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Hybridization of DNA at the surface of phospholipid monolayers. Effect of orientation of oligonucleotide chains

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Abstract

We studied the properties of lipid monolayers formed at the air–water interface composed of dioleoylphosphatidylcholine (DOPC) with incorporated short (19-mer) oligonucleotides. These oligonucleotides were modified by oleylamine at both (3′ and 5′) terminals or only at one (3′) terminal. Interaction of single-stranded (19-mer) oligonucleotides without oleylamine with DOPC monolayers resulted only in slight increase of surface pressure and the area per phospholipid molecule, while more substantial and significant increase of these values were observed following incorporation of oligonucelotides modified by oleylamine. This influence is similar for both types of oligonucleotide modifications. However, considerable differences in changes of monolayer properties took place after hybridization with complementary oligonucleotides. The hybridization of oligonucleotides with the DNA modified by oleic acid at both 3′ and 5′ terminals at the surface of lipid monolayer resulted in further increase of surface pressure and in the increase of the area per phospholipid molecule, while decrease of both the surface pressure and the area per phospholipid molecules were observed for hybridization with DNA modified by oleic acid at 3′ terminal. It is possible that in latter case, the hybridization caused the loss of hybridized molecules from monolayers. Interaction of noncomplementary chains with DOPC monolayers with incorporated oleyl acid-modified DNA also influenced the properties of monolayers, but the effect was weaker in comparison with that observed for complementary chains.

Keywords: DNA; Oligonucleotide orientation; Hybridization; Langmuir–Blodgett technique; DOPC monolayers

1. Introduction

Gene detection has a growing importance in medical diagnostics. This fact is connected with necessity to detect point mutations that are linked to or are responsible for inherited diseases [1]. Hybridization of DNA has become the most frequently used method for clinical laboratory testing of genetic and infectious diseases [2]. Most common are indirect methods based on modification of the DNA probe, usually a short (about 15–20 base) single-stranded oligodeoxynucleotide, by a radioisotope or optical label [3]. The most elegant approach would be direct detection of hybridization, e.g. electrochemically, gravimetrically or utilizing surface properties of lipid films. There is increasing research being done in this direction [4–7], but definitive data justifying this approach is yet to emerge. In this work, we studied the peculiarities of DNA hybridization at a surface of monomolecular lipid films formed at the air–water interface depending on orientation of oligonucleotide chains. We synthesized 19-mer oligonucleotides modified by oleylamine at 3′ or at both at 3′ and 5′ terminals. The chemical modification by oleylamine allowed us to prepare nucleic acids + phospholipid mixed monolayers at the air–water interface and to show possibility to detect hybridization of DNA by means of measurements the pressure-area isotherm. The changes of condensation of monolayers depended on orientation of oligonucleotides relatively to the monolayer surface and were stronger for monolayers con-

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tained by oligonucleotides modified by oleylamines at both terminals.

2. Experimental

2.1. Chemicals, synthesis and modification of oligonucleotides

2′-Deoxyribonucleoside 5′-*O*-dimethoxytrityl-3′-(*N*,*N*-diisopropylamido)-β-cyanoethyl-phosphites for oligonucleotide synthesis were purchased from Glen Research (USA). All other reagents for oligonucleotide chemistry were purchased from Fluka (Germany).

Synthesis of oligonucleotides (ODN) with oleyl-containing units was carried out on an Applied Biosystems 380B automated DNA synthesizer (USA) by the phosphoramidite method as described in detail elsewhere [8,9]. The sequences of synthesized oligonucleotides are presented in Table 1. The sequence of oligonucleotide I is characteristic for fragment of *Salmonella typhimurium* gene. This oligonucleotide is complementary to the oligonucleotides modified by oleylamine at both (3′, 5′) terminals (II) or at one (3′) terminal (III). The oligonucleotide of the same composition like II and III but not modified with oleylamine (IV) was used as noncomplementary ODN.

