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The influence of poly(ethylene glycol) on the molecular dynamics within the glycocalyx

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Interaction of polymers with cell surfaces is a question of general interest for cell aggregation and fusion. The molecular dynamics within the surface coat of human erythrocytes as well as alterations of membrane protein arrangement (IMPs) in the presence of poly(ethylene glycol) (PEG) were investigated by EPR spin labeling techniques and freeze-fracture electron microscopy, respectively. At PEG concentrations which induce aggregation of erythrocytes the surface coat and the protein arrangement is not disturbed by the polymer. This implicate an exclusion of the polymer from the cell surface.

Introduction

Poly(ethylene glycol)-induced cell-cell fusion is now a standard technique in biological, medical and biotechnical practice. Important features of PEG-induced fusion are the high fusion efficiency, its simple application as well as the low toxicity. Although contaminations of commercial available PEG enhance the fusion extent [1], it is now well accepted that PEG per se is able to nucleate fusion [2].

However, the interaction of PEG with biological membranes and the mechanism of membrane and cell fusion are not well understood. There were different attempts to explain the mechanism of PEG promoted aggregation and fusion of cells and vesicles, respectively. A direct interaction of the polymer was often supposed leading to alterations of membrane structure which nucleate fusion of closely apposed membranes [3,4]. Aggregation preceeding fusion was thought to be caused by bridging of cells or vesicles by PEG [5].

However, our recent results gave experimental evidence that PEG is excluded from the membrane surface [6,7]. Therefore, we suggested that PEG exerts its influence on membrane systems in an indirect manner due to changes of the physicochemical properties of the aqueous phase [6,8–11]. Fusogenic concentrations of PEG caused a decrease of the dielectric constant of the suspension medium [8,9] important for stability of the membrane bilayer. PEG creates a high osmotic pressure [11]. The considerable dehydrating potency [10,12] makes PEG suitable for membrane fusion, since there is convincing evidence that a prerequisite of membrane fusion is to overcome the hydration force [13]. Furthermore, it has been shown that dehydration of bilayers composed of lipids with different hydration properties can cause phase separations within the membrane [14]. Thus, without a direct interaction PEG is able to affect structure and organization of membranes important for fusion. Also aggregation of cells and vesicles, respectively, can be explained on the basis of the volume exclusion mechanism [15] and the resulting osmotic force [16] without assuming a direct contact of PEG with membrane components.

Electrophoretic measurements of human erythrocytes demonstrated that the concentration of higher molecular weight PEG ($> = 4000$) near the membrane surface (glycocalyx) is reduced compared to the bulk phase [7]. The present paper is thought to be a first attempt to investigate molecular alterations within the surface coat of human erythrocytes in the presence of PEG using electron spin resonance spectroscopy. The molecular motion in the glycocalyx was studied by (a) covalently labeling of sialic acid residues of glycoproteins and gangliosides and by (b) incorporating a charged spin probe into the erythrocyte membrane which is sensitive

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to structural changes directly on the outer membrane surface. It has been shown that binding at the cell surface has distinct and pronounced effects on the physical state of membrane proteins of the erythrocyte [17,18]. Recently, we have shown that both labeling approaches are sensitive to alteration within the membrane surface coat, e.g. to electrostatic interactions and to virus adsorption [19–21]. Lipid fluidity was explored by fatty acid spin labels. Rearrangement of integral membrane proteins under influence of PEG were followed by electron microscopy.

Materials and Methods

Human erythrocytes of different donors were used (blood-bank, Berlin-Lichtenberg) not later than 48 h after blood sampling. After removal of plasma and buffy coat ($500 \times g$, 5 min) three washings with 5 vol. of suspension medium followed at $2000 \times g$ (10 min). Erythrocyte ghosts were prepared according to the procedure of Dogde et al. [22].

PEG treatment. To avoid strong aggregation of the erythrocytes and to guarantee an intensive interaction of the polymer with the cells, PEG (Ferak, molecular weigth 6000) dissolved in a modified Eagle's medium was mixed with erythrocytes by means of a adapted rotation viscosimeter [23]. Under laminar shear conditions aggregates with less than 20 cells were obtained at hematocrit values up to 30% in concentrated polymer solutions. Cells were incubated for 5–7 min in the polymer solution at 37°C. Thereafter the suspension was diluted slowly with 10 volumes of Eagle's medium and collected for further investigation by short centrifugation at $300 \times g$.

