

Signals of seminal vesicle autoantigen suppresses bovine serum albumin-induced capacitation in mouse sperm

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Abstract

Capacitation is the prerequisite process for sperm to gain the ability for successful fertilization. Unregulated capacitation will cause sperm to undergo a spontaneous acrosome reaction and then fail to fertilize an egg. Seminal plasma is thought to have the ability to suppress sperm capacitation. However, the mechanisms by which seminal proteins suppress capacitation have not been well understood. Recently, we demonstrated that a major seminal vesicle secretory protein, seminal vesicle autoantigen (SVA), is able to suppress bovine serum albumin (BSA)-induced mouse sperm capacitation. To further identify the mechanism of SVA action, we determine the molecular events associated with SVA suppression of BSA's activity. In this communication, we demonstrate that SVA suppresses the BSA-induced increase of intracellular calcium concentration ($[Ca^{2+}]_i$), intracellular pH (pH_i), the cAMP level, PKA activity, protein tyrosine phosphorylation, and capacitation in mouse sperm. Besides, we also found that the suppression ability of SVA against BSA-induced protein tyrosine phosphorylation and capacitation could be reversed by dbcAMP (a cAMP agonist).

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During epididymal transit, sperm progressively acquire the ability to move, but they are still fertilization incompetent. Fertilization capacity is gained after residence in the female reproductive tract for a finite period of time, and the physiological changes in sperm during this period are collectively called “capacitation.” Capacitation is a complex process first described and defined independently by Chang [1,2] and Austin [3,4]. The capacitation processes involve changes in membrane properties and dynamics, enzyme activities, elevation of $[Ca^{2+}]_i$, pH_i , and the cAMP level. It leads to energy consumption and hypermotility, and eventually an acrosome reaction by sperm [5,6].

Sperm capacitation occurs in the oviduct or uterus, depending on the species [6]. The process of sperm capacitation is tightly regulated by suppression factors (in the epididymis and seminal vesicles) and capacitation factors (in the female reproductive tract). Serum albumin is abundant in the female reproductive tract. It is thought to serve as a cholesterol-binding protein to remove sperm membrane cholesterol, by which to destabilize the sperm membrane and induce sperm capacitation [7–9]. Serum albumin has also been demonstrated to regulate the T-type Ca^{2+} channel of sperm, induce extracellular Ca^{2+} and bicarbonate ion influx [10], and elevate $[Ca^{2+}]_i$ and pH_i . The increase of $[Ca^{2+}]_i$ and pH_i upregulates the cAMP-dependent signaling and enhances the protein tyrosine phosphorylation, ultimately inducing hyperactivation and capacitation of sperm [5,11].

The suppressive effect of capacitation by suppression factors is referred to as “decapacitation” [12]. Without suppression regulation, most of the sperm would undergo a

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spontaneous acrosome reaction. An acrosome-reacted sperm lose its acrosome cap which is required for sperm binding to the zona pellucida of the egg. Thus, sperm ultimately lose their fertilization ability even though they still have hypermotility [6]. It has been reported that the epididymis and seminal plasma contain decapacitation activity [12–23]; the presence of suppression factors (decapacitation factors) may prevent the unfruitful capacitation of sperm and allow effective fertilization of an egg at the right time and place [17]. Currently, several decapacitation factors are known. Fraser et al. [18] suggested that the decapacitation mechanism involves fucose residues and a GPI-anchored receptor on sperm in the epididymis. A low molecular weight N-glycosidically linked oligomannosidic glycopeptide (MGp) isolated from the autoprolysis products of human seminal plasma was reported to prevent premature sperm exocytosis [19]. Studies by Villemure et al. [21] revealed the gelatin-binding proteins from goat seminal plasma play a role in sperm decapacitation. A sperm adhesion family of boar accessory sex gland fluids is also supposed to consist of decapacitation factors [22]. In addition, the platelet-activation factor, acetylhydrolase (PAF-AH), was also suggested to play a role in decapacitation by hydrolysis of PAF to lyso-PAF [23]. However, the mechanisms of these potential factors in decapacitation have not been well defined.

Recently, we demonstrated that serum obtained from male and female mice immunized with seminal vesicle secretion (SVS) fluid is immunoreactive to an androgen-responsive glycoprotein [24,25], and it was designated seminal vesicle autoantigen (SVA). SVA is a 19-kDa protein secreted from luminal epithelium cells of seminal vesicles and contribute to the dominant component of seminal plasma (~300 μM) [26]. SVA binds Zn^{2+} [27] and choline-containing phospholipids, such as phosphatidylcholine and sphingomyelin [26]. SVA has been demonstrated to suppress BSA-induced zinc ion removal from the sperm membrane, sperm hypermotility, protein tyrosine phosphorylation, and capacitation [28]. In this communication, we further demonstrate that SVA suppresses BSA-induced $[\text{Ca}^{2+}]_i$, pH_i , the cAMP level, and PKA activity in mouse sperm. In addition, the suppressive effects of SVA on BSA-induced protein tyrosine phosphorylation and capacitation in mouse sperm can be reversed by a cAMP agonist.

