[Frontiers in Bioscience E1, 228-241, June 1, 2009]

Different strategies for controlling DNA conformation: compaction and decompaction

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1. ABSTRACT

In the present review we summarize different strategies to induce DNA compaction and decompaction. DNA compaction is achieved using different cationic co-solutes, such as trivalent ions, surfactant, and polycations. In addition, single-chained DNA compaction can also be achieved in solvents with low dielectric constants and by confinement. The decompaction strategies depend, naturally, on the method used for the compaction and can be accomplished by, for example, heparins, cyclodextrins, non-ionic or anionic surfactants.

2. INTRODUCTION

Since the discovery of the DNA structure in 1953 by Watson and Crick (1) much work has been done in order to understand the mechanisms involved in the life cycle of the cell. The understanding of such mechanisms has deep implications since it is the first step to control and mimic them. The possibility to treat diseases, hereditary diseases in particular, by the insertion of genes into human cells and tissues, so called gene therapy, has encouraged many studies in the last years (2). This promising new technology is still in its infancy and the therapeutic gene delivery

requires a better knowledge of the precise control of both the uptake into the cell nucleus (transfection) and posterior synthesis of RNA (transcription).

2.1. Gene delivery

The necessity to control DNA compaction by using different chemical agents is the basis of using non-viral vectors for gene delivery. Non-viral vectors are expected to have lower toxicity than the viral vectors and can be designed to avoid the immune system (2, 3). Furthermore, there is no limitation concerning the size of the DNA to be transfected while viral particles have the capacity of only around 40,000 base pairs. For these reasons non-viral vectors have generated increased interest (4-6). However, despite the efforts in synthesizing new molecules and testing new formulations, the efficiency of the non-viral vectors needs to be improved in order to make them a viable alternative.

Both controlled DNA compaction and decompaction, are needed for a successful delivery. In the first step DNA must be taken up by the cell membrane. Here, the compaction of DNA, together with the reduction of its charges, is believed to facilitate the uptake of the nucleic acids. Once inside, DNA in a compacted state is essential to protect it from the nucleases and allow it to reach the nucleus. For the compaction requirements, it is clear that cationic co-solutes are the best approach. The most commonly studied transfection vehicles are cationic liposomes and polycations. Cationic lipids have been very popular since the pioneer work of Felgner and co-workers (7) where DNA was successfully transfected into mammalian cells (a number of recent reviews can be found in *Curr. Med. Chem.* 11,133–220 (2004) and *Curr. Med. Chem.* 10, 1185–1315 (2003)).

When DNA arrives in the nucleus it should be accessible to the cell enzymatic machinery. This is only possible if decompaction occurs or if the degree of compaction of the DNA is not too high such as to hinder this step. The degree of compaction is therefore an important property. This has been shown, for example, for DNA-lipid complexes (lipoplexes) where the lipid bilayers with a large charge density transfected less efficiently (8). Also for the DNA–poly-L-lysine system the efficiency of transfection was higher for complexes with moderate DNA compaction (9).

Many studies are currently in progress to provide clues about the transfection mechanism (s) and how the composition of the vectors can improve their efficiency.

2.2. Gene regulation

The organisation of the nucleosome in hierarchical structures appears to provide a mechanism to modulate the stability of histone-DNA complexes and to facilitate or impede transcription. The mechanisms for gene regulation *in vivo* are not well known but transcription is believed to be related to the acetylation of certain residues in some histones, or their tails.

Several studies have been performed with the intent of regulating the gene expression at the transcriptional level, the so-called anti-gene strategy. This was successfully achieved using, for example, triplex-forming oligonucleotides (10-14), zinc-finger proteins (15-17), peptide nucleic acids (18-21), and synthetic polyamides (that bind to specific DNA sequences) (22, 23). These approaches rely on the base specificity of the promoter region for the binding of the agents that will inhibit transcription, interfering with either the transcription initiation or elongation. This is difficult to implement since the appropriate DNA sequence to target needs to be found. However, the potential for this sort of treatment is enormous. For other applications, such as protein production in large scales, this level of sophistication is unnecessary.

Following the indications that the conformation of DNA and its biological function are closely related, a number of studies have been conducted using non-specific binding agents. Dendrimers (24), lipids (25, 26), and polyamines (27, 28) have been used to control the transfection of DNA with different degrees of success. Polyamidoamine dendrimers, for example, were shown to provide protection against DNase activity and also to inhibit the transcription up to a certain extent, depending on the DNA to dendrimer molar charge ratio (24). It was also shown that the great majority of the transfection was carried out by low-density, soluble, complexes that represented only 10-20% of the total complexed DNA (24).

