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CD26 and Adenosine Deaminase Interaction: Its Role in the Fusion Between Horse Membrane Vesicles and Spermatozoa[1]

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ABSTRACT

Membrane vesicles of horse seminal plasma present at their surface a highly specific serine-type protease, dipeptidyl peptidase IV/CD26, a surface antigen known to characterize human prostasomes.

Horse sperm cells expressed at their surface A_1 adenosine receptors (A_1AR) and ecto-adenosine deaminase (ecto-ADA), both detected by immunoblot analysis, whereas CD26 was visualized at the equatorial segment by immunofluorescence microscopy. In addition to CD26, horse membrane vesicles showed ecto-ADA. The fusion process between horse sperm cells and vesicles was evidenced by confocal microscopy, which showed the localization of CD26 at the postacrosomal region and at the midpiece of the spermatozoa after incubation with vesicles. Moreover, a similar localization of CD26 and ecto-ADA on the spermatozoa was evidenced after fusion.

Our results suggest that the interaction CD26/ecto-ADA might be responsible for fusion. Since A_1ARs are said to be second receptors for ecto-ADA to form ecto-ADA/A_1AR complexes, and since horse spermatozoa have A_1ARs at their surface, the interaction CD26/ecto-ADA/A_1AR during the fusion process cannot be ruled out.

INTRODUCTION

The seminal plasma of several mammals contains membrane particles of different anatomic derivation. In humans these particles derive from the prostate by exocytosis and diacytosis [1], whereas in bulls they originate from seminal vesicles [2]. Therefore these particulate elements are named prostasomes in human and vesiculosomes in bovine ejaculates. Membrane vesicles have been described in horse seminal plasma [3, 4]. These vesicles present at their surface a highly specific serine-type protease, which cleaves N-terminal dipeptides from peptides with a proline or alanine at the penultimate position (dipeptidyl peptidase IV, DPPIV/CD26). DPPIV/CD26, a surface antigen found in several cell types, is known to characterize human prostasomes [5]. Thus there is a strong possibility that these horse particles may be of prostatic origin. It has been hypothesized that prostasomes, by closely interacting with spermatozoa, can modify the sperm microenvironment and assist its fertilizing potential [6–9]. Sperm cells were shown to be capable of acquiring the GPI-anchored proteins CD59, CD55, CDw52, and CD46, present at the prostasome surface, partly by the adherence of prostasomes to cells and partly by a second mechanism that may involve micellar intermediates [10–14]. We have already described an event between horse sperm cells and vesicles that can be properly defined as a fusion-like process [4]. This event, starting with the formation of a clearly visible bridge between the two membranes, gradually proceeds to the total embedding of the vesicle in the sperm cell membrane. A fusion between human prostasomes and spermatozoa was also observed [15–17]. This fusion, which seems to be pH dependent, requires at least one protein at the sperm surface, but proteins at the prostasomal surface also seem to be important [17]. Ecto-adenosine deaminase (ecto-ADA) is known to be capable of association with the ADA-binding protein CD26 [18], although the functional role of this ecto-ADA/CD26 complex is not completely clear [19]. CD26 has been thoroughly studied in lymphocytes, where its physiological role seems to be related to cell activation [19]. CD26 has been shown to be an ecto-ADA anchoring protein [18], but neither the protease nor the deaminase activity is required for the association between CD26 and ecto-ADA [20]. It has also been shown that ecto-ADA can interact with A_1 adenosine receptor (A_1AR) and that both proteins are functionally coupled [21, 22]. This was the first evidence of an interaction between a degradative ectoenzyme and a receptor whose ligand is the substrate of the enzyme. The interaction ecto-ADA/CD26 might be important for transducing A_1AR signals [23, 24], and the role of ecto-ADA differs depending on adenosine concentration. Ecto-ADA has been shown to have hydrophobic patches around the active site [21, 25] that might interact with CD26 and A_1AR. In addition, ecto-ADA might participate in neuroregulatory events by interacting with A_1AR [21, 26–29] and CD26 [19]. Ecto-ADA has a role in cell-to-cell contacts that might be essential to the development of lymphoid tissues and to the maturation of lymphocytes via two possible cell-to-cell contacts, i.e., CD26/ecto-ADA/CD26 and CD26/ecto-ADA/A_1AR [19]. Therefore ecto-ADA could be the modulator between different cell types.

