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The effect of hexadecaprenyl diphosphate on phospholipid membranes

Tadeusz Janas [a,*], Teresa Janas [b], Krystyna Walińska [a]

[a] *Department of Biophysics, Pedagogical University, Monte Cassino 21 B, 65-561 Zielona Góra, Poland*
[b] *Department of Physics, Technical University, Podgórna 50, 65-246 Zielona Góra, Poland*

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Abstract

In the present study we investigated phospholipid bilayer membranes and phospholipid vesicles made from dioleoylphosphatidylcholine (DOPC) or its mixture with the phosphate ester derivative of long-chain polyprenol (hexadecaprenyl diphosphate, C_{80}-PP) by electrophysiological and transmission electron microscopy (TEM) techniques. The membrane conductance-temperature relationships and the membrane breakdown voltage have been measured for different mixtures of C_{80}-PP/DOPC. The current-voltage characteristics, the membrane conductance, the activation energy of ion migration across the membrane and the membrane breakdown voltage were determined. Hexadecaprenyl diphosphate decreases the membrane conductance, increases the activation energy and the membrane breakdown voltage for the various values of C_{80}-PP/DOPC mole ratio. The analysis of TEM micrographs shows several characteristic structures, which have been described. The data indicate that hexadecaprenyl diphosphate modulates the surface curvature of the membranes by the formation of aggregates in liquid-crystalline phospholipid membranes. The properties of modified membranes can result from the presence of the negative charges in the hydrophilic part of C_{80}-PP molecules and can be modulated by the concentration of this compound in membranes. We suggest that the dynamics and conformation of hexadecaprenyl diphosphate in membranes depend on the transmembrane electrical potential. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Phospholipid membrane; Polyprenyl diphosphate; Membrane conductance; Membrane breakdown voltage; Activation energy; Transmission electron microscopy

1. Introduction

Polyisoprenols are natural products and derivatives of a common C_5 isoprene unit. Their phosphoryl derivatives, polyprenyl phosphates and dolichyl phosphates, function mainly as carriers of glycosyl units across membranes in the glycosylation reactions [1–4]. The occurrence of phosphate esters of polyisoprenols in membrane fractions from prokaryotic and eukaryotic cells is frequently reported [4–7]. Unicellular eukaryotes, fungi, animal and some plant tissues contain phosphoryl derivatives of α-saturated polyisoprenols (dolichyl monophosphates, dolichyl diphosphates) [6,7]. Bacterial membranes and leaves of some plants contain α-unsaturated polyisoprenols (polyprenols) and their phosphoryl derivatives [5,8,9].

The peptidoglycan layer of bacterial cell walls is

* Corresponding author. Fax: +48 (68) 3265449; E-mail: tjanas@asia.aw.wsp.zgora.pl

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biosynthesized using a lipid carrier undecaprenyl diphosphate to assemble and transport the disaccharide-pentapeptide precursor [4,8]. Similar lipid-linked cycles are involved in the biosynthesis of bacterial lipopolysaccharides and eukaryotic glycoproteins, the latter involving the structurally related dolichyl phosphate as a lipid carrier. The lengthy polyisoprenoid chain seems an important property for the lipid acceptors, and this probably relates to their ability to fluidize locally the membrane bilayer [4,10].

The molecule of hexadecaprenyl diphosphate (C_{80}-PP) consists of a hydroxyl group, which is esterified with two phosphate groups: a hydrophilic part with negative charges, and a hydrophobic part, a long unsaturated isoprenyl chain, mainly of poly-*cis* configuration. This molecule is composed of 16 isoprene units with the structure $\omega t_2 c_{12} \alpha PP$ where ω is an isoprene residue farthest from the esterified hydroxyl group, t is a *trans*-isoprene residue, c is a *cis*-isoprene residue, α is an unsaturated, terminal *cis*-isoprene residue and P is the phosphate group [11]. Long-chain polyprenols isolated from plant photosynthetic tissues of Spermatophyta contain di-*trans*-, poly-*cis*-prenols with the general structure $\omega t_2 c_n \alpha OH$, where the number of isoprene units *n* varies usually from 6 to 30–40, depending on the plant species [9]. The phosphoryl derivatives of these long-chain poly-*cis*-prenols have been present in small amounts in leaves of many species of Gymnospermae and Angiospermae [5].

In the present study we investigated bilayer lipid membranes (BLM) and lipid vesicles made from dioleoylphosphatidylcholine (DOPC) or its mixture with long-chain polyprenol by electrophysiological and transmission electron microscopy (TEM) techniques. The lipid bilayer has often served as an experimental model of biological membranes [12–18] due to its similarity to biological membranes. The current-voltage characteristics, the membrane conductance-temperature relationships and the membrane breakdown voltage have been measured for DOPC and different mixtures of C_{80}-PP/DOPC. The membrane conductance for DOPC and C_{80}-PP/DOPC bilayers, the membrane hydrophobic thickness and the activation energy of ion migration across these membranes have been determined. Structures of lipid vesicles prepared from DOPC and C_{80}-PP/DOPC mixtures have been studied.

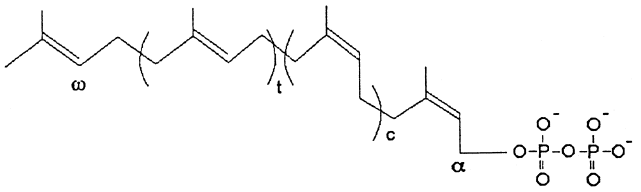


Fig. 1. The structure of hexadecaprenyl diphosphate (C_{80}-PP): $\omega t_2 c_{12} \alpha PP$, where ω is an isoprene residue farthest from the esterified hydroxyl group, t is a *trans*-isoprene residue, c is a *cis*-isoprene residue, α is an α-unsaturated, terminal *cis*-isoprene residue and P is the phosphate group.

