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THE COMPONENTS CONTAINED IN POLYETHYLENE GLYCOL OF COMMERCIAL GRADE (PEG-6,000) AS CELL FUSOGEN

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Summary: By reprecipitation with ether and/or by dialysis in water completely disappeared the fusion activity of PEG-6,000 in commercial grade with keeping the whole activity of cell aggregation. It was concluded that PEG-6,000 in commercial grade contains at least two components, one of which has the activity of cell aggregation and the other of which has the activity of perturbation of phospholipid bilayer. The former is PEG-6,000 itself and the latter is considered to be a catalyst or terminator of polymerization of ethylene oxide and/or an antioxidant for PEG.

INTRODUCTION

Cell fusion is a widely applied technique in genetic engineering, cell technology and other fields. There are two ways for cell fusion: virus-induced and chemically-induced methods. The latter has been developed by Lucy et al.(1) and other groups (2-5), and it is believed to be superior to the former because of the following reasons: (1) wide potential for various cell lines, (2) easy handling and (3) stability of the fusogens.The chemicals which induce cell fusion are called "fusogen". There are several types of fusogens. The typical chemicals are (A) fat-soluble substances (fatty acids containing long chains of saturated or unsaturated alkyl groups and liposomes consisting of various phospholipids) (1-a,6,7), (B) water-soluble polymers (polyethylene glycol (PEG), polyvinyl alcohol (PVA), dextran, dextran sulfate, gelatin, lectin, and

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so on) (1-c,2-5,8),and (C) others (9). The present authors have focussed their attention on the fusogenicity of water-soluble synthetic polymers, because amongst various types of fusogens they have a higher fusogenic activity with a lower lytic activity for plasma membranes. Novel polymers which are more fusogenic than commercial grade of PEG or PVA have been prepared by the chemical modification of PEG with various lipids in our laboratory (10). We report here an interesting and important finding that the fusogenicity of commercial grade of PEG disappeared when it was purified by reprecipitation and/or dialysis. This became a trigger for the elucidation of the mechanism of PEG-induced cell fusion and also for the synthesis of a series of new type of fusogens.

MATERIALS AND METHODS

Heparinized human venous blood was collected from healthy donors. Supernatant and buffy coat were completely removed by centrifugation (3,000 rpm x 5 min at 5 °C) and erythrocytes were further washed with Eagle's basal salt solution. Washed erythrocytes were re-suspended in Eagle's b.s.s. at 1 % of hematocrit and 1 ml of this suspension was centrifuged (1,200 rpm x 5 min at 5 °C). The supernatant was almost completely removed and then 50 wt% of PEG solution (1 ml) was mixed with the precipitates. After this suspension was incubated at 37 °C for 1-3 min, 10 ml of Eagle's b.s.s., which prewarmed at 37 °C, was added to it and further incubated at 37 °C for 5 min. Then, fused erythrocytes were collected by centrifugation (1,200 rpm x 5 min at 25 °C) and fixed with 1 % glutaraldehyde for light and scanning electron microscopies.

Purification of PEG: Commercial grade of PEG-6,000 was purified by reprecipitation according to the following method:10 g of PEG was dissolved in 80 ml of chloroform and reprecipitated into 2,000 ml of diethyl ether. Dialysis of PEG was carried out as follows: 5 % of PEG solution was dialyzed against distilled water for 22 h using Spectrapor Membrane® (Mw cut off 3,500, Spectrum Medical Industries Inc.)

The fusion index was calculated as the ratio of fused cells to total cells in several different fields of microphotographs with 500-600 cells counted.

RESULTS AND DISCUSSION

Generally, commercial grade of PEG contains several kinds of contaminants, for example, the initiator of polymerization of ethylene oxide, oligomeric products of PEG, oxidative decomposition products like aldehyde or ketone (11), and/or antioxidants. One kind of commercial grade of PEG had UV-absorption band and the other had no UV-absorption band as shown in Table I. The UV-absorption band disappeared due to the reprecipitation with ether as described

Table I. Changes of fusion index of PEG-6,000 supplied by different manufacturers before and after purification.

