Biochimica et Biophysica Acta, 551 (1979) 282—294

BBA 78290

VIRAL AND NON-VIRAL INDUCED FUSION OF PRONASE-DIGESTED HUMAN ERYTHROCYTE GHOSTS

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(Received May 30th, 1978)

Key words: Pronase digestion; Membrane fusion; (Erythrocyte ghost; Sendai virus)

Summary

Human erythrocyte ghosts were incubated with the proteolytic enzyme pronase under isotonic (iso-human erythrocyte ghosts) or hypotonic (hypo-human erythrocyte ghosts) conditions. Gel electrophoresis and electron microscope (freeze-etching) studies revealed that most of the erythrocyte membrane polypeptides were hydrolyzed by pronase under hypotonic conditions.

Sendai virus readily agglutinated both pronase-digested iso-human erythrocyte ghosts and hypo-human erythrocyte ghosts but was able to fuse only iso-human erythrocyte ghosts.

Iso- and hypo-human erythrocyte ghosts were fused by the non-viral fusogenic agent glyceromonooleate.

Freeze-etching studies revealed that during fusion the membranes of pronase-digested human erythrocyte ghosts are intermixed.

Introduction

Membrane fusion is a well known phenomenon which may be induced by enveloped viruses of the paramyxovirus group [1], and by non-viral fusogenic agents such as various derivatives of phospholipids [2], phospholipase C [3], polyethylene glycol of different molecular weights [4], or by internal Ca^{2+} (combination of Ca^{2+} and the ionophore A-23187) [5,6].

Based on recent experiments from various laboratories [7,8], it may be suggested that the membrane fusion process is composed of the following steps: (1) Induction of tight contact between membranes destined to fuse; (2)

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; Tos-Arg-OMe, toluene sulfonyl-L-arginine-O-methylester; SDS, sodium dodecyl sulfate; EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetra-acetic acid; PCMBS, *p*-chloromercuribenzene sulfonate.

Exposure of membrane phospholipids of adjacent cells and their mutual melting; and (3) Remasking of the exposed membrane phospholipids and restoration of the original ultrastructure of the cell membrane.

Induction of membrane fusion by enveloped viruses is always preceded by cell agglutination [1], a step which is always brought about by the association between the viral agglutinin (a glycoprotein of mol. wt. 67 000) and sialic acid residues of the cell's membrane [9]. Viruses of the paramyxovirus group fail to agglutinate and to fuse cells from which the sialic acid residues have been removed by treatment with neuraminidase [9]. However, non-viral fusogenic agents such as glyceromonooleate or internal Ca^{2+} promote a higher degree of fusion in desialized cells than in control untreated cells [2,5]. Due to the removal of the negatively charged groups of the membrane sialic acid, the desialized cells can establish a much better contact and thus the fusion process is substantially enhanced. Therefore, it appears that the sialic acid residues and probably the sialoglycoproteins are not directly involved in the membrane fusion process.

Recently we have shown that, as opposed to intact human erythrocytes, human erythrocyte ghosts can only be agglutinated but not fused by *Sendai* virus [10,11]. These results indicate that association between active viral particles and the membrane's sialic acid residues does not necessarily lead to induction of membrane fusion. A certain membrane organization, probably altered during hemolysis, is required to allow the process to occur [11]. Indeed, previous work shows that leakage of hemoglobin from human red blood cells is accompanied by significant changes in the arrangement of both membrane phospholipids and proteins [12]. Membrane fusion can be induced, however, in virus-agglutinated human erythrocyte ghosts by the addition of various SH-blocking reagents [11]. It has been shown that the SH-blocking reagents exert their effect by modifying the erythrocyte membrane polypeptides and not the viral particles [11]. Thus it appears that for a better understanding of the molecular mechanism of the membrane fusion process it is important to know whether, beside the added fusogenic agents, also any of the cell membrane polypeptides play an active role in this process.

Incubation of the proteolytic enzyme pronase with intact erythrocytes or resealed erythrocyte ghosts results in hydrolysis of external membrane polypeptides, while its incubation with leaky erythrocyte ghosts cause hydrolysis of both externally and internally located membrane polypeptides [13]. Thus, pronase-treated erythrocyte ghosts serve as a suitably system for studying the involvement of the different membrane polypeptides in the fusion process. In the present work we have treated human erythrocyte ghosts with pronase and then studied their ability to agglutinate and fuse. Both *Sendai* virus and the non-viral fusogenic agent glyceromonooleate were used for induction of fusion. Pronase hydrolyzed sialoglycoproteins but not sialoglycolipids from erythrocyte membranes [14]. Hence, by using pronase-treated human erythrocyte ghosts, we were also able to study the question of whether, besides membrane sialoglycoproteins, sialoglycolipids may also serve as receptors for virus particles.