The particle were counted using dark field microscopy ensuring also counting of eventually present ghosts. The aggregation index was determined as follows:

$$\mathrm{AI} = \frac{\text{number of cells in aggregates}}{\text{whole cell number}} \times 100\,(\%) \qquad (1)$$

Fusion index was calculated according to Ahkong et al. [24]. Extent of fusion was measured 30 min after dilution. Longer incubation did not enhance the amount of fused cells.

Spin labeling. Spin labeling of erythrocytes with the fatty acids I (10, 3) (2-(3-carboxypropyl)-2-decyl-4,4-dimethyl-3-oxazolidinyloxyl, Reanal Budapest) and I (1, 14) (2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl, Syva, Palo Alto) and of erythrocytes with the cationic spin probe CAT 16 (1-oxyl-2,2,6,6-tetramethyl-4-dimethyl aminopiperidinecethyl bromide, Inst. Org. Chemistry, Bulgarian Academy of Science, Sofia) was performed as already described [19,25]. In the case of CAT 16 suspension media contained 1 mM $K_3Fe(CN)_6$ to prevent a loss of signal intensity by chemical reduction of the NO-group caused by slight hemolysis. Line shape was not effected at such a concentration of $K_3Fe(CN)_6$.

In case of DPPC vesicles (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, SERVA) the label CAT 16 was added in a ratio of 1 : 100 prior to sonication.

Sialic acid residues of glycoproteins and glycolipids of erythrocyte ghosts were covalently labeled with Tempamine (2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl, Reanal Budapest) [20] following the procedure of Feix and Butterfield [26]. In all cases excess of spin label was removed by repeated washings. It should be mentioned that labeling of sialic acid residues did not abolish negative charges.

Evaluation of EPR spectra. For the spin label CAT 16 the external peak distance $2A_z$ was used in the low temperature region (5°C) as a relative measure of mobility of the probe [19,20]. At 37°C with fast motion of the label the apparent correlation time τ_{CAT} [19,20] according to

$$\tau_{\mathrm{CAT}}(\mathrm{ns}) = 6.65 \cdot 10^{-10} \cdot \Delta H_{+1}(\mathrm{G}) \cdot (\{I_{+1}/I_{-1}\}^{1/2} - 1) \qquad (2)$$

was estimated with ΔH_{+1} the linewidth of the low-field component and I_{+1} and I_{-1} the heights of the low- and high-field line, respectively (see Fig. 4).

In the case of spin-labeled sialic acid residues the following correlation times were calculated from the spectra [20,26,27]:

$$\tau_{\mathrm{B}}(\mathrm{ns}) = -5.9 \cdot 10^{-10} \cdot \Delta H_0\,(\mathrm{G}) \cdot (\{I_0/I_{+1}\}^{1/2} - \{I_0/I_{-1}\}^{1/2}) \qquad (3)$$

$$\tau_{\mathrm{C}}(\mathrm{ns}) = 6.65 \cdot 10^{-10} \cdot \Delta H_0\,(\mathrm{G}) \cdot (\{I_0/I_{+1}\}^{1/2} + \{I_0/I_{-1}\}^{1/2} - 2) \qquad (4)$$

ΔH_0 and I_0 are the linewidth and the amplitude of the mid-field line, respectively (see Fig. 2).

The outer hyperfine splitting $2T_{\parallel}$ of $I(10,3)$ and the apparent correlation time τ_{B} of $I(1,14)$ were determined for investigating the response of membrane fluidity to the addition of PEG.

ESR measurements. EPR spectra were recorded on an ESR 231 spectrometer (Ctr. Sci. Instr., Acad. Sci., G.D.R.) with a variable temperature equipment. The temperature was measured by a small thermistor inserted into the flat quartz cell used (accuracy $\pm$ 0.2°C).

Freeze-fracture electron microscopy. Erythrocytes, without further pretreatment or fixed with phosphate-buffered 1% glutardialdehyde and impregnated with 20% glycerol, were sandwiched between double-replica copper supports and rapidly frozen by plunging into liquid propane. The samples were fractured at −150°C in a BLAZERS BAF 400D apparatus and immediately replicated by platinium/carbon evaporation. The repli-

cas, cleaned with sodium hypochlorite bleached and washed with distilled water, were examined in a TESLA BS 500 electron microscope. Micrographs were orientated with shadow direction from bottom to top.

Statistics. If not stated otherwise the two-tailed t-test for paired observations was used at a significant level of $\alpha = 0.01$. Generally, the average and the standard deviation were given.

