Chiral DNA packaging in DNA-cationic liposome assemblies

Nicolaas J. Zuidam[a,1,*], Yechezkel Barenholz[a,1], Abraham Minsky[b,1]

[a] *Department of Biochemistry, The Hebrew University, Hadassah Medical School, P.O. Box 12272, Jerusalem 91120, Israel*
[b] *Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel*

Received 24 June 1999

Abstract Recent studies have indicated that the structural features of DNA-lipid assemblies, dictated by the lipid composition and cationic lipid-to-DNA ratio, critically affect the efficiency of these complexes in acting as vehicles for cellular delivery of genetic material. Using circular dichroism we find that upon binding DNA, positively-charged liposomes induce a secondary conformational transition of the DNA molecules from the native B form to the C motif. Liposomes composed of positively-charged and neutral 'helper' lipids, found to be particularly effective as transfecting agents, induce – in addition to secondary conformational changes – DNA condensation into a left-handed cholesteric-like phase. A structural model is presented according to which two distinct, yet inter-related modes of DNA packaging coexist within such assemblies. The results underline the notion that subtle changes in the components of a supramolecular assembly may substantially modulate the interplay of interactions which dictate its structure and functional properties.

Key words: Chiral DNA packaging; DNA-cationic liposome assemblies

DNA-cationic liposome assemblies became the subject of intensive studies due to their unique properties and their high efficiency in acting as vehicles for DNA delivery into eukaryotic cells [1]. Two inter-related processes were shown to occur upon interaction between positively-charged liposomes and DNA: a DNA-induced membrane fusion and a liposome-mediated DNA condensation [2]. High resolution studies indicated the formation of DNA-lipid complexes whose lamellar [3,4] or columnar inverted-hexagonal [5,6] structures depend upon the lipid composition and the cationic lipid-to-DNA ratio. Intriguingly, the columnar inverted-hexagonal complexes, obtained when the cationic liposomes contain the neutral helper lipid dioleoyl-phosphatidylethanolamine (DOPE), were found to be particularly effective as transfecting agents [5]. This may be related to the fact that DOPE introduces: better matching in charge density [8] between lipid and DNA surfaces, easier counter-ion release of the lipid surface by the DNA [7], lower hydration of the lipid surface (Hirsch-Lerner and Barenholz, unpublished). These DOPE effects prompted us to examine the conformational modulations exhibited by DNA molecules upon their binding to liposomes composed of the cationic dioleoyl-trimethylammonium-propane (DOTAP) in the presence and absence of DOPE.

*Corresponding author. Fax: +972 (2) 6411663.
E-mail: nicolaas@cc.huji.il

[1] All authors contributed equally.

We find that DNA molecules bound to liposomes containing pure DOTAP sustain a B-to-C secondary conformational change, whereas the presence of DOPE induces both secondary and tertiary DNA transitions. Significantly, DOTAP-DOPE liposomes affect DNA collapse into a tightly-packed phase characterized by a long-range chiral order.

Circular dichroism (CD) studies were conducted on DNA-liposome complexes at various lipid compositions and lipid-to-DNA ratios (Fig. 1). In the absence of cationic lipids, supercoiled plasmid DNA molecules exhibit short-wave negative and long-wave positive CD bands of similar magnitudes, typical of B-DNA conformation. The DNA ellipticities are not affected by liposomes composed of the neutral lipid DOPC (Fig. 1C, D). Substantial changes of the CD spectra are, however, observed upon mixing DNA with liposomes containing the cationic lipid DOTAP or a 1/1 (mole ratio) mixture of DOTAP and DOPE. Specifically, upon addition of either DOTAP or DOTAP/DOPE (1/1) liposomes at DOTAP/DNA ratios ≤ 1 the negative CD band slightly increases (i.e. becomes more negative) while the magnitude of the positive band decreases. At DOTAP/DNA charge ratios >1.0 the magnitude of the spectral changes as a function of DOTAP/DNA ratio significantly differ for DOTAP and DOTAP-DOPE liposomes. For liposomes composed solely of DOTAP the spectral modifications are continuous, starting with 10% modification relative to B-DNA at DOTAP/DNA = 0.2 and gradually increasing to a maximum of 30% at a ratio of 4.0. For DOTAP/DOPE (1/1) liposomes the CD changes exhibited at DOTAP/DNA ratios >1.0 are substantially larger and show a clear discontinuity. The largest change effected by DOTAP/DOPE occurs at DOTAP/DNA ratio of 1.5, followed by an attenuation at higher ratios (Fig. 1).

