Pharmacology

Chemotherapy

Chemotherapy 2006;52:196–199
DOI: 10.1159/000093270

Received: November 30, 2004
Accepted after revision: September 6, 2005
Published online: May 10, 2006

DNA-Spermine and DNA-Lipid Aggregate Formation Visualized by Fluorescence Correlation Spectroscopy

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Key Words
Lipid-DNA aggregates · Plasmid condensation · Fluorescence correlation spectroscopy

Abstract
Background: Fluorescence correlation spectroscopy (FCS) can be used for the determination of diffusion coefficients of single molecules. Since diffusion coefficients are correlated with size and shape of the labeled species, FCS provides information on conformational changes in plasmids aggregates. ***Methods:*** A 10-kbp plasmid stained with PicoGreen® was condensed by spermine or liposomes formulated from cationic lipid and egg phosphatidylcholine. ***Results:*** The diffusion coefficient of DNA increases from 1.0×10^{-12} m^2/s to 3.2×10^{-12} m^2/s by the addition of spermine, whereas the addition of cationic liposomes leads to complexes characterized by diffusion coefficients with values ranging from 1.7 to 1.9×10^{-12} m^2/s. ***Conclusions:*** FCS experiments allow determining the diffusion coefficients of DNA-containing aggregates which provide information regarding the topology and homogeneity of the aggregate.

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Presented at the 1st World Conference on Magic Bullets – Ehrlich 2004, Nürnberg, Germany, September 9–11, 2004.

Introduction

The condensation of DNA involves a dramatic decrease in the molecular volume and is of immense biological and practical importance [1]. For example, the assembling of supramolecular aggregates intended to serve as vectors in gene therapy requires precise control of the process and complete characterization of the sample [2, 3]. The non-viral vectors for gene therapy usually carry excess of positive charge which interact with a negative-charged DNA molecule, leading to its condensation into nanoscale complexes. This DNA condensation provides protection from enzymatic hydrolysis and contains the right size to enter cells [4]. It has been shown that fluorescence correlation spectroscopy (FCS) is a direct tool for the visualization of DNA condensation induced by a variety of cationic compounds [5].

Materials and Methods

Chemicals
PicoGreen® dye was purchased from Molecular Probes (Leiden, The Nederlands), spermine from Sigma (St. Louis, Mo.), and N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethyl ammonium methylsulfate (DOTAP) and egg yolk phosphatidylcholine (egg PC) from Avanti Polar Lipids (Oregon, USA). A 10-kbp plasmid (pHβApr-1-neo, 3.4 μm contour length) was prepared according to the protocol described elsewhere [5]. The small unilamellar vesicles were prepared according to standard procedures. The final step of vesicle

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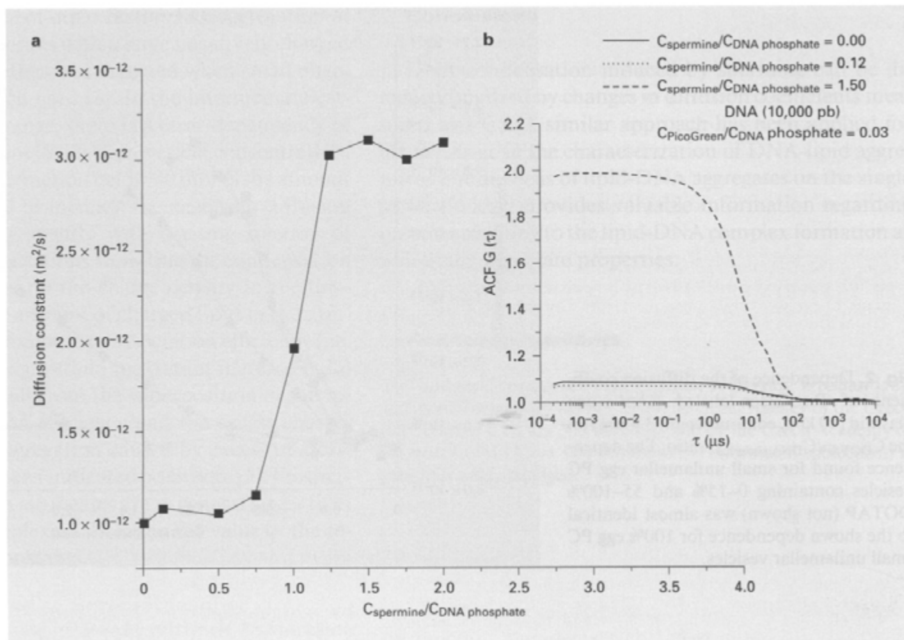