Materials and methods

Materials. Fatty acid-free BSA, polyvinylalcohol, and Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) were from Sigma (St. Louis, MO). Antiphosphotyrosine monoclonal antibody (clone 4G10) was from UBI (Lake Placid, NJ), horseradish peroxidase (HRP)-conjugated anti-mouse IgG was from Jackson ImmunoResearch Lab (West Grove, PA), Percoll, chemiluminescence detection ECL plus, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, and the cAMP assay kit (RPN 255) were from Amersham-Pharmacia Biotech (Buckinghamshire, UK), Fluo-3-AM and BCECF-AM were from Molecular Probes (Eugene, OR), dituyrllyl cAMP (dbcAMP), Rp-cAMPS, and IBMX were from Research Biochemicals International (Natick, MA), and H-89 was from LC Laboratories (Woburn, MA). Phosphocellulose Units SpinZyme Format for the radioactive kinase assay was from Pierce (Rockford, IL),

and the scintillation counting cocktail was from Merck (Darmstadt, Germany). All other chemicals were of reagent grade.

Sperm preparation and cytological observations. Outbred CD-1 mice purchased from Charles River Laboratories (Wilmington, MA) were bred in the Animal Center at Taipei Medical University School of Medicine. Animals were handled in accordance with institutional guidelines on animal experimentations.

The culture medium used throughout these studies was modified Krebs–Ringer bicarbonate HEPES medium (HM) as described previously [28]. In brief, modified HM contains 120.0 mM NaCl, 2.0 mM KCl, 1.20 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.36 mM NaH_2PO_4 , 15 mM NaHCO_3 , 10 mM HEPES, 5.60 mM glucose, 1.1 mM sodium pyruvate, and 1.7 mM CaCl_2 . The pH of the medium was adjusted to 7.3–7.4 with humidified air/ CO_2 (95:5) in an incubator at 37 °C for 48 h before use. Polyvinylalcohol (1 mg/ml) was added to serve as a sperm protectant. Mature mouse sperm were harvested by a swim-up procedure from the caudal epididymides and isolated with a 20–80% Percoll gradient. The viability and progressive motility of the sperm fraction used in the present study were more than 95%. The population of the capacitated stage in sperm was analyzed by the CTC staining method as described previously [29].

Flow cytometry. $[\text{Ca}^{2+}]_i$ of sperm was determined using fluo-3 AM by flow cytometry (FACScan, BD). In brief, Percoll-separated sperm were loaded with fluo-3 AM (10 μM) for 10 min. After 10 min incubation, sperm were washed twice with modified HM to remove any free fluo-3 AM. Fluo-3 AM-loaded sperm (10^6 cells/ml) were treated with SVA (0–66 μM) in the presence or absence of BSA (0.3%) at 37 °C for 90 min and then analyzed by epifluorescence microscope and flow cytometry.

pH_i of sperm was determined using BCECF-AM. In brief, Percoll-separated sperm were loaded with BCECF-AM (2 μM) for 10 min and then sperm were washed twice with modified HM to remove any free fluore. The fluore-loaded sperm were treated with BSA (0.3%) \pm SVA (2, 20, and 66 μM) and then analyzed by flow cytometry. For pH_i calibration, a nigericin/high K^+ calibration protocol was used to derive the pH_i values as described previously [30]. The fluorescence of fluo-3 was excited at 488 nm and measured via a 515–540 nm filter, and the fluorescence of BCECF was excited at 510 nm and measured via a 564–606 nm filter. PMT voltages and gains were set to optimize the dynamic range of the signal. The fluorescence intensity of sperm was quantified for 10,000 individual cells.

cAMP assay. The amount of cAMP produced in living, intact sperm was determined using a nonradioactive enzyme immunoassay kit according to the manufacturer's instructions.