It is relevant to point out here that the term DNA condensation has been used in the literature indiscriminately for single molecule collapse and aggregation of several DNA molecules. In this review we will mostly discuss the folding of single DNA molecules that we will denote as compaction, condensation will be used when the complexes are formed by more than one DNA molecule. It is not the objective of this work to give an exhaustive report of this subject and, as such, many interesting references are left out. Many other articles are referenced in previous reviews on the subject of DNA condensation (29-32).

3. COMPACTION STRATEGIES

The importance of the compaction or condensation of DNA is undeniable as discussed in the introduction.

Different approaches can be utilised for the compaction of DNA, including compaction by cationic species, solvents with low dielectric constants and by means of confinement. These have naturally different mechanisms. In this section we will briefly review them and direct the reader to relevant literature.

3.1. Cationic co-solutes

The compaction of DNA induced by cationic species is driven by ion correlation effects. The DLVO theory describes interactions between charged colloids in solution (33, 34); here the attractive van der Waals interactions are balanced by the repulsive electrostatic interaction, treated with the Poisson-Boltzmann (PB) mean

field approximation. However, large deviations are found from the PB approximation for highly charged systems at short separations. In fact, in some cases a net attractive force between equally charged surfaces is found instead (35-37). This is a consequence of the fact that the ions are discrete entities that can correlate with the ion cloud condensed at the opposite wall.

In the case of large DNA molecules the presence of multivalent species, such as trivalent metal ions, induces an effective attraction between different parts of the molecule, leading to the DNA compaction. In fact, Monte Carlo simulations have shown that the compacted polyelectrolyte has smaller dimensions than the corresponding neutral polymer (38).

Cationic co-solutes are, for obvious reasons, the most used strategy to compact DNA; therefore, examples are numerous. We will here focus on the ones most commonly used.

3.1.1. Histones

The compaction of DNA in eukaryotic cells is achieved by small positively charged proteins called histones (39). Two copies of the core histones (H2A, H2B, H3 and H4) form the histone octamer complex around which 146 base pairs (bp) of DNA are wrapped in ~1.65 turns. This structure is called the nucleosome and it constitutes the main building block of the chromatin. H1, the so-called linker histone, binds to the nucleosome at the entry and exit sites of the DNA locking the DNA in position. The nucleosomes are spaced by 10-60 bp of "linker" DNA, forming a "beads on a string" conformation, which, in turn folds into higher order structures.

Despite the high degree of compaction in the cell, the DNA molecule has to remain accessible to the molecules involved in processes such as replication and transcription. The chromatin is therefore a dynamic structure and it is believed that the packaging and unpackaging of the DNA is dictated by the flexible chains that protrude from the nucleosomes (40, 41). The histone "tails" have positive charges, due to lysine and arginine residues, that can, for example, be turned off through acetylation and deacetylation.

The interaction of DNA with histones and, specially the "structure vs. function" of the nucleosomes, has been subject of a number of experimental, simulation and theoretical studies; for reviews see Refs (42-44) and references within.

3.1.2. Multivalent ions

In aqueous solution co-solutes of charge +3 or more are generally required to compact DNA (45). There are however some exceptions; the compaction of DNA was induced using quaternary diammonium salts (divalent cations) with different intercharge distances, lengths, branching, and chemical nature of the hydrophobic substituents (46).

Among the most used multivalent ions for the condensation of DNA is the inorganic cation hexamino cobalt (III), $(Co(NH_3)_6)^{3+}$ (47, 48), but many other coordination compounds of Co^{3+}, Ru^{3+}, and others have been synthesized and used. Metal ions have also been popular condensing agents for DNA, such as Al^{3+} (49, 50), lanthanide ions (La^{3+}, Eu^{3+}, Tb^{3+}) (51), Ga^{3+} (50), and Cr^{3+} (52, 53).

A large number of computer simulations of DNA compaction have been presented in the literature. The complexity of the DNA molecule and the length needed to achieve some degree of compaction (around one thousand base pairs) demands a coarse grain approach. Simulations have shown attraction between charged cylinders (54, 55) and stiff polyelectrolytes (56) in the presence of multivalent ions. As mentioned above, this attraction has been ascribed to correlated fluctuations of the ions (35). If the DNA molecule is sufficiently large, the short-range attraction acts between different parts of the same molecule, resulting in a chain that is more compacted than a corresponding neutral polymer, i.e., the compaction of DNA does not result from a trivial neutralisation of the phosphate charges (38), as it is often suggested in the literature.

