

FORMATION OF DNA COMPLEXES WITH SPERMINE AND POLYLYSINE DERIVATIVES

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RESUMEN

En este trabajo se estudió la interacción entre ADN y moléculas con cargas positivas (surfactantes y polímeros) a través de técnicas de difusión dinámica (DLS) y estática (SLS) de la luz. Contrario a la creencia común respecto a los complejos polielectrolitos, se determinó que los complejos de ADN preparados en este trabajo contienen sólo una molécula del polianión. Al formar complejos con el copolímero de bloques polilisina-b-poliétilen glicol en medio acuoso, 100% de las cargas del ADN fueron neutralizadas con un pequeño exceso del catión.

Palabras Claves: complejos de polielectrolitos, ADN, masa molar, eficiencia de formación de complejos.

ABSTRACT

The interactions between DNA and positively-charged molecules (i.e. surfactants and polymers) have been studied in aqueous solutions through Dynamic (DLS) and Static (SLS) Light Scattering techniques. Contrary to the common believe about polyelectrolyte complexes, it was determined that the DNA complexes prepared in this studied contained only one polyanion molecule. When complexes are formed with polylysine-b-polyethylene glycol block copolymer in aqueous solution, 100% of the DNA charges are neutralized with a small excess of the cation.

Keywords: polyelectrolyte complexes, DNA, molar mass, efficiency of complex formation

1. INTRODUCTION

The formation of complexes with DNA (for applications such as gene therapy [1]), is characterized by multimolecular structures [3, 4]. The main objective of this work is to form DNA complexes consisting of only one polyanion. For this purpose, DNA complexes were formed in aqueous solutions with cations containing a hydrophilic block in order to obtain water-soluble complexes even after the neutralization of the DNA charges.

2. EXPERIMENTAL SECTION

Complex formation. An aqueous solution of DNA pUC19 c=0.05g/l was titrated directly in a light scattering cuvette with one of the multivalent cations shown in figure 1. The ionic strength of the solution is given by a 5 mM phosphate buffer pH=7.0.

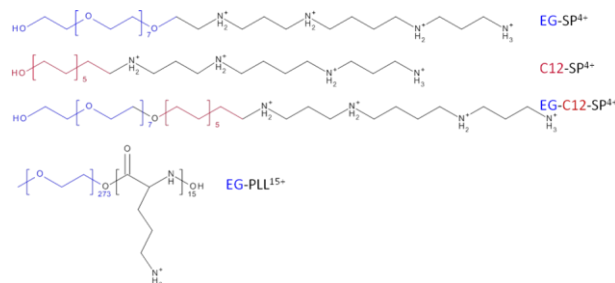


Figure 1. Multivalent cations used to form complexes with DNA in aqueous solutions: (a) polyethylene glycol-b-spermine (EG-SP⁴⁺), (b) dodecylspermine (C12-SP⁴⁺), (c) polyethylene glycol-b-dodecylspermine (EG-C12-SP⁴⁺), polyethylene glycol-b-polylysine (EG-PLL¹⁵⁺) (EG-PLL received from Prof. Kataoka, Tokyo University).

Static and dynamic light scattering. Static light scattering (SLS) measurements were performed with an ALV-SP86 goniometer, an ALV-3000 correlator, a Uniphase HeNe laser (25 mW output power at $\lambda=632.8$ nm wavelength) and ALV/High QE APD avalanche diode fiber optic detection system. For dynamic light scattering (DLS) a multiangle equipment (8 detectors separated by 17° each) consisting of an ALV/CGS/-8F DLS/SLS 5022F goniometer, 7004 correlator, a APD avalanche photodiode optic detection system and a

Uniphase HeNe ion laser (21 mW output power at $\lambda=632.8$ nm wavelength) was used. The complex solutions were typically measured from 30 to 150° in steps of 5° (SLS) or with a multiangle light scattering apparatus positioning the first detector at 30° and at 39°. Samples were filtered through a: GHP 200 nm DNA and spermine derivatives (a, b, and c from figure 1) and GS 220 nm for EG-PLL.

In order to evaluate properly the data, the following equation was deduced

$$\frac{Kc_{DNA}}{R} = \frac{1}{\left(\frac{M_{cation}^2}{M_{DNA}} f^2 + 2M_{cation}f + M_{DNA} \right)}$$

Where M_{cation} and M_{DNA} are the molar masses of the cation and the DNA respectively, c_{DNA} is the concentration of DNA without the bound cations and f is the number of cations per DNA molecule.

3. RESULTS AND DISCUSSIONS

In figure 2 it could be observed that the molar mass of the complexes remains below or equal to the maximum theoretical molar mass calculated for DNA with 100% of charge neutralized at any mixing charge ratio N+/P-. Thus, it is evidence that the complexes contain only one polyanion molecule and therefore there is no aggregation due to, for instance, hydrophobic interactions, or inter complex bridging. Also, the fact only one of the complexes with the studied cations achieve the maximum theoretical molar mass even after adding an excess of more than five-folds of positive charges and after having reached a plateau in the molar mass suggests that there is an equilibrium of the cations between the free and bound (to DNA) states. Steric hindrance or changes in the DNA conformation could be two possible explanations for the binding equilibrium of the cations.

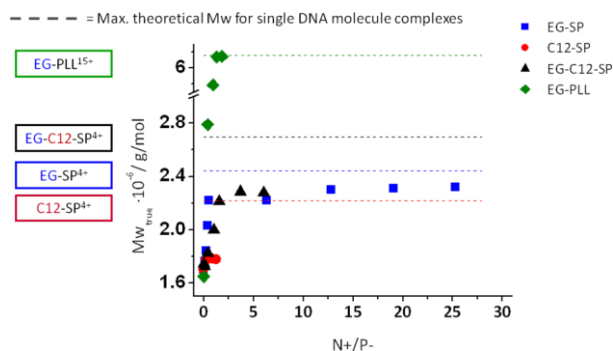


Figure 2. Molar mass of DNA complexes against mixing charge ratio N+/P-. The dashed lines represent the maximum theoretical molar mass for single-DNA-molecule complexes

Only in the case of the spermine derivative with a hydrophobic tail (C12-SP⁴⁺) precipitation was observed.

4. CONCLUSIONS

Except for DNA+C12-SP⁴⁺, all of the formed complexes consist of one DNA molecule. The EG-PLL seems to be the “ideal” condensing agent for DNA. The requirements of complex formation with 100% of the DNA charges neutralized with a very high efficiency (more than 80%) and consisting of only one DNA molecule were fulfilled.

5. REFERENCIAS

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