Assay of protein kinase A activity. Protein kinase A activity was measured using Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) as the specific substrate. Sperm (10^7 cells/ml) were incubated under different experimental conditions, such as BSA (0.3%), SVA (66 μM) or BSA supplemented with SVA. DbcAMP (a cAMP agonist, 1 mM) plus IBMX (a phosphodiesterase inhibitor, 100 μM) were used to be a positive control, and H-89 (a PKA inhibitor, 30 μM) served as a negative control. After incubation at 37 °C for 90 min, the sperm suspension (10 μl) was mixed with an equal volume of 2 \times assay cocktail (10 μl) and incubated at 37 °C for additional 15 min. The final concentration of the assay components was 100 μM kemptide, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (6000 Ci/mmol) (2×10^6 cpm/assay), 100 μM ATP, 1% (v/v) Triton X-100, 1 mg/ml BSA, 10 mM MgCl_2 , 40 mM β -glycerophosphate, 5 mM *p*-nitrophenyl phosphate, 10 mM Tris–HCl (pH 7.4), 10 μM aprotinin, and 10 μM leupeptin. The reactions were stopped by an equal volume of 20% TCA, and reaction mixtures were cooled on ice for 20 min and followed by centrifugation at 10,000g at room temperature for 3 min. Twenty-five microliter of the resultant mixture was applied onto an affinity support of the phosphocellulose unit and washed with 500 μl of 75 mM phosphoric acid for four times (10 min/each time). The washed-sample bucket was then transferred into a scintillation vial for counting.

Detection of protein tyrosine phosphorylation. Sperm (5×10^6 cells/ml) were incubated with BSA (0.3%) in the absence or presence of SVA (66 μM). In some experiments, BSA and SVA supplemented with dbcAMP (a cAMP agonist, 1 mM) plus IBMX (a phosphodiesterase inhibitor, 100 μM), or BSA and H-89 (a PKA inhibitor, 30 μM) or Rp-cAMP (a cAMP antagonist, 1 mM) were added. The reactions were incubated at 37 °C. After 90 min incubation, the cell lysate was prepared

according to Visconti et al. [31], subjected to a 10% SDS–PAGE, and then transferred to a PVDF membrane for Western blot analysis. The monoclonal anti-phosphotyrosine IgG (clone 4G10) (1 $\mu\text{g}/\text{ml}$) was used as the primary antibody and HRP-conjugated anti-mouse IgG (1:2000) served as the secondary antibody. The enzyme activity of HRP was detected by the ECL system according to the manufacturer's instructions.

Statistical analysis. All experiments were repeated at least three times with three different pooled sperm samples from four or five male mice. The data were expressed as means \pm SD. Difference in means was assessed by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer multiple comparisons test.

Results

SVA suppresses BSA-stimulated capacitation in mouse sperm

BSA, like serum albumin, is a well-known putative capacitation factor used for stimulating mouse sperm capacitation in vitro. Its related capacitation events involve the removal of membrane-bound zinc ions, elevation of $[\text{Ca}^{2+}]_i$, pH_i , the cAMP level, sperm hypermotility, and protein tyrosine phosphorylation [5]. To determine the molecular events of SVA suppression of sperm capacitation, we detected the SVA effect on BSA (0.3%)–induced capacitation signaling. The capacitation states of mouse caudal epididymal sperm under different experimental conditions were determined by the CTC assay [29]. As shown in Fig. 1, fewer than 15% of sperm underwent capacitation in the absence of BSA with or without SVA. BSA (0.3%) induced sperm capacitation by $53 \pm 8\%$. SVA (66 μM) suppressed BSA-induced capacitation in mouse sperm from $53 \pm 8\%$ to $30 \pm 4\%$. This effective concentration of SVA on BSA activity is well below the physiological concentration in semen ($\sim 300 \mu\text{M}$). These observations further confirmed our previous report that SVA suppressed BSA-induced mouse sperm capacitation [28]. In addition, adding dbcAMP plus IBMX reversed the suppressive effect of SVA on BSA-induced mouse sperm capacitation, suggesting a role of cAMP in SVA suppression signaling.

SVA suppresses BSA-induced elevation of $[\text{Ca}^{2+}]_i$ and pH_i in mouse sperm

Elevation of $[\text{Ca}^{2+}]_i$ and pH_i has been shown to be associated with mouse sperm capacitation induced by BSA [5,32]. $[\text{Ca}^{2+}]_i$ and pH_i have also been demonstrated to upregulate membrane/soluble adenylate cyclase activity, by which to produce cAMP, and activate PKA activity, inducing protein tyrosine phosphorylation and capacitation in mouse sperm [32]. Since SVA is capable of suppressing BSA-induced capacitation, we decided to determine the effect of SVA on $[\text{Ca}^{2+}]_i$ and pH_i in sperm stimulated by BSA. $[\text{Ca}^{2+}]_i$ was determined by epifluorescence microscopy and flow cytometry using fluo-3-AM. As shown in Fig. 2A, BSA (0.3%) increased $[\text{Ca}^{2+}]_i$ (Fig. 2A, panel b vs. panel a). SVA not only suppressed the BSA-induced elevation of $[\text{Ca}^{2+}]_i$ (Fig. 2A, panel d vs. panel b), but also

