DETERGENT-LIKE PROPERTIES OF POLYETHYLENEGLYCOLS IN RELATION TO MODEL MEMBRANES

R. SÁEZ, A. ALONSO, A. VILLENA[+] and F. M. GOÑI*

Department of Biochemistry, Faculty of Science, PO Box 644, Bilbao and [+]*Department of Histology, Faculty of Medicine, Bilbao, Spain*

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1. Introduction

Various polyethyleneglycols (PEG) have been extensively used as chemicals inducing cell fusion ([1,2] and references therein). In order to obtain information on the molecular mechanisms by which PEG induces the fusion of biological membranes, various studies have been carried out on the interaction of PEG with monolayers and model membranes [1–3]. These authors conclude that PEG acts by altering the physical state of the membrane water, decreasing the surface potential and modifying the transition temperature (T_c) of the lipid bilayers.

Previous work from this laboratory [4,5] has dealt with the interaction of Triton X-100 and other detergents with liposomes. It was found that many soluble amphiphiles produce an increase in size of sonicated unilamellar phospholipid vesicles, giving rise to large, multilamellar liposomes, probably through a mechanism of 'lysis and reassembly' [6]. Similar detergent concentrations, when acting on non-sonicated liposomes, produce only the gradual solubilization of the lipid bilayers.

Considering the extensive use of PEG in cell fusion experiments, a study was undertaken in order to determine the effects of PEG on the size, permeability and solubilization of sonicated and non-sonicated phospholipid vesicles. This paper reports our results concerning PEG 1000 and PEG 6000. It is found that these reagents produce very similar effects to those caused by detergents, although at much higher concentrations than the latter.

* To whom correspondence should be sent. Present address: Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP, England

2. Materials and methods

PEG 1000 and PEG 6000 were purchased from Merck (Darmstadt) and used without further purification. Egg-yolk lecithin was prepared according to [7].

Aliquots of the phospholipid in chloroform (11 μmol) were evaporated to dryness and liposomes prepared as in [4,5]. The vesicle suspensions were mixed with the appropriate amounts of PEG to obtain a final phospholipid level of 1 mM, and final PEG levels of 0–70% (w/v), and the various samples were left to equilibrate for 30 min at room temperature.

Turbidity measurements and electron microscopy observations were done as in [4]. Release of vesicle contents (6-carboxyfluorescein) was monitored at 25°C in a Perkin Elmer MPF-3 spectrofluorimeter, according to [8].

3. Results

When non-sonicated liposomes are treated with increasing concentrations of PEG 1000 or PEG 6000, the turbidity of the suspensions decreases following a hyperbolic pattern (fig.1a). (Liposome suspensions containing a PEG 6000 final concentration >30% could not be reliably prepared.) On the other hand, the addition of low concentrations of PEG 1000 or PEG 6000 to the initially clear suspensions of sonicated liposomes produces a considerable increase in turbidity, 50–70-times the original value (fig.1b). Higher polymer concentrations, at least in the case of PEG 1000, lead again to a decrease in turbidity of the sonicated liposome suspension. Polymer concentrations giving peak turbidities are 20% (w/v) for PEG 1000 and 8% (w/v) for PEG 6000. These PEG effects are remarkably similar to those shown, at much lower concentrations, by Triton X-100 [4].

