Review

Collapse of DNA in packaging and cellular transport

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ABSTRACT

The dawn of molecular biology and recombinant DNA technology arose from our ability to manipulate DNA, including the process of collapse of long extended DNA molecules into nanoparticles of approximately 100 nm diameter. This condensation process is important for the packaging of DNA in the cell and for transporting DNA through the cell membrane for gene therapy. Multivalent cations, such as natural polyamines (spermidine and spermine), were initially recognized for their ability to provoke DNA condensation. Current research is targeted on molecules such as linear and branched polymers, oligopeptides, polypeptides and dendrimers that promote collapse of DNA to nanometric particles for gene therapy and on the energetics of DNA packaging.

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1. Introduction

Remarkable improvement in the infectivity of poliovirus in the presence of cationic proteins such as histones and protamine facilitated research on cellular transfection of exogenous RNA and DNA in the presence of multivalent cations [1]. Electron microscopic characterization of DNA condensates formed in the presence of polyamines resulted in the first visualization of DNA nanoparticles [2]. Parallel developments of these areas of research have now culminated in nonviral gene delivery vehicles and nanomedicine, mimicking viral activities in the delivery of nucleic acids [3].

In the presence of multivalent cations, cationic polymers, proteins or peptides, DNA collapses from its extended coiled conformation to ordered nanometric particles (Fig. 1) [4–8]. The major prerequisite for the collapse of DNA is a sufficient degree of neutralization of DNA negative charges by positive ions, in order to overcome the repulsive forces between negatively charged DNA molecules, resulting in attractive forces [9,10]. Attractive and repulsive hydration forces are also involved in DNA–DNA interaction. The phenomenon of DNA condensation/collapse to nanoparticles has important implications in the spatiotemporal dynamics and functions of DNA in the cell [11].

The collapse of DNA to nanoparticles has biotechnological importance in nonviral gene delivery because DNA condensation by polycationic delivery vehicles facilitates the transport of DNA through the cell membrane [3,6,7]. Early investigators in this field developed model systems and theoretical calculations to determine the mechanism and energetics of DNA packaging in a small volume compared to the length of DNA [4,12–14]. Biogenic polyamines (Fig. 2), spermidine and spermine were considered to be the major driving force in the collapse of DNA in bacteriophage heads [2,4,12]. Polyamines are essential for cell growth and cell differentiation, and play important roles in gene regulation and carcinogenesis [15–17]. In vitro, they are known to stabilize duplex and triplex structures as well as unusual DNA conformations, and protect DNA from external radiation and reactive oxygen species [8,18–21].

Synthetic pentamines, hexamines and polyethyleneimine (PEI) are more effective than natural polyamines in condensing DNA [5,22–24] (Figs. 3 and 4). In polyamines, the positive charges are separated by methylene bridging regions. In order to determine the role of positive charge versus chemical structure of DNA condensing cations, Widom and Baldwin [25] examined the effectiveness of an inorganic cation, cobalt hexamine $(Co(NH_3)_6^{3+})$ to collapse DNA. This molecule has the same number of positive charges as that of spermidine^{3+}, and hence it can serve as a comparator to the trivalent polyamine. Thomas and Bloomfield [13] found $Co(NH_3)_6^{3+}$ provoked DNA condensation in a manner similar to that of spermidine, although it was ~5-fold more effective than spermidine. Arginine peptide was used to condense DNA as a model for DNA packaging in sperm and analyze the attractive and repulsive forces involved in DNA condensation [26]. Oligo- and poly-lysines were also very effective to collapse DNA to nanoparticles [27,28]. Considering DNA as a negatively charged biological polymer, its condensation is an example of coil–globule transition that occurs when approximately 89% of the polyanionic charge is neutralized by multivalent cations [12,13,29]. Although several cations have been shown to condense DNA to ordered structures such as toroids, spheroids and rods, extensive work on DNA condensation has been carried out using natural polyamines, polyamine analogues, and cobalt hexamine [4,6,7,8,12,14,21–23,30–32].