2.2. π-A isotherms

Surface pressure monolayer area (π-A) isotherm measurements were performed on computer-controlled miniature LB trough 601 M (NIMA Technology, Coventry, UK) with a Teflon through (volume 50 ml, the whole surface area was 86 cm^2) equipped with Wilhelmy plate pressure sensor Nima PS4. Langmuir monolayers of phospholipids were formed by spreading a small amount (50 μl) of chloroform solution of the dioleoyl phosphatidylcholine (DOPC) (Fluka) on a subphase of ultrapure water (resistance >15 MΩ cm, ELIX 5, Millipore, El Paso, USA), pH 6. The temperature of the subphase (25 ± 0.02 °C) was maintained constant by thermostat Lauda RE206 (Köningshofen, Germany). After 15 min, when chloroform was allowed to evaporate, the phospholipid isotherms were measured by compression of two mobile Teflon barriers (barrier speed 15 cm^2/min). In special experiments, we studied also π-A isotherms for a mixed monolayer composed of DOPC and oleic acid (Fluka). In this case, both DOPC and oleic acid were dissolved in chloroform and mixed at various molar ratio. Modification of lipid monolayer by oligonucleotides was performed by addition of 50 μl (stock solution 5 μM oligonucleotides dissolved in water) into the water subphase (final concentration of oligonucleotides in the subphase was 5 nM) at fully extended monolayer, i.e. at surface pressure $P = 0$. Because the subphase was not stirred, the stock solution of oligonucleotides were added in several drops (total volume 50 μl) in different places of subphase, closely to the surface of the monolayer (in a special experiment, when pure water of the same volume but without ODN was added in analogical manner, we proved that no disturbance of monolayer took place). After addition of oligonucleotides, we waited for approximately 1 h for the equilibration of the monolayer (see also Ref. [10], where similar experimental conditions were used). Similar procedure was also applied for the DNA hybridization study. In this case, the probe (i.e. ODN modified by oleyl chain) was added into the water subphase together with complementary or noncomplementary oligonucleotides (final concentration 5 nM). In this case, the probe was incubated during 30 min with complementary or noncomplementary ODN prior to addition to the water subphase at 25 °C.

We also studied the interaction of dextran sulfate (DS, MW 500,000) (Sigma) with DOPC monolayers. DS is a negatively charged polymer, which can be used as a model of the negatively charged sugar-phosphate backbone of oligonucleotide. Polymer has been dissolved in the water subphase at different concentrations (1, 10 and 100 nM) prior to the formation of lipid monolayer.

Each series of experiments was repeated at least four times at identical conditions after careful cleaning in the LB trough. Statistical analysis of the obtained results was performed by Student *t*-test.

Table 1
The sequences of oligonucleotides used

No.	Sequences (3′ → 5′)
I	TGGAACGACTTTAAAAGGG
II	C*CCTTTTAAAGTCGTTCC*A
III	C*CCTTTTAAAGTCGTTCCA
IV	CCCTTTTAAAGTCGTTCCA

The sequence of oligonucleotide (ODN) I is characteristic for fragment of *S. typhimurium* gene. This oligonucleotide is complementary to the oligonucleotides modified by oleylamine at both (3′, 5′) terminals (II) or at one (5′) terminal (III). (cytidines modified by oleyl residues are indicated by *). Oligonucleotides with sequence (IV) were used as noncomplementary to II and III types oligonucleotides.

3. Results and discussion

The π-A isotherm of lipid monolayer composed of DOPC formed at water subphase had a typical shape (Fig. 1, curve 1) characteristic for phosphatidylcholine monolayers [11,12]. We can see three different regions on curve 1 that correspond to the different structural state of the monolayer. The liquid-expanded structure (A) turns more condensed (B) at the surface pressure of ~7 mN/m, while multilayer regions appear after the collapse of the monolayer above the surface pressure of ~47 mN/m. The area per molecule, determined by extrapolation of the linear region of the isotherm, was 0.57 ± 0.02 nm^2, which agrees well with previously reported data [12]. The presence of the unmodified oligonucleotide IV (see Table 1) at the water subphase does not cause substantial changes of the shape of

