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Occurrence of novel Cu^{2+}-dependent sialic acid-specific lectin, on the outer surface of mature caprine spermatozoa

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Abstract Effects of several bivalent metal ions on the autoagglutination event in mature caprine epididymal sperm cells have been investigated using a chemically defined medium. This study demonstrates for the first time that Copper (Cu^{2+}) ion (300 μM) has high specificity for autoagglutination of mature cauda-epididymal sperm. Head-to-head interaction of the male gametes is responsible for this event. Studies on the effect of various sugars reveal that the autoagglutinated cells can be dissociated specifically with neutralized sialic acid (50 mM), which also inhibits the sperm cell autoagglutination phenomenon. Blood serum protein fetuin, that contains terminal sialic acid residue, showed high efficacy for inhibiting this autoagglutination event at 4 μM concentration. However, asialofetuin is not capable of inhibiting this Cu^{2+}-dependent cellular event. Mature sperm cells bound with caprine erythrocytes at their head region in presence of Cu^{2+} ion. The purified sperm membrane fraction isolated by aqueous two phase polymer method showed high efficacy to agglutinate erythrocytes. These sperm-erythrocyte interactions as well as sperm membrane induced haemagglutination were strongly blocked by neutralized sialic acid (50 mM). The results confirm the occurrence of unique Cu^{2+} dependent, sialic acid-specific lectin on the outer surface of a mammalian cell using caprine sperm as the model. The observed Cu^{2+}-mediated cellular autoagglutination is caused by the interaction of the cell surface lectin with the lectin receptor on the surface of the neighboring homologous cell.

Keywords Mature spermatozoa · Copper · Autoagglutination · Sialic acid · Lectin

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Introduction

Animal lectins are classified as C-type lectins, which are Ca^{2+}-dependent and contain the highly conserved disulfide bridge, and galectins (previously known as S-type lectins), which are independent of divalent metal ions, are thiol group dependent, and specifically bind β-galactoside residues [1, 2]. Most of the animal lectins are C-type lectins and comprise a vast group of proteins differing widely in their sugar specificity. An important family of these classes of proteins is selectins. These are sialic acid-specific transmembrane glycoproteins dependent on Ca^{2+} for their biological activities, and constitute a family of cell adhesion molecules (CAMs) [3, 4]. Selectin is involved in attachment processes including those of leukocytes to the vascular endothelium, lymphocytes to the high endothelial venules of lymph nodes [5], human melanoma cells [6], and of colorectal tumor cells to endothelial cells [7].

Few studies were reported about bivalent cation dependence of external cell surface sialic acid for binding with specific biomolecules in mammalian systems. Häyrinen *et al.* [8] demonstrated *in vitro* evidences about the presence of polysialic acid containing adhesion molecules in the neural cell; it showed increasing affinity for adhesion in presence of bivalent cations. Most of the reported studies about the role of the external cell surface lectins in cell-substratum and cell-cell adhesions in mammalian organs primarily center on the attachment of one cell type with another cell type, *i.e.* heterologous cell-cell adhesion [9–12]. Sialic acid containing receptors or sialic acid-specific lectins are purposely expressed on the surfaces of male and female gametes and attach to their corresponding ligands during sperm-oocyte fusion, an example of heterologous cell-cell adhesion [13, 14]. Kadirvel *et al.* [15] observed that sperm cell primarily attached 6-sialylated biantennary glycans to oviduct and this is essential for normal adhesion. A mammalian cell model (using liver parenchymal cells) has been developed in our laboratory for investigating

the direct role of a cell-surface lectin in homologous cell-cell adhesion in a mammalian tissue [16].

During epididymal maturation, spermatozoa pass through caput and corpus parts of epididymis, and the mature sperm are stored in the cauda end of the tissue. When incubated in a chemically-defined medium, the maturing spermatozoa derived from ram corpus-epididymis autoagglutinate by some unknown mechanism [17]. In case of guinea pig, sperm–sperm adhesion occurs during epididymal maturation, which results in the formation of rouleaux *in vitro* [18]. Upon incubation in a chemically defined medium, goat epididymal spermatozoa undergo autoagglutination during epididymal transit at distal corpus stage [19, 20]. Further studies have demonstrated that divalent calcium ion is essential for this cellular agglutination process and its optimal concentration is 1 mM [21]. A Ca^{2+} dependent galactose-specific lectin is located on distal corpus sperm surface that specifically interacts with its receptor of the neighboring homologous cells to cause this autoagglutination event. Failure of the pre- and post-distal corpus sperm to show this cell-cell agglutination is due to lack of the Ca^{2+}-dependent lectin molecule and its receptors, respectively. Maturing sperm at distal corpus stage acquire the lectin molecule; this is followed by sharp disappearance of its receptor, and this event is synchronously associated with the initiation of sperm forward motility that is essential for fertilization *in vivo* [21].

