ELSEVIER

Biochimica et Biophysica Acta 1369 (1998) 34–38

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The structure of DNA complexes with cationic liposomes-cylindrical or flat bilayers?

Nily Dan *

Dept. of Chemical Engineering, University of Delaware, Newark, DE 19716, USA

Received 7 April 1997; accepted 7 July 1997

Abstract

DNA complexes with cationic lipids promise to be versatile and effective synthetic transfection agents. Recent experiments identified both flat lamellar structures, where DNA strands are sandwiched between lipid bilayers, and cylindrical ones where the DNA is coated by a curved bilayer. Using a simple model we compare the stability of the two structures, and find that flat-bilayer aggregates are always more stable than the cylindrical ones. The different experimental observations are explained within the framework of the model predictions. © 1998 Elsevier Science B.V.

Keywords: DNA; Cationic liposome; Self assembly; Transfection

1. Introduction

The search for synthetic DNA transfection agents is prompted by the increasing needs of gene therapy techniques. One of the more viable alternatives to the currently predominant viral vectors is that of DNA complexes with cationic liposomes [1,2].

Most studies of DNA complexes with cationic liposomes concentrate on transfection characteristics [1–3]. While some systems display relatively high transfection efficiency, different lipids or cell lines show enormous differences in effectiveness [1]. The causes of these variations are unknown, since neither the structure of the complexes or the transfection mechanism were, as a rule, examined. Yet, one expects that the geometry of the DNA complex will influence the interactions between complexes and cells, and thus the transfection process.

In Fig. 1, we show three possible geometries of DNA complexes with cationic liposomes. The simplest case (Fig. 1A) is one where the liposomes adhere, as is, to the DNA strand in a 'beads on string' like manner, similar to that of micelle complexes with oppositely charged polyelectrolytes [4,5]. The second possibility is one where DNA adsorbs between the liposome bilayers in an alternating, locally flat lamellar structure, as shown in Fig. 1B. In both these cases the integrity of the original liposomes (which are multi-lamellar vesicles) is more or less preserved. The last configuration involves the breakup of the liposomes to coat the DNA by a cylindrical bilayer (Fig. 1C).

Recent experiments identified all three structures. Scattering [6,7] and fluorescence [8] experiments found multi-lamellar aggregates in several lipid systems and at various DNA to lipid ratios. 'Bead on string'-like complexes, as well as DNA strands coated by cylindrical bilayers, have been seen using freeze–fracture electron microscopy [9].

* Fax: +1-302-8311048.