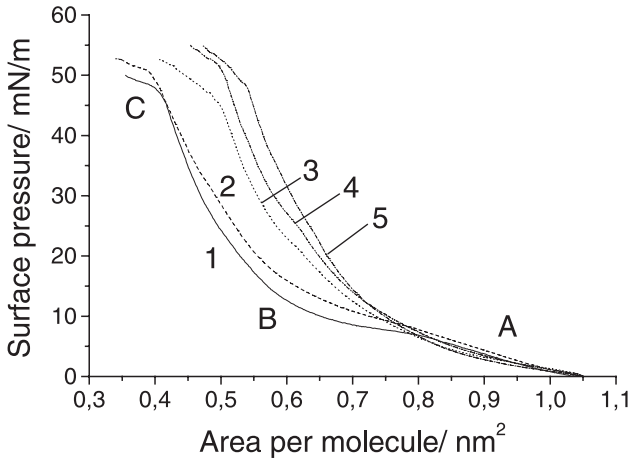

Fig. 1. π-A isotherms of: (1) an unmodified monolayer composed of DOPC, DOPC monolayer at presence of (2) ODN IV, (3) ODN II, (4) ODNs II and IV, (5) ODNs II and I at the deionized water subphase at concentration of 5 nM (the probe ODN II was modified by oleylamine at both 3′ and 5′ terminals; see Table 1).

the isotherm. However, the monolayer is characterized by higher surface pressure (Fig. 1, curve 2). Correspondingly, the transition between structural states of monolayers also takes place at higher pressure: 8 mN/m (transition: A → B) and 50.5 mN/m (transition: B → C). In addition, the area per molecule significantly ($P<0.01$) increases (Table 2). This effect is, however, concentration dependent and higher changes of surface pressure and area per molecule were observed at higher concentrations of ODNs (results are not shown). Addition of the ODN modified by hydrophobic chains at both 3′ and 5′ terminals (ODN II, Table 1) substantially influences the properties of the monolayers (Fig. 1, curve 3). At extended-liquid state of the monolayer (region A), the surface pressure is lower in comparison with that for pure DOPC, i.e. certain condensation effect takes place. However, the surface pressure is higher at condensed region (B) in comparison with pure DOPC, i.e. repulsive forces play dominant role in this case. The transition between A and B structural states is less expressed and occurs at surface pressure ~21 mN/m, which is substantially higher than for pure DOPC monolayer. Significant ($P<0.001$) increase of average area per molecule accompanies the interaction of ODN II with the DOPC monolayer (Table 2). It is interesting that collapse of the monolayer takes place at surface pressure similar to monolayers composed of pure DOPC. Addition of noncomplementary ODN IV further resulted to increase in surface pressure, area per molecule and the surface pressure at which the collapse of the monolayer takes place (Fig. 1, curve 4). The presence of complementary ODN I had the most pronounced effect on increase of the area per molecule (Table 2) and on the properties of the monolayer (Fig. 1, curve 5). In this case, it is difficult to distinguish the transition between the regions A and B. In addition, collapse of the monolayer takes place at higher pressure than for pure DOPC, but it is lower than for nonspecific interaction of ODN IV with the monolayer. It is interesting that at the region of surface pressure below 7 mN/m, the surface pressure for specific interaction is lower in comparison with that for the nonspecific one. It is very probable that the changes of the properties of lipid monolayers caused by noncomplementary ODN are due to the interaction with phospholipid head groups and not with ODNs II anchored at the DOPC monolayer. As we mentioned in Section 2.2, the probe ODN was preincubated with complementary or noncomplementary oligonucleotides prior to the addition to the water subphase. However, this preincubation has no substantial effect on the shape of the π-A isotherms. In a comparative experiment, we showed that the shape of π-A isotherm was similar also when first the lipid monolayer was modified by probe and then the target ODN was added into the water subphase.

The question arises concerning the physical nature of the observed changes. From the results presented above, it is clear that modification of ODN by hydrophobic chain has a crucial effect on the properties of DOPC monolayers. The area of phospholipid molecule in a monolayer is determined by an equilibrium of attractive and repulsive forces between lipid molecules. Attractive forces arise from hydrophobic effect and the van der Waals interactions between the atoms, while repulsive forces are caused by the electric charges, hydration effects and steric repulsion between atoms [13]. The increase of the average area per lipid molecule in the presence of ODN could be due to repulsive forces between negatively charged sugar-phosphate backbone and negatively charged phosphate groups of the lipids. Electrostatic repulsive forces between lipid monolayer and ODN without the hydrophobic chains are probably most responsible for the increase in the area per phospholipids. However, certain contribution into the interaction with lipid monolayer of the more hydrophobic nucleotide residues cannot be excluded. The latter could facilitate the adsorption of ODN to the monolayer surface. The stronger influence of ODN modified by hydrophobic chain on an increase of the area per lipid molecule could be the result of the contribution both