3.1.3. Polycations

Cationic polymers are the most efficient condensing agents. In fact, the most compacted DNA is found in sperm heads where the condensing agents are protamines, arginine-rich linear proteins. This is due to the fact that polycations are generally highly charged. Also, on the contrary to trivalent ions where the effect on DNA is very local, limited to a few consecutive monomers (bases) (57), polycations interact with DNA bases that are significantly far apart, promoting bridging between different sites in the DNA chain or between different DNA chains (58). This leads to the formation of DNA-polycation complexes even in very dilute solutions. These complexes are considered to be promising DNA vehicles in gene therapy, which has prompted the development of novel cationic polymers and the study of their interaction with DNA (see Ref. (59) and references within).

The importance of the compaction degree for transfection of DNA was mentioned in the introduction. It has been shown by molecular simulations that an increase in the interaction strength between two oppositely charged polyeletrolytes of equal length leads to more compacted structures (60). Also the degree of compaction depends on the size of the polycations (58). When short chains (3 and 4 monomers) were used, the polyanion chain was rather extended and not all the polycations were associated to it, which suggests that an excess of short polycations may be necessary to induce DNA compaction, in agreement with microscopy studies in very diluted solutions (61). On the other hand, by using sufficiently long polycations compaction can be achieved at or before charge neutralisation (27, 58, 62).

Cationic dendrimers can be considered a special case of polycations. Their popularity is due to the fact that they are monodisperse; furthermore, it is possible to functionalise the arms so that the compaction can be controlled by using, for example, pH, light or redox sensitive dendrimers. Amongst the more used are poly (amido amine) (PAMAM) dendrimers of generation 4.

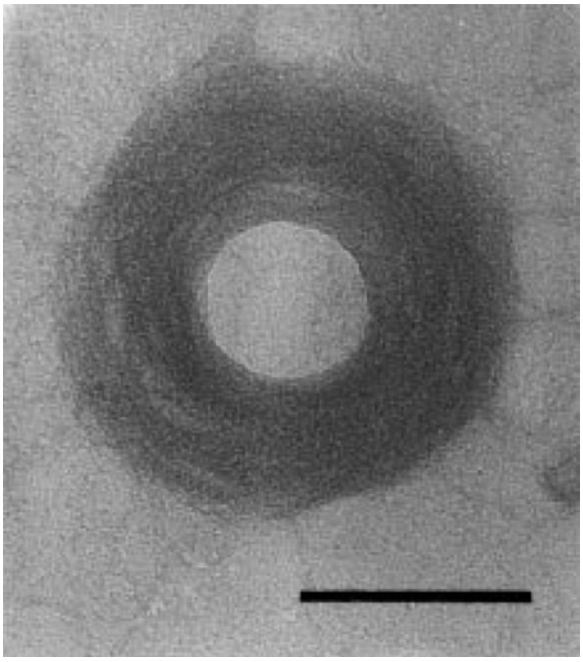

Figure 1. Transmission electron microscopic image of spermidine-collapsed T4 phage DNA. Scale bar is 100 nm. Reprinted with permission from (152).

These present a similar size and charge as the histone complex. However, on the contrary to the histone octamers, where the wrapping path of DNA is defined by grooves, ridges and binding sites (63-65), DNase I cleavage assays do not show a clear positioning of the dendrimers suggesting a random binding and probably some sliding along the DNA molecules (66).

3.1.4. Polyamines

Spermidine and spermine are among the most known and used condensing agents for DNA (Figure 1). In fact, the first DNA-spermidine complexes were observed already in 1976 using electron microscopy (67).

Flexible polyamine molecules are believed to interact in an irregular manner with DNA, with no definite binding sites, forming bridges between different helices, and showing no regular order; therefore they are not usually detected in X-ray studies even though they are present in high concentrations. Fluorescence microscopy studies have shown, not surprisingly, that a higher concentration of spermidine (three charges) was necessary to induce the compaction of DNA when compared with spermine (four charges) (61). As mentioned above, Monte Carlo simulations have shown that short polycations do not associate so strongly to DNA, in fact some of the molecules remain in solution. Therefore an excess of short-chained polyamines is generally required to compact DNA in diluted solutions.

When comparing spermidine with trivalent ions, it was found that the metal ions are more efficient condensing agents than the polyamines, where the charges are distributed along a chain (55, 68, 69).