Bearing this in mind and because of the presence of CD26 at the surface of the horse membrane vesicles [4] and the existence of A_1AR in the spermatozoa [30], we have hypothesized that the interaction-fusion-like process, already shown to occur between horse spermatozoa and these membrane vesicles, can be mediated by the CD26/ecto-ADA and/or A_1AR interaction.

MATERIALS AND METHODS

Hepes, benzamidine, aprotinin, leupeptin, soybean trypsin inhibitor, BSA, dimethyl sulfoxide (DMSO), fluorescein isothiocyanate, Pronase, *N*-succinyl-$(Ala)_3$-*p*-nitroanilide (Succ-$(Ala)_3$-pNA), Gly-Pro-p-NA, 5-carboxyfluorescein diacetate (CFDA), propidium iodide (PI), and N^6-cyclopentyladenosine (CPA) were from Sigma Chemical Co. (St. Louis, MO). Penicillin G and streptomycin were from Gib-

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co BRL (Oakville, ON, Canada). Acrylamide/*bis* (40%) w:v solution was obtained from Ambion (Austin, TX). Adenosine deaminase was from Boehringer-Mannheim (Indianapolis, IN).

Sephadex G-200, Sephadex G-25M (PD 10), ECL-Plus, Rainbow colored protein molecular weight markers, and (−)-N^6-R-[G-^{3}H]phenylisopropyladenosine (^{3}H-R-PIA) (44 Ci/mmol) were from Amersham Pharmacia Biotech (Rainham, UK). The Bio–Rad Protein Assay kit was from Bio-Rad Laboratories (Hercules, CA). Immuno-fluore mounting medium was from ICN Biomedicals (Costa Mesa, CA). All other reagents were of the highest quality available.

Semen Samples and Sperm Cell Preparations

Horse semen was obtained, using an artificial vagina, from eight stallions of proven fertility, 10–12 yr old, stabled at ARAM (Associazione Regionale Allevatori Marche; Dr. G.M. Corsalini, Contrada Lornano, 9, Macerata, Italy). Ejaculate samples were pooled and centrifuged (800 × *g* for 10 min at 22°C) to harvest spermatozoa, and the supernatant (S1) was used to prepare membrane vesicles. The pellet was suspended in 95.7 mM NaCl, 2.6 mM KCl, 0.7 mM K_2HPO_4, 26 mM $NaHCO_3$, 0.5 mM $MgCl_2$, 12.8 mM glucose, 1.3 mM sodium pyruvate, 7.6 mM sodium lactate, 10 U/ml penicillin G, 0.1 mg/ml streptomycin, 20 mM Hepes, pH 7.4 (Tyrode's modified medium, TALP). Spermatozoa number was determined in a Thoma chamber (Brand Gmbh. Co., Wertheim/Main, Germany), and viability was evaluated by fluorescent microscopy (Olympus CH-2; Tokyo, Japan) with CFDA and PI for each pooled sample.

Preparation of Sperm Membranes

Pooled spermatozoa were washed by centrifugation at 800 × *g* for 10 min at room temperature and suspended in TALP containing 1 μg/ml aprotinin, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, and 5 mM benzamidine. Sperm cells were frozen at −20°C and after thawing were sonicated on ice with six bursts of 30 sec (with 15-sec intervals). The preparation was centrifuged at 70 000 × *g* for 40 min at 4°C and then resuspended in 50 mM Tris-HCl, pH 7.4, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, and 5 mM benzamidine to a final concentration of 5 mg protein per milliliter (Bio-Rad Protein Assay kit) [31].

Preparation of Membrane Vesicles

The supernatant (S1), diluted (1:1, v:v) with 30 mM Tris-HCl and 130 mM NaCl, pH 7.6, was centrifuged at 5000 × *g* for 30 min at 4°C to eliminate cell debris and residual spermatozoa. The new supernatant was then centrifuged at 105 000 × *g* for 2 h at 4°C. The pellet, containing vesicles and amorphous material, was suspended in 30 mM Tris-HCl, 130 mM NaCl, pH 7.6, up to 1.0–1.5 mg of protein per milliliter.

