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The dielectric properties of aqueous solutions of poly(ethylene glycol) and their influence on membrane structure

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The dielectric constant of water is reduced drastically on addition of poly(ethylene glycol). The behaviour is not described by a linear mixture equation. The decreased dielectric constant can lead to the general perturbation of the membrane structure which is necessary in such a manner that a strong aggregation of membranes would lead to their fusion. The changed cation permeability in the presence of poly(ethylene glycol) can be explained as the effect of the lowered dielectric constant on the transfer energy.

Poly(ethylene glycol) (PEG) is a water-soluble synthetic polymer with interesting properties which make wide applications in technology, biology and medicine possible.

Besides the use of poly(ethylene glycol) for the fusion of cells [1] there are applications as a fractional precipitating agent for protein purification and isolation, DNA and protein crystallization [2,3] and for the cryopreservation of biological material.

Investigating the mechanism of cell fusion induced by poly(ethylene glycol) we studied the alteration of the polarity of the external phase in the presence of the polymer using the fluorescence properties of ANS [4] and the EPR spectra of spin label molecules [5,6]. These experiments and the results of other groups [7–9] demonstrated that poly(ethylene glycol) makes the solvent less polar and that the partition of hydrophobic as well as polar molecules between the membrane and the external phase is changed. In this way poly(ethylene glycol) can influence the conformational properties of macromolecules or membrane components without interacting in a direct manner with these molecules.

A more quantitative parameter to characterize the polar properties of a solution is the dielectric constant.

Measurements of the static dielectric constant of pure poly(ethylene glycol) in the liquid state have given values in the order of $\varepsilon \approx 10$ [10,11], which is relatively low for a molecule with a good solubility in water. If aqueous solutions of poly(ethylene glycol) behave as normal mixtures of dielectrics a drastic decrease of the dielectric constant from $\varepsilon \approx 80$ for water should occur in solutions with poly(ethylene glycol). The decrease of the dielectric constant observed in our experiments can give some explanations for changes of membrane properties under the influence of poly(ethylene glycol).

Measurements were made at 20°C using a DC meter type 60 GK (Franz Küster Nachf. KG,

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Abbreviation: ANS, 1-anilinonaphthalene-8-sulfonate.

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Dresden, G.D.R.) with a quartz frequency of 7 MHz. Twice distilled water with a specific conductivity of about 5 μS/cm was used. The DC meter was calibrated with different dioxane/water and methanol/water mixtures of known dielectric constants [12]. PEG 400 (Serva), PEG 6000 (Ferak), PEG 20000 (Serva) and PEG 40000 (Ferak) were used without further purification. The measurement of the static dielectric constant ε as a function of the poly(ethylene glycol) concentration and the molecular weight of the polymer is given in Fig. 1. With increasing polymer concentration the dielectric constant decreases slowly between 0 and 20 wt.% of poly(ethylene glycol) and in a more linear manner between 20 and 50 wt.%. In the case of PEG 400 there is a slight increase of the dielectric constant at low polymer concentrations. The dielectric constant of pure PEG 400 which is in the liquid state at room temperature was measured to be 11.6. Linear extrapolation of the concentration dependence of the dielectric constant gives this value also for the other molecular weights at a polymer concentration of 100 wt.%.

For pure poly(ethylene glycol) in the liquid state the time of dielectric relaxation was determined to be about $4 \cdot 10^{-10}$ s and it was found that the relaxation behaviour is only weakly dependent on the molecular weight [10]. Thus, the measuring frequency of 7 MHz is lower than the rate of dielectric relaxation and the decrease of the dielectric constant measured cannot be caused by relaxation processes. The concentration dependences of the dielectric constant on poly(ethylene glycol) of different molecular weights are nearly the same (Fig. 1) indicating that the effect of the polymer depends on the concentration of its subunits (Table I). Taking into account the molecular weight of the subunit ($-CH_2-CH_2-O-$) of 44, the different poly(ethylene glycols) 400, 6000 and 40000 consist of 9.1, 136 and 909 subunits, respectively. The ranges of molecular weights are 380–420, 5000–7000 and 35000–40000, respectively. In Table I the molar concentrations c_{PEG} at different weight percentages of poly(ethylene glycol) (mol. wt. 400, 6000 and 40000) and the concentrations of the subunits c_{Sb} (average of the values determined for PEG 400, 6000 and 40000) are given. The concentrations of the subunits at the same wt.% of poly(ethylene glycol) are independent of the molecular weight of poly(ethylene glycol).