As reviewed above, earlier workers have considered the role only of Ca^{2+} in the autoagglutination of the maturing epididymal sperm. Consequently, occurrence and isolation of Ca^{2+} dependent galactose-specific lectin on maturing sperm surface had been presented. To this date there is no report on the autoagglutination of the mature sperm. A vast range of reported and unreported glycoproteins are present in mature sperm surface glycocalyx. This glycoproteome could be modulated with altering external microenvironment or event specific biochemical signals. In the present study, the effects of multiple divalent metal ions including Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} on the autoagglutination of mature caprine epididymal sperm have been investigated. This study reports for the first time that Cu^{2+} has high specificity for autoagglutination of mature epididymal sperm and this autoagglutination event is mediated by a novel Cu^{2+}-dependent sialic acid-specific lectin located on the mature sperm surface.

Materials and methods

Chemicals

Sialic acid [*N*-acetylneuraminic acid (Neu5Ac)] (Code A2388), D-raffinose (Code R0250), D-galactose (Code G0750), L-arabinose (Code A91906), fetuin (Code F3004), polyethylene glycol (Code D 81300), and dextran (Code D 7265) [avg. mol. wt. 228 kDa] were purchased from Sigma, USA. D-mannose (Code 134917), *N*-acetyl-D-glucosamine (Code 0148204), and *N*-acetyl-D-galactosamine (Code 0149202) were obtained from Sisco Research Lab, India. D-glucose, $Cu(II)SO_4.5H_2O$, $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, and $Mn(II)SO_4.4H_2O$ were procured from Merck, Germany. Asialofetuin (Sigma A4781) was received from Dr. Kalyan K. Banerjee, Biochemistry division, NICED.

Fresh epididymides of adult goats were obtained from the local slaughterhouse. Spermatozoa were extracted from these tissues within 2–4 h of slaughtering.

Isolation of spermatozoa

Mature caudal sperm cells from caudal region of goat epididymis were isolated by the method of Mandal *et al.* [22] with minor modifications. Spermatozoa were extracted at 37 °C in a modified Ringer solution (RPS medium without calcium) containing 119 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 10 mM D-glucose, and 16.3 mM K-phosphate at pH 7.5 and penicillin 50 units/ml. Cells were washed twice at 250 × *g* and finally dispersed in the same buffer. The sperm preparation was highly pure as judged by phase contrast microscopy.

For sperm erythrocyte interaction, sperm cells were extracted in PBS (pH 7.4). This nullified the probability of the effect (if any) of other divalent metal ions (except copper) on sperm erythrocyte interaction.

Isolation of sperm plasma membrane

Sperm plasma membrane was isolated according to the method of Rana *et al.* [23] with minor modification. In brief, the intact spermatozoa were subjected to hypotonic shock with 1.25 mM EDTA and the suspension was stirred constantly at 4 °C for 1 h. Then the sample was centrifuged at 18,000 × *g* for 15 min and the supernatant fluid was discarded. The pellet so obtained was dispersed in a two phase polymer system (1:1 by volume) consisting of a lower dextran phase (MW 228 kD) and an upper poly ethylene glycol phase (MW 20 kD). The suspension was centrtifuged at 9,700 × *g* for 30 min and the plasma membrane was collected from the interphase. The isolated fraction was dialyzed in 50 mM Tris–HCl of pH 7.2 to remove EDTA and dissolved in the same buffer. The sperm membrane fractions thus obtained, devoid of cytosolic content, were measured with Bradford reagent under standard condition and used within 30 min of preparation for haemagglutination assay.