Results

PEG-mediated aggregation and fusion of erythrocytes

Investigating the response of the glycocalyx to the polymer such concentration of PEG were selected at which aggregation started and fusion was observed, respectively. In Fig. 1 the aggregation behaviour of washed human erythrocytes as a function of the added amount of PEG is shown. Obviously, aggregation was observed at polymer concentrations as low as 7 wt%. Although the presence of Ca^{2+} (5 mM) favoured erythrocyte aggregation even at low PEG concentrations, investigation of membrane surface coat by spin labeling was performed in the absence of Ca^{2+} to avoid Ca^{2+}-triggered membrane alterations [18] interfering with those caused by PEG.

At polymer concentrations above 40 wt% fusion of erythrocytes was established (not shown). The fusion extent was about 10% using 45 to 50 wt% of PEG. Under these conditions aggregation was independent of the PEG concentration (Fig. 1).

On the basis of these results spectroscopic investigation of membrane surface coat was done at 7 wt% and 45 wt%, respectively.

Effect of PEG on molecular dynamics in the glycoclayx

EPR spectra of covalently spin-labeled sialic acid residues on human erythrocyte membranes in the absence of PEG are shown in Fig. 2. The shape of the spectra measured at 5°C and 37°C indicated a rapid

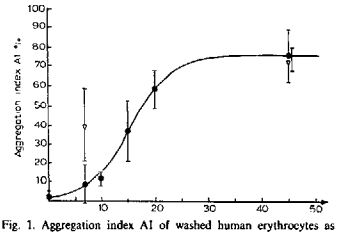

Fig. 1. Aggregation index AI of washed human erythrocytes as a function of the PEG concentration in the medium. ●, Eagle's solution without Ca^{2+} (0.5 mM EDTA); ▽, Eagle's solution with 5 mM Ca^{2+}.

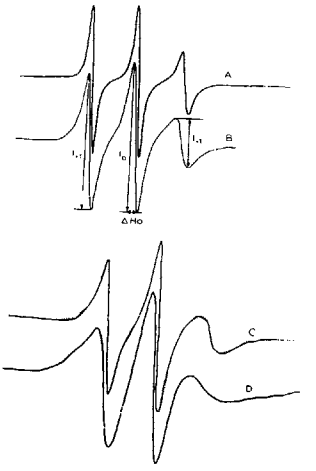

Fig. 2. EPR spectra of Tempamine-labeled sialic acid residues of the glycocalyx of human red cell ghosts. A, control cells at 37°C. The dotted line represents cells suspended in 7 wt% PEG 6000. B, cells suspended in 7 wt% PEG 6000 after centrifugation. I_{+1}, I_0, I_{-1} are the heights of the low-, mid- and high-field line, respectively, ΔH_0 is the linewidth of the mid-field line. C, control cells at 5°C. D, cells suspended in 7 wt% PEG 6000 after centrifugation.

isotropic motion (narrow lines) even at low temperature (at 5°C: $\tau_B = 1.89 \pm 0.21$ ns, $\tau_C = 2.5 \pm 0.35$ ns; at 37°C: $\tau_B = 0.69 \pm 0.04$ ns; $\tau_C = 0.77 \pm 0.05$ ns; $n = 6$).

In agreement with previous results [20] no immobilized component (broad components in the spectrum), especially at low temperature, was observed. The difference between both correlation times $\Delta\tau = \tau_C - \tau_B$ indicated at least a small anisotropy of label motion [28] increasing with decreasing the temperature.

It is important to note that the covalently bound label reflected a hydrophilic environment of the glycocalyx because the isotropic hyperfine splitting a_{iso} was similar to that of the free label in aqua dest. (16.8 G). Therefore, this spin-labeling approach should be sensitive to interaction of PEG present in the suspension medium with glycocalyx molecules.

In the presence of 7 wt% PEG the shape as well as the correlation times τ_B and τ_C were not significantly different in comparison to control (not shown). However, after spinning down erythrocyte ghosts suspended in a medium containing 7 wt% PEG (15000 rpm,

Janetzki K-24 centrifuge, rotor 6 × 26, 15 min) a second, more immobilized component was detected over the whole temperature region investigated (5°C–37°C) (see Figs. 2B, 2D). This component was not observed by centrifugation of ghosts in the absence of PEG. Superposition of spectral components did not allow for determination of correlation times. Removal of PEG by ghost washing restored the shape of the spectrum and the correlation times corresponded to that of the control samples (not shown).

At 45 wt% of PEG the spectrum of covalently bound Tempamine was dramatically altered. The pronounced asymmetric shape of the spectrum at 5°C suggested a strong immobilization of sialic acid residues (Fig. 3) with an external peak distance of about 69 G. It was not possible to distinguish between spatial and/or temporal restriction of label motion. It is well known that these types of spectra are not very sensitive to alterations of label environment (Saturation-transfer ESR is recommended). Therefore, we could not deduce from the spectrum that it refered only to a single component (see below).

