Discrimination of Two Fusogenic Properties of Aqueous Polyethylene Glycol Solutions

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Investigations on the dose response of cell fusion, induced by ionfree aqueous polyethylene glycol (PEG) solutions, reveal distinct lowest fusogenic PEG concentrations for different permanently growing mammalian cell lines. Part of the requisite PEG can be replaced by carbohydrates, preserving the fusogenity of the solutions. This discriminates two effects of PEG solutions causing cell fusion: a) cell shrinkage, the required hyperosmolality of the solutions may be provided by PEG or by carbohydrates, is supposed to cause intracellular processes necessary for consolidating polycaryons; b) membrane alterations, which can not be induced by carbohydrates, enable intimate cell-cell contact via particle-free membrane areas. Depending on cell line salts can not only raise the osmolality of PEG solutions but are able to co-operate with PEG in generating membrane alterations.

The polyethylene glycol-(PEG)-induced cell-cell fusion, as many other fusion events, consist of several distinct stages: (a) cells come to very close proximity, their adjacent membranes contact via particle-free lipid areas, (b) intercellular cytoplasmatic bridges develop at these contact sites and (c) the cytoplasmatic bridges expand and stable conditions in membrane and cytoplasma of the polycaryons are restored (for ref. see [1, 2]). By which properties of PEG solutions the necessary cell alterations are induced was still an open question [1, 3]. In this paper I demonstrate, how in ionfree fusogenic PEG solutions PEG can be replaced in part by carbohydrates and/or salts. These findings discriminate at least two properties of PEG solutions necessary for cell fusion. There is evidence that one of them accounts for membrane alterations enabling intimate cell-cell contact via particle-free lipid areas whereas the other induces intracellular processes involved in consolidating polycaryons. The high affinity of PEG molecules to water is supposed to be basicly responsible for all fusogenic properties of aqueous PEG solutions.

Confluent monolayers of permanently growing mammalian cell lines (HeLa, HE, BT5C1, BICR/M1R-K) were used in standardized fusion experiments carried out at first with ionfree aqueous PEG solutions [2]. Cytotoxic effects remained negligible in all experiments presented. The fusion yield expressed by the fusion index (F.I. = nuclei per 100 plasma bodies) was determined in May-Grünwald-Giemsa stained preparations fixed 3–4 h after PEG treatment. Each plotted F.I. represents the mean of at least 5 countings including 200–1000 nuclei in one plastic dish. For untreated cells very reproducible F.I.'s of 100–103 were ascertained; F.I.'s > 105 therefore indicate a fusogenic treatment. Each curve (Fig. 1–3) represents results obtained from a distinct batch of cells. Different batches of one cell line treated identically cause different F.I.'s when high PEG concentrations were used. But for each cell line one distinct lowest fusogenic PEG concentration (between 27.5% and 32.5%; Fig. 1) could be proved in many experiments. Investigating PEG-induced cell alterations, described to be essential for cell fusion [4–7], I found, that PEG concentrations below this threshold also evoked cell agglutination; aggregated protein particles and particle-free lipid areas in cell membranes were present in HeLa and BICR/M1R-K-cells treated with more then 20% PEG. However, direct measurement of the height of HeLa-cells revealed, that non-fusogenic aqueous solutions containing 20% PEG were isoosmotic to the cytoplasma whereas a fusogenic 50% PEG solution reduced the cell height by more than one third. To proof whether dehydration of the cells is a stimulus for cell fusion, the osmolalities of PEG solutions were raised by addition of sucrose. Indeed, PEG solutions led to higher fusion yields when containing sucrose. Moreover, the lowest effective dose of PEG for cell fusion decreased to 20–25% depending on

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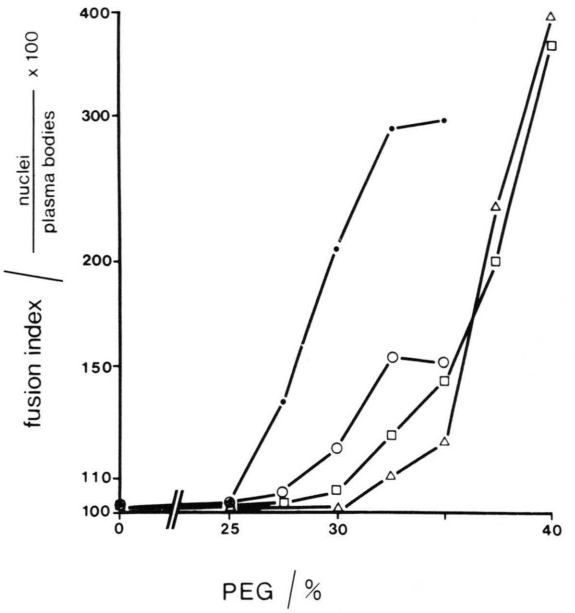