The modifications of DNA ellipticities exhibited by liposomes composed of DOTAP at all lipid-to-DNA ratios, or of DOTAP-DOPE at DOTAP/DNA ratios lower than 1.0 indicate a DNA secondary structural transition from the native B-DNA to the C motif. As shown in Fig. 2A, the 'limit' form of C-DNA is characterized by a very small positive CD band and a negative signal whose shape, location and magnitude are essentially identical to those revealed by the B motif [9,10].

The liposome-mediated B-to-C transition could be anticipated on the basis of the high positive charge density and reduced relative humidity that prevail near the surface of the charged lipid bilayer, and has indeed been previously reported [11]. The further increase in the magnitude of the negative CD band, as well as the non-continuous nature of the spectral changes revealed by the DOTAP-DOPE-DNA system as the DOTAP/DNA ratio is increased are, however, intriguing. Such patterns are incompatible with a further conversion of the DNA into the C motif since a gradual increase of the ionic strength effects a continuous, non-cooperative B-to-C

PII: S0014-5793(99)01053-4

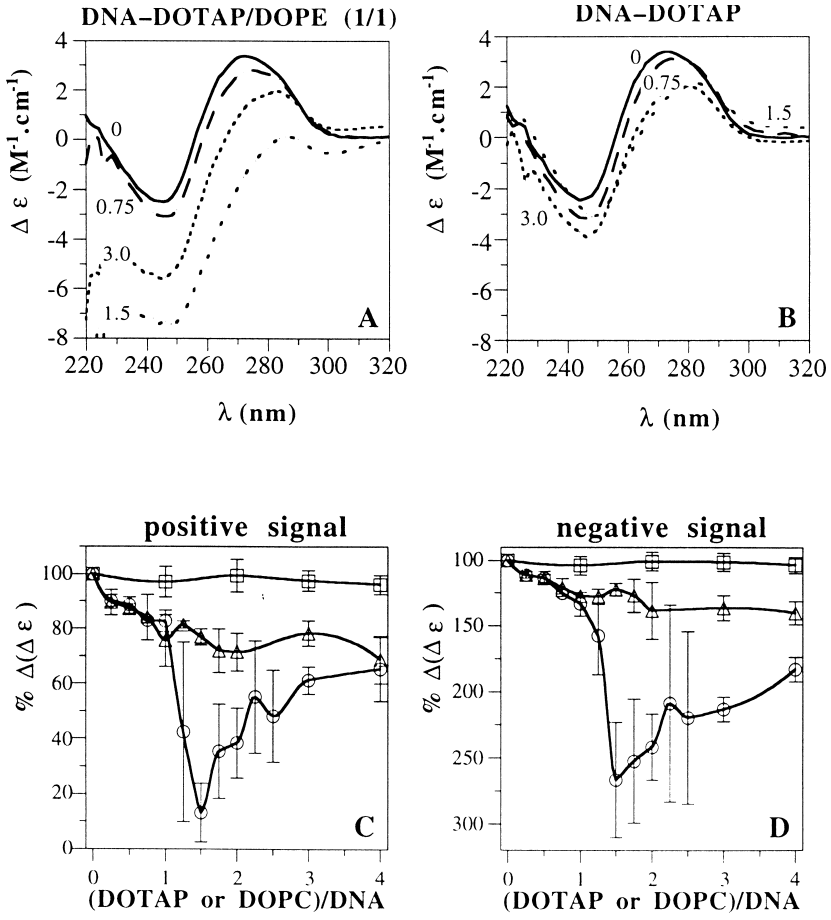

Fig. 1. A, B: CD spectra of DNA-DOTAP/DOPE (1/1) and of DNA-DOTAP complexes, respectively. The molar ratios of DOTAP/DNA are indicated. C, D: Changes in the maxima of the positive (top) and negative (bottom) CD signals exhibited by the DNA upon addition of DOTAP/DOPE (1/1) (○), DOTAP (△) or DOPC (□). The changes in the ellipticity are expressed as % of its value for native B-DNA. Notably, the significant variations in the amplitude of the signals indicate the presence of asymmetric, optically active aggregates of various sizes. For experimental details see [18].

transition which proceeds until a limit C-DNA is obtained. We propose that the enhanced negative ellipticity relative to a limit C form and the non-continuous nature of the spectral changes reflect a partial DNA collapse into a chiral ψ-phase. In this cholesteric-like phase DNA molecules are tightly-packed together to form a toroidal superhelical bundle whose chiral sense is defined by the intrinsic DNA handedness. Specifically, right-handed secondary DNA conformations such as the B and C motifs stabilize a left-handed tertiary conformation [12,13]. Such a tightly-packed left-handed DNA organization exhibits negative CD signals whose magnitude is larger than that characterizing dispersed DNA molecules which lack a tertiary structure [14] (Fig. 2).