Membrane vesicles were purified from amorphous material by chromatography on a Sephadex G-200 column (1.5 cm × 30 cm) preequilibrated with 30 mM Tris-HCl, 130 mM NaCl, pH 7.6. Membrane organelles, not retained by the column, were collected with the void volume. Fractions, examined for absorbance at 280 nm and endopeptidase activity, as the marker enzyme for prostasomes [32], were pooled and centrifuged at 105 000 × *g* for 2 h. The pellet, suspended in 30 mM Tris-HCl, pH 7.6, was kept at −196°C until use. Vesicle concentration was determined by Bio-Rad Protein Assay kit [4, 7, 31].

Membrane Vesicle Staining

Fluorescein isothiocyanate was dissolved in anhydrous DMSO under N_2 flux at a concentration of 5 mg/ml. Fluorescein isothiocyanate solution (10 μl) was added to vesicles (400 μg); diluted in 50 mM H_3BO_3, 200 mM NaCl, pH 9.2; and then incubated for 2 h at room temperature. Stained vesicles were chromatographed on a PD10 column equilibrated and eluted with PBS buffer, pH 7.4.

Enzyme Activities

Endopeptidase (EC 3.4.2.1) hydrolysing Succ-$(Ala)_3$ p-NA was assayed according to Laurell et al. [33]. Dipeptidyl peptidase IV (DPP, EC 3.4.14.5), hydrolysing Gly-Pro-p-NA, was assayed according to Nagatsu et al. [34]. Protein concentration was determined by Bio-Rad Protein Assay kit [31].

Radioligand-Binding Assay

Radioligand-binding assay was carried out as previously reported [30, 35]. Briefly, prostasome preparations (5 mg/ml) were preincubated with 2 U/ml adenosine deaminase for 30 min at 25°C, pH 7.4, to remove contaminating adenosine. The binding assay was performed in triplicate in a total volume of 600 μl containing 40–300 μg of vesicle preparation, 1–40 nM ^{3}H-R-PIA, 50 mM Tris-HCl, 1% DMSO, pH 7.4, and 100 μM of adenosine agonist CPA to compete with ^{3}H-R-PIA binding. After incubation at 25°C for 3 h, the assay was terminated by filtration on Whatman GF/B (Clifton, NJ) glass fiber filters using a modified cell harvester (Cell Harvester M-24R; Brandel, Gaithersburg, MD). The filters were presoaked in 0.3% polyethylenimine (2–4 h, pH 10) to improve the filtration process. Filter disks, washed 5 times in 3 ml of ice-cold 50 mM Tris-HCl, pH 7.4, were allowed to elute overnight in 2.5 ml of scintillant (Ultima Gold; Canberra Packard, Pongbourne, Australia) and counted in a Packard Tri-Carb scintillation counter at 60% efficiency.

Antibodies

Affinity-purified polyclonal antipeptide antibody against A_1AR (PC21) and affinity-purified polyclonal anti-ADA antibody were a gift from Prof. Franco (Departament de Bioquimica y Biologia Molecular, Universitat de Barcelona, Spain) and have been characterized elsewhere [36, 37]. Fluorescent monoclonal anti-CD26 Ta1 antibody was purchased from Coulter Clone, Coulter Immunology, Hialeah, FL [38, 39]. Rabbit IgG and fluorescent rabbit IgG were from Sigma.

Western Blotting

Sperm membrane preparations (20 μg) and membrane vesicles (40 μg) were added to electrophoresis sample buffer. Samples, analyzed at constant voltage at 180 mV for 40 min on 12% polyacrylamide gels, were transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad) using a constant voltage of 100 V for 60 min. Nonspecific sites were blocked by incubating the blots overnight at 4°C in 10% (w:v) low-fat dried milk in 50 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.4 (TBST-I), containing 0.1% NaN_3. Blots were washed twice with 10 mM Tris, 500 mM

NaCl, 0.5% Tween 20, pH 7.4 (TBST-II), and incubated for 2 h at room temperature with specific antibodies (PC21 or anti-ADA) diluted at concentration of 10 μg/ml in TBST-I containing 0.1% NaN_3. The PVDF membrane was washed four times with TBST-II and incubated for 60 min at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG diluted 1:10 000 in TBST-I. The blot was washed four times with TBST-II, then revealed by the ECL-Plus detection system. Molecular weight was determined by Rainbow colored protein molecular weight markers (low molecular weight range). Nonimmune rabbit IgG were used as negative controls.

