Transfer of CD26/dipeptidyl peptidase IV (E.C. 3.5.4.4) from prostasomes to sperm

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Abstract Prostasomes are vesicles present in human semen. They are secreted by the prostate and contain large amounts of cholesterol and sphingomyelin. Some of their proteins are enzymes. Prostasomes are involved in a number of biological functions. In previous papers we demonstrated that lipid can be transferred from prostasomes to sperm by a fusion process occurring at neutral or slightly acidic pH. In this paper we demonstrate that CD26/dipeptidyl peptidase IV, an enzymatic activity absent in sperm, is transferred to sperm from prostasomes. This may be of particular interest since, by this procedure, sperm may acquire new membrane-bound enzymes and modify the catalytic activity of their surface.

Key words: CD26; Membrane fusion; Prostasome; Dipeptidyl peptidase; Sperm

1. Introduction

Prostasomes are vesicles secreted by the prostate gland [1]. They are rich in cholesterol [2], Ca^{2+}, ADP and GDP [3,4]. Moreover, many proteins of their surface possess catalytic activity [3] or are involved in the immune response [5,6]. They also contain synaptophysin [7]. We would cite, among their physiological roles, the enhancement of sperm motility [8] and immunosuppression [9,10].

CD26 is a surface antigen that has been found in several cell types. It is a type II integral membrane protein [11] and possesses a peptidase activity (dipeptidyl peptidase IV). This highly specific serine-type protease cleaves N-terminal dipeptides from peptides with a proline or alanine at the penultimate position [12]. It is present in human semen where it is bound to prostasomes and has been purified from this material, after disrupting vesicles with detergent [13]. Only two aminopeptidases, aminopeptidase P and dipeptidyl peptidase IV, have specificity against the X–Pro motif in trans conformation [14]. This is shared by several cytokines and growth factors [14] and it may serve as a structural determinant for biological activity or as a protection against degradation. Therefore, it is not surprising that dipeptidyl peptidase IV is involved in a number of biological functions [12,14].

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Abbreviations: DMSO, dimethyl sulfoxide; Gly–Pro–4-Me–2-NA, glycyl–proline–4-methoxy–2-naphtylamine; HEPES, *N*-[2-hydroxyethylpiperazine]-*N'*-[2-ethanesulfonic acid]; Mes, 2-[morpholino]ethanesulfonic acid; 4-Me–2-NA, 4-methoxy–2-naphtylamine; PBS, phosphate-buffered saline (1.2 mmol·l^{-1} KH_2PO_4, 8.1 mmol·l^{-1} Na_2HPO_4, 2.7 mmol·l^{-1} KCl, 138 mmol·l^{-1} NaCl,; pH 7.2)

Prostasomes fuse to sperm at neutral or slightly acidic pH values, as shown by the method exploiting the relief of the octadecyl–rhodamine G (R_{18}) self-quenching [15]. The use of R_{18} gives an indication about the transfer of lipid from a membrane to another, but it is not suitable to measure the transfer of other membrane components. The transfer of some CD antigens (not of CD26) between seminal plasma and sperm has been described [6]; in this paper, we study whether the CD26/dipeptidylpeptidase IV is transferred to sperm (that are devoid of it) through the pH-dependent fusion mechanism [15]. This may have two main implications: (a) it would be a proof that not only lipid, but also protein, is transferred by H^+-dependent fusion and (b) the acquisition of a new enzymatic activity may give new properties to sperm and intervene in the processes that lie between the emission of semen and the fertilisation of the ovum.

2. Materials and methods

2.1. Materials

4-Me–2-NA (4-methoxy–2-naphtylamine) and Gly–Pro–4-Me–2-NA (glycyl–proline–4-methoxy–2-naphtylamine) were products of Sigma Chemical Co. (St. Louis, MO). R-phycoerythrin (R-PE)-conjugated mouse anti-human CD26 monoclonal antibody dissolved in PBS (also containing 0.1% (w/v) sodium azide and 0.2% bovine serum albumin) was a product of PharMingen (San Diego, CA). Other reagents were purchased from common commercial sources.

2.2. Sperm and prostasome preparation

Fresh human semen was collected from apparently healthy donors (aged 20–35 years) and left at room temperature to liquefy (30–40 min). Normospermic samples [16] were centrifuged (800×*g* for 10 min) to harvest sperm. The supernatant was used to prepare prostasomes [15]. Sperm and prostasome preparations were immediately used for further procedures. For cytofluorimetric determinations, sperm were collected by a swim-up method [17].

