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Short communication

THE BREAKDOWN OF BILAYER LIPID MEMBRANES BY DENDRIMERS

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Abstract: The BLM-system for studying the electrophysical properties of bilayer lipid membranes (BLM) was applied to investigate interactions between polyamidoamine (PAMAM) dendrimers and lipid bilayers. The cationic PAMAM G5 dendrimer effectively disrupted planar phosphatidylcholine membranes, while the hydroxyl PAMAM-OH G5 and carboxyl PAMAM G4.5 dendrimers had no significant effect on them.

Key words: PAMAM dendrimer, Planar bilayer lipid membrane, Conductivity, Voltammetry

INTRODUCTION

Dendrimers are new artificial polymers that have properties typical for small organic molecules (determined composition, monodispersity, relatively low viscosity at high concentration in solution) and characteristics typical for polymers (high molecular weight, multitude of physical-polymer properties) [1-2]. Their unique properties permit their application in targeting, microarray systems, catalysis and drug delivery systems [3-5]. Polyamidoamine (PAMAM) dendrimers are based on an ethylenediamine core and branched units built from methyl acrylate and ethylenediamine. In this paper, the fifth generation of water-soluble polyamidoamine dendrimers is used. They possess 128 amino groups on a surface, while PAMAM-OH G5 dendrimers have the same number of hydroxyl groups at their chain-ends. PAMAM G4.5 dendrimers possess 128 carboxyl groups on their surface. The molecular weights for PAMAM G4.5,

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PAMAM G5 and PAMAM-OH G5, which have similar diameters of around 5.3 nm, are 26258 Da, 28825 Da and 28951 Da, respectively [1, 20]. The interaction between dendrimers and lipids, liposomes or lipid membranes is an important issue in need of study. Lipid-dendron assemblies and lipid dendrimers are successfully used in medicine for the overall uptake of drugs through membranes [3-5]. The technique of voltammetry is extensively used with bilayer lipid membranes (BLMs) to study different interactions [6-9]. Specific electrodes, biosensor devices, biomolecular electronic devices and other measurement instrumentation are applied in such studies. BLMs are useful models as they have a number of characteristics that can be changed upon membrane modification, such as resistance, capacitance, breakdown voltage, area of bilayer and stability. Moreover, BLMs are successfully used as models of biological membranes [6-15]. This paper is devoted to the application of the BLM system and planar BLMs in the study of interactions between dendrimers and the lipid bilayer.

MATERIALS AND METHODS

Our experiments involved a customized multifunctional BLM-system [6] that allows the study of volt-ampere characteristics up to the picoampere level, the kinetics of electric conductivity, and pH-metry of planar BLMs at different temperatures. Two chambers of a Teflon cell, each containing platinized electrodes for measurments, were separated by a septum with a hole of 1.5 mm diameter [6, 10]. The teflon cell was temperature-controlled and intended for electrochemical analysis. A second pair of pH standard electrodes for parallel pH measurements was also attached [6, 10]. The formation of the BLM over the hole was monitored both via the appearance of a membrane potential and visually using a microvideo system to be able to see the membranes in real-time mode on a PC monitor and save the results on video files. The system was managed by a PC. The data analysis was performed by means of in-house software [6-9]. There were two components in the current through the membrane: the charging current I_C, and resistance current I_R. They can be determined as follows:

$$I_c = C_m \cdot \frac{dU}{dt} = C_m \cdot V_t \quad (1); \qquad I_r = \frac{U}{R_m} \quad (2); \qquad I = I_r + I_c = \frac{U}{R_m} + C_m \cdot V_t \quad (3)$$

In equations (1)-(3), V_t is the scan rate in $mV \cdot s^{-1}$, C_m is the membrane capacitance and R_m is the membrane resistance. The capacitance current I_C through the membrane is constant [7-9]. Equation (3) shows that increasing the scanning voltage results in an increase in the current through the resistor. In the case of a constant scan rate V_t, with fixed values of C_m and R_m, the current I has a linear relationship with the sweeping potential U. Thus, the slope reflects the value of R_m, while C_m can be determined by measuring I_C according to the graph of the I=f(U) response. In these experiments, the membrane resistance R_m [7-9] was studied.

Pure egg yolk phosphatidylcholine, PAMAM G5, PAMAM-OH G5 and PAMAM G4.5 dendrimers were obtained from Sigma-Aldrich. Phosphatidylcholine was dissolved in nonane at 40-50 mg per mL [10]. Phosphate buffered saline (PBS) containing 25 mM Na-phosphate buffer, pH 7.4, and 125 mM NaCl was used as the electrolyte. Planar BLMs (Muller-Rudin type) were obtained by bubbling the phosphatidylcholine solution towards the hole with a potential of -60 mV across it. The formation of the membranes was accelerated by applying short impulses of a given voltage [7-8]. Dendrimers were simultaneously added into both chambers of the electrochemical cell. The measurements were carried out at room temperature (approx. 20ºC) in different voltage and time ranges. The time interval between the application of a voltage and the onset of the rupture was measured on the basis of volt-ampere characteristics using the scan rate in $mV \cdot s^{-1}$. All the results were expressed as a mean value ±S.D. of n experiments.

