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RNA and DNA interactions with zwitterionic and charged lipid membranes — A DSC and QCM-D study

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ABSTRACT

The aim of the present study is to establish under which conditions tRNA associates with phospholipid bilayers, and to explore how this interaction influences the lipid bilayer. For this purpose we have studied the association of tRNA or DNA of different sizes and degrees of base pairing with a set of model membrane systems with varying charge densities, composed of zwitterionic phosphatidylcholines (PC) in mixtures with anionic phosphatidylserine (PS) or cationic dioctadecyl-dimethyl-ammoniumbromide (DODAB), and with fluid or solid acyl-chains (oleoyl, myristoyl and palmitoyl). To prove and quantify the attractive interaction between tRNA and model-lipid membrane we used quartz crystal microbalance with dissipation (QCM-D) monitoring to study the tRNA adsorption to deposit phospholipid bilayers from solutions containing monovalent (Na^+) or divalent (Ca^{2+}) cations. The influence of the adsorbed polynucleic acids on the lipid phase transitions and lipid segregation was studied by means of differential scanning calorimetry (DSC). The basic findings are: i) tRNA adsorbs to zwitterionic liquid-crystalline and gel-phase phospholipid bilayers. The interaction is weak and reversible, and cannot be explained only on the basis of electrostatic attraction. ii) The adsorbed amount of tRNA is higher for liquid-crystalline bilayers compared to gel-phase bilayers, while the presence of divalent cations show no significant effect on the tRNA adsorption. iii) The adsorption of tRNA can lead to segregation in the mixed 1,2-dimyristoyl-sn-glycerol-3-phosphatidylcholine (DMPC)-1,2-dimyristoyl-sn-glycero-3-phosphatidylserine (DMPS) and DMPC–DODAB bilayers, where tRNA is likely excluded from the anionic DMPS-rich domains in the first system, and associated with the cationic DODAB-rich domains in the second system. iv) The addition of shorter polynucleic acids influence the chain melting transition and induce segregation in a mixed DMPC–DMPS system, while larger polynucleic acids do not influence the melting transition in these system. The results in this study on tRNA–phospholipid interactions can have implications for understanding its biological function in, e.g., the cell nuclei, as well as in applications in biotechnology and medicine.

1. Introduction

It is becoming increasingly evident that RNA plays a crucial role in the regulation of cellular events [1]. In the cell nucleus, DNA and RNA together with proteins form the chromatin complex, which packages and controls the expression of DNA in the cell. During the last years, it has been revealed that this complex also includes phospholipids [2,3], and that RNA indeed co-localizes with these lipids [3]. The presence of substantial amounts of phospholipids in the isolated cell nuclei has also been demonstrated from mass-spectroscopy studies [4], and it has been shown that the major lipid classes are saturated zwitterionic phosphatidylcholines (PC) and phosphatidylethanolamine (PE) as well as anionic phosphatidylinositol (PI) and phosphatidylserine (PS) [4]. The endonuclear lipids have been related to structural function, signal transduction and in stimulating RNA or DNA synthesis [5–9], although their precise function is still not fully understood. It is likely that the endonuclear lipids interact with RNA and DNA, and thus influence cellular processes.

Another system where DNA and RNA has been found to directly interact with lipid membranes is RNA and DNA viruses in the *Cystoviridae* and *Tectiviridae* families, where phospholipid membranes are present on the inside of the viral protein capsid [10,11]. This membrane is composed mainly of zwitterionic phospholipids like PE and anionic phospholipids like phosphatidylglycerols (PG), promoting close interactions between RNA/DNA and the membrane inside the viral capsid [11]. The membrane is believed to be involved in the assembly of the viral capsid as well as in the infection mechanism where the nucleic acids are transferred to a new host [12,13].

Even though the RNA–phospholipid interactions may have important implications to biological function and in gene therapy

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and other applications in biotechnology and medicine, few studies have been dedicated to the physico-chemical aspects of RNA interaction with phospholipids. In model membrane systems, RNA and lipid membrane interactions have been demonstrated for model membranes composed of phospholipids and a cationic lipid [14] and for zwitterionic phospholipid model membranes in the presence of divalent cations [15,16]. It has also been reported that the permeability of phospholipid membranes increases after association with RNA [15]. In all these examples, the interaction is mainly attributed to electrostatic attractions between RNA and an oppositely charged bilayer or to the zwitterionic PC bilayer mediated by divalent cations, and strong analogies can be drawn to the well characterized attractive interaction between DNA and cationic membranes or PC membranes in the presence of divalent cations [17–20]. Strong association of specially designed network-forming RNA's (RNA9–10) to phospholipid membranes has also been demonstrated in the presence of divalent cations [21–23].

The aim of the present study is to establish whether tRNA associates with lipid bilayers also in the absence of divalent cations, and to explore how the RNA–lipid interaction can influence the lipid bilayer, which in turn can provide information of how and where in the bilayer the association takes place. We have therefore investigated a set of model zwitterionic and anionic membrane systems, as these are relevant model systems for biological membranes [24–26], with varying charge densities and with fluid or solid acyl-chains together with tRNA or DNA of different sizes and degrees of base pairing. It is noted that RNA is generally not as inert or structurally constrained as DNA. In biological systems, the RNA molecules are also in almost all cases smaller than the DNA molecules. The chemical difference between RNA and DNA basically lies in that RNA nucleotides contain ribose while DNA contains deoxyribose, and that RNA has the base uracil instead of the base thymine that is present in DNA. The RNA backbone is more sensitive to nuclease degradation than the DNA backbone, especially under alkaline conditions. Another important difference is that RNA occurs more often than DNA in the single-stranded form, where the apolar parts of the bases are obviously prone to hydrophobic interaction with other species. The attractive interactions between DNA and hydrophobic surfaces have previously been demonstrated by Cardenas et al. by means of ellipsometry [27]. Their study showed that both ds and ss DNA adsorb to hydrophobic surfaces, and that the highest adsorption was shown for small single-stranded DNA molecules. The results were explained by hydrophobic interactions between the nitrogen bases of the DNA and the hydrophobic surface.

The RNA used in the present study is tRNA from baker's yeast and contains 70–90 bases. This includes both paired and single-stranded segments. To further explore how the size and the degree of base pairing affect the interaction with the lipid bilayers, parallel studies with different DNA molecules that are either purely single-stranded or double-stranded have also been performed. This comparison includes a 63 base pair single-stranded DNA ($ssDNA_{63}$), a 2000 base pair double-stranded DNA ($dsDNA_{2000}$), and a 2000 base pair single-strand DNA ($ssDNA_{2000}$). The $ssDNA_{63}$ has a poly-A chain and can therefore not form any double-stranded sequences. The $ssDNA_{2000}$ is obtained by heat induced unfolding of the $dsDNA_{2000}$. Two techniques have been used investigating the system, differential scanning calorimetry (DSC) and quartz crystal microbalance with dissipation (QCM-D). The model-membrane systems used in the DSC studies were multilamellar vesicles (MLV's) or large unilamellar vesicles (LUV's) with a diameter of 100–200 nm. For the QCM-D studies, we used bilayers on solid support, formed by deposition from mixed surfactant–phospholipid micellar solutions by approaching phase-separation limit according to Tiberg et al. [28,29].