2.3. Determination of dipeptidyl peptidase IV activity

The activity of dipeptidyl peptidase IV was determined by using a synthetic substrate: Gly–Pro–4-Me–2-NA [18]. The incubation mixture contained in a final volume of 130 μl: Tris-HCl buffer (40 mmol·l^{-1}, pH 8.3), the biological material (≈1–2 μg protein) and 1.5 mmol·l^{-1} of substrate (Gly–Pro–4-Me–2-NA in 10 μl of DMSO). The reaction was performed at 37°C for 20 min and stopped by adding 1 ml of citrate buffer (pH 4; 100 mmol·l^{-1}). Fluorescence (ex 340 nm; em 425 nm, slit widths 18 nm) was measured within 1 h with a Jasco spectrophotofluorimeter model FP-920. Controls were made by adding citrate buffer before the substrate. The calibration was performed with a standard solution of 4-Me–2-NA. The response was linear with concentration from 1.5 to 35 μmol·l^{-1}. This range was never exceeded.

2.4. Reaction with the anti-CD26 monoclonal antibody

Sperm (≈1.6×10^6 cells) were mixed to the monoclonal antibody anti-CD26 (labelled with R-phycoerythrin; 20 μl). The mixture was kept at 4°C for 30 min in the dark, washed with 1 ml of PBS also containing 1% (w/v) bovine serum albumin and harvested by centrifugation at 600×g for 10 min. The pellet was suspended in 300 μl of

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the same buffer and assessed cytofluorimetrically. Observations were performed with a FACS Analyser flow cytometer (Becton-Dickinson, Sunnyvale, CA) and the signal emitted on the FL2 channel (585 ± 26 nm) analysed with a Convert 30 program.

2.5. Analyses

Protein was determined as described [19].

3. Results and discussion

3.1. Characterisation of prostasomes

We checked the prostasome preparations used in this work and concluded that they were similar to that utilised by others [1,2,15] as demonstrated by lipid composition and particle size. Prostasomes were very rich in cholesterol and showed a cholesterol/phospholipid molar ratio of about 2. In addition, sphingomyelin accounted for ~50% of lipid phosphorus and phosphatidylcholine for ~11%. The size of prostasomes was determined by quasi-elastic light scattering and it was ~150–200 nm.

3.2. Dipeptidyl peptidase activity

The optimal pH for this activity ranged between 8 and 9. The K_m versus the substrate was 130 μmol·l^{-1} and the activity was linear versus both protein and time in our experimental conditions (Fig. 1). These results agreed with [18]. In addition, the proteolytic activity was inactivated by 90% upon exposure to the anti-CD26 antibody. These findings confirmed that the activity studied in this paper was the E.C. 3.5.4.4. and that it corresponded to the CD26 antigen.

Sperm were practically devoid of activity that was entirely bound to prostasomes, as suggested by the finding that the protein fractions usually discarded during prostasome preparation did not possess any activity.

3.3. Transfer of dipeptidyl peptidase activity

The proteolytic activity was transferred to sperm upon incubation with prostasomes at slightly acidic pH values (Fig. 2). The transfer was the highest at pH 5 and it decreased upon increasing pH values. It was practically absent at pH 8. At pH 5, the amount of transferred peptidase was about 8% in 15 min, with a sperm/prostasome protein ratio of about 0.5 (average value found in human semen). Values below pH 5.0 were not investigated because of sperm sensitivity to acidic milieu. Obviously, adhering prostasomes could interfere. We washed off prostasomes from sperm and found than no decrease of sperm activity (on protein bases) occurred upon