RESULTS AND DISCUSSION

The membranes ruptured at +300 to 500 mV. The resistance of the intact membranes was $7.9 \pm 0.7 * 10^6$ $\Omega \cdot cm^{-2}$, and the C_m was -0.6±0.07 $\mu F \cdot cm^{-2}$. For pre-rupture voltages, the formation of metastable single pores [10, 11] was observed in ~32% of cases (n = 46) (Fig. 1, curve 1). The addition of PAMAM-OH G5 and PAMAM G4.5 dendrimers at different concentrations had no effect on the electrical characteristics of the membrane (Fig. 2). By contrast, the addition of the PAMAM G5 dendrimer led to an immediate decrease in the membrane resistance in a concentration-dependent manner (Fig. 1, curves 2-4, and Fig. 2). The insert in Fig. 2 shows the time interval between the application of voltage and the onset of the rupture (for the PAMAM G5 dendrimer at 0, 5 and 10 µM), or I_{max} (for the PAMAM G5 dendrimer at >15 µM). When PAMAM G5 was >10 µM, metastable pore formation was observed to be deficient (n = 10) (Fig. 1, curve 3). The results obtained can be explained as follows. Under our conditions, the PC molecules forming the BLMs are in the gel phase. The interaction between the positively charged PAMAM G5 dendrimer and the lipid membrane can induce the formation of holes (as was shown for PAMAM G7 dendrimers [17]), the inclusion of dendrimers into the lipid membrane and the disturbance of the bilayer (the formation of hexagonal phases, and so on) [4-5, 17], or, in some cases, the exiting of lipid molecules from the bilayer and the formation of dendrimer-lipid vesicles [18]. Furthermore, as polyelectrolytes, PAMAM dendrimers (like proteins and DNA) are able to form domains in a bilayer [19]. These interactions lead to significant changes in the conductivity of lipid membranes. As is known, the conductivity of the unmodified lipid bilayer at moderate electric field strengths is provided by voltage-induced metastable single pores (with lifetimes of ~3 ms [11, 14] and a theoretical pore radius of ~0.6 nm [13]) and described by the integrated and modified Nernst-Planck equation [11, 13]. At pre-breakdown voltages, these pores stabilize and form
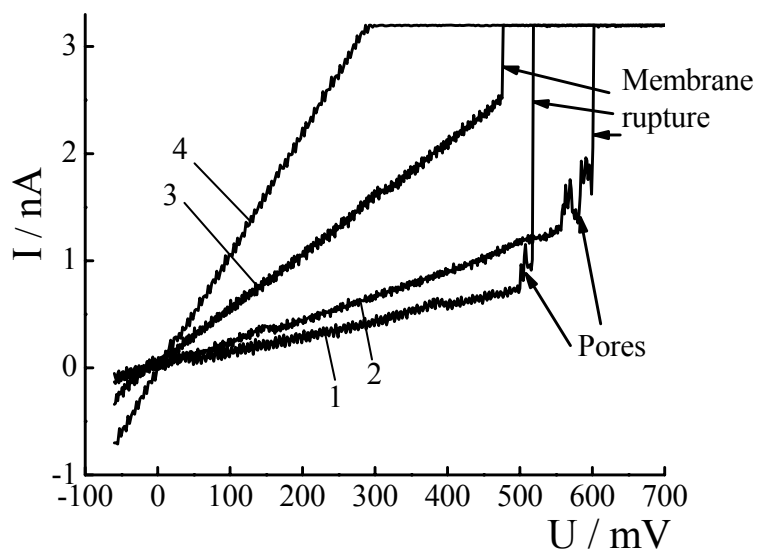
Fig. 1. Volt-ampere curves for planar BLMs in the absence (1) and presence (2-4) of the PAMAM G5 dendrimer at different concentrations: 1 – [G5] = 0 μM, 2 – [G5] = 5 μM, 3 – [G5] = 10 μM, and 4 – [G5] = 15 μM, in 25 mM sodium-phosphate buffer, pH 7.4, and 125 mM NaCl. 20ºC. The recording time was 20 s.
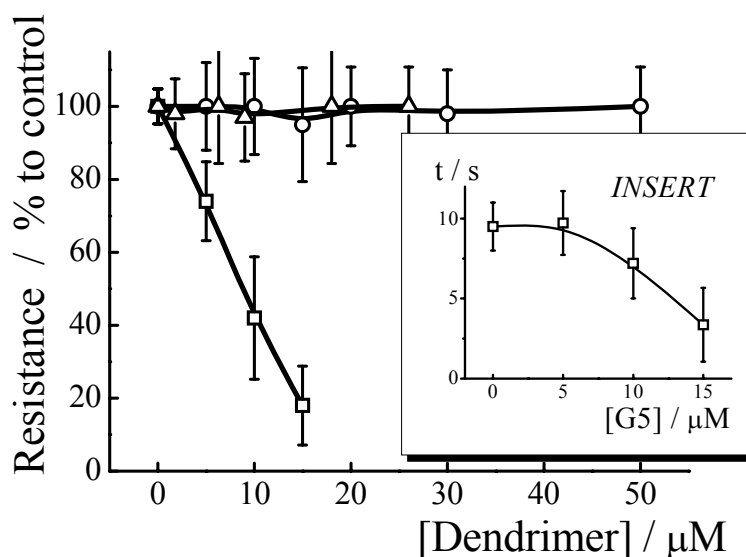
Fig. 2. The effect of PAMAM G5 (squares), G4.5 (triangles) and PAMAM-OH G5 (circles) dendrimers on the resistance of planar BLMs. The insert shows the time interval between the application of voltage and the onset of the rupture (or I_{max}) for the PAMAM G5 dendrimer. 25 mM sodium-phosphate buffer, pH 7.4, and 125 mM NaCl. 20ºC. The recording time was 20 s.

