Effect of Water Soluble Polymer, Polyethyleneglycol, and Glass-Forming Compounds on Cell Fusion

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The cell fusion of Molt T-cells was investigated in the presence of water soluble polymer, polyethyleneglycol (PEG) and glass-forming monomers. In cell fusion with PEG only, inactivation of the cell occurred within 2 min. However, in the presence of PEG and water soluble polymer, most of the fused and unfused cells lived even after fusion times of more than 10 min. It was observed that water soluble polymer prevented the inactivation of cells during fusion as a protectant. As the result, ratio of fused cells increased in the presence of water soluble polymers.

Some glass-forming monomers used as new fusogens such as M-23G ($n = 23$) and M-50G ($n = 50$) monomers having long oxyethylene chains in the methoxypolyethyleneglycol methacrylate, $CH_3O-(-CH_2CH_2-O)_n-CO-C(CH_3)=CH_2$ as well as PEG #2000 indicated large promoting and protecting effects on cell fusion.

Introduction

Cell fusion has been studied actively as one of the useful techniques in cell technology. Two methods for cell fusion are generally known [1, 2]. One is the method using Sendai Virus (virus-induced cell fusion). The other is the chemical method using polyethyleneglycol (PEG) [3] and Ca^{2+} or Mg^{2+} as a fusogen (Chemically-induced cell fusion) [4]. The latter method is a very simple one and is generally used for the fusion of animal and plant cells. Fusion of erythrocytes by fatty acids and esters was reported by Lucy *et al.* [5] and it was found that long chain esters such as glycerol monooleate had high fusion activity.

Recently, we found that microbial cells [6, 7] and tissue cells [8–10] could be immobilized stably by means of radiation-induced polymerization of glass-forming monomers having long oxyethylene structures at low temperature. For example, cells such as chloroplast [8] and erythrocyte [9] were immobilized, keeping high activity for a long period using long chain monomers such as polyethyleneglycol dimethacrylate and methoxypolyethyleneglycol methacrylate as a carrier. Moreover, we found that protein and water soluble polymers were effective on immobilization as protectants preventing inactivation of cells in the previous paper [10]. When various cells are fused using PEG, inactivation of cells occurs quickly and the fusion is stopped. Therefore, it is expected that cell fusion is stabilized and promoted in the presence of various additives such as water soluble polymer and glass-forming monomers.

In this report, the effects of these additives combined with PEG on cell fusion were investigated.

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Materials and Methods

Molt T-cells of human lymphocyte tumors were used for fusion. Cell fusion was carried out as follows: Molt T-cells growing in Eagle's minimum essential medium (MEM) including 10% fetal calf serum (FCS) under an atmosphere of 5% CO_2–95% air at 37 °C were collected at the bottom of plastic tubes of 10 mm diameter by centrifuge at 800–1000 rpm. Then, mediums including fusogens and protectants of 0.5–1.0 ml were poured slowly on the collected cells ($100-200 \times 10^5$ cells/ml), and they were incubated for determined times at 37 °C. When fusogen was poured on the collected cells, the tube was rotated somewhat. After cell fusion, MEM of 10 ml were added to tubes containing cells and the fusogen and protectant were removed by rinsing with medium. Fused cells were observed by optical microscopy and phase contract microscopy. The ratios of fused cells and living (fused and unfused cells) to total cells were determined after counting

cells within the field of the eye-piece of the microscope. Fused cells contained two or more cell nuclei.

Polymers used were commercially available polyvinylprrolidone (Average molecular weight = 10000, 24500 and 40000), polyethyleneglycol (#1000, 2000 and 6000), polyvinylalchol, polyacrylamide and poly-DL-Alanine. Glass-forming monomers such as 9G ($n = 9$) and 14G ($n = 14$) of polyethyleneglycol dimethacrylate, $[CH_2=C(CH_3)-CO-(-O-CH_2CH_2-)_n-O-CO-C(CH_3)=CH_2]$, M-9G ($n = 9$), M-23G ($n = 23$) and M-50G ($n = 50$) of $[CH_3O-(-CH_2CH_2-O-)_n-CO-C(CH_3)=CH_2]$, vinylprrolidone, 2-hydroxyethyl methacrylate, acrylamide, dimethylaminoethyl methacrylate, calcium methacrylate, diacetone acrylamide and 2-hydroxypropyl methacrylate were used as a fusogen of Molt T-cell.

