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DOTAP/DOPE and DC-Chol/DOPE lipoplexes for gene delivery studied by circular dichroism and other biophysical techniques

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Abstract

Cationic liposomes give rise to stable complexes with DNA molecules (*lipoplexes*) that are of great interest for gene delivery applications. In particular, liposomes made up by a cationic lipid (DOTAP or DC-Chol) and a zwitterionic lipid (DOPE), produce stable adducts with single and double-stranded DNA oligonucleotides. Formation of these lipoplexes has been further addressed here by circular dichroism spectroscopy (CD) and by other independent biophysical methods. Titration of DNA oligonucleotides with cationic liposomes resulted into significant modifications of their circular dichroic bands. Such spectral modifications were ascribed to progressive DNA condensation and loss of native conformation, as a consequence of the electrostatic interactions taking place between the phosphate groups of DNA and the positively charged head groups of cationic lipids. In all cases, the loss of the CD feature characteristic of the native DNA conformation closely matched the inflection point of Zeta potential profiles. The resulting adducts showed peculiar and non-canonical CD spectra, while exhibiting appreciable stability at physiological pH.

Keywords: Lipoplexes; Cationic liposomes; DNA; Circular dichroism; Zeta potential; Gel electrophoresis

1. Introduction

Gene transfer represents an important treatment option for several genetic and acquired diseases with concrete perspectives of clinical application [1–3]. Cationic lipids and cationic polymers have been extensively studied for gene transfer experiments, during the last two decades [4–9]. In spite of a few limitations, such as relatively low transfection rate and some intrinsic cell toxicity, these cationic agents are considered as a valid alternative to viral vectors [10–12]. Indeed, cationic polymers and liposomes are generally non-immunogenic, easy to produce, and non-oncogenic. They are biodegradable, highly biocompatible, and form stable complexes with DNA fragments (*lipoplexes*), owing to the occurrence of sufficiently strong electrostatic interactions [13–15].

The formation of lipoplexes between cationic liposomes and short oligonucleotides was formerly investigated in our laboratory mainly from the lipid side [5,16]; here, the analysis has been extended to the point of view of the DNA component. Such analysis largely relies on the application of circular dichroism spectroscopy. CD results were then compared with those obtained at the mesoscale level by Photon Correlation Spectroscopy, Zeta potential and gel electrophoresis measurements.

It is widely accepted that CD is one of the most powerful methods to investigate the solution structure of nucleic acids and the conformational changes elicited by ligand binding and/or by modifications of the experimental conditions [17,18]. CD spectra of nucleic acids arise from exciton-coupling between the electronic transitions of nucleobases. The latter are *stacked* in single-strand DNA and *are both stacked and hydrogen-bonded to the complementary base* in double-strand DNA. Such strong base–base interactions give rise to intense CD spectra that are very sensitive to the overall solution conformation of these biomolecules. A number of reviews and books have appeared, during the last 30 years, specifically dealing with these topics [19–22]. Some clear correlations between a few characteristic CD spectral features and the solution structure of various DNA molecules (B-DNA; A-DNA; C-DNA and so on) have been unambiguously established.

Recently, CD has been largely employed to characterise DNA complexes that might be useful for gene delivery [23–28]. CD is particularly sensitive to the precise nature of the helical state of DNA, even when complexed, and has the potential to

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highlight subtle structural changes. Previous CD studies of cationic DNA/lipid systems were interpreted in terms of DNA conformational transitions. In particular, Patil et al. have studied the interactions between various oligonucleotides and a plasmid DNA with anionic liposomes as a function of Ca^{2+} concentration [17,26]. In the resulting complexes these authors evidence a characteristic transition from the native B-DNA conformation to the more compact left-handed Z-DNA conformation. On the contrary, cationic lipids appear to induce a structural transition from B-DNA to C-DNA [11,14,29]; notably, the 'limit' form of C-DNA is characterised by a very small positive CD band (~280 nm) and a negative signal, around 250 nm. The shape, location and magnitude of the latter band are essentially identical to those revealed by the B motif [25]. In case of extreme dehydration, C-DNA is shown to collapse into an even more condensed and compact chiral Ψ-DNA (polymer salt induced DNA), showing a further enhanced negative ellipticity around 240 nm (or at least properties similar to it) [14].