PII S0005-2736(97)00171-5

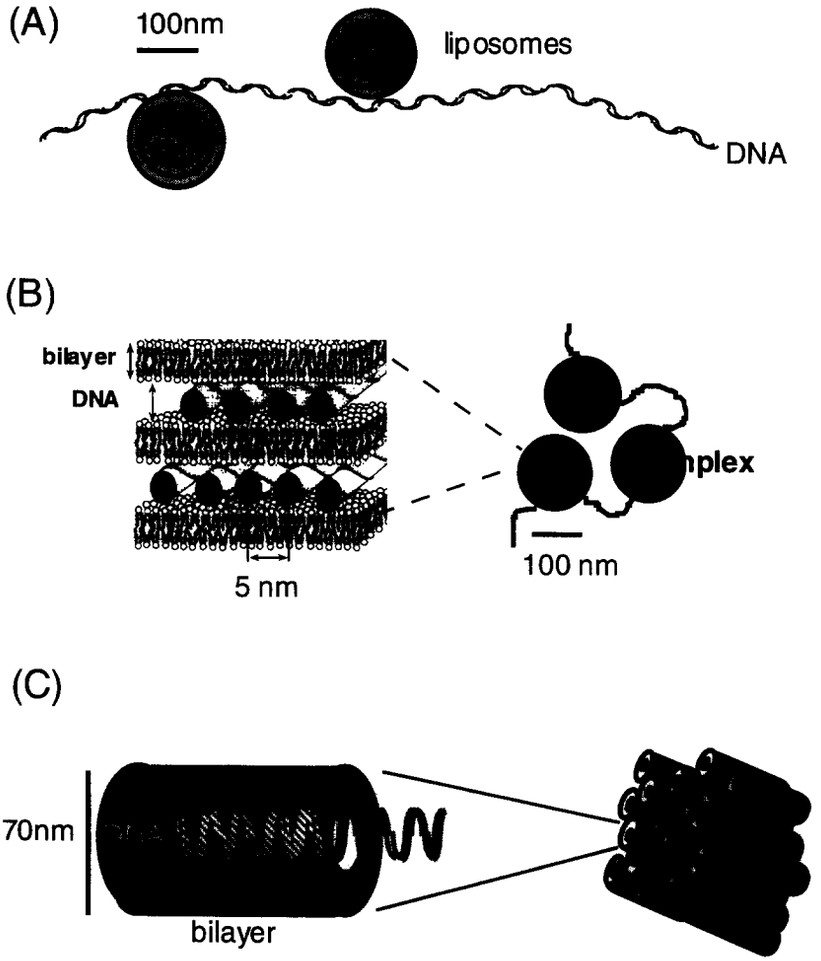

Fig. 1. Structures of DNA complexes with cationic liposomes: (A) 'Bead on string' complexes. The positively charged liposomes, which are multi-lamellar vesicles, adhere as an intact bead on the negatively charged DNA. (B) Lamellar complexes. Spherical macro-aggregates [9] are composed of flat lipid bilayers, with DNA packed between them in a 'sandwich' like structure. The macro-aggregates are sometimes joined by a DNA strand. (C) Cylindrical complexes between DNA and cationic lipids. The cationic lipids coat the DNA stand with a cylindrical-shaped bilayer.

In this paper, we utilize a simple model for lipid self assembly [10,11] to investigate the properties of DNA complexes with cationic lipids. Understanding the parameters determining the structure of DNA-lipid complexes would enable control of the complex structures and, thus, design of effective DNA carriers.

2. Model

Let us first review the mechanism controlling the self-assembly of lipids into aggregates. The interfacial area per lipid, Σ, is a function of the lipid chemistry and structure [10,11]. The thickness of the hydrophobic tail layer can be calculated by equating the volume of the tails (v) to the geometrical volume available to a chain in the given aggregate. In a flat monolayer, the thickness of the tail region, h_l, is equal then to v/Σ. Due to symmetry, the thicknesses of the two monolayers composing a flat bilayer are equal (Fig. 2A).

Similarly, we can calculate the thickness of each of the monolayers comprising a cylindrical bilayer. However, in this case the thickness of the inner monolayer is different from that of the outer mono-

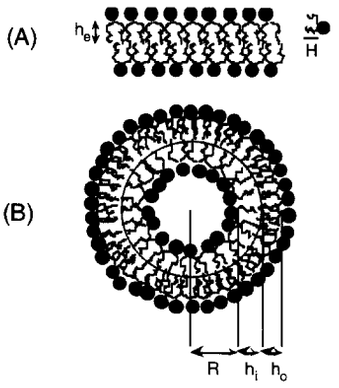

Fig. 2. Lamellar and cylindrical aggregates: (A) Packing of lipids in lamellae: The area per lipid is Σ. The thickness of the hydrophobic tail region is h_l, and is determined by equating the volume of a tail (v) to the geometrical volume Σh_l. The thickness of the unperturbed tail, H, is larger than h_l since packing into the lamellae enforces tail stretching. (B) Packing of lipids in cylindrical bilayers: R denotes the radius of curvature at the inner layer's head–tail interface. The thickness of the inner layer (h_i) is determined, as in the lamellar case, by equating the tail volume to the volume of a lipid in a cylinder of radius R. Similarly, the thickness of the outer layer is determined by equating the tail volume to the volume of a lipid in a cylinder of radius $R + h_i + h_o$.

layer, due to the interfacial curvature (see Fig. 2). Comparing the volume of a tail to the geometrical volume yields, for the inner and outer layers, respectively

$$h_i = R\left[(1+2\eta)^{1/2} - 1\right] \quad (1a)$$

$$h_o = R(1+2\eta)^{1/2}\left[(1+2\eta)^{1/2} - 1\right] \quad (1.b)$$

where R denotes the radius of the interior hydrophilic–hydrophobic interface, and $\eta \equiv h_l/R$. The number of lipids in each of the layers, per unit length, is also not the same and can be calculated by dividing the area (per unit length) of the inner and outer monolayers by Σ, the area per lipid:

$$n_i = 2\pi R/\Sigma \quad (2a)$$

$$n_o = 2\pi(R + h_i + h_o)/\Sigma = 2\pi R(1+2\eta)/\Sigma \quad (2b)$$

Let us now consider DNA complexes with cationic lipids. The driving force for DNA complexation with cationic lipid is due to the release of counter-ion entropy upon complexation [12]. This energy is very high, of order 1 $kT/$Å. Therefore, we expect that DNA will always form some sort of complex with cationic lipids.

The relative stability of DNA–lipid aggregates is determined by the difference in their free energy. This energy is composed of three contributions: The head–head interaction energy, the tail–tail interaction energy, and the interfacial energy.

Both the head–head interactions and the interfacial energy are a function of the surface density only [10,11]. Due to electrostatic screening effects, the surface density of lipids in a complex with DNA will vary as a function of DNA concentration [12]. Similarly, the surface density of the inner and outer layers in a cylindrical DNA–lipid complex will be different. For simplicity, however, we neglect these effects and assume that the surface density of both lamellar and cylindrical aggregates is uniform [1] and given by Σ. Since we take the surface density to be the same in both lamellar and cylindrical aggregates, the head–head and interfacial energies are equal in both.

