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Action of Polyethylene Glycol on the Fusion of Human Erythrocyte Membranes

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Summary. Factors affecting the polyethylene glycol (PEG)-induced membrane fusion were examined. Human erythrocyte membrane "ghosts," cytoskeleton-free vesicles budded from erythrocytes, mechanically disrupted erythrocyte vesicles, and recombinant vesicles from glycophorin and egg phosphatidylcholine were used as models. Fusion was monitored by dark-field light microscopy and by freeze-fracture electron microscopy. Osmotic swelling was found necessary for fusion between membrane ghosts following PEG treatment. The sample with the highest fusion percentage was sealed ghosts incubated in hypotonic media after at least 5 min of treatment in >25% PEG. At similar osmolarity, glycerol, dextran and PEG produced progressively more pronounced intramembranous particle (IMP) patching, correlating with their increasing fusion percentages. The patching of IMP preceded cell-cell contact, and occurred without direct PEG-protein interaction. The presence of cytoskeletal elements in small vesicles had no significant effect on fusion, nor on the aggregation of intramembranous particle (IMP) upon PEG treatment. Disrupting the membrane by lysolecithin, dimethylsulfoxide, retinol or mild sonication resulted in the fragmentation of ghosts without an increase in fusion percentage. The purity of the commercial PEG used had no apparent effect on fusion. We concluded that the key steps in PEG-induced fusion of cell membrane are the creation of IMP-free zones, and the osmotic swelling of cells after the formation of bilayer contacts during the PEG treatment. Cell cytoskeleton affects PEG-induced fusion only to the extent of affecting IMP patching.

Key Words polyethylene glycol · membrane fusion · erythrocyte · osmotic swelling · freeze fracture · cytoskeleton

Introduction

Many studies have been made to optimize the efficiency of polyethylene glycol (PEG)-induced cell fusion (Davidson et al., 1976). However, the mechanism of PEG-induced membrane fusion is still not well understood. Recently there has been much effort in elucidating the molecular mechanism of PEG-induced membrane fusion. Using liposomes as models, many researchers (Ahkong et al., 1975; Tillock & Fisher, 1979, 1982; Boni et al., 1981*a*,*b*; Arnold et al., 1983; Boni et al., 1984) have found that PEG aggregates and fuses lipid bilayers by a combination of dehydration and bilayer disruption process. The latter is associated with structural defects in the bilayer and the apolar environment created by PEG (Arnold et al., 1983; Boni et al., 1984). The fusion of biological membranes involves additional factors. The occurrence of membrane patches devoid of intramembranous particles (IMP) in regions of cell-cell contact (potential fusion sites) is common to PEG-induced fusions of erythrocytes (Knutton, 1979), HeLa cells (Krahling, 1981) and LM cells (Robinson et al., 1979). Roos et al. (1983) found that cells resistant to PEG-induced fusion also lack the ability to form IMP-free patches on their plasma membranes at low temperature. Whether the patching of IMP precedes or follows cell attachment remains to be solved. The cytoskeleton-mediated IMP aggregation has been suggested as a step in calcium phosphate-induced cell fusion (Zakai et al., 1977) and virus-induced cell fusion (Asano & Sekiguchi, 1978; Volsky & Loyter, 1978). Increased microtubule and actin stress fibers have been reported after PEG-induced fusion (Fuseler et al., 1978). Yet ultrastructural studies revealed no apparent involvement of microtubules and microfilaments in PEG-induced LM cell fusion (Robinson et al., 1979). Osmotic swelling, an important step in lipid vesicle fusion (Zimmerberg et al., 1980) and in virus-induced erythrocyte fusion (Knutton & Bachi, 1980), has been taken for granted as part of the PEG-induced fusion process (Knutton, 1979; Krahling, 1981), but the importance of this process in PEG-induced fusion has not been critically evaluated. Meanwhile, the need for certain impurities in commercial PEG for cell fusion remains an unknown factor (Honda et al., 1981*b*; Smith et al., 1982). This study is to elucidate the relative importance of these factors in PEG-induced membrane fusion, using human erythrocyte membrane and its modified or reconstituted forms as models.

Materials and Methods

Purification of PEG

Polyethylene glycol (molecular weight = 6000) was purchased from Fisher Scientific Company and used in most experiments without further purification. The purification procedure followed that reported by Honda et al. (1981*b*). Briefly, PEG (10 g) was dissolved in 100 ml chloroform and poured into 2000 ml of diethyl ether. The resulting white precipitate was separated by filtration and dried in vacuum over P_2O_5. The precipitated PEG was redissolved in water and dialyzed in Spectrapor membrane tubing (mol wt cutoff approx. 3500) against 6 × 2000 ml distilled water. The ^{31}P NMR spectrum of the product showed no phosphorus contaminant, which was present in unpurified compounds. The osmolarity of the solution was measured by a Wescor (Logan, Utah) 5100 osmometer.

