Field-Induced Interactions of Phospholipid Vesicles

Dietmar Porschke
Max-Planck-Institut für biophysikalische Chemie, 34 Göttingen, FRG

Received April 9, 1985

ABSTRACT: The effects of electric field pulses on aqueous solutions of bilayer vesicles formed from dimyristoylphosphatidic acid are studied by measurements of the light-scattering intensity. A field-induced rise of the scattering intensity is shown to increase with the field strength and also with the vesicle concentration. The rise-time constants observed under electric field pulses exhibit a unique dependence on the field strength E. When E is below about 10 kV/cm, the time constants decrease with increasing E, whereas higher E values lead to an increase of the time constants. In the range of $E \geq 20$ kV/cm, the reciprocal rise-time constants are a linear function of the vesicle concentration as expected for a relaxation reflecting an association reaction. Evaluation of the measured time constants according to an isodesmic reaction scheme leads to association rate constants of (2–4) $\times 10^{10}$ M^{-1} s^{-1} depending upon the number of lipid molecules per vesicle used for the calculation. The association rate constant shows relatively little dependence upon the field strength and is at the limit of a diffusion-controlled encounter of the vesicles used for the present measurements. The dissociation rate constants strongly decrease with increasing electric field strength and thus demonstrate that the field pulses induce interactions between the vesicles. This conclusion is consistent with the strong increase of the light-scattering intensity in the presence of high electric fields. The field-induced interactions of the vesicles are shown to increase with decreasing ionic strength. The present observations are very similar to those obtained previously for field-induced interactions of nucleic acid double helices. It is suggested that the field-induced interactions are a general phenomenon for particles with high induced dipoles. A close similarity to the phenomenon of pearl chain formation reported for microscopic particles like bacterial cells suggests that pearl chain formation and field-induced interactions of vesicles are of very similar nature.

Electric field pulses are known to induce not only physical effects of orientation (Fredericq & Houssier, 1973; O'Konski, 1976) but also reactions like dissociation of ion complexes or conformation changes (Onsager, 1934; Neumann, 1981; Porschke, 1985). These effects are very useful for the analysis of biopolymers and their reactions. Furthermore, the characterization of field-induced effects provides valuable information of an understanding of bioelectric phenomena. The reactions induced by electric field pulses in biopolymers frequently involve dissociation of ligands and destruction of ordered conformations (Porschke, 1985). Since dissociation and destruction are not always useful effects, the characterization of different types of field-induced reactions is of particular interest. A special example of an unusual electric field effect has been observed recently for nucleic acid double helices (Porschke et al., 1984). From a detailed analysis of the time constants associated with a field-induced increase of the light-scattering intensity, it could be demonstrated that electric fields lead to interactions between double helices. The dependence of the effect upon the electric field strength is consistent with interactions driven by a change of a dipole moment. More information on the nature of these interactions may be obtained by investigations of related biopolymers or macromolecular structures. Obviously, the field-induced effects of polynucleotides mainly result from their polyelectrolyte character. Thus, useful information may be obtained by a comparison of field-induced effects on structures sharing the polyelectrolyte property, but made from different constituents and having different shapes. Appropriate candidates for such investigations are phospholipid vesicles. The present measurements were performed on vesicles from dimyristoylphosphatidic acid, since these vesicles are associated with a particularly high charge density and can be prepared relatively easily. The changes of light scattering observed for the vesicles under electric field pulses are closely corresponding to those found previously for nucleic acids (Porschke et al., 1984). These results reveal a general mechanism leading to interactions of particles in electric fields.

MATERIALS AND METHODS

The disodium salt of L-β,γ-dimyristoyl-α-phosphatidic acid (DMPA) and dimyristoyl-α-lecithin were obtained from Fluka (Neu-Ulm, FRG). DMPA vesicles were prepared by the procedure of Elamrani & Blume (1983). It was demonstrated by thin-layer chromatography that the alkali treatment used for preparation of the vesicles did not lead to any detectable hydrolysis of the phospholipid. The vesicle suspensions were made up in a buffer containing 5 mM NaCl, 5 mM sodium cacodylate, pH 7.0, and 1 mM ethylenediaminetetraacetic acid (EDTA) (denoted 5 × NCE buffer) and were adjusted to the appropriate concentrations used for the measurements. A dilution of the 5 × NCE buffer by a factor of 5 results in a buffer denoted by 1 × NCE; other dilution factors are denoted correspondingly. The DMPA concentration was controlled by phosphate determinations according to the method of Eibl & Lands (1969). The dimyristoyl-α-lecithin vesicles were prepared by sonication under nitrogen followed by annealing at 60 °C for 30 min and filtration via a 0.45-μm filter.