2. Materials and methods

2.1. Chemicals

DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) was purchased from Sigma. It gave a single spot on Silica Gel TLC plates (Merck) in chloroform/methanol/water (65:25:4, v/v/v) and in chloroform/methanol/acetic acid/water (50:30:8:4, v/v/v). Hexadecaprenol (C_{80}) was isolated from leaves of *Picea abies* [19]. It gave a single spot on Silica Gel G TLC plates (Merck) in ethyl acetate/toluene (5:95, v/v) and on RP-18 HP TLC plates (Merck) in acetone. Hexadecaprenyl diphosphate (C_{80}-PP) (Fig. 1) was made by chemical phosphorylation of hexadecaprenol based on the method of Danilov et al. [20]. It gave a single spot on Silica Gel G TLC plates (Merck) in chloroform/methanol/water (65:25:4, v/v/v). *n*-Decane and butanol were purchased from Aldrich and Fisher, respectively.

2.2. Membrane formation

Bilayer lipid membranes in the form of hemispheres were formed according to the technique described previously [10] on a Teflon capillary tube in unbuffered (pH 6) aqueous solution of 0.1 M and 0.2 M NaCl (inside and outside, respectively). DOPC and C_{80}-PP/DOPC mixtures used for membrane formation were dissolved in *n*-decane/butanol (3:1, v/v) to obtain a concentration of 10 mg of lipid per ml of solvent. The area of the macrovesicular bilayer lipid membrane was about 50 mm^2.

2.3. Electrical measurements

Silver chloride electrodes were used to detect the

electric potentials and the currents. The area of the membrane, S, was determined by an optical measurement of membrane dimensions (the precision 0.03 mm). The temperature, T (the precision of measurements 0.1 K), was controlled by water circulating from an external bath. Electrical conductance of the membrane, G, was calculated from current-voltage characteristics (the precision of voltage and current measurements: 0.1 mV and 0.01 nA, respectively). To obtain the values of the breakdown voltage, V_B, the applied voltage was increased by a scan rate of 10 mV s^{-1}. The membrane rupture was reflected by a rapid increase of current [10]. The activation energy (the temperature-dependent part) of ion migration across the membrane, E_A, was determined from Arrhenius plots of normalized conductance of bilayer lipid membranes [21]:

$$(\ln[(G/C)/(G_0/C_0)]) = (E_A/R)[(1/T)-(1/T_0)] \quad (1)$$

where $\ln[(G/C)/(G_0/C_0)]$ is the normalized conductance of the membrane, G_0 and C_0 are membrane conductance and membrane capacitance, respectively, at temperature T_0, R is the gas constant. The normalization of membrane conductance (with respect to the membrane capacitance measured simultaneously) corrects any variations in the bilayer conductance which are due to variations in bilayer thickness or bilayer area. The capacitance of the membrane, C, was determined (the precision of measurement 0.1 nF) from recorded membrane discharge curves [22]. Dielectric constant equal to 2.1 was assumed for the calculations of the hydrophobic thickness of the bilayer capacitor.

2.4. Electron microscopy

Lipid vesicles were prepared from DOPC or the mixture of C_{80}-PP/DOPC. Small amounts of lipids (0.3 mg of DOPC or 0.112 mg/0.2 mg of C_{80}-PP/DOPC respectively, for mole ratio 0.2) mixed in chloroform were dried under nitrogen at 45°C until a thin film of dry lipid formed on the wall of the test tube. The lipid suspensions were obtained by addition of water from a Millipore water system. The concentration of lipid was 1 mg per ml of water. Lipids were hydrated at 45°C for 24 h. The vesicle dispersions were obtained by vortexing hydrated lipids using a laboratory vibrator (Janke and Kunkel, IKA Labortechnik VF 2) for 5 min at moderate modes.

A droplet of vesicle dispersion was put on a microscope copper grid (400 mesh) covered previously with Formvar membrane and a thin evaporated layer of carbon. The samples were dried at room temperature and negatively stained by depositing a drop of 1% aqueous uranyl acetate solution for 1 min, then grids were rinsed in water from a Millipore water system. The dried grids were again covered with a thin evaporated layer of carbon. Samples were analyzed in the transmission electron microscope JEOL JEM 1200EX at 80 kV.

3. Results

The behavior of hexadecaprenyl diphosphate/DOPC membranes as a function of applied potential was studied by performing voltammetric experiments and lipid vesicles prepared from C_{80}-PP/DOPC were analyzed in the transmission electron microscopy.

As presented in Fig. 2, the current-voltage characteristics are symmetric and linear for values of the potential in the range −20 to +20 mV. The values of the curve slope are smaller for C_{80}-PP/DOPC bilayers in comparison with the slope for DOPC membranes.

