Publication II

Sanna Hakkarainen, Anna-Kaisa Kontturi, Kyösti Kontturi, and Arto Urtti. 2004. Electrochemical and calorimetric study of oligonucleotide complexation with cetylpyridinium chloride. European Journal of Pharmaceutical Sciences, volume 23, numbers 4-5, pages 371-377.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutical Sciences 23 (2004) 371–377
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

Electrochemical and calorimetric study of oligonucleotide complexation with cetylpyridinium chloride

Sanna Hakkarainen[a], Anna-Kaisa Kontturi[a,*], Kyösti Kontturi[a], Arto Urtti[b]

[a] *Laboratory of Physical Chemistry and Electrochemistry, Helsinki University of Technology, P.O. Box 6100, FIN-02015 HUT, Finland*
[b] *Department of Pharmeceutics, University of Kuopio, Kuopio, Finland*

Received 29 March 2004; received in revised form 17 August 2004; accepted 31 August 2004
Available online 30 October 2004

Abstract

The complexation of oligonucleotides and antisense drugs with a cationic surfactant, cetylpyridinium chloride (CPC), has been studied using electrochemistry at a micrometer sized liquid/liquid interface. This method can be used to investigate the effect of chemical and structural modifications on the complexation behaviour of oligonucleotides. For the interaction between CPC and oligonucleotides the effect of phosphorothioate derivatisation upon binding characteristics has been examined. Phosphoromonothioate modification causes the onset of binding to occur at a lower free surfactant concentration. Calorimetric studies show that surfactants are strongly bound to oligonucleotides and the binding is driven by entropy. The enthalpy change for the formation of oligonucleotide-surfactant complex is negative in all cases at 25 °C indicating exothermic reaction.

Keywords: Oligonucleotide; Antisense drugs; Complexation; Ion transfer; Isothermal titration calorimetry

1. Introduction

Gene medicines are an emerging field in the drug treatment. Gene medicines are divided into gene therapy and gene inhibition. The aim is to use oligonucleotide or polynucleotide structures to modify expression of target by bringing in transgene or by inhibiting the expression with antisense or anti-gene oligonucleotides, ribozymes or RNA. A key stage in this process is the transport of DNA, RNA, oligonucleotide or their modifications across the cell membrane and into the cell, where it can alter cellular processes by either preventing or promoting specific gene expression (Schätzlein and Uchegbu, 2001).

One of the major obstacles to the clinical application of gene therapy is in the development of an efficient non-viral system of delivering the DNA into the cell (Segura and Shea, 2001). Under normal physiological conditions, oligonucleotides and DNA have a high negative charge density that causes electrostatic repulsion with negative charges of cellular membranes. In order to overcome this transport barrier, charges of DNA must be shielded, or even inversed to facilitate the cellular uptake by endocytosis. The interaction between synthetic ionic polymers and surfactants of opposite charge has been studied extensively over the last 50 years (Rodenhiser and Kwak, 1998). More recently the challenge of generating non-viral vectors as gene delivery systems to solve the transport problems of DNA and oligonucleotides has involved their complexation with amphiphilic molecules such as cationic surfactants and lipids (Safinya, 2001; Harashima et al., 2001; Torchilin, 2001). This complexation has been shown to result in condensation and charge neutralisation of the DNA (Bathaie et al., 1999; Hyvönen et al., 2000, 2002).

Even though cationic surfactants, amphiphiles and lipids have been widely used to augment oligonucleotide and DNA delivery, their interactions are not well understood. These interactions are of utmost importance, but currently the

* Corresponding author. Tel.: +358 9 4512573; fax: +358 9 4512580.
E-mail address: anna.kontturi@hut.fi (A.-K. Kontturi).

0928-0987/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.ejps.2004.08.010

formation of the complexes, their cellular entry and dissociation are not governed in controlled manner. The overall aim is to generate complexes in a controlled way reproducibly. The complexes should retain their on given properties during the storage, they should be resistant against the competition of extracellular polyanions, they should entry the cells, and release their oligonucleotide or DNA cargo in the cells in predetermined way. All these factors depend on the formation of the complexes and if this process is not mastered, the above-mentioned goals cannot be reached. Lack of sophisticated methods is a limiting complexation studies between oligonucleotide/DNA and their cationic counterparts.