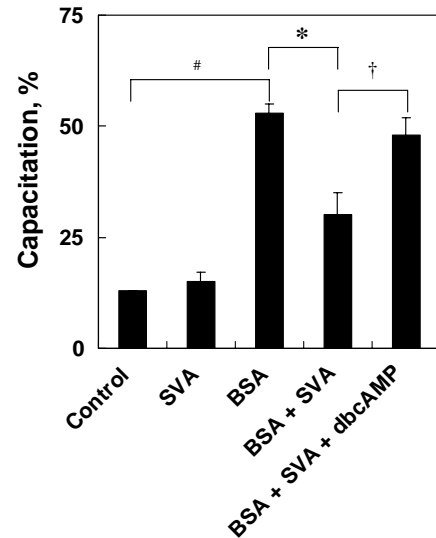


Fig. 1. cAMP agonist reverses SVA suppression of BSA-induced capacitation in mouse sperm. Capacitation stage of sperm (5×10^6 cells/ml) under different experimental conditions was determined using the CTC fluorescence method. Each data point is the mean \pm SD of three independent determinations. Data obtained from sperm treated with BSA alone were compared with that of control sperm, or data obtained from sperm treated with BSA and SVA were compared with that of sperm treated with BSA but without SVA, or data obtained from sperm treated with BSA and SVA in the presence of dbcAMP plus IBMX were compared with that of sperm treated with BSA and SVA but in the absence of dbcAMP plus IBMX, respectively, by one-way ANOVA, Tukey–Kramer multiple comparison test. ($\#p < 0.001$, $*p < 0.001$, $\dagger p < 0.001$).

decreased the basal $[\text{Ca}^{2+}]_i$ in mouse sperm (Fig. 2A, panel c vs. panel a). Besides, the relative $[\text{Ca}^{2+}]_i$ of sperm was also detected by flow cytometry and expressed as percentages in comparison with that of control cells. As shown in Fig. 2B, BSA elevated $[\text{Ca}^{2+}]_i$ to $153 \pm 6\%$. SVA suppressed $[\text{Ca}^{2+}]_i$ with or without BSA in a dose-dependent manner.

Elevation of pH_i has been correlated with BSA-induced sperm capacitation [33]. To determine the SVA effect on pH_i in mouse sperm, sperm pre-loaded with BCECF-AM were incubated with SVA and analyzed by flow cytometry. The pH_i of sperm was estimated by a pH standard curve which was calibrated according to a pH 6–7 standard solution (Fig. 3A) as described previously [30]. As shown in Fig. 3B, BSA elevated the pH_i from 6.5 to 6.9, and SVA suppressed the BSA-induced elevation of pH_i . At 20 μM , SVA significantly suppressed the BSA-induced elevation of pH_i to basal level of mouse sperm. In addition, SVA was also shown to decrease the basal level of pH_i of mouse sperm (our unpublished results).

SVA suppresses BSA-induced elevation of the cAMP level in mouse sperm

Since $[\text{Ca}^{2+}]_i$ and pH_i have been shown to be the upstream regulators of adenylate cyclase activity, we then determined the effect of SVA on the BSA-induced elevation