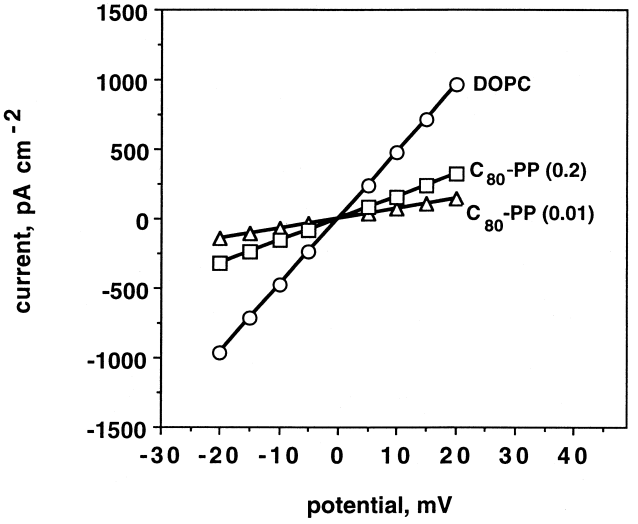


Fig. 2. Current-voltage steady-state characteristics of bilayer lipid membranes versus the C_{80}-PP/DOPC mole ratio. Experiments were performed at 25 ± 1°C.

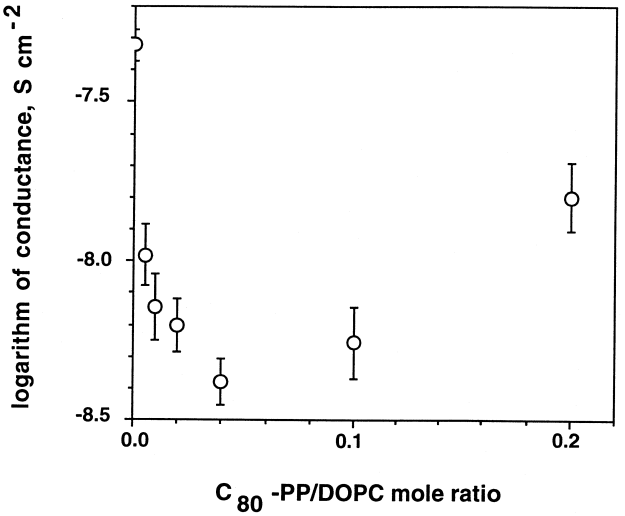


Fig. 3. The ionic conductance versus the C_{80}-PP/DOPC mole ratio. The values of membrane conductance were derived from the *I/V* curves by the least squares fitting. Each point represents the mean value (±S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25±1°C.

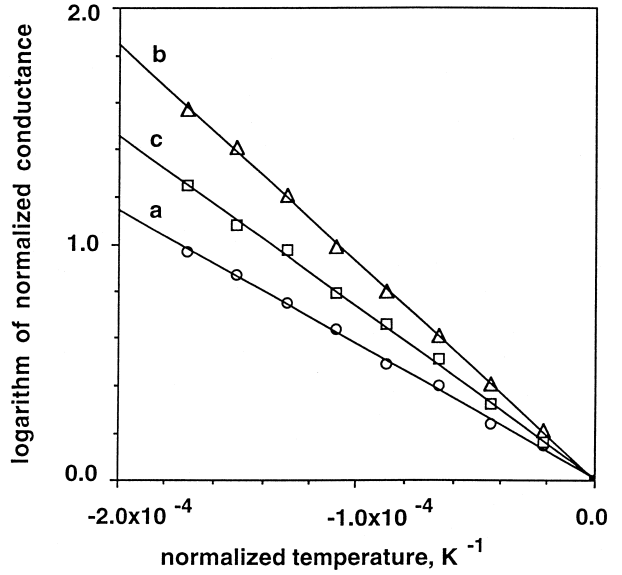


Fig. 4. Arrhenius plots of normalized conductance of macrovesicular bilayer lipid membranes made from: DOPC (a); C_{80}-PP/DOPC, mole ratio 0.01 (b); C_{80}-PP/DOPC, mole ratio 0.1 (c). The logarithm of normalized conductance was calculated as: $\ln[(G/C)/(G_0/C_0)]$, where G and C represent membrane conductance and membrane capacitance, respectively, for the temperature studied; G_0 and C_0 are membrane conductance and membrane capacitance, respectively, at 298 K. Normalized temperature was calculated as: temperature^{-1} (K^{-1})−(298 K)$^{-1}$.

The effect of hexadecaprenyl diphosphate on membrane specific conductance of bilayer lipid membranes formed from various mixtures of C_{80}-PP/DOPC is illustrated in Fig. 3. The values of the conductance were derived from the *I/V* curves by the least squares fitting. The dependence is presented on a semilogarithmic scale. The values of the membrane conductance decrease for small concentrations of C_{80}-PP in the bilayer in the range of the mole ratio 0–0.04 with a maximal 9-fold drop for the mole ratio 0.04 and then increase with increasing the concentration of hexadecaprenyl diphosphate. The maximal rise, over 4-fold, is observed for concentrations of C_{80}-PP/DOPC, from the mole ratio 0.04 to the mole ratio 0.2, in comparison with DOPC membrane.