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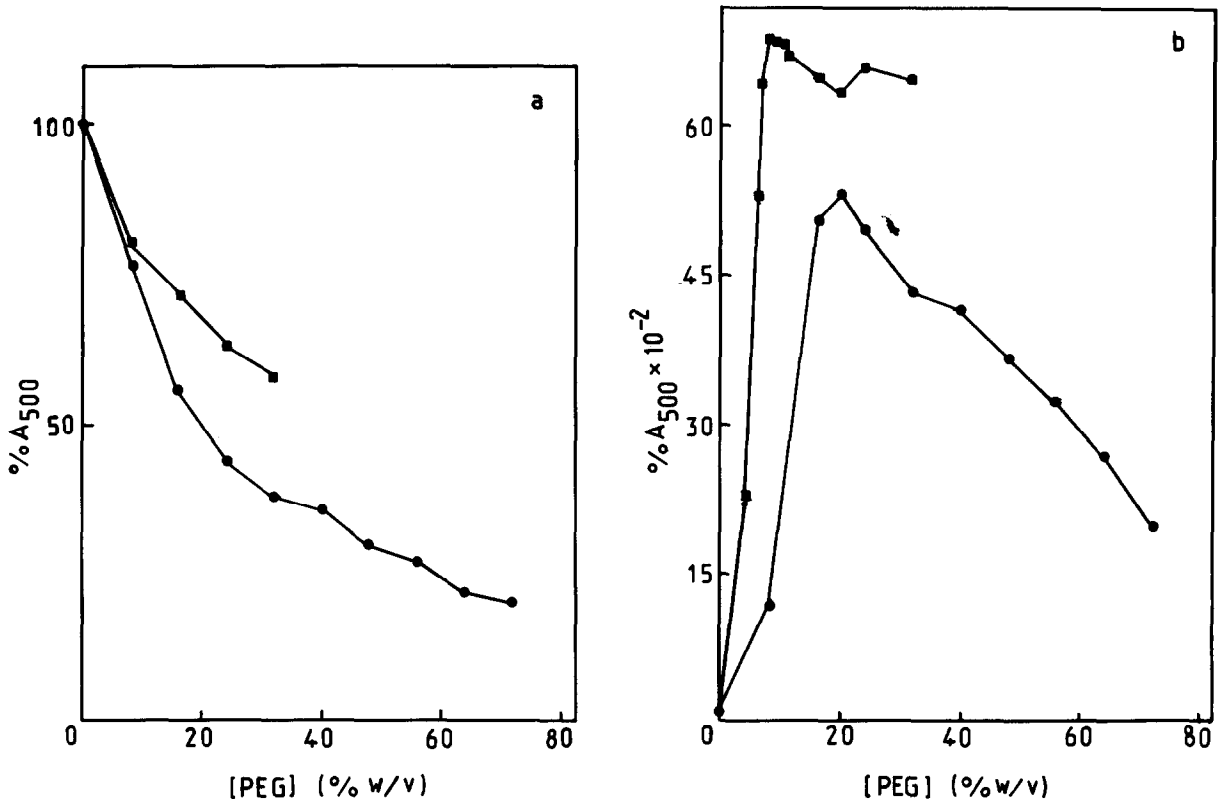
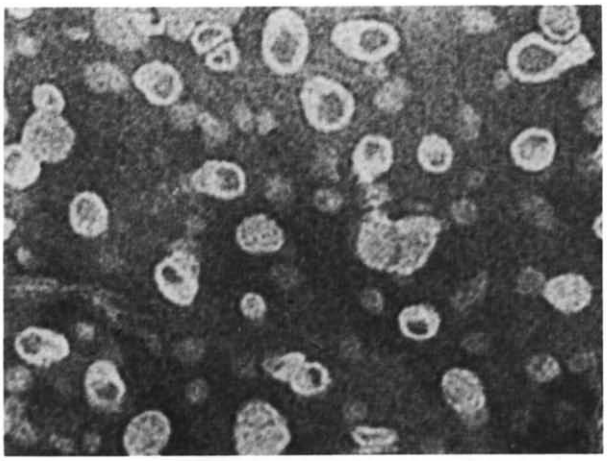


Fig.1. Changes in turbidity (A_{500}) of phosphatidylcholine liposome suspensions in the presence of (•) PEG 1000 and (▪) PEG 6000: (a) non-sonicated liposomes; (b) sonicated liposomes.

An examination of the unsonicated liposomes under the electron microscope by the negative-staining technique reveals large, multilamellar structures. Their appearance is not changed in the presence of PEG 1000 or PEG 6000, although their number decreases with increasing polymer concentrations (not shown). This is in accord with previous ideas of liposome solubilization by detergents [4,9]. The increase in turbidity of the sonicated vesicle suspensions can be due to liposome shrinking, or aggregation, or increase in size. Negative-staining electron micrographs show (fig.2) that PEG induces first aggregation and then an apparent fusion, giving rise to large multilamellar structures, much similar to the unsonicated liposome preparations. Maximum vesicle aggregation is found with 20% PEG 1000 and 8% PEG 6000, i.e., the polymer concentrations giving peak turbidity. The maximum observed increase in size of sonicated vesicles takes place around 40% PEG 1000 and 30% PEG 6000; the concomitant decrease in turbidity suggests that these phenomena occur simultaneously with bilayer solubilization. The increase in size of sonicated liposomes in the presence of PEG is very similar, from the morphological point of view, to what happens in the presence of detergents, although vesicle aggregation is not observed in the latter case [4,5].