Sample	Before purification	After purification		UV absorption
		Ether precipt. %	Water dialysis %	(commercial grade) nm
A	8.2 ± 3.2	1.7 ± 0.6	—	290
B	8.6 ± 2.8	5.5 ± 3.7	0.9 ± 0.2	290
C	6.6 ± 3.3	0.5 ± 0.2	—	n.d.
D	6.1 ± 2.1	0.6 ± 0.1	—	n.d.
E	8.8 ± 0.9	0.5 ± 0.3	—	245,269,275,282,293,302
F	4.3 ± 2.1	4.5 ± 2.1	0.5 ± 0.2	290

Cell density 10^7 cells/ml, in Eagle's b.s.s.(Eagle's M.E.M.(Nissui Seiyaku Co., Ltd.) 9.4 g/l + sodium cacodylate 1.6 g/l + glucose 1.0 g/l), pH 7.4 at 37 °C. Eagle's b.s.s. contains 1.8 mM of calcium ions (a final concentration). 50% of PEG was contacted with the cells for 1 min at 37 °C and then diluted to 4% for further incubation (5 min, 37 °C) in Eagle's b.s.s.

in Table I. Regarding the fusogenicity of commercial grade of PEG, it was fully reduced in the cases of the samples A and E, but in the others (samples B and F) scarcely reduced by the reprecipitation with disappearing the UV-absorption band near 290 nm. A certain kind of PEG (samples C and D), although it had no UV-absorption bands, was normally fusogenic. In any case, a combination of the reprecipitation with ether and the dialysis in water completely reduced the fusogenicity of commercial grade of PEG without reducing cell agglutinating capability.

When the fraction which had been removed from commercial grade of PEG by the reprecipitation was added again to the purified PEG after evaporation of the solvent, the fusogenicity was shown to some extent (see Fig.1 and Table II). In order to clarify the structure of the fusogenic substance, we examined the restoration of fusogenicity of such reconstituted PEG as consists of purified PEG and oligomeric PEG or antioxidants. Oligomeric PEG which has a relatively low molecular weight (Mw=44-400) had no effect on hemolysis and fusion of erythrocytes in the concentration range (< 100 μg/ml) applied in this experiment. However, when a certain kind of fatty acid like oleic acid