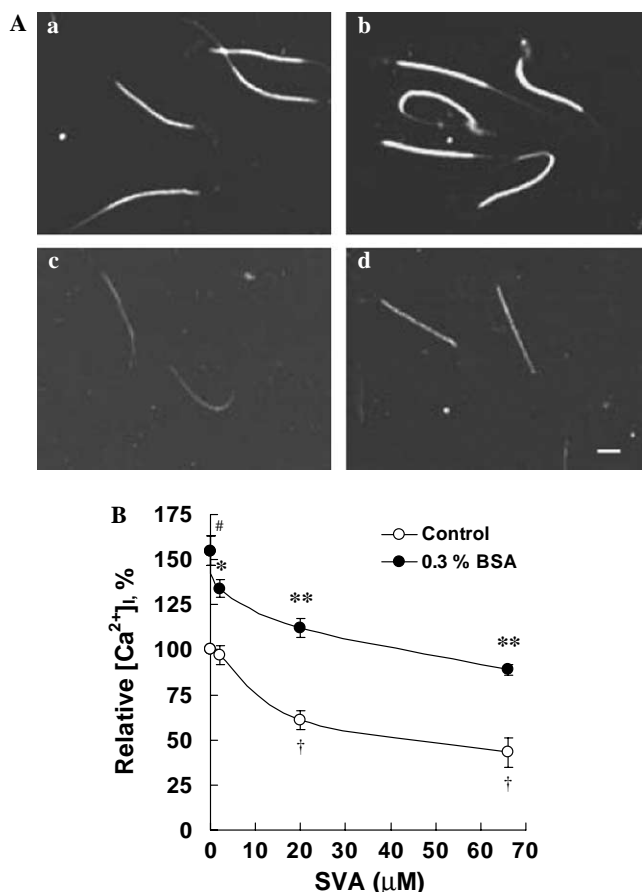


Fig. 2. SVA suppresses BSA-induced elevation of $[Ca^{2+}]_i$ in mouse sperm. Sperm (5×10^6 cells/ml) were loaded with fluo-3 AM to detect $[Ca^{2+}]_i$. Fluorescence images of sperm under different experimental conditions were shown in (A): (a) control (HM only), (b) BSA (0.3%), (c) SVA (66 μ M), and (d) BSA + SVA. Fluo-3-loaded sperm were incubated with increasing concentration of SVA (0–66 μ M) in the presence (closed circles) or absence (open circles) of BSA (B). After 90 min incubation, sperm were subjected to a flow cytometry to analyze $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ of sperm was shown as percentages in comparison with that of control sperm. Each data point is the mean \pm SD of five independent determinations. Data obtained from sperm treated with BSA or SVA alone were compared with that of control sperm ($^{\dagger}p < 0.001$, $^{\#}p < 0.001$), or data obtained from sperm treated with BSA and SVA were compared with that of sperm treated with BSA but without SVA ($^*p < 0.01$, $^{**}p < 0.001$), respectively, by one-way ANOVA, Tukey–Kramer multiple comparison test. Scale bar, 10 μ m.

of the cAMP level in mouse sperm. As shown in Fig. 4, BSA (0.3%) increased the cAMP level to $153 \pm 11\%$ as compared with that of control sperm. SVA suppressed the BSA-induced elevation of the cAMP level in a dose-dependent manner with the maximum effect at 66 μ M (from $153 \pm 11\%$ to $93 \pm 10\%$). Besides, SVA (66 μ M) decreased the basal level of cAMP to $43 \pm 8\%$ in mouse sperm. This observation is in coincidence with our previous results that SVA suppressed the basal $[Ca^{2+}]_i$ and pH_i .

SVA suppresses BSA-induced the PKA activity in mouse sperm

cAMP has been demonstrated to affect PKA activity and regulate protein tyrosine phosphorylation of mouse

sperm [34]. Since SVA is able to suppress the BSA-induced elevation of the cAMP level in mouse sperm, we suggested that SVA may suppress the BSA-induced PKA activity. A PKA-specific substrate, Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly), was used to detect the PKA activity of sperm. As shown in Fig. 5, dbcAMP plus IBMX served as a positive control, it enhanced the PKA activity to near 400% as compared with that of the control sperm. A PKA inhibitor H-89 was used to be a negative control; it suppressed the PKA activity to $\sim 75\%$. BSA (0.3%) significantly enhanced the PKA activity to $155 \pm 6\%$ and SVA both suppressed the BSA-induced and the basal level of PKA activity of sperm. These results support our previous observations that SVA suppresses both the BSA-induced and the basal $[Ca^{2+}]_i$ and the cAMP levels (Figs. 2–4). Furthermore, the suppressive effect of SVA (200 μ M) on the PKA activity of mouse sperm could be overcome by 10% BSA. This observation further supports our previous report that 10% BSA reversed the SVA's suppressive effect on 0.3% BSA-induced protein tyrosine phosphorylation and capacitation in mouse sperm [28].

cAMP agonist reverses SVA's suppression of BSA-stimulated protein tyrosine phosphorylation in mouse sperm

Protein tyrosine phosphorylation has been demonstrated to the molecular evidence of capacitation, and it is correlated with the cAMP level and PKA activity in mouse sperm [34]. Since SVA decreased the cAMP level in sperm, we therefore detected the effect of a cAMP agonist on SVA's suppression of BSA-induced protein tyrosine phosphorylation. As shown in Fig. 6A, two proteins of M.W. ~ 120 and ~ 50 kDa were tyrosine-phosphorylated in control sperm which showed normal motility. The M.W. 120-kDa protein did not respond to the BSA stimulation and was similar to p95/106 hexokinase identified by Kalab et al. [35] (Fig. 6A, indicated by an arrowhead). BSA (0.3%) treatment of sperm resulted in significant enhancement of protein tyrosine phosphorylation in the range of MW 50–100 kDa (Fig. 6A, lane 3, indicated by arrows), and this phenomenon was suppressed by Rp-cAMP and H-89 (Fig. 6A, lanes 4 and 5). These results further confirm the previous report that the cAMP-PKA pathway is involved in BSA's activity of mouse sperm [5,34]. Treatment of sperm with SVA reduced motility [26] and the tyrosine phosphorylation of the MW 50 kDa protein of control sperm (Fig. 6B, lane 2). Furthermore, the BSA-induced protein tyrosine phosphorylation of sperm was also significantly suppressed by SVA (Fig. 6B, lane 4).