Materials and Methods

Preparation of human erythrocyte ghosts. Human blood, type O^+, aged 3—6 weeks, was used. The blood was washed three times in solution A (see Media), and the buffy layer containing the white cells was discarded. The final pellet was hemolyzed according to Fairbanks et al. [15] by the addition of 40 volumes of fresh cold hemolyzing buffer (see Media). After five washings, the final pellet was washed once with either 5 mM sodium phosphate, pH 7.4, or with solution A, and then resuspended either in the above phosphate buffer (hypo-human erythrocyte ghosts) or in solution A (iso-human erythrocyte ghosts) to give a concentration corresponding to 20% (v/v) of the original erythrocytes. The same was done in all further experiments. The human erythrocyte ghosts obtained were completely white without any residual hemoglobin.

Hydrolysis of membrane sialic acid by neuraminidase. Iso-human erythrocyte ghosts and hypo-human erythrocyte ghosts were washed once with acetate buffer, pH 5.6, with or without NaCl, respectively, (see Media), and then suspended in the same buffer to give 20% (v/v) concentration. To each ml of human erythrocyte ghosts' suspension, 15 μl of soluble neuraminidase (*Vibrio comma* Boehringer, 500 U/ml) were added, and the human erythrocyte ghosts were incubated for 2 h at 37° C with gentle shaking. Sialic acid was determined by the thiobarbituric acid method [16] on 0.2-ml samples of the supernatant.

Incubation of human erythrocyte ghosts with pronase. Human erythrocytes, iso-human erythrocyte ghosts or hypo-human erythrocyte ghosts (all 20%, v/v), were suspended in solution A or 5 mM phosphate buffer, pH 7.4, respectively, and incubated, if not otherwise stated, with 0.5 mg/ml pronase for 30 min at 37° C with shaking. In some experiments hypo-human erythrocyte ghosts were incubated with 0.5 mg pronase for 12 h at 37° C (hypo-human erythrocyte ghosts II). At the end of the incubation period the different systems were centrifuged and 0.2 ml of the supernatant were removed for sialic acid estimation [16]. In experiments where the polypeptides of pronase-digested human erythrocyte ghosts were analyzed by gel electrophoresis, proteolysis was terminated by the method previously described [13] with some modifications. At the end of the incubation period a methanolic solution of phenylmethylsulfonyl fluoride (PMSF) (200 mM) was added to give a final concentration of 2 mM, and the human erythrocyte ghosts' suspension was further incubated for 15 min at 37° C. After centrifugation the pellet was washed twice with 10—20 volumes of solution A containing 2 mM PMSF, and then suspended in 10 volumes of the above medium and incubated for 15 min at 37° C. After two additional washings with the above medium, the human erythrocyte ghosts were washed once more with 10 volumes of the above buffer to which 5 mM toluene sulfonyl-L-arginine-*O*-methylester (Tos-Arg-OMe) were added. The final pellet was suspended in water to which 2 mM PMSF and 5 mM Tos-Arg-OMe were added. Samples of 100 μg protein were subjected to SDS-polyacrylamide gel electrophoresis [17] in the presence of 8 M urea.

For all other experiments, pronase was removed from the human erythrocyte ghosts as follows: A pellet of pronase-treated human erythrocyte ghosts was suspended in half a volume (to give 40%, v/v) of solution A containing 1%

bovine serum albumin, and the suspension obtained was incubated for 10 min at 37°C with gentle shaking. After the incubation period, the human erythrocyte ghosts were washed twice in solution A and the final pellet was suspended in solution A to give 10% (v/v) concentration.

Estimation of free and membrane-bound sialic acid. After neuraminidase or pronase treatment, the free sialic acid was measured in 0.2-ml samples of the first supernatant by the thiobarbiturate method [16]. Membrane-bound sialic acid was hydrolyzed by 0.05 M H_2SO_4, and then the sialic acid was isolated by a modification of the Warren method using Dowex 50-X8 and estimated as described before [16].

Virus-induced fusion of human erythrocyte ghosts. Sendai virus was isolated and its hemagglutinating titer was determined as previously described [10]. For fusion, intact erythrocytes, iso-human erythrocyte ghosts or hypo-human erythrocyte ghosts (10%, v/v) were agglutinated by *Sendai* virus in the cold and then incubated at 37°C with gentle shaking. In systems containing intact cells, samples were removed for phase microscopy observation after 45—60 min of incubation, while from systems containing pronase-treated human erythrocyte ghosts the samples were removed after 15—30 min of incubation. Fusion was determined as described before [10,11].

Fusion of pronase-treated human erythrocyte ghosts by glyceromonooleate. An ethanolic solution of glyceromonooleate (50 mM) (Sigma) was added to human erythrocyte ghosts' suspension (20%, v/v) to give a final concentration of 1—4 mM, and the suspension was incubated for 1—2 min at 37°C. After the incubation, samples were removed for phase and electron microscopy observations.

Freeze-etching and electron microscopy. Human erythrocyte ghosts were fixed at room temperature by addition of glutaraldehyde (Ladd Research Inc.) to give a final concentration of 1% (v/v) and then freeze-etching was performed as previously described [18]. Micrographs were obtained with Philips EM 300 operating at 80 kV.