It should be mentioned that the spectrum of the free Tempamine-label in an aqueous solution of 45 wt% PEG reflects a rapid isotropic motion (not shown; e.g. at 5°C: $\tau_B = 6.18 \cdot 10^{-10}$ s at 45 wt% PEG, $\tau_B = 0.19 \cdot 10^{-10}$ s control).

At elevated temperatures (cf. Fig. 3, 20°C and 37°C) the anisotropic character of the spectrum of labeled sialic acid residues was partially reduced. Obviously, at least two components could be distinguished differing significantly in their motional characteristic. Again, due to restricted motion and superposition of different spectral components as well the formalism of calculating correlation times was not applicable. We did not succeed in subtracting the spectrum of the control (Figs. 2A and C) from that of the sample suspended at 45 wt% of PEG. This suggested that also the more mobile component of the spectrum measured in the presence of fusogenic polymer concentration was restricted in comparison to the control. Although the rapid isotropic motion of spin-labeled sialic acid residues was restored by removal of PEG the correlation times τ_B and τ_C were significantly enhanced (e.g. $\tau_B = 2.26 \pm 0.01$ ns, $\tau_C = 3.00 \pm 0.17$ ns at 5°C) even after repeated washings. This effect was less pronounced, but significantly present at 37°C, too. No loss of signal intensity was established.

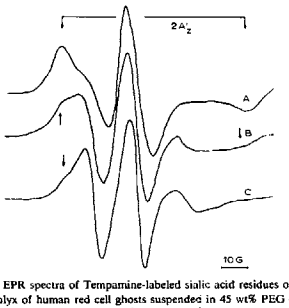

Fig. 3. EPR spectra of Tempamine-labeled sialic acid residues of the glycocalyx of human red cell ghosts suspended in 45 wt% PEG 6000 at 37°C (A), 20°C (B) and 5°C (C). $2\ A'_z$ is the external peak distance.

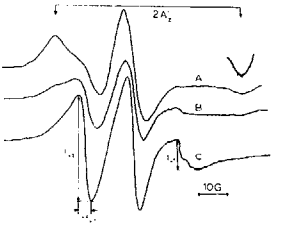

Fig. 4. EPR spectra of CAT 16 spin label in human erythrocyte membranes with the parameters used for evaluation of spectra (see Fig. 2 and Materials and Methods). A, control cells at 5°C. B, cells incubated in 45 wt% PEG 6000 at 37°C. C, control cells at 37°C.

Typical spectra of the positively charged spin probe CAT 16 incorporated into membranes of intact erythrocytes at different temperatures are shown in Fig. 4. Because the N-O group of CAT 16 was located in the vicinity of phospholipid headgroups [19,29], this label is sensitive to alterations on the membrane surface [29–31]. The tempoylgroup is able to rotate about the C-N bond, but in membranes this motion is restricted by steric as well as electrostatic interactions [29,31], especially with the negatively charged sialic acids of the glycocalyx. As shown previously [19] the charged headgroup of CAT 16 is localized exclusively on the outer surface of the erythrocyte membrane.

Because of the complex shape of the spectra at room temperature [19] spectra were recorded at 5°C and 37°C in order to better approximate slow and fast motional line shape, respectively. Being aware that anisotropic motion occurred to a certain extent [20] the apparent correlation time τ_{CAT} was calculated for spectra measured at 37°C because they did not differ significantly from those of isotropic motion (in the absence of PEG $\tau_{CAT} = (2.95 \pm 0.42)$ ns, $n = 12$).

At 37°C incubation of erythrocytes in the presence of 45 wt% PEG changed the spectra markedly as com-