Confocal Microscopy

Fluorescein-conjugated membrane vesicles were incubated with sperm cells at 37°C at pH 7.4 for various intervals up to 30 min. The mixture was centrifuged at 800 × *g* for 10 min and washed twice with TALP; the cells, resuspended in 50 μl of immuno-fluore mounting medium (ICN Biomedicals) and mounted on a slide, were used for microscopy observations.

Sperm cells, after incubation with vesicles, were adhered to glass coverslips, rinsed in PBS, fixed, and permeabilized with methanol at −20°C for 3 min.

These preparations were washed in PBS and incubated for 15 min with PBS, 1% BSA, 0.05% NaN_3. Fluorescent anti-CD26 staining was performed with anti-CD26 Ta1-rhodamine-conjugated RD1 (50 μg/ml). Double immunofluorescence staining was performed by treatment with a mixture of two antibodies (45 min, 37°C), i.e., anti-CD26 Ta1-fluorescein isothiocyanate conjugated (FITC) (50 μg/ml) and anti-ADA-tetramethylrhodamine isothiocyanate conjugated (TRITC) (70 μg/ml). The coverslips were then rinsed for 40 min in PBS, 1% BSA, 0.05% NaN_3 and mounted with immuno-fluore mounting medium. Negative controls were obtained by treating the preparations with nonimmune rabbit FITC- and TRITC-antibodies. Microscope observations were performed with a Leica TCS 4D (Leica Laser TechniK GmbH, Heidelberg, Germany) confocal scanning laser microscope adapted to an inverted Leitz (Leitz Wetzlar GBH, Wetzlar, Germany) DMIRBE microscope.

RESULTS

ADA, CD26, and A_1ARs in Sperm and Extracellular Organelles

Ecto-ADA, CD26, and A_1AR were shown in horse sperm and in horse seminal plasma membrane vesicles by means of immunostaining, immunoblotting, and/or binding assays. Horse sperm cell membranes, treated with PC21 and anti-ADA antibodies, showed the existence of A_1AR and ecto-ADA (Fig. 1; lanes A and B). Western immunoblots showed the existence of ecto-ADA in the membrane vesicles (Fig. 1; lane C). When treated with nonimmune rabbit IgG, these samples did not produce any immunostained band (negative controls). Ecto-ADA was also recognized in sperm cells by immunocytochemical analysis with anti-ADA-TRITC antibody (Fig. 2a). CD26 on sperm cells was detected by immunocytochemical analysis with anti-CD26 Ta1-RD1 monoclonal antibody (Fig. 2b). Western blotting of CD26 was not performed with the fluorescent antibody.

No A_1AR was found by Western immunoblotting on horse seminal plasma extracellular organelles. This result was confirmed by the lack of specific ^{3}H-R-PIA binding to a preparation of membrane vesicles (data not shown). Owing to their small size, it was not possible to perform immunostaining experiments or phase-contrast observations with these membrane vesicles.