Protein was determined according to Lowry et al. [19].

Media. The following media were used: Solution A contained 150 mM NaCl, 5.3 mM KCl, 0.8 mM $MgCl_2$, and 20 mM Tricine-NaOH, pH 7.4. The hemolyzing buffer contained 5 mM sodium phosphate buffer, pH 8.0. Treatment with neuraminidase was performed in a medium containing 50 mM sodium acetate, pH 5.6, and 9 mM $CaCl_2$ (hypotonic medium). In experiments where iso-human erythrocyte ghosts were treated with neuraminidase, 110 mM NaCl were added to the acetate buffer (isotonic medium).

Pronase, type VI, from *Streptomyces griseus* (Sigma) was dissolved in solution A to give 10 mg/ml and dialyzed in the cold overnight against solution A, pH 7.8, to remove its Ca^{2+} content. PMSF was obtained from Sigma, and Tos-Arg-OMe from Miles Yeda. All chemicals used were commercially obtained and were of analytical grade.

Results

Viral- and non-viral-induced fusion of pronase-treated human erythrocyte ghosts

Treatment of human erythrocyte ghosts with either neuraminidase or

pronase, releases virtually the same amount of sialic acid residues from the erythrocyte membranes. About 80—86% of the total membrane sialic acid was released by treatment of human erythrocyte ghosts with either of these enzymes under isotonic or hypotonic conditions. It should be mentioned that after gel electrophoresis, the polypeptides of pronase-treated erythrocytes, as of neuraminidase-treated cells, were not stained by the Schiff-periodate stain (not shown), indicating that most of the membrane's sialic acid residues were removed by this treatment.

Figs. 1B and 1C show the polypeptide profiles of pronase-digested iso- and hypo-human erythrocyte ghosts, respectively. As was already observed before

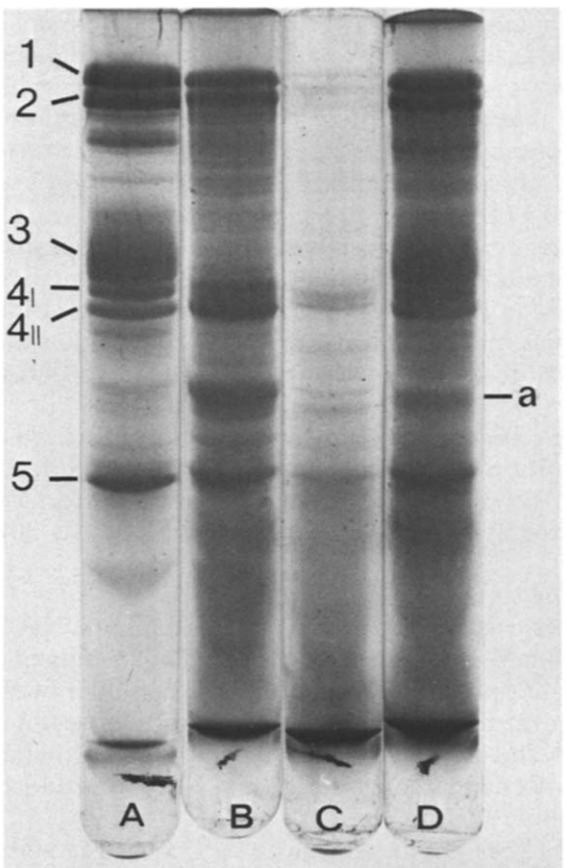

Fig. 1. Separation of pronase-digested human erythrocyte ghosts by gel electrophoresis. Iso- or hypo-human erythrocyte ghosts were incubated with pronase and subjected to gel electrophoresis, as described in Materials and Methods. (A) Control untreated iso-human erythrocyte ghosts. (B) Pronase-digested iso-human erythrocyte ghosts. (C) Pronase-digested hypo-human erythrocyte ghosts (hypo-human erythrocyte ghosts were incubated for 30 min at 37°C with 0.5 mg/ml pronase). (D) A mixture of pronase-digested hypo-human erythrocyte ghosts II and untreated human erythrocyte ghosts (50 μg of each sample were mixed and subjected to gel electrophoresis). The profile of the polypeptide is similar to that obtained from control iso-human erythrocyte ghosts (A). The erythrocyte polypeptides are numbered according to the nomenclature proposed by Steck [25]. The polypeptide marked as (a) has a mol. wt. of 35 000 and is the proteolytic product of band 3 [20]. A second proteolytic product of band 3, a polypeptide of mol. wt. 65 000 [20], comigrates with band 4.2.

[20], only band 3 was hydrolyzed in iso-human erythrocyte ghosts (Fig. 1B), while in hypo-human erythrocyte ghosts most of the polypeptides were digested by pronase (Fig. 1C). Fig. 1C shows that only a few faint polypeptide bands could still be observed in the electrophoresis patterns of hypo-human erythrocyte ghosts which were digested by pronase for 30 min at 37° C. Practically no membrane polypeptides were left after proteolysis of hypo-human erythrocyte ghosts for 12 h at 37° C (hypo-human erythrocyte ghosts II) (not shown, see Fig. 4B).