Preparation of "Pink" Ghosts

"Pink" membrane ghosts were prepared from freshly drawn human blood. The method followed that of Dodge et al. (1963) except for replacing the phosphate buffer with balanced salt solution (BSS) (in mM: NaCl 125, KCl 5, $CaCl_2$ 3.8, $MgCl_2$ 2.5, TRIS 5, at pH 7.4). Washed blood was lysed four times in 1/10 BSS for 20 min at room temperature, followed by centrifugation. The "pink" ghost pellet was suspended in equal volume of BSS, and one half was used as unsealed ghosts. The other half was diluted with 4 volumes of BSS and incubated at 37°C for 1 hr to make sealed ghosts.

In experiments calling for the treatment with membrane-disruptive agents, suspensions of ghosts were mixed with equal volumes of solutions of respective membrane-disruptive agents in BSS, and incubated at 37°C for 15 min. The mixtures were centrifuged at 12,000 rpm for 20 min and the pellets were suspended in 1/10 BSS before PEG treatment.

Cytoskeleton-Free Vesicles (CFV)

Cytoskeleton-free vesicles from human erythrocytes were prepared according to Leonards and Ohki (1983). The CFV were produced from washed erythrocytes by "budding." This was done by successively titrating the cell suspensions with EDTA and $CaCl_2$ in increments. CFV were separated from the remaining cells by sucrose gradient centrifugation. The purified CFV, which formed a red band at the aqueous/30% sucrose layer interface, were removed and washed twice and resuspended in 150 mM NaCl with 10 mM Tricine (pH 7.4). The remaining erythrocytes, the so-called mother cells (MC), were recovered from the sucrose gradient at the 60%/40% interface. The mother cells are slightly smaller than erythrocytes when observed under the microscope. The mother cells were broken up into vesicles of similar size as the CFV by being repeatedly drawn and injected through a #24 syringe. The product is termed vesiculated mother cells (VMC).

Glycophorin Recombinants (GPR)

Egg phosphatidylcholine (Avanti Polar-Lipids) vesicles were reconstituted with glycophorin, an intrinsic protein from human erythrocytes. Glycophorin was isolated from human erythrocyte ghosts by the lithium diiodosalicylate (LIS) extraction procedure (Yeagle & Romans, 1981). The anhydrous protein and phospholipid were co-solubilized in 2-chloroethanol, dried under vacuum, hydrated in 1 mM histidine, 100 mM sodium chloride (pH 8) overnight, applied to a 0 to 40% continuous sucrose gradient and centrifuged overnight at 130,000 × *g*. Three bands appeared with the recombinant in the middle band.

Fusion Assay by Dark-Field Microscopy

Monolayers of ghosts were prepared by allowing a suspension of ghosts to settle on alcian blue-coated glass coverslips. Unattached ghosts were removed by washing with BSS. The coverslip, with an attached monolayer of ghosts, was gently immersed into a petri dish containing 35% PEG in BSS (wt/wt) and let stand for 5 min, followed by washing once with BSS and incubation at 37°C in 1/10 BSS for 1 hr. The coverslip was inverted over a supporting microscope slide, bridging over the space between two grease-attached coverslips, on the slide. This is to ensure that membrane ghosts will not become dislodged during mounting of the coverslip. The closely adjacent monolayers of membrane ghosts were observed with a dark-field light microscope (American Optics, Buffalo). Dark-field polaroid micrographs were taken and the number of fused and unfused ghosts were counted. The method is similar to that described by Knutton and Bachi (1980) using polylysine-treated coverslips and phase-contrast microscopy.

Freeze-Fracture Electron Microscopy

Samples were frozen by the rapid-freeze sandwich method as described previously (Boni et al., 1981*a*). Briefly, 0.1 μl of the sample was sandwiched between two thin copper plates and quickly plunged in liquid propane without cryoprotectant. The samples were fractured at −120° in a Polaron E7500 unit. For size measurements, random fields were photographed, and all vesicles within the fields were counted. The size of the vesicles were derived from the apparent diameter using the method of Weibel and Bolender (1973).

Results

Duration of PEG Treatment

Sealed membrane ghosts were treated in 50% PEG (wt/vol in BSS) for various lengths of time at room temperature, before washing (in BSS) and incubating (in 1/10 BSS) at 37°C for 1 hr. Untreated samples consisted entirely of individual membrane ghosts (Fig. 1*a*). Samples treated with PEG for more than 1 min showed many clusters of membrane ghosts. About 6 to 10% of the ghosts fused and became interconnecting membrane sacs. These sacs (Fig. 1*b* larger arrow) showed no boundary between the original membrane ghosts, and were easily discernible by dark-field microscopy. The clusters (Fig. 1*b*, arrowhead), on the other hand, were formed by ag-