The experimental approach taken is to

1) Prove and quantify the attractive interaction between tRNA and model-lipid membrane. For this purpose we used quartz crystal microbalance with dissipation (QCM-D). The aim is to confirm the adsorption of tRNA at the surface of fluid liquid-crystalline (L_α) or solid gel-phase (L_β) zwitterionic phosphatidylcholine (PC) bilayers in the presence of monovalent or divalent cations.
2) Reveal how polynucleic acids influence the lipid phase transitions and segregation in the model-lipid membrane. For this purpose we performed differential scanning calorimetry (DSC) studies. We studied model membrane systems of zwitterionic PC with fluid liquid-crystalline (L_α) or solid gel-phase (L_β) bilayers in the presence and absence of divalent cations. We also investigated the effect of bilayer charge density by mixing DMPC (1,2-dimyristoyl-sn-glycerol-3-phosphatidylcholine) with an anionic lipid, DMPS (1,2-dimyristoyl-sn-glycero-3-phosphatidylserine), or a cationic surfactant, DODAB (dioctadecyl-dimethyl-ammoniumbromide).

2. Material and methods

DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine), DMPC (1,2-dimyristoyl-sn-glycerol-3-phosphatidylcholine) and DODAB (dioctadecyl-dimethyl-ammoniumbromide) with 99% purity were purchased from Avanti polar lipids Inc. (Alabaster, USA). DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine) and DMPS (1,2-dimyristoyl-sn-glycero-3-phosphatidylserine) with 99% purity were purchased from Larodan fine chemicals (Malmö, Sweden). DDM (β-D-dodecyl-maltoside), 99.5% purity was purchased from Anatrace (Ohio, USA). Double-stranded salmon sperm DNA (2000 ± 500 bp) was purchased from Invitrogen Life Technologies (California, USA). NaBr, $CaCl_2$, MES and EDTA with 99% or higher purity, as well as, Ammonium hydroxide (25%), Molybdenum blue, spray reagent, PEG35000 and bakers' yeast tRNA (60–90 bp) was purchased from Sigma Aldrich (Missouri, USA). Short ss DNA (poly-A 63 bp, HPLC purified) was synthesized by MWG Biotech (Ebersberg, Germany). Agarose gel electrophoresis was used to check for degradation products in the DNA and tRNA samples. Chloroform, Methanol (both 99.8% purity) and TLC Aluminum sheets, 20×20cm silica gel 60 F_{254}, were purchased from Merck (Darmstadt, Germany). All chemicals were used as received without further purification. All solutions were prepared using ultrapure water from a Milli-Q® Ultrapure water purification system from Millipore (Massachusetts, USA). All glassware were soaked in 10% hydrochloric acid for several hours and then washed 10 times with Milli-Q water.

For all experiments the polynucleotides were dissolved in 10 mM NaBr aqueous solution. As tRNA is very sensitive to nuclease degradation, glassware used for tRNA studies were sterilized at 200 °C for 8 h and all solutions were autoclaved (121 °C for 20 min) prior to use. Control experiments were also done in a buffer with slightly acidic pH and completely free from multivalent ions, conditions where the tRNA is stable. For those control studies the buffer chosen was a 10 mM MES buffer pH 6.5 containing 1 mM EDTA. We did not observe any differences in the results obtained when using these different solutions, and we therefore presume that tRNA is stable to degradation under the experimental conditions used in this study.

2.1. Sample preparation

Mixtures of DMPC/DMPS and DMPC/DODAB were prepared as stock solutions in chloroform/methanol (Merck, HPLC grade) 2:1 and stored at −20 °C until use. To create multilamellar vesicles (MLV's), a small amount of lipid from one of the stock solutions was placed in a pear shaped glass flask and dried under nitrogen until all solvent had evaporated. For mixtures containing DMPS the solution must be heated above the T_m for the DMPS i.e. above 38 °C during removal of the solvent in order to keep the components homogeneously mixed. Samples were left under vacuum over night to remove the remaining solvent. The lipid film was suspended in freshly sterilized aqueous

solutions containing either 10 mM sodium bromide, NaBr, with pH measured to 6.5, or a 10 mM MES 1 mM EDTA solution with pH set to 6.5 or a 10 mM MES 2 mM $CaCl_2$ solution.

The solution was subjected to a freeze thawing cycle (10 times, freezing in CO_2(s) + ethanol, thawing in hot water bath 60 °C), in order to create MLV's. For preparation of large unilamellar vesicles (LUV's), the solution was heated above the chain melting transition of the lipids and extruded 10 times through a filter with pore size 100 nm (Acrodisc®, 25 mm∅ non pyrogenic sterilized syringe filter, Pall Corporation, Missouri, USA). The size of the LUV's was approximately 120 nm (checked with single angle light scattering, ZetaSizer, Malvern Instruments Ltd., UK). The LUV's were freshly prepared the same day as used. Since material is lost during extrusion, the lipid concentration of each sample was determined by phosphorus analysis [30], or from the melting enthalpy in the pure lipid system using the enthalpy data for pure DMPC and DMPS [31]. When cationic DODAB was incorporated in the vesicles, the measurements were performed on MLV's since the DODAB appears to interact with the syringe filter used for extrusion and completely clogged the filter. The lipid concentration was 0.16 ± 0.03 mM in all samples investigated.

The DMPC and DMPC/DMPS vesicles were mixed with the polynucleic acid at different concentrations. After thoroughly mixing, samples were placed in a 40 °C oven for 3 h before experiments were started since the association process may need some time [32]. No such time effects were observed for the DMPC/DODAB vesicles, and these systems were therefore mixed with tRNA immediately before the start of the measurement.

2.2. QCM-D

Quartz crystal microbalance with dissipation (QCM-D) measurements were performed using a Q Sense E4 system from Q Sense (Gothenburg, Sweden) with four measurement cells. The silica crystals used for the experiments were purchased from Q Sense. Prior to use the silica crystals were cleaned in 1% SDS solution for 1 h, washed with MQ water, dried under nitrogen and thereafter treated in a plasma cleaner from Harrick Scientific (New York, USA) for 5 min. Crystals were stored in ethanol until use. Before each measurement, the crystals were allowed to equilibrate with hydrochloric acid (0.1 mM) for 20 min before deposition of the bilayer. The bilayer was deposited on the surface according to the protocol developed by Tiberg et al. [28,29], using a solution of mixed micelles containing lipid and DDM at a ratio of 1:6, in hydrochloric acid (0.1 mM) at a concentration 0.114 g/dm^3. The method is based on gradually approaching the two-phase region of the DOPC–DDM aqueous system, thereby causing the deposition of a DOPC bilayer. This was achieved in several steps starting with adsorption of the micelle solution followed by rinsing with 0.1 mM HCl. The procedure was repeated using 10× and 100× diluted micellar solutions in order to generate a bilayer with high (>98%) surface coverage. The adsorption steps were performed at a flow rate of 50 µl/min and the rinsing at 100 µl/min. After formation of the bilayer, the bulk solution was changed to 10 mM NaBr solution or a solution with 10 mM NaCl and 1 mM $CaCl_2$. Polynucleic acids (in 10 mM NaBr or 10 mM NaCl/1 mM $CaCl_2$ solution) were then added continuously with a flow of 10 µl/min and the adsorption was studied during 20–80 min. Experiments were performed at 22 °C.