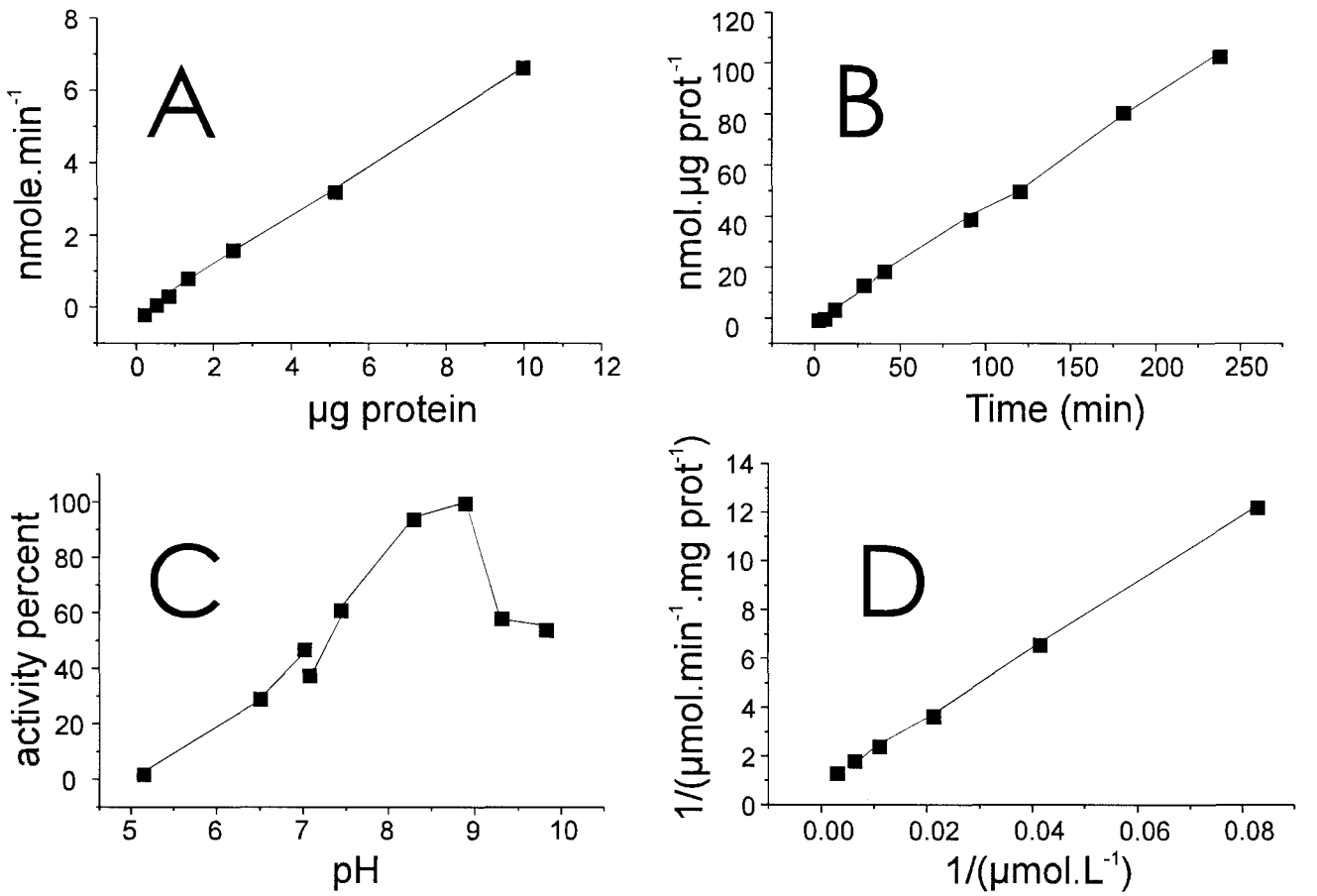

Fig. 1. Some parameters related to the dipeptidyl peptidase IV activity. The activity was measured as described under Section 2. The incubation mixture contained in a final volume of 130 μl: Tris-HCl buffer (40 mmol·l^{-1}, pH 8.3), the biological material (≈1–2 μg protein) and 1.5 mmol·l^{-1} of substrate (Gly–Pro–4-Me–2-NA). The reaction was performed at 37°C for 20 min and stopped by adding 1 ml of citrate buffer (pH 4; 100 mmol·l^{-1}). A: Effect of protein concentration. The final concentration of protein is indicated. Other parameters were left unchanged. B: Effect of time. The reaction was performed for the indicated times. C: Effect of pH. For pH values in the range 7–9.5, Tris-HCl (40 mmol·l^{-1}) and in the range 5–7 Mes (40 mmol·l^{-1}) buffers were used. DL Effect of substrate concentration.

washing more than twice. The very low transfer at pH 8 also confirmed that the phenomenon was pH dependent and that it could not be due to unwashed prostasomes.

3.4. Transfer of CD26 antigen

The transfer of CD26 was studied by using a fluorescent monoclonal antibody directed against human CD26. Upon mixing sperm and prostasomes at slightly acidic pH values, the fluorescence of sperm increased (Fig. 3). This confirmed a H^+-dependent transfer of the antigen form prostasomes to sperm. Therefore the data obtained by measuring the enzymatic activity agreed with those obtained immunologically and the properties of the transfer were similar to those already described for lipid [15].