Electroanalytical techniques have been used successfully to determine the binding isotherms of surfactant-oligonucleotide complexes by measuring the concentration of free surfactant throughout the binding process to obtain the binding constant (Liu et al., 1999; Bloor et al., 1996). The free surfactant concentration is obtained either potentiometrically using a surfactant-selective membrane electrode (Hayakawa and Kwak, 1982) or by ion transfer voltammetry of the complexing agent (Hakkarainen et al., 2004). In this study, the free surfactant concentration was measured using ion transfer voltammetry at a micro sized interface between two immiscible liquids. All measurements were carried out at low surfactant concentrations, below the critical micelle concentration (cmc). This prevented the formation of large aggregates and hence phase separation.

The degree of binding between a polyanion and cationic surfactant is governed by both electrostatic and hydrophobic interactions. Binding isotherms were used to compare the binding mechanisms of the oligonucleotide and antisense samples, and hence to establish how the complexation process is affected by the structural and chemical modifications to the oligonucleotides. These data were supplemented by the results from titration calorimetry experiments to establish the thermodynamics of the oligonucleotide-surfactant binding.

2. Materials and methods

2.1. Chemicals

Organic solvents, surfactant, oligonucleotide and antisense samples were used as purchased without further purification. Aqueous solutions were prepared in 10 mM NaCl using Milli-Q water.

2.1.1. Surfactant

Cetylpyridinium chloride (CPC) (p.a. 99%) was purchased from Sigma–Aldrich.

2.1.2. Oligonucleotides

The oligonucleotides were purchased from sigma genosys. The base sequences were (5′-CCC CAT TCT AGC AGC CCG GG-3′), for abbreviation it is denoted by ODN1 and (5′-GCC GAG GTC CAT GTC GTA CGC-3′), which is denoted by ODN2. The modified oligonucleotides were structurally modified by replacing one of the non-bridging oxygens with sulphur in the sugar phosphate backbone of the oligonucleotide, thus producing a phosphoromonothioate compound from the naturally occurring phosphodiester. They have the same base sequence as their unmodified counterparts and hence are denoted by MOD1 and MOD2, respectively.

2.1.3. Electrolytes for the electrochemical cell

The organic solvents used were 1,2-dichloroethane (DCE, HPLC-grade) from Rathburn. The organic supporting electrolyte was tetraphenyl arsonium tetrakis-4-(chloro)phenylborate ($TPAsTPBCl_4$), which was synthesized from tetraphenylarsonium chloride (TPAsCl, 97%) and potassium tetrakis-4-(chloro)phenylborate ($KTPBCl_4$) as described earlier (Cunnane et al., 1988). Both of them were purchased from Aldrich. The aqueous supporting electrolyte was sodium chloride (NaCl, p.a.) purchased from Merck.

2.2. Ion transfer voltammetry

The transfer of surfactant ions across the liquid/liquid interface was induced electrochemically. The electrochemical cell used is shown below where || refers to the polarizable interface under study.

$$\begin{aligned}&\text{Ag}|\ \text{AgCl}|\ \text{(aqueous phase) 5 mM TPAsCl}|\ \text{(organic phase)}\\&\quad 2-10\ \text{mM TPAsTPBCl}_4\|\ \text{(aqueous phase) 10 mM NaCl}\\&\quad x\ \mu\text{M CPC} + y\ \text{mM ODN/MOD}|\ \text{AgCl}|\ \text{Ag}\end{aligned}$$

Sodium chloride and tetraphenyl arsonium tetrakis-4-(chloro)phenylborate ($TPAsTPBCl_4$) were used as aqueous and organic supporting electrolytes, respectively. The organic solvent was 1,2-dichloroethane. Two electrodes located in opposing phases controlled the applied potential across the liquid/liquid interface. As the current flow across the interface is in the nA range, two-electrode arrangement is sufficient with each electrode acting as counter and reference electrode for the respective phase.

