Adsorption of DNA and electric fields decrease the rigidity of lipid vesicle membranes

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The adsorption of calf-thymus DNA-fragments of 300 ± 50 base pairs (bp) to the outer membrane monolayer of unilamellar lipid vesicles in the presence of Ca²⁺ ions has been quantified by the standard method of chemical relaxation spectrometry using polarized light. The vesicles of radius \( r = 150 ± 45 \) nm are prepared from bovine brain extract type III containing 80–85% phosphatidylserine (PS) and palmitoyl-oleoyl-phosphatidylcholine (POPC) in the molar ratio PS : 2POPC; total lipid concentration \([L_t] = 1 \) mM in 1 mM HEPES buffer, pH 7.4 at \( T = 293 \) K (20 °C). The turbidity relaxations of vesicle suspensions, at the wavelength \( \lambda = 365 \) nm at two characteristic electric field strengths are identified as electroelongation of the whole vesicle coupled to smoothing of thermal membrane undulations and membrane stretching, and at higher fields, to membrane electroporation (MEP). The elongation kinetics indicates that the DNA adsorption renders the membrane more flexible and prone to membrane electroporation (MEP). Remarkably, it is found that the Ca-mediated adsorption of DNA (D) decreases both, bending rigidity \( k \) and stretching modulus \( k_r \) along an unique Langmuir adsorption isotherm for the fraction of bound DNA at the given Ca concentration \([Ca_t] = 0.25 \) mM. The characteristic chemomechanical parameter of the isotherm is the apparent dissociation equilibrium constant \( K(D,Ca) = 100 ± 10 \) µM (bp) of the ternary complex DCA of DNA base pairs (bp) and Ca binding to sites B on the outer vesicle surface. Whereas both \( k \) and \( k_r \) decrease in the presence of high electric fields (\( E \)), the key parameter \( K(D,Ca) \) is independent of \( E \) in the range \( 0 \leq E/(kV \text{ cm}^{-1}) \leq 40 \).

Introduction

The membrane electroporation (MEP) technique\(^{1-12,2005}\) is widely used for introducing gene DNA and drugs, in particular chemotherapeutica, into isolated cells and tissue.\(^{3-7,2005}\) Traditionally used for introducing gene DNA and drugs, in particular chemotherapeutica, into isolated cells and tissue.\(^{3-7,2005}\) The lipid part of cell membranes is modelled with lipid bilayer vesicles\(^{8,9,2005}\) and many characteristic properties of lipid vesicles scale very well with those of biological cells.\(^{10,2005}\) The elastic properties of membrane, such as the spontaneous curvature and the bending rigidity, play an important role in the interactions of charged liposomes with colloidal nanoparticles and in the adsorption of neutral and ionic polymers to the outer surface of vesicle membranes.\(^{11-13,2005}\) It is known that the elastic properties and permeability of the lipid membrane can be altered not only by polymer adsorption and sugar asymmetry but also by electric fields.\(^{12,14,15,2005}\) For instance, prior adsorption of the anionic DNA on the outer surface of cells facilitates the electrotransfer of genes into the cell interior.\(^{16,2005}\) On the other hand, DNA-vesicle interactions have been studied intensively with positively charged vesicles,\(^{17-21,2005}\) but rarely with anionic liposomes.\(^{22,2005}\) The complexation of cationic liposomes with DNA can lead to multimellar complexes, vesicle aggregation or even vesicle rupture at higher DNA concentration.\(^{23,24,2005}\) To avoid vesicle aggregation, we have used small anionic unilamellar vesicles at low total lipid and Ca²⁺ concentrations, respectively.\(^{25,2005}\) The Ca²⁺-ions on the membrane surface are known to bridge the negatively charged DNA phosphate groups with the negatively charged lipid head groups of the vesicles.\(^{16,2005}\) It is widely known that Ca-ions can significantly rigidify the lipid membrane, eventually impeding cell functions.\(^{26,2005}\) It has been observed that solid-surface-attached liposomes are less prone to distortion by a laser beam, when the Ca-concentration is \([Ca_t] = 5 \) mM.\(^{27,2005}\) Theoretical studies predict that, contrary to the effect of Ca²⁺-ions, the adsorption of polymers decreases the rigidity of lipid membranes.\(^{28,29,2005}\)