The change of light-scattering intensity under electric field pulses was measured as described by Porschke et al. (1984). Relaxation time constants were evaluated at the computer center of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH, Göttingen, using a deconvolution procedure described by Porschke & Jung (1985). The reference curves used for the deconvolution were obtained by using light pulses of fast light-emitting diodes driven by an 8004 A pulse gen-

0006-2960/85/0424-7981$01.50/0

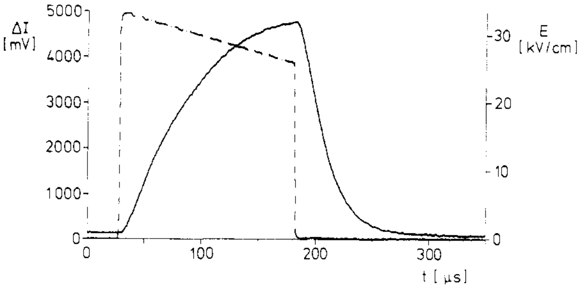

FIGURE 1: Change of light-scattering intensity (ΔI) of DMPA vesicles induced by an electric field pulse. The electric field strength (E) as a function of time (t) is shown as a dashed line (1×NCE buffer, 20 °C). The sigmoidal delays of the signal change at the beginning and the end of the field pulse are due to convolution with the response of the detector.

erator from Hewlett Packard.

RESULTS

Application of electric field pulses to aqueous suspensions of DMPA vesicles leads to a strong increase of the light-scattering intensity (cf. Figure 1). The increase may be attributed to various field-induced phenomena including deformation of vesicles, orientation of deformed vesicles, and interactions between vesicles. The observed effects may be assigned to one of these phenomena by quantitative analysis of the following parameters obtained by field jump experiments: (1) amplitudes, (2) "rise"-time constants observed under field pulses, and (3) "decay"-time constants observed after pulse termination. Each of these experimental parameters will be discussed separately.

Stationary Change of Light Scattering under Field Pulses. Due to the construction of the apparatus used for the present measurements, the field-induced changes of the light-scattering intensity could only be measured at a fixed angle of 90° relative to the illuminating light beam, and thus a detailed quantitative analysis of the light scattering at different angles was not possible. Furthermore, the aperture of the detector is particularly large (±20°) to increase the signal to noise ratio for kinetic measurements. Nevertheless, the stationary change of the light-scattering intensity induced by electric field pulses, shortly denoted amplitude, provides useful information. In some cases, the amplitudes could be read directly from the limit value of the scattering intensity approached at the end of the electric field pulse. However, in many cases, the scattering intensity still increased at the end of the pulse, and thus the amplitudes had to be determined by exponential fitting (cf. below). For convenience, the amplitudes are given relative to the intensity of scattered light measured at zero field strengths. As shown in Figure 2, the amplitudes are strongly dependent upon both the electric field strength and the concentration of the vesicles. At low vesicle concentrations, the amplitudes remain relatively small even at high field strengths. At high vesicle concentrations (c_v), the relative amplitudes observed at low field strengths are almost equivalent to those found at low c_v. However, at high c_v, the amplitudes strongly increase with increasing field strength, and thus at high field strengths, the amplitudes also strongly increase with increasing c_v. The strong concentration dependence is hardly compatible with any field-induced intramolecular effect like orientation or deformation and suggests the existence of an association equilibrium. It is remarkable that the field-induced increase of the scattering intensity apparently does not approach a limit value at high field strength or at high vesicle concentration.