The wet mass of DOPC bound to silica was calculated according to the Sauerbrey expression

$$\Delta m = \frac{C}{o_n}\Delta f \quad (1)$$

where $C \approx 17.7$ ng Hz^{-1} cm^{-2} for a 5 MHz crystal and o_n is the overtone number [33]. The validity of applying this equation to non-rigid biomolecular systems has been addressed by Höök et al. [34], concluding that the Sauerbrey expression is a good approximation for rigid, thin and evenly distributed adsorbed films. However, also an acoustically rigid film may trap solvent, which becomes sensed as an additional mass. Hence, only in cases when the amount of coupled water is low, the "Sauerbrey mass" corresponds to the adsorbed molecular weight. Planar supported lipid bilayers constitute one such example, as verified with other methods such as ellipsometry [35]. In the case of polynucleotide coupling to supported lipid bilayers, the situation is more complicated [36], but as long as the damping is low, relative differences can still be trusted with high accuracy. Justified by the fact the adsorption of the DNA and tRNA was observed to cause only minor change in the dissipation and since we mainly are concerned with relative differences in the amount of polynucleotides adsorbed to phospholipid bilayers with different lipid composition, we applied the Sauerbrey expression to quantify the adsorbed mass of tRNA and DNA.

2.3. DSC

Differential scanning calorimetry (DSC) experiments were performed using a vp DSC from MicroCal inc. (North Hampton, USA) with a cell volume of 0.5072 ml. LUV's with a lipid concentration of 0.32 ± 0.03 mM was mixed with polynucleic acid to reach a final concentration of 0.16 ± 0.03 mM phospholipid and with a nucleic acid concentration varying from 0.01 mg/ml to 1 mg/ml. Temperature was increased by 1 °C/min during the measurements. Unless otherwise stated, 3 heating cycles were performed starting at a temperature of 5 °C and with the final temperatures of 50 °C, 90 °C and 50 °C, respectively, for each cycle.

2.4. TLC

Thin film liquid chromatography (TLC) was used to test for degradation products in lipid samples that had been exposed to heat (50–60 °C) for times up to 30 h. Lipid containing samples were freeze dried for 2 days and then dissolved in a mixture of chloroform/methanol 2:1. The lipid solutions and a reference containing DMPC, DMPS and lysolipid were spotted onto silica plates and placed in the TLC chamber until the solvent level reached 1 cm from the top of the plate. Plates were migrated with two different solvent compositions; chloroform/methanol/water (65:25:4) and chloroform/methanol/ammonium hydroxide (65:25:4). To develop the plates, molybdenum blue spray reagent was used, which visualizes the lipids as dark green spots.

2.5. UV–Vis

The unfolding of tRNA to fully single-stranded tRNA was studied using UV–Vis (260 nm). For this purpose samples containing solutions of tRNA was subjected to several heating/cooling cycles from 15–90 °C. The temperature was raised with 0.3 °C/minute when temperature increases and decreased with 3 °C/minute as temperature is lowered. The melting temperature of the tRNA was determined to 55 °C which is well in agreement with previous studies.

3. Results

3.1. Model systems

In this study we investigate how tRNA and different DNA molecules influence lipid phase behavior. In order to compare the results from the studies using different polynucleic acids, the concentrations are chosen so that, for all comparisons made, the number of nucleotides is the same, irrespectively of molecule size, and whether the polynucleic acids are single-stranded or not. The phospholipid phase behavior can be altered either by changing the

temperature or the length and degree of saturation of the acyl-chains. In the present study we chose the L_α model membranes either as DMPC (C14:0) above chain melting temperature $T_m \approx 24$ °C or DOPC (C18: 1) at room temperature ($T_m \approx -20$ °C) [37]. The gel-phase model membranes are either DMPC at lower temperatures (below $T_m \approx 24$ °C) or DPPC (C16:0) at room temperature ($T_m \approx 41.5$ °C)[37]. The influence of tRNA on lipid phase behavior was investigated in aqueous solutions with only monovalent (Na^+) cations and in the presence of divalent (Ca^{2+}) cations, and for lipid systems with varying charge densities. In order to vary the lipid charge density, we used model-lipid membranes composed of binary mixtures of the zwitterionic DMPC with an anionic lipid, DMPS, or a cationic lipid, DODAB. The bilayer charge density was varied between a highly negatively charged bilayer with $\Phi_{PS} = 100 \cdot m_{PS}/(m_{PS} + m_{PC}) = 40\%$ (wt.%) to slightly positively charged bilayers with $\Phi_{DODAB} = 100 \cdot m_{DODAB}/(m_{DODAB} + m_{PC}) = 5\%$. The melting temperatures for DMPS and DODAB have previously been reported to 38 °C and 44 °C, respectively [37,38].

We will start by giving a quantitative estimate of the interaction between polynucleic acids based on results from QCM-D measurements. These results will support our DSC experiments that monitor the effect on the state of the lipid membrane.

3.2. tRNA adsorption to deposited phospholipid bilayers

QCM-D is a powerful technique for studies of adsorption to solid surfaces. When applied in aqueous solutions, the measurements rely on the changes in resonance frequency, f, and energy dissipation, D, of a quartz crystal upon adsorption of material to one of its interfaces. We used the QCM-D technique to answer the questions whether tRNA adsorbs to zwitterionic bilayers in gel and in liquid crystal phase and how the lipid phase affect the adsorption. We also study the effect of divalent Ca^{2+} ions on tRNA adsorption. The systems chosen for this study are bilayers formed from DOPC L_α liquid-crystalline phase and from DPPC L_β gel phase, with the temperature set to 22 °C. The bilayers were deposited at the solid surface from a mixed micellar solution with nonionic detergents [29] as described in Section 2. Successful deposition gives surface coverage that corresponds to an almost complete lipid bilayer as determined from the frequency shift using the Sauerbrey equation [29]. For DOPC this amounts to 4.00–4.50 mg/m^2, while the corresponding value for the DPPC bilayer was slightly higher, around 4.75 mg/m^2. The difference in the amount of lipid deposited is attributed to the denser packing in the DPPC L_β gel-phase bilayers compared to the DOPC liquid-crystalline L_α bilayer[37].

Fig. 1 shows representative QCM-D data for tRNA adsorption to L_α DOPC and L_β DPPC bilayers. In these experiments, the bilayer surface was exposed to a constant flow of a solution containing 0.1 mg/ml tRNA for 20–80 min and thereafter rinsed with 10 mM NaBr solution. For all systems investigated, the adsorption process is relatively slow, and it can be divided into two kinetic regimes (Fig. 1a). An increase in mass, Δm, is detected immediately after the addition of tRNA, and it continuously increases over 5–10 min. After this initial and major adsorption step, there is still a gradual, but small increase in Δm with time until the tRNA solution is replaced with 10 mM NaBr solution. The initial adsorption step gives a surface excess, Γ, of tRNA of 14 mg m^{-2} to the DOPC L_α bilayers and 6 mg m^{-2} to the DPPC L_β bilayer. The measured Δm for the adsorption of tRNA to the bilayer corresponds to one tRNA molecule per 1700 DOPC lipids, and one tRNA per 4300 DPPC lipids. From these results we conclude that the coverage of tRNA on the lipid surface is low but significant. No significant difference in adsorption upon addition of Ca^{2+} was observed. The adsorbed amount decreases when the tRNA solution is replaced with a solution containing only 10 mM NaBr (rinsing), which indicates tRNA desorption.