Table 2
The area per phospholipid molecule for DOPC monolayers without and with corresponding type of oligonucleotides (ODN) (see Table 1) and or different concentrations of dextran sulfate (DS) at the water subphase

Subphase composition	Area per molecule (nm^2)
Without ODN	0.57 ± 0.02
ODN IV	0.60 ± 0.02
ODN II	0.65 ± 0.02
ODN II + ODN IV	0.67 ± 0.02
ODN II + ODN I	0.70 ± 0.02
ODN III	0.68 ± 0.02
ODN III + ODN IV	0.66 ± 0.02
ODN III + ODN I	0.65 ± 0.02
DS (1 nM)	0.59 ± 0.01
DS (10 nM)	0.69 ± 0.01
DS (100 nM)	0.76 ± 0.01

The results represent mean ± S.D. calculated from four independent experiments in each series.

repulsive and attractive hydrophobic forces. The decrease of the surface tension at the extended-liquid part of the monolayer (region A, see Fig. 1) could be due to the incorporation of hydrophobic chain of oleylamine of modified ODN into the monolayer. This explanation is supported by different shapes of π-A isotherm in the presence of modified ODN II with that for unmodified ODN IV at the region A. We also performed a special experimental study of mixed monolayers composed of DOPC and oleic acid (see below). According to these experiments, the presence of oleic acid resulted the decrease of surface pressure at the A region of π-A isotherm. On the other hand, the presence of ODN resulted to an increase in surface pressure at the condensed region (B). This effect is very probably caused by repulsive forces between phospholipid head groups and negatively charged phosphate backbone of ODN. This has been proved in model experiment, when instead of ODN, dextran sulfate has been added into the subphase (see below). Thus, the hydrophobic chains of ODN provides anchoring of oligonucleotides to the lipid monolayer. However, parallel orientation of ODN to the monolayer surface on the other hand facilitates the increase of repulsive electrostatic forces between sugar-phosphate backbone of ODN and phosphate groups of the lipids.

Similar results have been obtained also in the case of interaction with DOPC monolayers of ODN III modified by hydrophobic chain only at one (3′) terminal. When ODN probe modified by oleylamine at 3′ terminal (III) was present in the subphase, the surface pressure (Fig. 2, curve 2) and area per molecule (Table 2) also increased substantially in comparison with the isotherm for DOPC without ODN. However, presence of the subphase of noncomplementary (IV) and/or complementary (I) ODN resulted in decrease of surface pressure (Fig. 2, curves 3 and 4, respectively) and the decrease of the area per molecule (Table 2). The changes

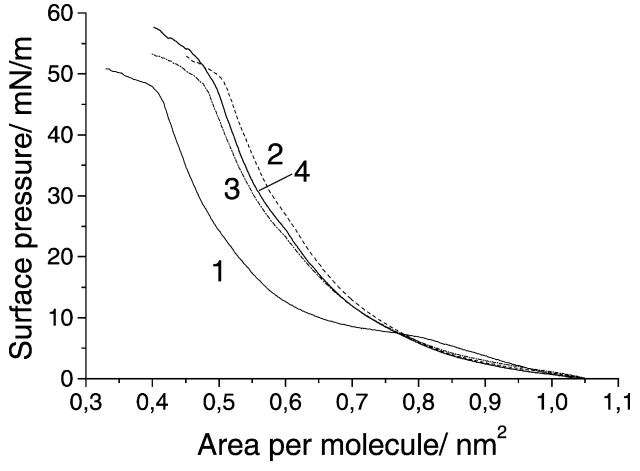

Fig. 2. π-A isotherms of: (1) an unmodified monolayer composed of DOPC, DOPC monolayer at presence of (2) ODN III, (3) ODNs I and III, (4) ODNs III and IV at the deionized water subphase at concentration of 5 nM (the probe ODN III was modified by oleylamine at 3′ terminal; see Table 1).

of both surface pressure and area per molecule were larger for complementary chain in comparison with that for noncomplementary chain. The calculated values of area per molecule for these two cases do not differ significantly (Table 2). However, the isotherms for complementary chain + probe were in all cases (four identical experiments) below the isotherms for noncomplementary chain + probe ODN.