In a recent study, Braun et al. [27] have examined the interactions of DDAB or DOTAP with a plasmid DNA using CD, FTIR and Raman spectroscopies; molecular dynamics simulations have also been performed to get details on base–base interactions in these complex systems. A global interpretation of the data suggests that the DNA component of these lipoplexes still remains in a variant B form in which, nonetheless, base–base interactions are deeply perturbed [27,30].

The CD results reported in the present paper aim at characterising the solution structure of selected synthetic and natural DNAs during their progressive engagement in complex formation with DOTAP/DOPE or DC-Chol/DOPE liposomes. The other biophysical methods here employed (Zeta potential, Photon Correlation Spectroscopy and gel electrophoresis) turned out to be very helpful in providing independent information on these systems.

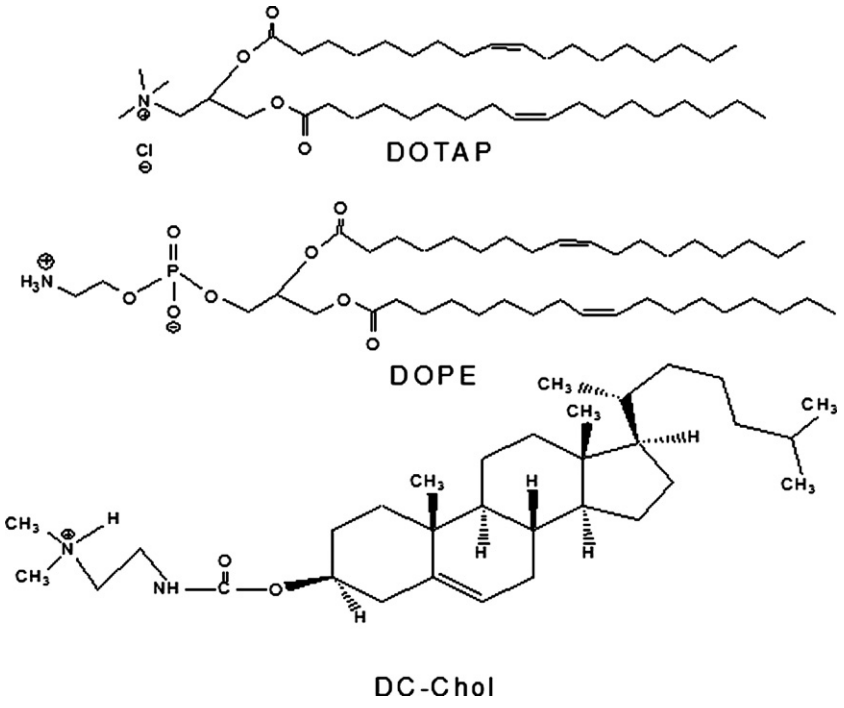
All the above mentioned physico-chemical methods, taken together, allowed us to monitor the evolution of the investigated lipoplexes and to provide a satisfactory, unified description of the occurring interactions, as well as of the formed adducts.

In detail, we report on the effects of adding increasing amounts of either DOTAP/DOPE or DC-Chol/DOPE cationic liposomes (Scheme 1) to short oligonucleotides and to chromosomal DNA molecules. CD spectra of 20-base single-strand polyA and polyT, and of the corresponding double-strand polyAT (dsAT hereafter) were measured in each step of this titration. CD spectra of genomic *calf-thymus* DNA were also recorded under the same experimental conditions, since we were interested in comparing short double-stranded linear oligonucleotides and long linear DNA fragments. *Calf-thymus* DNA was selected as it represents a very popular DNA model. However, it is worth noting that some significant differences might arise with respect to supercoiled circular plasmids that are routinely utilized in non-viral gene therapy.

2. Materials and methods

2.1. Materials

DOTAP (purity > 99%), DOPE (purity > 99%) and DC-Chol (purity > 99%) were purchased from Avanti Polar Lipids, Inc., Alabaster, AL, and used without further purification. The 20-mer oligonucleotides (ODNs), polyA, and polyT were obtained from QIAGEN Operon, Alameda, CA. The annealing



Scheme 1. Structure of the cationic lipids used in this study.

temperature was 39.9 °C, as indicated by the manufacturer. *Calf-thymus* DNA was purchased at Sigma (Code D3664). Phosphate Buffer Saline solution (PBS) was purchased from Sigma as tablets. After thawing, the pH value was tested and set to 7.4 with NaOH or HCl.