Fig. 1. a Changes in the diffusion coefficient of the βApr-1-neo plasmid (10 kbp, contour length 3.4 µm) as a dependence of $C_{spermine}/C_{DNA\ phosphate}$. The plasmid was labeled with PicoGreen. The $C_{dye}/C_{DNA\ phosphate}$ ratio was equal to 0.03. **b** Normalized fluorescence autocorrelation functions (AFCs) for the plasmid (βApr-1-neo 10 kbp, contour length 3.4 µm) labeled with PicoGreen ($C_{PicoGreen}/C_{DNA\ phosphate}$ = 0.03). Autocorrelation functions represented by solid, dotted, and dashed lines refer to DNA at the presence of spermine expressed in $C_{spermine}/C_{DNA\ phosphate}$.

formation was carried out with a LiposoFast small volume homogenizer (Avestin, Ottawa, Canada) with 100-nm pore size polycarbonate filters (Nucleopore, Pleasanton, Calif., USA). Finally, the liposome suspension was added to the plasmid suspension and, after short incubation, the fluorescence experiment was performed [5]. All concentration ratios are given in molar. All experiments were performed in a TE buffer (pH 7.95, 10 m*M* Tris, 1 m*M* EDTA).

FCS Experiments

Fluorescence measurements were performed on a ConfoCor® 1 (Carl Zeiss, Jena, Germany), as described by Kral et al. [5]. The FCS is based on a statistical analysis of the temporal behavior of spontaneous fluorescence intensity fluctuations [6] described by the autocorrelation function $G(\tau)$:

$$G(\tau) = \frac{[I(t)*I(t+\tau)]}{[I(t)]^2}$$

To obtain real physical parameters from the autocorrelation function, the appropriate physical model is needed. Assuming small point-like non-interacting molecules freely diffusing in a space much larger than the detection volume which are showing only triplet state dynamics, the $G(\tau)$ has the following form:

$$G(\tau) = 1+\left(1-T+Te^{-\tau/\tau_{tr}}\right)\left(\frac{1}{PN[1-T]}\right)\cdot\frac{1}{1+(\tau/\tau_D)}\left(\frac{1}{1+(\tau/\tau_D)(\varpi_0/\varpi_Z)^2}\right)^{1/2}$$

where T is a triplet fraction, τ_{tr} is a triplet decay time, PN is the apparent particle number, τ_D is a diffusion time and ω_0 and ω_Z are lateral and axial radii of the detection volume. The τ_D is related with macroscopic values of diffusion coefficient D via

$$\tau_D = \frac{\omega_0^2}{4D}.$$

Among other things, the diffusion coefficient depends on the shape of the macromolecules, and therefore, the change in the autocorrelation function should be observed during DNA condensation (fig. 1b).

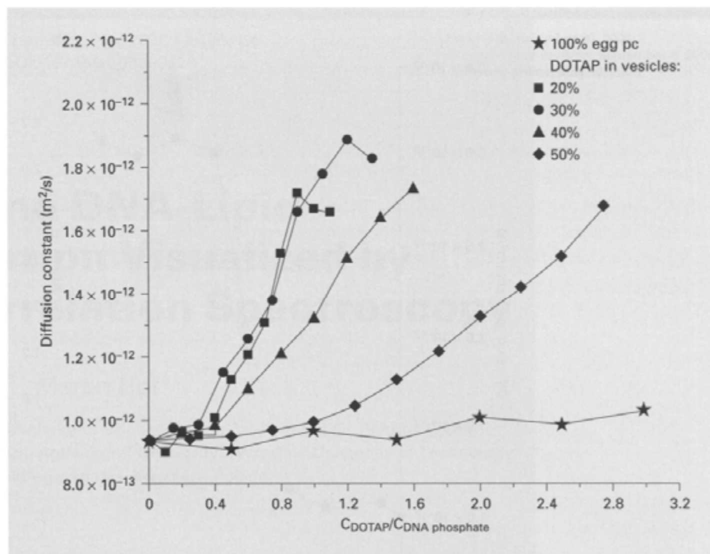

Fig. 2. Dependence of the diffusion coefficient of PicoGreen-labeled βApr-1-neo plasmid (10 kbp, contour length 3.4 μm) on the $C_{DOTAP}/C_{DNA\ phosphate}$ ratio. The dependence found for small unilamellar egg PC vesicles containing 0–15% and 55–100% DOTAP (not shown) was almost identical to the shown dependence for 100% egg PC small unilamellar vesicles.

Results and Discussion

DNA Condensation by Spermine

In order to follow the condensation process, the DNA molecule was labeled with PicoGreen. This dye has been shown to have excellent properties for the FCS applications. The condensation of the pHβApr-1-neo plasmid was induced by its titration with the cationic compound spermine. The increasing spermine concentration has caused a change of the diffusion coefficient from 1×10^{-12} m^2/s for naked DNA to about 3×10^{-12} m^2/s for the DNA-spermine complex (the condensed form) (fig. 1). From such a dependence, the amount of the condensing compound needed to complete the condensation process can be easily determined ($C_{spermine}/C_{DNA\ phosphate}$ = 1.5). The diffusion coefficient of plasmid in the presence of different spermine concentrations provides the quantitative values which may serve as an indication of DNA conformation when treated with other cationic compounds.