The normalized conductance of bilayer lipid membranes was measured as a function of temperature in the range of 25–42°C. Typical trends are reported in Fig. 4. An increase in normalized conductance was observed with increasing temperature. The Arrhenius plots were linear, the slope of the curves depending on the percentage of C_{80}-PP in the bilayer. It is unlikely that variations in the concentration of the solvents in the bilayer were responsible for the measured temperature dependence. This is because variations of the bilayer capacitance occurred only

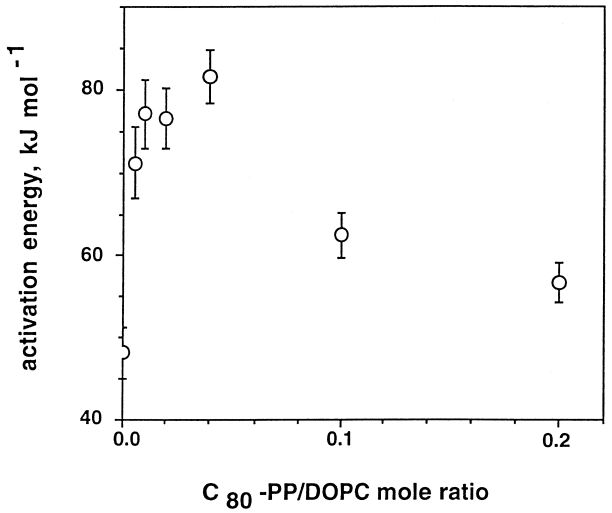


Fig. 5. The activation energy of ion migration versus the C_{80}-PP/DOPC mole ratio. The values of activation energies were derived from the Arrhenius plots by the least squares fitting. Each point represents the mean value (±S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes.

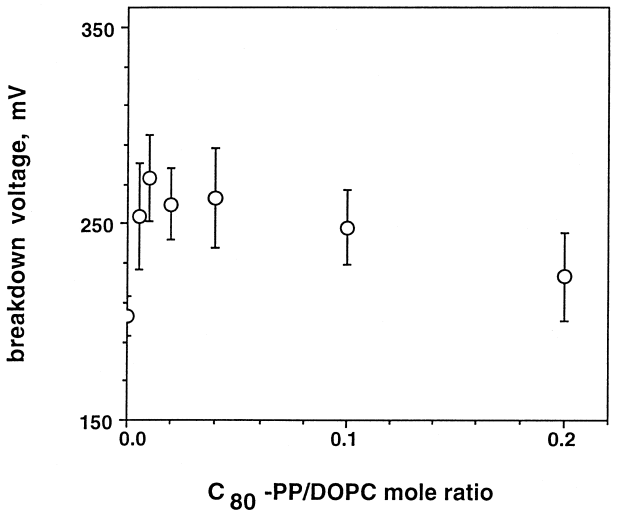


Fig. 6. The membrane breakdown voltage versus the C_{80}-PP/DOPC mole ratio. Each point represents the mean value (± S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25 ± 1°C.

slowly whereas the variations with temperature reported in our paper occurred rapidly. These results are in accordance with the observations reported by Smith et al. [21].

The relationship between the value of activation energy of ion transport across the membrane, E_A, and the percentage of hexadecaprenyl diphosphate in macrovesicular bilayers is shown in Fig. 5. The values of activation energies were derived from the Arrhenius plots by the least squares fitting. Arrhenius plots were linear in the temperature range 25–42°C (data not shown). For smaller concentrations of hexadecaprenyl diphosphate in the membrane, an increase of E_A was observed in comparison with DOPC bilayers. For higher concentrations of C_{80}-PP in the membrane a decrease of the value of E_A was observed. The E_A value increases from 48 ± 3 kJ mol^{-1} for DOPC bilayers to the maximal value, $E_{Amax} = 82 \pm 3.1$ kJ mol^{-1}, for bilayers prepared from the mixture C_{80}-PP/DOPC, mole ratio equals to 0.02, and then decreases to 57 ± 2.6 kJ mol^{-1} for the value of C_{80}-PP/DOPC mole ratio equals to 0.2. The values of the activation energy for electrical conduction with DOPC membranes found in our study are a little higher than those for egg lecithin/cholesterol membranes (35 ± 2 kJ mol^{-1}) [21].

Fig. 6 illustrates the effect of hexadecaprenyl diphosphate on the breakdown voltage of the membrane, V_B. The membrane electromechanical stability (proportional to the value of V_B) is modulated by the presence of hexadecaprenyl diphosphate in the bilayer lipid membrane. The increase of V_B is observed for small concentrations up to the mole ratio 0.01 of C_{80}-PP in the membrane, and than, for higher concentrations of C_{80}-PP in the membrane, a slight decrease in the value of V_B is observed. The value of membrane breakdown voltage increases from 203 ± 10 mV for DOPC bilayers to the maximal value $V_{Bmax} = 273 \pm 22$ mV for the bilayer prepared from the C_{80}-PP/DOPC mixture at 0.01 mole ratio.

Lipid vesicles prepared from DOPC and C_{80}-PP/DOPC mixtures (mole ratios 0.01 and 0.2), were analyzed in the transmission electron microscope. Examples of these vesicles are shown in Figs. 7–9. They were chosen from about 100 micrographs and represent the typical tendency of the modification of the vesicle structure. Fig. 7 presents typical DOPC dis-