Fig. 1. Dose response of cell fusion induced by ionfree aqueous PEG solutions. For HeLa- (□–□), HE- (△–△), BT5C1- (○–○) and BICR/M1R-K- (●–●) -cells distinct lowest fusogenic PEG concentrations are found.

cell line when solutions contained 0.3 mol/l sucrose (Fig. 2). PEG-induced cell agglutination and membrane alterations were not effected by this surplus of sugar [2]. Other carbohydrates, *e. g.* glucose, fructose, lactose and mannitol had an identical effect. From measurements of cell height (it was assumed that close cell-cell contact prevents lateral cell shrinkage and only water outflow takes place), the water contents of HeLa-cells under PEG solutions were calculated. It was found to decrease from 83% for cells in culture medium (for ref. see [8]) to 70% in 32.5% PEG solutions and to 20% in 50% PEG solutions. This estimation indicates a dehydration of the cells to such an extent that proteins denaturate and intracellular structures collapse. Freeze fracture observations and interference measurements support these conclusions [2]. After replacing fusogenic PEG solutions by culture medium, cell size and intracellular structures are restored by rapid water reuptake. Phase contrast observations and ultrathin sectioning revealed severe secretion proceses [4]. They probably excret irreversible denaturated cell components and excessively penetrated water. It is supposed that these intracellular processes support the enlargement of intercellular cytoplasmatic connections as well as the reorganisation of membranes and cytoplasma.

As a result there is evidence, that osmotically induced cell shrinkage is one essential effect of fusogenic PEG solutions; the proposed implication for the cell fusion event remains hypothetically.

Whereas carbohydrates, as mentioned above, do not affect PEG induced membrane alterations, there is evidence that salts (*e. g.* NaCl, $CaCl_2$, Na_2HPO_4) are able to co-operate with PEG in inducing these alterations. The lowest effective dose of PEG required for cell fusion decreased to 12.5% for BICR/M1R-K-cells when solutions contained 0.3 mol/l NaCl (Fig. 3). Most of the sodium chloride could be substituted by an isoosmotic amont of sucrose, so that a significant fusion yield (F.I. > 110) was obtained after treating BICR/M1R-K-cells with aqueous solutions containing 12.5% PEG, 0.06 mol/l NaCl and 0.3 mol/l sucrose. It is assumed that the amount of sodium chloride which can be replaced by sucrose substi-

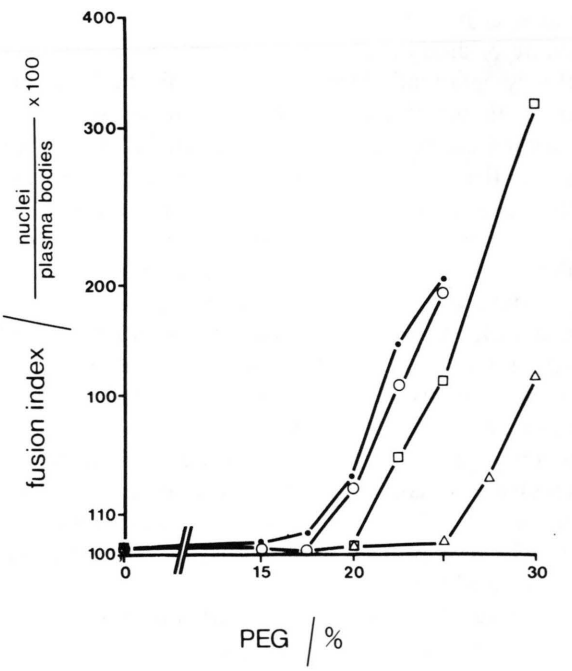

Fig. 2. Dose response of cell fusion induced by aqueous PEG solutions containing 0.3 mol/l sucrose. Fusion of HeLa- (□–□), HE- (△–△), BT5C1- (○–○) and BICR/M1R-K- (●–●) -cells can be induced with PEG concentrations below the thresholds shown in Fig. 1. In previous fusion experiments 0.3 mol/l sucrose was found be the optimal effective dose. Higher sugar concentrations cause increasing lateral cell shrinkage and cell fusion is more and more impeded because of the lack of cell-cell contacts.