By making the electrochemical potential of the aqueous phase positive relative to the organic phase, the cationic surfactant molecules transfer from the aqueous to the organic solution. The potential at the interface was controlled via two Ag/AgCl electrodes, one placed in each phase, so that the rate at which the cations transferred, measured as the current flowing, was controlled by the diffusion rate of the surfactant to the interface. For a micro sized interface, where diffusion is hemispherical, the relationship between the steady state current i, and the concentration of free surfactant is given by the following equation (Bard and Faulkner, 2001):

$$i = 4nFDrc_{\mathrm{f}} \tag{1}$$

where D is the diffusion coefficient and c_{f} the concentration of free surfactant ions, F the Faraday constant, n the ionic

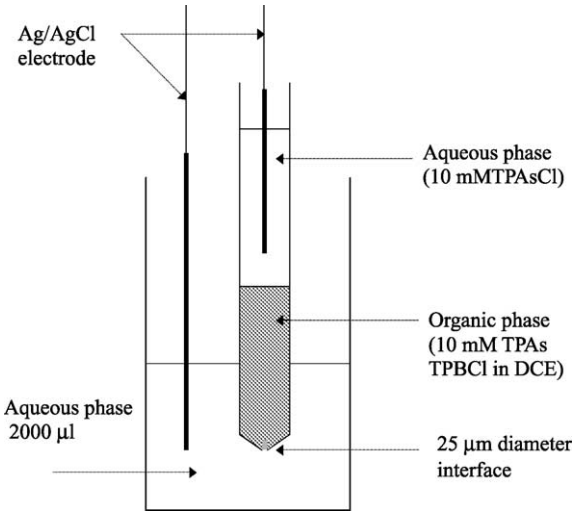

Fig. 1. A schematic illustration of the experimental set-up.

charge and *r* the radius of the microhole or micropipette tip. Within the available potential range (determined by the supporting electrolytes used), the surfactant molecules were the only species transferring across the interface. Therefore, the measured steady state current was directly proportional to the concentration of free surfactant. Also, the electric currents used were sufficiently small not to have an effect on the surfactant concentration. A schematic drawing of the experimental set-up is shown in Fig. 1.

2.3. Microinterface preparation

Micropipettes for the electrochemical measurement were prepared as described previously (Evans et al., 2000). The pipette was filled by drawing solution through the tip using a syringe attached at the other end. Initially, an aqueous reference phase was drawn inside the capillary followed by an organic phase so that the aqueous reference phase was situated on top of the organic phase. The Ag/AgCl electrode was placed into the upper aqueous phase. Placing the filled pipette in an aqueous solution resulted in a well-defined 25 μm diameter liquid/liquid interface being established at the tip of the capillary between the organic and aqueous phases. The second Ag/AgCl electrode was placed in the aqueous phase.

2.4. Titration procedure

The complexation was realised by stepwise addition of surfactant to a solution of the oligonucleotide. Both the concentration of the supporting electrolyte ($TPAsTPBCl_4$) in the organic phase (1,2-DCE) and that of the aqueous phase (NaCl) were 10 mM. Oligonucleotide solution of 20 μM was titrated with 10–20 μl injections of 2.8 mM surfactant. The volume of the aqueous phase was 2 ml. Steady state currents were then recorded at a fixed potential after each addition.

2.5. Isothermal titration calorimetry

Calorimetric measurements were carried out by using an isothermal titration calorimeter (Microcal™ VP-ITC, USA) at 25 °C and analysed with origin® 5.0 software (Microcal™, USA). The injections were typically 11 μl and the equilibrium time before each injection was 5 min. The concentration of CPC was 140 μM and it was chosen to be under critical micelle concentration, which is 180 μM by isothermal titration calorimeter. The concentration of the oligonucleotides was 0.75 μM. The volume of the cell was 1.44 ml and the stirring rate was 300 rpm. The heat caused by the dilution of the CPC solution was small and was subtracted from the results.

3. Results and discussion

3.1. Calibration

In Fig. 2, the steady state current is plotted against the total concentration of surfactant c_t in the absence of oligonucleotide. It is seen that there is a linear relationship between current and surfactant concentration, the slope of which can be used to determine the concentration of free surfactant in solution. The CPC concentration range chosen (0–0.15 mM) is below the critical micelle concentration (cmc) ensuring that all the added surfactant is present in monomeric form and is available to be transferred across the liquid/liquid interface.