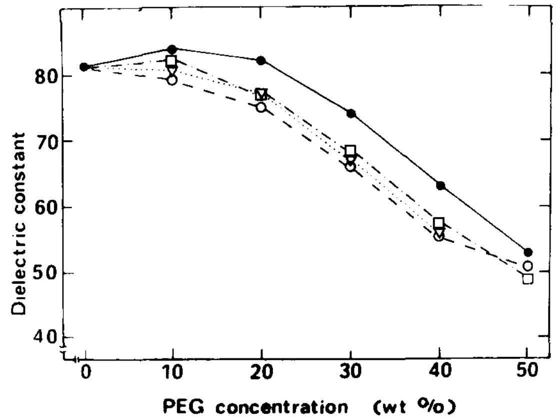

Fig. 1. Dielectric constant of aqueous poly(ethylene glycol) solutions as a function of concentration (● PEG 400 (Serva), □ PEG 6000 (Ferak), ○ PEG 20000 (Serva), ▽ PEG 40000 (Ferak)). The relative error of measurement is smaller than 0.6%.

TABLE I

MOLAR CONCENTRATIONS OF PEG (c_{PEG}) AND OF PEG SUBUNITS (c_{SB}) AT DIFFERENT WEIGHT PERCENTAGES OF AQUEOUS SOLUTIONS OF PEG FOR PEG 400, 6000 AND 40000

c_{SB} is the average of the molar concentrations determined for PEG 400, 6000 and 40000. c_{SB} was obtained by measuring the volume of aqueous PEG solutions and by taking into account the number of subunits per PEG molecule. Δc_{SB} is the absolute deviation of the single values from the average.

wt.% PEG	c_{PEG} (mM) of PEG			c_{SB} (M)	Δc_{SB} (M)
	400	6000	40000		
10	252.7	16.8	2.3	2.3	0.1
20	512.3	34.7	5.3	4.7	0.1
30	779.2	58.9	7.1	7.0	0.5
40	1130.1	74.1	10.7	10.0	0.3

Using the simple linear mixture equation of Silberstein (quoted in Oehme [12]) the dielectric constant of an aqueous solution can be determined from the dielectric constants of the pure components and their relative volume

$$\varepsilon = \varepsilon_{H_2O} \cdot \dot{V}_{H_2O} + \varepsilon_{PEG} \cdot \bar{V}_{PEG}$$

In Table II the calculated and experimental dielectric constants are given for PEG 400, with a dielec-

TABLE II

CALCULATION OF THE CONCENTRATION DEPENDENCE OF ε FOR PEG 400 ASSUMING AN IDEAL MIXING OF WATER AND PEG

wt.%	$\bar{v}$		ε	
	H_2O	PEG	Calcd.	Exp.
10	0.91	0.09	73.8	81
20	0.83	0.17	68.4	77
30	0.73	0.27	61.5	68
40	0.64	0.36	55.4	56

tric constant $\varepsilon = 11.6$ for the polymer and $\varepsilon = 81$ for water at 20°C. In all cases the experimentally observed dielectric constants are higher than the values calculated on the assumption of an ideal mixture of the components.

It is also known that poly(ethylene glycol) exerts a high osmotic pressure resulting for instance in cell shrinking [13] and in reducing the number of bound water molecules on hydrated phospholipid bilayers [4]. Therefore, it could not be expected that aqueous poly(ethylene glycol) solutions behave as normal mixtures of dielectrics.