Isolation of erythrocytes

Caprine whole blood samples were obtained from local slaughterhouses. Erythrocytes were isolated from whole blood

samples using an established procedure [21]. Briefly, whole blood collected in the presence of 0.1 % heparin was centrifuged in microfuge tubes (300 × *g*, 5 min). Leukocytes in the buffy coat were aspirated off. The red blood cell pellet was resuspended in sterile phosphate-buffered saline (PBS; pH 7.4) and centrifuged again (300 × *g*, 5 min). After removal of the supernatant, this washing procedure of resuspension, centrifugation, and supernatant removal was repeated twice more. The erythrocytes were then diluted to 1–2 % (v/v) in sterile PBS (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) and stored at 4 °C. All erythrocyte preparations were used in binding studies within 48 h of their isolation. Native caprine erythrocytes were used for haemagglutination and sperm-erythrocyte interaction assay. About 4×10^6 erythrocyte cells were used to observe haemagglutination and for sperm-erythrocyte interaction assay in presence of Cu^{2+}.

Assay of sperm autoagglutination

Sperm autoagglutination was measured by the method of Banerjee *et al.* [20]. Briefly, washed intact caprine mature spermatozoa free of epididymal plasma were tested for their efficacy to undergo autoagglutination in presence of 0, 200, 300, 400, 500 and 1,000 μM of Cu^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} dispersed in RPS medium. The standard assay medium contained 2×10^6 sperm cells in a total volume of 0.5 ml RPS medium and was incubated at 37 °C for 30 min. Cell autoagglutination characteristics were measured periodically under phase contrast microscope at 400× magnifications. The percentage of autoagglutination was calculated. Dose courses of Mg^{2+} were performed following the same protocol stated above except that RPS devoid of $MgSO_4$ was used. All experiments were performed in triplicate. In our laboratory caudal sperm cells were extracted in RPS medium containing 1 mM $CaCl_2$ for routine analysis [22, 24]. As no observable agglutination was seen during those studies we decided not to investigate the effect of Ca^{2+} (0–1 mM) dose course on mature sperm cell autoagglutination.

Haemagglutination assay

Sperm membrane fractions were isolated from the aqueous two phase polymer separation system. Membrane proteins, free from EDTA, were taken in 50 mM Tris–HCl (pH 7.2) and used in this assay. The reaction mixture contained 2×10^6 washed erythrocytes along with 0, 0.1, 0.2 and 0.4 mg/ml membrane protein in a total volume of 500 μl PBS (pH 7.4) solution in presence of 300 μM Cu^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}. Assay mixture devoid of plasma membrane was taken as control. Assays were done at 37 °C and degree of agglutination was examined timely for 1 h span. Haemagglutination activity was evaluated as the percentage of erythrocytes agglutinated after incubation for 30 min at 37 °C.

Sperm-erythrocyte binding assay

Washed mature caudal spermatozoa were extracted in PBS (pH 7.4). Around 2×10^6 sperm cells were mixed with 4×10^6 two-times washed erythrocytes in a total volume of 500 μl of PBS (pH 7.4) solution at 37 °C for 30 min in presence or absence of 300 μM Cu^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}. The sperm erythrocyte interactions were investigated at 600× by Olympus phase contrast microscope (CH 30). Images of RBC-bound spermatozoa were acquired through a camera attached to it.

Agglutination inhibition assay

The anti autoagglutination potency of different sugars (D-glucose, D-raffinose, L-arabinose, D-galactose, D-mannose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and sialic acid), fetuin or asialofetuin were estimated using goat epididymal mature sperm cells as the autoagglutination model according to the method of Banerjee *et al.* [5]. In sialic acid inhibition assay small amount of sodium bicarbonate was mixed to maintain the pH. Intact washed spermatozoa (10^6) were isolated in D-glucose-free RPS medium and incubated with 0, 10, 20, 30, 40, 50 mM of sugars or 0, 0.5, 1, 2, 3, 4 μM fetuin/asialofetuin in a total volume of 0.5 ml RPS medium at 37 °C for 15 min in presence of 300 μM Cu^{2+}. The degree of sperm aggregation was then assessed as described above. Anti autoagglutination potency of the sample was expressed as its efficacy (%) to inhibit sperm auto agglutination phenomenon after incubation for 1 h at 37 °C.

Statistical analysis

The results were expressed as means ± SEM. The data were statistically analyzed by Microcal Origin-pro version 8.0 (USA). Significance was tested using Student's t test.