It is recalled that the application of electric pulses to vesicle suspensions leads to smoothing of membrane undulations, membrane stretching, electroporation (MEP) and to elongation of the vesicles at the expense of an increase in the projected membrane surface.\(^{30,2005}\) Recently, electrodeformation and poration of giant vesicles has been visualized with high temporal resolution.\(^{31,2005}\) We use the relaxation kinetic of elongation of the vesicles in high electric field pulses to quantify the effect of the adsorption of DNA and of Ca²⁺-ions on the rigidity of lipid membrane. Here, we apply the standard electrooptical technique of chemical relaxation spectrometry,\(^{32,2005}\) using polarized light, in order to identify and quantify field-induced shape changes of the whole vesicles as well as local membrane electric pore formation (MEP) in connection with the viscoelastic membrane parameters. The main result is that the Ca-mediated adsorption of DNA decreases both, bending rigidity and stretching modulus along an unique Langmuir adsorption isotherm for the fraction of bound DNA at a given Ca concentration.\(^{33,2005}\)

Materials and methods

Materials

Synthetic POPC (palmitoyl-oleoyl-phosphatidylcholine) from Lipoid GmbH (Ludwigshafen, Germany) and bovine brain extract type III (containing 80–85% PS) from Sigma Chemie GmbH (Deisenhofen, Germany) in the molar ratio PS : POPC = 1 : 2 are used to prepare unilamellar vesicles of the average radius \( a = 150 ± 45 \) nm have been used to prepare unilamellar vesicles by the extrusion technique. The lipid mixture in the liquid crystalline phase at \( T = 293 \) K, which is well above the phase transition temperature, is known to be homogeneous.
The multifile extrusion process prevents any inhomogeneity.

The adsorption of added polydeoxyribonucleotides of 300 ± 50 base pairs (bp) of calf thymus DNA (DNA type I, Sigma Chemical GmbH) may lead to local repulsion between the polyanions and the anionic PS or attraction in the presence of bridging Ca ions. 33 The sample cell is thermostated at 293 K (20 °C) and the total concentration of DNA fragments per one vesicle is in the range 0 ≤ [Dt] /μM (bp) ≤ 42. Each sample is a separate mixture of liposomes and DNA fragments. The concentration notation refers in each case to the respective total volume of mixture (no dilution of vesicles). 34

Methods

In each case, one rectangular electric pulse with field strengths $E = 30$ or 40 kV cm$^{-1}$ and pulse duration of $t_0 = 10$ μs has been applied to the vesicle suspension between the two planar graphite electrodes of the measuring chamber by cable discharge. 34 The sample cell is thermostated at $T = 293$ K (20 °C). The sample chamber of about 1 ml is equipped with quartz windows with an optical path length of $l = 1$ cm. The field induced changes $\Delta OD_2$ in the optical density $OD_2$ for plane-polarised light are measured at the wavelength $\lambda = 365$ nm (Hg-line), see Fig. 1. The light intensity change $\Delta I^p$, caused by electric pulse and measured at the two polarisation angles $\sigma = 0^\circ$, 90° relative to the applied external field $E$, is related to the optical density change by:

$$\Delta OD^p = OD^p(E) - OD^p_0 = - \log \left(1 + \frac{\Delta I^p}{I_0^p}\right)$$

where $\Delta I^p = I^p(E) - I^p_0$ is the intensity change from $I^p_0$ (at $E = 0$) to $I^p(E)$ in the presence of $E$, and $OD^p(E)$ and $OD^p_0$ are the optical densities at $E$ and $E = 0$, respectively.

In the absence of an optical probe and outside the absorption band of the optical probe, the optical density OD is given solely by the turbidity $T$, hence $OD = T$. The field-induced change $\Delta T$ in $T$ may be decomposed into a deformational/ orientational part $\Delta T_{DR}$ and a structural-chemical part $\Delta T_{CH}$ according to $\Delta T^p = \Delta T_{DR} + \Delta T_{CH}$. 35 The reduced turbidity minus mode is defined by:

$$\frac{\Delta T^-}{T_0} = \frac{\Delta T_{DR}}{T_0} + \frac{\Delta T_{CH}}{T_0}$$

where $T_0$ is the turbidity by $E = 0$ and $\Delta T^\parallel = T^\parallel - T_0$ and $\Delta T^\perp = T^\perp - T_0$ are the field induced changes at the two light polarisation modes $\sigma = 0^\circ$ (∥, parallel to the direction of external field) and $\sigma = 90^\circ$ (∥, perpendicular to the direction of external field), respectively. The reduced turbidity plus mode is analogous to the respective absorbance term 36 and given by:

$$\frac{\Delta T^+}{T_0} = \frac{\Delta T_{CH}}{T_0} = \frac{\Delta T^\perp + 2\Delta T^\parallel}{3T_0}$$