From the data in Fig. 1a, we conclude that the adsorbed amount of tRNA to the fluid L_α DOPC bilayers is more than twice the adsorbed amount to the solid L_β DPPC bilayers (Fig. 1a). Fig. 1b shows a

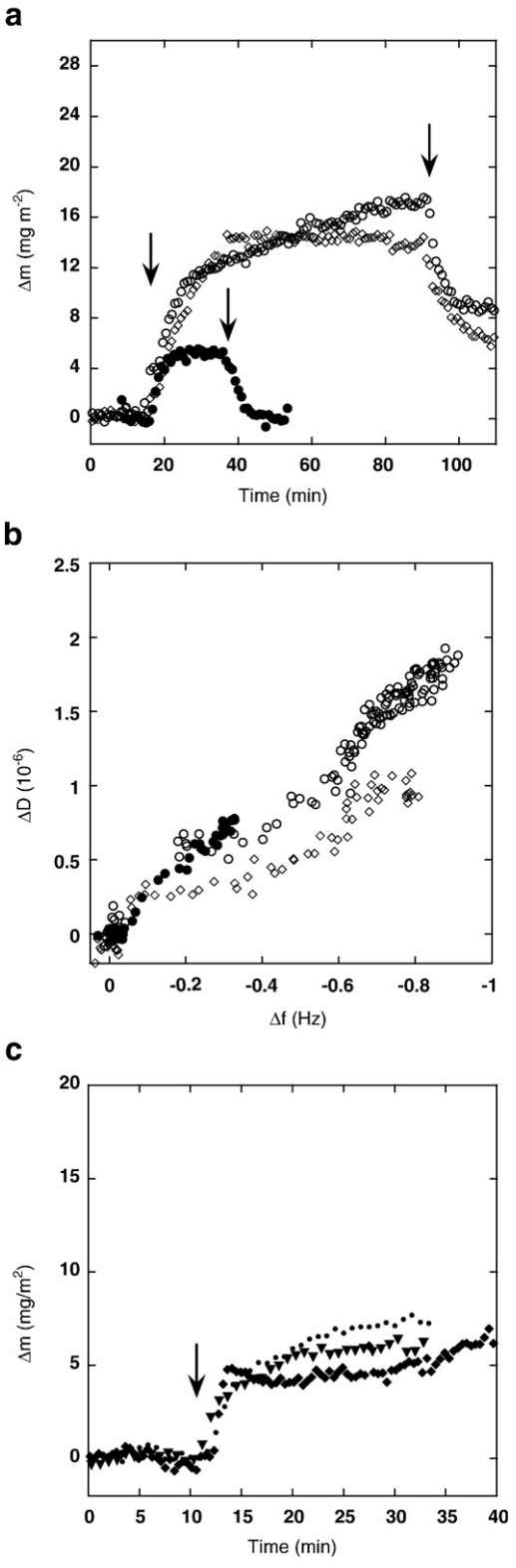

Fig. 1. Addition of tRNA to surface-deposited bilayers monitored by QCM-D. a) the adsorbed amount of tRNA to surface-deposited DOPC L_α bilayer from aqueous solution containing 10 mM NaBr (○) and 10 mM NaBr + 1 mM $CaCl_2$ (◇), and to DPPC L_β bilayer from an aqueous solution containing 10 mM NaBr (●) obtained from QCM-D data evaluated from the recorded frequency shift by using the Sauerbrey expression (for $o_n = 3$). The addition of tRNA and the start of the rinsing with 10 mM Na Br are indicated by arrows. b) A correlation plot of ΔD as a function of Δf ($o_n = 3$) for the same systems as in (a). c) A comparison between three different DPPC experiments is shown, the adsorbed amount of tRNA is small in all cases but the results are reproducible.

correlation plot of ΔD as a function of Δf for the same systems as Fig. 1a. Although the lower coverage of tRNA on the DPPC bilayers means that the complete curves cannot be compared over the whole data range, the initial parts of the curves obtained from the three different samples overlap and have similar slopes. This implies that the conformation of the adsorbed molecules does not seem to differ between these different systems. The low dissipation implies a rather flat conformation of tRNA parallel to the bilayer surface.

From the results shown in Fig. 1b, we conclude that the dissipation for all systems investigated is small and thus in the limit where the Sauerbrey equation should be applicable as reported by Höök et al. [34]. It should be noted that in addition to the "dry" tRNA mass, QCM-D measurements are sensitive also to the viscoelastic properties of the layer as well as the water hydrodynamically coupled to the film (wet mass) [34]. As the amount of tRNA at the surface is rather low, one would expect that the amount of water in the layer is small, which implies that the amount registered with QCM-D mainly reflect the tRNA. This is consistent with results from ellipsometry and QCM-D studies of dsDNA (2000–4300 base pairs) adsorption to PC bilayers that show a good agreement in adsorbed mass [39]. The study also showed that the surface coverage of dsDNA is fairly low and within a thin layer, therefore the amount of water coupled, measured by QCM-D should be small. Under all circumstances, since the change in dissipation was low for all cases (Fig. 1), the relative difference in tRNA adsorption to the two different bilayer systems can be trusted with high accuracy. We also note that, although the measured effects are small, they are reproducible. As an example of this, Fig. 1c shows the adsorbed mass as a function of time for tRNA adsorption to DPPC bilayers from three separate experiments.

Finally, we point out that QCM-D measurements were only performed for bilayer systems with only one lipid component because of the uncertainty regarding lipid distribution between the upper and lower leaflet of a deposited mixed bilayer. This uncertainty is particularly relevant for systems with charged lipid species as the silica surface is slightly negative and the lipid–surface interaction clearly play a role for the lipid distribution in the bilayer. In fact, it has been shown that the composition of the monolayer facing the solid support and the monolayer facing the solution can differ radically in composition in mixed lipid bilayers [40].

3.3. DSC studies of tRNA and mixed phospholipid bilayers

The interaction between the lipid bilayer and macromolecules like tRNA and DNA can potentially alter the properties of the lipid membrane, and influence lipid acyl-chain packing, phase transitions and lipid segregation. In this study, we investigated these aspects of tRNA–lipid interactions by means of DSC.

MLV's of DMPC dispersed in excess 10 mM NaBr aqueous solution exhibit two endothermic transitions upon heating, a lower enthalpy pre-transition (T_{pr}) at 14 °C, and a higher enthalpy main transition (T_m) at 24 °C (data now shown). The pre-transition arises from the conversion of a planar lamellar gel (L_{β}) phase to the rippled gel (P_{β}) phase and the main transition from the chain melting associated with the conversion between the P_{β} gel phase to the lamellar liquid-crystalline (L_{α}) phase. For LUV's, the pre-transition is generally not observed, which is also consistent with previous observations [41–43], and T_m for the L_{β}–L_{α} transition was measured to 24.0 °C (Fig. 2a). LUV's composed of binary mixtures of DMPC and DMPS also give rise to a single peak in the thermogram and the T_m varied between 24 and 29 °C depending on the PS content ($\Phi_{PS}=0\%$–$\Phi_{PS}=40\%$) (see Fig. 2). Thus, the lipids show good miscibility in both the L_{β} and in the L_{α} phases in this concentration regime, and the two-phase coexistence regions are rather narrow for all mixtures