3.1.5. Surfactants

Amphiphilic molecules are very popular in nanotechnology due to their self-assembly properties. Most common cationic surfactants bear only one charge in the polar headgroup and are therefore not sufficient to induce the compaction of DNA *per se*. However, due to their amphiphilic characteristics, surfactants self-assemble into aggregates in the vicinity of DNA that in turn lead to the compaction of DNA. Since the self-assembly of the surfactants is relatively easy to control it is in principle possible to control the compaction of DNA. In fact, this concept has been used with other positively charged agents to improve their efficiency and control. This was demonstrated with, for example, lipospermidine (70, 71) and lipopeptides (72-74).

The compaction of DNA is driven by the formation of the surfactant aggregates, that is, by a cooperative binding of the surfactant to DNA, as observed already in the 80s (75, 76). As a consequence, the compaction of DNA is mostly dictated by the properties of the surfactants; the critical micelle concentration (cmc) of the surfactants, i.e. the concentration at which the surfactants form micelles in DNA-free solutions is generally related to the critical association concentration (cac), the concentration at which the surfactants form aggregates in the presence of DNA. In the case of ionic surfactants the presence of an oppositely charged polyelectrolyte facilitates the association of the surfactants and the cac is much lower than the cmc, often by orders of magnitude (77).

Surfactants with longer chain lengths are more hydrophobic and have therefore lower cmc values, meaning that the amount of surfactant required to condense (75) and precipitate (78) DNA will also be lower for more hydrophobic surfactants. The same principle holds for surfactants with two hydrocarbon chains (79) or surfactants with more hydrophobic headgroups (80). A very clear example is given by gemini surfactants, in which the (hydrophobic) linker between the two charges in the headgroups was systematically increased (79). It was observed that there was a non-monotonic dependence of the onset of the compaction of DNA with the length of the spacer. For shorter spacers the surfactant acts as a divalent ion, being more efficient than the monovalent counterparts. On the other hand, longer spacers increase the hydrophobicity of the surfactant and enhance the self-assembly of the surfactants. Interestingly the cac and the cmc follow the same trend, and the surfactant that is the least efficient in compacting DNA is the one that presents the higher value of cmc.

3.1.5. Nanoparticles

The interaction of DNA with nanoparticles (NP) is of great interest. The large interest has derived not only from the possibility of understanding the driving forces in DNA packaging, for example, using simpler model systems, but also to the large possibilities these systems offer for the diagnosis and treatment of diseases. Functionalised NP are been currently developed and studied for quantification, imaging and drug delivery.

Much and interesting work has been performed in this area which would justify a review on itself. We will instead briefly mention a few different studies.

A few fundamental studies have been performed in order to better understand the condensation of DNA around the histone complex. For example, Baigl and co-authors have performed fluorescence microscopy studies where silica NP (made positive by adsorption of poly (L-lysine)) were shown to compact DNA molecules on a stepwise and progressive fashion at the single-chain level. The compaction was found to be more efficient for larger particles, since it was controlled by the ability of the DNA to wrap the NP (81). The composition and structure of the DNA-NP complexes were also investigated using transmission electron microscopy and molecular dynamics simulations and the complexation mechanism was found to be dependent on the size of the particles and ionic strength of the medium (82). In another work, Cárdenas and co-authors have also used (negatively charged) silica particles but, in this case, cationic surfactant was added to promote the interaction between the NP and the DNA. It is interesting to note here that, the adsorption of the surfactant on the NP occurs due to the presence of the DNA. Also, it is suggested that the structure of these complexes is similar to that formed onto planar surfaces for the same systems (83).

As mentioned previously NP have received much attention due to their potential applications in Nanomedicine. An interesting mini-review on the use of functional NP for cancer therapy and diagnosis can be found in Reference (84) . Other recent reviews can be found in Refs. (85, 86).

3.2. Solvent

The dielectric constant of the medium is a key factor on the conformation of DNA. When the dielectric constant of the solvent is lowered, the strength of the electrostatic interactions increases, which also enhances the ion correlation effects (87). Therefore, the concentration of multivalent ions necessary to compact DNA decreases in solvents with low dielectric constants. A study by Ascott and co-authors, for example, has shown that the concentration of hexaammine cobalt (III) needed to induce the condensation of DNA decreases in water/methanol, water/ethanol and water/isopropanol mixtures (88). The addition of ethanol also facilitates the condensation of DNA in the presence of divalent ions, lowering the critical concentration by orders of magnitude (89, 90). Furthermore, decreasing the dielectric permittivity of the medium can induce the compaction of DNA without the addition of cationic species. This was shown for the large T4 DNA molecules (87, 91) where the phase transition occurred at various weight fractions of organic solvents in aqueous solution, but at similar dielectric constants of the mixed solvents (87).