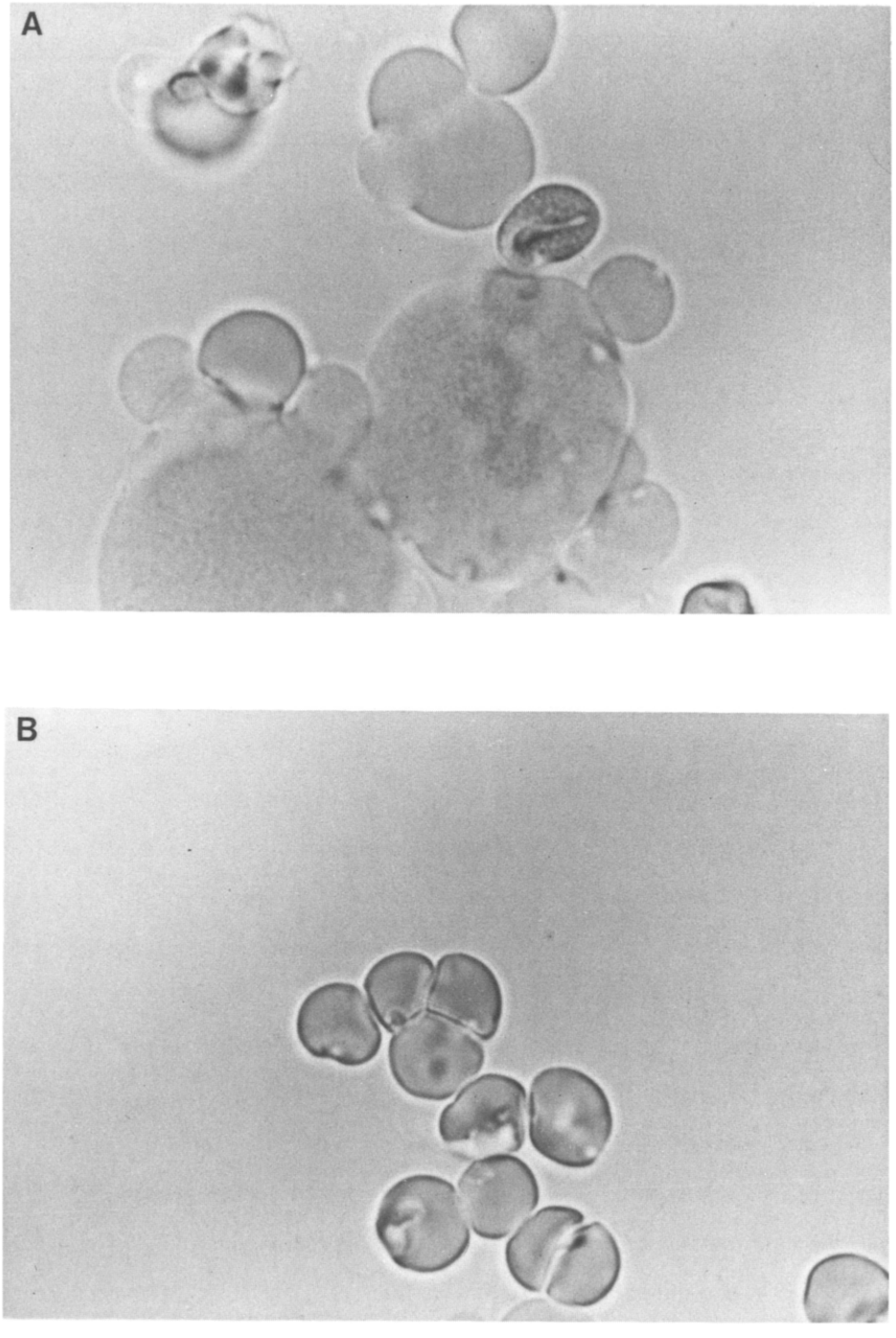

Fig.1 Light microphotographs of human erythrocytes after incubation with (a) commercial, (b) purified and (c) reconstituted PEG-6,000. magnification x 500.