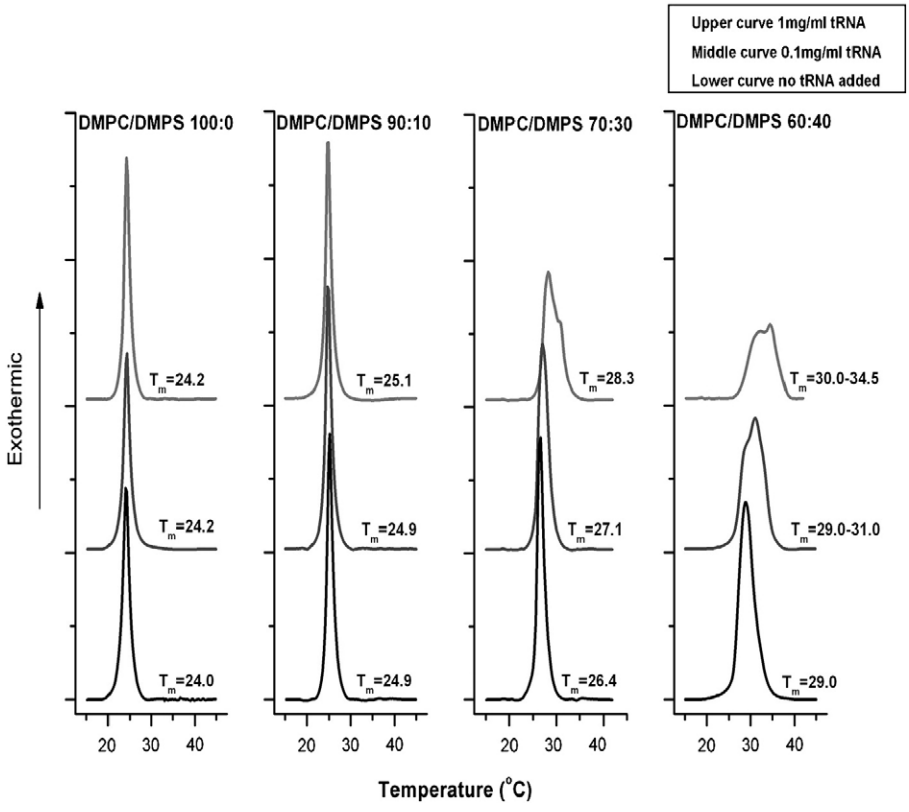

Fig. 2. Enthalpy traces obtained from DSC. The amount of anionic lipid increases from right to left in the figure and the tRNA concentration increases from bottom row to the top row. The transition temperature from gel to liquid-crystalline phase increase with increasing concentration of tRNA, this is most obvious for the bilayers containing 30 and 40% anionic lipid where also a splitting of the transition peak is observed.

investigated as implied from the narrow peaks. This is also consistent with previous studies of similar systems [31,44,45].

Fig. 2 shows a summary of the enthalpy traces obtained for DMPC LUV's and DMPC/DMPS LUV's ($\Phi_{PS}=0\%$ to $\Phi_{PS}=40\%$) after the addition of varying amounts of tRNA (only monovalent Na^+ cations). The data are also summarized in Table 1. The most striking observation is that the addition of tRNA only affects the lipid phase transition for the mixed lipid systems. The addition of tRNA to dispersions of DMPC LUV's does not affect T_m significantly, although it does cause a slight broadening of the transition peak (Table 1, Fig. 2). This has also been observed by others in similar systems [14]. On the other hand, addition of tRNA to the mixed DMPC/DMPS LUV's leads to an increase in T_m, a broadening of the transition peak and sometimes also splitting of the peak. This implies that the solid gel phase is stable at slightly higher temperatures in the presence of tRNA compared to the pure lipid system. The broadening and splitting of the peak imply reduced miscibility and segregation in the bilayer. No significant changes in transition enthalpy upon the addition of tRNA could be concluded for any of these systems.

As illustrated in Fig. 2, the transition temperatures increase with increasing concentration of tRNA, and this is most obvious for the bilayers with the highest content of anionic lipids. For vesicles with $\Phi_{PS}=10\%$, the increase in T_m is minor and only observed at the higher concentrations of tRNA. When the fraction of anionic lipid in the bilayer is increased, the effects are even more pronounced. At $\Phi_{PS}=30\%$, the addition of tRNA at concentration 0.1 mg tRNA/ml causes an increase in T_m of ca 1 °C as well as a broadening of the peak. When the concentration of tRNA is increased to 1 mg tRNA/ml, T_m increases further, and a splitting of the transition peak is observed. At $\Phi_{PS}=40\%$, the splitting of the transition peak is visible already at the lower concentrations of tRNA. The splitting of the transition peak can be explained by lipid segregation, that can be related to the formation of DMPC-rich domains and DMPS-rich domains with different melting temperatures.

The results in Fig. 2 and Table 1 were all obtained for solutions with only monovalent Na^+ present. To further explore the influence of adding divalent cations, experiments were performed in a solution containing 2 mM $CaCl_2$. The results for tRNA and DMPC vesicles in the presence of Ca^{2+} are shown in Table 2. The T_m for the DMPC system in this solution was 24 °C, and no effect on T_m was observed even at the higher concentrations of tRNA. The influence of Ca^{2+} on the systems of tRNA and mixed zwitterionic/anionic phospholipids was not investigated as the addition of Ca^{2+} lead to segregation and sometimes even precipitation in these lipid systems [46,47].

3.4. DSC studies of different polynucleic acids and phospholipid vesicles

tRNA is generally relatively short compared to DNA, and it contains both unpaired and paired bases. To further investigate which of the polynucleic acid properties it is that influences the phospholipid phase behavior, we conducted experiments where we compared tRNA, with other single and double-stranded polynucleic acids; $ssDNA_{63}$, $dsDNA_{2000}$ and $ssDNA_{2000}$. The results for the change in transition temperature are shown in Table 2. The main conclusion from Table 2 is that the completely single-stranded $ssDNA_{63}$, which is of similar size as tRNA, show similar influence on the lipid phase transition as tRNA. The longer DNA molecules do not seem to influence the lipid phase behavior at any concentration. None of the investigated polynucleic acids affected the melting transition of pure DMPC in any detectable way. The influences of the polynucleic acids on T_m at varying lipid compositions are further illustrated in Fig. 3. From these data we conclude that both tRNA and $ssDNA_{63}$ can induce lipid segregation, while $dsDNA_{2000}$ and $ssDNA_{2000}$ do not show any influence on the transition or the phase segregation. Together, this implies that the size of the polynucleic acid is crucial for its effect on the lipid phase behavior.

Table 2
Shown is the ΔT_m measured for DMPC and DMPC/DMPS (70:30) vesicles in the absence and in the presence of tRNA, $ssDNA_{63}$, $dsDNA_{2000}$ and $ssDNA_{2000}$ where the ΔT_m is the difference in T_m between the pure lipid samples and the RNA containing samples ($\Delta T_m = T_{m,lipid+RNA} - T_{m,lipid}$). The lipid concentration was 0.3 mM. Nucleic acid concentration was 0.1 mg/ml and 1 mg/ml.