2.2. Liposome preparation

DOTAP/DOPE and DC-Chol/DOPE liposomes were prepared at 1:1 mol ratio with 1.4×10^{-2} M total lipid concentration, as stock solution. Mixtures of dry lipid powders were dissolved in chloroform and the solvent was dried under vacuum overnight. The resulting mixed lipid film was swollen at room temperature with 10^{-2} M PBS buffer (pH=7.4). Upon vortexing, multilamellar vesicles were obtained, and were then submitted to eight freeze/thaw cycles. This method improved the homogeneity of the size distribution in the final suspension. Liposomes were subsequently downsized and converted to unilamellar vesicles by extrusion through 100 nm polycarbonate membranes (27 passages, LiposoFast apparatus, Avestin, Ottawa, Canada). Liposomes were stored at 4 °C.

The presence of high saline concentration was very important for liposome stability. In the presence of phosphate buffer alone, liposomes collapsed and started to flocculate, soon after extrusion. This phenomenon was probably due to the phosphate ions which are able to bridge the cationic polar heads of lipids. The presence of high saline concentration shielded these interactions, making the liposome suspension stable for more than 1 month. Therefore we decided to use PBS (one of the most common medium for cell manipulation) because in this medium DOTAP/DOPE and DC-Chol/DOPE liposomes showed enhanced stability.

2.3. Lipoplex preparation

Each lipoplex sample was prepared by addition of equal volumes of a stock solution of nucleic acid to a monodisperse suspension of liposomes at the desired lipid concentration, at room temperature. This yielded different −/+charge ratios r (from 0.05 to 5), where

$$r = -/+ = \frac{[\text{phosphate groups of DNA}]}{[\text{DOTAP molecules in liposomes}]}.$$

This way of preparing complexes avoided passing through a charge neutrality region where complexes tend to aggregate.

In each series of samples the concentration of nucleic acid was kept constant at 5.25×10^{-5} M for oligonucleotides, and 1.75×10^{-4} M for *calf-thymus* DNA, where concentration is expressed in terms of negative charges.

2.4. Circular dichroism studies

CD spectra of lipoplexes were recorded using a Jasco spectropolarimeter (J-800). Samples were prepared in PBS buffer at pH 7.4, and placed in a cuvette of 1 cm path length. Spectra were taken as the average of four accumulations from 200 to 500 nm. The scan rate was 50 nm/min. CD spectra were recorded at room temperature immediately after the addition of DNA to the liposome suspension ($t=0$). No significant spectral changes were detected over a period of days. The molecular ellipticity is reported as [deg × cm^2 × $dmol^{-1}$].

2.5. Zeta potential and Photon Correlation Spectroscopy study

Measurements of the Zeta potential and of the sizes of liposomes and lipoplexes were performed with a Coulter DELSA 440 SX (Coulter Corporation, Miami, FL, USA). The Zeta potential was automatically calculated from the electrophoretic mobility based on the Helmholtz–Smoluchowski relationship. Homemade hemispherical electrodes, covered by a thin gold layer, were used as the measurement cell. This allowed to reduce the oxidation that partially affects silver electrodes, normally used in this kind of instruments. Runs were performed using a current of 0.7 mA and a frequency of 125 Hz. Zeta potential was calculated for the following scattering angles: 8.6°, 17.1°, 25.6° and 34.2°.

Sizes were calculated by assuming light scattering modelling of Brownian diffusion [31]. During size measurement, sample time was changed according to the size of the scattering objects. Size measurements were only performed at the largest angle, i.e. 34.2°, in order to minimize artefacts due to possible interparticle interactions.

2.6. Electrophoretic mobility studies

20 μl of each sample analysed in this work were incubated for 10 min at room temperature with 5 μl of 6× non-denaturing gel loading buffer (37% glycerol, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mM Tris/HCl pH 8, 5 mM EDTA) followed by brief vortexing.