Results

Effects of divalent metal ions on sperm agglutination

Divalent cations, *viz*. Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} (0–1 mM) were studied for their role in sperm cell autoagglutination. The spermatozoa efficiently autoagglutinated when the concentration of Cu^{2+} was 300 μM. The percentage of agglutination increased with increasing concentration of the metal ion, reaching the saturation point at a concentration of 500 μM. Concentrations of Cu^{2+} higher than 500 μM did not promote further increase in agglutination. A low amount of non specific agglutination (no sugar dependent inhibition) was obtained at a higher concentration of Zn^{2+} (1 mM). Mg^{2+} and Mn^{2+} (0–1 mM) had no effect. At 300 μM concentration, only Cu^{2+} exerted appreciable effect on caudal

cell autoagglutination (Fig. 1); at 400 μM sperm cell autoagglutination started after 10 min and around 65 % cells autoagglutinated within 30 min of Cu^{2+} addition. Maximum autoagglutination (around 85 %) was achieved at 500 μM Cu^{2+} ($p<0.001$). The type of autoagglutination was random, although head to head interactions were predominant.

Effect of different sugars on sperm auto agglutination

Several sugars were tested for their anti-agglutination efficacy with a view to finding out the biochemical reasons behind the sperm-sperm adhesion process. D-glucose, D-raffinose, L-arabinose, D-galactose, D-mannose, *N*-acetyl D-glucosamine, and *N*-acetyl D-galactosamine at a concentration range of 0–50 mM did not possess appreciable anti-agglutination potency. However, sialic acid (neutralized with bicarbonate) was found to be an effective inhibitor of Cu^{2+} dependent mature sperm-sperm adhesion on the above stated concentration range (Fig. 2). The sialic acid mediated inhibition was dose dependent and maximal inhibition (approx 90 %, $p<0.001$) was noted at 50 mM concentration (Fig. 3). This data strongly supports the possibility of existence of sialic acid specific lectin in mature sperm cell membrane, which is activated in presence of copper. It was found to bind with its sialic acid containing receptor that is present on the sperm cell surface to promote cell-cell adhesion.

Interaction of mature sperm with erythrocytes

As shown in Fig. 4, erythrocyte was bound to sperm head region. Like sperm-sperm adhesion, sperm-erythrocyte interaction also took place in presence of 300 μM Cu^{2+}. In the reaction mixture erythrocyte was taken in excess in comparison to the number of sperm, so that sperm–erythrocyte interaction was predominant. The interaction was observed within 10 min of incubation. Image was taken with phase contrast microscope at 600× magnification. Microscopic data clearly displayed that sperm cell bound with erythrocyte maintained their horizontal movement. This observation confirmed that sperm-erythrocyte interaction was mediated by adhesion and was not due to direct physical contact. Sialic acid (50 mM)

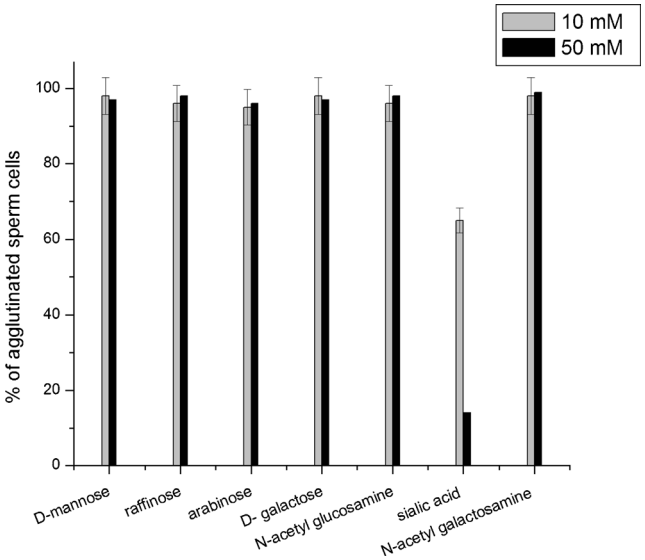

Fig. 2 Effects of different sugars on autoagglutination of mature sperm cell. Spermatozoa were isolated in RPS medium without D-glucose. Agglutination studies were performed under standard assay conditions except for the addition of various sugars at concentrations of 10 mM and 50 mM. The data were mean ± SEM of three different experiments, $p<0.001$