DbcAMP and IBMX have been reported to induce protein tyrosine phosphorylation and capacitation in mouse sperm [34]. To define the role of cAMP in SVA's suppressive effect, we determined the effect of dbcAMP plus IBMX on SVA suppression of BSA-induced protein tyrosine phosphorylation. As shown in Fig. 6B, the suppressive effects of SVA on BSA-induced protein tyrosine phosphorylation were overcome in the presence of dbcAMP plus

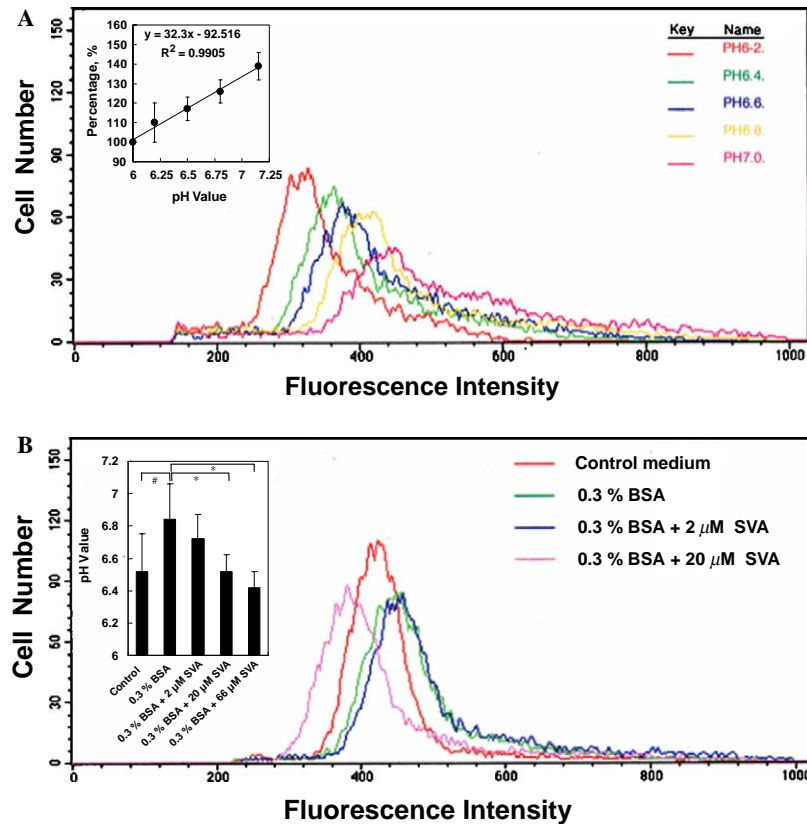


Fig. 3. SVA suppresses BSA-induced elevation of pH_i in mouse sperm. Sperm (5×10^6 cells/ml) pre-loaded with BCECF AM were calibrated with a pH 6–7 standard solution (A), or incubated with BSA (0.3%) in the presence or absence of SVA (2, 20, and 66 μM) (B). pH_i of sperm under different experimental conditions are shown as percentages of that of control sperm. Each data point is the mean \pm SD of three independent determinations. Data obtained from sperm treated with BSA alone were compared with that of control sperm, or data obtained from sperm treated with BSA and SVA were compared with that of sperm treated with BSA but without SVA, respectively, by one-way ANOVA, Tukey–Kramer multiple comparison test. ($\#p < 0.001$, $*p < 0.001$).

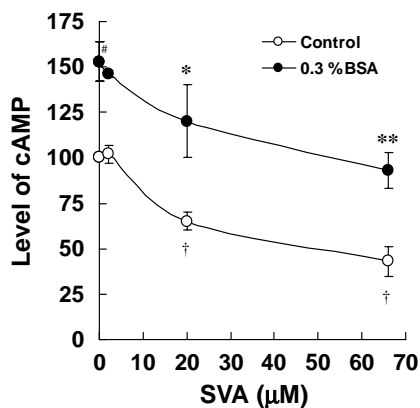


Fig. 4. SVA suppresses BSA-induced elevation of the cAMP level in mouse sperm. Sperm (5×10^6 cells/ml) were incubated with increasing concentration of SVA (0–66 μM) in the presence (closed circles) or absence (open circles) of BSA (0.3%). After 90 min incubation, the total cell lysate was extracted and the total intracellular cAMP amount was detected. Each data point is the mean \pm SD of five independent determinations. Data obtained from sperm treated with BSA or SVA alone were compared with that of control sperm ($\#p < 0.001$, $\dagger p < 0.001$), or data obtained from sperm treated with BSA and SVA were compared with that of sperm treated with BSA but without SVA ($*p < 0.01$, $**p < 0.001$), respectively, by one-way ANOVA, Tukey–Kramer multiple comparison test.

IBMX (Fig. 6B, lanes 5–7). This result further supports our previous observation that a cAMP agonist reversed SVA's suppression on capacitation of mouse sperm (Fig. 1). Together with the fact that SVA decrease the cAMP level and PKA activity, these observations strongly suggest that cAMP not only participates in BSA-induced protein tyrosine phosphorylation and capacitation but also involves in SVA's suppressive mechanism.