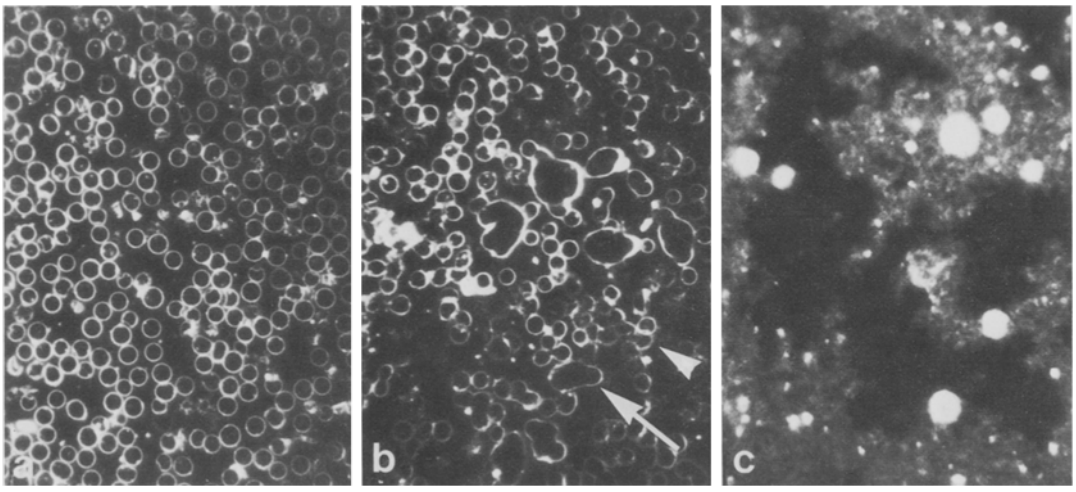

Fig. 1. Dark-field micrographs of erythrocyte membrane ghosts. (*a*) Untreated ghosts after incubation in 1/10 BSS for 1 hr at 37°C. (*b*) Ghosts treated in 35% PEG for 5 min followed by incubation as in (*a*). (*c*) Ghosts treated in 10 mM retinol as described in the text, followed by PEG treatment and incubation as in (*b*). Arrow points to fused cells whereas arrowhead indicates aggregated clusters

gregated but unfused ghosts. The numbers of fused, clustered and individual ghosts in samples treated in PEG for various durations were scored at random fields of view. The results are presented in Fig. 2. Although more than half of the individual ghosts aggregated after 1 min of PEG treatment, the percentage of fused ghosts remained constant at about 8 to 10% when the treatment time was 5 min or longer.

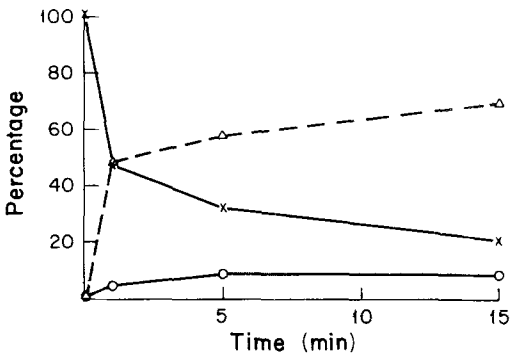

Fig. 2. Variation of the percentages of fused (○), aggregated (△) and single (×) membrane ghosts with the duration of treatment in 50% PEG

Concentration of PEG and Other Dehydration Reagents

Sealed ghosts on cover slips were treated with dextran, glycerol, and various concentrations of PEG for 5 min at room temperature. After incubation in 1/10 BSS at 37°C for 1 hr, the fused cells were scored. The results are presented in Table 1. The fusion percentages for PEG-treated samples seem to reach a saturated level at 25% PEG. At this concentration free water still exists (Arnold et al., 1983). The purity of PEG did not make a significant difference in the fusion capacity, in disagreement with Honda et al. (1981*b*), but agreed with the results of Smith et al. (1982) and with our findings on liposome fusion (Boni et al., 1984). At similar dehydration capacities to PEG (measured by osmolarity), dextran induced little or no fusion, whereas glycerol has no effect.

Osmotic Swelling

Both sealed and unsealed ghosts were used in this experiment to test the importance of osmotic swelling. Both types of ghosts collapsed during the PEG treatment. After the post-treatment incubation in BSS, unsealed ghosts remained a shell shape while sealed ghosts partially regained their original volume. When the post-treatment incubation was carried out in 1/10 BSS instead, the unsealed ghosts were still partially collapsed, whereas the sealed ghosts were swollen to full spheres. The difference in the swelling behavior is due to the fact that sealed ghosts are impermeable to most electrolytes. Therefore, when transferred from the isotonic washing solution (BSS) to a hypotonic incubation solution (1/10 BSS), a concentration gradient is created across the membrane. Unsealed ghosts quickly reach equilibrium and the osmotic swelling is transient. For sealed ghosts, the concentration gradient is compensated by swelling, which remains as long as the external medium is hypotonic.

Table 1. Fusion percentage resulting from treatments of dehydrating agents[a]

Reagent	Average molecular weight	Concentration	Osmolarity (mOsm/kg)	Fusion % (±1%)	Number of cells scored
PEG	6,000	10% w/v	340	—	>500
PEG	6,000	15% w/v	430	—	>500
PEG	6,000	20% w/v	550	5.4	798
PEG	6,000	25% w/v	710	10.2	850
PEG	6,000	28% w/v	875	9.2	621
PEG	6,000	30% w/v	940	10.5	456
PEG	6,000	35% w/v	1400	9.8	1542
Purified PEG	6,000	35% w/v	1400	11.8	1603
Dextran	10,300	33% w/v	700	—	>500
Dextran	10,300	40% w/v	875	—*	>500
Dextran	500,000	25% w/v		3.3	815
Glycerol	92	60% v/v	≫3000	—	>500

[a] Note: bar indicates no significant fusion was observed; * indicates some cell fragmentation was observed.