3.2. Binding mechanism

The stepwise addition of surfactant to oligonucleotide solution was used to determine the binding parameters. Typical voltammetric data is shown in Fig. 3a where the steady state current is plotted against the total concentration of added surfactant. Initially, virtually all of the surfactant is bound and therefore unavailable to transfer indicated by very small current. However, once the oligonucleotide is saturated with surfactant, further addition gives rise to free surfactant ions and the current starts to increase. Since the relationship between current and the concentration of free surfactant is known, the

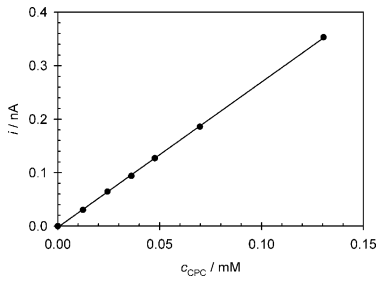

Fig. 2. A steady state current as a function of the concentration of CPC at 25 °C in the absence of polyelectrolyte.

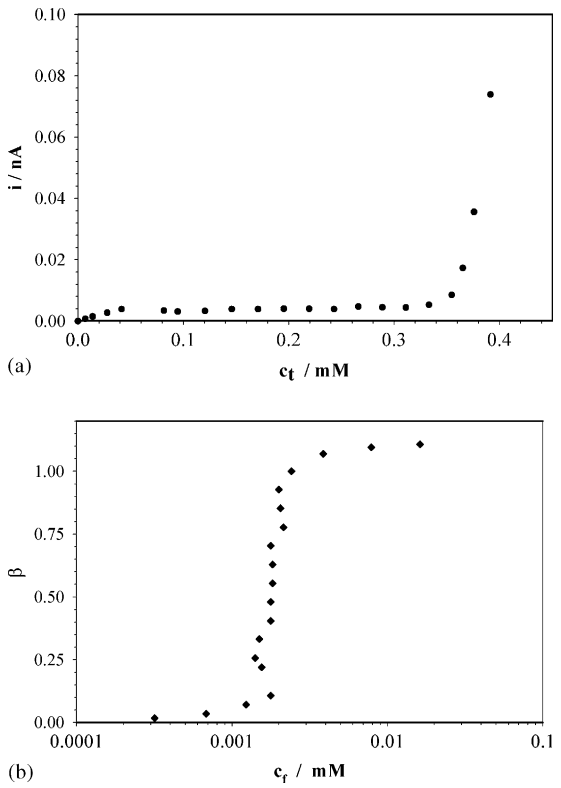

Fig. 3. (a) A steady state current as a function of total concentration of CPC for stepwise addition of 2.8 mM CPC to 20 μM MOD1 in 10 mM NaCl solution at 25 °C, (b) the binding isotherm corresponding the data in (a).

degree of binding, β, can be calculated using Eq. (2).

$$\beta = \frac{c_b}{zc_o} = \frac{c_t - c_f}{zc_o} \quad (2)$$

where c_b is the concentration of bound surfactant, c_o the concentration of oligonucleotide and z the number of charged binding sites of the oligonucleotide, which is the number of phosphate groups in the oligonucleotide. c_b is calculated from the difference between the concentration of total added surfactant, c_t, and the concentration of free surfactant, c_f.

The binding isotherm corresponding the voltammetric data of Fig. 3a is shown in Fig. 3b. The position of the isotherm along the x-axis is relative to the binding affinity. A shift to the right indicates a lower binding affinity, essentially increasing the critical aggregation concentration (cac), the free surfactant concentration at which the onset of binding occurs (Chu and Thomas, 1986). The final value of β as indicated by the plateau in the binding isotherm, and denoted by β_{max} indicates the maximum or saturation concentration of bound surfactant.