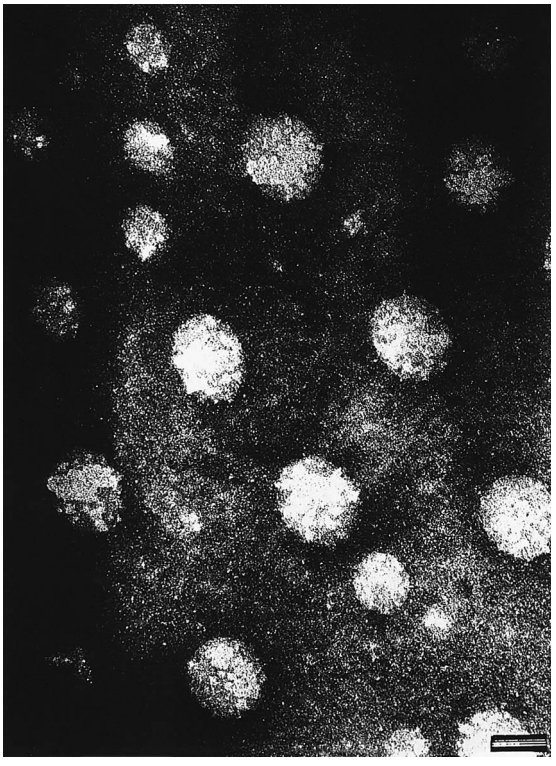
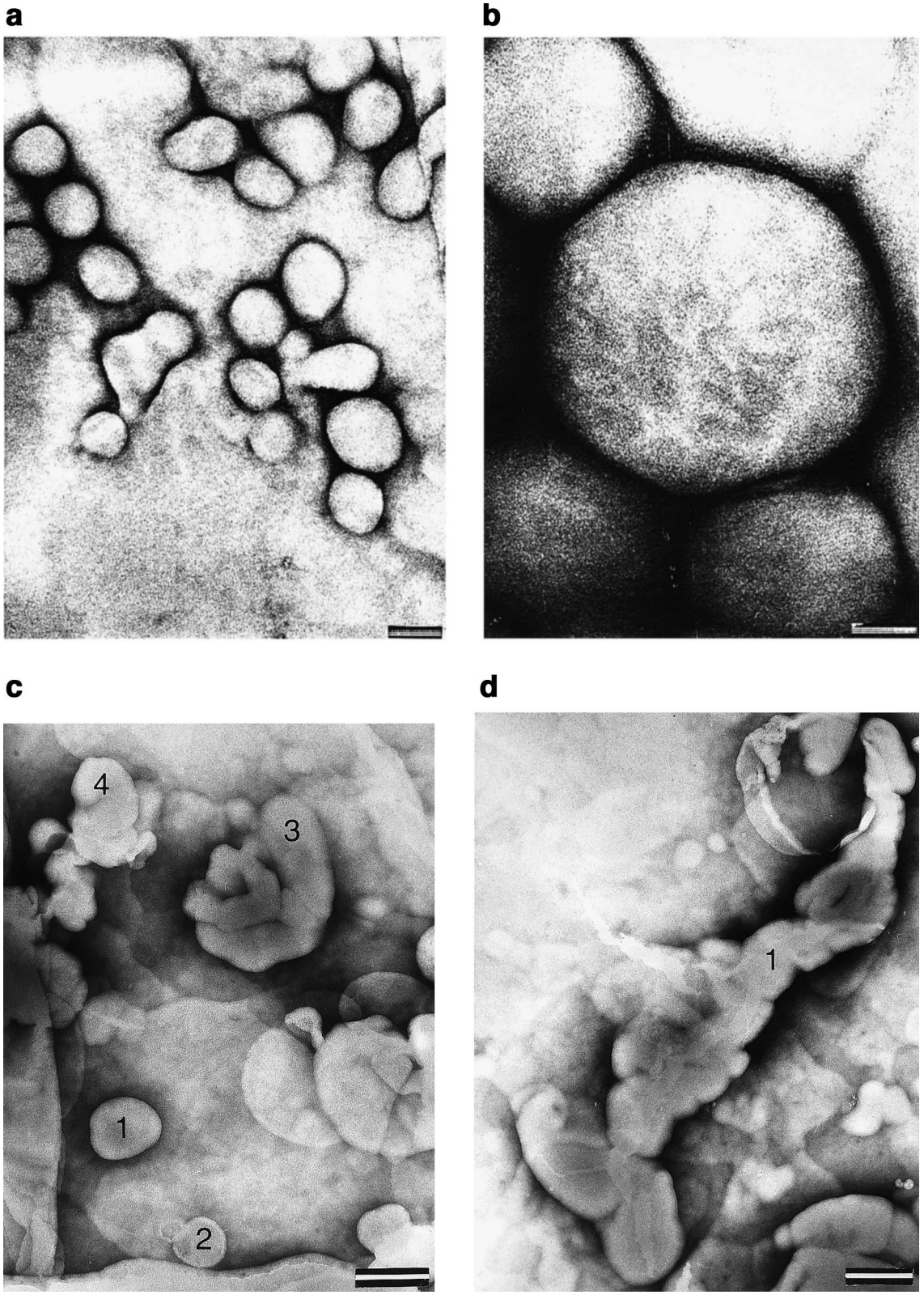