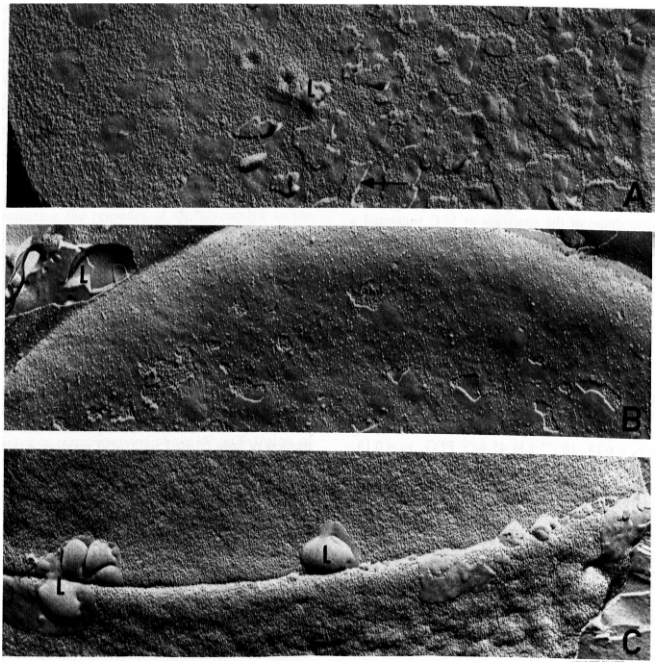

Fig. 5. Freeze-fracture electron micrographs showing membrane fracture faces of erythrocytes in presence of 45 wt% PEG 6000. (A) Region of close contact, predominantly PF (protoplasmic fracture face), with aggregation of intramembranous particles (IMP) and smooth particle-free areas. Where the fracture jumps into the apposed membrane complementarity of the smooth areas can be seen (arrow). A small separated lipid structure (L) is located within the planar contact region. (B) Region of close contact, predominantly EF (exoplasmic fracture face), showing the particle free areas and smooth structures representing lamellae of segregated lipids (L) localized outside of the contact region. In the vesicular structure two lamellae are visible. (C) Normal random distribution of particles on the PF outside of the contact region. The furrow is the border of the vertically oriented contact region and there are some smooth structures of segregated lipids (L). Magnification: 40000×.

pared to the control (Fig. 4) indicating partly immobilization of the label. Similar to the results presented above at least two components can be distinguished differing also in their motional characteristics. The more mobile component seemed to be comparable to the spectrum of the control sample. Removal of PEG restored the spectrum of the control ($\tau_{CAT} = (2.77 \pm 0.19)$ ns, $n = 6$).

At 5°C both components seen at 37°C merge into a single anisotropic spectrum which did not differ from that of the control sample. No significant differences between the external peak distances $2A'_z$ were established (control: (64.3 ± 0.72) G, $n = 12$; at 45 wt% PEG: (64.6 ± 0.94) G, $n = 9$). A slight reduction of $2A'_z$ was established after removal of PEG: $2A'_z = (63.4 \pm 0.78)$ ns. Again, we were not able to decide whether these is only one component contributing to the signal.

No significant influence of PEG at 7 wt% on spectra of CAT 16 was found.

Using DPPC-vesicles labeled with CAT 16 the spectrum did not split into two components at fusogenic concentrations of the polymer (not shown) even at

37°C. Interestingly, the apparent correlation time τ_{CAT} was not significantly enhanced in comparison to the control ($\tau_{CAT} \cong 2.1$ ns at 37°C). Also Gawrisch [32] did not observe different components of the spectrum of CAT 16 inserted into egg phosphatidylcholine membranes at 60 wt% PEG.

Under no circumstances a signal component of CAT 16 corresponding to free label in the suspension medium was observed. This suggest a strong anchoring of the label within the membrane even at high PEG concentrations.

Effect of PEG on lipid phase of human erythrocyte membranes

In accordance with previous investigations [33] we have found that the outer hyperfine splitting $2T_{\parallel}$ of the fatty acid spin label I(10,3) incorporated into the human erythrocyte membrane remained unchanged up to 60 wt%. The values obtained for $2T_{\parallel}$ were 54.2 ± 0.6 G and 55 ± 0.4 G at 37°C and 63 ± 0.3 G and 63.6 ± 0.4 G at 5°C for controll cells and cells incubated in 60 wt% PEG, respectively. Furthermore, removal of PEG did not effect $2T_{\parallel}$ at 5°C and 37°C, respectively. Similar conclusions could be drawn on the basis of the order parameter S of the spectra measured at 37°C calculated according to Griffith and Jost [33] (not shown).

Also, we failed to establish an effect of PEG on the lipid phase using the fatty acid spin label I(1,14) with the nitroxide located deeper within the hydrophobic tail of the membrane. Both the shape of the spectra and the apparent correlation time τ_R (about 1.5 ns at 20°C) remained unchanged in the presence of high polymer concentrations (not shown).