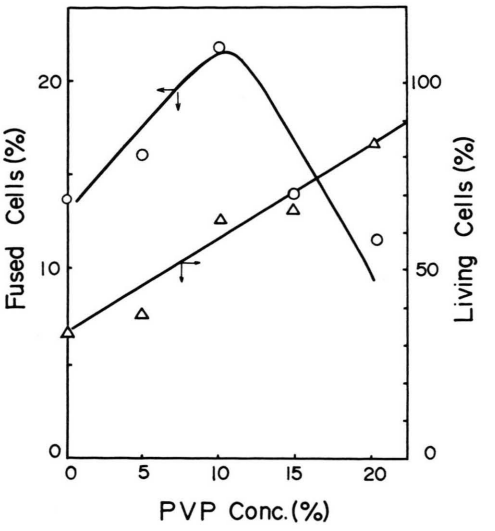


Fig. 1. Effect of polyvinylprroridone (PVP) on cell fusion by polyethylene glycol (PEG) #1000. (PVP + PEG): medium = 50%:50%; molecular weight of PVP, 40000; fusion time, 10 min.

Results and Discussion

Effect of water soluble polymer on cell fusion by PEG

PEG is widely used as an inter cellular matrix for cell fusion. But in cell fusion of lymphocytes, cells are easily inactivated (within 2 min) when they are immersed in a medium including PEG. As the result, the ratio of fused cells is not greatly increased. Therefore, a survey of protectants to prevent the inactivation of cells during cell fusion is greatly significant. The effect of the addition of water soluble vinylpolymers as protectants on cell fusion was investigated. Fig. 1 shows the effect of polyvinylprrolidone (PVP, Average molecular weight = 40000) concentration (%) on cell fusion of lymphocytes by PEG #1000. As shown here, the ratio of living cells increased with increasing PVP concentration, though 60% of the cells were inactivated without PVP. The ratio of cell fusion reached a maximum with 10% of PVP and was lowered in concentrations of PVP more than 10%. It was reported that in hybridization of 3T3 and R96 cells in mice, cell fusion occurred effectively with the mixture of 50% medium and 50% PEG [3]. According to microscopic observation, water soluble polymers such as PVP, polyvinylalchol and polypeptide in a medium caused aggregation of cells, and cell fusion by these polymers hardly occurred. When 20% PVP was added to the medium, the ratio of cell fusion was lower than in the case without PVP. This fact might be attributed to the decreased opportunity of cell membranes to contact and fuse with each other owing to the covering of cell membranes by PVP. Fig. 2 shows the effect of PVP addition on cell fusion and cell life. The ratio of living cells without PVP decreased with times, and all cells were inactivated after 30 min. On the other hand, in the presence of PVP, the inactivation of cells was much slower than in the case without PVP, and 40% of the cells remained

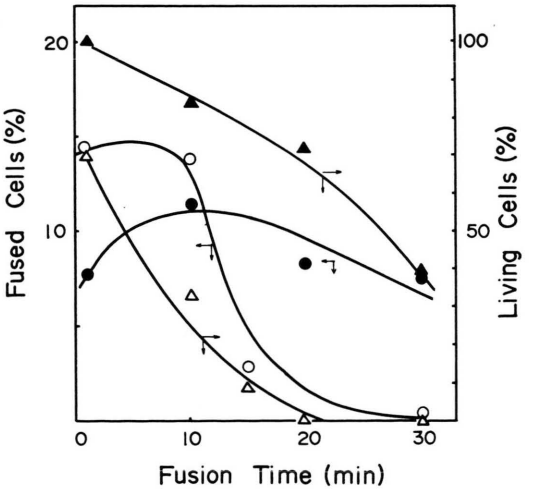


Fig. 2. Effect of PVP addition on cell fusion by PEG #1000. (○, △) PEG 50%-medium 50%; (●, ▲) PEG 30%-PVP 20%-medium 50%; molecular weight of PVP; 40000.