3.5. Possible physiological roles

Dipeptidyl peptidase IV is an integral membrane protein and therefore its transfer must involve a deep contact between donor and acceptor membranes. No activity was found in the supernatants and therefore it is unlikely that CD26 was transferred through a passage in the soluble fraction of semen, as it has been reported for other proteins [6]. In addition, the transfer was H^+-dependent and it appeared to be connected to H^+-depended fusion [15]. Although the phenomenon was better evident at pH 5 than at higher pH values, it was still present at pH 7. The normal pH of human seminal fluid is 7.6 [20] and the vaginal pH is neutral soon after coitus and becomes more and more acidic as the time passes by [21]. This would permit a transfer in vivo. Many biologically active peptides are substrates for dipeptidyl peptidase IV [14] and therefore, the acquisition of this enzyme may open the way to interesting speculation about the possible physiological role of the H^+-dependent transfer.

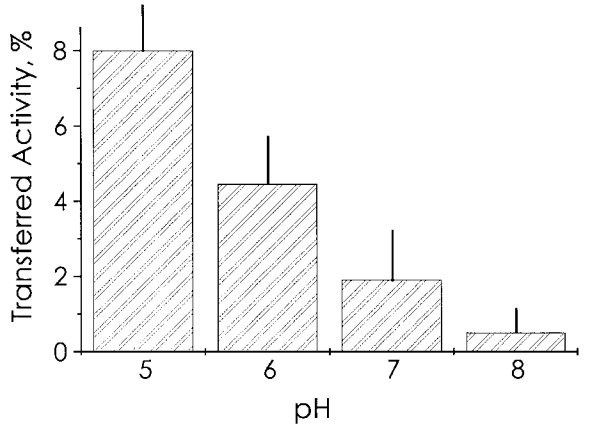

Fig. 2. Transfer of dipeptidyl peptidase IV activity form prostasomes to sperm upon mixing at various pH values. The dipeptidyl peptidase activity was measured in sperm after mixing with prostasomal preparations (sperm/prostasome protein ratio was ≈0.5) for 15 min at the indicated pH values, obtained with the following buffers: 0.32 M sucrose+2 mmol·l^{-1} HEPES (pH 6–8) or 20 mmol·l^{-1} Mes (pH 5–6). Prostasomes were removed from sperm by washing with 0.32 mol·l^{-1} sucrose+2 mmol·l^{-1} HEPES, pH 8, two times. Further washings did not decrease the activity as referred to protein content. Reported results represent the percentage of prostasomal activity transferred to sperm and are the average of three determinations ±SEM (vertical bars).

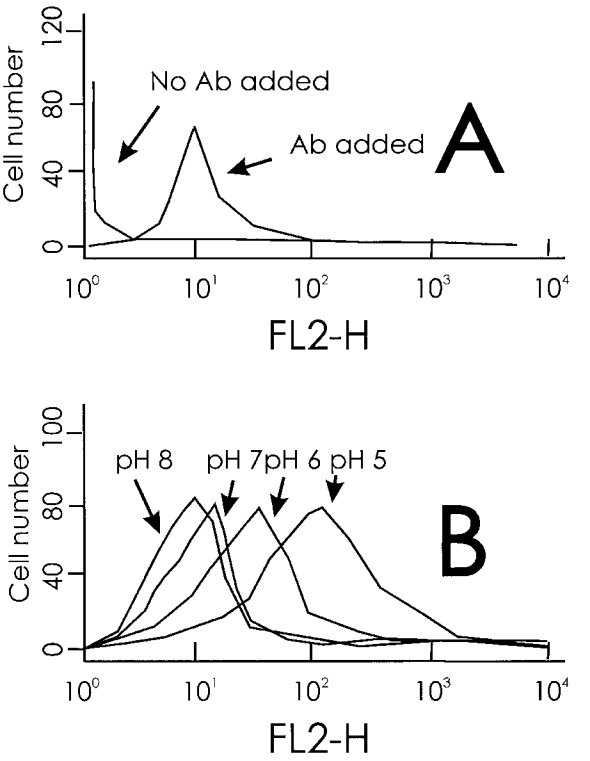

Fig. 3. Transfer of CD26 antigen as measured by the test with anti-human-CD26 fluorescent monoclonal mouse antibody. A: Antibody was added to isolated sperm not mixed to prostasomes. B: Antibody was added after mixing prostasomes and sperm at the indicated pH. Prostasomes were then removed. For details, see the legend to Fig. 2.

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