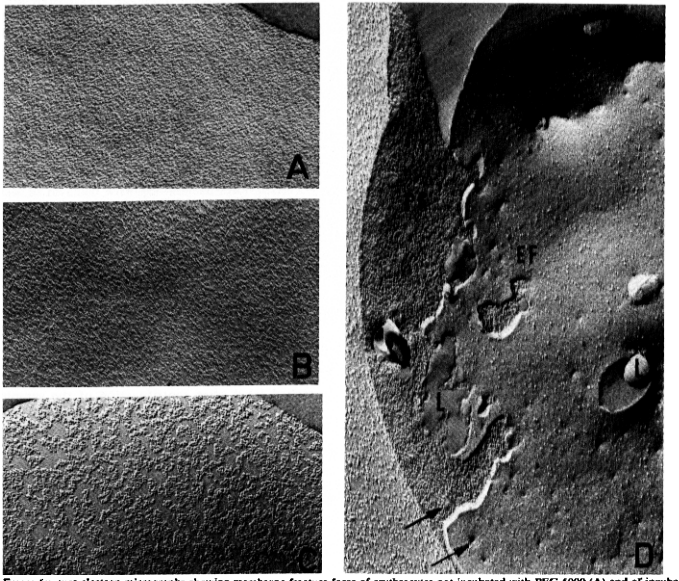

Fig. 6. Freeze-fracture electron micrographs showing membrane fracture faces of erythrocytes not incubated with PEG 6000 (A) and of incubated cells after removal of most of the polymer (B, C, D). (A) Normal random distribution of particles on the PF (control). (B and C) Distribution of particles on the PF, indicating a more clustered state compared to the control (PEG diluted from 45 wt% to ≤ 4wt%; incubated at 37°C for 75 min). (D) Contact region, PF and EF without the large particle-free areas. Only small 'contact points' (arrow) and separated lipid structures (L) are present (PEG diluted from 45 wt% to < 4 wt%; incubation of cells for 75 min at 37°C). Magnification: 40000×.

Protein rearrangement observed by electron microscopy

Membrane fracture faces of erythrocytes suspended in PEG solutions of 7 or 10 wt% (not shown) were comparable to those of the control showing a random distribution of intramembrane particles (IMP) (Fig. 6). However, incubation at 45 wt% PEG induced alterations of the lateral organisation of apposed membranes (Fig. 5) in the contact region of aggregated cells. The strong aggregation of particles (IMP) was accompanied by formation of smooth areas without particles. The particle-free areas of the two adjacent membranes were complementary. Furthermore, more or less separated smooth vesicular or flat structures were observed which are probably segregated membrane lipids. In some cases fracture revealed the involvement of two bilayers (Fig. 5B). These lipid structures were frequently localized at the peripheral zone of contact regions (Figs. 6B and 6C), but could be observed also within the contact region (Fig. 6C) and in form of separated vesicles.

The large IMP-free areas disappeared when the cells were incubated for 10 min at 37°C after dilution of PEG (Fig. 6D). However, aggregated particles in form of small clusters distributed over the whole membrane (Figs. 6B and 6C) were still visible after prolonged incubation (up to 60 min). These results are in accordance with those of Knutton [35].

Discussion

Since up to now there was no evidence for a direct interaction between PEG and membranes we proposed recently that the fusogenic activity of PEG arises from the altered physicochemical properties of the aqueous solution which lead to a strong aggregation of membranes, a destabilization of the membrane, changes of membrane structure and osmotic stress on cells. These effects may occur without any direct interaction between PEG and the membrane. The absence of a direct interaction of high molecular weight PEG with membranes is strengthened by different experimental evidence.

From NMR and neutron scattering data we have evidence that PEG of molecular weight higher than 400 does not interact with lipid membranes directly and is excluded from the area of close contact of two apposed membranes [32,36]. Recently, we have shown by means of electrophoretic mobility measurements of human erythrocytes that PEG of higher molecular weights form depletion layers near the cell surface [7] similar as described for dextran by Bäumler and Donath [37]. The viscosity of the double layer determining also the mobility of cells was lower than the bulk viscosity since the depletion layer was in the order of the thickness of the double layer or even higher. Further indication for an indirect action of PEG on membrane structure was given by Hui et al. [38] who observed IMP aggregation within multilamellar glycophorin/egg phosphatidylcholine recombinant vesicles in areas which are beyond the reach of PEG. Also McDonald [12] was able to induce vesicle fusion by separating PEG from the vesicle solution by a dialysis tube. Using spin-labeled PEG Boss [39] failed to establish a direct interaction between PEG and membrane lipids from plant protoplasts.