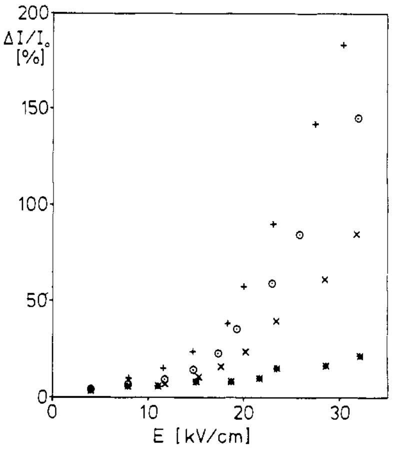

FIGURE 2: Relative change of light-scattering intensity ($\Delta I/I_0$) as a function of the electric field strength (E) (average value) at different DMPA concentrations: (+) 1 mM; (○) 0.5 mM; (×) 0.25 mM; (asterisks) 0.063 mM monomer units (1×NCE buffer, 20 °C).

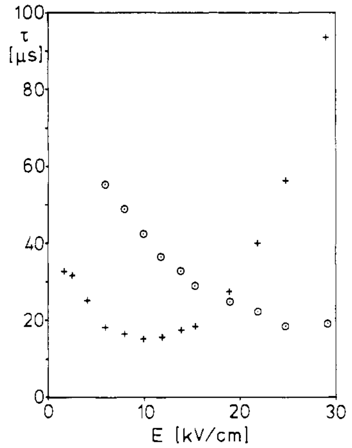

FIGURE 3: Rise- (+) and decay-time (○) constants observed for DMPA vesicles as a function of the electric field strength; 1 mM DMPA monomers in 1×NCE buffer.

Most of the measurements reported in this investigation have been performed with an illuminating light beam of 313 nm (mercury line). For comparison, the field-induced change of light scattering was also measured at a series of different wavelengths. At a given field strength and vesicle concentration, the relative change ($\Delta I/I_0$) turned out to be independent of the wavelength of the illuminating light beam. The amplitudes have also been measured at different salt concentrations. Both at reduced and at increased salt concentration (c_s), the dependence of the amplitudes upon the electric field strength (E) and the vesicle concentration (c_v) is very similar to that shown in Figure 2. However, the magnitude of the amplitudes at given values of E and c_v strongly increases with decreasing c_s, indicating an increasing extent of the field-induced effect at decreasing c_s values. A corresponding result is obtained quantitatively from the analysis of the time constants (cf. below).

Rise-Time Constants Observed under Field Pulses. The time constants observed under electric field pulses, shortly denoted rise-time constants (τ_r), show an unusual dependence upon the electric field strength. In the range of low field strengths ($E \leq 10$ kV/cm), the τ_r values decrease with increasing E (cf. Figure 3). Thus, in this range, the electric field accelerates the reaction of the DMPA vesicles. Corresponding effects are known for the orientation of dipolar particles (Schwarz, 1956) and also for, e.g., field-induced dissociation of ligands (Onsager, 1934). The familiar dependence is changed to a quite unusual one at higher field strengths. For E values increasing beyond 10 kV/cm, the rise-time constant increases and at $E = 30$ kV/cm is much larger than that observed at low field strength. A comparison of time constants and amplitudes demonstrates that the increase of τ_r values and the particularly strong increase of the amplitudes are observed in the same range of electric field strengths.

The time constants given in Figure 3 are obtained from single exponentials fits. The quality of the fits, in particular at high field strengths, can be improved by admitting a second exponential. In general, the two-exponential fits obtained for rise curves at high field strengths show a fast component with time constants $\tau_r^{I} \leq 5$ μs and amplitudes <10% with respect to the total amplitude together with a slow component (τ_r^{II}) representing the main part of the light-scattering change. The fast component observed at high field strengths appears to be equivalent to the process observed at low field strengths. At least the time constants and also the amplitudes associated with the effect observed at low field strength and the fast effect observed at high field strengths are approximately consistent with respect to the field strength dependence.