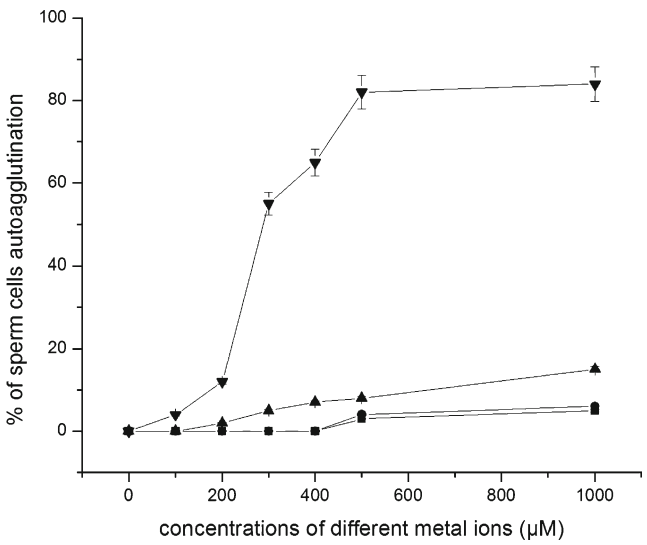

Fig. 1 Dose courses of Mg^{2+} (■), Mn^{2+} (●), Zn^{2+} (▲), and Cu^{2+} (▼) on mature sperm cells. Data represents autoagglutination after incubation for 30 min under standard assay condition. The data were mean ± SEM of three different experiments, $p<0.001$

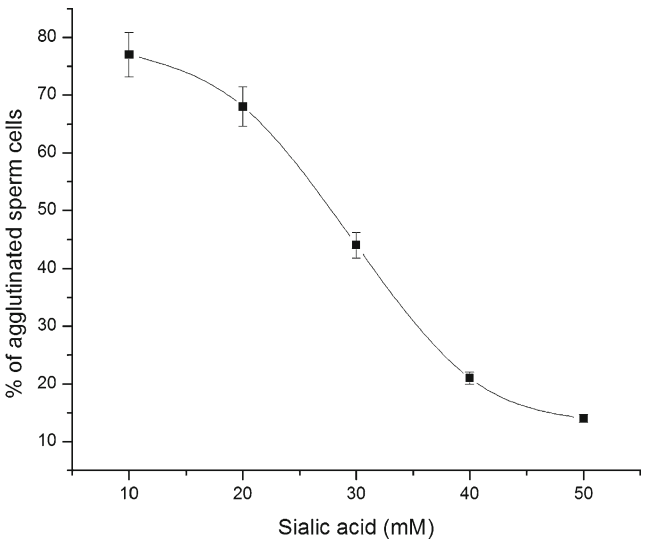

Fig. 3 Dose course of sialic acid on the autoagglutinated matured spermatozoa under standard assay condition. Anti autoagglutination potency was measured by the procedure described in "Materials and methods" section. The data were mean ± SEM of three different experiments, $p<0.001$

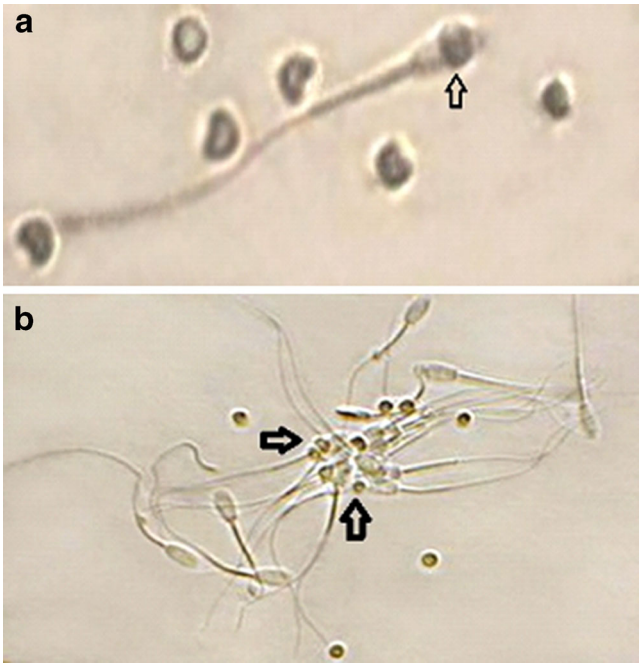

Fig. 4 Sperm-erythrocyte interaction study in presence of 300 μM Cu^{2+} after 10 and 30 min of incubation at 37 °C. Erythrocytes and mature spermatozoa were isolated by the procedure described in "Materials and methods" section. Micrographs were taken at 600× magnification. **a** was magnified for better vision. After 30 min of incubation sperm cells already attached with erythrocyte had a tendency to undergo self agglutination (**b**). The attachments of sperm cells and erythrocyte were indicated with *arrowheads*