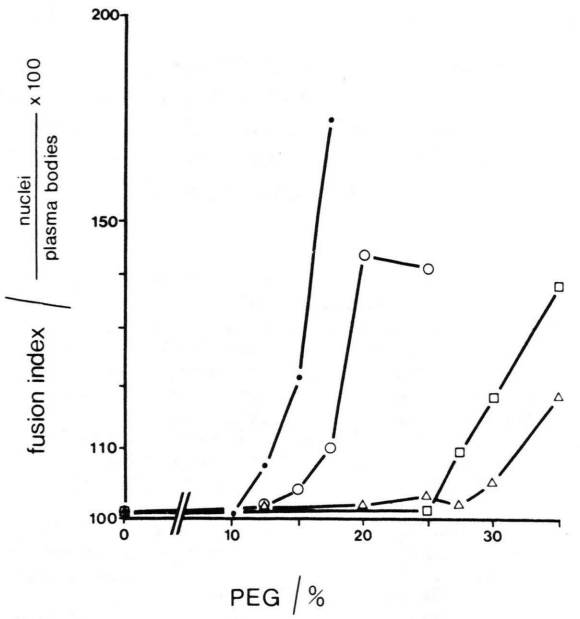

Fig. 3. Dose response of cell fusion induced by aqueous PEG solutions containing 0.3 mol/l NaCl. Fusion of BT5C1- (○–○) and BICR/M1R-K- (●–●) -cells but not of HeLa- (□–□) and HE- (△–△) -cells can be induced with PEG concentration below the thresholds shown in Fig. 1 + 2. In previous fusion experiments 0.3 mol/l NaCl was found to be the optimal effective dose. Higher salt concentrations tended to be cytotoxic. Note that for HeLa- and HE-cells the fusion yield even decreases if NaCl is added to fusogenic PEG solutions (compare with Fig. 1).

tutes PEG in generating the essential hyperosmolality (see above) in the solution. In addition, however, sodium chloride (the part which cannot be replaced by sucrose) enables even solutions containing 12.5% PEG to induce cell fusion if sufficient osmolality is provided. That indicated, that sodium chloride facilitates PEG induced membrane alterations. Preliminary freeze fracture investigations confirm this hypothesis. In principle these results are valid also for BT5C1-cells (Fig. 3). Using HeLa- or HE-cells, however, it was found that sodium chloride could hardly influence the fusogenic potency of aqueous PEG solutions (see Fig. 1 + 3), even if they contained sucrose. Other salts tested (results will be published in detail elsewhere) did not show substantial different effects.

The induction of close cell-cell contact demandes mechanisms to overcome electrostatic repulsion [9] and steric hinderance due to glycopolysaccharides [10] between adjacent membranes. The formation of particle-free membrane areas, which are the designated points of intercellular membrane fusion, requests lateral displacement of membrane particles. Taking into account previously described findings [1–3, 5, 7] as well as the results presented in this paper it is supposed that these necessary membrane alterations finally occur due to the lack of free water in fusogenic PEG solutions. Tilcock and Fisher calculated, that in aqueous solutions containing more than 13% PEG every water molecule is bound or at least "structured" [11]. Under suchlike PEG solutions glycopolysaccharides lose their water of hydration and collapse [1]. Due to the water deficit PEG molecules change to a conformation [12] more potent for complexing charge bearing molecules in the membrane. As a result close cell-cell contact is facilitated. Refering to the hypothesis desgned to explain the PEG induced protein precipitation [13, 14], membrane particles may aggregate because they seek for a hydrophilic environment under "water-free" fusogenic PEG solutions; particle-free membrane areas occur inevitably. Salts, as already described for protein precipitation, may diminish the charge of particles and thus facilitate their aggregation [15]. Solutions containing less PEG than 10%, which according to Tilcock and Fisher [11], possess "unstructured" water, do not cause cell fusion and are not effective in precipitating proteins [15], irrespectivly of their salt concentration. As shown above, salts rather obstruct PEG-induced fusion when HeLa- or HE-cells were used. Clearcut differencies between these two cell lines on the one hand and BICR/M1R-K- or BT5C1-cells on the other hand have been described previously [16, 17]. To investigate why these two groups of cell lines respond different in distinct cell fusion experiments gives hope for valuable information about cell properties and molecular mechanisms involved in fusion events.

In conclusion, a distinct part of the requisite PEG in aqueous fusogenic solutions can be replaced by carbohydrates. This discriminates two effects of PEG involved in inducing cell fusion. The part of PEG which can not be replaced by carbohydrates accounts for membrane alterations necessary for fusion. It is proposed that membrane alterations occur due to the lack of "free water" in the solutions. Depending on cell line, salts co-operate with PEG and facilitate membrane alterations. The part of PEG which can be replaced by carbohydrates provides hyperosmolality in the solutions and thus dehydrates

the cells. In consequence intracellular processes are induced which supposedly support the expansion of intercellular cytoplasmatic bridges and the consolidation of the polycaryons. The fusogenic effects of PEG would be basicaly an expression of its high affinity to water.

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