Fig. 7. The transmission electron microscopy micrograph of lipid bilayer structures consisting of DOPC. Magnification ×100 000; the bar represents 50 nm.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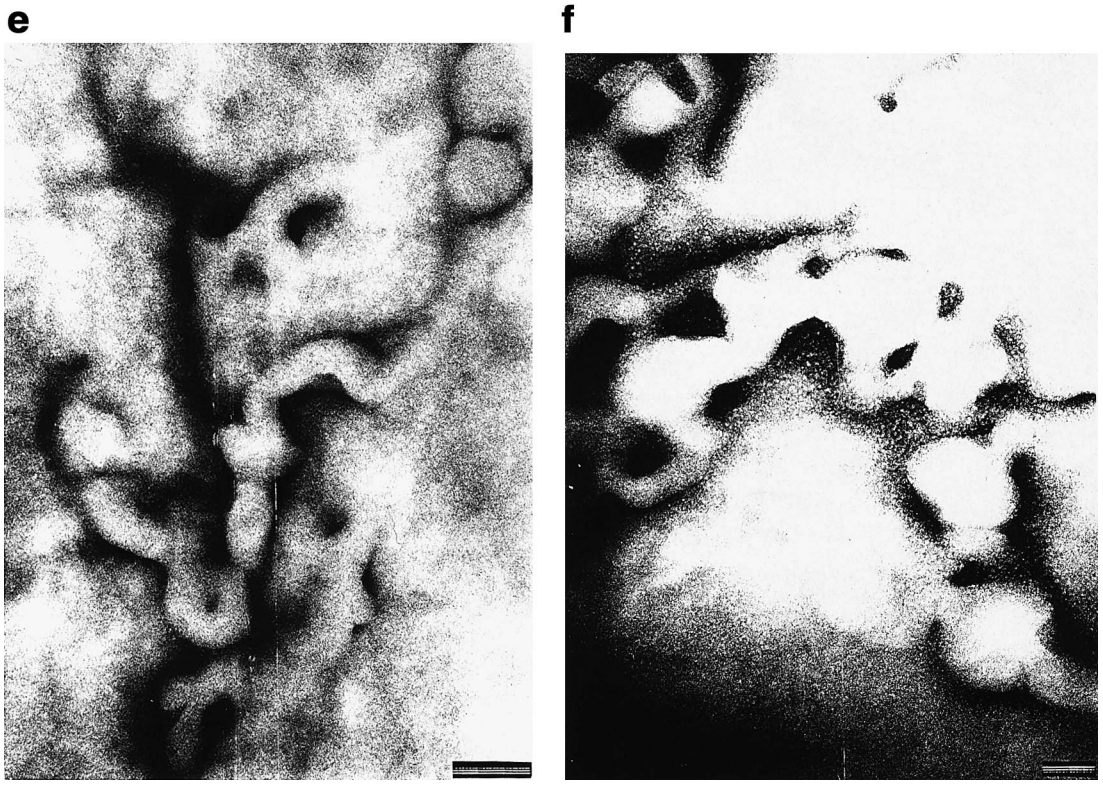

Fig. 8. Transmission electron microscopy micrographs of lipid bilayer structures consisting of C_{80}-PP/DOPC, mole ratio 0.01. Magnifications and scale bars: (a) ×60 000, 100 nm; (b) ×150 000, 50 nm; (c) ×25 000, 200 nm; (d) ×20 000, 200 nm; (e) ×75 000, 100 nm; (f) ×100 000, 50 nm.

persion of spherical vesicles of various sizes in the range from approx. 40 nm to approx. 300 nm. Fig. 8a–f show lipid vesicles prepared from C_{80}-PP/DOPC, mole ratio 0.01. The shapes of smaller vesicles presented in Fig. 8a are rather regular with diameters from 100 nm to 150 nm. Among the spherical vesicles there are some irregular vesicles with similar diameters. The spherical vesicles are shown in Fig. 8b. The diameters of these regular vesicles are approx. 170–300 nm. Among the regular vesicles prepared from C_{80}-PP/DOPC, mole ratio 0.01, there are some elongated, multi-bent structures (Fig. 8c,d). The micrograph presented in Fig. 8c shows some regular (structures 1 and 2) and some elongated vesicles (structures 3 and 4). Fig. 8d shows the large, elongated and bent vesicle (structure 1). The dimensions of this vesicle range from 200 nm to 2600 nm. Elongated vesicles in Fig. 8e,f are bent and they are geometrically similar to the torus. The part of structure 1 in Fig. 8e is similar to the handle of the jug. Structures 2 and 3 are also torus-like structures and their dimensions range from 100 nm to 300 nm. The micrograph presented in Fig. 8f shows a large, elongated torus-like structure with many 'handles'. The length of this structure is about 700 nm.

A new kind of structure, prepared from C_{160}/DOPC mixture, mole ratio 0.2, can be seen in Fig. 9a–d. A small bottle-like vesicle is presented in Fig. 9a. The light characteristic shape can be observed inside of the vesicle. The membranes of this vesicle are grainy. The dimensions of the vesicle range from 160 nm to 200 nm. Among the vesicles presented in Fig. 9b there are also some vesicles with grainy membranes. Structure 1 is similar to the budding structure. Structure 2 seems to be the effect of the fusion of two vesicles. Inside of these vesicles (structures 1 and 2) are seen dark, circular shapes. The vesicle presented in Fig. 9c is irregular and a budding vesicle. Membranes are grainy and the internal structure is heterogeneous. The width of this vesicle is about 170 nm. Some vesicles with grainy membranes are presented in Fig. 9d. One of them (structure 1)