Table 2. Osmotic effect on fusion of sealed erythrocyte membrane ghosts

Pre-PEG suspension media[a]	Post-PEG incubation media[a]	Fusion percentage[b]
1/10 BSS	1/10 BSS	1.8
1/10 BSS	BSS	2.1
1/10 BSS	BSS + sucrose	—
BSS	1/10 BSS	9.5
BSS	BSS	2.0

[a] Osmolarity of solutions: BSS = 260 mOsm/kg; 1/10 BSS = 44 mOsm/kg; Sucrose (8.5% wt/vol in BSS) = 538 mOsm/kg.
[b] At least 500 membrane ghosts were counted per sample. The experimental error is ± 1% for all measurements.

Table 3. Combination effects on PEG-induced fusion

Treatment prior to immersion in 35% PEG	Observation after incubation in 1/10 BSS	
	fusion percentage	ghost fragmentation[a]
None	9.6%	
1% DMSO	6%	
2% DMSO	3.5%	+
5% DMSO	—	+++
0.1 mM retinol	—	+, clumping
1 mM retinol	—	+, clumping
10 mM retinol	—	++
50 mM retinol	—	+++
1 mM lysolecithin	—	++, clumping
Polylysine (0.1% wt/vol)	—	−, clumping
Spermidine (0.1% wt/vol)	—	++, clumping
Mild sonication	—	++

[a] The degree of cell fragmentation is indicated by the number of plus signs. A minus sign indicates no fragmentation.

The sealed and unsealed ghosts provide a convenient way to study the importance of osmotic swelling in the fusion process. Table 2 summarizes the result of the osmotic swelling study. In spite of extensive aggregation, no fusion between unsealed ghosts was observed by dark-field microscopy, in agreement with the results by Knutton and Bachi (1980) using Sendai virus. When the sealed membrane ghosts were returned after the PEG treatment to BSS or to the same suspension media before the PEG treatment, the ghosts showed about 2% fusion. If the swelling was reduced by incubating the ghost in a sucrose medium (about twice the equilibrium osmolarity of the erythrocyte), fusion was reduced to practically zero. A significant increase in fusion was found when isotonically suspended ghosts were placed in a hypotonic incubation medium after PEG treatment. Apparently, the additional osmotic swelling led to higher fusion efficiency.

Membrane Disruption

Sealed membrane ghosts were treated with dimethylsufoxide (DMSO), lysolecithin, or retinol, all of which were known to alter the bilayer structure and cause fusion at high concentrations (Howell et al., 1973; Papahadjopoulos et al., 1976; Tilcock & Fisher, 1982). The effects of polylysine and spermidin, a polyamine, were also examined. These chemicals were used in conjunction with 15 and 35% PEG, to investigate if there are any synergistic effects on fusion. The observation is given in Table 3. None of the additional treatment resulted in enhancing the fusion efficiency. On the contrary, all additional treatments at higher concentrations resulted in membrane fragmentation upon immersion in PEG (Fig. 1c).

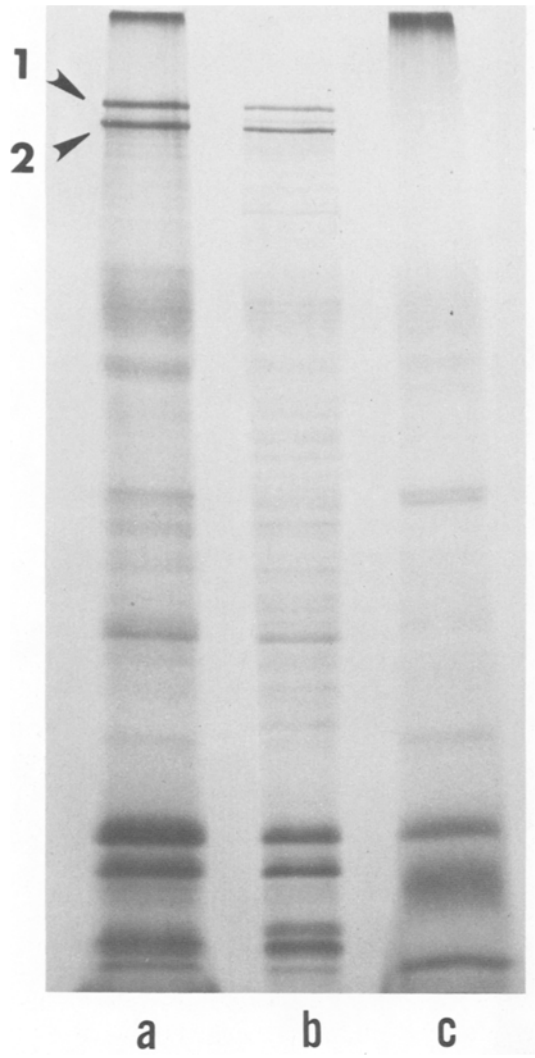

Fig. 3. SDS-polyacrylamide gel electrophoresis slabs of (*a*) mother cells, (*b*) vesiculated mother cells and (*c*) cytoskeleton-free erythrocytes vesicles. Bands 1 and 2 correspond to the cytoskeletal components