Nucleic acid	Concentration	DMPC ΔT_m(°C)	DMPC/DMPS 70:30 ΔT_m(°C)
tRNA	0.1 mg/ml	+0.1	+0.5
	1 mg/ml	+0.2	+2
			+4.5
tRNA in 2 mM $CaCl_2$	1 mg/ml	0	–
$ssDNA_{63}$	0.1 mg/ml	0	+0.4
dsDNA2000	0.1 mg/ml	+0.1	0
	1 mg/ml	–	+0.1
ssDNA2000	0.1 mg/ml	0	0

For the interpretation of the DSC data on lipid melting transition, it is important to realize that changes of the lipid phase transitions does not directly prove adsorption of the added molecules (the polynucleic acids), but could also be due to changes in the solution properties. For example, the addition of water-soluble non-interacting polymers, e.g. polyethyleneglycol (PEG) can cause a shift in phase transition due to dehydration (osmotic stress) of the lipid system [48]. In the case of DMPC, dehydration leads to an (small) increase in T_m, and we can expect a similar response for the mixed DMPC/DMPS system. As a control experiment, we therefore performed DSC experiments for DMPC/DMPS vesicles ($\Phi_{PS}=30\%$) in solution containing 2 mg/ml PEG solution, using a PEG of similar size as the tRNA ($Mw_{PEG}=35\,000$ g/mol). Addition of the PEG solution at this low concentration did not cause any measurable changes on the T_m, neither did it cause any segregation or broadening of the transition peak. From this control experiment, we conclude the observed effects are not simply due to lipid dehydration caused by the reduced water activity after addition of the polynucleic acid polymers.

The DSC was performed in three cycles with the starting temperatures of 5 °C and the final temperatures of 50 °C, 90 °C and 50 °C for each cycle, respectively. The data shown in Figs. 2, 3 and Tables 1–2 were obtained in the third cycle, which is considered to

Table 1
Shown is the T_m measured for DMPC and DMPC/DMPS vesicles in the absence and in the presence of tRNA. The values are given with a confidence interval where the temperature is calculated as a mean over several (>3) measurements. For DMPC/DMPS 60:40 and for DMPC/DMPS 70:30 containing tRNA two melting temperatures are given since the addition of tRNA gave rise to a splitting of the transition peak. The T_m given in the table was obtained from the 3rd heating scan. ΔT_m is the difference in T_m between the pure lipid samples and the RNA containing samples ($\Delta T_m = T_{m,lipid+RNA} - T_{m,lipid}$).

tRNA concentration	DMPC			DMPC/DMPS 90:10			DMPC/DMPS 70:30			DMPC/DMPS 60:40		
	T_m(°C)	ΔT_m	$T_{1/2}$(°C)	T_m(°C)	ΔT_m	$T_{1/2}$(°C)	T_m(°C)	ΔT_m	$T_{1/2}$(°C)	T_m(°C)	ΔT_m	$T_{1/2}$(°C)
0 mg/ml	24.0±0.1		1.6	24.8±0.2		1.6	26.6±0.5		2.3	27.9±0.6		3.5
0.1 mg/ml	24.1±0.1	+0.1	1.6	24.8±0.2	0	1.3	27.1±0.4	+0.5	2.5	29.6±0.9	+1.7	4.4
										30.8±0.9	+2.9	
1 mg/ml	24.2±0.1	+0.2	1.3	25.3±0.3	+0.5	1.8	28.6±0.6	+2	4.3	31.7±1.2	+3.8	5.1
							31.1±0.6	+4.5		34.1±1.2	+6.2	

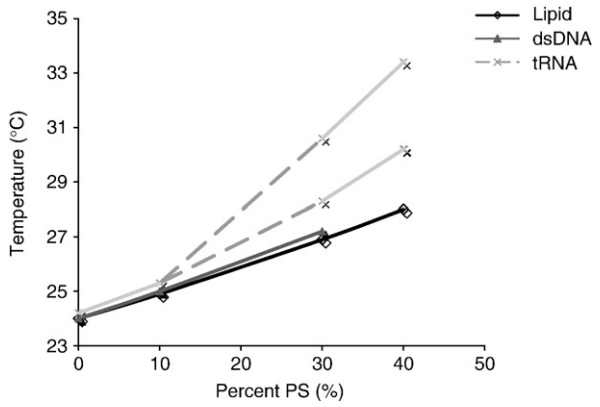

Fig. 3. Shown is how different nucleic acids influence the T_m as the lipid composition is changed, the nucleic acid concentration is in all cases 1 mg/ml. At the addition of dsDNA only a marginal change in T_m of the lipid is to be seen whereas when tRNA is added, segregation as well as an increase in T_m is visible.

correspond to steady-state conditions. Indeed, the DSC experiments showed on a gradual increase in T_m and peak broadening over the first heating cycles, while no further changes were observed after the third heating cycle. This implies that the system has reached steady-state (stable) conditions after three cycles. This behavior can be explained by slow kinetics and low adsorption, which is also supported by the QCM-D data for the pure PC bilayers (Fig. 1). It is also consistent with previous studies showing that the adsorption of ssDNA and dsDNA to a hydrophobic surface is a relatively slow process [27]. Furthermore, diffusion controlled adsorption processes is expected to be faster at the elevated temperatures. We also observed that T_m and peak splitting/broadening increased already in the first heating cycle when the samples were pre-equilibrated for longer periods (>12 h) at 50 °C (not shown), again supporting slow kinetics and diffusion controlled adsorption process. However, the long exposure to high temperature in slightly acidic solutions (pH 6–6.5) could also promote hydrolyses of the phospholipids, as also described for similar lipid systems [49], and the kinetics at elevated temperatures was therefore not further investigated. Finally, samples that had been studied in the DSC experiments in several heating cycles were analyzed by means of TLC, and no traces of hydrolysis products could be detected with this method.

3.5. Effects of bilayer charge density

The interaction between bilayers and the highly negatively charged polynucleic acids is strongly dependent on the bilayer charge density. We therefore investigated how tRNA influences the lipid phase behavior and segregation for bilayer systems containing both anionic lipids and cationic double chain surfactants. The data presented above involve neutral or anionic lipids, and the interaction is weak, reversible and exhibit slow kinetics. The situation is radically different if the bilayer instead carries a positive charge by inclusion of cationic lipids, as this add a strong electrostatic attractive contribution to the interaction force. In the present study, we used model-lipid membranes composed of DMPC and a small amount of DODAB.

Fig. 4 shows the DSC traces for mixed vesicles containing cationic or anionic lipids and how they are affected by the addition of tRNA. The DMPC/DODAB ($\Phi_{DODAB}=5\%$) vesicles give a DSC thermogram with a single and sharp transition peak at $T_m=26.7$ °C, which implies good miscibility of DMPC and DODAB in both the fluid and the solid phases (Fig. 4). This result is consistent with previous studies on binary mixtures of DODAB and PC's [50]. The addition of tRNA to the DMPC/DODAB vesicle dispersion leads to phase segregation as observed from the splitting of the transition peak. In the presence of tRNA the melting takes place in two steps, one melting event with higher enthalpy at slightly lower temperature (25.8 °C) than for DMPC/DODAB dispersion, and another melting event with lower enthalpy at higher temperature (34.4 °C). Both transitions occur between the transition temperatures of pure DMPC and pure DODAB. This implies the presence of DMPC-rich and DODAB-rich domains with different melting temperatures. Similar observations have previously been reported when DNA or RNA were added to mixed cationic–zwitterionic vesicles [14,51,52].