More information on the field-induced processes of DMPA vesicles is obtained from their concentration dependence. The rise-time constants observed at low electric field strengths are virtually independent of the DMPA concentration, whereas the reciprocal time constant $1/\tau_r^{II}$ associated with the slow component observed at high field strength strongly increases with increasing DMPA concentration. This observation demonstrates that the slow process at high E values reflects an association reaction of the vesicles. Since an association reaction of vesicles is probably not limited to dimers, an infinite association scheme appears to be appropriate. For simplicity, it will be assumed that subsequent association steps are equivalent to each other, corresponding to an isodesmic reaction scheme which is reflected by a reciprocal relaxation time constant

$$1/\tau = 2k^{+}c_{s} + k^{-}$$

where k^+ and k^- are the association and dissociation rate constants, respectively, and c_s is the site concentration [cf. Porschke & Eggers (1972)]. The evaluation is further simplified by assuming that the site concentration is equivalent to the total vesicle concentration c_v. The c_v value is calculated from the DMPA monomer concentration by using a number of 10^4 monomers per vesicle. This value may be estimated from the dimensions of the vesicles (cf. below) by using a surface area for one lipid molecule of about 60 Å^2. The value estimated by this procedure appears to be reasonable when compared to values determined for similar vesicles by, e.g., Huang & Charlton (1971) or Watts et al. (1978). On the basis of the isodesmic model, the association rate constants k^+ are around 4×10^{10} M^{-1} s^{-1}. Since the number of DMPA monomers per vesicle is not known exactly, the value of the association rate constant is subject to some uncertainty. If the number of DMPA monomers per vesicle is assumed to be 5000, for example, a k^+ value of 2×10^{10} M^{-1} s^{-1} is calculated.

As shown in Figure 4, the k^+ values are almost independent of the electric field strength, whereas the k^- values strongly decrease with increasing field strength. Since the unusual slow relaxation process can be characterized only for a restricted range of field strengths, the nature of the field-induced reaction mechanism cannot be established with certainty. However, as shown in Figure 5, the logarithm of the dissociation rate constants can be represented as a linear function of the square of the electric field strength and thus is consistent with a mechanism driven by a change of a dipole moment (Eigen & DeMaeyer, 1963). The strong decrease of the k^- values with increasing field strength clearly demonstrates the direction of the field-induced reaction: high electric field pulses induce an association reaction of vesicles. This conclusion is supported by the strong increase of the light-scattering intensity at high electric field strength.

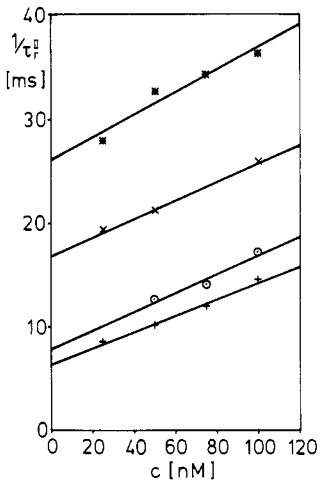

FIGURE 4: Reciprocal rise-time constant ($1/\tau_r^{II}$) as a function of DMPA concentration at different electric field strengths: (asterisks) 22.6, (×) 26.0, (O) 29.7, and (+) 33.2 kV/cm. The concentration is given in vesicle units assuming 10^4 monomers per vesicle (1×NCE7 buffer, 20 °C).

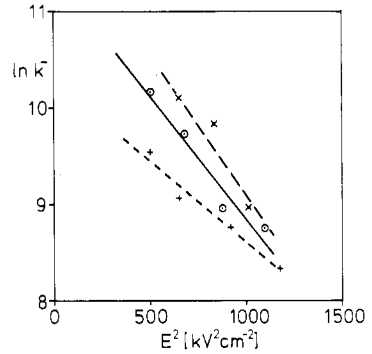

FIGURE 5: Logarithm of the dissociation rate constant (ln k^{-1}) as a function of the square of the electric field strength (E^2) at buffer concentrations of 0.75×NCE7 (+), 1×NCE7 (O), and 1.5×NCE7 (×) at 20 °C.

All these phenomena are very similar to those observed previously for nucleic acid double helices (Porschke et al., 1984). A close similarity is also found with respect to the ionic strength dependence. While the association rate constants are virtually independent of the ionic strength, the dissociation rate constants strongly decrease with decreasing salt concentration (cf. Figure 5). This result is again consistent with the light-scattering amplitudes which show a strong increase at decreasing salt concentration.