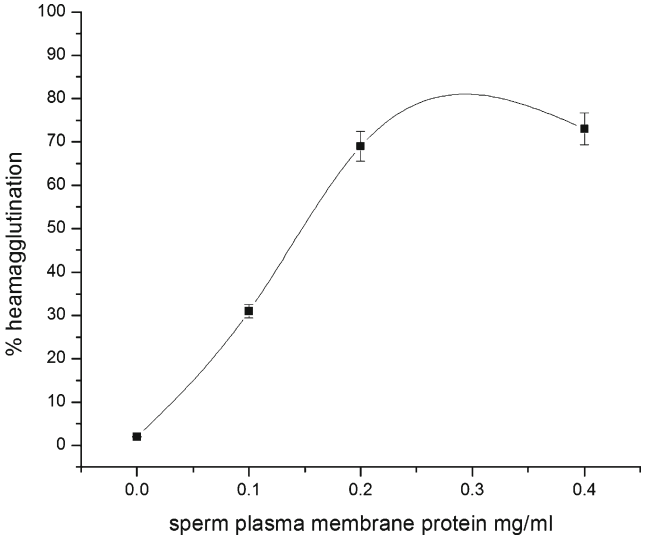

Fig. 5 Dose dependent haemagglutination of sperm plasma membrane after incubation for 30 min at standard assay condition. Protocol described in "Materials and methods" section. The data were mean ± SEM of three different experiments, $P<0.001$

when present in the assay medium markedly inhibited sperm-erythrocyte cell adhesion but other tested sugars had little influence on this cell-cell adhesion process (data not shown). Mg^{2+}, Mn^{2+} and Zn^{2+} up to 1 mM concentration did not provide any reportable data regarding sperm erythrocyte interaction.

Isolated sperm plasma membrane at 0.2 mg/ml concentration effectively agglutinated erythrocytes (Fig. 5a). Cu^{2+} alone was not able to agglutinate erythrocytes at 300 μM concentration after incubation for 1 h (Fig. 5b), although RBC agglutination did take place (data not shown) at higher concentration of Cu^{2+} (above 500 μM). After that, slight increment in % of agglutination occurred with the augmented membrane protein concentration (Fig. 6). The agglutinating activity is time dependent and occurred only in presence of Cu^{2+}. Around 75 % of the cells got agglutinated after 30 min of the reaction (Fig. 7). Sperm plasma membrane was unable to promote haemagglutination when co-incubated with 50 mM sialic acid in presence of Cu^{2+} (data not shown).

Effect of fetuin and asialofetuin on sperm cell autoagglutination

Fetuin is a glycoprotein containing sialic acid as the terminal sugar residue and asialofetuin, which is derived from fetuin by removing its terminal sialic acid, contains terminal galactose sugar. Dose course of anti-agglutination potency of fetuin and asialofetuin was examined at micro molar range on copper treated mature sperm cell. Mature sperm cells were incubated with different concentrations of fetuin and asialofetuin for 10 min at room temperature, then treated with 300 μM Cu^{2+} and incubated for further 60 min. Interestingly, fetuin significantly reduced the percentage of agglutinated cells ($p<0.001$) with increasing concentration. At 4 μM concentration, it completely (88 %) blocked sperm cell adhesion (Fig. 8). The phenomenon is reversible, *i.e.* when fetuin was removed from the assay medium by normal centrifugation, agglutination could be recovered by treatment of 300 μM Cu^{2+}. However asialofetuin at the same concentration (4 μM) was merely able to prohibit sperm autoagglutination by 11 % (Fig. 8). The result emphasized the sialic acid dependence of this autoagglutination.

Discussion

The present study demonstrates that Copper (Cu^{2+}) promotes head-to-head autoagglutination of intact mature caprine spermatozoa. It is possible that Cu^{2+} may interact electrostatically with intact spermatozoa, thereby causing neutralization of cell surface charges and subsequent self aggregation of cells. This, however, appears unlikely because other bivalent metal ions like Mg^{2+}, Mn^{2+} and Zn^{2+} showed relatively lower or undetectable affinity for sperm cell autoagglutination (Fig. 1). To elucidate the mechanism of this cell-cell adhesion process, the roles of multiple sugars in this autoagglutination event were