The increase in vesicle size may happen through a process of true fusion or through lysis and reassembly of the sonicated liposomes, the main difference being that in true fusion phenomena the vesicle contents are not spilled out to the surrounding medium. This point is important in order to elucidate the molecular mechanism of the interaction of PEG with sonicated liposomes and their increase in size. When sonicated liposomes loaded with 6-carboxyfluorescein are treated with PEG 1000 in the usual way, polymer additions produce an increase in the suspension fluorescence, up to a 20% PEG (fig.3a). This is the concentration producing maximum turbidity. The increase in fluorescence is interpreted as a liberation of 6-carboxyfluorescein from leaky vesicles [8], thus supporting a mechanism of lysis and reassembly, rather than true



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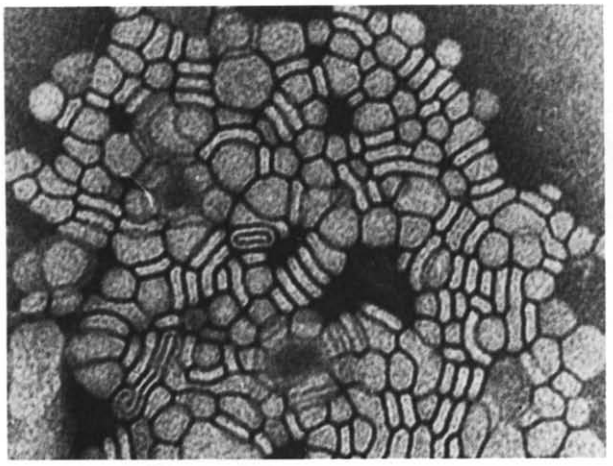

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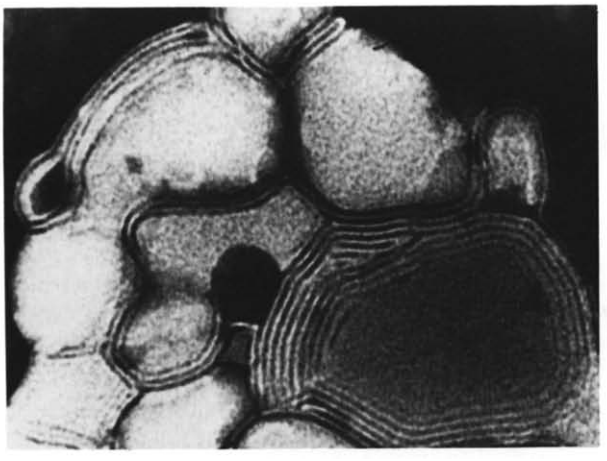

c

Fig.2. Electron micrographs of a negatively stained suspension of sonicated liposomes: (a) control; (b) in the presence of 20% PEG 1000; (c) in the presence of 40% PEG 6000; magnification, 269 000 ×.

fusion. All the vesicle contents seem to have been liberated at 20% PEG 1000. However, higher polymer concentrations do not leave unchanged the fluorescence intensity of the solution, but rather produce a clear decrease; this paradoxical behaviour was found to be due to a quenching effect of PEG on 6-carboxyfluorescein fluorescence (fig.3b). The intrinsic fluorescence of the vesicle preparations did not contribute to any extent to the observed changes (fig.3a), Similar results of 6-carboxyfluorescein liberation were found with sonicated and unsonicated vesicles in the presence of PEG 1000 or PEG 6000.

4. Discussion

The above results show that PEG 1000 and PEG 6000 solubilize phospholipid suspensions producing also the lysis and reassembly of sonicated liposomes into larger structures. There is a marked similarity between the effects of PEG and those of detergents [4,5] on phospholipid vesicles. Both groups of chemicals differ, however, in the range of concentrations required to exert their effects, PEG requiring 10^2–10^3-times higher levels than Triton X-100. Another interesting point is that vesicle aggregation could not be observed separately from fusion when detergents were used, probably because the surfactant concentrations producing aggregation and size increase are very close to each other.