Discussion

Successful fertilization is tightly regulated by capacitation and decapacitation processes. Sperm interact with suppression factors in the seminal plasma (e.g., seminal plasma proteins) and capacitation factors in the female reproductive tract (e.g., serum albumin) while they are ejaculated from the caudal epididymis to move to the female reproductive tract. Serum albumin is thought to stimulate sperm capacitation in the female genital tract. In vitro, $[\text{Ca}^{2+}]_i$ and pH_i have been demonstrated to be the upstream mediators of BSA-induced mouse sperm capacitation. $[\text{Ca}^{2+}]_i$ and pH_i regulate the membrane and/or cytosolic adenylate cyclase [5,36–39], modulate cAMP metabolism and PKA activity [36], and ultimately affect capacitation-associated

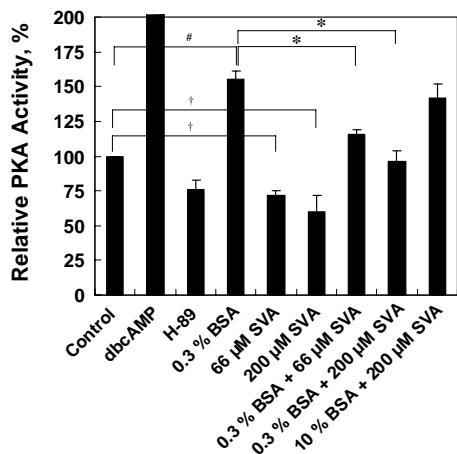


Fig. 5. SVA suppresses BSA-induced elevation of the PKA activity in mouse sperm. The PKA activities of sperm under different experimental conditions were determined, such as (1) control (HM only), (2) dbcAMP (1 mM) + IBMX (100 μM), (3) H-89 (30 μM), (4) BSA (0.3%), (5) SVA (66 μM), (6) SVA (200 μM), (7) BSA (0.3%) + SVA (66 μM), (8) BSA (0.3%) + SVA (200 μM), and (9) BSA (10%) + SVA (200 μM). Each data point is the mean ± SD of three-independent determinations. Data obtained from sperm treated with BSA or SVA alone were compared with that of control sperm, or data obtained from sperm treated with BSA and SVA were compared with that of sperm treated with BSA but without SVA, respectively, by one-way ANOVA, Tukey–Kramer multiple comparison test. (#*p* < 0.001, **p* < 0.001, †*p* < 0.001).

protein tyrosine phosphorylation of sperm [32,40]. Stimulation of tyrosine phosphorylation is an important event during mammalian sperm capacitation [34,41]. In somatic cells, the tyrosine-phosphorylated proteins mediate a variety of cellular functions such as growth regulation, cell cycle control, cytoskeletal assembly, ionic current regulation, and receptor regulation [42]. In human sperm, the capacitation-associated tyrosine-phosphorylated proteins have been demonstrated to involve the valosin-containing

protein (VCP), SNARE-interacting protein and protein kinas A-anchoring protein family, etc., suggesting the changes in regulatory enzymes of acrosomes and cytoskeletal elements during the sperm capacitation process [43].

An unregulated capacitation process causes sperm to undergo unfruitful capacitation prior to reaching the egg for fertilization. Seminal plasma is thought to contain some suppressive factors to regulate the capacitation processes [12,14–17,19–23,44–46]. The accessory sexual organ is also thought to play a role in maintaining an optimal calcium environment in seminal plasma, by which to regulate sperm function [45]. In supporting this hypothesis, Coronel et al. [46] reported that a calcium transport inhibitor, caltrin, is secreted by bovine seminal vesicles and binds with sperm to maintain sperm in a low cytosolic calcium concentration during ejaculation. In our results, as a major component of seminal plasma, SVA significantly suppresses BSA-induced mouse sperm capacitation and its related signaling. The SVA-affected molecular events include $[Ca^{2+}]_i$, pH_i , the cAMP level, PKA activity, and protein tyrosine phosphorylation. The observations that SVA decreases $[Ca^{2+}]_i$ and the cAMP level in sperm are in coincidence with our previous studies that SVA suppresses sperm motility [26]. The IC_{50} of SVA suppression on BSA-induced $[Ca^{2+}]_i$ and the cAMP level was less than 20 μM, a concentration which is well below the physiological concentration of SVA in semen (~300 μM).