It is recalled that the turbidity term $\Delta T^+/T_0$ contains also chemical contributions $\Delta T_{CH}$, but refers primarily to global elongations of the vesicles in the electric field pulse. The term $\Delta T^-/T_0$ relates to chemical changes in the scattering cross section, for instance, due to entrance of water and ions in the membrane as well as to changes of the vesicle volume. 37

The refractive indices at different wavelengths in the visible range are determined using an Abbe-refractometer at $T = 293$ K (20 °C) for different mole fractions $x_{opt}$ of the lipids in the lipid/water system. 34 The values of the refractive indices at the wavelength $\lambda = 365$ nm are calculated using the Cauchy dispersion law. The refractive index $n_{opt} = 1.3639 ± 0.0005$ of the pure lipid mixture PS : 2POPC is obtained experimentally by extrapolation of the refractive index of the mixture to $x_{opt} = 1$. The refractive index of the medium (buffer) is $n_{med} = 1.3483 ± 0.0005$.

Results and data analysis

DNA binding and turbidity

The initial optical density $OD_0$ in the absence of an electric field and at the given $[Ca]_c$ slightly decreases with increasing total concentration $[D_t]$ of DNA (Fig. 2a). There are no signs of vesicle aggregation at this low value of $[Ca]_c$ in the concentration range 0 ≤ $[D_t]_{[\muM (bp)]} ≤ 40$, i.e. 0 ≤ $n_0 ≤ 118$ DNA fragments per one vesicle. In the case of vesicle aggregation,
due to light scattering of larger particles, OD₀ should steeply increase with [D_l]. The observed decrease in OD₀ is instead rationalized by a decrease in the refractive index, n, of the vesicle membrane caused by the adsorption of DNA into the membrane surface layers. Concomitant water entrance into the lipid head group regions appears to decrease the refractive index, n, and thus decrease the OD₀. The concentration [Dₐ] of DNA base pairs (bp) bound to the outer monolayer of the vesicular lipid bilayer membrane (as ternary DNA–Ca–lipid complexes (DCaB) in which Ca bridges lipid binding sites B with DNA), is calculated according to

\[ [D_a] = [D_b] - [D_l]^{sup} \]  

In eqn (4), [Dₐ]^{sup} is the total DNA concentration in the supernatant of the pellet phase of the sedimented vesicles with bound DNA and Ca. In Fig. 2b it is seen that [Dₐ] increases with [D_l]. Here the total Ca-concentration [Caₐ] = 0.25 mM has been selected; note that the exemplary electrophoretic curves refer to the same [Caₐ].

**Turbidity relaxations**

The kinetic modes ΔT⁻⁻(t)/T₀ and ΔT⁻⁺(t)/T₀ at i = 365 nm of the vesicle suspension are opposite in sign and have different kinetics (Fig. 3), suggesting different processes. Specifically, the negative sign of the ΔT⁻⁻(t)/T₀ relaxations indicates a decrease in the refractive index, n, of the membrane, caused by field-induced entrance of water into the membrane. Neither the smoothing of the short-wave membrane undulations, a₀ ≪ a, nor the membrane thinning due to Maxwell’s electric stress can quantitatively rationalize the negative amplitude of ΔT⁻⁻/T₀ mode. The positive sign of ΔT⁻⁺(t)/T₀ relaxations is dominantly caused by elongation of the flexible vesicles in the direction of the external field vector. Visual inspection shows that the kinetics is changed in the presence of Ca and DNA and Ca, respectively, in a characteristic manner. This indicates that Ca and DNA slow down and reduce the extent of change.