'Fusion' between unilamellar phospholipid vesicles by PEG 6000 has been described in [10,11]. Our results with [10,11] although they do not distinguish between true fusion and lysis and reassembly [6] as possible mechanisms for increase in vesicle size, nor do they mention the effect of PEG on unsonicated liposomes. It is interesting, however, to note that these authors suggest the formation of structural defects in the lipid bilayers as a mechanism for PEG-induced membrane fusion [11]. The same hypothesis was put forward by us in relation to detergents and their effects on vesicle size [4,5].

Nevertheless, it is difficult to rationalize the similar behaviour of amphiphilic molecules, such as detergents, and extremely hydrophilic ones, such as PEG. The huge difference in concentration range required by both types of reagents is also difficult to explain. A clue to this problem may be presented in [12]. These authors are able, by standard organic chemistry methods, to purify commercial PEG 6000 so that the purified material has no longer any fusogenic activity.

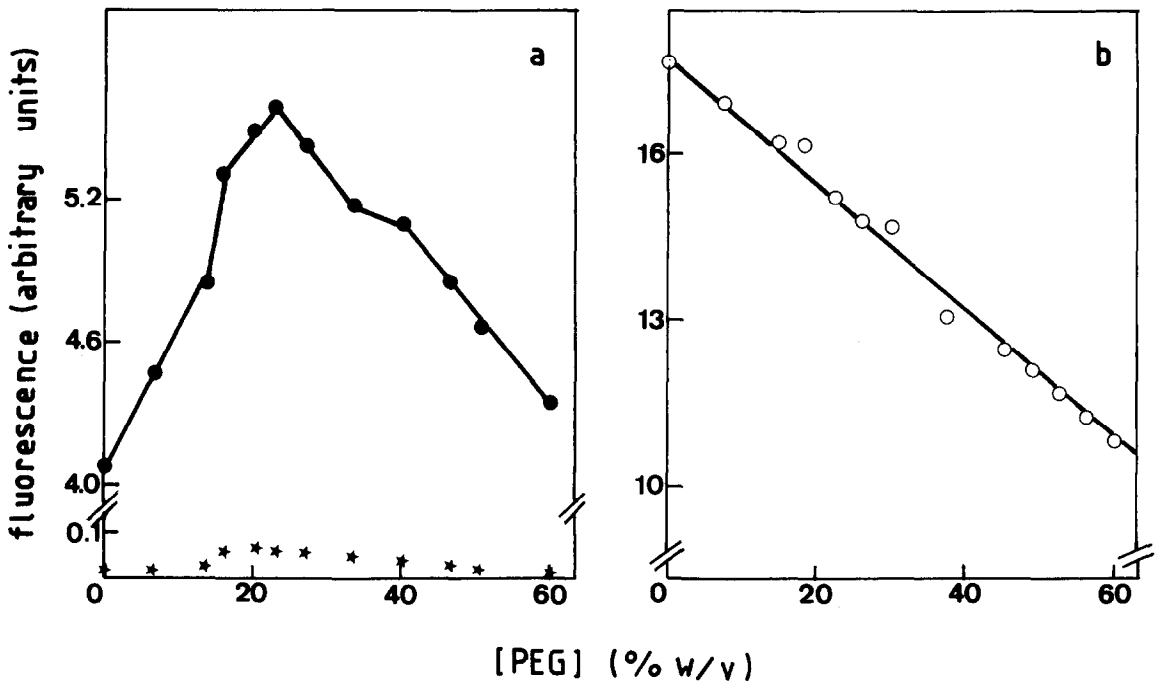

Fig.3. (a) (•) Fluorescence intensity of a sonicated liposome suspension containing entrapped 6-carboxyfluorescein, in the presence of various concentrations of PEG 1000. (*) Intrinsic fluorescence of a similar liposome suspension. (b) (○) Fluorescence intensity of a 0.05 mM aqueous solution of 6-carboxyfluorescein in the presence of various concentrations of PEG 1000.

The latter is restored by adding some of the impurities that had been removed. One of the additives that restores the fusogenic properties of PEG 6000 is polyoxyethylene oleate, 4.1×10^{-3} wt%, i.e., a water-soluble amphiphile in the concentration range at which other detergents produce an increase in liposome size.

Although these results do not invalidate previous research on PEG-induced cell fusion, some of the work may need re-examination from this point of view. A possible relationship between the degree of polymerisation of PEG and its optimum fusogenic concentration is now being examined by us in this context.

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