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Interactions of metal ions with DNA

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1 Introduction

Polyelectrolytes are polymers that consist of many monomer units bearing ionizable groups. In aqueous solutions they can dissociate into polyions and a large number of small ions of opposite charge (conuter ions). Polyelectrolytes are therefore similar to simple electrolytes, such as NaCl. While positive and negative charges can be separated when NaCl is dissolved in water, the negative charges in the case of polyelectrolyte, such as DNA are joined together and form a higly charged molecule. Disociiation of both, a simple electrolyte and a polyelectrolyte are presented shematically in figure 1.[1]

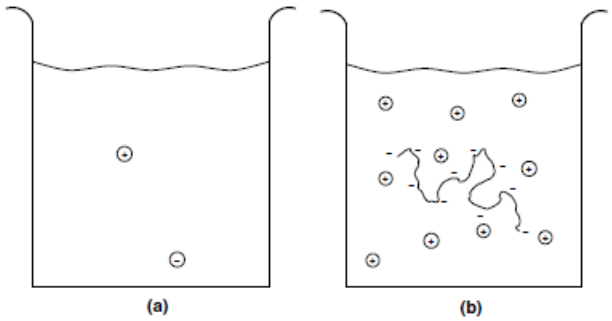

Figure 1: Dissociation of a) a simple electrolyte and b) a polyelectrolyte in water.[1]

2 DNA condensation[2,3,4]

DNA

Deoxyribonucleic acid (DNA) was first isolated in 1868 by the Swiss doctor Friderich Misch. At that time this molecule was quite irrelevant, since it wasn't known that contains the genetic instructions which are used in the development and functioning of almost all living organisms. After its three dimensional structure had been described by Watson and Crick, the DNA molecule became the center of almost infinite number of studies covering many different fields. The B-form of DNA is the most common conformation in vivo and consists of two long polymer chains of simple units called nucleotides. Nucleotides consist of nucleobase (guanine, adenine, thymine or cytosine), deoxyribose and phosphate group. Self-assembled complementary nucleobases are connected via hydrogen bonds and present the interior of the DNA molecule, whereas covalent backbone consists of deoxyribose and phosphate groups (figure 2).

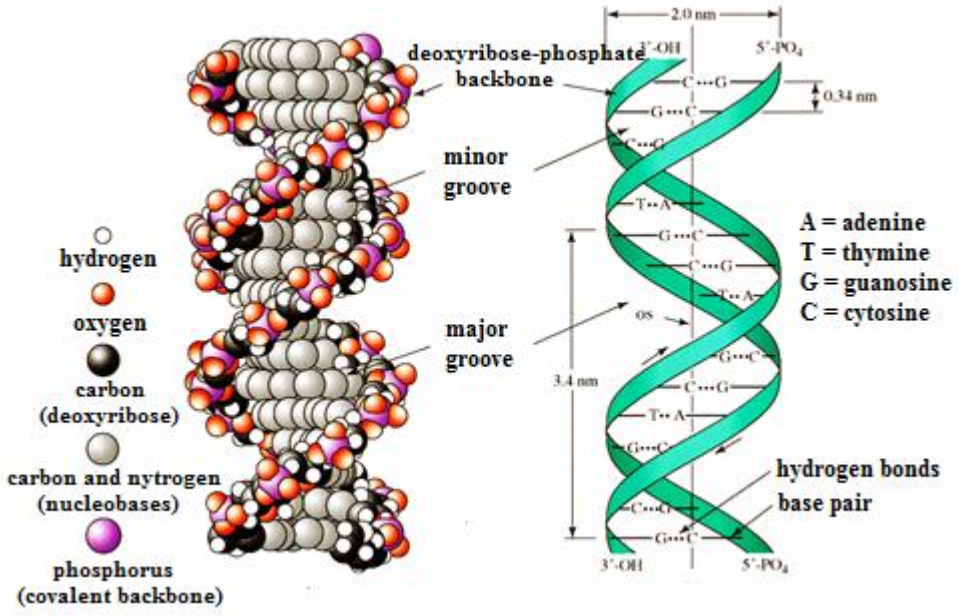

Figure 2: Dimensions of B-DNA.[5]

Double-stranded DNA (dsDNA) is a negatively charged polymer with a linear charge density of *-2e/0.34* nm that corresponds to two electrons per base pair (bp). The negative charge can be compensated by inorganic cations but also by positively charged organic molecules (e.g. amino acids, polyamines). Due to its structure, the dsDNA molecule is relatively stiff polymer having three independent degrees of freedom: twisting, bending, and contraction/extension. The persistence length of such a molecule is of order 500 Å or 150 bp and therefore long dsDNA chains are effectively modeled as worm-like random coil. Short dsDNA with a chain length of the order of persistence length are treated as rigid rods or cylinders and are often used in biophysical studies.

