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Surfactant enhancement of polyethyleneglycol-induced cell fusion

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B16 mouse melanoma cells in monolayers may be satisfactorily fused with 50% PEG 1500. However, pre-treatment with detergents in solution at low concentrations significantly increases PEG fusion, up to 8-fold in some instances, without impairing cell viability. The practical and mechanistical implications of this finding are discussed.

Cell fusion; Polyethyleneglycol; Surfactant; (B16 melanoma)

1. INTRODUCTION

Polyethyleneglycol (PEG) is commonly used in cell fusion protocols, although its mechanism of action is still unclear [1]. Studies with model membranes [2] have pointed out the detergent-like properties of PEG towards phospholipid vesicles. Moreover, it has been described [3] that the addition of small quantities of fusogenic amphiphiles (e.g. glycerol monooleate, retinol) to PEG enhances significantly its fusogenic properties.

Since we had previously shown [4–6] that a variety of commercially available detergents can induce the fusion (increase in size) of sonicated liposomes, we decided to explore the possiblility of enhancing PEG-induced cell fusion with detergents. B16 melanoma cells [7] in monolayers were chosen because of convenient culture conditions and reproducible fusion properties. Our results demonstrate the enhancing power of surfactants on PEG fusion, and raise a number of questions, both practical and mechanistical, that will be outlined below.

2. MATERIALS AND METHODS

CHAPS was purchased from Boehringer-Mannheim; all other detergents were from Sigma. All were used without further purification. PEG 1500 was 'for synthesis' quality, from Merck. The B16-F10 cell line from C57-B1/6 mouse melanoma was a gift of Dr M.F. Poupon (Villejuif, France). Cells were grown in monolayers on 25 cm^2 Falcon flasks according to Halfer et al. [8].

Approximately 24 h after subculture, cells were exposed to 50% PEG 1500 (w/w) for 1 min, then washed with serum-free growth medium [8]. When required, cells were treated with detergent solutions for 5 or 15 min, after which the detergent was decanted, and PEG added as above. 6 h after PEG treatment the cells were fixed with 1% (v/v) glutaraldehyde for 5 min, stained by the May-Grunwald-Giemsa method and examined under the light microscope. Fusion indexes were calculated as follows:

$$\text{Fusion index} = [(\text{No. of nuclei}/\text{No. of cells}) - 1] \times 100$$

In all cases, corrected fusion indexes are given, obtained by subtracting from the apparent fusion index the 'blank fusion index', i.e. fusion index in a monolayer not treated with PEG. Cell viability was assessed as described by Gerlier and Thomasset [18].

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Abbreviations: CHAPS, 3((3-cholamidopropyl) dimethylammonio)-1-propanesulphonate; CTAB, cetyltrimethylammonium bromide; PC, phosphatidylcholine; SDS, sodium dodecylsulphate

3. RESULTS

The effects of treating cell monolayers with sublytic detergent concentrations prior to PEG addition are described in detail in table 1. Results are expressed as percentages, 100% being the value obtained with 50% PEG 1500 alone. Data for both fusion and cell viability are included, for two different timelengths of detergent treatment, i.e. 5 and 15 min. Detergent concentrations, well below the corresponding critical micellar concentrations, have been selected so that cell viability is not impaired by the detergent itself [9]. Some selected results are highlighted in fig.1. In summary, it can be said that, under our conditions, certain detergents increase significantly, or even spectacularly, PEG-induced cell fusion. Non-ionic surfactants, Triton X-100 in particular, appear to have little activity in this respect. Na-cholate, CTAB or lysolecithin increase fusion indexes by about two-fold, CHAPS is even more active, and SDS is able to increase fusion, under certain conditions, by one order of magnitude. Thus, a scale of increasing power of fusion enhancement would be approximately as follows: non-ionic < lysophospholipids < bile derivatives < ionic.

It should be noted from the above results that cell viability never falls, after the combined PEG + detergent treatment, below 80% of the value obtained

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Table 1

Effect of detergents on PEG-induced cell fusion and cell viability[a]

	Detergent concentration (M)	Fusion index[b]		Cell viability[b]	
		5 min	15 min	5 min	15 min
Non-ionic					
Triton X-100	5×10^{-5}	94 ± 12[c]	119 ± 25	111 ± 32	87 ± 8
Triton X-100	7×10^{-5}	99 ± 6	111 ± 30	109 ± 23	93 ± 4
Octylglucoside	7×10^{-4}	154 ± 72	148 ± 26	132 ± 30	129 ± 31
Octylglucoside	10^{-3}	171 ± 47	154 ± 42	115 ± 25	127 ± 38
Lysophospholipids					
Lyso PC	2×10^{-6}	166 ± 45		102 ± 28	
Lyso PC	5×10^{-6}	255 ± 72		98 ± 25	
Bile derivatives					
Na-cholate	7×10^{-4}	203 ± 50	149 ± 72	112 ± 28	117 ± 33
Na-cholate	10^{-3}	226 ± 64	192 ± 46	115 ± 32	85 ± 21
CHAPS	10^{-3}	370 ± 54	327 ± 55	84 ± 26	89 ± 17
CHAPS	2×10^{-3}	373 ± 49	269 ± 98	93 ± 13	98 ± 17
Ionic					
CTAB	5×10^{-6}	211 ± 74	202 ± 73	91 ± 22	150 ± 17
CTAB	10^{-5}	249 ± 70	193 ± 86	81 ± 19	91 ± 14
SDS	2.5×10^{-5}	434 ± 107	159 ± 33	92 ± 25	84 ± 18
SDS	5×10^{-5}	835 ± 199	508 ± 185	116 ± 19	80 ± 17