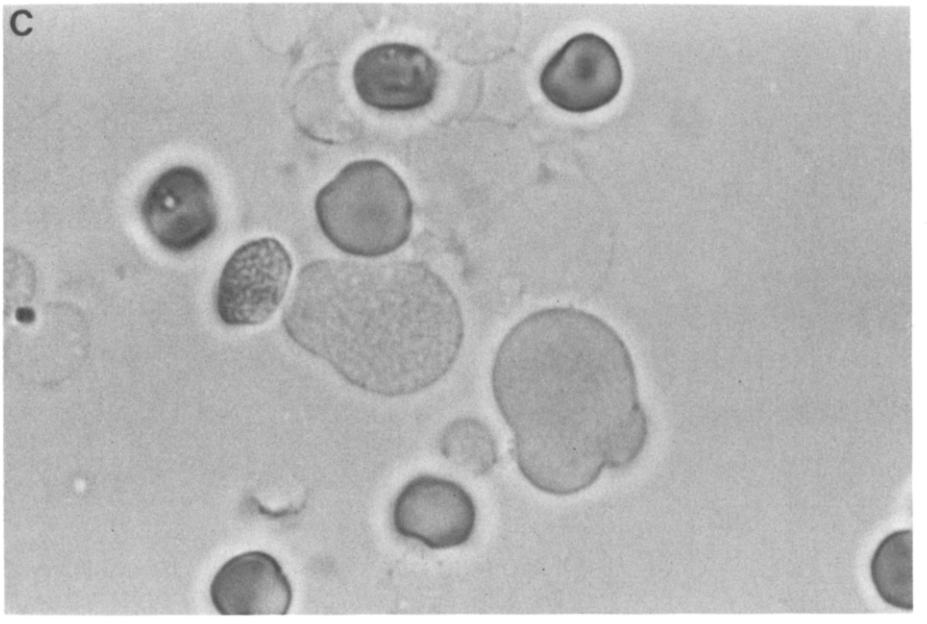

Fig. 1 - Continued

was introduced to oligomeric PEG at the terminal OH groups, the fusion index was increased approximately 1.5-fold compared to that of commercial grade of PEG (see O-23EO in Table II). In general, one of the antioxidants cited in

Table II. Fusogenicities of purified and reconstituted PEGs to human erythrocytes

Polymer	+ Additive	Fusion %	Hemolysis*[a] %	UV-absorption nm
Purified PEG		1.7 ± 0.6	12.2 ± 1.2	n.d.
"	+ Ether Extracts	5.1 ± 1.9	63.8 ± 19.2	—
"	+ α-TP*[b]	4.6 ± 0.6	7.6 ± 1.9	300
"	+ BHA*[c]	7.2 ± 2.1	33.1 ± 6.5	290
"	+ PG*[d]	1.8 ± 0.4	8.6 ± 2.3	278
"	+ O-23EO*[e]	11.8 ± 4.0	41.5 ± 5.5	—
Commercial PEG		8.2 ± 3.2	27.7 ± 8.8	290

*a) The absorbance at 578 nm of a supernatant of lysates which were prepared by osmotic shock with distilled water was used as 100%.
*b) α-TP: α-tocopherol, 4.8×10^{-3} wt%
*c) BHA: tert-butylated hydroxyanisole, 9.8×10^{-3} wt%
*d) PG: n-propyl gallate, 9.8×10^{-3} wt%
*e) O-23EO: polyoxyethylene oleate, 4.1×10^{-3} wt%

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7\text{-}\overset{\overset{O}{\|}}{C}\text{-}OCH_2CH_2O\text{-}(CH_2CH_2O)_{21}\text{-}CH_2CH_2OH$$

Table II or their combination is added to commercial grade of PEG for the protection against gradual oxidative degradation of PEG to aldehyde, ketone or carboxylic acid in atmosphere (11). It was already reported that α-tocopherol induces cell fusion in the presence of dextran (1-a). In fact, when it was added to the purified PEG, the fusion activity was partially restored. However, the UV-absorption band of the reconstituted PEG with α-tocopheral was different from that of commercial grade of PEG. Concerning UV-absorption spectra, tert-butylated hydroxyanisole (BHA) was very similar to the additives of commercial grade of PEG. In addition, the fusion activity was almost fully restored by addition of BHA. The similar effect could not be observed for n-propyl gallate (PG).

It is suggested from the above-discribed findings that there are two components contained in commercial grade of PEG concerning the activity of cell fusion: one is a high polymer and the other a small molecule. The former has a role of cell aggregation and the latter has a role of promotion of membrane fusion between aggregated cells. The intrinsic function of PEG itself is the cell aggregation by interpolymer complex formation between polymeric chains of PEG and polysaccharide chains of membrane-bound proteins through hydrogen bond (12). The small molecule of the contaminatant of PEG might interact with phospholipid bilayer and/or membrane-bound protein so that the resulting aggregated cells could be enhanced to be fused. There appear three following substances as the fusogenic contaminatant: (1) the substance which is soluble in ether and has UV-absorption band, (2) the substance which is soluble in ether but has no UV-absorption band, and/or (3) the substance which is soluble in water and has no UV-absorption band. These may be assignable either to phenolic antioxidants like BHA, the initiator or terminator of polymerization of ethylene oxide. At present, unfortunately, each substance has not exactly been identified yet.

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