great pores (single ion channels) [11]. Thus, the disturbance of a lipid bilayer by PAMAM G5 dendrimers at constant ionic pressure (initially, the formation of holes [17]) leads to a rapid decrease in the resistance, a stopping of the formation of spontaneous pores, and an increase in the breakdown voltage. The non-linear time dependence (Fig. 2, insert) indicates the non-uniform interaction of PAMAM G5 with the bilayer, initially by the continuous attachment of added dendrimer molecules to formed aggregates (= holes) [17]. As seen from Figs 1-2, under our conditions, PAMAM G5 affected BLM at micromolar (0-15 μM) concentrations. The effects of PAMAM G7 dendrimers at nanomolar (10-100 nM) concentrations are presented in [17]. Such a difference can be explained in several ways. First, PAMAM G7 dendrimers have 512 charged surface groups and a diameter of 6.7 nm [1, 20]. This means that the ratio of "charged groups per square surface" is 3.63 groups per nm^2 for G7 and 1.45 for G5 (calculations made based on [20]). Second, the structure of PAMAM G7 dendrimers is much more rigid than that of G5 dendrimers [20]. Third, in our previous experiments, the addition of dendrimers induced pH changes in the solution, which can also cause the described effect [21]. It can easily be predicted that the pH effect of PAMAM G7 dendrimers will be several times higher than that of G5 (512 *vs* 128 surface groups). In this study, we used a buffer to neutralize such an effect (see Materials and Methods). In [17], a buffer was not used. Fourth, we used phosphatidylcholine for the BLMs rather than dimyristoylphosphatidylcholine (DMPC), used in [17]. Fifth, our experiments were performed at 20ºC (below the transition temperature of PC), while those in [17] were at temperatures above 28ºC (above the transition temperature of the supported DMPC bilayers). All these reasons explain the 10- to 100-fold differences in dendrimer concentrations.

Electrostatic forces are predominant in interactions between dendrimers and lipid bilayers [4, 5, 17, 18], so the neutral PAMAM-OH G5 dendrimer cannot effectively interact with zwitterionic phosphatidylcholine-forming BLMs. One of the possible reasons for the absence of effective interactions between the anionic PAMAM G4.5 dendrimer and BLMs is the formation of different concentrations of LH^+ and LOH^- forms of phosphatidylcholine [22], which can also disturb the effective binding of Ca^{2+} ions by the phosphatidylcholine monolayer above pH 6.5 [22, 23].