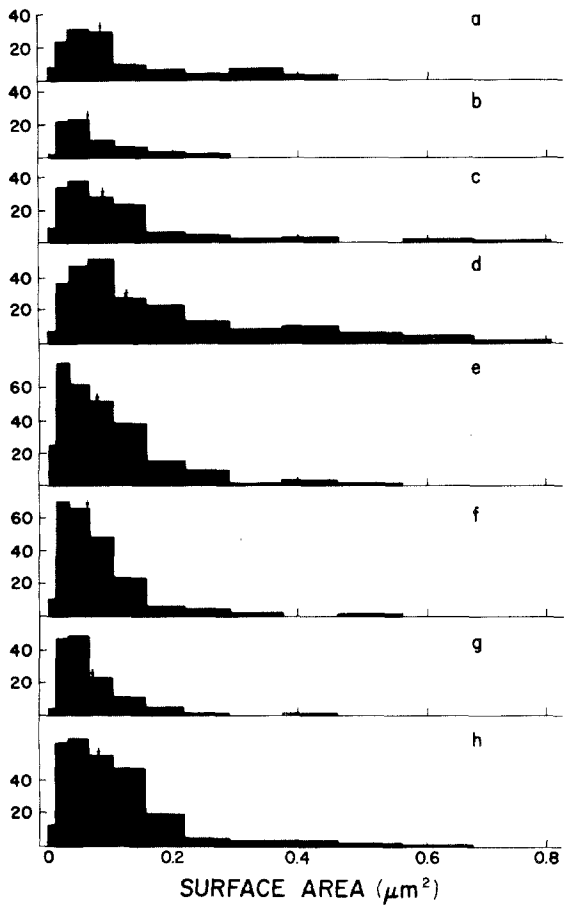

Fig. 4. Histograms of surface area per vesicle of respective control and PEG-treated VMC post-incubated in BSS (*a*, *b*) or in 1/10 BSS (*c*, *d*), control and PEG-treated CFV post-incubated in BSS (*e*, *f*) or 1/10 BSS (*g*, *h*). Arrows indicate mean surface area per vesicle for each sample

Integral Membrane Proteins and Cytoskeletal Elements

The following experiments were to investigate the factors affecting the formation of IMP-free areas, the effects of cytoskeleton in this process, and their relations to membrane fusion. Cytoskeleton-free vesicles (CFV) and vesiculated mother cells (VMC) were used. These vesiculated mother cells (VMC) contain cytoskeletal proteins band 1 and 2, as do the mother cells. The SDS gel electrophoresis analyses of CFV, MC and VMC are shown in Fig. 3. CFV contains virtually no band 1 and 2 proteins, the spectrin-containing cytoskeleton network (Leonards & Ohki, 1983).

The loose pellets of CFV, VMC and MC were treated in 35% of PEG at room temperature for 5 min, followed by dilution in BSS or in 1/10 BSS and incubated for 1 hr at 37°C. The sizes of untreated CFV and VMC varied over a wide range. All vesicles appeared spherical, and no collapsed vesicle was observed. The histograms of both PEG-treated and control populations are shown in Fig. 4. The surface areas were calculated assuming all the vesicles were spherical. If the total surface area of the vesicles is conserved after fusion, the increase in the mean surface area per vesicle indicates the percentage of vesicles fused. The fusion percentage is given by $(a_2 - a_1)/a_2(1 - 1/n)$ where a_1 and a_2 are the mean surface area per vesicle before and after fusion, and n is the mean number of vesicles which fused into one larger vesicle. The relation is only an approximation since the size dispersion is not ideal, and n may be 2 or more. Comparing PEG-treated vesicles (Fig. 4*b*, *d*, *f*, *h*) with their control populations (*a*, *c*, *e*, *g*), we found that for those samples post-incubated in 1/10 BSS, both VMC (*d*) and CFV