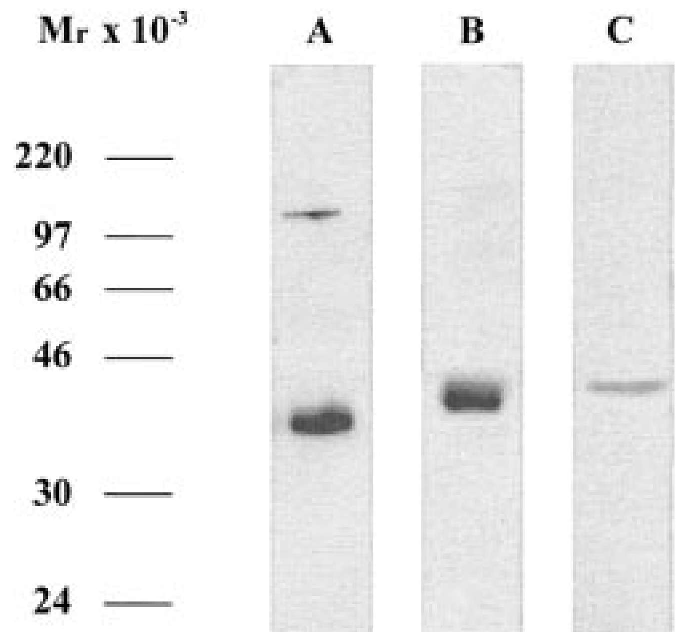

FIG. 1. Immunochemical detection of ADA and A_1AR. Sperm membrane preparations (20 μg) were analyzed by SDS-PAGE. PVDF membrane was treated with PC21 antibody (10 μg/ml; lane A) and anti-ADA antibody (10 μg/ml; lane B). Membrane vesicles (40 μg) were analyzed by SDS-PAGE and PVDF membrane treated with anti-ADA antibody (10 μg/ml; lane C). Immunoreactive bands were visualized by horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10 000) by ECL-Plus detection system.

Sperm/Extracellular Organelle Fusion

A precocious diffuse fluorescence localized at the midpiece was shown by horse sperm cells after incubation at 37°C and pH 7.4 for 10 min with fluorescein-conjugated vesicles (Fig. 3a). After 30-min incubation, i.e., at the time the fusion process has been completed, horse spermatozoa showed a diffuse fluorescence localized at the midpiece and at the postacrosomal region, where the fusion starts later (Fig. 3b).

Punctuate fluorescence, clearly visible at the principal segment of the acrosomal region, suggested a binding/adhesion process that did not proceed to fusion. After fusion with the membrane vesicles stained with anti-CD26 Ta1-RD1, sperm cells showed the fluorescence distribution observed with fluorescein-conjugated vesicles (Fig. 3c).

When vesicles stained with anti-CD26 Ta1-RD1 were added to horse sperm cells, no fusion could be detected; i.e., sperm cells did not acquire any fluorescence whatsoever.

Sperm cells immunostained with anti-CD26 Ta1-FITC and anti-ADA-TRITC after fusion with the vesicles showed the diffuse fluorescence indicative of true fusion as well as the localization of CD26 (green fluorescence) and ADA (red fluorescence) in the midpiece and in the postacrosomal region (Fig. 4).