It is also necessary to remind how DNA appears in the cell or a viral capsid. Genomic DNA is a very long molecule, but has a striking property to pack itself efficiently into a very small place inside a cell or a viral capsid. For example, the length of fully extended DNA molecule in T4 phage genome is around 54 μm (contour length), but the molecule can pack itself into a viral capsid about 100 nm in diameter. This corresponds to a 540-fold linear compression. The same happens in the case of larger DNA molecules, such as bacterial or human DNAs with contour lengths of several millimetres or several centimetres.

DNA condensation is defined as the collapse of extended DNA chains into compact, orderly particles containing only one or a few molecules.[3] Usually, several DNA molecules are incorporated into the condensed structure and therefore, it is difficult to distinguish between condensation and aggregation. The term

condensation is generally used when the aggregate is of finite size and orderly morphology.

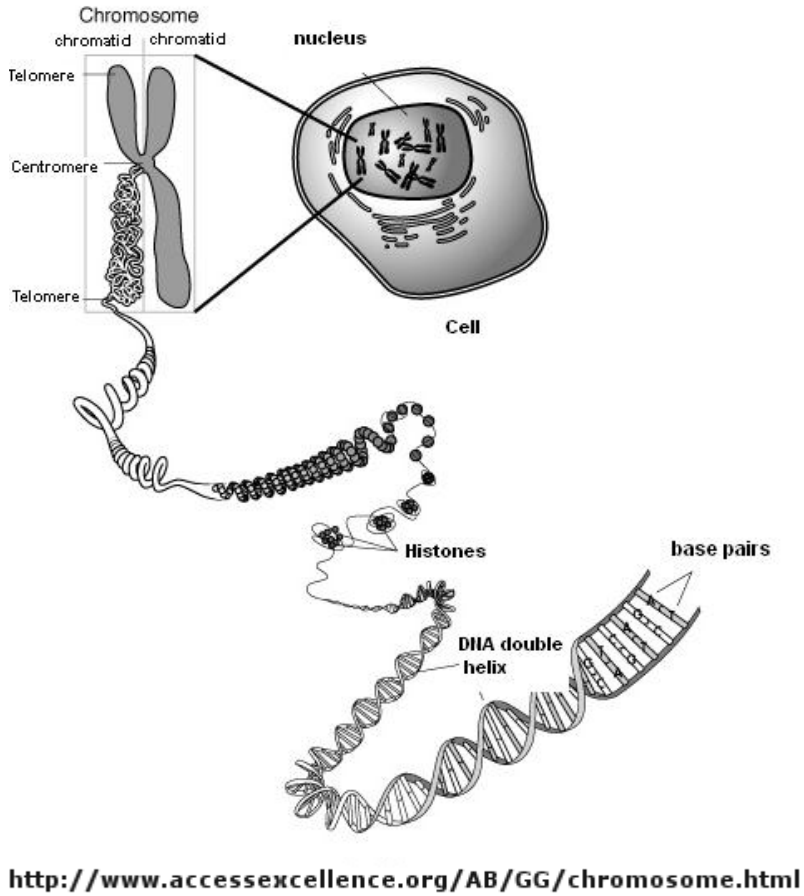

Figure 3: Packaging of eukaryotic DNA. Multiple proteins are involved in DNA packaging, notably positively charged histones that DNA encircles.

Considering the forces involved in DNA packaging – strong electrostatic repulsion between negatively charged phosphate groups, the loss of DNA configurational entropy, and deformation of the stiff DNA helix – there is no surprise that organisms expend substantial metabolic energy to accomplish the task. It has been estimated that about one ATP molecule is hydrolysed per two base pairs packaged.[6]

On the other hand, in vitro DNA condensation can occur spontaneously upon addition of low concentration of multivalent ions.

3 DNA condensation in vitro[3,4]

DNA condensation can be induced in vitro by applying condensing agents, including multivalent cations, basic proteins, polyamines, cationic liposomes, neutral crowding polymers and alcohols. They cause condensation either by decreasing repulsions between DNA segments (e.g., neutralizing of phosphate charge, and/or reorienting water dipoles near DNA surfaces by multivalent cations) or by making DNA-solvent interactions less favourable.

