Publication IV

Sanna Carlsson, Anna-Kaisa Kontturi, and Kyösti Kontturi. 2006. Improving membrane activity of oligonucleotides by cetylpyridinium chloride: An electrochemical study. European Journal of Pharmaceutical Sciences, volume 29, number 5, pages 451-459.

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 29 (2006) 451–459

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Improving membrane activity of oligonucleotides by cetylpyridinium chloride: An electrochemical study

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ARTICLE INFO

Article history:
Received 19 April 2006
Received in revised form
2 August 2006
Accepted 20 August 2006
Published on line 30 August 2006

Keywords:
Oligonucleotide
Phosphodiester
Phosphoromonothioate
Surfactant
Langmuir–Blodgett
Interfacial capacitance

ABSTRACT

The interaction of phospholipid membranes and oligonucleotides complexed with a positively charged surfactant is reported. Phospholipid membranes were assembled at the interface between an immobilized organic phase and an aqueous phase using the Langmuir–Blodgett (L–B) technique. The interaction and adsorption of the naked oligonucleotides and oligonucleotides complexed with cetylpyridinium chloride (CP) was studied electrochemically using cyclic voltammetry (CV) and ac-voltammetry. Interfacial capacitance, obtained indirectly from ac-voltammetry as a function of interfacial potential, was fitted to the theory based on the solution of the Poisson–Boltzmann equation. It was shown that both types of naked oligonucleotides (phosphoromonothioates and phosphodiesters) adsorb on the lipid monolayers poorly. The introduction of CP to the system increases the adsorption efficiency significantly. However, one phosphoromonothioate appeared to form a compact globule with CP instead of adsorbing to the lipid membrane. These results demonstrate that electrochemical methods are a powerful tool for probing the behavior of drugs in the vicinity of model cell membranes.

1. Introduction

Antisense oligonucleotides are short single-stranded DNA fragments that block undesired protein syntheses by making a duplex with the target mRNA, and thus activating RnaseH for hydrolyzation processing. Many setbacks have been encountered since the first introduction of the antisense strategy in 1970s (Zamecknik and Stephenson, 1978). These include poor serum resistivity of phosphodiesters, poor specificity, and inefficient systemic and cellular uptake. While resistivity problems have been overcome by modifications such as phosphoromonothioates and specificity problems have been tackled with empirical methods, delivery issues remain poorly understood. While it has been shown that oligonucleotides are effective and capable of rapidly moving into the cell nucleus when microinjected directly to the cytoplasm, substantial extracellular concentration is needed to obtain a sufficient biological response by extracellulary administered oligonucleotides. To overcome this limitation, carriers are introduced to improve the cellular delivery of oligonucleotides. The carrier agents that have been proposed are numerous and include liposomes (Zelphati and Szoka, 1996a), surfactants (DeLong et al., 1999), peptides (Lochmann et al., 2004), polymers (Chirila et al., 2002), dendrimers (Hussain et al., 2004), and polymeric nanoparticles (Weyermann et al., 2004). All have both advantages and drawbacks. Properties (such as toxicity and efficiency) of different carrier systems have been compared in an article by Weyermann et al. (2004).

The aim of this work is to study cellular delivery of naked oligonucleotides and those complexed with cetylpyridinium chloride (CP), a cationic surface-active agent. It has been previously shown that CP forms stable complexes with oligonu-

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doi:10.1016/j.ejps.2006.08.008

cleotides with binding degrees near one at the saturation point and should, consequently suit well for oligonucleotide delivery (Hakkarainen et al., 2004a). To study the membrane activity of these complexes, we used a model membrane, a phospholipid monolayer at the oil/water interface, prepared using the Langmuir–Blodgett method (L–B). The interaction of oligonucleotides complexed with a carrier agent was investigated using electrochemical methods: cyclic voltammetry (CV) and ac-voltammetry. These techniques are suitable for this purpose as CV enables a direct measure of charge delivery processes through the model membrane and ac-voltammetry can be used to measure the capacitance of the model membrane. The L–B method, used for transferring the lipid monolayer to the interface between aqueous and organic phases, was originally utilized to study the permeability of phosphatidylcholine monolayers as a function of surface pressure (Liljeroth et al., 2000) and was subsequently applied to polyelectrolyte multilayers (Slevin et al., 2003), peptide binding to mixed monolayers of DSPC and stearic acid (Mälkiä et al., 2003), dextran sulphate adsorption on lipid monolayers (Santos et al., 2005a) and nanoparticle binding to multilayers (Santos et al., 2005b). For the case considered here, the procedure can be described as follows. Lipid monolayers mimicking neutral and negatively charged natural cell membranes were assembled at the air–water interface and transferred to the interface between an immobilized organic phase and an aqueous phase by the L–B technique. Phosphorothioates or phosphodiesters were introduced to the aqueous phase representing the extracellular space of the natural cell membrane. The surfactant was added to the system and electrochemical measurements were performed after each surfactant addition, enabling estimation of the oligonucleotide adsorption efficiency as a function of the surfactant concentration. A model based on the solution of the Poisson–Boltzmann equation was used to analyze the data obtained from the electrochemical measurements and enabled the extraction of theoretical values for permittivity and surface charge of the lipid monolayer, thus providing very useful information on the final destination of the oligonucleotides.