On the other hand, it is possible to hinder DNA condensation by increasing the dielectric permittivity of the medium, as shown for DNA-spermidine and DNA-spermine systems in the presence of different aminocarboxylic acids (92). These were later used to unfold single DNA molecules previously compacted using spermine (93).

3.3. Confinement and crowding

DNA compaction can also be achieved by means of confinement and crowding. With the advances in visualizing and manipulating macromolecules on shorter and shorter length scales and the growing interest in manufacturing structures such as nanopores and nanochannels, the effect of confinement on biomacromolecules has gained increasing importance. Here we will only focus on DNA confinement in spherical geometries, such as viruses and equivalent synthetic systems.

In viruses, DNA is condensed due to its confinement, and generally the pressure that is created inside the capsids, due to the electrostatic repulsion between different parts of the DNA molecule, is used to inject the genetic material into the host cells.

Cells have crowded environments that certainly influence the conformation of the macromolecules that exist in it. In fact, it has been suggested that the molecular crowding within a bacterial cell is a critical factor for DNA condensation in bacteria nucleoids (94, 95).

3.3.1. Viruses

The assembly of virus particles, and consequent encapsulation and condensation of the nucleic acids, can be roughly divided into two very different processes. For viruses with single-stranded (ss) RNA genomes, the presence of the nucleic acid is a requirement for the self-assembly process of the viral particle. The capsid proteins have positive charges in the part that faces the interior of the capsid and the electrostatic interactions between the capsid and the nucleic acid is believed to be the driving force for encapsulation during the virus assembly (96). For viruses made of double-stranded (ds) DNA, and presumably due to its higher persistence length, the protein capsid is assembled first and afterwards the nucleic acid is inserted into the capsid by a molecular motor incorporated in the capsid.

The DNA inside the capsid has a spool-like conformation with a region of low density at the center, as shown by electron microscopy (97, 98) and X-ray diffraction (99) studies. A number of theoretical studies (100, 101) as well as Monte Carlo simulations have been performed on these systems (100, 102-105), confirming that the compaction of a stiff linear chain (such as dsDNA) into a spherical geometry leads to a spool-like conformation (Figure 2).

3.3.2. Reversed micelles and microemulsions

DNA condensation by confinement can also be achieved using reversed surfactant micelles or microemulsions.

Recently, phase diagram studies were performed using a “DNA-based cationic surfactant”, water and decanol (106). Even though the DNA-based surfactant