Binding occurs due to electrostatic interaction between the positively charged surfactant head group and the negatively charged groups on the polyanion, and hydrophobic interactions between the hydrocarbon chains of the surfactant. Hydrophobic interactions are indicative of a co-operative binding mechanism, which is characterised in the binding isotherm by a sharp increase in β at a given value of c_f. It is common to describe this kind of co-operative binding by a Zimm–Bragg model, where the polyelectrolyte is represented by a linear array of binding sites and where the effect of co-operative interactions between adjacent bound ligands is taken into account (Zimm and Bragg, 1959). Satake and Yang have developed the model further and show the binding degree and the free surfactant concentration to have the following relationship (Satake and Yang, 1976).

$$\beta = 0.5\left(1 + \frac{K_w c_f - 1}{\sqrt{(1 - K_w c_f)^2 + 4Kc_f}}\right) \quad (3)$$

where K is the intrinsic binding constant and is a function of the electrostatic interaction between the surfactant and the polymer. The position of the isotherm along the x-axis gives the binding affinity K_w. A shift to the right indicates a lower binding affinity, essentially increasing the critical aggregation concentration (cac). For co-operative binding, K_w can be calculated using Eq. (4).

$$c_f|_{\beta=0.5} = (K_w)^{-1} \quad (4)$$

where $c_f|_{\beta=0.5}$ is the free surfactant concentration when the degree of binding is 0.5. The slope of the binding isotherm determines a co-operativity parameter, w, which is a function of the hydrophobic interaction between adjacent bound surfactant molecules. The experimental normalized binding isotherms with the corresponding fitted Satake–Yang curves are shown in Fig. 4. As it is difficult to obtain a value for w directly from the slope of the isotherm, a value for K is firstly calculated by a least square fit of the binding isotherm to Eq. (3). The parameters obtained from the theory are given in Table 1.

Fig. 4 shows that the chemical modification, in which an oxygen atom in the phosphodiester linkage is replaced with sulphur, has a significant effect on the binding process. The binding starts at a lower free surfactant concentration for phosphorothioates, indicated by the increase in both the binding affinity K_w, and the equilibrium constant K. Although the phosphorothioate modification does not change the net charge of the phosphate backbone, the different size and polarizability has been shown to affect the charge distribution of the phosphate backbone (Pichierri and Sarai, 1999). In addition, different conformations of the natural and modified oligonucleotides might have an effect on the binding parameters.

Table 1
Co-operative coefficients and binding degrees

Oligonucleotide	K_w (M^{-1})	K (M^{-1})	w	β_{max}
MOD1	5.5×10^5	8000	70	1.1
MOD2	7.1×10^5	5700	124	1.0
ODN1	1.9×10^5	3700	51	1.1
ODN2	1.6×10^5	1300	122	0.9

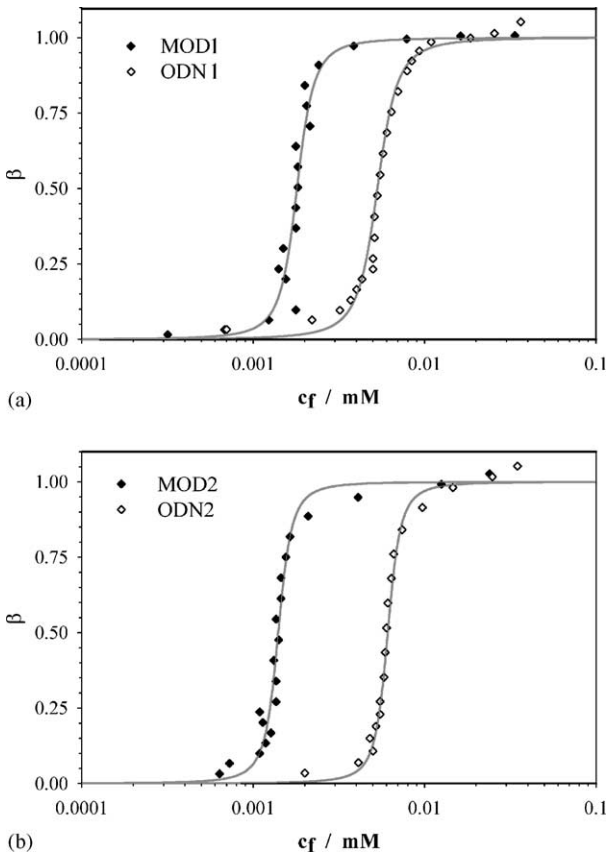

Fig. 4. The experimental binding isotherms and corresponding fitted Satake–Yang curves for (a) MOD1 and ODN1 and (b) MOD2 and ODN2.