Samples containing *calf-thymus* DNA were electrophoresed on 10% agarose gels containing 4 μl ethydium bromide (EtBr) in Tris-borate EDTA buffer (TBE), pH 8 at 100 V for 20 min [32]. Samples containing dsAT were electrophoresed on 20% non-denaturating acrylamide gel at 250 V, until the bromophenol blue had moved about 75% of the gel length. The corresponding gel was stained by soaking for 5–10 min in 1 μg/ml EtBr water solution. All the gels were visualized on an UV transilluminator.

Although the running buffer (TBE), used in electrophoresis experiments contained EDTA, it is not expected to disrupt lipoplexes because of the high ion strength in the investigated systems.

3. Results and discussion

3.1. CD measurements

In Fig. 1, the CD spectra of the polyA/DOTAP/DOPE system, recorded at different r ratios, are shown. The CD spectrum of single-strand polyA dissolved in PBS buffer (spectrum 1a) exhibited an intense positive band at 217 nm (with a shoulder at 235 nm), a negative band at 248 nm and two

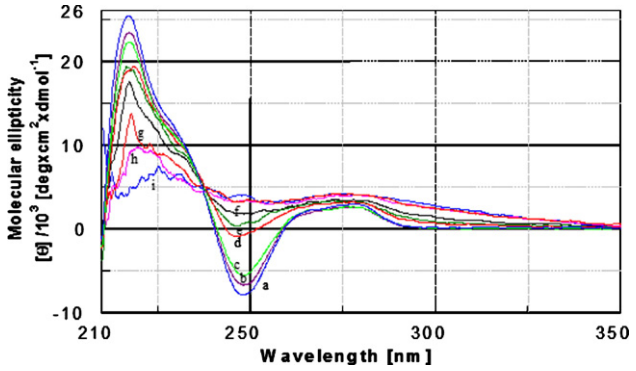


Fig. 1. CD spectra of single-strand 20-base polyA-DOTAP/DOPE lipoplexes at different −/+charge ratios *r*. In detail the spectra corresponds to: a: pure polyA; b: *r*=5; c: *r*=2.5; d: *r*=1; e: *r*=0.75; f: *r*=0.50; g: *r*=0.25; h: *r*=0.15; i: *r*=0.05.

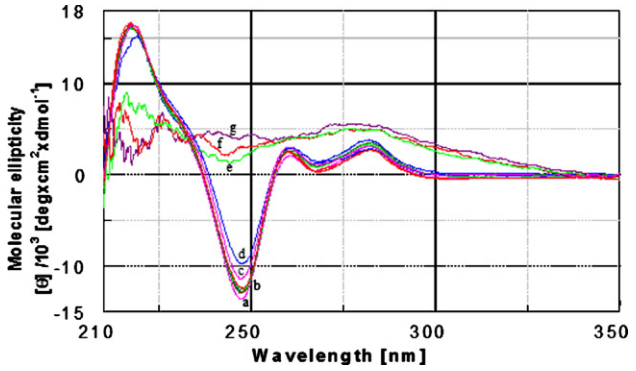


Fig. 3. CD spectra of double-strand 20-base polyAT-DOTAP/DOPE lipoplexes at different −/+charge ratios *r*. In detail the spectra corresponds to: a: pure dsAT; b: *r*=5; *r*=2.5; *r*=1; c: *r*=0.75; d: *r*=0.50; e: *r*=0.25; f: *r*=0.15; g: *r*=0.05.

additional (weaker) positive bands at 265 and 280 nm. Such spectral features were previously described and interpreted in terms of base stacking within a single-stranded right-handed helical conformation [33–35]. Addition of small amounts of cationic liposomes (with 5>*r*>2.5) affected the CD spectrum of polyA very scarcely. Only small decreases in the intensity of the positive band at 217 nm and of the negative band at 248 nm were indeed detected, the bands at 265 and 280 nm being almost unchanged. However, when *r* approached 1, the CD features of this nucleic acid were greatly perturbed, particularly in the region between 210 and 260 nm, with a marked intensity decrease of the two main bands. For lower charge ratios (0.5>*r*>0.05), the CD signals of this oligo disappeared almost completely, the final CD spectrum being characterised by two broad positive bands at 225 and 275 nm.