The absence of a direct interaction of PEG with membrane components and the existence of a depletion layer is in agreement with the results of our spin labeling investigations of the surface coat suspending erythrocyte membranes at 7 wt% of PEG where cell aggregation starts. Also labeling of sialic acid residues failed to detect any significant effect of PEG on molecular dynamics within the glycocalyx. One should be kept in mind that this amount of PEG corresponded to a rather high concentration of about 10 mM enhancing the bulk viscosity about three times [7] in comparison to that of the suspension medium without PEG. Butterfield et al. [18] found a 33% reduction in the apparent correlation time τ_C of the spin label attached to sialic acid by addition of 40 μM of the lectin wheat germ agglutinin binding to glycophorin A. Also attachment of influence virus to erythrocyte membranes increases τ_C [20]. The absence of any polymer–cell surface interaction was also supported by the finding that in the case of 7 wt% of PEG no changes of shape as well as $2A'_z$ and τ_{CAT} of the spectra of CAT 16 incorporated into erythrocyte membranes occur.

At fusogenic polymer concentrations spectra of spin-labeled sialic acid residues of the erythrocyte glycocalyx reflected at least two different label environments. We suggest that the more immobilized component corresponds to aggregates of IMP as observed with freeze-fracture electron microscopy and/or sialic acid residues entrapped between two closely apposed membranes. It was shown by several authors that IMPs consist of band 3 proteins and glycophorin as well [40]. This suggestion is confirmed (1) by the appearance of a additional more restricted component of covalently attached label after centrifugation of the sample in the presence of 7 wt% enhancing considerable cell aggregation and (2) that the disappearance of aggregates of IMPs after removing PEG was accompanied with a restoring of rapid isotropic motion of spin label attached to sialic acid residues. Also the formation of small clusters of particles over the whole membrane after removal of the polymer was in line with the significant enhanced apparent correlation times τ_B and τ_C observed in comparison to the controll.

The more mobile component at 45 wt% of PEG (Fig. 3) was assumed to belong to spin-labeled oligosaccharide chains not involved in aggregates of IMPs. It turned out by spectral subtraction (see Results) that this component was also restricted in comparison to control.

This can be explained quite easily. With increasing polymer concentration in the bulk phase the chemical potential of PEG becomes larger. Therefore, even if close approach of PEG to cell surface was statistically unfavourable [7,37], now, some of the PEG molecules are forced to occupy space within the glycocalyx. As a result label motion is restricted.

The spectra of the CAT 16 incorporated into erythrocyte membranes splitted also at least into two components at elevated temperatures. Probably, the more immobilized component was due to label associated with and/or intercalated in aggregates of IMPs. This assumption was supported by the absence of such a component in DPPC-membranes at fusogenic polymer concentrations. Furthermore, we obtained evidence for an electrostatic interaction between the positively choline group and the negatively sialic acid residues mainly attached to glycophorin [20]. The motional features of the more mobile component probably not associated with aggregates of IMPs seemed to be comparable to those of the control suggesting only a minor influence of PEG on phospholipid headgroup motion in agreement with our earlier results measuring headgroup motions on erythrocyte ghosts using ^{31}P-NMR [10]. We observed also no significant enhancement of τ_{CAT} in DPPC-membranes after addition of PEG.

The observed alterations of the molecular dynamics of CAT 16 and of covalently labeled sialic acid residues are not caused by the change of the physical state of lipid phase as revealed with spin-labeled fatty acids.

Summarizing, our results supported the existence of a higher molecular weight PEG depletion layer on the cell surface. It underlines that aggregation as well as fusion do not arise from a direct interaction between PEG and membranes, but rather from altered physicochemical properties of water surrounding the cells. Probably, aggregation of human erythrocytes is achieved by volume exclusion of cells from the polymer as proposed for protein precipitation [41,42] and vesicle aggregation [15].