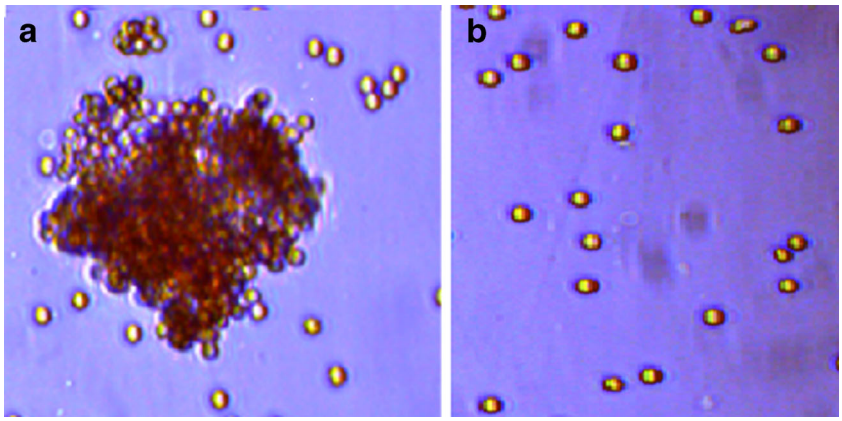

Fig. 6 Agglutination of goat erythrocytes with isolated sperm plasma membrane. Erythrocytes were isolated and agglutination studies were carried out by the procedure described in "Materials and methods" section. Micrographs of erythrocytes at 600×magnification; 6 **a** Erythrocytes+sperm membrane (0.2 mg / ml)+300 μM Cu^{2+}, 6 **b** Erythrocytes+300 μM Cu^{2+} (control)

tested. Of the sugars tested only sialic acid (Fig. 2) showed high efficacy to block the sperm-sperm autoagglutination phenomenon in a dose dependent manner (Fig. 3). The finding provides preliminary evidence supporting the occurrence of a Cu^{2+}-dependent sialic acid-specific lectin and its receptor on the external surface of mature cauda-epididymal spermatozoa. Lectins usually promote multivalent carbohydrate interaction [25, 26]. Here the lectin of one sperm cell possibly binds with the receptor molecules of the neighboring sperm cells and amplification of such interaction across the multiple neighboring cells lead to formation of variable sizes of cell clusters. This view is supported by the observation that intact live spermatozoa (Fig. 4) as well as highly purified sperm membrane (Fig. 5) efficiently agglutinate erythrocytes and these cellular agglutination events are sensitive to the action of exogenous sialic acid. Clark *et al.* [27] reported that sperm-erythrocyte interaction needs carbohydrate specificity. Caprine mature sperm cell alone was not capable of interacting with erythrocytes; but interaction occurred readily after addition of Cu^{2+} (Fig. 4). Isolated sperm plasma membrane also has copper dependency to execute time dependent haemagglutination (Figs. 6 and 7). In presence of Cu^{2+}, the lectin might be unmasked or acquire agglutinating activity by certain biochemical transformations that are able to detect sialic acid containing glycoprotein present on the cell surface of erythrocytes (Fig. 5). The above observation of the occurrence of a Cu^{2+} dependent sialic acid-specific lectin on sperm surface is strengthened further by the finding that fetuin with its terminal sialic acid [28] is a proven inhibitor of sperm autoagglutination whereas asialofetuin (a fetuin derivative lacking sialic acid moiety) does not show any inhibitory effect at all (Fig. 8).

Lectins are multivalent carbohydrate binding proteins; they show a high degree of specific affinity for carbohydrate groups and are widely distributed in plants and animals [26]. The

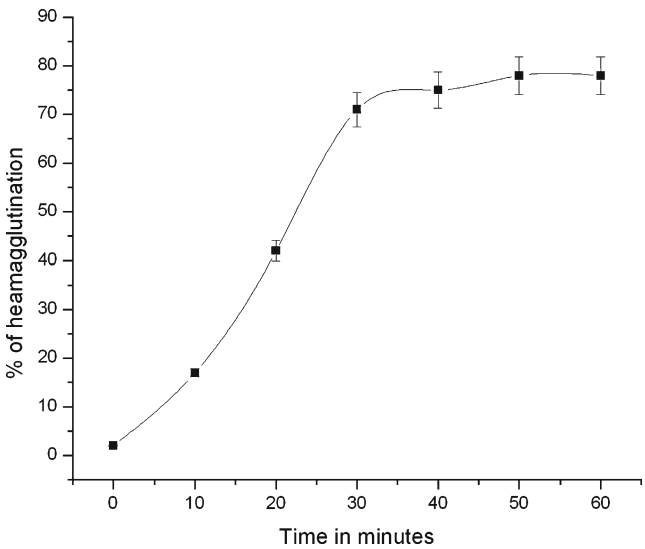

Fig. 7 Time course of 0.2 mg/ml sperm plasma membrane haemagglutination potency at standard assay condition for 60 min. Protocol described in "Materials and methods" section. The data were mean ± SEM of three different experiments, $P<0.001$