2. Experimental

2.1. Chemicals

All chemicals were used without further purification. The aqueous supporting electrolyte was sodium chloride (NaCl, Merck, p.a.) and the electrolyte in the aqueous reference phase was tetraphenyl arsonium chloride (TPAsCl, Aldrich, 97%). The organic supporting electrolyte, tetra phenyl arsonium tetrakis-4-(chloro)phenylborate (TPAsTPBCl), was synthesized from tetraphenyl arsonium chloride and potassium tetrakis-4-(chloro)phenylborate (KTPBCl4, Aldrich) as reported elsewhere (Cunnane et al., 1988). Lipids used for forming the monolayer, were 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC, Avanti), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, Avanti) and 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-L-serine] (sodium salt) (DPPS, Avanti). All lipid solutions were prepared in chloroform. Two different kinds of oligonucleotide backbones were used: naturally occurring phosphodiesters (ODNn, desalted) and synthetic phosphoromonothioates (MODn, desalted). Phosphoromonothioates have a modified sugar phosphate backbone, where one of the non-bridging oxygen is replaced with sulfur. The base sequence of ODN1 and MOD1 oligonucleotides was 5′-CCC CAT TCT AGC AGC CCG GG-3′ and the sequence of ODN2 and MOD2 was 5′-GCC GAG GTC CAT GTC GTA CGC-3′. Oligonucleotides were purchased from Sigma Genosys. The complexing agent was cetylpyridium (chloride salt) (CP, Merck, 99%).

2.2. Electrochemical cell

The schematic representation of the cell is shown in Fig. 1 and the cell configuration is given below.

Ag	AgCl	(aq.) 5mM TPAsCl	(NPOE, 5 m-% PVC) 10mM TPAsTPBCl	(aq.) 150 mM NaCl	AgCl	Ag

The organic phase was 2-nitrophenyl octylether (NPOE, Fluka, Selectophore®), which was immobilized by polyvinylchloride (PVC, Sigma, very high molecular weight). The immobilization was performed by heating the NPOE solution containing 5 wt.% of PVC to 120 °C and drop casting the mixture into the electrochemical cell as shown in Fig. 1 (Lee et al., 1997). Aqueous phases were prepared using ion-exchanged Milli-Q® water. The material of the cell was poly(tetrafluoroethylene) (Teflon®). The potential difference between the aqueous and organic phase was controlled via two Ag|AgCl—reference electrodes which were placed in Luggin capillaries as shown in Fig. 1. The potential difference was converted to Galvani potential scale $\Delta_o^w\phi$ before data analysis, where $\Delta_o^w\phi$ is the potential difference between the aqueous and organic phases. The current, which in this context means ion transfer across the liquid–liquid interface, was measured by two counter electrodes made of platinum located in opposing phases. The interface between the aqueous and organic phases acted as the working electrode and had an interfacial area of 28.3 mm^2.

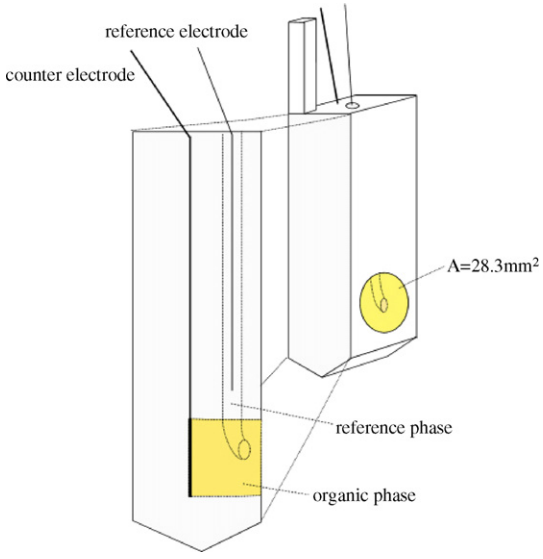


Fig. 1 – A schematic illustration of the electrochemical cell used in this study.

The applicable $\Delta_o^w\phi$ range where adsorption can be investigated is limited by the transfer of the aqueous and organic base electrolyte ions, from one phase to another at a $\Delta_o^w\phi$ determined by their relative hydrophilicities. This $\Delta_o^w\phi$ range, where the current is practically zero and no base electrolyte ion transfer occurs is called the potential window.

2.3. *Langmuir–Blodgett equipment*

The Langmuir–Blodgett equipment was purchased from KSV Instruments Ltd., Helsinki. The Langmuir trough was made of PTFE (Teflon) and the movable barriers of Delrin. The trough was equipped with a small well, which enabled dipping of the electrochemical cell. The surface pressure was measured by a Wilhelmy-plate made of sand blasted-platinum. The apparatus was thermostatted and the whole system was placed inside a Faraday cage.