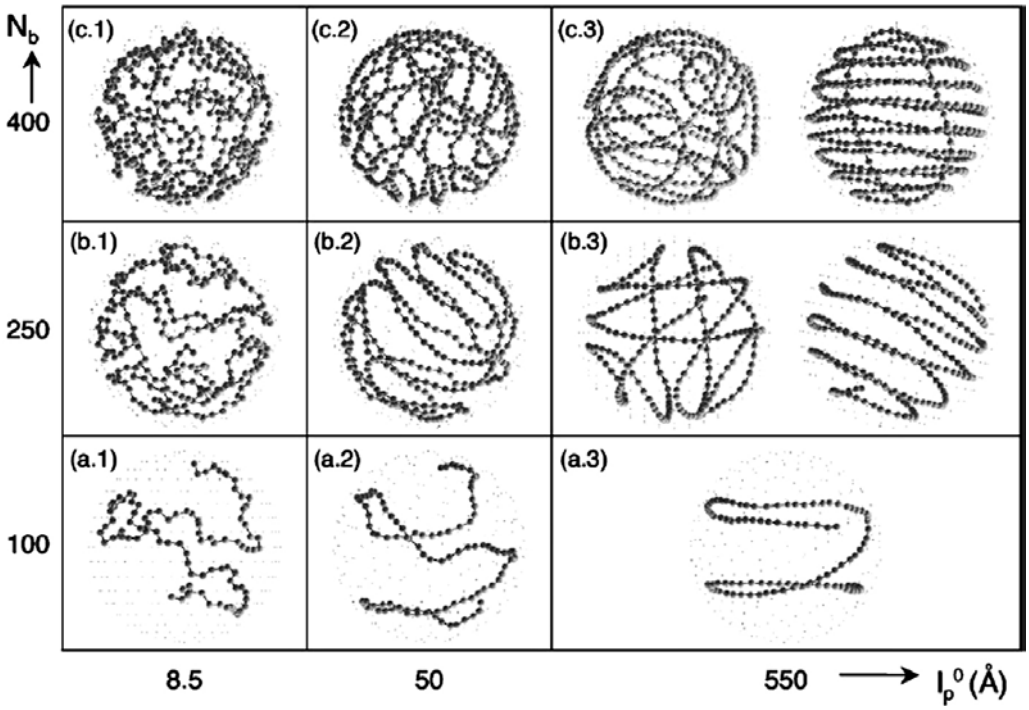

Figure 2. Snapshots showing encapsidated polyelectrolyte beads (connected gray spheres) and capsid charges (dots) at increasing number of beads N_b (bottom to top) and increasing bare persistence length from l_p^0 (left to right) taken from the end of the production simulations. Same length scale is used among the panels. In (b.3) and (c.3), the right snapshots show final configuration obtained from simulations starting with a spool-like initial chain configuration. The small ions are omitted for sake of clarity. Reprinted with permission from (105). Copyright (2006) by the American Physical Society.

could mix oil and water in a similar fashion as classical surfactants, the authors did not observe the formation of microemulsions since the film that the DNA-based surfactant forms is too rigid. Some systems, however, have been reported to form microemulsions in the presence of DNA: water-in-olive oil microemulsions (24), and emulsions composed of castor oil, a zwitterionic lipid, cholesterol and surfactants (107) were prepared for use as topical DNA transfection. Even though the condensation of DNA is not described in these studies, presumably the plasmid undergoes some condensation, since the reported size of the droplets is smaller than the contour length of the used DNA molecules. It has also been shown that the pre-condensation of the DNA with polycations (chitosan) improved the transfection rate (108).

Luisi and co-workers have incorporated DNA in bis-2-ethyl-hexyl sodium sulfosuccinate (AOT)/isooctane reverse micelles and showed that DNA is solubilized in the non-ionic surfactant micelles in a condensed form (109, 110). Also, reversed micelles of tetraethyleneglycol dodecyl ether ($C_{12}E_4$) in 2,2,4-trimethylpentane were used to entrap and condense plasmid DNA (111).

The condensation of DNA inside micelles and microemulsions can potentially be used to control the size of the DNA – co-solute complexes for gene transfection purposes, for example, by using the same concept as microemulsion polymerizations (107).

3.3.3. Non-ionic surfactants and polymers

Compaction of DNA can be achieved by osmotic exclusion or macromolecular crowding using neutral polymers or surfactants.

The surfactant Triton X-100 has been successfully used for the compaction of large DNA molecules (112). The concentration of surfactant required was rather high, much higher than the cmc of the surfactant.

The condensation of DNA by neutral polymers was reported already in 1971 (113). Poly (ethylene glycol) (PEG) was the polymer used then and remains as one of the most used crowding agent (see for examples (114-117)). It has been shown that DNA condensed by PEG is less compact and less rigid when compared to DNA condensed by cationic species (117). Furthermore, when DNA-binding agents are mixed with the crowding agents, less DNA binding and crowding agents are required for condensation.

4. DECOMPACTION STRATEGIES

As mentioned in the introduction, a precise control of the DNA morphology is of great biological and technological importance. The degree of compaction of the DNA mediates the extent of accessibility of the DNA sequences and therefore is indirectly responsible for the

control of processes such as gene expression, recombination and repair.

As shown above, many chemical agents have been successfully used for DNA compaction. Even though such agents are in general much simpler and interact with DNA in an irregular fashion without specificity they can, nevertheless, be used to mimic more complicated systems. For most applications DNA unfolding (or decompaction) is an equally important step.

In this section we will summarize different strategies that have been used for the redissolution of DNA complexes, with the decompaction and release of DNA. The strategies are obviously strongly dependent on the type of chemical used for the compaction of DNA.

4.1 Histone tail modifications and HMGN proteins

As mentioned previously the compaction of DNA in eukaryotic cells is achieved by cationic proteins called histones. Despite the high degree of compaction in the cell, the DNA molecule has to remain accessible to a myriad of molecules that are responsible for several cellular processes; therefore the chromatin has to go through structural changes. Despite the significant advances in the past years, there is still some debate regarding the chromatin dynamics.

One of the proposed mechanisms refers to the modification of the (cationic) flexible histone tails by, for example, methylation (118, 119) or acetylation (120, 121). The histone "tails" have positive charges, due to lysine and arginine residues that can be turned off through, for example, acetylation and deacetylation. This would presumably (reversibly) unwind a segment of the DNA necessary for a particular function. In addition to this, the cell nucleus contains numerous proteins, such as the High Mobility Group (HMG) proteins, which bind to the nucleosomes and decrease the chromatin folding, leading to an increase in the rates of transcription and replication (122, 123).