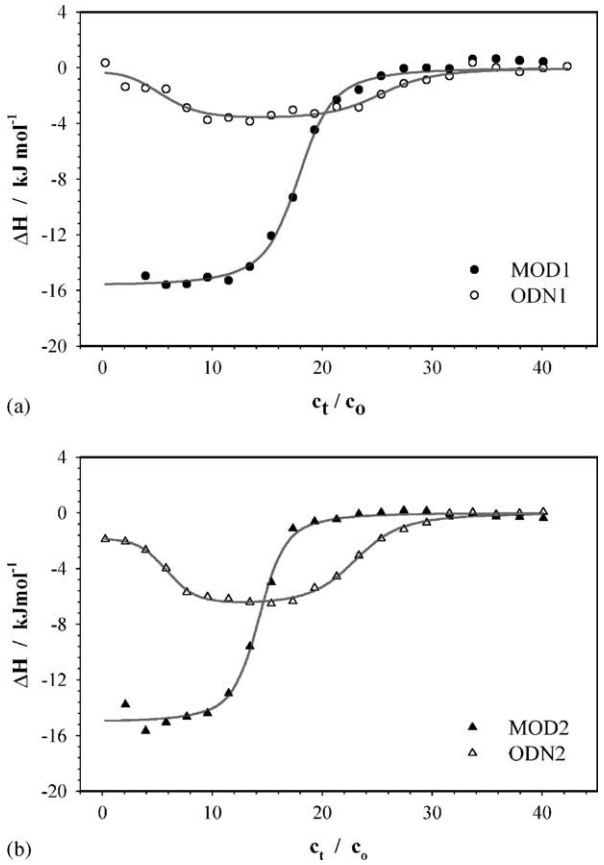

Fig. 5. The enthalpy change in calorimetric measurements as a function of concentration ratio c_t/c_o for complexation of (a) MOD1, ODN1 and (b) MOD2, ODN2 at 25 °C.

Also the base sequence of the oligonucleotide has an effect on the binding parameters. Co-operativity constant, w, is considerably higher in a case of MOD2 and ODN2 oligonucletides. This may be explained by the different tendency of the oligonucleotides to form dimers and hairpin loop structures, although both oligonucleotide base sequences may adopt such conformations. Molecules have to reorganize and possibly break hydrogen bonds between the base pairs when forming a complex with surfactants. This makes complex forming with ODN1 and MOD1 less co-operative compared to MOD2 and ODN2. Binding parameters obtained for unmodified oligonucleotides were rather similar to values (K_w = 93,000, w = 80) obtained by the same theoretical method for cetyltrimethylammonium bromide binding to DNA containing 1,66,000 basepairs (Mel'nikov et al., 1995). Thus the double helix-structure and huge size of the molecule does not seem to affect the surfactant binding.

3.3. Calorimetry

Surfactant binding to polyelectrolytes as well as binding of cationic lipids to DNA has been shown to be entropy driven processes (Shaw, 1992; Spink and Chaires, 1997; Lobo et al., 2001; Pozharski and MacDonald, 2002). In this work, the surfactant binding to a short 20 bases containing unmodified and modified oligonucleotides was studied with isothermal titration calorimetry. The resulting enthalpy curves are shown in Fig. 5 and the thermodynamic variables are shown in Table 2. Microcalorimetric measurements were performed at lower concentration ranges than amperometric measurements due to heat effects of CPC micelle deformation.