The third contribution to the free energy of aggregated lipids is due to the deformation energy of the hydrophobic tails. It is associated with their perturbation from an optimal length, which we will denote by H [11]. The perturbation is small, so that the energy (per lipid) can be written using the general form

[1] Two effects are neglected when assuming that Σ, the area per lipid, is the same in both flat and cylindrical aggregates. The first is the effect of interface curvature, and the second is due to screening of the electrostatic interactions between the cationic lipid head groups. Detailed analysis of the former [13–15] have shown that, spherical bilayers are more stable than flat one only if they are allowed to 'choose' an optimal curvature, and if the spontaneous curvature of the amphiphile is extremely high (in which case micelles are likely to form rather than bilayers). Similar results are found for cylindrical bilayers. As for electrostatic screening; in the flat bilayers we can crudely estimate that the DNA directly interacts with $4R/\Sigma$ lipids per unit length (i.e., two times the DNA diameter, as shown in Fig. 1), while in the cylindrical bilayer the DNA screens the number of lipids in the inner bilayer, i.e., $2\pi R/\Sigma$ per unit length. The small difference in the number of screened lipids is not enough to overcome the interfacial curvature constraints and the tail packing energy. Detailed analysis of the system, taking into account these effects, is currently in preparation.

$$F(h) \approx F(H) + \left.\frac{\partial F}{\partial h}\right|_H (h-H) + 1/2 \left.\frac{\partial^2 F}{\partial h^2}\right|_H (h-H)^2 \quad (3a)$$

where h is the length of the tail in the aggregate (see Fig. 2). Since we define H as the equilibrium length, $(\partial F/\partial h)|_H$ is by definition zero. The effective compressibility of the tails, i.e., their resistance to compression or expansion, is given by $B \equiv (1/2)(\partial^2 F/\partial h^2)|_H$. The free energy per lipid can therefore be written as

$$F(h) = F_0 + B(h-H)^2 \quad (3b)$$

where F_0 is the energy of the unperturbed state (H). The energy of a lipid in the lamellar aggregate is, then, $F(h_l)$. The energy of a lipid in a cylindrical aggregate is given by $F(h_i)$ or $F(h_o)$, for the inner and outer layers, respectively.

To compare the energy of the two types of geometries, let us imagine taking a total of $n_i + n_o$ lipids from a lamellar aggregate and inserting them into a cylindrical one: n_i molecules will go into the inner layer and therefore change their energy by $F(h_l) - F(h_i)$, and n_o molecules will change their energy by $F(h_l) - F(h_o)$. If the overall change is positive, the energy of a lamellar aggregate is higher than that of cylindrical ones;

$$\begin{aligned}\Delta F &= n_i\{F(h_l) - F(h_i)\} + n_o\{F(h_l) - F_l(h_o)\} \\ &= Bn_i\{(h_l - H)^2 - (h_i - H)^2\} + Bn_o\{(h_l - H)^2 \\ &\quad - (h_o - H)^2\}\end{aligned} \quad (4)$$

A positive ΔF indicates that cylinders are stable, while lamellae are favorable when ΔF is negative. For a given system, we can define a critical equilibrium tail length, H_c, at which the energy of the two geometries is equal and $\Delta F = 0$:

$$H_c = R \times \left(\frac{2+6\eta+7\eta^2+3\eta^3-2(1+2\eta)^{1/2}(1+2\eta+2\eta^2)}{2\eta(1+\eta-(1+2\eta)^{1/2})} \right) \quad (5)$$

When the tail rest length (H) is larger than this critical value H_c, hexagonal phases are favorable. When H is smaller than this value lamellae are the stable configuration.

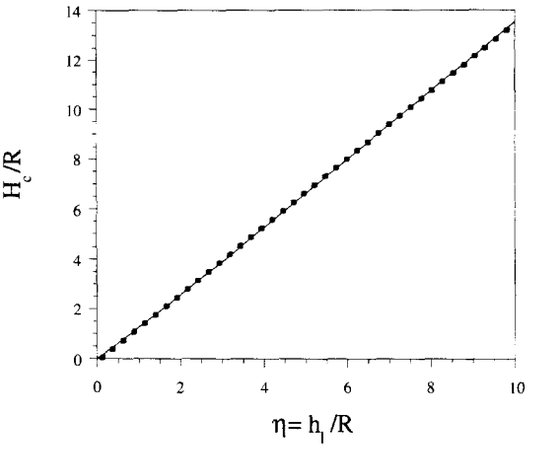

Fig. 3. The critical equilibrium tail length, H_c, as a function of the lamellar thickness, h_l (Eq. (5)). The solid line denotes the results of Eq. (5). The dashed line is a least-square fit to a linear function, with a slope which is ca. 1.34.