4.2. Monovalent and divalent salts

The association of cationic compacting agents to DNA and its compaction is, as mentioned above, primarily due to the strong electrostatic attraction between DNA and the co-solutes. Addition of large concentrations of simple (monovalent) salt does reduce the Debye length and therefore screens the attraction between the DNA and the co-solutes. For sufficiently high concentration, the simple salt will simply kill the interaction and DNA will no longer be in the compacted state.

The weaker association of divalent ions to DNA, when compared with trivalent ions, can be successfully used to control the DNA compaction. Yamasaki and co-authors have suggested the redox reaction of Fe^{2+}/Fe^{3+} for this purpose (124); whereas the transition from a coil to a compact globule is achieved with a concentration of Fe^{3+} of around 1–2 μM, there is no compaction of DNA with the addition of Fe^{2+} up to a concentration of 30 μM. The same principle was successfully adopted by using redox sensitive surfactants (see below).

4.3. Polyanions

The large interest in polyplexes for gene delivery was mentioned above. Once in the cell, however, the polyplexes must dissociate (at least to some extent) so that the DNA is accessible to the cell machinery. The dissociation of the polyplexes and the release of DNA can be achieved using polyanions. A few examples have been presented in the literature. For example, poly (sodium styrenesulfonate) has been used to decompact DNA previously compacted by poly- (allylamine hydrochloride) (125) and a block copolymer of N- (2-hydroxypropyl)methacrylamide with 2-(trimethylammonio)ethyl methacrylate (126). Also, poly (L)aspartic acid was used used to decompact DNA-poly (L)lysine (127-129) and DNA-poly (L)histidine (130) complexes; heparins can be used to dissociate DNA-chitosan (131, 132), DNA-poly (L)lysine (129) and DNA-dendrimer complexes (133).

The polyanion that is added to the DNA-polycation solution competes with the DNA for the polycation and when the polycation-polyanion association is more favorable the dissociation of the polyplexes will, naturally, occur.

Heparins, for example, are very efficient decompacting agents for polyplexes due to the high charge density and, more importantly, the fact that they are much more flexible than the DNA molecules (persistence length of approximately 5 nm (134)). Also, the degree of polymerization seems to be of importance for the formation of the polycation-polyanion complexes (127, 130).

Heparins have also been shown to induce the dissociation of complexes of nucleic acids and cationic lipids (135, 136).

4.4. Anionic and non-ionic surfactants

It is well known that the cmc of an ionic surfactant can be lowered by mixing with other types of surfactants (33). This synergetic effect is used in many industrial applications to improve the properties of single surfactants in solution, but can additionally be a useful tool to control the DNA conformation. When DNA is compacted by cationic surfactants it is possible to induce the dissociation of the DNA-surfactant complex and the release of DNA by adding anionic (65, 137) or non-ionic surfactants (138, 139).

A systematic study on the dissociation of DNA-alkyltrimethylammonium bromide surfactants by sodium alkylsulfates of different chain lengths has shown that anionic surfactants of longer chain length are more efficient in decompacting DNA. However, no dependence was found on the hydrophobicity of the cationic surfactant (137).

Non-ionic surfactants were also used to decompact and dissolve DNA-cationic surfactant

complexes (138). Studies were conducted on non-ionic surfactants of the series $C_{12}E_n$, where the headgroup size was varied (n=5,8,23). It was observed that the most hydrophilic surfactant, that is, the one with the largest headgroup was more efficient in decompacting DNA (140). This is presumably due to the fact that the inclusion of the cationic surfactant molecules on the non-ionic surfactant micelles, or in other words, the formation of the mixed micelles is more favorable for the longer hydrophilic surfactants, since the presence of the ionic surfactant reduces the steric repulsions between the hydrophilic headgroups.

Interestingly, it was also found that it is possible to predict the type of structures that the mixed surfactant system will form, with the knowledge of the phase diagram (137). This can be extremely advantageous for some applications. Oppositely charged surfactants form crystals when mixed at the charge equivalence. Crystals are relatively easy to separate from the DNA molecules in solution and can therefore be used for DNA separation and purification.

Furthermore, anionic liposomes can be used to dissociate DNA-lipid complexes (135, 141-146). The release of DNA was achieved using liposome compositions that mimicked the cell membrane and it was shown to be independent of ionic strength and pH but dependent on the physical state of the anionic liposomes; fluid membranes were a requirement (135).