In order to avoid any proteolysis of the erythrocyte membranes by pronase after termination of the incubation period, and especially during the electrophoresis process [13], it was essential to wash the pronase-digested human erythrocyte ghosts as described under Materials and Methods. It should be emphasized that in order to completely remove the enzyme and totally inhibit its action, it was essential to add the inhibitor PMSF, and the substrate Tos-Arg-OMe to the washing medium (see Materials and Methods)..

The fact that the band pattern seen in Fig. 1B (as well as the pattern seen in Fig. 1C) represents the real polypeptide profile of pronase-digested human erythrocyte ghosts, can be inferred from the results seen in Fig. 1D which shows polypeptide profiles obtained from a mixture of untreated human erythrocyte ghosts and pronase-digested hypo-human erythrocyte ghosts. The polypeptide of the untreated human erythrocyte ghosts remained intact and unaffected. Fig. 1D shows the complete polypeptide pattern of the control untreated human erythrocyte ghosts, despite the fact that they were mixed with pronase-digested hypo-human erythrocyte ghosts (compare Fig. 1A with Fig. 1D).

Incubation of hypo-human erythrocyte ghosts with pronase resulted in their disruption and in the formation of heterogeneous population of small vesicles of 1—2 μm in diameter (Fig. 2B). *Sendai* virus readily agglutinated pronase-treated iso-human erythrocyte ghosts or hypo-human erythrocyte ghosts at 4° C, the degree of which was slightly higher than in control untreated cells (Fig. 2C). Neuraminidase-treated cells were neither agglutinated nor fused by *Sendai* virus. Human erythrocyte ghosts that were treated with pronase under isotonic conditions (iso-human erythrocyte ghosts) could be fused by *Sendai* virus, while those treated under hypotonic conditions (hypo-human erythrocyte ghosts), although agglutinated, were not fused by it. Incubation of virus-agglutinated, pronase-treated hypo-human erythrocyte ghosts at 37° C resulted in their relatively rapid disagglutination (after about 10 min of incubation at 37° C) without the formation of polyghosts.

The non-viral fusogenic agent, glyceromonooleate, failed to fuse human erythrocyte ghosts unless they were treated either with neuraminidase or with pronase (Figs. 2D and 5). Pronase-treated human erythrocyte ghosts fused almost instantly after addition of glyceromonooleate at 37° C (1—2 min incubation), whereas about 60—90 min incubation at 37° C were required to induce fusion by glyceromonooleate in pronase-treated intact human erythrocytes.

Promotion of fusion in pronase-digested human erythrocyte ghosts by either *Sendai* virus or by glyceromonooleate did not require Ca^{2+} and occurred even in the presence of 4 mM EGTA. However, addition of Ca^{2+} to the fusion system slightly stimulated the process. Similar observations were reported

