Electron spin resonance study on the mechanism of polyethylene glycol–membrane interaction

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Polyethylene glycol *Fusogen* *Membrane structure* *Spin label* *ESR*

1. INTRODUCTION

There is a growing interest in the application of cell fusion in various fields of modern biology. Besides viruses, some chemicals are also effective in causing biological membranes to fuse. Among the most potent fusogens of this type appear to be polymers of ethylene glycol. Polyethylene glycol (PEG) has been used to fuse a great variety of cell types [1–3]. The wide applications of PEG-induced cell fusion stimulate research on the mechanism by which the polymer interacts with biological membranes. Most of the studies performed have been focussed on the effect of PEG on the organization of membrane phospholipids [4–13]. The molecular mechanism of PEG–membrane interaction and the sequence of events leading to cell fusion remain, however, not fully understood. Particularly little is known about the effect of PEG on the physical state of membrane proteins.

In this study, the interaction of PEG with human erythrocyte ghost membranes and lipid bilayer vesicles has been investigated by spin-labeling. It has been shown that the polymer exerts an ordering effect on lipids and produces marked changes in the organization of membrane proteins. These results are discussed in relation to the mechanism of PEG-induced cell fusion.

2. MATERIALS AND METHODS

Spin labels used in this study, 2,2,6,6-tetramethyl-4-maleimidopiperidine-1-oxyl (MSL) and 5-doxylstearic acid, were obtained from Syva (Palo Alto CA). Chromatographically pure egg yolk phosphatidylcholine and cholesterol were from Sigma (St Louis MO). Polyethylene glycol M_r 6000 (PEG 6000) was supplied by Loba (Vienna).

Human erythrocyte ghost membranes were prepared as in [14]. Protein spin-labeling with MSL and lipid spin-labeling with 5-doxylstearic acid were performed as in [15].

Spin-labeled lipid bilayer vesicles were prepared as follows. 5-Doxylstearic acid was added to chloroform solution of phosphatidylcholine or phosphatidylcholine–cholesterol mixture and the solvent was evaporated under a stream of argon. Tris–HCl buffer (25 mM, pH 7.4) was then added and the dispersion was sonicated for 30 min in an ice bath using an MSE probe type sonicator. In order to remove titanium particles and larger lipid aggregates the suspension was centrifuged at $40000 \times g$ for 20 min, and the clear supernatant was used for further studies. The final concentration of phosphatidylcholine amounted to about 30 mg/ml, and the spin probe content was 1 mol% of phospholipid.

Electron spin resonance (ESR) spectra were recorded at 20°C with an SE/X-28 ESR spectro-

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meter (Wroclaw Technical University) operating at 9.5 GHz.

3. RESULTS AND DISCUSSION

ESR spectra of 5-doxylstearic acid incorporated into the membranes of erythrocyte ghosts and model lipid bilayer vesicles reflect a rapid, highly anisotropic motion of the spin probe. They may be analysed using the formalism of order parameter, S [16] obtained from the anisotropic hyperfine splittings $A_{\parallel}$ and $A_{\perp}$ (fig.1) by using the equation [16]:

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - 1/2(A_{xx} + A_{yy})} \cdot \frac{a}{a'}$$

where:

A_{xx}, A_{yy} and A_{zz} = the principal values of hyperfine splitting tensor [17];

$a = 1/3(A_{xx} + A_{yy} + A_{zz})$;

$a' = 1/3(A_{\parallel} + 2A_{\perp})$.

Incubation of sonicated lipid vesicles and erythrocyte ghost membranes with PEG resulted in an increase in order parameter. Changes in the molecular organization of membrane lipids were relatively fast. As shown in fig.2, new values of order parameter were reached within <10 min after PEG addition and they remained constant for ⩾90 min. To compare the effect of PEG on different membrane systems, the increase in order parameter of model bilayers and erythrocyte ghost membranes has been plotted against polymer concentration (fig.3). It is seen that PEG is a very potent orderer of both phosphatidylcholine and phosphatidylcholine–cholesterol liposomes. The rigidity of human erythrocyte ghost membranes appears to be much less susceptible to the perturbation by the polymer. In this case the ordering effect is relatively small, about 3-times smaller than that observed for liposomes.

The close similarity in the response to PEG of the relatively fluid phosphatidylcholine bilayer and of the much more rigid phosphatidylcholine–cholesterol bilayer indicates that the smaller ordering effect of the polymer on erythrocyte membrane as compared to liposomes cannot be attributed to fluidity differences. Apparently membrane proteins are responsible for the relatively small susceptibility of erythrocyte-ghost-membrane fluidity to perturbation by PEG. As the surface of erythrocyte membrane is largely coated with protein and polysaccharide chains, the interaction between the polymer and lipid molecules may be hindered.