2.4. *Procedure*

The protocol for transferring a lipid monolayer to the interface between the aqueous and immobilized organic phases was first introduced by Liljeroth et al. and it is briefly described here. Lipid monolayers were prepared in a carefully cleaned Langmuir trough (3× ethanol and water rinses). The surface of the aqueous phase containing 150 mM NaCl was vacuum-cleaned and lipid solution was then pipeted onto the surface using a Hamilton syringe. Time (15–30 min) was then allowed for solvent evaporation. The monolayer was compressed to the desired surface pressure by the movable barriers (5 mm min^{-1}). Pre-dipping, the electrochemical cell was sonicated in chloroform for 15 min, rinsed with ethanol and allowed to dry followed by organic phase immobilization. The lipid monolayer was then transferred to the surface of the immobilized NPOE by dipping the electrochemical cell through the lipid monolayer at a constant downward velocity (2 mm min^{-1}). The transfer ratio varied from 0.9 (DSPC monolayers) to slightly over 1.0 (mixed monolayer of DPPC and DPPS). The surface pressure was kept constant during the dipping procedure by the movable barriers to ensure homogenous lipid monolayer formation. Oligonucleotides and/or CP were then added to the aqueous phase. ac-voltammograms (frequencies 5, 10, 15 and 25 Hz) and cyclic voltammograms (sweep rate 20 mV s^{-1}) were measured after each addition. All electrochemical measurements were taken using an Autolab potentiostat (Autolab® PGSTAT100, Eco Chemie, The Netherlands).

3. Theory

A simple electrostatic model based on the Poisson–Boltzmann equation describes the adsorption of oligonucleotides on the lipid membrane and enables the extraction of relevant membrane properties, such as permittivity, ion concentration and surface charge. The system is divided into three layers representing the immobilized organic phase, lipid monolayer and aqueous phase as shown in Fig. 2. Zwitterionic headgroups of DSPC and DPPC and the anionic headgroup of DPPS are assumed to lie parallel to the interface of the lipid monolayer

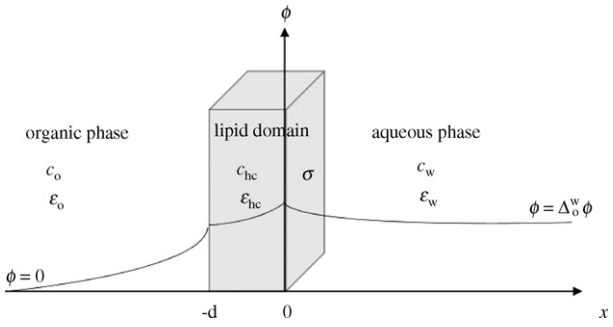


Fig. 2 – Boundary conditions for the potential distribution. c_o, c_w and c_{hc} refer to concentrations in the organic phase, aqueous phase and lipid domain, respectively and ε_o, ε_w, ε_{hc} refer to permittivity of these domains. σ represents the surface charge of the monolayer.

domain and the aqueous phase. The concentrations of these domains are designated as c_o, c_{hc} and c_{aq} and permittivities as ε_o, ε_{hc} and ε_{aq}, respectively. c_{hc} is defined as a product of partition coefficient and K_p is the concentration of the organic phase, $c_{hc} = K_p c_o$. The theory is an extension of a previously reported model (Liljeroth et al., 2000), and the modification made to the theory is discussed below.

In our model, the charge density, σ, of the interface is divided into two parts:

$$\sigma = (\alpha - \beta)\frac{e}{\text{mma}} \quad (1)$$

where e is the elementary charge and mma is the mean molecular area of the lipid monolayer. α is a potential dependent constant describing the amount of zwitterionic headgroups bound by sodium ions and is evaluated from the equation describing the law of mass-action for cation adsorption from the aqueous phase to the surface of the lipid monolayer (Liljeroth et al., 2000). The other constant, β, describes the negative charge distribution on the surface of the lipid monolayer due to naked and complexed oligonucleotides. β is assumed to be independent of the potential.

4. Results and discussion

4.1. *Isotherms*

The isotherms are shown in Fig. 3. The isotherm of DSPC monolayer is similar to those obtained previously (Liljeroth et al., 2000; Chou and Chu, 2003). The monolayer collapses slightly before 80 mN m^{-1} with a mean molecular area of approximately 36 $Å^2$. The monolayer exhibits a direct transition from the expanded to the condensed state already at a very low surface pressure and does not show a fluid phase. The isotherm obtained for the mixed monolayer of 90% DPPC and 10% DPPS is far more expanded and exhibits a wide fluid region with a gradual transition from the fluid to compressed phase. The monolayer collapses slightly before a surface pressure of 50 mN m^{-1}, which is reached with a mean molecular area of 30 $Å^2$. The isotherm obtained for DPPC mixed with DPPS resembles the recently published isotherm for a mixed mono-

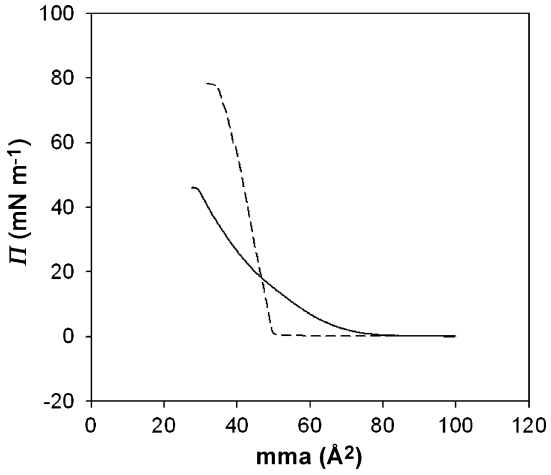


Fig. 3 – Isotherms measured at the air/water interface for a DPPC lipid monolayer with 10% DPPS (solid line) and a DSPC lipid monolayer (dashed line).

layer of DPPC and cholesterol, which is similarly expanded and shifts to the smaller mean molecular area (mma) as a function of the increased cholesterol content (Gong et al., 2002). It is important to note that these isotherms were measured at the air–water interface while all the electrochemical measurements have been undertaken at the liquid–liquid interface. It is probable that the monolayers are more disordered when transferred to the liquid–liquid interface.