Decay-Time Constants Observed after Pulse Termination. The relaxation time constants observed after pulse termination, shortly denoted decay times, are measured at zero field strength but nevertheless depend upon the field strength of the pulse inducing the change of light scattering. Although the amplitudes observed after field pulses below 5 kV/cm are relatively small, two relaxation processes can be distinguished. The "fast" process is associated with the major part of the amplitude and with time constants around 50 μs. The time constant of the slow process is about 2 ms. When the field strength is increased, the relative amplitude of the slow process decreases and finally at high field strength can hardly be identified anymore. The decay curves observed after field pulses exceeding 10 kV/cm can be represented with reasonable accuracy by single exponentials. The decay-time constants have been measured in this range at different vesicle concentrations (c_v) and did not reveal any dependence upon c_v within the limits of experimental accuracy. Measurements at different salt concentrations demonstrate an increase of the decay-time constant with decreasing ionic strength. The decay time observed after pulses of 31 kV/cm is about 12.4 μs in 1.5 × NCE and 18.3 μs in 0.75 × NCE. These time constants are expected to reflect essentially the "dissociation" of vesicles from the field-induced interacting state at zero field strength.

Characterization of Vesicles and Various Controls. The experiments reported above have been repeated for various vesicle preparations. While the general results could be confirmed for different preparations, some variation of amplitudes and time constants were observed, which could be traced back to details of the procedure used for the preparation of the vesicles. The DMPA vesicles were prepared according to the procedure described by Hauser & Gains (1981) and Elamrani & Blume (1982). The critical step of this procedure is exposure of DMPA to 0.01 M NaOH, leading to spontaneous vesiculation. Apparently, the equilibrium distribution of vesicles is formed in a relatively slow process. Exposure of DMPA to 0.01 M NaOH for a relatively short period of about 5 min led to a vesicle preparation which exhibited a broad spectrum of decay-time constants with a relatively large contribution in the millisecond time range. When DMPA was exposed to 0.01 M NaOH for about 45 min (at room temperature, the decay curves could be represented by single exponentials in the range around 20 μs with good accuracy. Even without a complete assignment of the relaxation effects, it is apparent that a broad spctrum of relaxation times reflects a relatively broad distribution of vesicles, whereas a narrow spectrum indicates a more homogeneous size distribution. Thus, field jump experiments may provide useful information for the control of vesicles. The exposure of DMPA to 0.01 M NaOH could not be prolonged too much because of alkaline hydrolysis. After treatment with 0.01 M NaOH for 60 min, hydrolysis of DMPA was noticeable by thin-layer chromatography.

It may be suspected that electric field pulses lead to strong and probably irreversible changes of the vesicle structure like breakdown of bilayers of fusion of vesicles (Zimmermann, 1982). However, the DMPA vesicles used for the present investigation appear to be quite stable. Even after a series of many field pulses, the amplitudes and time constants did not change by more than about 10%. The stability may be due to the relatively small size of the vesicles generated by the alkaline treatment.

The dimensions of the vesicles were controlled by dynamic light scattering. These measurements confirmed that the DMPA vesicles used for the present investigation are relatively homogeneous. The diffusion coefficient $D = 1.3 \times 10^{-7}$ cm^{-2} s^{-1} obtained at 20 °C in the standard buffer for a DMPA sample, that had been exposed to 0.01 M NaOH for 60 min, indicates a Stokes diameter of 330 Å. Another sample obtained by alkaline treatment for 45 min showed a Stokes diameter of about 400 Å. Electron microscopy revealed a mean particle size of the same magnitude.

As a test for the influence of vesicle charge on the field-induced reaction, some experments were performed with vesicles prepared from dimyristoyl-α-lecithin (DML), which is a zwitterion, and thus DML vesicles can be considered as electroneutral. The field-induced increase of the light-scattering intensity observed for DML vesicles did not exceed 5% even at a field strength of 36 kV/cm and a vesicle concentration of approximately 0.25 μM (2.5 mM monomer in 0.5×NCE7 buffer at 20 °C). The relaxation curves observed for the DML vesicles under electric field pulses did not exhibit a slow component and also did not show any unusual dependence of the time constants upon the electric field strength. Thus, the experimental data do not provide any evidence for a field-induced interaction of DML vesicles. These results indicate that the field-induced interactions are mainly observed for particles with a relatively high charge density, which is required for the induction of large dipole moments.