The difference of calorimetric behaviour between phosphodiester and phosphorothioate modification is remarkable. The negative enthalpy change of CPC binding to modified

Table 2
Thermodynamic values for binding

Oligonucleotide	K (M^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	$-T\,\Delta S$ (kJ mol^{-1})
MOD1	9.9×10^6	−39.9	−15.7	81.3	−24.2
MOD2	1.4×10^7	−40.7	−15.0	86.0	−25.6
ODN1	4.6×10^6	−38.0	−3.9	114	−34.1
ODN2	5.6×10^6	−38.5	−6.9	106	−31.6

phosphorothioate oligonucleotides is very similar to the case of cetylpyridinium chloride binding to polystyrenesulphonate (Hakkarainen et al., 2004) and the magnitude is same for both phosphorothioates. On the other hand, in the case of natural oligonucleotides enthalpy changes are so small that they were hardly measured. The enthalpy change curves for these two ODNs seem to have two binding regions, of which the first is limited to only a few binding sites. Since the first binding region is not seen for modified oligonucleotides possibly due to very low surfactant concentration where binding starts, we consider only the second, co-operative binding region. The interesting fact is that negative enthalpy change for ODN2 was almost twice the value for ODN1. This cannot be interpreted only by the base sequence of ODN1, since the same kind of effect is not seen for the phosphorothioate modifications of the same molecules. It might be due to the base sequence determined natural structures of the unmodified DNAs, which are not distorted like in the case of phosphorothioate modification (Smith and Nikonowicz, 2000).

Higher enthalpy values in a case of modified oligonucleotides are another indication of different charge distribution in modified and unmodified phosphate backbones, since the enthalpy change is usually thought to arise from the electrostatic interaction between polymer and ligand. Entropy values indicate that binding is driven by entropy and the formed complexes are entropically favourable. This is understood by the fact that hydrophobic tails of surfactants are freer inside the micelle-like oligonucleotide-surfactant complexes.

Equilibrium constants obtained by calorimetric method are higher than co-operative equilibrium constants obtained by ion transfer voltammetry. This is because of lower concentration of the oligonucleotide used in calorimetric measurements. The onset of binding is highly dependent on the polymer concentration due to the activity effects. Furthermore, the model that was used to interpret calorimetric data is based on set of identical and independent sites of charged groups of the polyelectrolyte, ignoring the mutual interaction of these groups.

4. Conclusions

The present study has shown that electrochemistry at microsized liquid/liquid interfaces is a useful tool for monitoring the complexation reaction of oligonucleotides with cationic surfactants. It has been shown that replacing one of the nonbridging oxygens with sulphur in the sugar phosphate backbone of the oligonucleotide has a strong effect on the binding affinity as well as on the co-operativity of binding. Phosphoromonothioate modification causes the onset of binding to occur at a lower free surfactant concentration. Also, calorimetric measurements show the difference between modified and unmodified oligonucleotides. The complexation reaction of CPC with modified oligonucleotides is more exothermic than the reaction with unmodified oligonucleotide. In both cases the reaction is entropy driven and the complexes formed are entropically favourable.

Acknowledgements

National Technology Agency in Finland is greatly thanked for the financial support. Ilpo Jääskeläinen from University of Kuopio is thanked for the fruitful co-operation in the course of this project.

References

Bard, A.J., Faulkner, L.R., 2001. Electrochemical methods. In: Fundamentals and Applications. John Wiley and Sons, USA.

Bathaie, S.Z., Moosavi-Movahedi, A.A., Saboury, A.A., 1999. Energetic and binding properties of DNA upon interaction with dodecyl trimethylammonium bromide. Nucl. Acid Res. 27, 1001–1005.

Bloor, D.M., Mwakibete, H.K.O., Wyn-Jones, E., 1996. EMF studies associated with the binding of cetyltrimethylammonium bromide to the polymers poly(propylene oxide), poly(vinylmethylether), and ethyl(hydroxyethyl) cellulose. J. Colloid Interface Sci. 178, 334–338.

Chu, D.Y., Thomas, J.K., 1986. Effect of cationic surfactants on the conformation transition of poly(methacrylic acid). J. Am. Chem. Soc. 108 (20), 6270–6276.

Cunnane, V., Schiffrin, D.J., Beltran, C., Geblewicz, G., Solomon, T., 1988. The role of phase transfer catalysts in two phase redox reactions. J. Electroanal. Chem. 247, 203–214.

Evans, N.J., Gonsalves, M., Gray, N.J., Barker, A.L., Macpherson, J.V., Unwin, P.R., 2000. Local amperometric detection of K^+ in aqueous solution using scanning electrochemical microcopy ion-transfer voltammetry. Electrochem. Commun. 2 (3), 201–206.