**Theory and discussion**

**Farafanov numerical code analysis**

The two experimental relaxation modes, ΔT⁻⁻/T₀ and ΔT⁻⁺/T₀ (Fig. 3), are used for the computation of the deformation ratio \( p(t) \) (Fig. 4a) and of the relative refractive index \( n_{rel} = n_{lip}/n_{med} \). Here \( n_{lip} \) and \( n_{med} \) are the refractive indices of the lipids and of the medium (buffer), respectively. (c) The relative increase \( \Delta S(t)/S₀ \) of the membrane surface area calculated from the axis ratio \( p(t) = c/b \) according to eqn (5) of the text, where \( \Delta S = S₀ - S(t) \) is the increase in the projected surface area of membrane. The solid lines represent the theoretical curves for membrane stretching and smoothing calculated with the membrane stretching modulus: \( K = 0.225 \) N m⁻¹, the membrane bending rigidity \( \kappa = 3.5 \times 10^{-19} \) J and the initial surface tension \( \sigma₀ = 2.1 \times 10^{-2} \) N m⁻¹ for the case (c). The relative refractive index \( m(t) = n_{rel}^{-1} \) of the vesicle membrane as a function of time t at the two characteristic field strengths \( E = 30 \) kV cm⁻¹ (1, 2) without MEP and \( E = 40 \) kV cm⁻¹ (3, 4) and for the total DNA concentrations [Dₐ]μM (bp) = 0 (1, 3) and 142 (2, 4), respectively. Here \( n_{lip} \) and \( n_{med} \) are the refractive indices of the lipid and of the medium (buffer), respectively. (c) The relative increase \( \Delta S(t)/S₀ \) in the membrane surface area calculated from the axis ratio \( p(t) = c/b \) according to eqn (5) of the text, where \( \Delta S = S₀ - S(t) \) is the increase in the projected surface area of membrane. The solid lines represent the theoretical curves for membrane stretching and smoothing calculated with the membrane stretching modulus: \( K = 0.225 \) N m⁻¹, the membrane bending rigidity \( \kappa = 3.5 \times 10^{-19} \) J and the initial surface tension \( \sigma₀ = 2.1 \times 10^{-2} \) N m⁻¹ for the case (c); see Fig. 3. For (c): \( K = 0.83 \) N m⁻¹, \( \kappa = 1.29 \times 10^{-18} \) J and \( \sigma₀ = 4.0 \times 10^{-3} \) N m⁻¹ and \( \sigma₀ = 1.0 \times 10^{-8} \) N m⁻¹ (Fig. 4b), applying the numerical code analysis of Farafanov et al., respectively. The linearly polarized light is scattered differently when the plane of polarization is parallel to the main axis of the ellipsoidal vesicle as compared to the orthogonal polarization. The difference in the scattering amplitudes of the two polarization modes increases with the degree of vesicle elongation. It has been shown previously that the scattering difference mode \( \Delta T^-/T₀ \) yields the axis ratio \( p \) by Mie-type numerical code analysis. The total reduction of the intensity of the scattered light, as reflected in the \( \Delta T^-/T₀ \) mode,
refers dominantly to the decrease in the refractive index $n_{\text{tip}}$ of the membrane. In the absence of an electric field the vesicles prepared by multifold extrusion technique are mostly spherical.30 Indeed, the very rapid ($\approx 1$ ms) and major part of the after-field relaxation of the turbidity mode $\Delta T/T_0$ (data not shown) excludes that the optical signal is due to orientational changes of non-spherical vesicles in an external field.31 Note that ellipsoidal vesicles with the long axis in the order of $a = 150$ nm would orientationally relax in the ms-time range. This is, however, not observed experimentally.

In the short pulse duration of 10 μs, the electrophoretic displacements can be too small to alter vesicle orientation, vesicle shape or refractive index. The electrophoretic relaxations are reproducible for several consecutive pulse applications (data not shown). There is no indication of an irreversible electrophoretic precipitation of the vesicles on the electrodes. Note that the approach time of vesicles in an electric field, due to induced electrical dipole moments, is in the ms-range, i.e., a factor of 10$^3$ slower than the pulse time of 10 μs.42

The kinetic features of the turbidity modes are reflected also in the deformation ratio $p(t) = c/h$, where $c$ and $h$ are the semi-axes, and in the $m(t)$ relaxation mode. Both membrane modes increase with increasing total concentration $[D]_t$ of DNA and with the field strength $E$. Because of the short pulse time $t_E = 10$ μs, there is no measurable efflux of electrolyte from the vesicles.42,43 hence the vesicle volume remains constant. The characteristic time of membrane smoothing is given by:30

$$\tau_{\text{SU}} \approx \frac{4c^2 a^2}{\eta} \left(1 + \frac{\kappa}{\sigma_0 + \kappa L}ight)$$

where $\eta$ is the solution viscosity.