4.2. *Electrochemical studies*

Interaction studies were performed utilizing cyclic and ac-voltammetry. Cyclic voltammograms (CVs) obtained for all the studied oligonucleotides were very similar. Typical CVs obtained in the presence of ODN2 are given in Fig. 4. The increase in the current at the positive end of the potential window is due to sodium ions transferring through the monolayer

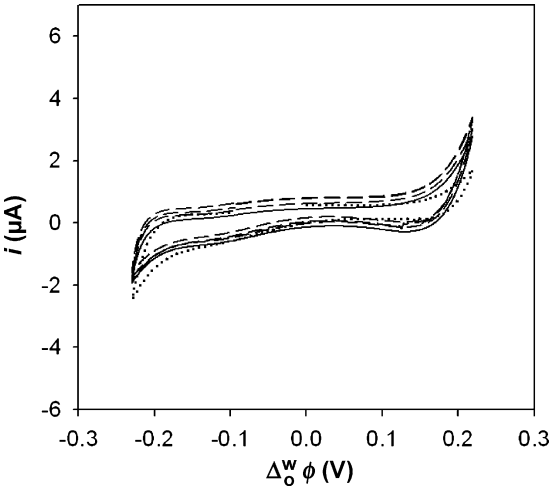


Fig. 4 – CVs obtained at the lipid modified NPOE–water interface in the presence of supporting electrolytes (dotted line), ODN2 naked (solid line) and ODN2 complexed with different ratios of surfactant (dashed lines).

to the organic phase as the aqueous phase becomes positive compared to organic phase. The increase in the negative end is a result of $TPAs^+$ transfer to the aqueous phase. As the current is practically zero at $\Delta^{w}_{o}\phi$-scale in between, no base electrolyte transfer occurs at that scale. Thus, the negligible increase in the Faradaic current after the addition of each component indicates that the studied substances, oligonucleotides and CP–oligonucleotide complexes, do not transfer from the aqueous phase through the lipid monolayer to the organic phase in the $\Delta^{w}_{o}\phi$ range studied. A small amount of free CP is always present in the system and it transfers across the interface throughout the potential window (Hakkarainen et al., 2004b). However, the concentration of CP is low as long as the complex remains unsaturated, and thus its effect on the Faradaic current is minimal.

The current increase at the positive $\Delta^{w}_{o}\phi$ end of the CV is due to the transfer of the oligonucleotide-counterion. Since the capacitance study is limited to $\Delta^{w}_{o}\phi$ scale from −0.15 to 0.05, this increase is not analyzed further.

ac-voltammetry was used to obtain the interfacial capacitance as a function of $\Delta^{w}_{o}\phi$. For this purpose, the Randles' equivalent circuit was used and it was assumed that the resistance of the solution was perfectly compensated by the positive-feedback provided by the potentiostat. The admittance of the equivalent circuit is

$$Y = \frac{\sqrt{\omega}(1+j)}{2\sigma} + j\omega C \tag{2}$$

where ω is the angular frequency of the potential applied on the interface, σ the coefficient of the Warburg impedance and C is the interfacial capacitance. The interfacial capacitance can be calculated from the real and imaginary components of the measured admittance.

$$\omega C = Y'' - Y' \tag{3}$$

where Y'' denotes the imaginary component and Y' the real component of the admittance.

The experimental interfacial capacitance curves obtained are shown in Fig. 5. Details on the relationship between the properties of the monolayer and the capacitance are presented in Appendix A. The presence of a DSPC monolayer compressed to 60 mN m^{-1} has a clear effect on the capacitance (Fig. 5A): the minimum of the parabola shaped curve shifts to more negative $\Delta^{w}_{o}\phi$ and the capacitance is significantly lowered. The origin of this negative shift has been discussed in detail by Liljeroth et al. They concluded that it was mainly governed by cation binding to zwitterionic phosphatidylcholines, since the binding of anions to phosphatidylcholines is negligible. In this study, we adopt the same view. The decrease in capacitance is understood to be governed by the partial ion exclusion from the lipid domain.

Capacitance curves obtained in the presence of naked and complexed oligonucleotides are presented in Fig. 5A–D. All the naked oligonucleotides gave similar responses. The critical part of the capacitance information for naked oligonucleotides is beyond the applicable $\Delta^{w}_{o}\phi$ range, however some conclusions concerning the membrane activity of naked oligonucleotides can be drawn. Compared to the capacitance curve