A slight opalescence of the solution was observed in the latter range of *r* values, indicating some aggregate formation. However, the scattering contribution to the CD spectra due to opalescence turned out to be small in the analysed systems.

The CD spectra of single-strand polyT/DOTAP/DOPE system are reported in Fig. 2. The CD spectrum of single-strand polyT in PBS buffer (spectrum 2a) showed a main negative band at 250 nm and a positive one at 275 nm, in agreement with previous observations [33,34]. Based on crystallographic data obtained on sodium thymidylyl-(5′→3′)-thymidylate-(5′) hydrate (pTpT) it has been proposed that poly(dT) adopts a right-handed helical conformation in solution [36]. The CD spectral features of single-strand poly(dT) were previously interpreted in terms of limited stacking within the single-strand helical structure [37]. Upon addition of small amounts of DOTAP/DOPE liposomes (5>*r*>2.5) a slight, progressive decrease of the intensity of these two bands was observed. However, as *r* approached unity, the negative band at 250 nm progressively decreased in intensity, while the positive band at 280 nm was broadened. Notably, at low *r* values, the CD spectra become very noisy in the spectral region below 250 nm.

The CD spectra of dsAT/DOTAP/DOPE system are shown in Fig. 3. The spectrum of the double-stranded oligonucleotide, at the concentration of 5.25×10^{-5} M in terms of negative charges (spectrum 3a), showed a positive band at 217 nm with a shoulder at 235 nm, a negative band at 247 nm and two less intense peaks at 260 and 280 nm respectively, in line with previous reports [33,34]. Upon addition of DOTAP/DOPE liposomes, for *r* values in the 5–0.5 range, only minor variations of the dsAT spectrum were observed, consisting in a slight intensity decrease of the negative band at 247 nm and of

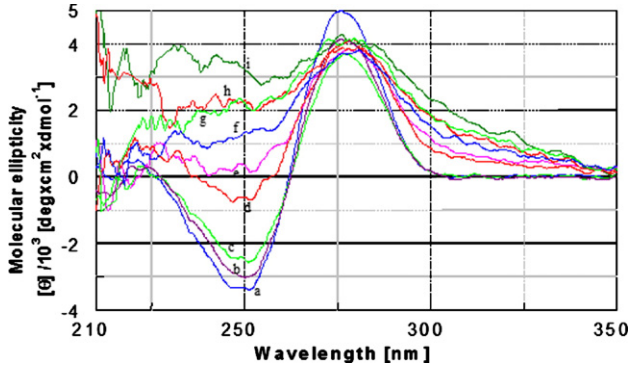


Fig. 2. CD spectra of single-strand 20-base polyT-DOTAP/DOPE lipoplexes at different −/+charge ratios *r*. In detail the spectra corresponds to: a: pure polyT; b: *r*=5; c: *r*=2.5; d: *r*=1; e: *r*=0.75; f: *r*=0.50; g: *r*=0.25; h: *r*=0.15; i: *r*=0.05.

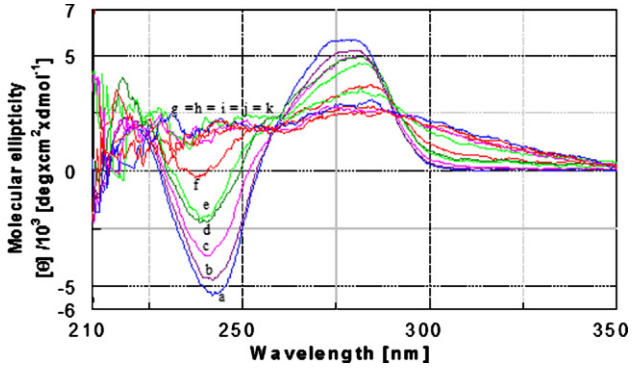


Fig. 4. CD spectra of *calf-thymus* DNA-DOTAP/DOPE lipoplexes at different −/+charge ratios *r*. In detail the spectra corresponds to: a: pure *ct*DNA; b: *r*=5; c: *r*=2.5; d: *r*=2; e: *r*=1.5; f: *r*=1; g: *r*=0.75; h: *r*=0.50; i: *r*=0.25; j: *r*=0.15; k: *r*=0.05.

the positive band at 217 nm, as well as in small perturbations of the peaks at 260 and 280 nm. However, for lower charge ratios, a marked decrease of all CD bands was observed within a relatively narrow r interval, suggesting a generalized, rapid "collapse" of the native solution conformation. The final spectrum g was dominated by a broad and unstructured positive signal centered at 280 nm.