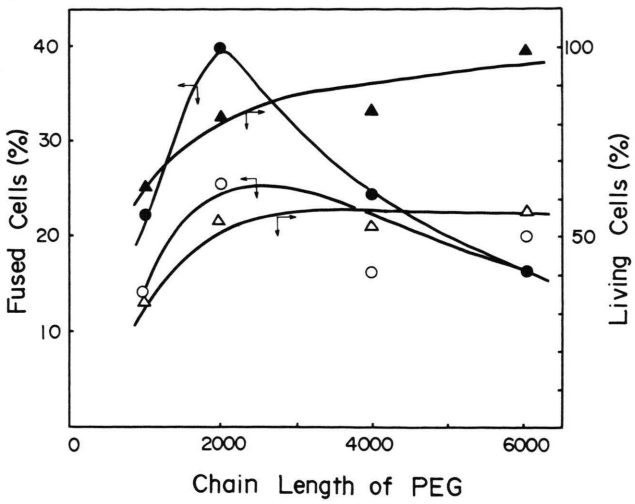
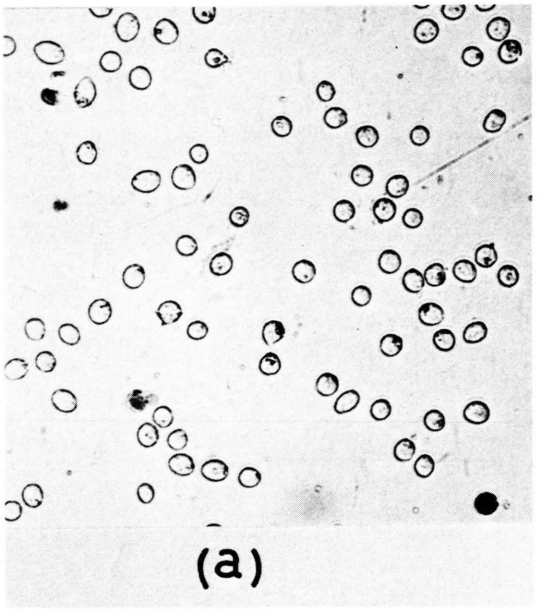


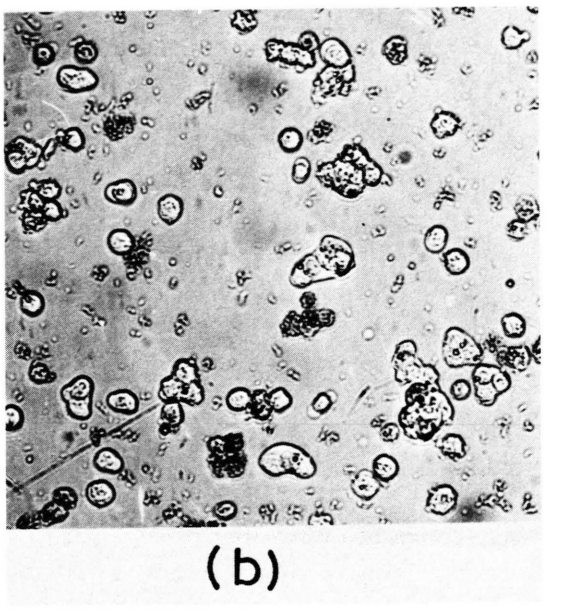
Fig. 3. Effect of chain length of PEG on cell fusion in PEG-PVP and PEG alone. (○, △) PEG 50%-medium 50%; (●, ▲) PEG 40%-PVP 10%-medium 50%; molecular weight of PVP; 40000; fusion time, 10 min.