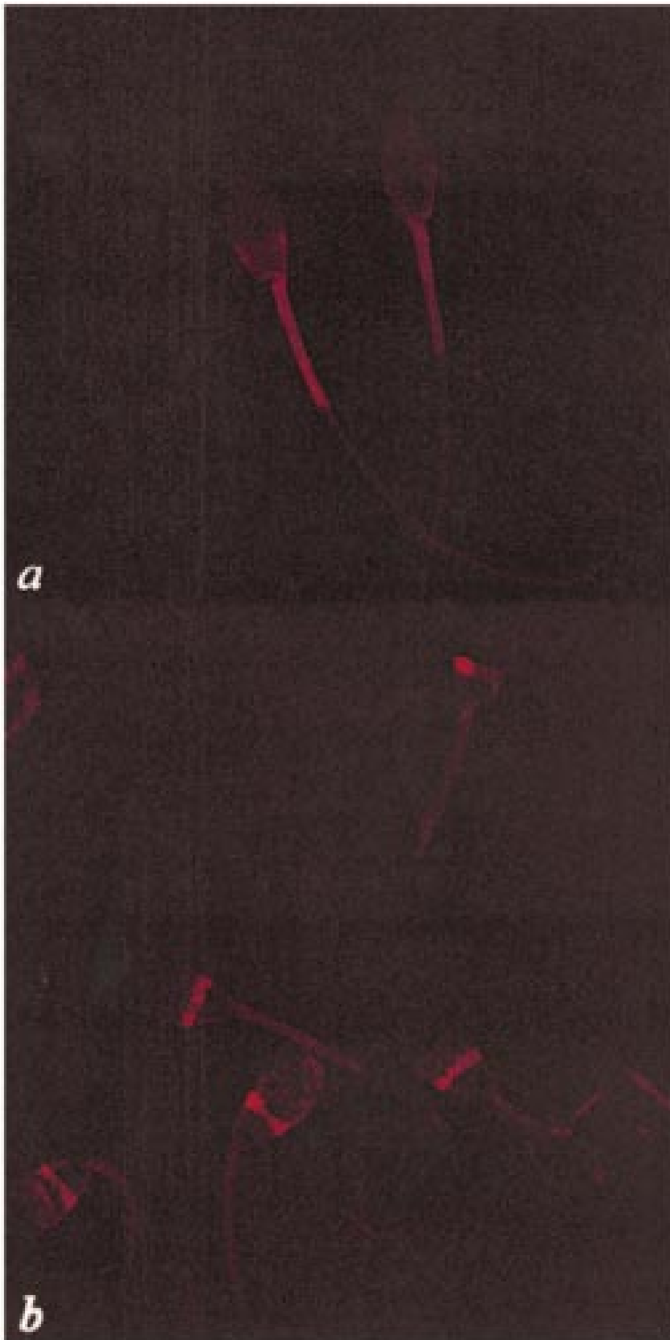

FIG. 2. Expression of ecto-ADA and CD26 in horse sperm cells. Spermatozoa were adhered to glass coverslips, rinsed in PBS, fixed, and permeabilized with methanol at −20°C for 3 min. Immunofluorescence staining was performed, as described in *Materials and Methods,* by using an anti-ADA-TRITC (70 μg/ml) antibody (**a**) and anti-CD26 Ta1-RD1 (50 μg/ml) antibody (**b**). Fluorescence at the cell surface was observed by confocal microscopy. Representative images corresponding to a horizontal section at the middle of the cell are shown.

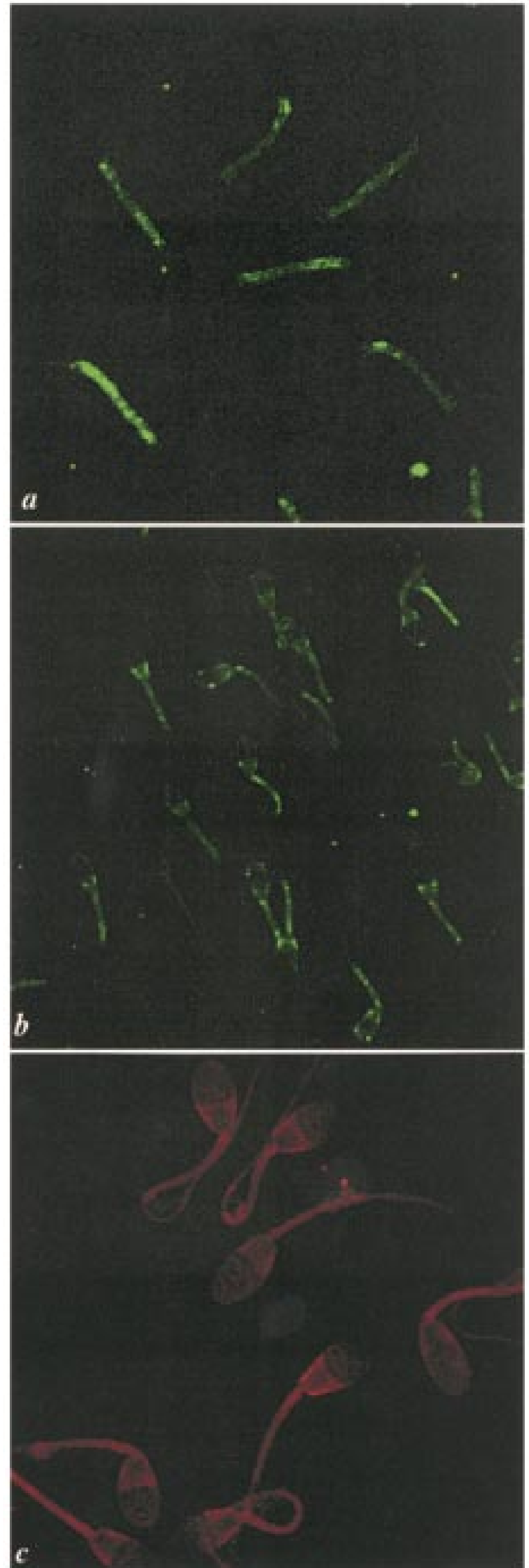

FIG. 3. Fluorescence labeling patterns of horse sperm cells after incubation with membrane vesicles. Fluorescein-conjugated membrane vesicles were incubated with sperm cells at 37°C and pH 7.4 for 10 min (**a**) and 30 min (**b**). Sperm cells, rinsed with TALP, were resuspended in the immunofluorescence mounting medium and mounted on a slide for confocal microscopic observations. Fluorescent anti-CD26 staining was performed with anti-CD26 Ta1-RD1 (50 μg/ml) (**c**). Sperm cells after incubation with vesicles were adhered to glass coverslips, rinsed in PBS, fixed, and permeabilized with methanol at −20°C for 3 min.