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References

1 Honda, K., Maeda, Y., Sasakawa, S., Ohno, H. and Tsuchida, E. (1981) Biochem. Biophys. Res. Commun. 100, 442–447.
2 Smith, T.C., Akhong, Q.F., Fisher, D. and Lucy, J.A. (1982) Biochim. Biophys. Acta 692, 103–114.
3 Boni, L.T., Hah, J.S., Hui, S.W., Mukherjee, P., Ho, J.T. and Jung, C.Y. (1984) Biochim. Biophys. Acta 775, 409–418.
4 Ohno, H., Maeda, Y. and Tsuchida, E. (1981) Biochim. Biophys. Acta 642, 27–36.
5 Ohno, H., Shimidzu, N., Tsuchida, E., Sasakawa, S. and Honda, K. (1981) Biochim. Biophys. Acta 649, 221–228.
6 Arnold, K., Herrmann, A., Gawrisch, K. and Pratsch, L. (1988) in Molecular Mechanisms of Membrane Fusion (Ohki, S., ed.), Pergamon Publishing, New York.
7 Pratsch, L. and Donath, E. (1987) Stud. Biophys. 120, 113–122.
8 Herrmann, A., Pratsch, L., Arnold, K. and Laßmann, G. (1983) Biochim. Biophys. Acta 733, 87–94.
9 Arnold, K., Herrmann, A., Pratsch, L. and Gawrisch, K. (1985) Biochim. Biophys. Acta 815, 515–518.
10 Arnold, K., Pratsch, L. and Gawrisch, K. (1983) Biochim. Biophys. Acta 728, 121–128.
11 Herrmann, A., Arnold, K. and Pratsch, L. (1985) Biosci. Rep. 5, 689–696.
12 Mc Donald, R.I. (1985) Biochemistry 24, 4058–4066.
13 Rand, R.P. (1981) Annu. Rev. Biophys. Bioeng. 10, 277–314.
14 Arnold, K., Lösche, A. and Gawrisch, K. (1981) Biochim. Biophys. Acta 645, 143–148.
15 Tilcock, C.P.S. and Fisher, D. (1982) Biochim. Biophys. Acta 688, 645–652.
16 Evans, E. and Needham, D. (1988) in Molecular Mechanisms of Membrane Fusion (Ohki, S., ed.), Pergamon Publishing, New York.
17 Feix, J.B., Green, L.L. and Butterfield, D.A. (1982) Life Sci. 31, 1001–1009.
18 Butterfield, D.A., Farmer, B.T. and Feix, J.B. (1983) Ann. N.Y. Acad. Sci. 414, 169–179.
19 Laßmann, G. and Herrmann, A. (1984) Stud. Biophys. 103, 113–118.
20 Herrmann, A., Laßmann, G., Groth, T., Donath, E. and Hillebrecht, B. (1986) Biochim. Biophys. Acta 861, 111–121.
21 Herrmann, A., Groth, T., Laßmann, G., Ladhoff, A.-M. and Hillebrecht, B. (1986) Biosci. Rep. 6, 45–55.
22 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119–130.
23 Lerche, D., Hessel, E. and Donath, E. (1979) Stud. Biophys. 75, 95–106.
24 Ahkong, Q.F., Blow, A.M.J., Botham, G.M., Launder, J.M., Quirk, S.J. and Lucy, J.A. (1978) FEBS Lett. 95, 147–152.
25 Herrmann, A., Arnold, K., Laßmann, G. and Glaser, R. (1982) Acta Biol. Med. Germ. 41, 289–298.
26 Feix, J.B. and Butterfield, D.A. (1980) FEBS Lett. 115, 185–188.
27 Buuer, K.W. and Smith, I.C.P. (1978) Can. J. Biochem. 56, 117–122.
28 Schreier, S., Polnaszek, C.F. and Smith, I.C.P. (1978) Biochim. Biophys. Acta 515, 375–436.
29 Coughlin, R.T., Hang, A. and Mc Groarty, E.J. (1983) Biochim. Biophys. Acta 729, 161–166.
30 Lepock, J.R., Morse, P.D. Mehlhorn, R.J., Hammerstedt, R.H., Snipes, W. and Keith, A.D. (1975) FEBS Lett. 60, 185–189.
31 Hubbell, W.L., Metcalfe, J.C., Metcalfe, S.M. and Mc Connel, H.M. (1970) Biochim. Biophys. Acta 219, 415–427.
32 Gawrisch, K. (1986) Thesis, 'Molekulare Mechanismen und Membranveränderungen bei der durch PEG induzierten Zellfusion', Karl-Marx-University, Leipzig.
33 Herrmann, A., Arnold, K., Pratsch, L. and Laßmann, G. (1983) Biomed. Biochim. Acta 9, 1151–1155.
34 Griffith, O.H. and Jost, P. (1976) in Spin Labeling - Theory and Applications (Berliner, L.J., ed.), pp. 454–523, Academic Press, New York.
35 Knutton, S. (1979) J. Cell Sci. 36, 61–72.

36 Arnold, K., Lvov, Y.M., Szögyi, M. and Györgyi, S. (1986) Stud. Biophys. 113, 7–14.
37 Bäumler, H. and Donath, E. (1987) Stud. Biophys. 120, 113.
38 Hui, S.W., Isac, T., Boni, L.T. and Sen, A. (1985) J. Membr. Biol. 84, 137–146.
39 Boss, W.F. (1983) Biochim. Biophys. Acta 730, 111–118.
40 Verkleij, A.J. and Ververgaert, P.H.J.T. (1978) Biochim. Biophys. Acta 515, 303–327.
41 Arakava, H. and Timasheff, S. (1985) Biochemistry 24, 3756–3762.
42 Atha, D.H. and Ingham, K.C. (1981) J. Biol. Chem. 256, 12108–12117.