Fig. 4 shows the CD spectral changes experienced by *calf-thymus* DNA upon addition of increasing amounts of DOTAP/DOPE. As expected [20], *calf-thymus* DNA, dissolved in PBS buffer, showed the typical CD spectrum of B-type DNA with a negative band at 242 nm and a positive peak around 273 nm (spectrum 4a). Following addition of cationic liposomes ($5 > r > 1.5$), the positive peak showed a marked intensity decrease, accompanied by a red shift (the maximum absorption shifted toward 283 nm). In the same range of r values, the negative band at 242 nm progressively decreased. At $r = 1$ only a weak CD spectrum was observed. For r values ranging between 0.75 and 0.05 the negative band completely disappeared, while a residual intensity of the positive peak around 280 nm was maintained.

Analogous CD titrations were carried out on the corresponding DNA/DC-Chol/DOPE lipoplexes under identical experimental conditions. Remarkably, these results closely matched those obtained for DOTAP/DOPE lipoplexes.

Fig. 5 reports the percentage of changes in molar ellipticity [θ], at 242 nm, as a function of the DNA/DC-Chol/DOPE molar ratio. A progressive disappearance of the characteristic CD signals of the various DNA molecules was observed, according to spectral patterns very similar to those reported for the corresponding DOTAP/DOPE systems. It must be noted, however, that higher DC-Chol/DOPE to DNA ratios were needed to obtain comparable spectral perturbations with respect to DOTAP/DOPE liposomes.

The analysis of the experimental CD patterns allows the identification of a few common trends that emerged in the course of lipoplex formation: i) in all cases, the progressive perturbation and weakening of the CD feature characteristic of the starting DNA molecules were observed; ii) the original CD spectral patterns of the individual nucleic acids were eventually replaced by broad and poorly structured positive signals; iii) in all cases, limited aggregate formation occurred in the region of the charge neutrality, accompanied by a slight opalescence.

Table 1
Zeta potential values (mV) of DNA/DOTAP/DOPE and DNA/DC-Chol/DOPE lipoplexes at different charge ratio

Sample	$r=0$	$r=0.20$	$r=0.50$	$r=1$
polyA/DOTAP/DOPE	37±7	40±5	10±9	4±10
polyT/DOTAP/DOPE	37±7	38±5	12±7	−10±10
dsAT/DOTAP/DOPE	37±7	39±6	−30±10	−39±8
*ct*DNA/DOTAP/DOPE	33±6	32±6	0±15	−32±5
polyA/DC-Chol/DOPE	17±3	14±3	6±4	−3±4
polyT/DC-Chol/DOPE	23±7	16±5	5±4	5±6
dsAT/DC-Chol/DOPE	23±7	16±7	−39±6	−29±7
*ct*DNA/DC-Chol/DOPE	17±3	15±4	8±4	−35±6

$r=0$ column: pure liposomes; $r=0.20$ column: plateau before inversion; $r=0.50$ column: mid point; $r=1$ column: plateau after inversion.

It is straightforward to attribute the progressive perturbation of the native CD features of these nucleic acids to important electrostatic interactions taking place between the negatively charged phosphate groups and the cationic groups of the lipid heads, as commonly assumed for the interactions of DNA with various cationic compounds. Based on careful inspection of the CD spectra and on results obtained by independent spectrophotometric measurements, we might rule out that absorption flattening and light scattering effects, previously described [27,38], play a major role in affecting the CD signals.