[a] Cell monolayers were treated with the appropriate surfactant concentrations for 5 or 15 min; the detergent solution was then decanted and 50% PEG 1500 added for 1 min. See text for details

[b] Fusion indexes and cell viabilities are given here as percent values, 100% being the absolute value obtained after treatment with 50% PEG 1500 alone. These absolute values are 20 ± 9 for fusion indexes and 71 ± 19% for cell viabilities

[c] Values ± SD ($n = 3$)

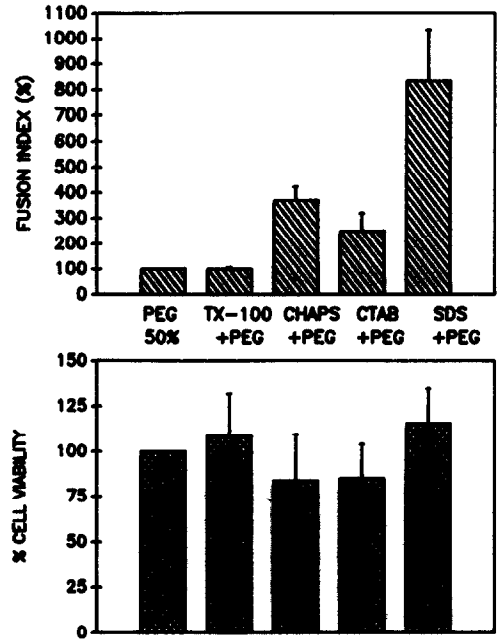

Fig.1. The effect of detergents on PEG-induced cell fusion (upper box) and cell viability (lower box). Results expressed as percentages (± SD, $n = 3$), 100% being the value obtained with 50% PEG alone.

for PEG alone. Indeed in some cases viability is higher than 100%, indicating that the combined treatment may be less harmful than PEG alone. Detergents by themselves, i.e. without any further PEG treatment, do not induce significant amounts of fusion (data not shown).

The possible implication of PEG impurities [10] in PEG fusion or its enhancement by detergents was tested

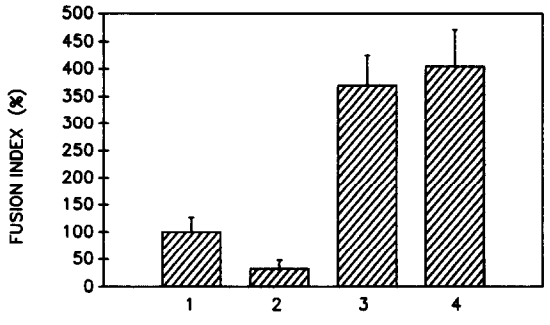

Fig.2. The effect of PEG impurities on PEG-induced cell fusion. Fusion obtained with: (1) commercial PEG; (2) PEG purified according to [10]; (3) 2.5×10^{-5} M SDS, then commercial PEG; (4) 2.5×10^{-5} M SDS, then purified PEG.

Table 2

Effect of using various experimental protocols in cell fusion induced by PEG + surfactants

Protocol[a]	Fusion index[b]	Cell viability[b]
A	49 ± 9[c]	96 ± 17
B	103 ± 17	75 ± 20
C	63 ± 20	96 ± 18
D	327 ± 73	93 ± 19
E	432[d]	74[d]

[a] See text for description of each protocol
[b] Fusion indexes and cell viabilities are given here as percent values, 100% being the value after treatment with PEG alone
[c] Values ± SD ($n = 3$)
[d] One experiment

by comparing the fusogenic abilities of commercial and purified (according to [10]) PEG, in the presence and absence of 2×10^{-3}M CHAPS.

The data in fig.2 demonstrate that, under our conditions, PEG purification significantly reduces its fusogenic capacity; on the other hand, pre-treatment with CHAPS clearly increases the fusion indexes, irrespective of the use of commercial or purified PEG.