For poly(ethylene glycol) concentrations larger than about 20 wt.% a drastic decrease of the dielectric constant is observed. This behaviour agrees qualitatively with the polarity alterations which were measured by means of EPR using a small nitroxide label molecule [5]. In a first approximation it can be concluded that the dielectric constant of aqueous solutions of poly(ethylene glycol) of different molecular weights depends on the concentration of the subunit ($-CH_2-CH_2-O-$) (Table I).

The changes of the dielectric constant cannot be explained by a simple mixture of two dielectrics because deviations from a linear mixture behaviour appear at poly(ethylene glycol) concentrations below 30 wt.% where free and bound water coexist. Deviations seem to be more pronounced for polymer of low molecular weight. This could be caused by the different water binding capacities of poly(ethylene glycols) of various molecular weights. Tilcock and Fisher [14] measured that between 1.8 to 2.7 water molecules are bound per subunit in dependence on the molecular weight which agrees with values reported earlier [13]. Baran [15] pointed out that besides 3 to 4 bound water molecules at each subunit, additionally, 12 water molecules are influenced per subunit.

For the thermal and electrical conductivity it was also found that the behaviour cannot be explained by mixture theories [16]. A change of the dielectric properties of the external phase should have a drastic influence on the hydrophilic-hydrophobic balance at surfaces of macromolecules and membranes. However, for proteins in aqueous solution it was found that the presence of poly(ethylene glycol) does not change their conformation [3]. As demonstrated on the basis of thermodynamic considerations poly(ethylene glycol) generates a phase separation in these systems so that it is excluded from the surface of the macromolecules. These properties of poly(ethylene glycol) are discussed in terms of the concept of excluded volume [2] which was also employed for the explanation of the poly(ethylene glycol)-induced aggregation of liposomes [14,17]. The aggregation of liposomes and cells is observed at relatively low concentrations of poly(ethylene glycol) [13,14] where only minor changes of the dielectric constant occur. Therefore, we have to assume that for the aggregation other mechanisms are responsible (e.g., volume exclusion) and changes of the dielectric properties are unimportant.

However, fusion becomes important for concentrations higher than 45 wt.% [13,18]. There are some contradictious studies about the role of impurities of poly(ethylene glycol) in inducing the fusion process because a general perturbation of the membrane structure is necessary in order that a strong aggregation of membranes should lead to their fusion [17,19]. Recently, it was shown that poly(ethylene glycol) is itself able to fuse cells, and some contaminating substances can enhance the fusogenic activity of commercial poly(ethylene glycol) [20]. The decreased dielectric constant of the external medium can also lead to such a destabilization of the membrane structure. Elworthy and McIntosh [21] demonstrated that the dielectric constant influences the self-assembling of phospholipids in such a manner that a minimum aggregate size occurs at about $\varepsilon = 33$. From other experiments it was concluded that the lowest value for the formation of bilayers from phospholipids in polar solvents is about 35 [22]. The high rates of hemolysis and the influence of poly(ethylene glycol) on the partition of membrane components between the membrane and the external phase [4] demonstrated the destabilizing effect of poly(ethylene glycol) on the membrane. Such properties were discussed as a detergent-like behaviour of poly(ethylene glycol) [23].

The increase of the cation permeability of cell membranes which was observed in the presence of poly(ethylene glycol) [21,24] can be explained as an effect of changed dielectric constant on the transfer energy. The influence of the dielectric constant can be estimated from a pore model which was proposed by Parsegian [25] to explain the ion transport across low dielectric membranes. In view of this model it can be estimated that the energy of transfer of a charge from the external medium into the membrane (pore) is lowered by about 11% (pore radius 5 Å) or 21% (pore radius 10 Å) by decreasing the dielectric constant from 80 to 50 ($\varepsilon_{\text{pore}} = 80$, $\varepsilon_{\text{hc}} = 2$, $a = 2$ Å in Ref. 25).

Since there is no evidence for a direct interaction between poly(ethylene glycol) and phospholipid membranes [1,2,26] we suggest that the observed effects of poly(ethylene glycol) on the physical state of membranes as well as the fusogenic activity of poly(ethylene glycol) arise from altered physicochemical properties of water which lead to a strong osmotic compression of membranes and changed dielectric and polar properties of the external phase.

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