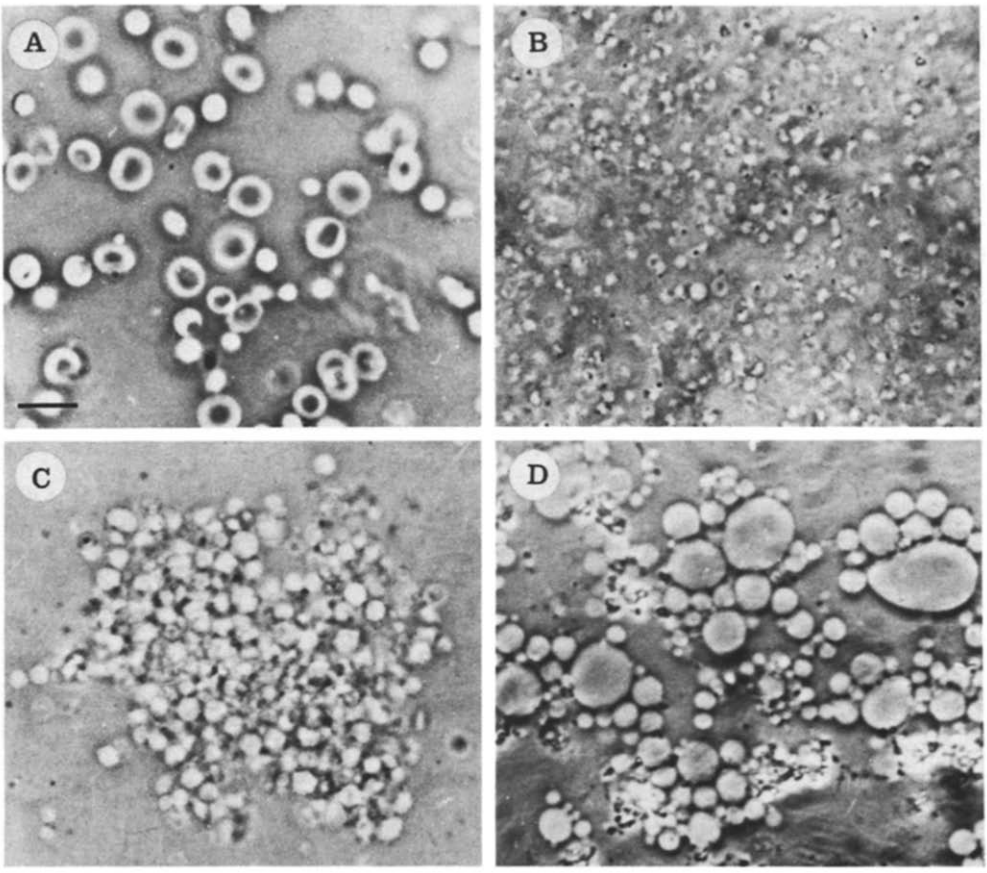

Fig. 2. Phase micrographs of pronase-digested human erythrocyte ghosts agglutinated by *Sendai* virus or fused by glyceromonooleate. (A) Control untreated human erythrocyte ghosts. (B) Pronase-digested hypo-human erythrocyte ghosts. (C) Large agglutinates obtained after incubation of pronase-digested hypo-human erythrocyte ghosts with 800 hemagglutinating units of *Sendai* virus at 4°C. (Incubation at 37°C caused dissociation of the erythrocytes without induction of membrane fusion.) (D) Fused erythrocyte ghosts formed after incubation of pronase-digested hypo-human erythrocyte ghosts with 2.4 mM glyceromonooleate for 2 min at 37°C. Since it is difficult to obtain clear pictures of the human erythrocyte ghosts in the phase microscope, a drop of 10% bovine serum albumin was added to the suspension of the human erythrocyte ghosts before examination in the microscope in order to increase their contrast, as described by Low et al. [26]. Addition of bovine serum albumin also prevented bursting of the fused erythrocyte ghosts which occurs during their prolonged incubation at 37°C. Bar = 10 μm.

before for virus induced fusion of intact human erythrocytes [10].

Fig. 3 shows the effect of increasing concentrations of *Sendai* virus on the degree of fusion of intact human erythrocytes and different preparations of human erythrocyte ghosts. Intact human erythrocytes were fused readily by low titer *Sendai* virus, while human erythrocyte ghosts failed to fuse unless high amounts of virus particles were added (Fig. 3). Between 800 and 1200 hemagglutinating units of *Sendai* virus were sufficient to induce a high degree of fusion in intact human erythrocytes, while 8000 hemagglutinating units were required to induce the same degree of fusion in untreated human erythrocyte ghosts or in pronase-digested iso-human erythrocyte ghosts. Fusion could not be induced, however, in pronase-treated hypo-human erythrocyte ghosts,

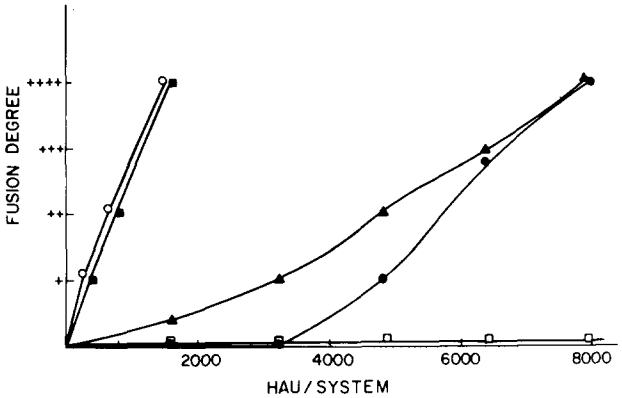

Fig. 3. The effect of increasing concentrations of *Sendai* virus on the degree of fusion of intact erythrocytes, human erythrocyte ghosts, and pronase digested human erythrocyte ghosts. Fusion was induced by *Sendai* virus, as previously described [10], and in Materials and Methods. ○———○, intact erythrocytes; ●———●, human erythrocyte ghosts; ▲———▲, pronase-digested iso-human erythrocyte ghosts; □———□, pronase-digested hypo-human erythrocyte ghosts; ■———■, human erythrocyte ghosts fused in the presence of 1 mM PCMBS [11]. Symbols of fusion essentially as described before [11].

even with high concentrations of *Sendai* virus (Fig. 3).

The addition of SH-blocking reagents greatly increased the fusion potentiality of human erythrocyte ghosts [11]. Fig. 3 shows that 800—1600 hemagglutinating units of *Sendai* virus, that failed to fuse control human erythrocyte ghosts, were sufficient to induce a high degree of fusion in human erythrocyte ghosts incubated in the presence of *p*-chloromercuribenzene sulfonate (PCMBS). The addition of PCMBS, however, did not have any effect on the fusion process of pronase-treated iso-human erythrocyte ghosts nor did its addition promote fusion in virus-agglutinated hypo-human erythrocyte ghosts (not shown).