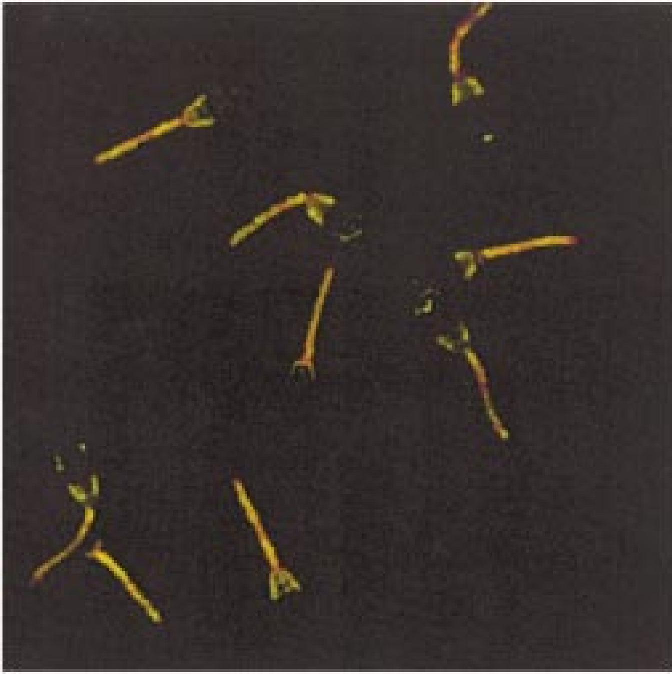

FIG. 4. Localization of ADA and CD26 on horse sperm cells after fusion with membrane vesicles. Sperm cells, after incubation with vesicles at 37°C and pH 7.4 for 30 min, were adhered to glass coverslips, rinsed in PBS, fixed, and permeabilized with methanol at −20°C for 3 min. Double immunofluorescence staining, as described in *Materials and Methods,* was performed by treatment with a mixture of anti-CD26 Ta1-FITC (50 μg/ml) and anti-ADA-TRITC (70 μg/ml) at 37°C for 45 min. The coverslips were used for confocal microscopy observations.

DPPIV/CD26 Activity During Fusion

The transfer of CD26 activity from vesicles to sperm cells during fusion was followed by the measurement of dipeptidyl peptidase (DPPIV) activity using 1 mM Gly-Pro-p-NA as substrate (Table 1). Washed spermatozoa showed scarcely detectable DPPIV activity, whereas membrane vesicles showed high activity that was not affected by Pronase treatment (800 μg/mg protein). DPPIV activity was transferred to sperm cells upon incubation with the vesicles at 37°C and pH 7.4 for up to 30 min. There was evidence of a time-dependent transfer, since the maximum of enzyme activity in the sperm was reached after 20 min. At this time of incubation, the activity in sperm did not increase (data not shown).

In sperm cells treated with a relatively high concentration of Pronase (2.6 μg/10^6 cells), the transfer of enzyme activity was lowered by 40%. The concomitant treatment with Pronase of sperm cells and membrane vesicles lowered the enzyme activity transfer by 50%. The treatment of sperm cells with 6 μg of anti-ADA antibody resulted in a 25% reduction of enzyme transfer. On the other hand, when 1 mg of commercial ADA from calf intestine was added to sperm cells that were then incubated with the vesicles, the CD26 transfer was reduced by 40%.

TABLE 1. CD26/DPPIV enzyme activity.*

Treatment	U/mg prot/min
Control	
Sperm cells	$5.7 \pm 2.2 \times 10^{-3}$
Membrane vesicles	5.6 ± 1.5
Pronase-treated vesicles	5.6 ± 1.5
After fusion	
Sperm cells	$36 \pm 9 \times 10^{-3}$
Pronase-treated cells	$22 \pm 8 \times 10^{-3}$
Pronase-treated sperm cells and vesicles	$18 \pm 4 \times 10^{-3}$
Anti-ADA treated sperm cells	$27 \pm 9 \times 10^{-3}$
Commercial ADA treated cells	$22 \pm 6 \times 10^{-3}$

* Values are the mean ± SD of 4 experiments in triplicate; statistical analysis was performed by Student's *t* test (paired-two tailed, $p \leq 0.05$).