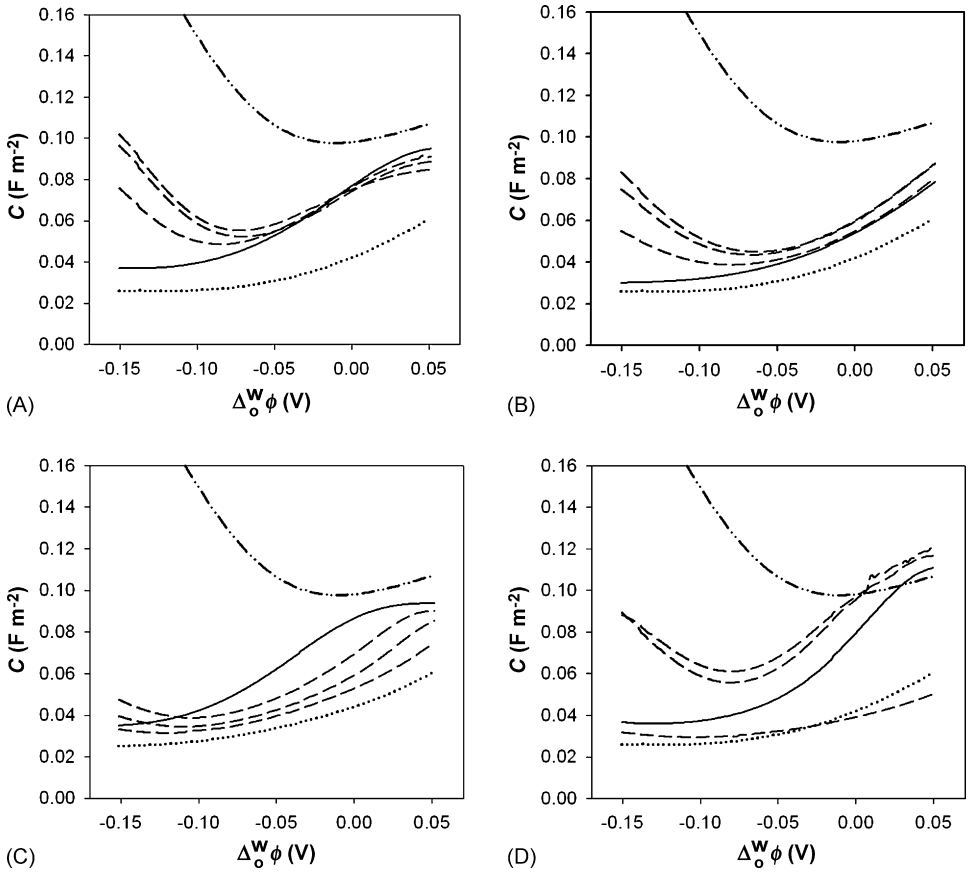


Fig. 5 – Experimental capacitance curves: (A) ODN1. The solid lines represent data obtained in the presence of naked oligonucleotide and the dashed lines represent those obtained in the presence of the complexed oligonucleotides with the CP/oligonucleotide ratios of 0.24, 0.71 and 0.96 from bottom to top. The dotted lines represent the response for the compressed lipid monolayer with only base electrolytes in the aqueous compartment and the dash-dot-dot line shows the bare interface without lipid monolayer, (B) ODN2, (C) MOD1 and (D) MOD2. The lines are assigned as in (A).

for the plain lipid interface, the curves are shifted to the negative direction. The surface charge, σ, of the monolayer is thus increased upon oligonucleotide addition. This might arise from the packing of oligonucleotide counterions at the interface and is a clear indication the negative oligonucleotides having practically no interaction with the monolayer, as the adsorption of the negative oligonucleotide on the lipid domain should decrease the surface charge of the membrane.

When CP is added to the aqueous phase, its binding to the oligonucleotide is supposed to improve the interaction between the lipid monolayer and oligonucleotide. Fig. 5A and B (dashed lines) show the effect of increasing total surfactant ion concentration in the aqueous phase. It is seen that the surface charge of the lipid monolayer decreases as a function of increased CP concentration. This can be explained by adsorption of the CP–oligonucleotide complex at the interface, as the total charge of the CP–oligonucleotide complex is negative. The observed capacitance response is unlikely to be due to dissociation of CP from the complex following by adsorption at the interface, as this would result in an increase in the current response in the CV-measurements. This was not seen in Fig. 4. Another confirmation of this is the negligible shift between the concentration ratios of 0.72 and 0.96, which can be explained by the adsorbing CP–oligonucleotide complex being nearly saturated. In that case, the total charge of the adsorbing complex is only slightly negative, and thus the surface charge of the lipid domain remains practically the same. The increase in the capacitance at the minimum is suggested to be a consequence of the CP–oligonucleotide complexes internalizing into the lipid domain and leaving the charged parts on the outer lipid layer. This increases the permittivity of the lipid domain and supports the previous study, which showed that the DNA–surfactant complex incorporates into the hydrophobic interior of the membrane (Hianik and Labajova, 2002). The internalization of the surfactant–oligonucleotide complex occurs, even if the total charge of the complex is considerably negative, which may prove useful for practical purposes.

The capacitance curves for the complexed modified oligonucleotides are shown in Fig. 5C and D. There is a considerable difference between the capacitances of the lipid domain interacting with the complexed MOD1 and MOD2. Apart from the lowest CP/oligonucleotide charge ratio, the capacitance of lipid domain with complexed MOD2 follow the trend noted for the complexed unmodified oligonucleotides. On the contrary, complexed MOD1 has only a weak effect on the interfacial capacitance of the lipid domain and shifts the curve minimum only slightly. This indicates that MOD1 complexes internalize poorly into the lipid domain. It is noteworthy that the lowest