living even after fusion times of 30 min. In the case of no PVP addition, the ratio of cell fusion reached 12.5%, however, it decreased with the increasing of the inactivated cells in the latter stage. In the presence of PVP, the ratio of cell fusion was slightly lower than in the case of no PVP and they increased during the first 10 min of incubation. Even after 30 min, the ratio of cell fusion was almost the same as it was in fusion time of early stage. It is obvious that PVP in medium retarded the inactivation of cells during fusion. Fig. 3 shows the effect of the chain length of PEG on cell fusion and cell life. In the absence of PVP, the ratios of living cells and fused cells were similar for 2000 and 6000 of PEG, but they were larger in the case of PEG 1000. In the presence of PVP, the ratio of living cells increased extremely with increasing molecular weight of PEG, and cells of 100% were living in fusion by PEG 6000. Cell fusion by PEG 1000 and 2000 including PVP were promoted remarkably in comparison with the cases without PVP, and fusion ratio by PEG 2000 and PVP reached 40%. This fact suggested that the ratio of fused cell increased since the inactivation of cells during fusion was retarded by PVP. In the case of fusion by PEG 6000, the ratios of cell fusion were not affected solely by the presence or absence of PVP, although all cells were living. Viscosity in the medium including PEG 6000 became very high due to its high molecular weight, so mutual contact of cells might have been retarded. This might be the reason for the decreased fusion ratio. The effect of the molecular weight of PVP on fusion of Molt T-cell by PEG 2000 was investigated, and PVP of average molecular weight of 10000, 24500 and 40000 were used. In the case of PVP 10000 addition, living cells were 98% and fused cells were 47% in fusion times of 10 min. PVP 10000 was more effective than PVP of the higher molecular weights for cell fusion by PEG 2000. The effects of other water soluble polymers as a protectant such as polyvinylalchol, polyacrylamide, poly-DL-Alanine and poly-L-Lysine HB_r on cell fusion and cell life by PEG 2000 were studied. These polymers retarded the inactivation of cells and promoted the fusion of cells by PEG. Polypeptides polymers such as poly-DL-Alanine and poly-L-Lysine HB_r were more effective as a protectant for fusion and inactivation of cells by monomers, as described later. Fig. 4 shows optical microphotographs of fused cells. Intact cells had circular shapes. The black cells in the pictures were inactivated cells. They could be colored by the addition of dye due to the destruction of their membranes. As seen in Fig. 4b, black cells were rarely found even after a fusion time of 10 min, and many fused cells were observed in the pictures. This fact showed evidently that inactivation of cells was retarded and cell fusion was promoted by PVP addition. On the other hand, many cells were inactivated during fusion, and the ratio of cell fusion decreased in the absence of PVP (Fig. 4c).

Cell fusion by glass-forming monomers

Surveys for other chemical compounds as fusogen except for PEG were reported by Lucy *et al.* [5].





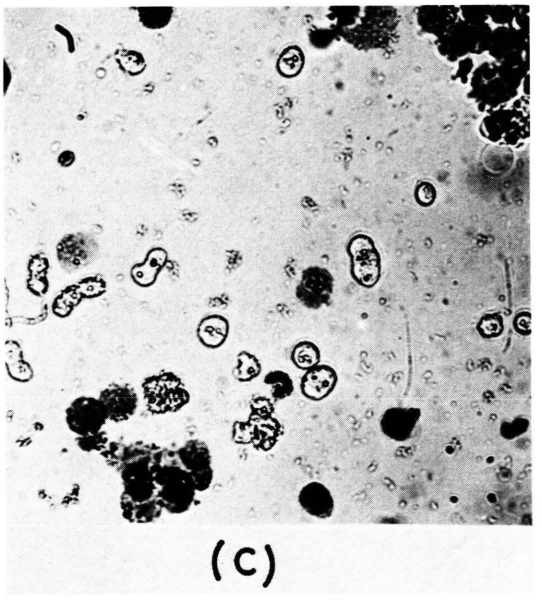


Fig. 4. Optical microphotographs of intact and fused cells. (a) intact; (b) fusion by composition of PEG #2000 40%-PVP 10%-medium 50%; (c) fusion by PEG #2000 alone (PEG 50%-medium 50%); fusion time, 10 min.