The ionic strength and relative humidity conditions that effect a B-to-C transition and the formation of a tertiary ψ-phase are shown in Fig. 2B. We find that the C motif can gradually transform into a chiral ψ-DNA phase. This observation suggests a cationic liposome-mediated sequential process in which DNA molecules undergo an initial B-to-C transition for mixture of 1/1 DOTAP/DOPE liposomes that is followed by an intermolecular collapse into a condensed ψ-phase as the DOTAP/DNA ratio is increased.

A packed chiral ψ-DNA phase is indicated by the large negative CD signal, a further enhancement of this signal in the presence of EtOH (data not shown), as well as by the significant variability that is observed in the amplitude of the signal as experiments are repeated (Fig. 1C, D). Notably, dehydrating agents were found to promote DNA collapse (Fig. 2), and the variability in the signal amplitude has been shown to characterize condensed DNA phases [15]. The helper lipid DOPE which dries the liposome surface (Hirsch-Lerner and Barenholz, unpublished) may play the same role dehydrating agents playing in DNA collapse in solution. The variability in signal amplitude results from the presence of chiral, asymmetric aggregates at various sizes. The mechanism by means of which positively-charged liposomes affect chiral packaging is, however, not straightforward. For DNA molecules to form a condensed ψ-phase two requirements must be met: a tight DNA organization that allows inter-helical couplings between chromophores, and a long-range twist of the closely packed double-stranded DNA [14]. Yet, neither the lamellar nor the columnar inverted-hexagonal DNA-liposome complexes (both are major forms of the lipoplex-rich polymorphism) fulfil these prerequisites. Within the columnar assembly the DNA molecules are packed in a 2-dimensional (2D) smectic phase; this organization is, however, non-chiral and, due to the separation between the 2D layers, not sufficiently dense to allow efficient DNA-DNA couplings. The relatively low DNA density and the intrinsic lack of a long-range chiral twist are even more pronounced in the columnar