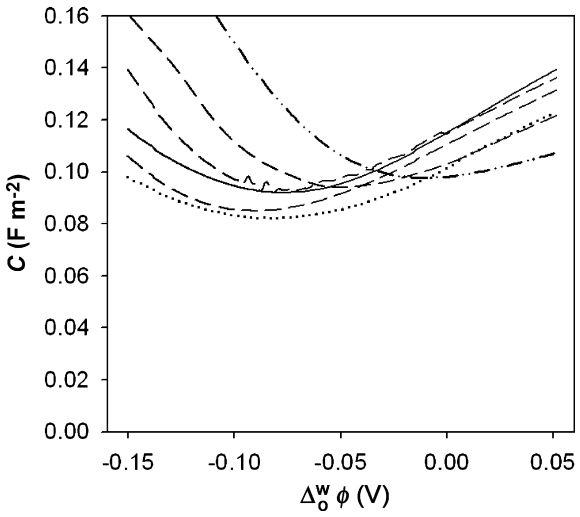


Fig. 6 – Experimental capacitance curves obtained for mixed monolayer of DPPC (90%) and DPPS (10%). The solid line shows that response obtained in the presence of naked ODN2 and the dashed lines that obtained in the presence of the complexed oligonucleotides with CP/oligonucleotide monomer concentration ratios of 0.24, 0.71 and 0.96 from bottom to top. The dotted line shows the response for the compressed lipid monolayer and bare interface with base electrolytes only in the aqueous phase and dash-dot-dot lines shows the bare interface without lipid monolayer.

concentration ratio of MOD2 complex actually gives a MOD1 complex-like capacitance response, but when the ratio of the CP ions is increased, the membrane activity approaches the activity of complexed ODN1 and ODN2. Thus, we believe that phosphoromonothioate modification is the main reason for the different membrane properties of MOD1 complexes. It is probable that MOD1 and CP form such compact complexes with the hydrophobic core and hydrophilic shell that they do not have a strong tendency to adsorb to the lipid membrane.

To get an idea of the role of negatively charged lipids in natural membranes on oligonucleotides transfer through a lipid membrane, the experiment was also carried out with a mixed monolayer of DPPC containing 10% of DPPS lipids (Fig. 6). The lipid monolayer was compressed to a surface pressure of 40 mN m^{-1} before transferring it on the immobilized organic phase. Higher surface pressure could not be used because the monolayer collapses at ca. 48 mN m^{-1} as shown in Fig. 3. The interfacial capacitance curves for the mixed monolayer interacting with both naked and complexed ODN2 are shown in Fig. 6. The presence of the mixed lipid monolayer does not have such a significant effect on the interfacial capacitance compared with the zwitterionic DPPC monolayer. There are two reasons for this. Firstly, since the mixed monolayer is studied at a rather low surface pressure (40 mN m^{-1}), the partial ion exclusion is not considerable. Secondly, the surface of the mixed monolayer of DPPC and DPPS is not as positive as the surface of the DSPC monolayer, because of negatively charged phosphatidyl serines.

The addition of the naked ODN2 and CP to the aqueous phase gives capacitance responses, which follows the trend noted for the same oligonucleotide studied in the vicinity of the DSPC monolayer (Fig. 5B). Thus, it is concluded that CP increases the partition of ODN2 to the mixed lipid monolayer.

These results show that cationic surfactants enhance the internalization of the oligonucleotides into the phospholipid membrane with possibly a very similar mechanism to that shown for oligonucleotide delivery via liposomes (Zelphati

Table 1a – Values of K_p used in fitting procedure

	ODN1	ODN2	MOD1	MOD2	ODN2 (mixed monolayer)
Naked oligonucleotide	0.02	0.015	0.02	0.03	0.035
CP/ON-monomer conc. ratio 0.24	0.065	0.035	0.025	0.02	0.035
CP/ON-monomer conc. ratio 0.72	0.075	0.07	0.035	0.1	0.05
CP/ON-monomer conc. ratio 0.96	0.075	0.09	0.045	0.08	0.075

Table 1b – Values of ε_{hc} used in fitting procedure

	ODN1	ODN2	MOD1	MOD2	ODN2 (mixed monolayer)
Naked oligonucleotide	10	8	9	9	60
CP/ON-monomer conc. ratio 0.24	15	11	9	7	50
CP/ON-monomer conc. ratio 0.72	17	12	10	17.5	60
CP/ON-monomer conc. ratio 0.96	18	12.5	11	22	65

Table 1c – Values of β used in fitting procedure

	ODN1	ODN2	MOD1	MOD2	ODN2 (mixed monolayer)
Naked oligonucleotide	−0.5	−0.3	−0.6	−0.5	0.5
CP/ON-monomer conc. ratio 0.24	0.45	0.45	0.2	0.2	0.35
CP/ON-monomer conc. ratio 0.72	0.6	0.6	0.3	0.45	0.55
CP/ON-monomer conc. ratio 0.96	0.62	0.67	0.3	0.45	0.65