During the early phases of the CD titrations, similar spectral changes were observed for the various DNA molecules, usually consisting in moderate intensity decreases of the main CD bands and in the occurrence of small red shifts. On the other hand, at high ligand/DNA ratios, large and substantially different spectral modifications were observed. Noteworthy, some of the DNA molecules tested in this study seemed to oppose a greater resistance to the structural perturbations induced by cationic liposomes. For instance, an evident alteration of the solution structure of dsAT was observed for both kinds of liposomes only in the presence of relatively high lipid concentrations, implying a higher structural stability toward

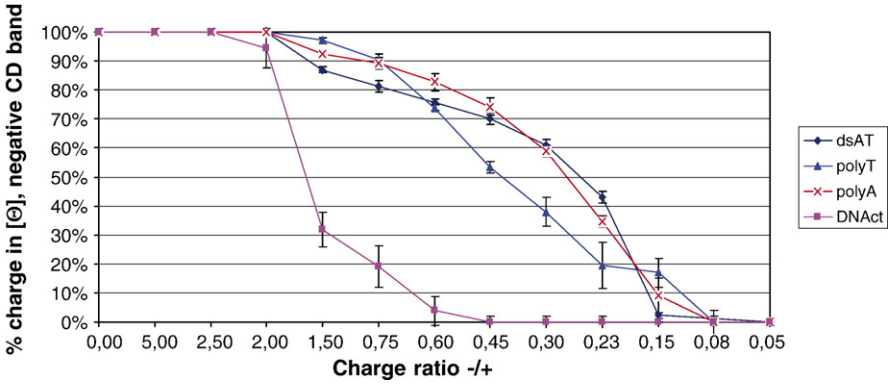


Fig. 5. Percentage change of molecular ellipticity [θ] for the negative CD band of the DNA/DC-Chol/DOPE lipoplexes studied in this work, as a function of DNA/lipid charge ratio r (−/+).

electrostatic perturbations. In any case, the CD spectra of the final adducts were broad and poorly structured, and differed substantially from those reported for similar systems. At variance with previous studies [11,14,29], no evidence was obtained here for the formation of characteristic "C type" or "Ψ type" DNA conformations [39,40]. Thus, our results were in agreement with those recently reported by Braun et al. [27] Choosakoonkriang et al. [30] suggesting the existence of a variant B-DNA form in which base–base interactions are deeply perturbed.

3.2. Zeta potential and Photon Correlation Spectroscopy measurements

The surface charge of lipoplexes, dissolved in PBS, was measured as a function of *r*. Table 1 reports the Zeta potential values of the lipoplexes at representative charge ratios studied in this work. In all cases the inversion point was found to occur when the total negative charges added as DNA phosphate groups approximately equalled half the concentration of cationic lipids. This behaviour was similar to the lipoplex/Tris/HCl and lipoplex/culture medium systems previously described [5,16]. This meant that roughly half the cationic lipids were available to interact with DNA and only the external surface of the lipidic aggregates interacted with the DNA phosphate groups.

There was an important correspondence between the variations of CD spectra and those of Zeta potential. When the CD signal was weak and very noisy ($0.50 > r > 0.05$) the Zeta values of lipoplexes were all positive and almost the same of pure liposomes.

In the case of DC-Chol/DOPE lipoplexes, the inversion point occurred at $r = 0.4$, rather than $r = 0.5$ and the titration curve was smoother. This difference was attributed to the nature of DC-Chol, which is a weak base, and not all its head groups have a net positive charge. In fact, a smaller amount of DNA negative phosphate was able to neutralize the DC-Chol/DOPE liposome surface.

The size range for the above described lipoplexes was measured with Photon Correlation Spectroscopy. Pure DOTAP/DOPE or DC-Chol/DOPE liposomes had a mean diameter in the 100–120 nm range. In some cases this value was roughly doubled, because the phosphate groups in PBS could act like a glue between liposomes, bridging the cationic polar heads of different lipidic spherical objects.

With double-strand DNA (viz. dsAT and *ct*DNA), the complex size was rather small (200–300 nm) in the presence of large amounts of liposomes, that is before the inversion point. Close to the inversion point, the autocorrelation curves of lipoplexes indicated the presence of large (>1 μm) and very polydisperse aggregates. At $r > 0.5$ the mean size reached a plateau at ~400–500 nm.

Photon Correlation Spectroscopy was not able to measure the size of single-strand DNA lipoplexes, since the scattering intensity of these samples was not high enough to give reproducible results.