Hakkarainen, S., Gilbert, S.L., Kontturi, A.K., Kontturi, K., 2004. Amperometric method for determining the degree of complexation of polyelectrolytes with cationic surfactants. J. Colloid Interface Sci. 272, 404–410.

Harashima, H., Shinohara, Y., Kiwada, H., 2001. Intracellular control of gene trafficking using liposomes as drug carriers. Eur. J. Pharm. Sci. 13, 85–89.

Hayakawa, K., Kwak, J.C.T., 1982. Surfactant-polyelectrolyte interactions. 1. Binding of dodecyltrimethylammonium ions by sodium dextransulfate and sodium poly(styrenesulfonate) in aqueous solution in the presence of sodium chloride. J. Phys. Chem. 86, 3866–3870.

Hyvönen, Z., Plotniece, A., Reine, I., Chekavichus, B., Duburs, G., Urtti, A., 2000. Novel cationic amphiphilic 1,4-dihydropyridine derivatives for DNA-delivery. Biochim. Biophys. Acta 1509, 451–466.

Hyvönen, Z., Ruponen, M., Rönkkö, S., Suhonen, P., Urtti, A., 2002. Extracellular and intracellular factors influencing gene transfection mediated by 1,4-dihydropyridine amphiphiles. Eur. J. Pharm. Sci. 15, 449–460.

Liu, J., Nakama, M., Takisawa, N., Shirahama, K., 1999. Binding of dodecyloxyethylpyridinium bromide to polymer: the effect of molecular geometry of surfactant. Colloids Surf. A 150, 275–281.

Lobo, B.A., Davis, A., Koe, G., Smith, J.G., Middaugh, C.R., 2001. Isothermal titration calorimetric analysis of the interaction between cationic lipids and plasmid DNA. Arch. Biochem. Biophys. 386 (1), 95–105.

Mel'nikov, S.M., Sergeyev, V.G., Yoshikawa, K., 1995. Transition of double-stranded DNA chains between random coil and compact globule states induced by co-operative binding of cationic surfactants. J. Am. Chem. Soc. 117, 9951–9956.

Pichierri, F., Sarai, A., 1999. Properties of phosphorothiate analogs. An ab initio study of prototype model linkages derived from dimethylphosphate anion. Theochemistry 460, 103–116.

Pozharski, E., MacDonald, R.C., 2002. Thermodynamics of cationic lipid–DNA complex formation as studied by isothermal titration calorimetry. Biophys. J. 83 (1), 556–565.

Rodenhiser, A.P., Kwak, J.C.T., 1998. Polymer-surfactant systems: introduction and overview. In: Kwak, J.C.T. (Ed.), Polymer-Surfactant Systems. Marcel Dekker, New York, pp. 1–20.

Safinya, C.R., 2001. Structures of lipid–DNA complexes: supramolecular assembly and gene delivery. Curr. Opin. Struct. Biol. 11, 440–448.

Satake, I., Yang, J.T., 1976. Interaction of sodium decyl sulfate with poly (L-ornithine) and poly (L-lysine) in aqueous solution. Biopolymers 15, 2263–2275.

Schätzlein, A.G., Uchegbu, I.F., 2001. Non-viral vectors in gene delivery. Drug Deliv. Syst. Sci. 1, 17–23.

Segura, T., Shea, K.D., 2001. Materials for non-viral gene delivery. Annu. Rev. Mater. Res. 31, 25–46.

Shaw, D.J., 1992. Introduction to Colloid and Surface Chemistry, fourth ed. Butterworth–Heinemann Ltd., London.

Smith, J.S., Nikonowicz, E.P., 2000. Phosphorothioate substitution can substantially alter RNA conformation. Biochemistry 39, 5642–5652.

Spink, C.H., Chaires, J.B., 1997. Thermodynamics of the binding of a cationic lipid to DNA. J. Am. Chem. Soc. 119 (45), 10920–10928.

Torchilin, V.P., 2001. Structure and design of polymeric surfactant-based drug delivery systems. J. Control. Rel. 73 (2–3), 137–172.

Zimm, B.H., Bragg, J.K., 1959. Theory of the phase transition between helix and random coil in polypeptide chains. J. Chem. Phys. 31, 526–535.