Plotting the critical tail thickness as a function of $\eta = \eta_l / R$ (Fig. 3), we see that H_c is almost linearly proportional to h_l. Since least square analysis shows that the slope value is somewhat larger than unity (See Fig. 3), cylindrical aggregates of any curvature will be more stable than lamellae only if $H_c > h_l$. This condition cannot be fulfilled, however; H defines the length of the unperturbed tail. Aggregation into lamellae will always stretch it [11]. Therefore, we conclude that cylindrical bilayer aggregates are always meta-stable when compared to lamellar structures.

3. Discussion

The simple model derived here for DNA complexes with cationic liposomes shows that these should only form flat bilayer aggregates. Although the model is oversimplified, it captures the essential features of the system (Dan, in preparation). This analysis is in agreement with recent experiments [6–8], where only flat lamellar aggregates are found, in several DNA–lipid mixtures [6–8] and at all DNA to lipid concentration ratios [6].

Yet, Sternberg et al. [9] clearly see cylindrical and 'bead on string' arrangements, which seems to contradict the model predictions [2]. Or does it?

Let us review the experimental data. In both scattering [6] and microscopy [9] experiments, globules of order 100–200 nm were observed. Radler et al. [6] have been able to show, using scattering techniques, that these globules actually consist of multi-lamellar DNA–lipid stacks. We suggest that the globules observed by Sternberg et al. [9] in their freeze–fracture images are similar multi-lamellar aggregates, and not pure lipid liposomes as supposed by the authors. Moreover, in both cases [6,9] the globules were found to be connected by a DNA 'string' (see Fig. 2B). These appear because of packing and bending constraints on the long DNA molecules in the confined multi-lamellae. Due to the high interactions energy between DNA and cationic lipids [12], the exposed DNA sections will be covered by a meta-stable cylindrical bilayer, as indeed observed by Sternberg et al. [9].

In conclusion, we show here that the only stable geometry of DNA–cationic lipid aggregates is a multi-lamellar one, regardless of the lipid properties. The only exception is lipids who form hexagonal II phases [10], where the DNA is coated by a single monolayer rather than a bilayer, and the cylinders are closely packed. Recent calculations by May and Ben-Shaul [16] have shown that these, 'honeycomb' like structures are more stable than the cylindrical ones, and probably, in some cases, preferable to flat aggregates as well.

[2] The discrepancy cannot be explained by rapid formation of meta-stable cylindrical aggregates, who slowly re-assemble into stable lamellar structures: Radler et al. [6] observe rapid kinetics of lamellar assembly (within 30 min), while Sternberg et al. [9] see cylindrical and bead-like structures even after 24 h of incubation.

Acknowledgements

I would like to thank Avinoam Ben Shaul, Robijn Bruinsma, Bill Gelbart, Cyrus Safinya, Tim Salditt and Phil Pincus for useful discussions.

References

[1] J.P. Behr, Bioconjugate Chem. 5 (1994) 382–389.
[2] P.L. Felgner, Y.J. Tsai, L. Sukhu, C.J. Wheeler, M. Manthorpe, J. Marshall, S.H. Cheng, Ann. NY Acad. Sci. 772 (1995) 126–139.
[3] E. Fortunati, A. Bout, M.A. Zanta, D. Valerio, M. Scarpa, Biochim. Biophys. Acta 1306 (1996) 55–62.
[4] B. Cabane, R. Duplessix, J. Phys. (Fr.) 43 (1982) 1529–1542.
[5] B. Cabane, R. Duplessix, J. Phys. (Fr.) 48 (1987) 651–662.
[6] J.O. Radler, I. Koltover, T. Salditt, C.R. Safinya, Science 275 (5301) (1997) 810–814.
[7] D.D. Lasic, H. Strey, M.C.A. Stuart, R. Podgornik, P.M. Frederik, J. Am. Chem. Soc. 119 (1997) 832–833.
[8] D. Hirsch-Lerner, Y. Barenholz, Biophys. J. (1997) in press.
[9] B. Sternberg, F.L. Sorgi, L. Huang, FEBS Lett. 356 (1994) 361–366.
[10] R.B. Gennis, Biomembranes-Molecular Structure and Function, Springer-Verlag, Berlin, 1989.
[11] J. Israelachvili, Intermolecular and Surface Forces, 2nd ed. Academic Press, New York, 1992.
[12] N. Dan, Biophysical J. 71 (1996) 1267–1270.
[13] A. Ajdari, L. Leibler, Macromolecules 24 (1991) 6803–6805.
[14] Z.G. Wang, Macromolecules 25 (1992) 3702–3705.
[15] N. Dan, S.A. Safran, Macromolecules 27 (1994) 5766–5772.
[16] S. May, A. Ben-Shaul, Biophysical J. (1997) in press.