Effects at Low Electric Field Strength and/or Low Vesicle Concentrations. Because of their large amplitudes, the effects observed at high field strengths and high vesicle concentrations can be characterized relatively easily. As shown in Figure 2, the effects at low field strength and low vesicle concentrations are much smaller, and thus, their analysis is more difficult. In addition, these effects have not been exactly reproducible and apparently depend upon details of the vesicle preparation, which are not yet under full control. Further investigations will be necessary for a complete characterization. The available data demonstrate that the rise time observed at low vesicle concentration c_v decreases with increasing field strength E. For E values above 15 kV/cm, the rise time at low c_v is shorter than the time constant of the detection unit (10 μs) and cannot be identified anymore. The time constant τ_r^{I} observed at high E values and high vesicle concentrations is also clearly faster than the detection time constant. Since the amplitudes associated with τ_r^{I} are approximately of the same magnitude (5–10%) as those found at low field strength, it is likely that these effects reflect the same process. The rise-time constants observed at low electric field strengths are independent of the vesicle concentration and thus indicate an intramolecular process. This process may be, for example, a deformation of the vesicles induced by the polarization of their ion atmosphere. Measurements performed with various vesicle preparations indicate that these processes are dependent upon the size distribution [cf. Ruderman et al. (1984)] and thus may be useful for the characterization of vesicles.

DISCUSSION

The magnitude of the light-scattering intensity observed at an angle of 90° with respect to the illuminating light beam does not provide enough information required for an interpretation of a field-induced effect. A detailed interpretation of light-scattering intensities requires measurements at various angles, which are not possible by the instrument available for the present investigation. Thus, the assignment of the field-induced effects observed for DMPA vesicles is mainly based on relaxation time constants, which are not dependent on the optical geometry. The concentration dependence of the rise-time constants observed at high electric field strength directly demonstrates that the change of light scattering observed in this range reflects interactions between DMPA vesicles. Since the details of the interaction scheme cannot be specified from the concentration dependence with certainty, the data are evaluated by a relatively simple interaction model. Interactions between vesicles probably do not lead only to the formation of pairs, and thus, an infinite interaction scheme is postulated as would be expected, e.g., for a phenomenon like pearl chain formation (cf. below). For simplicity, it is assumed that the interactions are identical and can be described by an isodesmic reaction scheme. The rate constants evaluated by this model provide further information on the process induced by the electric field. The formation of pairs is a very fast process with rate constants at the limit of diffusion control and with a relatively small dependence upon the electric field strength. A much stronger dependence is observed for the dissociation rate constants, which decrease with increasing electric field strength and thus demonstrate that the field pulses induce interactions between vesicles. This conclusion is consistent with the observation that electric field pulses lead to an increase of the light-scattering intensity which is particularly strong at high vesicle concentrations.

All the results obtained by field jump experiments on DMPA vesicles are equivalent to those obtained recently for DNA double helices (Porschke et al., 1984). In both cases, electric field pulses induce interactions between polyelectrolytes, suggesting that the nature of the interactions is similar. A similarity is also observed with respect to the ionic strength dependence: a decrease of the ionic strength leads to increasing interactions for both vesicles and helices. It is well-known that electric fields induce large dipole moments in polyelectrolytes which increase with decreasing ionic strength. Since dipoles are known to interact with each other, the field-induced interactions probably result from the induction of dipole moments. This is supported by the dependence of the dissociation rate constant upon the electric field strength, which corresponds to that expected for a reaction driven by a change of dipole moments.