Eqns (6)-(7) are applied to describe the rapid part of the relaxation $\Delta S(t)/S_0$ (Fig. 4c), yielding the numerical values of the elastic constants $\kappa$ and $K$, displayed in Fig. 5 as a function of $[D]$. The analysis of the slower kinetic mode of $\Delta S(t)/S_0$ using eqns (8) and (9), yields $\sigma_0$ as a fitting parameter. At a given $[D]$, both $\kappa$ and $K$ are lower at the higher field strength. The presence of a higher field thus decreases $\kappa$ and $K$, also in the presence of Ca-mediated adsorption of DNA.

**Chemical thermodynamics of DNA adsorption**

Straightforward chemical thermodynamics describes the DNA binding to the vesicle surface sites (lipid head groups) B and CaB with the overall scheme:7

$$D + (B + \text{CaB}) \rightleftharpoons (DB + \text{DCaB})$$

where the DNA-free sites (B and CaB) and occupied sites (DB and DCaB) are lumped together, respectively. The respective overall apparent dissociation equilibrium constant is defined as:

$$K(D, \text{Ca}) = [D] \frac{[B] + [\text{CaB}]}{[DB] + [\text{DCaB}]} = [D] \frac{1 - f_D}{f_D}$$

The bending rigidity $\kappa$ (a) and the stretching modulus $K$ (b) of the lipid membrane of the vesicles at $[\text{Ca}^2+] = 0.25$ mM as a function of the total DNA concentration $[D]$ at the two characteristic field strengths: $E = 30$ kV cm$^{-1}$ (□) and $E = 40$ kV cm$^{-1}$ (○), respectively, calculated with eqns (6)–(9) of the text from the $\Delta S(t)/S_0$ relaxations in Fig. 4c. Other experimental conditions are as in the legend to Fig. 2. The solid lines for $\kappa$ and $K$ are the respective Langmuir adsorption isotherms at the two field strengths.
Analogous to eqn (14), the respective ansatz for the slopes \( \frac{dD}{d[D_b]} \) and \( \frac{d[D_{b}]}{d[D_b]} \):

\[
-f_D = \frac{[D_b]}{[D_{b}]_{\text{max}}} = \frac{[D]}{[D] + K(D, Ca)}
\]  

(11)

where \([D_{b}]_{\text{max}}\) refers to the maximum of the sum \([D] + [D_{b}A]\).

The concentration \([D]\) of free DNA is expressed in terms of mass conservation:

\[
[D] = [D]_0 - [D]_1 = [D]_1 - \gamma_0[D_{b}]_{\text{max}}
\]  

(12)

It is instructive to apply eqn (12) to the half maximum \([D_{b}]_{\text{max}}/2\) of bound DNA, where \(f_D = 0.5\) and \(K(D, Ca) = [D]_0.5\). Hence, \(K(D, Ca)\) can be determined from

\[
[D]_{b,0.5} = K(D, Ca) + [D_{b}]_{\text{max}}/2
\]  

(13)

Data fit (Fig. 2b) using eqn (11) and eqn (13) yield: \(K(D, Ca) = 100 \pm 10 \mu M\) (bp). The apparent equilibrium constant \(K(D, Ca)\) is very close to the ternary complex constant \(K_{(D, Ca)} = 110 \pm 10 \mu M\) (bp), obtained from the analysis of titration experiments. Hence, at the same conditions of \([Ca]\) and \([Ca]_0\), the identity \(K(D, Ca) = K_{(D, Ca)}\) holds true.