The finding that PEG 6000 increases the order

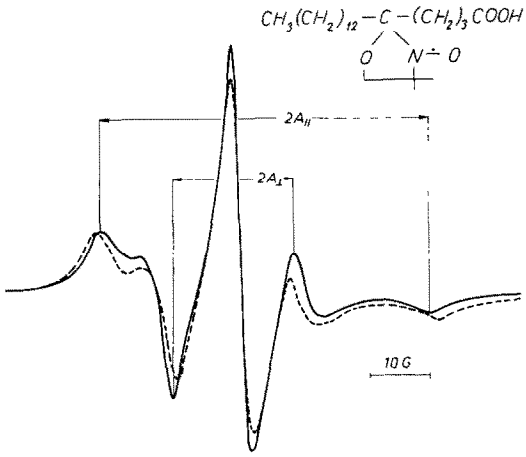

Fig.1. Electron spin resonance spectra of 5-doxylstearic acid spin probe incorporated into phosphatidylcholine liposomes without, and in the presence of PEG; (——) control liposomes; (– – –) liposomes in 42 wt% PEG.

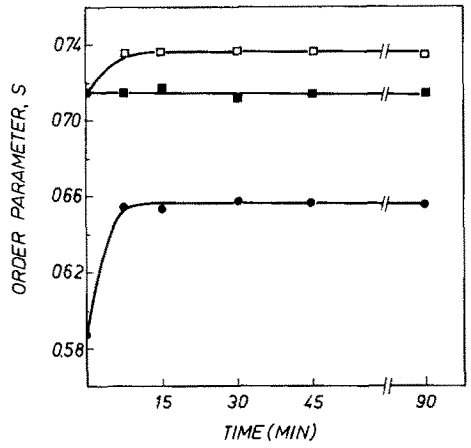

Fig.2. Time course of PEG-induced changes in order parameter: (■) human erythrocyte ghost membranes in 3 wt% PEG; (□) human erythrocyte ghost membranes in 42 wt% PEG; (●) phosphatidylcholine liposomes in 42 wt% PEG.

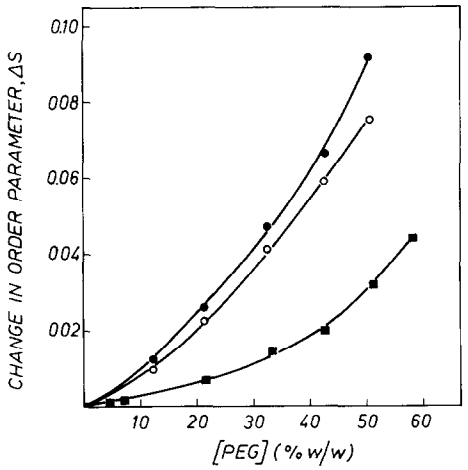

Fig.3. The effect of PEG on the order parameter change of 5-doxylstearic acid spin-labeled membranes: (●) phosphatidylcholine liposomes; (○) phosphatidylcholine–cholesterol (1:0.8 molar ratio) liposomes; (■) human erythrocyte ghost membranes. Each point represents the mean of 3 or 4 expts. The mean order parameters for control membranes were 0.589, 0.692 and 0.716 for phosphatidylcholine liposomes, phosphatidylcholine–cholesterol (1:0.8 molar ratio) liposomes, and human erythrocyte ghost membranes, respectively.

parameter of spin-labeled lipid bilayer vesicles is, in general, consistent with the results of recent ^{1}H-NMR and fluorescence spectroscopy studies [8,9]. On the other hand, a considerable discrepancy appears when the results of various studies on erythrocyte ghost membranes are compared. Based on fluorescence polarization measurements [9,10], it has been suggested that the fluidity of human erythrocyte ghost membrane is temporarily increased by PEG, with the maximal fluidizing effect occuring about 15–30 min after addition of the polymer. A mechanism has been also proposed [9] assuming this temporal membrane fluidization as an essential initial stage of erythrocyte fusion. Results of the present spin-labeling study apparently contrast with the above suggestion. Addition of PEG to the suspension of erythrocyte ghosts results in an increase in ESR-derived order parameter, with no indication of the temporal membrane fluidization.

The extent to which the observed ordering effect of PEG on lipids contributes to membrane fusion is not fully clear. According to previous considerations on the mechanism of cell fusion [8,9,18] the ability of liposomes to fuse should rather be decreased than enhanced when the mobility of lipid molecules is suppressed. However, there is experimental evidence [6,11,12] that liposomes containing lipids in the liquid–crystalline state are fused by PEG. A possible explanation, combining the ordering effect of PEG with its fusogenic activity, could be that with progressing interaction between the polymer and lipid bilayer some transient local boundaries between domains of increased order and those of unaltered order are produced. Such regions of increased instability could be the likely sites of membrane fusion.