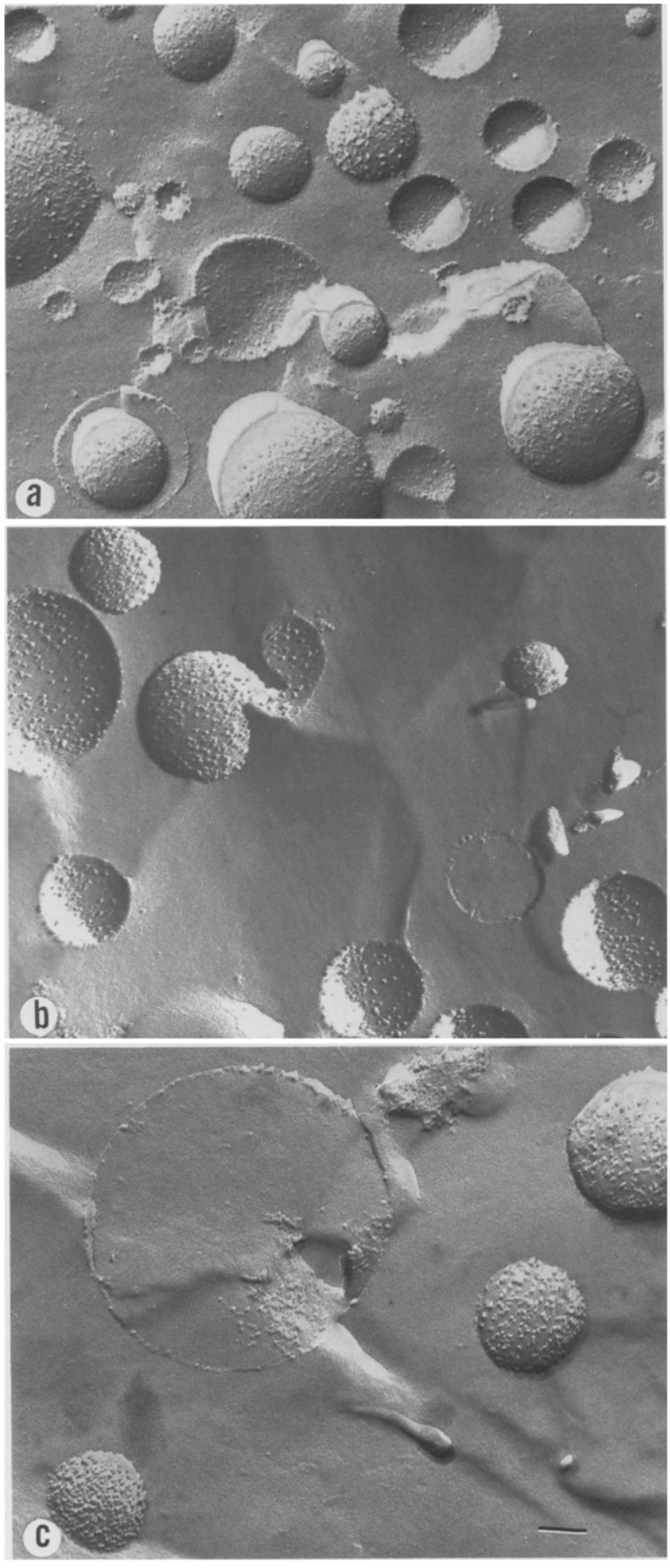

Fig. 5. Freeze-fracture electron micrographs of control (*a*) glycophorin/egg PC recombinants, (*b*) cytoskeleton-free erythrocyte vesicles and (*c*) vesiculated mother cells, all were frozen from 20°C. Bar = 0.1 μm