They found the effect of glyceryl monooleate including dextran as a fusogen in the cell fusion of erythrocytes by fatty acids, esters, retinol and α-tocopherol. Dextrane was used as a protectant to prevent inactivation during fusion. The effects of glass-forming monomers and solvents as fusogen were investigated. Fig. 5 shows the effect of PVP or poly-DL-Alanine on fusion of T-cell by methoxypolyethyleneglycol methacrylate, M-23G. No cell fusion occurred by M-23G alone since all cells were inactivated. M-23G induced cell fusion when inactivation was prevented by water soluble polymer addition. However, it was necessary that cell fusion by M-23G including PVP should be carried out within 1 min since inactivation occurred rapidly. With the addition of poly-DL-Alanine, cells were relatively stable

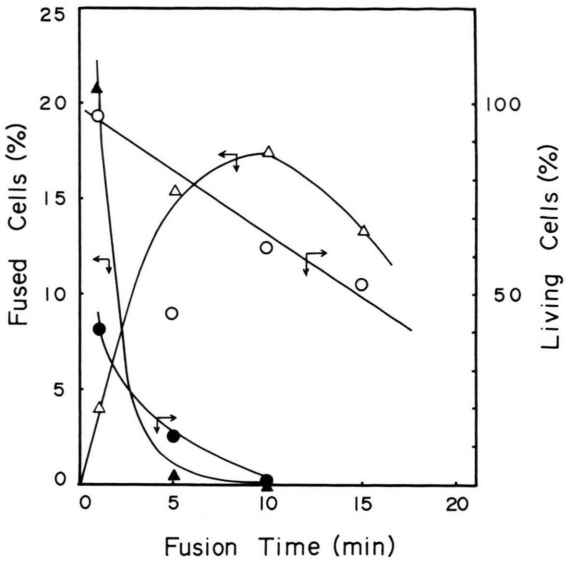


Fig. 5. Effect of PVP and poly-DL-Alanine on cell fusion by M-23G monomer. (○, △) M-23G 45%-medium 50%-Poly-DL-Alanine 5%; (●, ▲) M-23G 40%-medium 50%-PVP 10%; PVP, 10000.

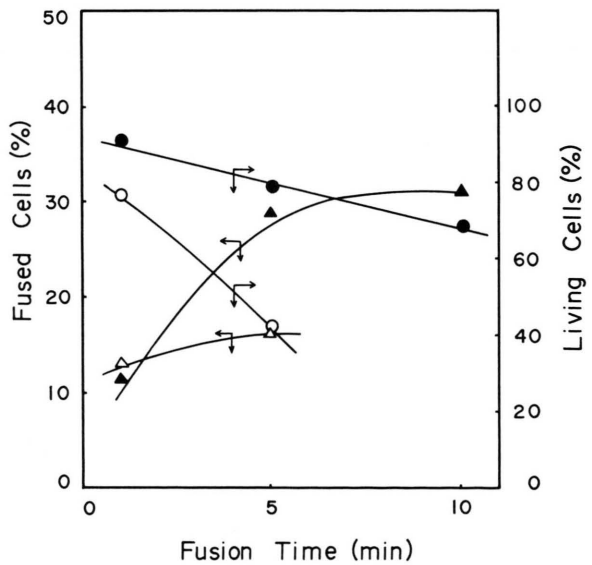


Fig. 6. Effect of PVP on cell fusion by M-50G monomer. (○, △) M-50G 50%-medium 50%; (●, ▲) M-50G 45%-medium 50%-PVP 5%, PVP, 10000.

for a long time. The ratio of fused cells increased up to 10 min, and then it decreased.