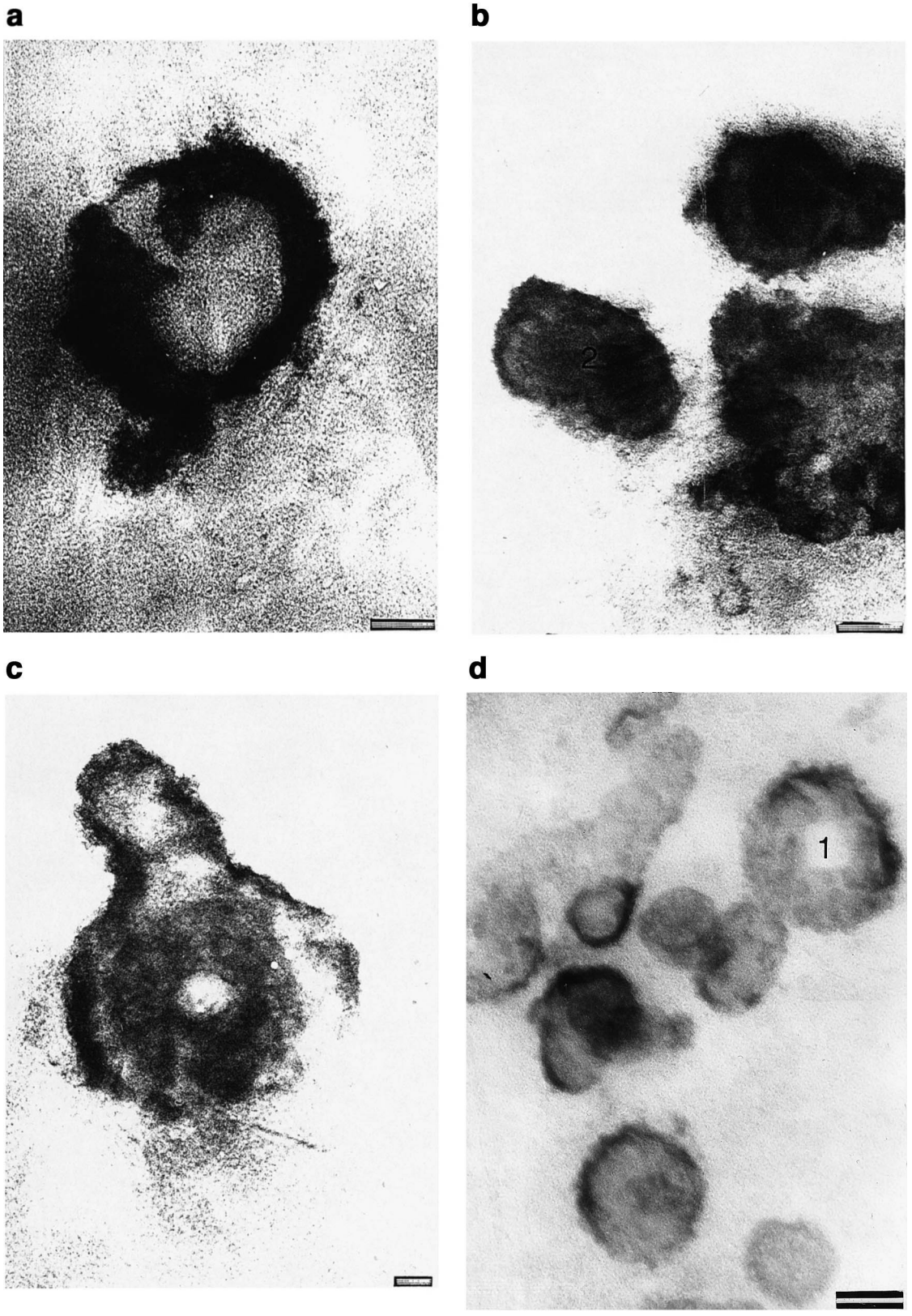

Fig. 9. Transmission electron microscopy micrographs of lipid bilayer structures consisting of C_{80}-PP/DOPC, mole ratio 0.2. Magnifications and scale bars: (a) ×150 000, 50 nm; (b) ×75 000, 100 nm; (c) ×2 000 000, 20 nm; (d) ×60 000, 100 nm.

seems to be a torus-like structure. Numerous granulations are seen inside of these vesicles.

The electron micrographs show that lipid vesicles prepared from DOPC are regular whereas lipid vesicles prepared from C_{80}-PP/DOPC, besides regular structures, form several irregular characteristic structures.

4. Discussion

Our investigations show that the lipid bilayers modified by hexadecaprenyl diphosphate (C_{80}-PP) modulate electrical, transport and mechanical properties of these membranes. The conformational changes of a negatively charged lipid molecule can be induced electrostatically, by changing membrane surface charge density [23]. The molecule of hexadecaprenyl diphosphate contains a long, hydrophobic isoprenyl chain and two phosphate groups and it seems that changes of modified membranes are mainly caused by a negative electrical charge of the phosphate groups. All the studied membranes were in the liquid-crystalline state, because the phase transition temperature for dioleoylphosphatidylcholine bilayers is below −20°C [24] and the hydrocarbon chain of hexadecaprenyl diphosphate contains several double bounds in *cis*-conformation [11]. No phase transition of α-saturated polyprenol/DOPC bilayers could be detected between 12°C and 82°C [25].