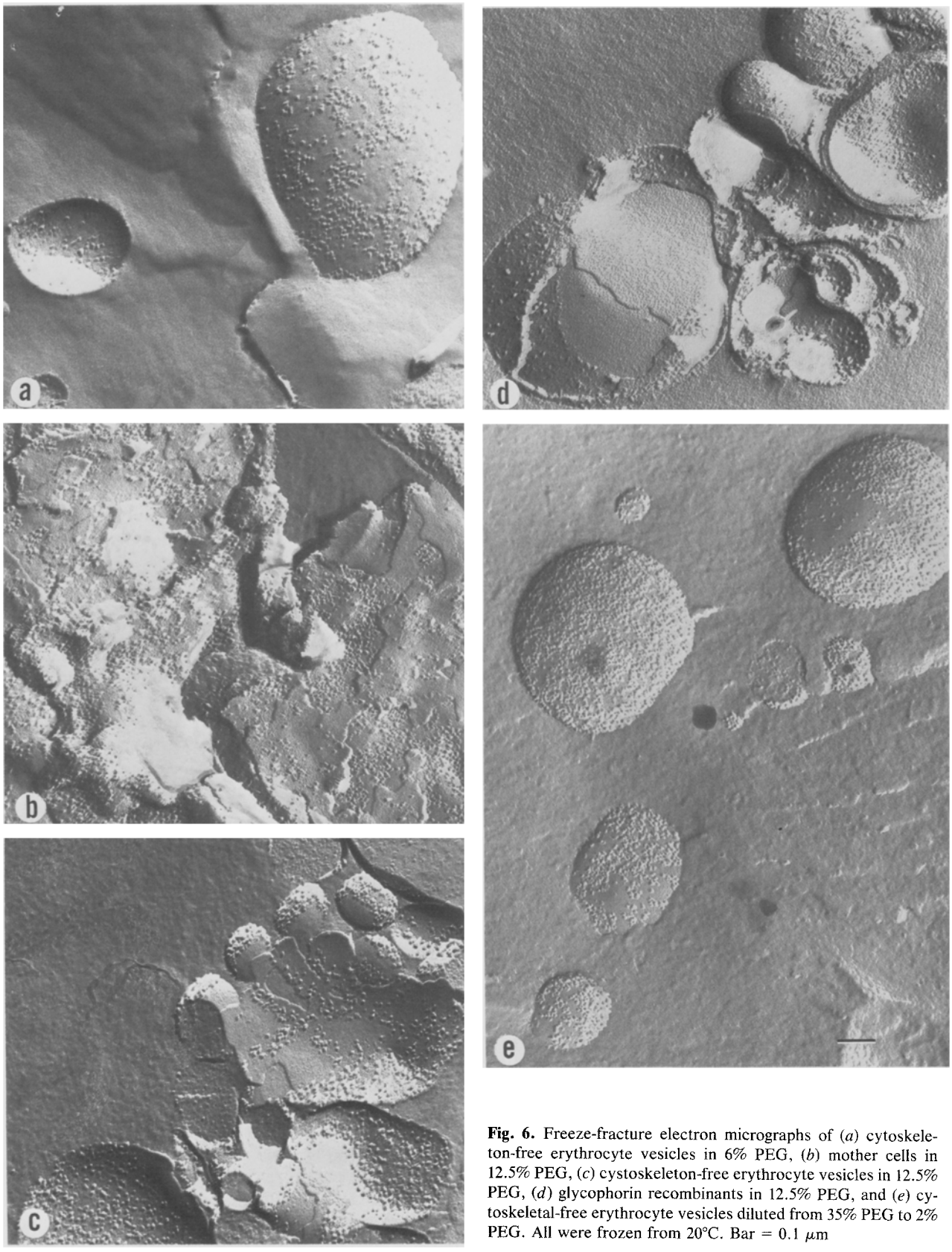

Fig. 6. Freeze-fracture electron micrographs of (*a*) cytoskeleton-free erythrocyte vesicles in 6% PEG, (*b*) mother cells in 12.5% PEG, (*c*) cystoskeleton-free erythrocyte vesicles in 12.5% PEG, (*d*) glycophorin recombinants in 12.5% PEG, and (*e*) cytoskeletal-free erythrocyte vesicles diluted from 35% PEG to 2% PEG. All were frozen from 20°C. Bar = 0.1 μm

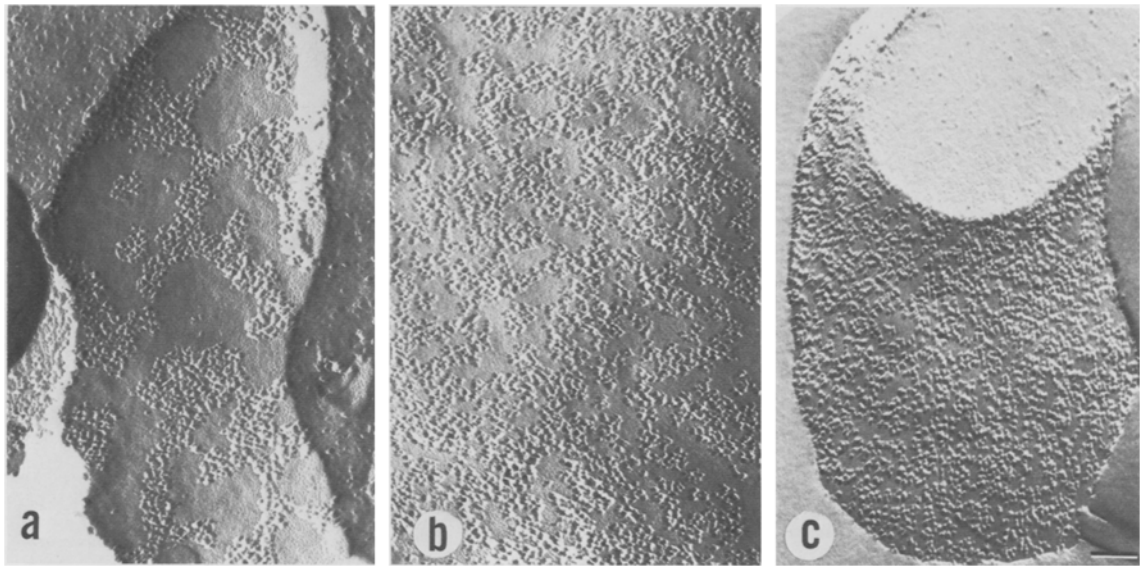

Fig. 7. Freeze-fracture electron micrograph of erythrocyte membrane ghosts in (*a*) 35% (wt/vol) PEG, (*b*) 25% (wt/vol) dextran (mol wt = 500,000) and (*c*) 60% (vol/vol) glycerol. Bar = 0.1 μm

Table 4. Reversibility of IMP aggregation after PEG treatment

	PEG concentration (% wt/vol)		
	6	12.5	35
GPR	*R*	*R*	*R*
CFV	*R*	*R*	*I*
VMC	*R*	*R*	*I*
MC	*N*	*R*	*R*

(*h*) show a shift to large sizes in comparison to their respective controls (*c*, *g*). Those samples post-incubated in BSS (*b*, *f*) showed a slight reduction in size distribution in comparison to the control populations (*a*, *e*), indicating some vesicle fragmentation. Assuming $n = 2$, the fusion percentages for VMC and CFV in 1/10 BSS are calculated to be 30 and 15%, respectively.

To examine the role of cytoskeleton in the PEG-induced patching of IMP, the glycophorin/egg PC recombinant (GPR) was used in addition to CFV, VMC and MC. Prior to PEG treatment, all samples showed a random distribution of IMP on the freeze-fracture faces (Fig. 5*a–c*). Pelleted cells and vesicles were incubated for 5 min in 6, 12.5 and 35% PEG. Both 6 and 12.5% PEG solutions were well below the fusogenic threshold value. Freeze-fracture samples were taken with the vesicles in PEG, as well as after dilution to a final PEG concentration of 1, 1 and 2%, respectively. A summary of the freeze-fracture results is presented in Table 4, where (*I*) indicates an irreversible aggregation and the formation of bare patches, (*R*) indicates a reversible aggregation of the IMP which randomize following the dilution step and (*N*) indicates no IMP aggregation.