In summary, we have shown that tRNA induces lipid segregation in all types of mixed lipid systems investigated. There are clear differences between different systems. As expected the tRNA–lipid interaction is much stronger when the vesicles include cationic lipids, as shown from the observed concentration dependence. In the experiments with cationic surfactants, the effects of tRNA on the thermal transition was observed already at a tRNA concentration of 0.01 mg/ml, which is 10 times lower than the lowest concentration where effects were observed for the mixed DMPC/DMPS systems. We are aware that this relates to the total tRNA concentration, and not the tRNA concentration at the vesicle surface. In other words, it is possible that the partitioning of tRNA to the vesicle is much higher for the cationic vesicles, while the amount of tRNA on the surface needed to induce segregation does not have to differ between the systems. For the DMPC/DODAB system, the charge ratio between cationic lipid and tRNA was 0.95, and higher ratio of tRNA leads to precipitation. It is also noted that the effects of tRNA addition to the positively charged vesicle system appear to occur faster as steady-state is reached already during the first heating cycle, which was not the case for the negatively charged vesicles.

The data shown in Fig. 4 are obtained for MLV's. The LUV's used to obtain the results presented in Figs. 2–4 were prepared by extrusion through a 100 nm syringe filter. We could not use the same protocol for the DODAB-containing vesicles as for the zwitter- and anionic vesicles, as loss of material occurred during the extrusion due to the strong interaction between the cationic surfactant and the extrusion membrane. Therefore, control experiments were also performed for DMPC and DMPC/DMPS MLV's and the results showed the same effects of tRNA as described above for LUV's.

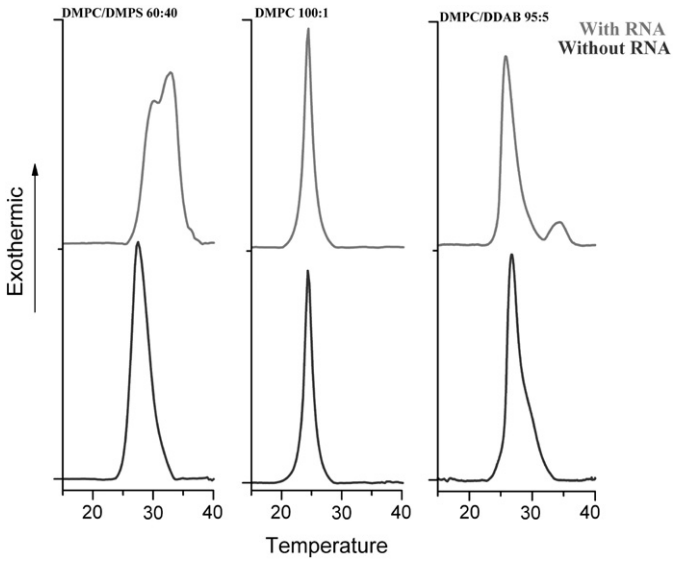

Fig. 4. Enthalpy traces obtained from DSC for three different lipid mixtures, with anionic lipids to the left, zwitterionic in the middle and cationic to the right. Melting of the mixed lipid bilayers are clearly affected by the presence of tRNA while the zwitterionic bilayer remains unchanged. In both cases of mixed membranes a segregation of the melting transition is visible. In the DODAB-containing bilayer the melting in the presence of tRNA takes place in two steps. Note that the tRNA concentration is significantly lower in the mixed DMPC/DODAB membrane than in the DMPC/DMPS membrane.

3.6. Unfolding of tRNA and DNA

The unfolding of the polynucleic acids, that is the separation of the double-stranded polynucleic acid segments into single-stranded chains ("melting"), was studied by means of DSC and UV–Vis. The unfolding temperature of $dsDNA_{2000}$ was determined to be 62 °C (data not shown), which is consistent with previous reports [53–55]. We were not able to measure the heat of unfolding of the tRNA with DSC due to the low enthalpy of the transition. The unfolding temperature was instead studied by means of UV–Vis. Samples containing solutions of tRNA was subjected to several heating/cooling cycles from 15–90 °C. The unfolding temperature was determined to be ca 55 °C, which also agrees with previous studies [56,57]. From the UV–Vis experiments, we could also conclude that the unfolding of the tRNA molecule appears to be reversible when temperature is lowered below the unfolding temperature. It is, however, possible that the effects of tRNA on the DMPC/DMPS phase transitions might be related to the unfolding of tRNA during the DSC experiment at temperatures above 55 °C, thus giving rise to an increased number of unpaired bases, which potentially is more prone to interact with the lipid membrane. This might explain the similar responses observed for tRNA and $ssDNA_{63}$.

4. Discussion

4.1. tRNA adsorption to phospholipid bilayers

RNA and DNA are highly negatively charged polyelectrolytes that adopt an extended conformation in solution of low ionic strength due intramolecular electrostatic repulsion. They form complexes with oppositely charged molecules, which in turn can lead to conformational changes of the polyelectrolyte and compaction. The interaction is driven by electrostatic attraction, and it has been extensively studied during the last decades due to applications as gene delivery systems [58–60]. The main focus of the present study lies on far less explored aspects of more subtle interactions with neutral and anionic lipids that are abundant in the cell membrane and cell nuclei [22,61], and that may be responsible for the RNA–lipid co-localization in the chromatin complexes [2,3].

A first important conclusion from the present study is that tRNA adsorbs to zwitterionic liquid-crystalline and gel-phase phospholipid bilayers both in the absence and presence of divalent cations. The interaction is weak and reversible, and it cannot be explained only on the basis of electrostatic attraction. The higher adsorption to the fluid L_α than to the solid and L_β bilayer indicates that the lipid chain packing influence the adsorption. These results agree with previous studies of oligonucleotide and DNA adsorption to bilayers with different chain packing [62,63]. Here it is noteworthy that even dsDNA show low but significant adsorption to hydrophobic surfaces [27], and with zwitterionic lipid bilayers also in the absence of divalent cations [39,62]. DNA confinement in PC multilamellar membranes has also been proposed from small angle X-ray scattering (SAXS) studies on vesicle systems [64,65].

The QCM-D studies show adsorption of tRNA both to L_α and L_β PC bilayers. Still, the DSC experiments on the similar systems show that tRNA does not influence the chain melting transition from the L_β phase to the L_α phase. The same conclusion is drawn for tRNA–PC systems both in the presence and absence of divalent Ca^{2+}. Similarly, $dsDNA_{2000}$ did not affect the melting transition in the zwitterionic lipid system, even though $dsDNA_{2000}$ has also been shown to adsorb to zwitterionic phospholipid membranes (Ainalem et al. In Press, Langmuir). At a first glance, these QCM-D and DSC results might appear contradictive. However, here should be pointed out that DSC does not directly give a quantitative measure of the interaction, but rather shows how the interaction influences the lipid phase behavior. The combination of QCM-D and DSC can therefore be used to say something about how the tRNA adsorbs on the bilayer. Our results do show that tRNA adsorbed to PC bilayers, but it does not influence the lipid chain packing in a major way. If there was significant penetration of tRNA into the hydrophobic core of the bilayer interior, melting point-depression would have been expected [66]. We therefore propose that the adsorbed tRNA molecules are present in the lipid–aqueous interfacial region of the bilayer and that the apolar bases of tRNA interacts with exposed apolar parts of the bilayer interface. Furthermore, the highly charged phosphate groups of the polynucleic acids are very unlikely to penetrate deeper into the apolar region of the bilayer.