Morphological studies of the fusion process in pronase-treated human erythrocyte ghosts

Fig. 4A is a freeze-fractured membrane showing that intramembrane particles in the PF face [29] of pronase-digested iso-human erythrocyte ghosts are significantly clustered. Intramembrane particles were completely absent, however, from the PF face of fractured membranes obtained from human erythrocyte ghosts digested with pronase under hypotonic conditions for 12 h (hypo-human erythrocyte ghosts II, Fig. 4B). Only huge smooth areas almost completely devoid of intramembrane particles could be identified in freeze-fractured membranes of these cells (Fig. 4B). Interestingly, virus particles can be found attached to the intramembrane particle-free areas in freeze-fractured membranes obtained from virus-agglutinated pronase-digested hypo-human erythrocyte ghosts II (Figs. 4C and 4D).

The PF faces of fused pronase-digested iso-human erythrocyte ghosts are seen in Fig. 5. Figs. 5A—5C show human erythrocyte ghosts' membranes in the process of fusion, while Fig. 5D is the PF face of a fused erythrocyte ghost. As can be seen, one of the cells in Fig. 5 (cell No. 1 in A and B) shows a PF face which is crowded with intramembrane particles, while the PF face of the adjacent cell (cell No. 2) is almost devoid of intramembrane particles. The

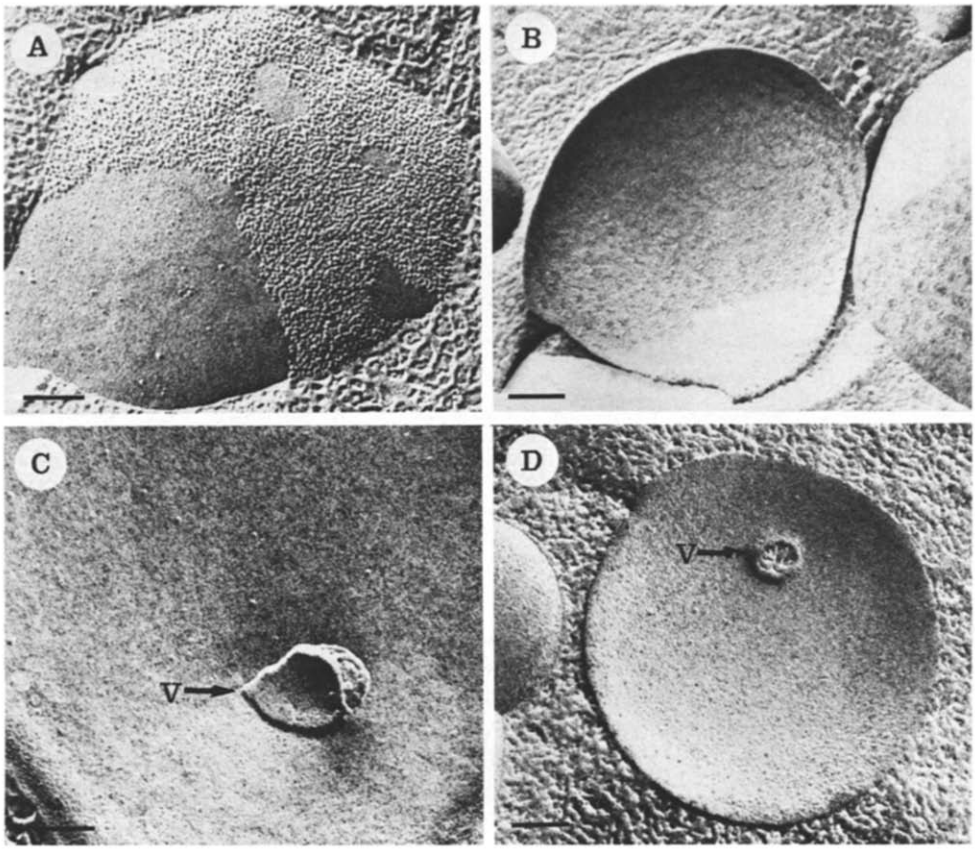

Fig. 4. Freeze-fractured membranes of pronase-digested human erythrocyte ghosts. (A) The PF face of pronase-digested iso-human erythrocyte ghosts. Intramembrane particles are significantly clustered. (B) PF face of pronase-digested hypo-human erythrocyte ghosts II. Smooth membranes completely devoid of intramembrane particles were obtained. (It should be mentioned that the PF face of hypo-human erythrocyte ghosts obtained by incubation with pronase for 30 min at 37° C contained only a few intramembrane particles, not shown.) (C and D) PF face of pronase-digested hypo-human erythrocyte ghosts incubated with *Sendai* virus at 4° C. Note the appearance of virus particles in the smooth, intramembrane particles'-free regions. V = virus particles. Iso- and hypo-human erythrocyte ghosts were obtained as described in Materials and Methods. Bars in A—D = 0.15 μm.