3.3. Gel electrophoresis measurements

The same samples used for CD and Zeta potential measurements were analysed for electrophoretic mobility. This method allowed to evaluate the amount of free DNA, which retained the ability to migrate through an agarose or polyacrilamide gel [32]. Only the double-strand DNA could be evidenced by ethidium bromide, which is a typical fluorescent intercalating agent. Lipoplexes, i.e. complexed DNAs, were not able to move from the well, their size being too large with respect to the gel network. Therefore, this method allowed to detect the presence of not complexed DNA in different samples [17,41,42]. Photos of dsAT/DC-Chol/DOPE or *ct*DNA/DC-Chol/DOPE lipoplex gels are reported in Fig. 6 as representative examples. Panel A in Fig. 6, showed a visible band that could migrate above the inversion point ($r > 0.4$). For dsAT it was not possible to evidence any DNA trapped into the well: dsAT was too short and compact onto the liposomes to incorporate ethidium bromide. For *ct*DNA, the presence of DNA into the lipoplexes was visible also in the case of positive Zeta potential (Fig. 6B). In the central region of the investigated charge ratios there was practically no fluorescent signal in the gel, since the samples tended to precipitate. For DOTAP/DOPE liposomes the gel electrophoresis results were exactly the same, the only difference being the *r* value at the inversion point.

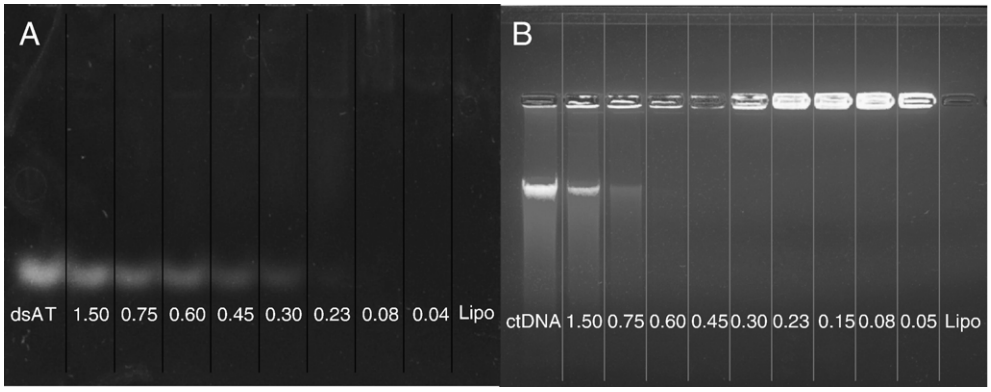


Fig. 6. A: Non-denaturating acrylamide gel of dsAT/DC-Chol/DOPE lipoplexes with C_M dsAT 5.25×10^{-5} M in phosphate groups; B: agarose gel of *ct*DNA/DOTAP/DOPE with C_M *ct*DNA 1.75×10^{-4} M in phosphate groups.

4. Conclusions

CD spectroscopy represents a suitable and powerful method to investigate the structure of various types of DNA bound to standard liposomes. In the present investigation CD titrations of DNA oligonucleotides with increasing amounts of either DOTAP/DOPE or DC-Chol/DOPE cationic liposomes revealed some common spectral patterns consisting in the progressive loss of the CD signal characteristic of native, right-handed, helical DNA conformations. Eventually, all spectra consisted of a broad and poorly structured CD positive bands at 260–300 nm. The latter CD features, that were assigned to the resulting lipoplexes, did not corresponded to DNA structures previously reported to originate from DNA condensation processes. Zeta potential measurements showed that the observed loss of native DNA structure closely corresponded to the inflection point. This finding supported the hypothesis that the observed DNA structural perturbations were primarily due to direct electrostatic interactions. The structure of either DOTAP/DOPE or DC-Chol/DOPE liposomes was substantially preserved during lipoplex formation, so that it could be inferred that the DNA molecules wrapped the positive charged surface of liposomes. Information on the size of the liposomes and lipoplexes was obtained by Photon Correlation Spectroscopy. The presence of free DNA, after the Zeta potential inversion point, was unambiguously detected by electrophoretic measurements.

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