Utilization of the condensation process has advanced gene therapy applications of polycations such as PEI [24,33]. PEI's strong transfection ability is due to its effectiveness in condensing DNA to nanoparticles. Another common agent used in transfection is cationic liposomes, a spherical vehicle with at least one lipid layer [34]. Cationic liposomes spontaneously react with negatively charged oligodeoxynucleotides and plasmid DNA that are used in gene therapy, resulting in the formation of self-assembled complexes. Highly branched polymers or dendrimeric molecules from poly(amidoamine) (PAMAM), polypropyleneimine (PPI), poly(L-lysine)(PLL), and carbon-silanes have been explored for the cellular delivery of oligodeoxynucleotides and siRNA [35].

2. Electrostatic nature of DNA binding with polyamines and other cations

The theoretical framework for DNA condensation by multivalent cations was initially developed using polyamine-DNA interaction as a model system [12,13]. Polyamines are positively charged under physiological ionic and pH conditions [4]. For polyamines with more than one amine group, there exists a pK$_a$ value for each center of ionization depending on the type of the amine (primary, secondary or tertiary), steric and electronic factors, position of the amine group, and number of carbon atoms between the neighboring amines. At a physiological pH of 7.2, pK$_a$ values for natural polyamines are as follows: putrescine, 10.8 and 9.4; spermidine, 10.8, 9.94 and 8.4; spermine, 10.9, 10.1, 8.9 and 8.1 [36]. Hence the predominant mode of polyamine interaction with DNA is electrostatic in nature. Electrostatic interaction leads to the release of bound mono-valent ions from the DNA, with a net gain in entropy due to the release of a monovalent ion, such as Na$^+$ into solution:

$$spermine^{4+} + DNA \cdot nNa^+ \rightarrow DNA \cdot (n-4)Na^+ \cdot spermine^{4+} + 4Na^+$$

When the bulk Na$^+$ concentration is increased in the medium, the net gain in entropy on releasing the bound Na$^+$ to the solution decreases and an increased concentration of polyamines is required to compete with Na$^+$ and collapse DNA.

The ionic strength dependence of monovalent ions versus multivalent ions during DNA condensation can be calculated using the counterion condensation theory [12,37,38]:

$$1 + \ln(1000\theta_1/c_1 v_{p1}) = -2z_1\xi(1 - z_1\theta_1 - z_2\theta_2)\ln(1 - e^{-\kappa b}) \quad (1)$$

$$\ln(\theta_2/c_2) = \ln(v_{p2}/1000e) + (z_2/z_1)\ln(1000\theta_1 e/c_1 v p_1) \quad (2)$$

In these equations, c_1 and c_2 are the concentrations of counterions of charges z_1 and z_2 contributing to fractional charge neutralization of θ_1 and θ_2 and occupying volumes v_{p1} and v_{p2}, respectively. When they are bound to DNA and κ is the Debye screening parameter, $\xi = q_p^2/\varepsilon kTb$ where q_p is the charge of the proton, ε is the bulk dielectric constant, and b is the average linear charge spacing of the polyelectrolyte in the absence of any associated ions. In other words, the parameter ξ is given by the ratio between the Bjerrum length and the average axial charge spacing, that is, the contour length divided by the number of charge groups. For double-helical B-DNA, $\xi = 4.2$, while for the single-stranded DNA, $\xi = 1.8$. These values were calculated by Manning [37] and Record et al. [38] whereas Olson and Manning [39] provided a configurational interpretation of this result. κ is given by the equation:

$$\kappa = 3.29 z^{1/2} c_1^{1/2} (nm^{-1}) \quad (3)$$

Using the value of κ in Eq. (1) and introducing the first term of Eq. (1) into Eq. (2), the following equation can be derived after rearrangement:

$$\ln c_2 = [\ln\theta_2 - \ln(v_{p2}/1000e) + 2z_2\xi(1-r)\ln(3.29bz^{1/2})] + z_2\xi(1-r)\ln c_1 \quad (4)$$

In Eq. (4), $r = z_1\theta_1 + z_2\theta_2$, and the approximation of $\kappa b \sim 1 - e^{-\kappa b}$ has been introduced in Eq. (1). The maximum extent of charge neutralization of DNA by a combination of Na$^+$ and spermine^{4+} was calculated to be ~91% [12–14]. Substituting this value for r, the slope of a plot of $\ln c_2$ against $\ln c_1$ can be calculated to be 1.5 from Eq. (4). Vijayanathan et al. [14] found the slope value of such a plot to be close to this value for a series of spermine homologues, although