In cell fusion in the presence of M-23G, the protecting effect of poly-DL-Alanine on cell fusion was larger than that of PVP. Cell fusion by M-50G having a longer oxyethylene unit than M-23G was tried and the results are shown in Fig. 6. Cell fusion occurred even by M-50G alone. This fact suggests that virulence for cells of M-50G was smaller than that of M-23G. The ratio of fused cells increased and that of inactivated cells decreased during fusion by M-50G in the presence of PVP, and about 30% cells in all cells were fused. Fig. 7 shows the effect of poly-DL-Alanine and PVP concentration on cell fusion by M-50G. With the addition of poly-DL-Alanine, the ratio of inactivated cells decreased with increasing poly-DL-Alanine concentration. Cells of 30% fused in a lower concentration of poly-DL-Alanine 0.5%, but

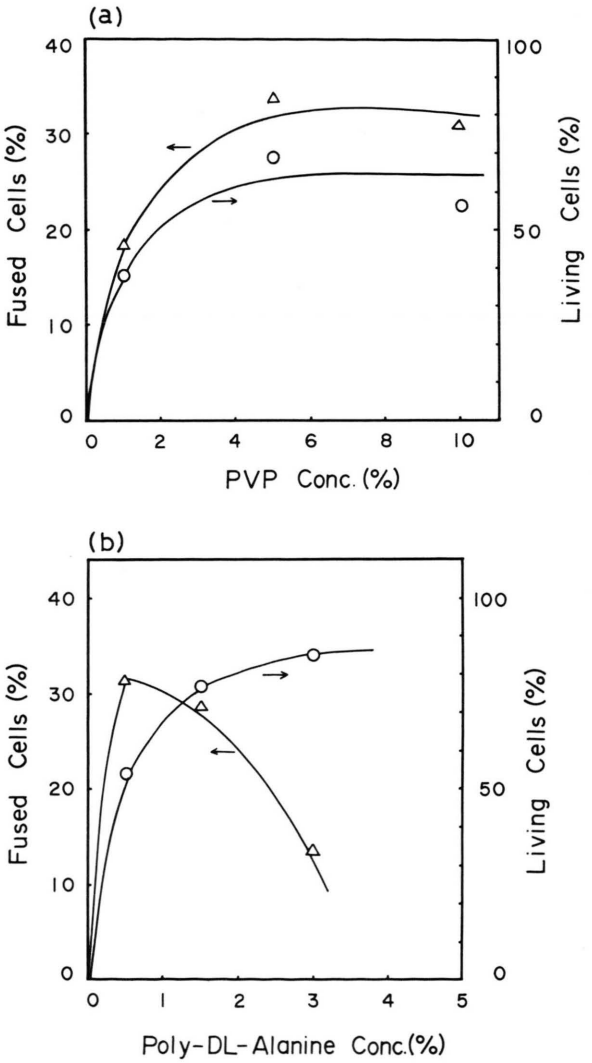


Fig. 7. Effect of PVP (a) and poly-DL-Alanine (b) on cell fusion by M-50G monomer. Fusion time, 10 min; PVP, 10000.

in the presence of more than poly-DL-Alanine 1%, the ratio of fused cells decreased greatly. In the case of PVP addition, the ratio of fused and living cells increased with increasing PVP concentration, though they saturated at a PVP concentration of 5%. Fusion cells reached 30% and cells of 80% lived. Optimum concentrations of the protectant for cell fusion by M-50G were 0.5% for poly-DL-Alanine and 5% for PVP and were quite different by the kind of polymer. From these results, cell fusion occurred easily in the presence of protectants, though inactivation of 20–25% cells was inevitable. Cell fusion hardly occurred in the presence of additives having an excess protective effect on cell membrane. Rapid inactivation of cells by the addition of fusogens such as PEG and monomer was retarded in the presence of water soluble polymer as a protectant. Compounds such as M-9G having shorter oxyethylene chain length than M-23G and M-50G showed no fusion activity. Fusion activity increased with increasing oxyethylene length in this monomer series according to the order of M-9G < M-23G < M-50G. In other hydrophilic monomers such as 9G, 14G, vinylprroridone, 2-hydroxyethyl methacrylate, acrylamide, dimethylaminoethyl methacrylate, calcium methacrylate, diacetone acrylamide and 2-hydroxypropyl methacrylate showed no fusion activity even in the presence of protectants.

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