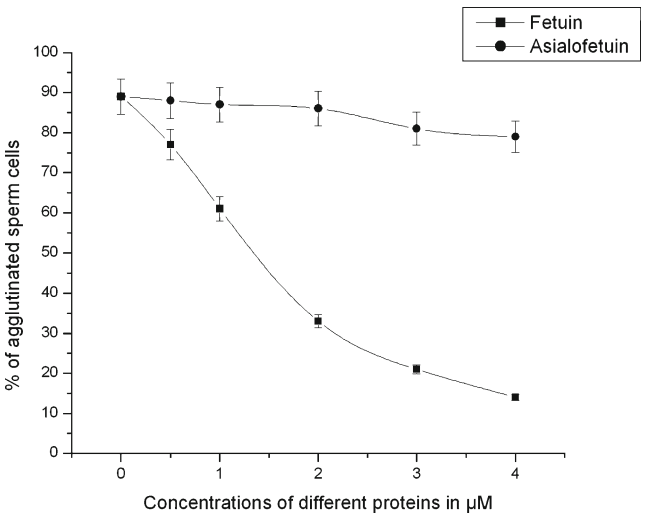

Fig. 8 Dose course of fetuin and asialofetuin (Sigma-Aldrich) on sperm autoagglutination under standard assay condition. Proteins were dissolved in PBS at pH 7.4. Different μM concentrations of fetuin and asialofetuin were included in the assay medium from 200 μM stock solution. The detailed protocol was described in "Materials and methods" section. The data were mean ± SEM of three different experiments, $P<0.001$

present study, using caprine sperm as the cell model, reports for the first time the occurrence of a Cu^{2+}-dependent lectin on a mammalian cell surface. This is altogether a new class of lectin as no Cu^{2+}-dependent lectin has ever been reported. It may be a new variety of selectin, which is sialic acid–specific but is not activated by Ca^{2+}.

Cu^{2+} is an essential micronutrient for all eukaryotic organisms, as it has a markedly positive role in physiological and reproductive processes [29–32]. Copper is essential for the activity of a wide range of enzymes such as copper/zinc-superoxide dismutase, dopamine β-monooxygenase, and lysyl oxidase [33]. As copper possesses high redox potential, it serves as a cofactor for proteins involved in a variety of biological reactions including photosynthesis and respiration, connective tissue formation, iron metabolism, free radical eradication and neurological function [34]. A variety of Cu^{2+} containing metalloenzymes and proteins mediate their actions through this trace element in the biological systems. Based on their functions and biochemical characteristics, copper dependent-proteins have been classified in the following categories: (*i*) Cu bearing proteins (ceruloplasmin, albumin, PrP^{C} etc.), (*ii*) cuproenzymes (Cu/Zn superoxide dismutase, cytochrome c oxidase, lysyl oxidase etc.), (*iii*) Cu transporters (Ctr1, P-type ATPase etc.), and (*iv*) Cu chaperone proteins (metallothionein, amyloid precursor protein [APP] etc.) [35]. The present study introduces a new class of outer cell surface protein, a Cu^{2+}-dependent sialic acid-specific lectin that may participate in the propagation and amplification of the biological functions of copper in the mammalian cells by modulating cell surface lectin-sugar interactions.

Conclusion

Homologous, lectin dependent cell-cell interaction is a comparatively new concept. Several lines of evidence have been presented to support the occurrence of a D-galactose-specific lectin on the outer-surface of liver parenchymal cells that bind with the neighboring parenchymal cells to cause autoagglutination of these homologous cells [16]. The well-known cell surface adhesive proteins [36] may serve as the secondary force to strengthen the homologous cell-cell contacts, triggered by lectin-receptor interactions. The present finding on the involvement of mature sperm surface Cu^{2+}-dependent lectin and its receptor in homologous sperm-sperm aggregation strengthens the above notion further. This novel Cu^{2+}-lectin, one of the possible physiological functions of which is to participate in homologous cell-cell adhesion in the tissues, may as well be present in other mammalian cells also.

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