The experimental data reported above do not provide any direct information on the arrangement of the molecules in the interacting state. However, a model for the field-induced arrangement of molecules may be deduced by comparison with a related phenomenon observed for various microscopic particles. It was found many years ago (Krasny-Ergen, 1936; Schwan & Sher, 1969) that particles like silicone globules, polystyrene spheres, *Escherichia coli* bacteria, and erythrocytes in aqueous solutions are aligned by electric fields to "pearl chains". The pearl chains can be seen by microscopy and are formed by alignment of particles into chains of various length directed parallel to the field vector. The formation of pearl chains has usually been studied by application of high-frequency alternating-current field pulses, but pearl chains are also formed by direct-current pulses (Schwan & Sher, 1969; Hu & Barnes, 1975; Zimmermann, 1982). It has been shown that the formation of pearl chains can be used to induce fusion of living cells and produce hybrid cell lines (Zimmermann et al., 1984). Apparently, it is relatively difficult to obtain quantitative information on pearl chains and their formation by microscopic observations. However, some of the reported observations are quite useful for a comparison with the field-induced interactions of vesicles. It has been described that the formation of pearl chains requires an electric field strength exceeding a threshold value. In close analogy, the field-induced interactions of vesicles can only be demonstrated when the electric field strength E exceeds a threshold value. The time constants observed for pearl chain formation decrease with increasing E. When E is above the threshold, the time constants decrease with E^2 (Schwan & Sher, 1969). This observation is also in close analogy to the field-induced interactions of vesicles and helices. Apparently, pearl chain formation and its time constants have not been investigated as a function of the particle concentration. It is surprising that the dependence upon the particle concentration c_p is not even discussed in the standard literature available on pearl chains, although pearl chain formation clearly is an intermolecular process and thus should be dependent upon the c_p value.

The formation of pearl chains has been attributed to attractive forces between induced dipoles on neighboring particles (Schwan & Sher, 1969; Hu & Barnes, 1975; Sauer, 1983). These forces should be very general for all kinds of particles with induced dipoles. Thus, pearl chain formation should not be restricted to microscopic particles like bacteria or erythrocytes but should also be observed for, e.g., macromolecules. The results obtained for the field-induced interactions of vesicles and helices are clearly consistent with this expectation.

Although the experimental observations can be explained by the model presented above, some problems remain which require further discussion. It may be suspected, for example, that the interaction of dipoles in a pearl chain cannot be described by an isodesmic model, since the overall dipole moments of the pearl chains may increase with chain length and thus the reaction may be cooperative. The experimental data do not provide an indication for the existence of cooperativity. However, some chain length dependence of the reaction parameters cannot be excluded, and the observed parameters may represent an average of the actual parameters. The existence of a relatively narrow distribution of time constants would remain undetected because of a technical problems. The field pulses required to induce interactions of vesicles are relatively long, and thus, the field strength cannot be maintained at a constant level. This effect leads to some distortion of the rise curves and to a systematical error in the rise-time constants. Due to the conductivity of the samples, the accuracy of the measurements cannot be increased by an extension of the pulse length. It is also not possible to study a potential saturation of the field-induced effect at higher vesicle concentrations, since the ionic strength would not remain constant, if the lipid concentration is increased beyond the values used for the present measurements.

For a simple qualitative test of the reaction model, the distribution of chain lengths may be calculated from the "equilibrium" constant k^+/k^-. Such calculations demonstrate that the average length of the pearl chains remains relatively low under the conditions of the present experiments, which is in qualitative agreement with the observed increase of the light-scattering intensity. This increase may be simulated according to the distribution of chain lengths calculated from the k^+/k^- values and assuming an "ideal" increase of the scattering intensity with molecular weight. Such a simulation shows reasonable agreement with the experimental values but is not discussed further because of the crude approximations involved.

The association rate constant k^+ obtained according to the isodesmic model is relatively high. The k^+ value may be compared with the theoretical value obtained for a diffusion-controlled reaction (von Smoluchowski, 1916) by using the diffusion coefficient evaluated from the measurements of dynamic light scattering. When the reaction distance is assumed to correspond to the diameter of the vesicles, the calculated rate constant is about 7.5×10^9 M^{-1} s^{-1}. Since the dipole moments of the reactants are unusually high, it may be expected that the effective reaction distance is much larger than the diameter of the vesicles. As discussed under Results, the experimental k^+ value depends upon the number of DMPA molecules per vesicle. If this number is assumed to be 5000, the experimental k^+ value is 2×10^{10} M^{-1} s^{-1}. Thus, it may be concluded that the observed association rate constant is consistent with a diffusion-controlled reaction. The association rate constants may be expected to increase with the electric field strength E, if the dipole moments increase with E. However, it is known that the dipole moments induced in polyelectrolytes are saturated at surprisingly low field strengths [cf. Diekmann et al. (1982) and Porschke (1985)]. A similar saturation effect for the case of vesicles may provide an explanation for the observed small dependence of the k^+ values on the electric field strength.