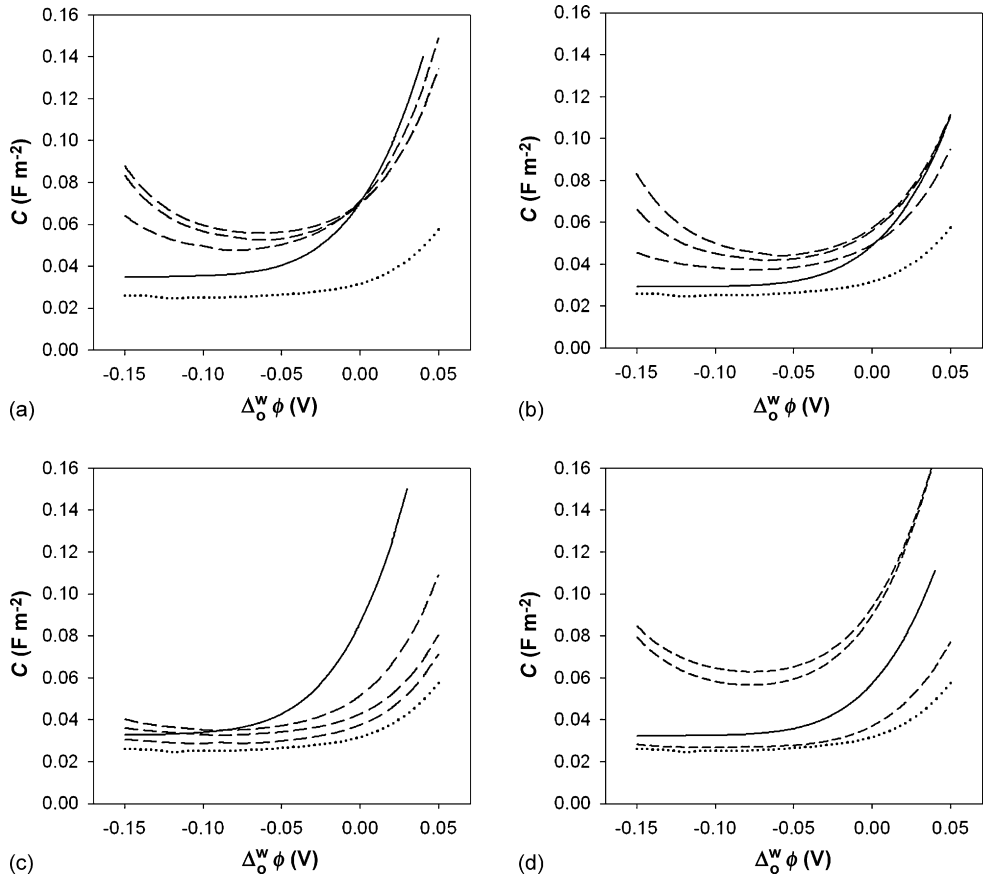
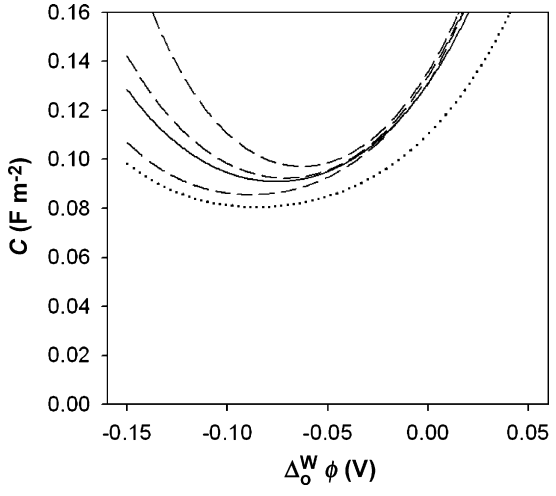


Fig. 7 – Theoretical capacitance curves: (A) ODN1. The solid line shows the predicted response for the case of the naked oligonucleotide and the dashed lines show the responses for the complexed oligonucleotides with the CP/oligonucleotide ratios of 0.24, 0.71 and 0.96 from bottom to top. The dotted line shows the response predicted for the compressed lipid monolayer with only base electrolytes in aqueous compartment, (B) ODN2, (C) MOD1 and (D) MOD2. The lines are assigned as in (A).

and Szoka, 1996b). However, results do not show whether the oligonucleotides can escape the lipid vesicles, which has been a problem with many delivery systems (Liang et al., 1996). Nonetheless, this may not be an issue with a surfactant based delivery system, since the inner cell surface is rich with negative lipids, which associate with positive surfactants and possibly act as oligonucleotide releasers.



The calculated capacitance curves are shown in Figs. 7 and 8. The parameters that were varied in the fitting procedure are shown in Tables 1a–c. The following parameters were kept constant. The mean molecular areas were extracted from the isotherms (45 Å^2 for DSPC monolayer and 40 Å^2 for mixed monolayer of DPPC and DPPS). The binding constant of sodium ions (500 M^{-1}) gave the appropriate shift in the capacitance minimum for the DSPC curve. The thickness of the hydrocarbon domain (20 Å) was chosen to be the approximately the lipid length. Permittivities of NPOE and water are 24.2 and 78, respectively (Ammann, 1986). The surface charge (σ) was divided into two parts: one describing a sodium ion adsorption (α) and the other one

Fig. 8 – Theoretical capacitance curves of mixed monolayer of DPPC (90%) and DPPS (10%). The solid line shows the response predicted for naked ODN2 and the dashed lines the responses predicted for the complexed oligonucleotides with CP/oligonucleotide monomer concentration ratios of 0.24, 0.71 and 0.96 from bottom to top. The dotted line and dash-dot line show the responses predicted for the compressed lipid monolayer and bare interface with base electrolytes only in the aqueous phase, respectively.

describing partition of the negatively charged complex to the lipid domain (β). Surface charge affects the position of the capacitance curve minimum (see Appendix A). To keep the fitting procedure simple, only β was altered while K_b, which determines the value of α, was kept constant assuming it is not responsible for the shift. The capacitance increase is governed by the increased permittivity of the hydrocarbon domain (ε_{hc}) as well as the increased ion concentration in the lipid domain (ions are only partially excluded from the lipid monolayer), seen as an increased partition coefficient, K_p. The interfacial capacitance curves calculated from the theory follow the experimental trends very well with the fitting parameters strengthening the conclusions drawn from the experiments. Complexation of oligonucleotide with CP clearly increases the oligonucleotide partition to the lipid membrane in most cases, which can be concluded from a significant increase in β, ε_{hc} and K_p-values upon CP addition.

