (0–1 M).

nal fluid. These bovine seminal plasma proteins (BSP proteins) bind to sperm membrane choline phospholipids and potentiate in vitro capacitation induced by heparin and HDL [27, 29–32]. In this regard, we demonstrated that BSP proteins bind to heparin [29] and stimulate cholesterol and phospholipid efflux from spermatozoa [33] and human fibroblasts [35].

In the present investigation we report for the first time that the second type II domain of BSP-A1/-A2 proteins is an autonomous unit that binds choline phospholipids and heparin. Previously, Banyai et al [13] have reported that Db binds to gelatin. Our experiments with affinity chromatography showed that gelatin-Agarose bound BSP-A1/-A2 proteins and Db fragment could be eluted by choline chloride (data not shown). Our unpublished observations also indicate that gelatin and choline binding sites are the same or are at least in proximity. Furthermore, heparin- and choline-binding sites are likely to be the same since HSP-1 and pB1 [39] that are analogs of BSP-A1/-A2 proteins bound to heparin-Sepharose and are eluted with choline or phosphorylcholine. Therefore, BSP-type II domains bind gelatin, choline phospholipids and heparin. In line with this, the tertiary structure determination and NMR analysis of the second type II domain of BSP-A1/-A2 proteins revealed a flexible structure that may bind various ligands [40, 41]. Indeed, data is available which indicate multiple protein-binding properties of the BSP proteins. In particular, BSP proteins interact with different genetic types of collagens (types I, II, IV, V), fibrinogen [25], heparin [29], calmodulin [42], insulin-like growth factor II [43], apoA-I and apoA-I associated with HDL [30]. The present results reinforce the hypothesis that the type II domains of BSP-A1/-A2 proteins are multifunctional binding sites for a variety of ligands.

Using equilibrium dialysis, we previously estimated

TABLE 2

Cholesterol Efflux from Fibroblasts in the Presence of BSP-A1/-A2 Proteins and Db Fragment

Conditions		Cholesterol efflux (%)
Control		3.43 ± 0.49
BSP-A1/A2	9 μg/ml	8.89 ± 1.18[a]
Domain b	9 μg/ml	2.47 ± 0.43
	27 μg/ml	2.86 ± 0.48
	50 μg/ml	3.04 ± 0.14

Note. Labeled ([^{3}H]cholesterol) cells were incubated with Db, BSP-A1/-A2 or in culture medium (control). The values represent the mean ± S.E.M. of the cholesterol efflux at 3 h for 3 independent experiments.

[a] $P<0.001$, compared to condition without protein.

TABLE 3

Cholesterol Efflux from Epididymal Spermatozoa in the Presence of Db Fragment

Conditions	Cholesterol efflux (%)
Control	6.50 ± 1.49
BSP-A1/-A2 (120 μg/ml)	17.46 ± 2.01[a]
Domain b (120 μg/ml)	7.46 ± 2.68

Note. Labeled ([^{3}H]cholesterol) spermatozoa (50×10^6 cells) were incubated for 6 h in the presence of BSP-A1/-A2 proteins, Db or in mTALP medium (control). The cholesterol efflux was determined by measuring the radioactivity in the medium following centrifugation of the cell suspension. The values represent the mean ± S.E.M. of 3 experiments.

[a] $P<0.001$, compared with control condition.

TABLE 4

Epididymal Sperm Capacitation in the Presence of Db Fragment

Conditions	Percentage acrosome reaction		
	Control	HDL	Heparin
mTALP	12.5 ± 0.8	17.2 ± 0.8[a]	15.0 ± 0.6
BSP-A1/-A2	11.8 ± 1.0	28.8 ± 1.1[b]	32.8 ± 1.3[c]
Domain b	12.8 ± 0.8	17.3 ± 0.7	14.2 ± 0.8

Note. Cauda epididymal sperm (25×10^6 cells) were preincubated for 20 min with 40 μg/ml of BSP-A1/-A2 proteins, 50 μg/ml of Db fragment or in protein-free mTALP medium (mTALP). Then the cells were washed and incubated for 5 h with 12 μg/ml of heparin or for 8 h with 160 μg/ml of HDL. The values represent the mean ± S.E.M. of percentage of sperm that were acrosome-reacted from 3 individual experiments.