With respect to the electrical properties, the measurements showed that hexadecaprenyl diphosphate increases the activation energy for ion migration and membrane breakdown voltage. Furthermore the presence of hexadecaprenyl diphosphate in the phospholipid bilayer results in changes in the slopes of *I*/*V* curves and also in a decrease of membrane specific conductance. The membrane specific conductance (Fig. 3) increases above the C_{80}-PP/DOPC, mole ratio 0.04, although values of conductance of modified membranes are smaller than the values of conductance of DOPC bilayers. McCloskey and Troy [26] noted that neutral spin-labeled α-saturated polyprenyl phosphate-dolichyl phosphate (Dol-P) did not aggregate at concentrations smaller than 0.03. In our case the aggregation of hexadecaprenyl diphosphate seems to occur at concentrations higher than 0.04. The aggregation leads to domain formation with phosphate groups in the hydrophilic part of the membrane. Similar properties of Dol-P were presented by Valtersson et al. [25]. The authors suggested the formation of Dol-P-hexagonal structures in the lipid vesicle dispersion. The decrease in membrane conductance at smaller C_{80}-PP concentrations can result from a restriction of the movement of DOPC molecules in bilayers. The following increase in membrane conductance can arise from a creation of ionic pathways in the boundary region of C_{80}-PP aggregates. Differences in properties of modified bilayers for various concentrations of C_{80}-PP in membranes have also been confirmed in the case of investigations of the activation energy of ion migration across the membrane, the breakdown voltage and the membrane thickness. The large increase, over 2-fold, in the activation energy upon incorporation of the C_{80}-PP is most interesting. The values of the activation energy of bilayers formed from lecithin/cholesterol [21] were found to be similar to those for DOPC bilayers investigated by us. The dependence of the activation energy on the percentage of C_{80}-PP in the bilayer (Fig. 4) has a maximum at C_{80}-PP/DOPC, mole ratio 0.04, at the same concentrations as for the minimum of membrane conductance (Fig. 3). The value of the hydrophilic-lipophilic balance (HLB) of hexadecaprenyl diphosphate is expected to be smaller than 1 and it seems to influence the permeability of C_{80}-PP/DOPC bilayers for ions.

Hexadecaprenyl diphosphate increases the value of the breakdown voltage of DOPC bilayers. This phenomenon reflects the stabilization effect of the interaction of the potential gradient with the C_{80}-PP headgroup on DOPC bilayers. As shown in Fig. 6, the values of breakdown voltage increase substantially for C_{80}-PP/DOPC, mole ratio range 0–0.04, and then decrease, although the values of the breakdown voltage are higher than in the case of DOPC bilayers. The inclusion of long chained hydrophobic chains in lipid bilayers is known to increase their stability [27]. This is probably related to the increase in the breakdown voltage in the case of our study. The observed increase in the breakdown voltage can also be related to the decrease in the size of pores. As analyzed in the paper of Smith et al. [21] the increase in the activation energy is related to the decrease in the radius of the transmembrane pore. For the value of the activation energy equals 18 kJ/mole the au-

thors estimated the minimum pore radius to be about 1 nm. For C_{80}-PP/DOPC bilayers, with the activation energy for ion transport about 4–5-fold bigger, the minimal pore radius can be estimated to be in the range 0.2–0.3 nm. The action of C_{80}-PP seems therefore to effect the formation of these pores or dramatically reduce their size.

Hexadecaprenyl diphosphate molecules consist of a long unsaturated, mainly poly-*cis* configuration isoprenoid chain with phosphate groups bonded to the unsaturated α-isoprene residue. The *cis* geometry enables the chain to be more compact and fold into a shorter length than poly-*trans*-isoprenoids. Hexadecaprenyl diphosphate molecules seem to orient in the membrane with their phosphate headgroups at the hydrophilic interface. Similar properties were experimentally detected by McCloskey and Troy [26], Valtersson et al. [25], and de Ropp et al. [17] in the case of lipid bilayers modified by dolichyl phosphate. Contrary to the behavior of C_{80}-PP, polyprenyl alcohols increase the membrane specific conductance, and all values of conductance of modified membranes are higher than the value of conductance of DOPC bilayers, decrease activation energy of ion migration, breakdown voltage and membrane thickness [10]. The aggregation of neutral polyisoprenoid in phospholipid membranes was observed even at relative concentrations less than 0.005 [17]. These aggregates can modulate the permeability and stability of polyisoprenol-phospholipid membranes.

The dispersions of vesicles prepared from DOPC or C_{80}-PP/DOPC mixtures were investigated by transmission electron microscopy. Analysis of lipid vesicles in TEM indicates the changes of the membrane elasticity for liposomal membranes modified by hexadecaprenyl diphosphate, respectively to the concentrations of C_{80}-PP in bilayers. In the case of C_{80}-PP/DOPC dispersions both spherical and non-spherical vesicles were observed. There were multi-budding structures, torus-like structures, elongated vesicles, and fused vesicles. Vesicles were smooth or rough. The membrane texture of rough vesicles was grainy. On the basic of these micrographs we infer that hexadecaprenyl diphosphate changes the membrane fluidity and elasticity and modulates the surface curvature of modified lipid membranes, especially for relative concentrations of C_{80}-PP in the membrane higher than 0.03. The effect of hexadecaprenyl diphosphate on the surface curvature of the membranes can result from its molecular shape with a considerable hydrophobic part in comparison with the small hydrophilic part with the phosphate groups. The variety of structures of phosphatidylcholine vesicles has been reported by Klösgen et al. [28]. They demonstrated the existence of superstructures of phospholipid bilayers. The existence of the superstructure can be regarded as the evidence that lipids may directly influence the activity of biological membranes by changing their fluid-crystalline properties. Some functions of the phosphate ester derivatives of long-chain polyprenols in bilayers are connected with the modification of some properties of the membranes.

The data obtained by electrophysiological investigations and analysis by transmission electron microscopy show that electrical, mechanical and transport properties of lipid membranes change under the influence of hexadecaprenyl diphosphate. The results indicate that hexadecaprenyl diphosphate can modulate the surface curvature of the membranes by the formation of microdomains. The properties of modified membranes can result from the presence of the negative charges in the hydrophilic part of hexadecaprenyl diphosphate molecules and are modulated by the concentration of this compound in membranes. We suggest that the dynamics and conformation of the phosphate ester derivatives of long-chain polyprenols in membranes depend on the transmembrane electrical potential.

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