REFERENCES

1. Tomalia, D.A., Naylor, A.M. and Goddard III, W.A. Starburst dendrimers: Molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter. **Angew. Chem. Int. Ed.** 29 (1990) 138-175.
2. Newkome, R., Moorfield, C. and Vögtle, F. **Dendritic Molecules-Concepts, Synthesis, Perspectives.** VCH, Weinheim, 1996.
3. Cloninger, M.J. Biological applications of dendrimers. **Curr. Op. Chem. Biol.** 6 (2002) 742-748.
4. Purohit, G., Sakthivel, T. and Florence, A.T. Interaction of cationic partial dendrimers with charged and neutral liposomes. **Int. J. Pharm**. 214 (2001) 71-76.
5. Ottaviani, M.F., Turro, N.J., Jockusch, S. and Tomalia, D.A. Interactions between starburst dendrimers and mixed DMPC/DMPA-Na vesicles studied by the spin label and the spin probe techniques, supported by transmission electron microscopy. **Langmuir** 18 (2002) 2347-2357.
6. Loban, V.A., Drapeza, A.I., Khmelnitski, A.I., Lisichenok, A.N., Cherenkevich, S.N. and Vasilevskaya, N.V. Apparatus-program tools for studying electrophysical characteristics of planar bilayer membranes, in: **Book of articles of XIV Conference "Sensor 2002"**, Minsk, 2002, p. 252.
7. Tien, H.T., Barish, R.H., Gu, L.-Q. and Ottova, A.L. Supported bilayer lipid membranes as ion and molecular probes. **Anal. Sci.** 14 (1998) 3-14.
8. Tien, H.T and Ottova, A., Eds. **Planar Lipid Bilayers (BLMs) and their Applications.** Elsevier Sci., Amsterdam, New York, 2003.
9. Naumowicz, M., Petelska, A.D. and Figaszewski, Z.A. Impedance analysis of phosphatidylcholine–cholesterol system in bilayer lipid membranes. **Electrochim. Acta** 50 (2005) 2155-2161.
10. Cherenkevich, S.N., Khmelnitski, A.I., Drapeza, A.I. and Bakovich, I.A. Single ionic channels and macroscopic conductivity of bilayer lipid membranes. **Biofizika** 34 (1989) 45-48.
11. Weaver, J.C. and Chizmadzhev, Yu.A. Theory of electroporation, **Bioelectrochem. Bioenerg.** 41 (1996) 135-160.
12. Hianik, T., Kaatze, U., Sargent, D., Krivánek, R., Halstenberg, S., Pieper, W., Gaburjaková, J., Gaburjaková, M., Pooga, M. and Langel, U. A study of the interaction of some neuropeptides and their analogs with bilayer lipid membranes and liposomes. **Bioelectrochem. Bioenerg.** 42 (1997) 123-132.
13. Kakorin, S. and Neumann, E. Ionic conductivity of electroporated lipid bilayer membranes, **Bioelectrochem.** 56 (2002) 163-166.
14. Melikov, K.C., Frolov, V.A., Shcherbakov, A., Samsonov, A.V., Chizmadzhev, Y.A. and Chernomordik, L.V. Voltage-induced nonconductive pre-pores and metastable single pores in unmodified planar lipid bilayer. **Biophys. J.** 80 (2001) 1829-1836.
15. Karoonuthaisiri, N., Titiyevskiy, K. and Thomas, J.L. Destabilization of

fatty acid-containing liposomes by polyamidoamine dendrimers. **Colloids Surf. B: Biointerfaces** <u>27</u> (2003) 365-375.

16. Klajnert, B. and Epand, R.M. PAMAM dendrimers and model membranes: differential scanning calorimetry studies. **Int. J. Pharm.** <u>305</u> (2005) 154-166.

17. Mecke, A., Uppuluri, S., Sassanella, T.M., Leeb, D.-K., Ramamoorthy, A., Baker Jr., J.R., Orr, B.G. and Banaszak Holl, M.M. Direct observation of lipid bilayer disruption by poly(amidoamine) dendrimers. **Chem. Phys. Lipids** <u>132</u> (2004) 3-14.

18. Zhang, Z.-Y. and Smith, B.D. High-generation polycationic dendrimers are unusually Effective at Disrupting Anionic Vesicles: Membrane Bending Model. **Bioconjugate Chem.** <u>11</u> (2000) 805-814.

19. MacDonald, P.M., Crowell, K.J., Franzin, C.M., Mitrakos, P. and Semchyschyn, D.J. Polyelectrolyte-induced domains in lipid bilayer membranes: the deuterium NMR perspective. **Biochem. Cell Biol.** <u>76</u> (1998) 452-464.

20. Svenson, S. and Tomalia, D.A. Dendrimers in biomedical applications – reflections on the field, **Adv. Drug Deliv. Rev.** <u>57</u> (2005) 2106-2129.

21. Shcharbin, D., Klajnert, B. and Bryszewska, M. The Effect of PAMAM dendrimers on human and bovine serum albumins at different pH and NaCl salt concentrations. **J. Biomater. Sci., Polymer Edn.** <u>16</u> (2005) 1081-1093.

22. Brzozowska, I. and Figaszewski, Z.A. The influence of pH on phosphatidylcholine monolayer at the air/aqueous solution interface. **Colloids Surf. B: Biointerfaces** <u>27</u> (2003) 303-309.

23. Quinn, P.J. and Dawson, R.M. The pH dependence of calcium adsorption onto anionic phospholipid monolayers. **Chem. Phys. Lipids** <u>8</u> (1972) 1-9.