[a] $P<0.01$, compared with sperm incubated without capacitation factor.

[b] $P<0.001$, compared with sperm incubated without BSP-A1/-A2 proteins and with HDL.

[c] $P<0.001$, compared with sperm incubated without BSP-A1/-A2 proteins and with heparin.

that BSP-A1/-A2 protein have two binding sites for choline [28]. Since each BSP protein contains two similar tandem type II structures, it is more likely that each type II motif of BSP proteins constitutes a choline-, gelatin- and heparin-binding site. By affinity chromatography, we observed that concentrations of choline required to elute Db fragment were less than that required for BSP-A1/-A2 proteins. This is expected since there are two competing binding sites in BSP-A1/-A2 proteins for eluting agent whereas Db contains only one binding site.

Our results indicate that both type II structures are essential for the expression of the biological function of BSP proteins. The second type II domain of BSP-A1/-A2 per se is not sufficient to promote lipid efflux or potentiate bovine sperm capacitation induced by heparin and HDL. The preincubation of epididymal spermatozoa with BSP proteins potentiate the capacitation induced by heparin by increasing the number of binding sites for heparin at the sperm surface [44]. The present studies indicate that the second type II domain of BSP-A1/-A2 proteins bind to choline phospholipids and to heparin. However, simultaneous binding of these two ligands to one type II domain seems unlikely since heparin-Sepharose bound proteins can be eluted by phosphorylcholine [39]. Therefore, Db bound to the sperm surface cannot bind simultaneously to heparin and is unable to potentiate heparin-induced capacitation. Thus, the presence of two type II domains in BSP proteins confer the ability to BSP proteins to bind to the surface of the spermatozoa by one domain and leave the second domain accessible to heparin binding. In this process, BSP proteins serve as an anchoring proteins for heparin. It should be noted that high concentration of heparin inhibit sperm capacitation [44]. It is possible in this case that heparin displaces BSP proteins from the sperm membrane by binding to both type II domains.

The absence of stimulation of cholesterol efflux from fibroblasts and spermatozoa by Db fragment along with the lack of potentiation of HDL-induced spermatozoa capacitation suggest that BSP proteins enhance sperm capacitation by favouring membrane cholesterol efflux. Our previous results indicate that preincubation of BSP proteins with choline inhibits cholesterol efflux suggesting that type II domains of BSP proteins are involved in this process [35]. The present results indicate that the Db exhibited binding ability for choline but did not, however, stimulate cholesterol efflux. This apparent discrepancy indicates that both type II domains are necessary for cholesterol efflux. Furthermore, we and other investigators [45, 46] have reported the aggregation tendency of BSP-A1/-A2 proteins into multiple oligomers (60,000–150,000 daltons). Dissociation of BSP-A1/-A2 oligomers occurs upon increasing the concentration of choline or phosphorylcholine suggesting that type II domains of BSP proteins are implicated in the formation of oligomers. The choline inhibition of BSP-mediated cholesterol efflux may reside in the prevention of protein oligomerization. The importance of oligomerization of apoA-I in cholesterol efflux has been demonstrated previously. In aqueous solution, apoA-I forms several oligomers, including dimers, trimers, etc and Jis et al. [47] have reported that the C-terminal domain of apoA-I is crucial for self-association and initial lipid binding for liposome formation. Mendez et al [48] reported that cooperativity between two or more helical repeats is required to remove cellular cholesterol. Synthetic amphipathic helical peptides with a single helix has little or no ability to induce cholesterol efflux but peptides containing two tandem repeats promote efficient lipid efflux from cells. As in apoA-I, the aggregation tendency of BSP proteins may be implicated in cholesterol efflux.

In summary, the type II domains of BSP-A1/-A2 proteins are autonomous binding sites for choline phospholipids and heparin, and are involved in sperm capacitation by anchoring heparin to the sperm surface and by stimulating sperm cholesterol efflux. The oligomerization tendency of BSP-A1/-A2 proteins may be important in their lipid efflux potential.

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