Effect of Cationic Liposomes on the DNA Condensation

Cationic lipids are frequently used for transfection formulations in vitro, as well as being a potential adjuvant for gene therapies [7]. In our case, for the correlation between the lipid properties and DNA-containing aggregate topology, sample uniformity needs to be precisely determined and correlated with the transfection efficiency.

When the DNA molecule interacts with the lipid aggregate, a number of factors need to be evaluated, including the total amount of cationic lipid and the fraction of charged lipids in each vesicle. It has been previously established that when oligonucleotides are exposed to lipid aggregates, 30 mol% of cationic lipid is needed to ensure the complete binding [8]. We intended to establish if a similar dependence can be found for large DNA molecules. For that reason, the plasmid labeled with PicoGreen was titrated with liposomes of fixed sizes but with varied fraction of cationic lipid (fig. 2). When the plasmid was titrated with neutral liposomes, the diffusion coefficient was constant throughout the whole range of lipid concentrations used. This result shows that FCS is capable of providing straightforward information regarding supramolecular interactions which are difficult to obtain with other techniques. Similar results have been obtained when the amount of cationic lipid in liposome was lower than 15 mol% and, surprisingly, at a concentration above 60 mol% (data not shown). In the first case, the charge density on the lipid surface is too low to ensure permanent binding of large DNA molecules. The apparent lack of interaction at a higher cationic lipid fraction may result

from the interference of other factors like aggregation of highly charged liposomes with a large negatively charged plasmid. A similar effect was detected when small oligonucleotides have been used [8]. In the intermediate cationic lipid fraction range, there is a clear dependency of the DNA diffusion coefficients on vesicle concentration. For the cationic lipid fraction below 30 mol%, the amount of liposomes needed to increase the aggregate diffusion coefficient decreases slightly with a rising fraction of DOTAP. These observations show that the condensation effect depends rather on the charge density in the liposome and not on the amount of charged lipid in the sample. The apparent maximum of association efficiency (i.e. minimum lipid concentration, maximum increase in D) at 30 mol% may result from the superposition of the increasing condensation efficiency and the excess charge-induced liposome aggregation caused by excess of a cationic charge, as has been indicated elsewhere [2]. Furthermore, the lipid-DNA aggregates are in general larger than DNA-spermine complexes, reflecting the value of the respective diffusion constants.

Conclusions

DNA condensation induced by spermine can be directly visualized by changes in diffusion coefficients measured by FCS. A similar approach has been applied for the first time in the characterization of DNA-lipid aggregates. The analysis of lipid-DNA aggregates on the single molecule level provides valuable information regarding processes leading to the lipid-DNA complex formation as well as the aggregate properties.

Acknowledgements

The financial support by the Institute of Physics, Wrocław University of Technology (to M.L.), by the Grant Agency of the Czech Republic (to T.K. via 203/05/2308) and by the Czech Academy of Sciences (to M.H. via 1ET400400413 – informacni spolecnost) is gratefully acknowledged.

References

1 Bloomfield VA: DNA condensation. Curr Opin Struct Biol 1996;6:334–341.
2 Kennedy MT, Pozharski EV, Rakhmanova VA, MacDonald RC: Factors governing the assembly of cationic phospholipid-DNA complexes. Biophys J 2000;78:1620–1633.
3 Scarpignato C, Pelosini I: Somatostatin analogs for cancer treatment and diagnosis: an overview. Chemotherapy 2001;47(suppl 2):1–29.
4 Byk G, Dubertret C, Escriou V, Frederic M, Jaslin G, Rangara R, Pitard B, Crouzet J, Wils P, Schwartz B: Synthesis, activity, and structure-activity relationship studies of novel cationic lipids for DNA transfer. J Med Chem 1998;41:224–235.
5 Kral T, Langner M, Benes M, Baczynska D, Ugorski M, Hof M: The application of fluorescence correlation spectroscopy in detecting DNA condensation. Biophys Chem 2002;95: 135–144.
6 Elson E, Magde D: Fluorescence correlation spectroscopy. 1. Conceptual basis and theory. Biopolymers 1974;13:1–27.
7 Miller AD: Cationic liposomes for gene therapy. Angew Chem Int Ed Engl 1998;37:1769–1785.
8 Jurkiewicz P, Okruszek A, Hof M, Langner M: Associating oligonucleotides with positively charged liposomes. Cell Mol Biol Lett 2003;8: 77–84.