The results of decrease of surface pressure reported here are similar with that obtained earlier by Zeng et al. [6] on egg phosphatidylcholine monolayers contained 5′-hexadecyl-deoxythymidic acid icosanucleotide modified by palmitic acid ($dT_{20}-C_{16}$). This phenomenon has been interpreted by these authors as a loss of hybridized molecules from the surface of the lipid monolayer. We suppose that this explanation is also applicable for results presented here.

Thus, the changes of the properties of DOPC monolayers following DNA hybridisation substantially depend on the type of modification of ODN by oleylamines. It is evident that when ODN is anchored to the phospholipid monolayer by two oleyl chains, the system is more stable. Instead of desorption, further increase of surface pressure and area per molecule took place following hybridization with complementary ODN.

However, if we compare the shape of isotherms, the changes of surface pressure and the area per molecule for DOPC modified by ODN II and III (see Figs. 1 and 2 and Table 2), we can see that the results are very similar and do not differ significantly. This result is not surprising. Certainly, the ODN III modified by oleylamine at one (3′) terminal is also oriented preferably parallel to the surface of the monolayer and interacts electrostatically with the choline groups of DOPC molecules. The presence of complementary ODN I at the water subphase, however, caused changes of the conformation of ODN III probe due to hybridization. It is known that persistent length of double-stranded DNA is approximately 50 nM [14], which is larger then the length of double-stranded DNA composed of I and III types ODN (approximately 6.6 nm). Thus, the hybridized DNA (i.e. ODNs I + III) should be oriented preferably perpendicular to the plane of the DOPC monolayer.

The ODN II, which is parallel to the monolayer surface, is stabilized by two oleylamines at both 3′ and 5′ terminals. Therefore, hybridization with complementary chain should not change the orientation of this ODN.

We already mentioned the possible role of hydrophobic chains of ODNs in the decrease of surface pressure at region A of π-A isotherm (see Fig. 1). As we noted above, this suggestion is also supported by results obtained for π-A isotherms composed of DOPC with different molar ratios of oleic acid (Fig. 3). We can see that at presence of oleic acid for all molar ratios studied, the surface pressure decreases at the extended region (A) of the isotherm. However, at condensed part of the isotherm (B), the shape of the isotherm for the monolayer composed of pure DOPC and

that of mixed monolayers is significantly different only at higher molar ratios of DOPC/oleic acid (>5). At experimental conditions used in this work and at maximal possible adsorption of ODN modified by hydrophobic chain, the molar ratio of DOPC/oleylamine does not surpass 140 for ODN modified by two oleylamine molecules. Therefore, the changes of the surface properties of lipid monolayers at the condensed region B of the monolayer could be caused mainly by the repulsive forces between negatively charged sugar-phosphate backbone and the surface of lipid monolayer. The maximum accessible molar ratio of DOPC/nucleotides at used experimental conditions is approximately 15 for single-stranded and 7.5 for double-stranded ODNs. Thus, we could expect about 6–12% changes of measured values due to the electrostatic effects. This corresponds to the changes of average area per phospholipid molecule 0.03–0.07 nm^2, which is in coincidence with obtained results (see Table 2).

The increase of surface pressure of monolayer composed of neutral phospholipid diphytanoylphosphatidylcholine following addition of plasmid DNA (5.6 kbp) has been reported also by Spassova et al. [15]. This effect was explained by the interaction of DNA with lipid head groups leading to less-dense lipid packing. They pointed out that in the DNA/lipid complex, there is a large distance between the lipids and this causes an increase in the surface pressure.