absence of intramembrane particles in these fractured membranes might be explained by the fact that either a few iso-human erythrocyte ghosts in the population were leaky and the treatment with pronase caused hydrolysis of most of the membrane polypeptides or, alternatively, that the intramembrane particles of these membranes were clustered on the unfractured side of the membranes. The fusion process was completed in these pronase-digested iso-human erythrocyte ghosts after a short incubation period with glyceromono-oleate at 37° C, and the fused erythrocyte ghosts formed became completely round (ref. 10 and Fig. 2D). The intramembrane particles in fused erythrocyte ghosts obtained after fusion of pronase-digested iso-human erythrocyte ghosts appeared evenly distributed (Fig. 5D), while the intramembrane particles in whole pronase-digested iso-human erythrocyte ghosts appeared clustered

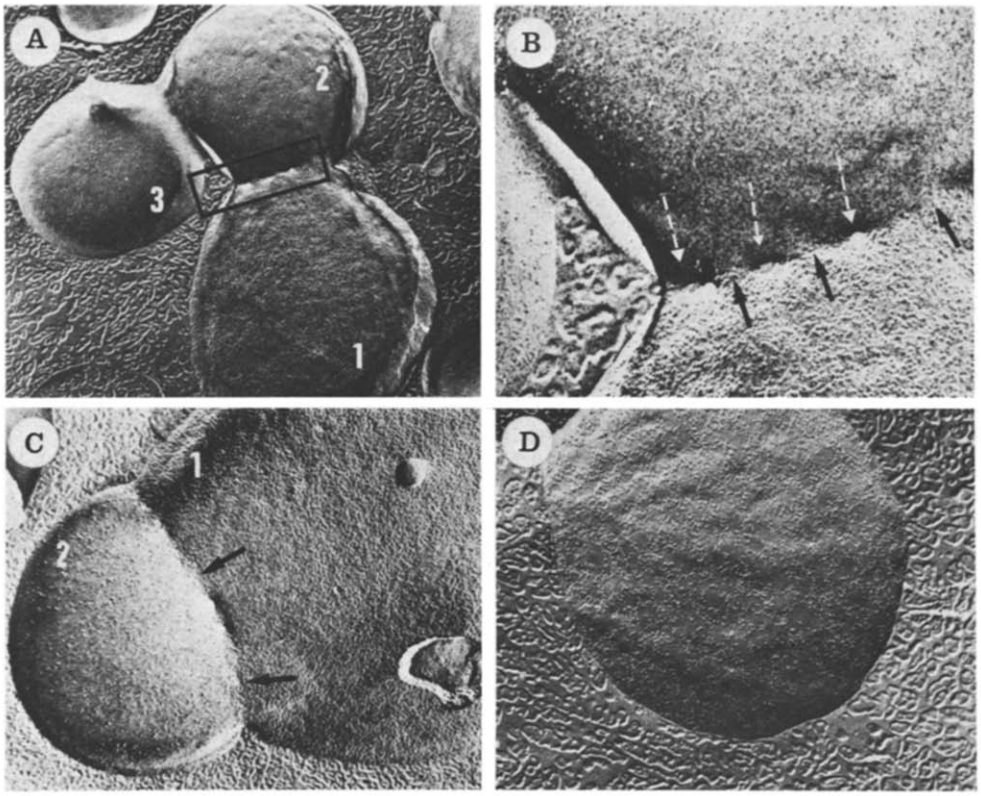

Fig. 5. Fusion of pronase-digested iso-human erythrocyte ghosts. (A) Fractured membranes of fused pronase-digested iso-human erythrocyte ghosts. Three cells in the process of membrane fusion can be seen. Cell No. 1 contains few intramembrane particles, while the PF faces of cells No. 2 and No. 3 are devoid of intramembrane particles. The membranes of cells No. 2 and No. 3 are probably intermixed, while in the region connecting cells No. 1 and No. 2 pores can still be seen. Bar = 0.3 μm. (B) The enlargement of the fusion area of cells No. 1 and No. 2 of picture A. Note the pore (white arrows) between the cells, probably indicating that the fusion process was not completed. Intramembrane particles in the process of migration between the two cells can also be observed (black arrows). Bar = 0.1 μm. (C) Fractured membranes of fused iso-human erythrocyte ghosts. Migration of intramembrane particles from the PF face of cell No. 1 (crowded with intramembrane particles) to cell No. 2 can clearly be observed (arrows). Bar = 0.25 μm. (D) Fused erythrocyte ghosts obtained from pronase-digested iso-human erythrocyte ghosts. Note that the few intramembrane particles seen in the PF face of the fused erythrocyte ghosts are almost evenly distributed. Bar = 0.25 μm. Fusion was induced in all cases by 3 mM glyceromonooleate, as described in Materials and Methods.

(Fig. 4A). This may indicate that during the fusion process and after its completion, the intramembrane particles move laterally and become equally distributed between the membranes which compose the fused erythrocyte ghosts. Initiation of the lateral movement of intramembrane particles during membrane fusion can probably be seen in the freeze-fractured membranes of Fig. 5C. Many intramembrane particles (arrows) are seen on the margin between the fused cells (cells No. 1 and 2 in Fig. 5C), giving the impression that they are in the process of moving from the crowded area of cell No. 1 to the smooth area of the adjacent cell (cell No. 2). Small pores, probably representing cytoplasmic bridges which penetrate the joints between two fused cells, can be observed in Figs. 5B and 5C (white arrows).