Except MC in 6% PEG, all other samples showed nonrandom IMP distribution with empty patches, typically represented in Fig. 6*a* for CFV. The individual vesicles, however, were not aggregated. This implied that IMP aggregation did not occur solely in regions of close cell apposition due to electrostatic displacement as proposed by Knutton and Pasternak (1979). MC in 12.5% PEG contained many bare patches, some of which appeared to represent contact points of cells (Fig. 6*b*) as indicated by the double layers. Cell attachment was infrequent. Attachments of VMC, CFV and GPR vesicles were extensive in 12.5% PEG, and occurred only between surfaces denuded of IMP (Fig. 6*c*,*d*). In up to 12.5 % PEG, both vesicle attachment and IMP clustering were reversible upon dilution of the PEG. Identical results were obtained when purified PEG was used.

Cells or vesicles in 35% PEG exhibited extensive aggregation. Diluting the samples to 2% and incubating for 1 hr did not randomize the IMP distributions in VMC or CFV, as shown in Fig. 6*e*.

The potential of various dehydrating agents to induce IMP patching in ghost membrane is illustrated in Fig. 7. The degree of IMP patching varies from extensive, for samples in 35% PEG, to unnoticeable, for samples in 60% glycerol, in correspondence with their fusion efficiency (Table 1).

Discussion

An obvious difference between fusion of liposomes and fusion of cells is that liposomes completely fuse in PEG (Boni et al., 1981*a*,*b*, 1984) while cell fusion is not completed until the subsequent dilution and incubation steps. The disruption of the bilayer structure is a vital step in membrane fusion (Hui et al., 1981). It is expected that the bilayer structure in liposomes breaks down when the water polarity is drastically reduced in concentrated PEG; but with asymmetrically located integral proteins and a cytoskeleton, cell membranes can better maintain their integrity in PEG despite possible local disruptions of the bilayer; a subsequent swelling step is required to open up the connecting sites to complete the fusion process. Most ultrastructural studies showed cytoplasmic connection between cells occurred only in the incubation step (Knutton, 1979; Robinson et al., 1979; Wojcieszyn et al., 1983). The PEG treatment thus serves a dual role as a means to induce bilayer attachment and disruption, as well as to induce swelling. Since unsealed ghosts do not swell after the initial shock, fusion among them is minimal. Retinol (Smith et al., 1982) and DMSO (Akhong et al., 1975) have been reported to enhance cell fusion, but we found that none of the lipid-soluble fusogens enhance the fusion of membrane ghosts. It could be that the membrane ghosts are more susceptible than intact cells to fragmentation when subjected to a combined treatment of PEG and lipid-soluble fusogen, and the fragmentation of ghosts abrogates the effect of osmotic swelling.

The creation of IMP-free zones in the membrane to facilitate bilayer attachment seems to be prerequisite for fusion of biological membranes. Comparing the fusion efficiencies of PEG, dextran and glycerol, we found that their abilities to induce IMP patching rather than their dehydration capacities are the determining factors. Significantly, we found that the patching of IMP precedes instead of being a consequence of membrane attachment. How does PEG induce patching of IMP? Possible mechanisms are: (1) PEG is known to lower the pH of solutions (Kao & Michayluk, 1974) and increase the local concentration of electrolytes (Glasstone & Lewis, 1960). Both of these effects have been noted to cause IMP free regions in erythrocyte ghosts, with or without spectrin (Elgsaeter et al., 1976). (2) PEG can interact with proteins directly, as with albumin (Atha & Ingham, 1981), and has been proposed as its mode of action in IMP aggregation (Honda et al., 1981*a*). This, however, appears not to be the case, since IMP-free patches appear within the multilamellar vesicles of GPR (Fig. 6*d*), in areas which are beyond the reach of PEG (Aldwinckle et al., 1982). (3) The excluded volume effect causing proteins to be excluded from areas occupied by the solvent could cause precipitation or flocculation of proteins (Laurent, 1963; Maroudas, 1975; Atha & Inghams, 1981), and lead to IMP aggregation. (4) Changing the lipid structure of the membrane would also allow for IMP aggregation (Speth & Wunderlich, 1973; Power et al., 1978; Hui et al., 1980). Previous studies (Boni et al., 1984) have revealed the tendency of PEG to make lipid dispersions more gel-like. The temperature-dependent IMP patching observed by Roos et al. (1983) could also be a lipid phase separation phenomenon. The different reversibilities of IMP patching in GPR and in CFV after being treated in 35% PEG could be a consequence of PEG interacting differently with their particular lipids.

The similar results on CFV and VMC indicate that the presence of cytoskeleton elements in VMC has no apparent effect on fusion or IMP distribution. Perhaps the major function of cytoskeletal elements on PEG-induced cell fusion is the preservation of a normal IMP distribution against patching. However, the loss of patching restriction in the VMC could be a result of a mechanically disrupted spectrin network, or a consequence of the higher degree of curvature which makes the cytoskeletal constraints on IMP less effective. We find no evidence of any active participation of cell cytoskeleton in the PEG-induced fusion process.

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