The role of the contribution of electrostatic interaction between negatively charged sugar-phosphate backbone of ODN and the phospholipid head groups for changes of the properties of lipid monolayers has been proved in additional experiments. We studied the interaction of DOPC monolayers with dextran sulphate, i.e. negatively charged polymer, that could serve as a model of negatively charged sugar-phosphate backbone of ODN. The π-A isotherms of DOPC monolayers without and with three different concentrations (1, 10 and 100 nM) of DS at the water subphase are presented in Fig. 4. The presence of DS caused an increase

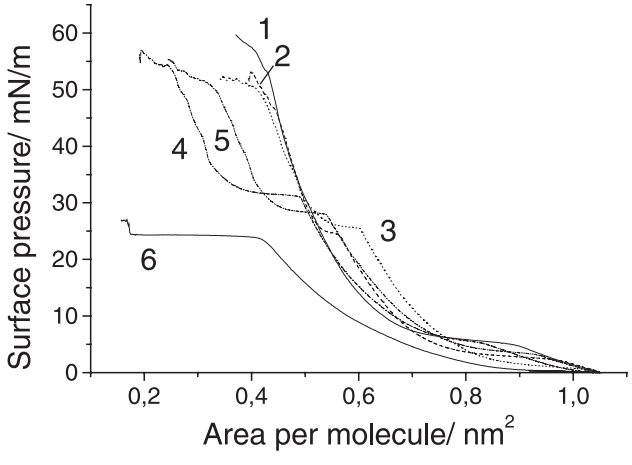

Fig. 3. π-A isotherms of monolayers composed of DOPC and oleic acid (OA) at different molar ratios (DOPC/OA): (1) 1:0, (2) 10:1, (3) 5:1, (4) 2:1, (5) 1:1, (6) 0:1.

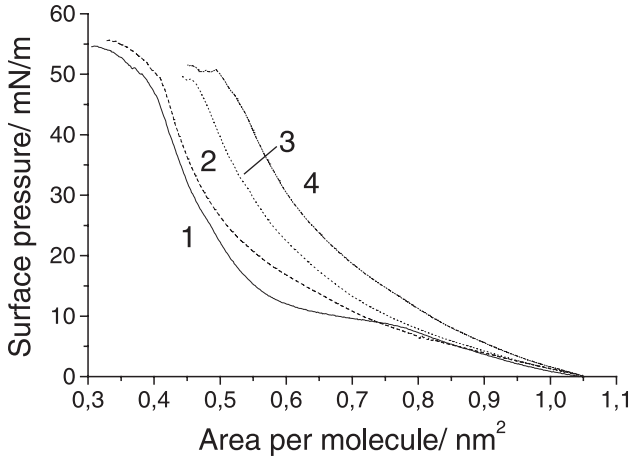

Fig. 4. π-A isotherms of: (1) an unmodified monolayer composed of DOPC in a pure water at presence of dextran sulphate at the water subphase at concentrations (2) 1 nM, (3) 10 nM, (4) 100 nM at pH 6.

in the surface pressure and the area per phospholipid molecule in a manner similar to unmodified ODN. We can see that changes of surface pressure and area per molecule (Table 2) are more expressed with increasing of the DS concentration. Similar results have been reported earlier and explained by penetration of DS deep into the lipid monolayer [16], while reduced surface pressure was observed when binding of DS to the phospholipid monolayer was mediated by Ca^{2+}. In this case, the DS molecules bind to the lipid head groups by forming Ca^{2+} bridges without penetrating the hydrophobic region [17].

Our recent results of the study the changes of specific volume of unilamellar liposomes composed of dimyristoyl phosphatidylcholine (DMPC) during temperature phase transition and at the presence of DS support the abovementioned explanation based on penetration of DS into the monolayer. We showed that changes of specific volume of DMPC liposomes above the phase transition temperature (T>24 °C) are lower by 10% in the presence of 1 μM DS. This can be caused by a restriction in the increase of specific volume of DMPC due to partial penetration of DS in lipid bilayer.

4. Conclusion

The approach based on Langmuir–Blodgett monolayers allowed us to study some peculiarities of the interaction of short oligonucleotides with monolayers. We showed that surface pressure is influenced by the incorporation of oligonucelotides modified by oleylamine. This influence is stronger for oligonucleotide modified by oleylamine at both 3′ and 5′ terminals in comparison with that modified at only one (3′) terminal. The orientation of oligonucelotides had a substantial effect on the hybridization of DNA. The methodology can be useful for the detection DNA hybridization at the surface of phospholipid monolayers.

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