Current Opinion in Colloid & Interface Science 26 (2016) 66–74

Contents lists available at ScienceDirect

Current Opinion in Colloid & Interface Science

journal homepage: www.elsevier.com/locate/cocis

Cooperative action in DNA condensation

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ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form 19 August 2016
Accepted 21 September 2016
Available online 28 September 2016

Keywords:
DNA condensation
Polyelectrolytes
Electrostatics
Depletion forces
Crowding
Confinement
Cooperativity

ABSTRACT

In this review we address the two main sets of strategies for DNA compaction and condensation, those based on electrostatic interactions and those based on space restrictions, including confinement and excluded volume. Focus will be given to work in which these overall strategies are combined in multi-factorial approaches, thus promoting cooperative and synergistic effects. Discussion includes bio-motivated and bio-inspired work. Recent work is reviewed, and an effort is made to extract general trends and characterize the overall behaviour.

1. Brief historical perspective

Despite more than four decades of study, DNA compaction still remains an intriguing and elusive subject. The first studies began in the early 1970s, employing essentially multications and polycations as condensing agents [1,2]. At the same time, Lerman [3] reported the collapse of DNA through the combined action of salt and neutral polymers, such as polyethylene glycol (PEG) and polyethylene oxide (PEO), a phenomenon known as polymer and salt-induced condensation (ψ-condensation). However, the role of crowding on DNA condensation in prokaryotes was suggested only in the 90s [4].

In the same decade, and after different charged agents had been tested, it was recognized [5] that, in aqueous solution, a compacting agent acting individually would only be effective in condensation, if its positive charge was equal to or in excess of +3.

Later, using single molecule visualization and molecular simulation techniques, the perspective of all-or-none folding transition upon addition of some efficient cationic agents, emerged [6,7]. This mechanism was revised for some systems, and the concept of intrachain segregation [8] used instead. Single-molecule morphologies, such as globules, toroids, chains internally segregated, and bundles composed of several chains were observed in different mixtures of protamine/DNA for a fixed final concentration [9]. Also observed was the expansion of the globules in situations indicating overcharging. Contributions involving different compacting agents, such as surfactants and dendrimers, have also been explored and were recently reviewed [10,11•].

Meanwhile, aspects pertaining to the confinement of DNA molecules started being considered using geometrical constraints. Simulation work have both address the measurement of packaging/ejection forces or the cost for imposing confinement on polyelectrolytes resorting to free energy calculations [12–14].

More recently, the recognition that the small and crowded cellular environment can strongly affect all biological processes has boosted the interest on studying the effects of crowding and confinement in DNA compaction mechanisms. Excellent reviews on the topic have also been published recently [15–19].

In the last decade, researchers have made efforts to consider the simultaneous contribution of two or more factors, in order to create more realistic systems to model the intracellular environment.

2. The intricate nature of DNA condensation

Inspired by biological evidences, the inclusion of multi-factor effects in the study of DNA compaction is of paramount importance. The dramatic reduction in size imposed to DNA to fit into cell nucleus anticipates the level of complexity of the compaction phenomenon. Depending on the nature of living organisms the requirements for DNA storage differ. In eukaryotic cells, DNA is enclosed in the nucleus while, in prokaryotic, this organelle is absent. Notwithstanding, DNA is also highly compacted in prokaryotes, in a structure denoted the nucleoid, which occupies only 15% to 25% of the total cell volume. It is generally accepted that in both types of cells, DNA compaction is

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http://dx.doi.org/10.1016/j.cocis.2016.09.014
1359-0294/

partially provided by DNA-binding proteins, the histones in eukaryotes and the nucleoid associated proteins (NAPs) in prokaryotes. In the eukaryotes, only half of the negative charge of DNA is neutralized by histones, forming a compact and dynamic large nucleoprotein complex with multiple levels of folding, denoted chromatin. Due to its polyelectrolyte nature, these higher order complexes are highly sensitive to the ionic environment, and their structure can be modulated electrostatically by the action of cationic agents (e.g. Na^+, Ca^{2+}, Mg^{2+}, spermidine and spermine), thus providing the regulation of chromatin shape and gene expression [20]. Similarly, it has been postulated that in bacteria NAPs do not act alone [17,21].

The intracellular environment contains high concentrations of large polymers and small solutes (30–40% *w*/w), that although not interacting directly with DNA, highly affect diffusion, conformation, and reactivity of the biomolecules inside the cell, due to crowding effects that decrease the available space [22]. This fact is intimately related to the confinement imposed, e. g. in the cell nucleus, elucidating why these two topics are often jointly addressed. [16,19,23].

In a clearly different, although related, approach, DNA condensation studies have also been conducted aiming at the development of effective gene delivery systems. In this field, a large number of innovative transfection mediators have been used. These must be able to respond to the adverse and labile cellular environmental conditions, such as endosomal pH variations, macromolecular crowding, high ionic strength and competing interactions with charged cellular biomolecules. To meet these requirements, functionalized condensing agents able to mediate the gene interaction with cell membrane or trigger the disassembly of the vector cargo in a specific cell compartment have been synthesized [24,25]. Additionally, the combination of condensing agents with distinct properties has also been considered as a promising approach to attain good levels of gene transfection [26]. Despite the vast work on this topic, in this review they will only be analysed from a fundamental point of view, focusing on the DNA condensation stage promoted by biomimetic condensing agents.