The precise protocol for the combined use of PEG and surfactants was found to be critical for obtaining good fusion enhancement. This was shown by a separate series of experiments, based on 50% PEG + 2.5×10^{-5}M SDS, in which the following protocols were used: (a) SDS (5 min), wash, PEG (1 min), wash; (b) PEG (1 min), wash, SDS (5 min), wash; (c) SDS + PEG (1 min), wash; (d) SDS (5 min), decant, PEG (1 min), wash; (e) SDS (1 min), decant, PEG (1 min), wash. Protocol (d) is the standard procedure used in the experiments of table 1 and figs 1 and 2. All washings were carried out with serum-free medium. The results of using the various protocols are summarized in table 2. It is clear that fusion enhancement only occurs when: (i) the detergent is added prior to PEG, and (ii) the surfactant is not completely washed away: only the excess is removed by decanting.

4. DISCUSSION

The above results clearly demonstrate that, under certain conditions, pre-treating B16 melanoma cells with low concentrations of some surfactants greatly enhances PEG-induced cell fusion. This may be of considerable practical importance, since PEG fusion is currently used e.g. in the construction of hybridomas for monoclonal antibody production [11]. Of course, specific applications may require performing preliminary experiments in order to optimize the particular method to be used, although our results (tables 1 and 2) suggest that the conditions of detergent treatment (concentration, time) are far less critical than the order of addition of the reagents or the chemical structure of the surfactant.

In addition to the practical applications, our results are also important from the point of view of the mechanisms of PEG-induced fusion. It is generally accepted that an early effect of PEG treatment on cell membranes is the redistribution of intramembrane particles, giving rise to large 'bare' areas in the plasma membrane [1]. Formation of particle-free areas may be interpreted as a localized protein precipitation phenomenon, due to the combined action of dehydration and decrease in polarity of the medium [12,13]. Detergents may well cause these two effects with high efficiency even at low concentrations, within a localized area. Both effects would be clearly reversed after detergent removal, which explains the need to avoid washing off the surfactant (table 2, protocol A). It is also clear that the detergent plays its role at an early stage of the fusion process, since their effects are not detected unless added prior to PEG (table 2, protocol B). Years ago, Lucy [14] suggested the role of amphipathic compounds in membrane fusion, and, more recently, Lucy and Ahkong [15] pointed out the importance of osmotic forces in cell fusion. Note that detergents may also perturb the osmotic properties of cell membranes. In addition, amphiphiles have been shown to increase the yield of electrically-induced fusion [16]. Finally, we have reported recently that in situ generation of amphiphiles (by the action of phospholipase C) induces liposome fusion [17]. The hypothesis that amphiphiles produce changes in the water activity and solvent polarity of the intracellular space may provide a unified explanation for the above phenomena.

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REFERENCES

[1] Boni, L.T. and Hui, S.W. (1987) Cell Fusion (A.E. Sowers, ed.) pp. 301–330, Plenum, New York.
[2] Sáez, R., Alonso, A., Villena,, A. and Goñi, F.M. (1982) FEBS Lett. 137, 323–326.
[3] Smith, C.L., Ahkong, Q.F., Fisher, D. and Lucy, J.A. (1982) Biochim. Biophys. Acta 692, 109–114.
[4] Alonso, A., Villena, A. and Goñi, F.M. (1981) FEBS Lett. 123, 200–204.
[5] Alonso, A., Sáez, R., Villena, A. and Goñi, F.M. (1982) J. Membr. Biol. 67, 55–62.
[6] Urbaneja, M.A., Goñi, F.M. and Alonso, A. (1988) Eur. J. Biochem. 173, 585–588.
[7] Fidler, I.J. (1970) J. Natl. Cancer Inst. 45, 773–782.
[8] Halfer, C., Del Pin, D. and Dell'Oro, A. (1987) Genetica 75, 173–187.
[9] Partearroyo, M.A., Ostolaza, H., Goñi, F.M. and Berberá-Guillem, E. (1989) submitted.
[10] Honda, K., Maeda, Y., Sasakawa, S., Ohno, H. and Tsuchida, E. (1981) Biochem. Biophys. Res. Commun. 101, 165–171.
[11] Campbell, A.M. (1984) Monoclonal Antibody Technology, pp. 120–134, Elsevier, Amsterdam.

[12] Hui, S.W., Isac, T., Boni, L.T. and Sen, A. (1985) J. Membr. Biol. 84, 137–146.
[13] Glasstone, S. and Lewis, D. (1960) Elements of Physical Chemistry, pp. 559–599, Van Nostrand, New York.
[14] Lucy, J.A. (1970) Nature 227, 815–817.
[15] Lucy, J.A. and Ahkong, Q.F. (1986) FEBS Lett. 199, 1–11.
[16] Nea, L.J., Bates, G.W. and Gilmer, P.J. (1987) Biochim. Biophys. Acta 897, 293–301.
[17] Nieva, J.L., Goñi, F.M. and Alonso, A. (1989) Biochemistry 28, 7364–7367.
[18] Gerlier, D. and Thomasset, N. (1986) J. Immunol. Methods 94, 57–63.