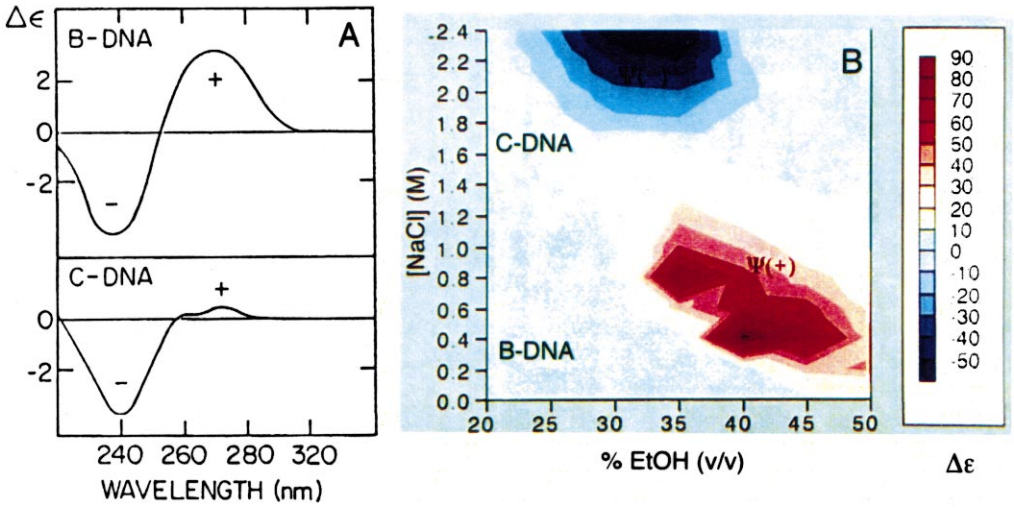

Fig. 2. A: CD spectra exhibited by sonicated calf thymus DNA (3000–4000 base-pairs) taken at conditions that stabilize the 'limit' forms of B-DNA (10 mM NaCl, 10 mM Tris, pH 7.0) and C-DNA (6.0 M LiCl, 10 mM Tris, pH 7.0). B: Phase diagram of B-C transition and DNA condensation as a function of NaCl and EtOH. Transitions are monitored by CD; data points represent signal magnitudes.

inverted-hexagonal structure [5], for which ψ-DNA packaging is observed (Fig. 3).

Our interpretation for the liposome-mediated chiral DNA condensation derives from features that were detected in assemblies composed of DNA and positively-charged micelles [16]. In this system a novel DNA packaging mechanism was discerned where DNA molecules are partially embedded in a hexagonal micellar scaffold and partially condensed into a highly packed chiral structure. We propose that very similar structural and spectral phenomena occur within the DNA-DOTAP-DOPE assembly (Fig. 3). Specifically, in a lamellar packaging (e.g. liposomes composed of pure DOTAP) only secondary structural DNA modifications are observed. Such modifications result from the ionic environment and dehydrating conditions prevailing near the lipid surface. In contrast, the inverted-hexagonal assembly acts as a pre-packaging scaffold that provides the DNA with a given spatial organization and a fixed directionality. At moderate lipid-to-DNA ratios, small segments of the DNA molecules remain free of lipids; due to their hexagonal directionality dictated by the complexed regions, such 'naked' segments are capable of converging into a highly condensed bundle. The left-handed chirality exhibited by these toroidally-packed bundles and indicated by the negative ellipticities, results from the DNA right-handed secondary conformations, as discussed above. The proposed structure provides a straightforward interpretation of the spectral features, as well as of the differential interference contrast images of the complexes, where connected blobs were detected [5]. Moreover, the attenuation of the ψ-CD signal at high DOTAP/DNA ratio can be directly assigned to a more extensive coverage of the DNA by the lipids which negates a lipid-free DNA collapse. The transformation between a lipid-DNA complex in which part of the DNA is in a ψ-tertiary structure and a lipid-DNA complex lacking the tertiary DNA structure is fast and reversible, and dependent on the DNA-to-lipid ratio [18].

The results presented in this study point toward an assembly in which two distinct, yet causally inter-related DNA packaging modes coexist. This system provides a unique example of the notion that subtle changes in the components of a supramolecular assembly may substantially modulate the interplay of interactions which dictate its structure. In the case of the DNA-lipid complexes these modulations are likely to have considerable effects upon the transfection efficiency.