Elastic membrane parameters \(\kappa\) and \(K\)

It is recalled that the presence of Ca ions increases both \(\kappa\) and \(K\), respectively, and that the rigifying effect of Ca has been rationalized by the electrostatic bridging by Ca of anionic head groups.46 The bridging effect of Ca has also been invoked for the ternary complex between the anionic phosphate groups of DNA and anionic lipid head groups.16 At the unusually high Ca concentration \([Ca]_0 = 5 \mu M\), where liposomes in solution usually aggregate and precipitate, solid-attached single liposomes are so rigidified, that they cannot be distorted at moderate power of a laser beam.25 Differently to \(\kappa\) and \(K\), the presence of Ca decreases the surface tension \(\gamma_0\), consistent with the reduction of the anionic lipid head group repulsion by Ca-complexation. In the case of lipid mixture PS : 2POPC, \(\kappa\) and \(K\) is hardly justifiable by a Ca-induced phase transition from gel-crystalline to fluid-crystalline.46 Remarkably, the decrease of \(\kappa\) and \(K\) with increasing \([D_b]\) and at higher electric field (Fig. 5) is consistent with Langmuir-like adsorptions isotherms.

Selecting, for instance, \(\kappa\), the data suggest the linear differential ansatz for the slopes \(d\kappa/d[D_b]\) and \(d[D_b]/d[D_b]\):

\[
-d\kappa = b \times d[D_b]
\]  

(14)

where \(b\) is a constant. Appropriate boundary conditions are that \(\kappa_0\) refers to \([D_b] = 0\), \(\kappa\) to \([D_b]_0\) and \(\kappa_{\text{max}}\) to \([D_{b}]_{\text{max}}\). Pairwise integrations of eqn (14) and rearrangements using the definition of \(f_D = [D_b]/[D_{b}]_{\text{max}}\) yield:

\[
f_D = \frac{\kappa - \kappa_0}{\kappa_{\text{max}} - \kappa_0} = \frac{K - K_0}{K_{(D, Ca)} - K_0}
\]  

(15)

Analogous to eqn (14), the respective ansatz for \(K\): \(-dK = b d[D_b]\) has been used.

The calculated parameters for eqn (15) are: \(\kappa_0(30) = (17.15 \pm 0.86) \times 10^{-20} J\), \(\kappa_{\text{max}}(30) = (8.75 \pm 0.44) \times 10^{-20} J\); \(\kappa_0(40) = (14.35 \pm 0.72) \times 10^{-20} J\), \(\kappa_{\text{max}}(40) = (5.95 \pm 0.30) \times 10^{-20} J\); \(K_0(30) = 0.17 \pm 0.06) N m^{-1}\), \(K_{(D, Ca)}(30) = (0.44 \pm 0.02) N m^{-1}\); \(K_0(40) = 0.92 \pm 0.05) N m^{-1}\), \(K_{(D, Ca)}(40) = 0.38 \pm 0.02) N m^{-1}\) (see Fig. 5).

In Fig. 6 it is demonstrated that both data sets, that of \(\kappa\) and that of \(K\), are consistent with DNA Langmuir binding, characterized by one (unique) binding or adsorption isotherm at a given Ca concentration.

Remarkably, although \(\kappa\) and \(K\) are dependent on the field strength, the adsorption isotherms characterized by the Langmuir constant \(K(D, Ca) = 100 \pm 10 \mu M\) (bp) at \([Ca]_0 = 0.25 \mu M\) is independent of \(E\) up to \(E \leq 40 \text{ kV cm}^{-1}\) within the margin of experimental error.

Membrane electroporation

In this study, chemical relaxation spectrometry52 and chemical normal mode analysis32 are used to evaluate the mechanical membrane parameters \(\kappa\) and \(K\) from field-induced vesicle electroelongation. If we restrict the choice of the field strength to a range \(0 < E \leq 30 \text{ kV cm}^{-1}\), there is no sign of membrane electroporation (MEP). At the higher field strength \(E = 40 \text{ kV cm}^{-1}\), MEP is indicated by a delayed further increase in the membrane surface \(\Delta \Sigma(S)\) (Fig. 7), at about 4 \(\mu s\) after the onset of the rectangular electric field pulse. Analogous to the data interpretation for low fields (\(E \leq 30 \text{ kV cm}^{-1}\)), the additional normal mode for the range \(t \geq 4 \mu s\) at \(E = 40 \text{ kV cm}^{-1}\) and