Both in studies devoted to the understanding of the DNA compaction inside cells, and in those related to gene delivery, it is hard to identify mechanisms in which either electrostatics or volume do not play a definite role. Although electrostatics is inherent to these systems, volume restrictions may dominate in crowding and confinement situations, but a strong interplay with electrostatics (including ion condensation) is usually present [27]. Some efforts to identify the magnitude of each thermodynamic contribution have been made, resorting mainly to simulation and using simple models [28–30•].

In this brief review, that does not attempt an extensive coverage of the topic, focus will be given to DNA compaction/condensation promoted by proteins and polycations, with their charge modulated by pH and ionic strength. Higher valence salts may be also seen as an additional condensing agent. Crowding and confinement situations will also be duly addressed. It should be mentioned that, in the literature, compaction and condensation have been frequently and ambiguously used as synonymous. In what follows, when possible, the former term will be used mainly for phenomena at the single chain level, while condensation will arise essentially in connection to more concentrated systems.

3. When electrostatics is dominant

Different architectured polycations have been used as model systems to provide insight on the mechanisms of condensation of the giant DNA molecule inside the cell, aiming at improving gene delivery. In this context, there have been recent advances on the understanding of the roles of polycation structure, charge density and concentration in the condensation of nucleic acids. Herein, special attention will be given to the influence of media conditions, including ionic strength and pH, which cannot promote DNA condensation per se, but can regulate the magnitude of the electrostatic interaction between DNA and condensing agents.

Most of the synthetic condensing agents are positively charged, commonly incorporating primary and/or secondary and/or tertiary amine groups in their backbones, which according to the amine chemical environment and to pH value of the medium may display different degrees of protonation. Thus, experimentally, variations in the pH of the medium are directly related with modulation of the effective charge density of the condensing agent. On the other hand, theoretical models introduce the effect of pH by means of the imposed positive charge on relevant sites, or by the explicit consideration of protons, when using coarse-grained or all-atom simulations, respectively. Regarding the control of ionic strength, in experimental techniques, the concentration of ions in solution is often provided by the addition of a buffer or salt. Computationally, the ions may be introduced explicitly or implicitly by the use of screened-Coulomb potentials. Similarly to pH and ionic strength, there are other extrinsic factors that are able to regulate DNA conformation, such as light, temperature and solvent polarity, but these will not be considered, because they fall mostly outside the scope of features available to the cellular machinery under physiological conditions. In what follows, the three main groups of natural and synthetic condensing agents namely chromatin/nucleoid components, hyperbranched polycations and linear/branched polycations will be reviewed in the context of DNA condensation. Potential synergies that may arise from the use of more than one condensing agent will also be addressed.

3.1. Chromatin/nucleoid components

The first level of DNA compaction inside cells includes linear domains of DNA (145–147 bp) complexed to an octamer of histones (1.75-turn superhelix), called nucleosomes. Due to its negative charge, the mechanisms of nucleosome neutralization promoted by complexation with positively charged species and self-assembly may exhibit the same behaviour as other polyelectrolyte systems. Livolant and coworkers [31,32] reported a number of interesting results in this field, detailing the phase-behaviour of the regular central part of nucleosome, known as nucleosome core particle (NCP), in solution. They observed a pronounced sensitivity of the NCP/NCP interaction to the ionic environment and, in addition, suggested that histone tails were able to mediate this interaction in a salt-dependent fashion.

More recently, in a experimental work, Nordenskiöld and coworkers [33•] systematically studied the effect of potassium and sodium ions, combined with magnesium in chromatin compaction. It was observed that, in the presence of magnesium, the addition of sodium promotes folding of the 10 nm beads-on-string fibres array into 30-nm fibres, whereas in mixtures of potassium and magnesium, folding does not occur. Moreover, it was concluded that self-association (aggregation) of nucleosome arrays in mixed salt solutions is synergistically promoted by magnesium and monovalent ions, but sodium is slightly more efficient than potassium. The phenomenon of NCPs self-association in the presence of multivalent salt was also observed using a new coarse-grained model that includes the crystal structure of the NCP [34]. Based on these results, it is now established that the ability of NCPs to aggregate depends on the charge and nature of the counterions. The interaction between NCPs evolves from repulsive in the presence of monovalent ions, to progressively attractive in the presence of divalent and trivalent ions (see Fig. 1 reprinted from [34]). This new model also reveals the strong influence of NCP–NCP bridging promoted by the positively charged and highly flexible histone tails. Experimental studies also support the importance of the histone tails in NCP ordered phases, highlighting the contribution of the respective branched structure and charge density to promote stacking and bridging between the lateral surfaces of contacting NCPs [35]. The advances made in chromatin modelling in the past decades and the remaining challenges are addressed in Ref. [36]