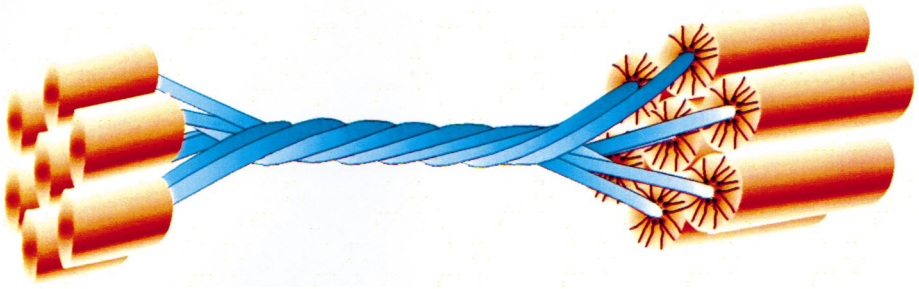

Fig. 3. Schematic representation of a model depicting a small section of the DNA-liposome complex where two DNA packaging modes coexist. Most of the DNA is embedded within the lipid columnar inverted-hexagonal assembly [5] which provides the DNA with a given spatial organization and a fixed directionality. Short unbound segments of the DNA which appear along the hexagonal assembly converge into a highly condensed toroidal ψ-structure whose left-handed sense is dictated by the DNA secondary structure and indicated by negative non-conservative CD signals [12,13,17].

Acknowledgements: This work was supported in part by EC Biotech, Contract No. PL 97002 to Y.B.

References

[1] Lasic, D.D. and Barenholz, Y. (1996), Vol. IV, CRC Press, Boca Raton, FL.
[2] Gershon, H., Ghirlando, R., Guttman, S.B. and Minsky, A. (1993) Biochemistry 32, 7143–7151.
[3] Lasic, D.D., Strey, H., Stuart, M.C., Podgornic, R. and Frederic, P.M. (1997) J. Am. Chem. Soc. 119, 832–833.
[4] Radler, J.O., Koltover, I., Salditt, T. and Safinya, C.R. (1997) Science 275, 810–814.
[5] Koltover, I., Salditt, T., Radler, J.O. and Safinya, C.R. (1998) Science 281, 78–81.
[6] May, S. and Ben, S.A. (1997) Biophys. J. 73, 2427–2440.
[7] Zuidam, N.J. and Barenholz, Y. (1998) Biochim. Biophys. Acta 1368, 115–128.
[8] Zuidam, N.J. and Barenholz, Y. (1997) Biochim. Biophys. Acta 1329, 211–222.
[9] Ivanov, V.I., Minchenkova, L.E., Schyolkina, A.K. and Poletayev, A.I. (1973) Biopolymers 12, 89–110.
[10] Hanlon, S., Brudno, S., Wu, T.T. and Wolf, B. (1975) Biochemistry 14, 1648–1660.
[11] Akao, T., Fukumoto, T., Ihara, H. and Ito, A. (1996) FEBS Lett. 391, 215–218.
[12] Rudall, K.M. (1955) Lect. Sci. Basic Med. 5, 217–230.
[13] Reich, Z., Wachtel, E.J. and Minsky, A. (1994) Science 264, 1460–1463.
[14] Keller, D. and Bustamante, C. (1986) J. Chem. Phys. 84, 2972–2980.
[15] Huey, R.M. and Mohr, S.C. (1981) Biopolymers 20, 2533–2552.
[16] Ghirlando, R., Wachtel, E.J., Arad, T. and Minsky, A. (1992) Biochemistry 31, 7110–7119.
[17] Reich, Z., Levin, Z.S., Gutman, S.B., Arad, T. and Minsky, A. (1994) Biochemistry 33, 14177–14184.
[18] Zuidam, N.J., Hirsch-Lerner, D., Margulies, S. and Barenholz, Y. (1999) Biochim. Biophys. Acta, in press.