DISCUSSION

The occurrence of a fusion between horse spermatozoa and membrane vesicles has already been described [4]. This process starts with formation of a bridge and proceeds to the total embedding of the vesicle in the horse spermatozoa membrane.

Our results in this study confirm that horse sperm cells bind to and subsequently fuse with horse prostasome-like vesicles. This fusion process was followed by fluorescence microscopy, since the occurrence of fusion could be readily distinguished from that of binding. The latter shows a punctuate fluorescence pattern, whereas diffuse fluorescence is indicative of true fusion [13, 14]. Measurements of CD26/DPPIV activity transfer were mostly run in parallel. Enzymatic determinations showed that the fusion occurred both at pH 5.5 and pH 7.4, although the extent of the process was lower at pH 7.4. However, considering that the mare vaginal pH is about 8.4, we decided to study the process at the probable physiological pH value. In addition, treatment of both sperm cells and vesicles with Pronase did not completely eliminate the fusion process. Therefore the event described here is certainly different from that reported for human semen. It may be that the characteristics of horse vesicles are different from those of prostasomes, and it is accepted that the use of different types of vesicles may lead to different results [12, 14, 15]. CD26, a cell-surface glycoprotein with known DPPIV activity on its extracellular domain, was found either in the vesicles or in the spermatozoa, although in the sperm cells the expression was so low that the protein could be detected only by immunostaining procedures at the equatorial segment. This area of the sperm cell has already been described as the primary fusogenic domain. The presence of CD26 in the postacrosomal and midpiece regions of the spermatozoa after fusion with vesicles showed that sperm cells acquired the surface antigen that characterizes the seminal vesicles. The temporal fluorescence labeling pattern described in spermatozoa, after incubation with fluorescein-conjugated vesicles, demonstrated the existence of regional differences in the lipidic composition of the sperm cell [40]. These different lipidic distributions might facilitate the binding of the vesicles, although true fusion occurs only in the presence of appropriate protein structures. The importance of a glycoprotein in the binding and subsequent fusion of rabbit seminal plasma membrane vesicles to rabbit sperm cells has been described previously [41].

It is known that CD26, a type II membrane protein with only six amino acids in its cytoplasmic region, must be associated with other signal-transducing molecules. The extracellular domain has been shown to interact both with CD45, a protein tyrosine phosphatase, and with ecto-ADA, proteins capable of functioning in a signal transduction pathway [42].

Moreover, ADA, at the cell surface, is involved in an important immunoregulatory mechanism. This multifunctional molecule may be implicated in cell migration and

human immunodeficiency virus-1-associated loss of CD4+ cells via the process of programmed cell death. Ecto-ADA was coexpressed with CD26 in PHA blast cells and in CD26-transfected Jurkat T-cell lines [43]. It is of great interest (mainly for the physiological implications to be investigated shortly) that in sperm cells, CD26 and ecto-ADA are localized in the same region after fusion with horse vesicles.

Besides the functional role of this hypothetical complex CD26/ecto-ADA, the interaction between CD26 and ecto-ADA seems to be responsible for fusion. This suggestion is supported by the fact that the block of the vesicle CD26 by anti-CD26 monoclonal antibody completely inhibited fusion. CD26 is known to be the ecto-ADA receptor. Therefore the addition of commercial ADA to the sperm cells and the subsequent binding to the CD26 molecules reduced the fusion process. The treatment of sperm cells with anti-ADA polyclonal antibody caused a lower degree of reduction.

It has been suggested [23] that in DDT_1MF-2 cells, the A_1AR may act as a second receptor for ecto-ADA and that an interaction between ecto-ADA and A_1AR occurs at the cell-surface level in intact cells. We have reported the existence of A_1AR at the surface of horse sperm cells. Preliminary studies of their localization (unpublished results) seem to be indicative of such interaction at the sperm cell surface. Therefore the postulated interaction, in which ecto-ADA allows specific contacts between a cell expressing CD26 and another expressing A_1AR, seems to be a plausible event in the fusion process between horse sperm cells and prostasome-like vesicles.

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