4.5. Cyclodextrins

Cyclodextrins (CD) are well known for their ability to bind and solubilize hydrophobic molecules (*e.g.* drugs and surfactants) in water, by forming an inclusion complex. If the association constant of the cyclodextrin and the cationic surfactant used to compact DNA is sufficiently large it is possible to decompact DNA. This strategy has been recently used to dissolve DNA-CTA complexes and release the DNA into solution (147).

At low CTAB concentration, but ensuring that all DNA molecules were compacted, and hence no coexistence between coils and globules was present in the solution, DNA can be decompacted using α- and β-cyclodextrins. The critical concentration of CD necessary to decompact DNA-CTAB complexes was lower for the case of β-CD than for α-CD. It is interesting to note that no coexistence region was observed when the decompaction was achieved; instead large aggregates were observed at the transition point. This is, as far as we are aware, the only system that shows this behavior and suggests some kinetic effects.

4.6. pH, light and redox sensitive surfactants

The manipulation *in situ*, of the structure of the surfactants used to compact DNA, is another successful strategy to decompact DNA. There are a few interesting examples of this methodology.

Dodecyldimethylamine oxide (DDAO) is a pH-sensitive surfactant (pK ~ 5.0), which can exist either in non-ionic or cationic (protonated) form depending on the pH of the aqueous solution. It is then possible to control the compaction of DNA by changing the pH of the solution (62, 80).

Hays *et al* (148) have recently shown that the conformational state of lambda phage DNA (λDNA) can be reversibly controlled using a redox-active surfactant (11-ferrocenylundecyl)trimethylammonium bromide (FTMA)). If FTMA is in the reduced state, DNA can be compacted using the appropriate surfactant concentration. However, if FTMA is in the oxidated state, the DNA remains decompacted.

Le Ny *et al* (149) explored the possibility of controlling the condensation of DNA using a light-responsive surfactant. The surfactant undergoes a reversible photoisomeration when exposed to visible or UV light, presenting a more hydrophobic (trans) isomer or a more hydrophilic (cis) isomer, respectively. It was therefore possible to tune the compaction and precipitation of λDNA.

4.7. Single-chain elongation

It is also possible to induce the unfolding of previously compacted single DNA molecules by stretching the molecules. This has been performed using two different methodologies, optical tweezers and microfluidics.

The elastic response of single DNA molecules can be probed using optical tweezers. It is possible, using this approach, to follow (and hinder) the condensation of DNA (150) and to induce the unfolding of the condensed structures (68). The elongation of the DNA molecules hinders the compaction of the molecules since it does not allow intrachain attraction and concomitant loop formation.

It is known that DNA molecules undergo elongation inside microfluidic devices (151). This same approach has been used to study the effect of such constrain on a compacted DNA molecule. It was interesting to note that when the DNA molecules were compacted using spermidine they dynamically unfolded while traveling through the micropillars. On the other hand, for the molecules compacted using a polycation, poly (L-lysine) of molecular weight 30,000-70,000, no unfolding was observer, even when the applied electric field was as high as 1000 Vcm^{-1}. This difference in behavior clearly indicates the enormous difference in the strength of the interaction between the DNA and the different condensing agents.

5. CONCLUSIONS AND PERSPECTIVES

In a time where nanotechnology and biomedicine are of growing importance, it is important to have a good knowledge regarding the manipulation of DNA molecules.

While most of the current technological applications (e.g. molecular motors and sequence recognition) are performed with short oligonucleotides which evidently do not undergo single-molecule compaction, there is a growing interest to understand and manipulate large DNA molecules, which is of crucial importance for applications such as large-scale protein

production, gene therapy, and DNA purification. As each application will demand a different DNA size and compaction degree, some co-solutes will be undesirable; it is of paramount importance to have a wide variety of methods that are available both for compaction and decompaction.

6. ACKNOWLEDGEMENTS

A. Gonzalez-Perez is thankful to the EU Research Training Network, CIPSNAC (contract number: MRTN-CT-2003-504932). R. S. Dias acknowledges the Fundação para a Ciência e a Tecnologia (FCT), Portugal (SFRH/BPD/24203/2005). We thank Björn Lindman for fruitful discussions.

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Key Words: DNA, Compaction, Decompaction, DNA Release, Histones, Trivalent Ions, Polycations, Polyamides, Surfactants, Cyclodextrins, Heparins, Review

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