Since Ca^{2+} is the upstream regulator of sperm capacitation, the fact that $[Ca^{2+}]_i$ decreases apparently plays an important role in SVA's activity. We hypothesized that there are two possibilities for SVA suppression of $[Ca^{2+}]_i$ of mouse sperm. SVA may block the calcium influx or SVA may play a role in calcium clearance of mouse sperm. The calcium influx may not be affected by SVA because SVA is effective in lowering $[Ca^{2+}]_i$ in Ca^{2+} -free medium as well as in Ca^{2+} -containing medium (unpublished results). Besides, in view of

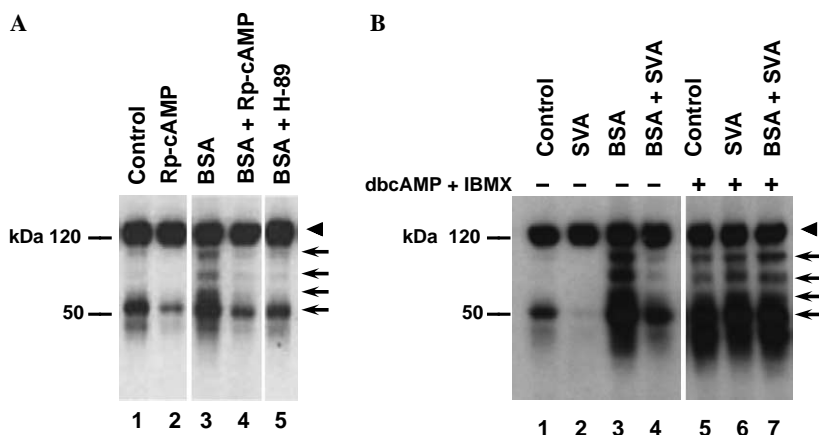


Fig. 6. cAMP agonist reverses the SVA inhibition of BSA-induced protein tyrosine phosphorylation in mouse sperm. The pattern of protein tyrosine phosphorylation in mouse sperm (5×10^6 cells/ml) incubated in different experimental conditions was detected (A): (1) control (HM only), (2) Rp-cAMP (1 mM), (3) BSA (0.3%), (4) BSA + Rp-cAMP, and (5) BSA + H-89 (30 μM). The suppressive effect of SVA on BSA-induced protein tyrosine phosphorylation was detected and is shown in (B): (1) control (HM only), (2) SVA (66 μM), (3) BSA (0.3%), (4) BSA + SVA, and (5–7) BSA + SVA + cAMP agonist (dbcAMP plus IBMX). The arrows indicate the location of capacitation-related tyrosine-phosphorylated proteins, and the arrowhead denotes the location of a capacitation-unrelated 120 kDa tyrosine-phosphorylated protein.

intracellular Ca^{2+} clearance, there are four major mechanisms that have been reported in most cell types [47]. Two are on the plasma membrane: the plasma membrane Ca^{2+} -ATPase (PMCA, which exports a cytoplasmic Ca^{2+} ion while importing one or two extracellular protons), and the plasma membrane Na^{2+} - Ca^{2+} exchanger (NCX, which exports an intracellular Ca^{2+} ion and imports approximately three Na^{+} ions). The other two clearance sites are in the intracellular organelles: sarcoplasmic endoplasmic reticulum Ca^{2+} -ATPase (SERCA pumps) and mitochondrial Ca^{2+} uniporter (MCU). In mouse sperm, PMCA and NCX are considered to be the most important molecules for maintaining low $[\text{Ca}^{2+}]_i$, but MCU just play a minor role and the SERCA pumps are not thought to be essential in mediating Ca^{2+} clearance in sperm [47]. In consideration of that SVA decreases both the BSA-induced and basal $[\text{Ca}^{2+}]_i$ and pH_i (Figs. 2 and 3) and the PMCA's dominant role in exporting Ca^{2+} and importing extracellular protons, we suggest PMCA is likely the site where SVA exerts its action. However, this hypothesis needs to be further examined.

BSA and SVA are both phospholipid binding proteins. The interactions of BSA and SVA with sperm membrane lipid components may play roles in regulating capacitation signaling. Recent studies by Sleight et al. [48] conclude that BSA-induced cholesterol efflux alters the lipid raft domain on sperm membrane, by which to initiate the capacitation signaling. Lipid rafts are highly enriched in cholesterol, gangliosides, and sphingolipids, and are thought to recruit specific types of proteins to serve as the cholesterol traffic centers for signal transduction pathway originating at the plasma membrane [49]. PMCA has also been shown to be concentrated in the caveolae/raft and mediated by sphingolipids [50]. As SVA is capable of binding choline-containing phospholipids and sphingolipid [26], the possible interaction of SVA with these specific phospholipids/sphingolipids on sperm membrane lipid-rafts may mediate the Ca^{2+} signal to suppress the BSA-induced capacitation in mouse sperm. This suppressive effect of SVA (in semen) on capacitation may enable sperm to avoid unfruitful capacitation before encountering the egg. Sperm may undergo capacitation at or near the site where the egg resides, and the concentration of SVA is low in the uterus and oviduct.

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