Discussion

The results of the present work indicate that besides sialoglycoproteins, membrane sialoglycolipids may also serve as receptors for *Sendai* virus. This is inferred from the observation that human erythrocyte ghosts, digested with high concentrations of pronase for a long period, were still agglutinated by *Sendai* virus. Under these conditions, virtually all the external membrane polypeptides, including the sialoglycoproteins, were hydrolyzed. It is conceivable, therefore, that agglutination of pronase-digested human erythrocyte ghosts is due to the interaction of the virus with the membrane sialoglycolipids which were left intact after incubation of human erythrocyte ghosts with pronase. It should be mentioned here that Haywood [21] already reported that *Sendai* virus is able to interact and to fuse with liposomes containing sialoglycolipids. In addition, Gordon et al. [27] recently reported that erythrocyte ghosts digested with pronase, under virtually identical conditions as those reported in the present work, have agglutinated with soybean agglutinin, wheat germ agglutinin, and phytoagglutinin. This treatment also led to formation of small vesicles devoid of intramembrane particles [27].

Further indications for the interaction between membrane sialoglycolipids and *Sendai* virus were obtained from electron microscopy studies. The PF face of pronase-digested hypo-human erythrocyte ghosts and, especially, of pronase-digested hypo-human erythrocyte ghosts II, appeared smooth and almost completely devoid of intramembrane particles. Such intramembrane particles-free areas of fractured membranes are considered as regions of naked phospholipids [18]. Also, the analysis of hypo-human erythrocyte ghorsts' membranes by polyacrylamide gel electrophoresis revealed that most of their polypeptides are missing. From our microscopic observations, it is clar that *Sendai* virus is able to interact with the membrane sialoglycolipids which are embedded in these smooth regions.

Freeze-fractured membranes of pronase-digested hypo-human erythrocyte ghosts are ultrastructurally very similar to those obtained from phospholipid liposomes [23]. In spite of that, the hypo-human erythrocyte ghosts could not be fused by Ca^{2+} alone, which was shown to induce fusion in liposomes [23].

One of the main steps in the membrane fusion process has been suggested to be the intermixing of exposed phospholipids of two adjacent cells [7,8,28]. As mentioned above, these regions could be recognized in freeze-fractured membranes as smooth intramembrane particles'-free areas [18,23]. One should assume, therefore, that pronase-digested hypo-human erythrocyte ghosts and hypo-human erythrocyte ghosts II, the membranes of which are almost completely devoid of integral proteins, would fuse better than intact cells. This, indeed, is the case when fusion is promoted by glyceromonooleate. However, incubation of virus-agglutinated hypo-human erythrocyte ghosts and hypo-human erythrocyte ghosts II at 37°C did not result in induction of membrane fusion. It appears, therefore, that certain polypeptides are specifically required for the productive interaction of the erythrocyte membranes with the virus, although not necessary for the membrane fusion process itself. It may be speculated that besides the sialic acid-containing glycoproteins, other receptors exist in the cell membrane which interact specifically with the viral glycopro-

tein, designated as 'fusion factor' [9]. One of the erythrocyte membrane polypeptides might be required to integrate the viral 'fusion factor' into the human erythrocyte ghosts' membranes, a step that is essential for the induction of membrane fusion by *Sendai* virus [24]. Treatment of hypo-human erythrocyte ghosts with pronase probably hydrolyzes this hypothetic receptor. Evidently, the presence of intact band 3 is not essential for the promotion of membrane fusion by *Sendai* virus. Pronase-digested iso-human erythrocyte ghosts, band 3 of which is cleaved [2], were fused by incubation with *Sendai* virus.

The fact that *Sendai* virus is able to fuse pronase-digested iso-human erythrocyte ghosts but not hypo-human erythrocyte ghosts, makes this system a suitable model for studying the molecular mechanism of virus-cell and cell-cell fusion. Gradual proteolysis of human erythrocyte ghosts' polypeptides, followed by the study of their fusion ability, might establish a correlation between certain membrane polypeptides and the ability of the membranes to be fused by the virus. Such information would be most valuable for the elucidation of the first molecular steps of virus-cell interaction and of infection of cells by enveloped viruses. Currently, experiments are carried out in our laboratory to study the question, through the use of ferritin-conjugated antiviral antibody, whether *Sendai* virus is able to penetrate into the membranes of pronase-digested hypo-human erythrocyte ghosts, despite the fact that it does not promote cell-cell fusion in these hypo-human erythrocyte ghosts.

Acknowledgements

This work was supported by a grant from the Israel Commission for Basic Research and the Israel Academy of Sciences and Humanities. The authors wish to thank Dr. Julius A. Gordon of the University of Colorado School of Medicine, Denver, CO, whose discussions on the subject initiated the present work, and Mrs. Yehudit Reichler for her excellent assistance with the electron microscope.

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