One important finding in the present study is that the shorter polynucleic acids tRNA and $ssDNA_{63}$ can induce segregation in the mixed lipid bilayers, which indicates phase separation and heterogeneous distribution of the lipids. The data are consistent both with the formation of microdomains in the bilayer and with macroscopic segregation. In the present case, no larger aggregates or precipitation was visible even after several days although we cannot completely rule out the presence of a small amount of aggregates that are not visible by a naked eye. The most likely explanation for the data in Figs. 2 and 3 are lateral segregation and formation of domains with different composition in the bilayer. The anionic polynucleic acids are likely associated with the zwitterionic DMPC-rich domains and excluded from the anionic DMPS-rich domains due to electrostatic repulsion. We note that T_m for both the DMPS-rich and DMPC-rich domains are higher than T_m for the DMPC/DMPS mixtures in the absence of tRNA. This result is perhaps a bit surprising as the addition of tRNA to the pure DMPC vesicles did not cause any change in T_m. It is possible that the short polynucleic acids may accumulate in the domain boundaries, and thereby stabilize the domain structure as a line-active agent in a similar way as has been previously shown for, e.g., cholesterol and some proteins [67,68]. This could lead to stabilization of the PC-rich domain and may thus explain the increase in T_m. Additional studies of the system employing surface or imaging techniques would be useful to further elucidate this effect.

Segregation is also observed for the situation when the bilayer contains cationic DODAB together with zwitterionic DMPC. In this case, we expect that tRNA mainly associate with the DODAB-rich domains. Complex formation between the negatively charged DNA and positively charged lipids and surfactant has been extensively studied [59,69–71]. The strong association is due to electrostatic attraction between the oppositely charged components [69]. In lamellar complexes formed by DNA, DODAB and zwitterionic dilauryl phosphatidylcholine (DLPC), solid-state NMR studies have shown on local segregation of the lipids in the bilayer and formation of DODAB-rich domains with the headgroups close to the DNA helix [72]. Similar association could be expected in the more dilute DMPC–DODAB vesicle system investigated here. Still, the melting temperatures measured for the DMPC/DODAB vesicles in the presence of tRNA (Fig. 4) imply that phase segregation is not complete. The lower temperature transition in Fig. 4 is interpreted as melting of the DMPC-rich domains and the fact that the transition temperature is higher than that of pure DMPC vesicles indicate also the presence of DODAB in these domains. The increased melting temperature of the DMPC-rich domains can also be related to the stabilization of domain interfaces as suggested for the segregated DMPC/DMPS systems. The higher temperature transition is presumably due to melting of DODAB-rich domains. The transition temperature is clearly lower than that of pure DODAB, implying that the domains contain also tRNA and possibly also some DMPC. Similarly, it has been reported that the addition of DNA to mixed DOPE/DODAB bilayers ($\Phi_{DODAB} =$ 50%) lead to lowering of T_m [73]. On the other hand, for pure cationic vesicles of DODAB or DMTAB, the addition of DNA leads to increasing T_m [51,73].

From the studies of different polynucleic acids together with DMPC/DMPS vesicles, we can draw some conclusions on the relation

between polynucleic acid properties and their influence on the phospholipid phase behavior. Here we note that all the experiments were performed so that the number of bases for the different types polynucleic acids, and thus also the charge ratio, were comparable between the different systems and varied between 2 and 20 bases per lipid, where 0–40% of the lipids carried negative charges. From Fig. 3 together with the data in Table 2, it is clear that tRNA and $ssDNA_{63}$, affect the melting transition and the segregation in similar manner, while the larger polynucleic acids, $dsDNA_{2000}$ and $ssDNA_{2000}$ do not influence the melting transition. Together, this indicates that the size of the polynucleic acids is important in determining its influence on the lipid phase behavior. The comparison between the short polynucleic acids, tRNA, which have both double-stranded and single-stranded segments, and $ssDNA_{63}$ that is completely single-stranded suggests the existence of unpaired bases, but it is not necessarily only the number of unpaired bases, that plays a role in determining extent of the observed effects. Here we also would like to point out that it is possible that unfolding of tRNA at $T \approx 55$ °C might affect the interaction with the lipid bilayer observed in the DSC studies. The observation that the long unfolded $ssDNA_{2000}$, does not show any influence on the phase transition may be explained by the fact that it is more difficult for the large polymer due to entropic "steric" (and electrostatic) repulsive forces, to adsorb or penetrate into the lipid bilayer. Similar trends related to polynucleic acid size have previously been reported for small (146 bp) and large (2000 bp) single and double-stranded DNA adsorption to hydrophobic surfaces, where substantially higher adsorption of the short chain ssDNA compared to long chain ssDNA on hydrophobic surface was demonstrated by means of ellipsometry [27]. In the same study, it was also shown that the small DNA molecules adsorbs flat along its entire length instead of having loops and tails as in the case of long DNA. It is reasonable to assume a similar conformation at the bilayer interface, and we therefore expect more close contacts and thereby stronger interaction for the shorter polynucleic acids.

One fundamental question in this work is the nature of the driving force for the tRNA–lipid interaction. The association of polynucleic acids with cationic bilayers is clearly driven by electrostatic attraction. The attraction to the membranes that contain zwitterionic or binary zwitterionic/anionic lipid bilayers, on the other hand, is not completely understood. The difference in adsorption to the fluid L_α bilayer compared to the solid L_β bilayer show that the lipid chain packing influence the adsorption, and it is possible that the observed difference is due to the interactions between the hydrophobic bases of tRNA and the exposed hydrophobic parts of the fluid L_α bilayer. The results from the QCM-D studies can also be compared to the theoretical model by Dan et al. that describe interactions between dsDNA and L_α phase bilayers, suggesting that the adsorbed DNA perturbs the lipid packing and thereby introduce an attractive membrane interaction [74].

We suggest that hydrophobic interaction between the polynucleic acids with exposed single bases and the hydrophobic parts of the bilayer is one important contribution to the attraction. In a fluid bilayer system, the bilayer interface is dynamic and there is clearly water–hydrocarbon contact even for a bilayer with a high surface coverage. This is inherited in the system and has been demonstrated in molecular dynamics and coarse grain simulations [75, 76]. Another issue is the presence of defects in the bilayer, which cannot be ruled out. Such defects also exist in biological systems. This agrees with the observation that ssDNA adsorb stronger to hydrophobic surfaces compared to dsDNA, while there is still adsorption also of the double-stranded DNA species [27]. There are also several studies of DNA and oppositely charged amphiphiles demonstrating that the association is not solely controlled by the electrostatic attraction, but that also the hydrophobicity of the surfactant moiety affects its interaction with DNA [77]. Another possible reason for the attractive interaction can be local polarization of the zwitterionic PC headgroup when large polyanionic molecules approach the surface [78,79]. It is possible that the positively charged parts of the PC headgroup are more exposed in the bilayer interface and enable electrostatic attraction to the tRNA or DNA, and the tilt might also differ between the L_α and L_β phases [80,81]. However, the higher adsorption to the fluid L_α than to the solid and L_β bilayer indicate that interaction is not solely controlled by an attractive interaction with the lipid headgroups. If it was solely controlled by the headgroup, then one would expect the adsorption of polynucleic acids to give rise to the opposite trend due to the higher surface density of PC headgroups in the densely packed L_β bilayer. We finally point out that the present results obtained mainly in the presence of only monovalent salt should be distinguished from several reports in the literature as well as the present data of attraction between DNA or RNA and zwitterionic PC bilayers in the presence of divalent ions, like Ca^{2+} [18,21,22]. In the latter situations, the divalent ions are supposed to associate with the PC bilayer [18,82] and thus the interaction is suggested to be dominated by the electrostatic attraction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2009.12.009.

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