In parallel with lipid spin-labeling experiments, the effect of PEG on the molecular organization of erythrocyte membrane proteins has been examined. This study was performed using a maleimide spin label (MSL), known to bind mainly to crysteine residues of membrane proteins [19–22]. A typical spectrum of MSL attached to human erythrocyte ghost membranes is shown in fig.4a. Similar spectra have been described as reflective of at least two classes of spin-label binding sites: one strongly immobilized (S) and one weakly immobilized (W) [15,19–22]. The h_W/h_S ratio of signal height of MSL attached to weakly immobilized sites may be used as a convenient monitor of protein organizational, and/or conformational changes in membranes.

Fig.4 illustrates the effect of increasing PEG concentrations on the spectra of erythrocyte membranes labeled with MSL. The concentration-dependent decrease in h_W/h_S ratio (fig.5) indicates the PEG causes a conversion of weakly immobilized spin-label binding sites to strongly immobilized ones. The time course of PEG-induced changes in the physical state of membrane proteins closely resembles that of membrane fluidity changes. New values of h_W/h_S ratio are reached within less than 10 min after PEG addition and they remain constant for at least 1 h.

The majority of studies on PEG-induced fusion have put emphasis on the interaction of the polymer with lipid bilayer. These data show that, besides lipids, membrane proteins are also perturbed by PEG. The above described erythrocyte membrane protein conformational changes may play a significant role in the chain of events leading to erythrocyte fusion. Particularly, these effects may

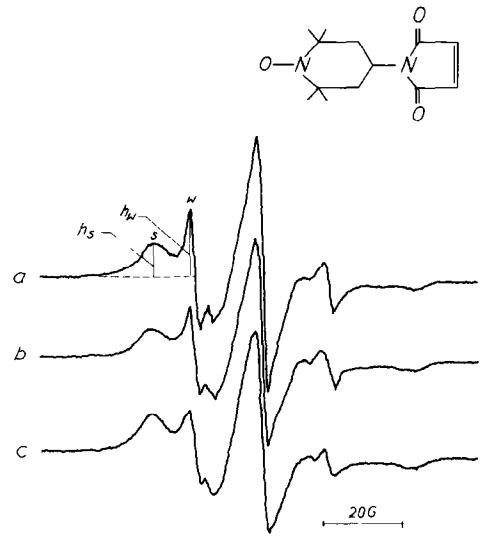

Fig.4. Electron spin resonance spectra of erythrocyte ghost membranes (2–2.5 mg protein/ml) labeled with MSL: (a) no PEG; (b) 5 wt% PEG; (c) 17 wt% PEG.

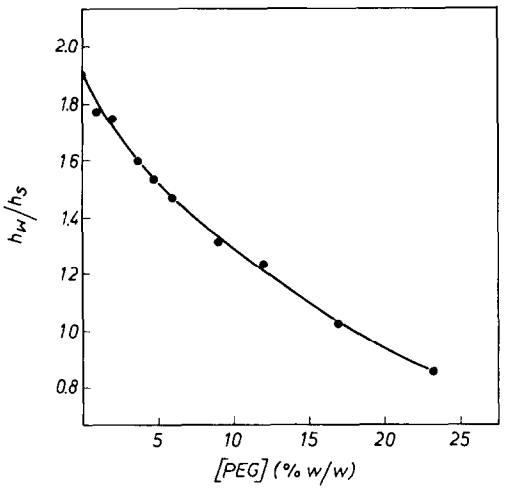

Fig.5. The effect of PEG on the mobility parameter h_W/h_S derived from electron spin resonance spectra of human erythrocyte ghost membranes labeled with MSL. Each point represents the mean of 3 or 4 expts.

be related to the aggregation of intramembranous particles induced by PEG. Such an aggregation has been observed by freeze-fracture technique and an important role in the mechanism of PEG-induced erythrocyte fusion has been ascribed to it [2].

Lipid spin-labeling experiments show that PEG produces an increase in the order parameter of both model lipid bilayers and erythrocyte ghost membranes. These experiments apparently fail to confirm suggestions [9,10] that temporal membrane fluidization occurs at the initial stage of PEG-induced erythrocyte fusion. However, it is demonstrated that, besides perturbing the molecular organization of lipids, PEG produces marked alterations in the physical state of membrane proteins. This observation may give a clue to a better understanding of the mechanism of PEG-induced cell fusion.

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