Although field-induced processes in phospholipid vesicles may be important for an understanding of bioelectricity, there are not many systematical investigations of vesicles under high electric field pulses with direct optical detection. Thus, a comparison of the present results with related data is hardly possible. Alternative explanations of the present results in terms of processes that are known to be associated with changes of the light-scattering intensity (e.g., phase transition) appear to be unlikely. An analysis of phosphatidylserine vesicles by the temperature jump technique in the presence of phenol red as indicator (Hammes & Tallman, 1970) revealed a process with an increase of the reciprocal time constant upon increasing vesicle concentration. Although the concentration dependence appears to be similar, this process is probably not due to a field-induced interaction. The electric field applied for standard temperature jump experiments decays already in a few microseconds and thus will hardly be sufficient to induce any large extent of the field-induced reaction described above. However, the present results clearly demonstrate the strong power of high electric field pulses to induce unusual reactions. Experimental data obtained by application of high field pulses should always be examined very carefully for indications of field-induced reactions. The field strengths used for the present investigation are in the range observed in biological systems, and thus, the field-induced interactions of lipid vesicles may well be of biological significance.

ACKNOWLEDGMENTS

I am greatly indebted to J. Ronnenberg for his expert technical assistance. The controls of the vesicle preparations by dynamic light scattering and electron microscopy were generously performed by C. Koitz and P. Kaufmann-Kolle, respectively.

Registry No. L-β,γ-DMPA, 28874-52-4.

REFERENCES

Eibl, H., & Lands, W. E. M. (1969) *Anal. Biochem. 30*, 51–57.

Eigen, M., & DeMaeyer, L. (1963) *Tech. Org. Chem. 8*, 895–1054.

Elamrani, K., & Blume, A. (1983) *Biochim. Biophys. Acta 727*, 22–30.

Fredericq, E., & Houssier, C. (1973) *Electric Dichroism and Electric Birefringence*, Clarendon Press, Oxford.

Hammes, G. G., & Tallman, D. E. (1970) *J. Am. Chem. Soc. 92*, 6042–6046.

Hauser, H., & Gains, N. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1683–1687.

Hu, C. J., & Barnes, F. S. (1975) *Radiat. Environ. Biophys. 12*, 71–76.

Huang, C. H., & Charlton, J. P. (1971) *J. Biol. Chem. 246*, 2555–2560.

Krasny-Ergen, W. (1936) *Hochfrequenztech. Elektroakust. 48*, 126–133.

Neumann, E. (1981) *Top. Bioelectrochem. Bioenerg. 4*, 113–160.

O'Konski, C. T., Ed. (1976) *Molecular Electro-optics*, Marcel Dekker, New York.

Onsager, L. (1934) *J. Chem. Phys. 2*, 599–615.

Porschke, D. (1985) *Annu. Rev. Phys. Chem.* (in press).

Porschke, D., & Eggers, F. (1972) *Eur. J. Biochem. 26*, 490–498.

Porschke, D., & Jung, M. (1985) *J. Biomol. Struct. Dyn. 2*, 1173–1184.

Porschke, D., Meier, H. J., & Ronnenberg, J. (1984) *Biophys. Chem. 20*, 225–235.

Ruderman, G., Jennings, B. R., & Lyle, I. G. (1984) *Int. J. Biol. Macromol. 6*, 99–102.

Sauer, F. (1983) in *Coherent Excitation in Biological Systems* (Fröhlich, H., & Kremer, F., Eds.) pp 134–144, Springer-Verlag, West Berlin.

Schwan, H. P., & Sher, L. D. (1969) *J. Electrochem. Soc. 116*, 22C–26C.

Schwarz, G. (1956) *Z. Phys. 145*, 563–584.

von Smoluchowsky, M. (1916) *Phys. Z. 17*, 585–599.

Watts, A., Marsh, D., & Knowles, P. F. (1978) *Biochemistry 17*, 1792–1801.

Zimmermann, U. (1982) *Biochim. Biophys. Acta 694*, 227–277.

Zimmermann, U., Vienken, J., & Pilwat, G. (1984) in *Investigative Microtechniques in Medicine and Biology* (Chayen, J., & Bilensky, L., Eds.) Vol. 1, pp 89–167, Marcel Dekker, New York.