5. Conclusions

It has been shown that the Langmuir–Blodgett technique combined with electrochemical measurements is a powerful tool for understanding events in a lipid monolayer during the oligonucleotide internalization. Two different kinds of phosphatidylcholine monolayers were successfully transferred to the interface formed between the aqueous phase and immobilized organic phase, one of them containing 10% of negative phosphatidyl serine. The main observation was that complexation of oligonucleotide with cetylpyridinium chloride (CP) clearly increases the oligonucleotide partition to the lipid membrane in most cases. This was concluded from the modified electrochemical properties of the lipid domain. However, in one case, CP seemed to form compact globules with phoshoromonothioates, which do not have a tendency to internalize into the lipid domain, and thus do not have a significant effect on the interfacial capacitance. The result indicates that the base sequence and chemical modification of the oligonucleotide have a significant influence on the success of cellular delivery of the oligonucleotide–surfactant complex. A simple theory based on the Poisson–Boltzmann potential distribution gave further clarification of the reasons for the observed phenomena.

These results illustrate the potential of a simple cationic surface-active agent for oligonucleotide delivery. This work will be complimented by other techniques (calorimetry, etc.) and varying the type of surfactant (cationic gemini surfactants).

Acknowledgments

We thank the Academy of Finland for financial support and Dr. Bernadette Quinn for proof reading this manuscript.

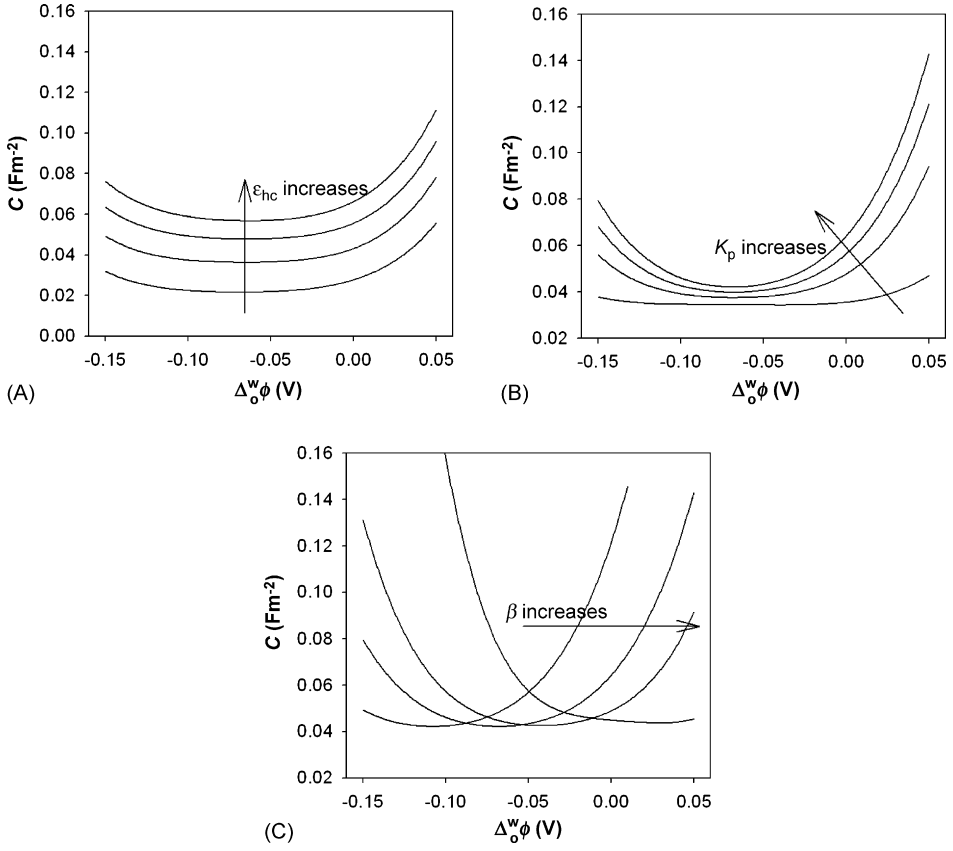


Fig. A1 – Effect of the fitting parameters on the interfacial capacitance (A) influence of permittivity of the lipid domain (ε_{hc}), (B) influence of K_p, a constant linearly dependent on the concentration of the electrolytes in the lipid domain and (C) influence of β, a unit less constant describing the amount of the negatively charged oligonucleotides or their complexes adsorbing on the surface of the lipid domain. When β is increased, the surface charge of the lipid monolayer is decreased.

Appendix A

The interfacial capacitance is given as follows:

$$C = \frac{\partial Q}{\partial \Delta_o^w \phi}$$

where Q is thermodynamical charge. The effect of the fitting parameters on the interfacial capacitance are shown in parts (A)–(C) of Fig. A1.

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