Fig. 6 The (unique) Langmuir adsorption isotherm; fraction \(f_D = [D_b]/[D_{b}]_{\text{max}}\) of bound DNA as a function of DNA concentration \([D_b]\) at \([Ca]_0 = 0.25 \mu M\). Characterized by the apparent equilibrium dissociation constant \(K(D, Ca) = 100 \pm 10 \mu M\) (bp) of the ternary DNA/Ca/lipid complex; ( ) \(f_D = (k - k_0)/(k_{c} - k_0)\) for \(E = 30 \text{ kV cm}^{-1}\); ( ) \(f_D = (k - k_0)/(k_{c} - k_0)\) for \(E = 40 \text{ kV cm}^{-1}\); ( ) \(f_D = (k - k_0)/(k_{c} - k_0)\) for \(E = 40 \text{ kV cm}^{-1}\).

Other experimental conditions are as in the legend to Fig. 2.

Fig. 7 The relaxations of the relative increase \(\Delta \Sigma(t)/\Sigma_0\) in the membrane surface area for the two characteristic field strengths \(E = 30 \text{ kV cm}^{-1}\) ( ) and \(E = 40 \text{ kV cm}^{-1}\) ( ) at zero DNA and Ca. Other experimental conditions are as in the legend to Fig. 2. The solid lines refer to the first relaxation phase of membrane stretching and smoothing of undulations with the overall time constant \(t_0 = 0.7 \mu s\). At \(E = 40 \text{ kV cm}^{-1}\), the new (electroporation) phase \(\Delta \Sigma(t)/\Sigma_0\) starts at about 4 \(\mu s\) after the start of the pulse (indicated by “MEP” and the arrow). This second relaxation phase reflects the formation of membrane pores. The time constant is estimated as \(t_1 = 3.5 \pm 0.5 \mu s\). The amplitude is \(\Delta \Sigma(t)/\Sigma_0 \approx 0.25\Delta \Sigma(t)/\Sigma_0\), i.e., about 25% of the total increase of the \(\Delta \Sigma(S)\) term.
the further decrease in $\kappa$ and $K$, as reflected, too, in the further decrease in $m(t)$, are consistent with the loss of membrane stiffness due to field-driven entrance of water molecules into the surface of the outer layer of the vesicle pole cap regions.27,41

Conclusion

It is shown that chemical relaxation kinetics in high electric fields can be used to quantify the dependence on Ca and adsorbed polymers of the mechanical and the electromechanical membrane parameters $\kappa$ and $K$. In the case of DNA, the dependence of $\kappa$ and $K$ on the total DNA concentration is described in terms of a unique Langmuir isotherm at a given Ca concentration.

Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$a$</td>
<td>Vesicle radius</td>
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<tr>
<td>$[Ca]_0$, $[D_t]$</td>
<td>Total concentrations of Ca$^{2+}$ and DNA, respectively</td>
</tr>
<tr>
<td>$E$</td>
<td>External applied field</td>
</tr>
<tr>
<td>$[D_n]$</td>
<td>Concentration of bound DNA</td>
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<tr>
<td>$K(D,Ca)$</td>
<td>Apparent dissociation equilibrium constant</td>
</tr>
<tr>
<td>$\kappa$, $K$</td>
<td>Membrane bending rigidity and stretching modulus, respectively</td>
</tr>
<tr>
<td>$[L]_n$</td>
<td>Total lipid concentration</td>
</tr>
<tr>
<td>$n_{lip}$, $n_{meas}$</td>
<td>Refractive index of the lipid membrane and medium, respectively</td>
</tr>
<tr>
<td>$m = n_{lip}/n_{meas}$</td>
<td>Relative refractive index of the vesicle membrane</td>
</tr>
<tr>
<td>MEP</td>
<td>Membrane electroporation</td>
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<tr>
<td>$OD_0$</td>
<td>Initial optical density at the wavelength $\lambda = 365$ nm</td>
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<tr>
<td>$p$</td>
<td>Axis ratio of the elongated vesicle</td>
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<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>POPC</td>
<td>Palmitoyl-oleoyl-phosphatidylcholine</td>
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<tr>
<td>$\sigma_0$</td>
<td>Initial lateral tension of the membrane</td>
</tr>
<tr>
<td>$t_E$</td>
<td>Pulse duration</td>
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<td>VET</td>
<td>Vesicle extrusion technique</td>
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