Let us examine the various types of condensing agents.

Condensation by multivalent cations

In the literature was reported that in aqueous solutions, a cation valence of charge +3 or greater is required to cause DNA condensation. At the simplest schematic level, multivalent ions are thought of as electrostatic bridges between negatively charged DNA segments and thus they can induce a formation of ordered condensates. The ions most commonly used in the condensation studies are the inorganic cation $Co(NH_3)_6^{3+}$, polylysine, basic proteins (histones) and the naturally occurring polyamines spermidine^{3+} and spermine^{4+}.

On the other hand, divalent metal cations do not induce DNA condensation in water solutions, but they do so if a small amount of alcohol is added. It was shown that Mn^{2+} ions can produce toroidal condensates of supercoiled plasmid DNA, while in the case of linearized plasmid toroids are not formed.

High concentrated alcohols are usually used to precipitate DNA, but at low concentrations they can also provoke DNA condensation to toroids or rods, especially in the presence of $Co(NH_3)_6^{3+}$ ions and at low ionic strength of the solution.

Condensation by neutral polymers

Condensation can be also achieved by the addition of neutral polymer (e.g., polyethylene glycol – PEG) in the presence of monovalent salt. Due to the entropic random collisions with crowding polymers, DNA segments are pushed together. In this case the presence of monovalent salt is required to neutralize charges and decrease DNA-DNA repulsion.

Condensation by cationic liposomes

Cationic liposomes complexed with DNA are promising carriers for transfection of eukaryotic cell. This is because the condensed state of the DNA protects it from nucleases, while the lipid coat on DNA facilitates its permeability through cell membranes.

4 Morphology of condensed particle[2,3]

Condensation agents cause DNA molecules to assemble into nanometerscale structures, with toroid being the most common morphology. The toroids have a similar size distribution regardless of DNA length. This indicates that either one large molecule or several small ones are incorporated in a single particle. Torroids with an outer diameter of 50 nm and inner diameter of 15 nm have been observed with DNA lengths from 400 to 56000 base pairs (bp).

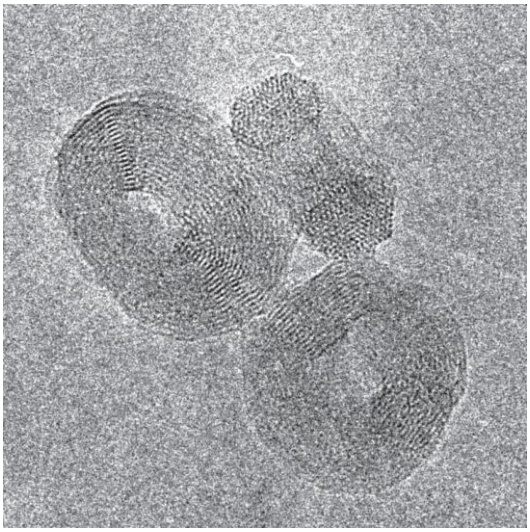

Figure 4: DNA in toroids. The image was taken with cryo-electron microscope.[2]

By changing conditions under which condensation takes place, non-toroidal shapes can be also formed. For example, the addition of alcohol tends to produce more rod-like structures, especially when $Co(NH_3)_6^{3+}$ ions are also present. Presumably this happens due to the synergetic behaviour of these two agents.

As the alcohol concentration becomes higher, the formation of ramified fibrous aggregates occurs. Cooperative behaviour of alcohol and $Co(NH_3)_6^{3+}$ ions cause that

the DNA firstly undergoes a B-DNA to A-DNA transition. The A-DNA then strongly self-adheres and rapidly aggregates into fibrous networks.

It was found that DNA segments that are shorter than about 400 base pairs do not condense into toroidal aggregates. At very high concentrations around 200 mg/mL, short dsDNA spontaneously form a liquid crystalline phase. The transition to a liquid crystalline phase appears due to repulsive excluded volume interactions between the rod-like molecules. In the presence of spermidine or spermine the short DNA strands also form liquid crystalline aggregates, either hexagonal or cholesteric. In this case, a transition to the ordered phase is caused due to attractive interactions between the parallel rods.

Short DNA duplexes form anysotropic units (cylinders) by end to end adhesion stacking when they are surrounded by either monovalent or divalent ions. These units then, depending on the DNA concetration, self-organize into a nematic or a columnar phase (figure 5).

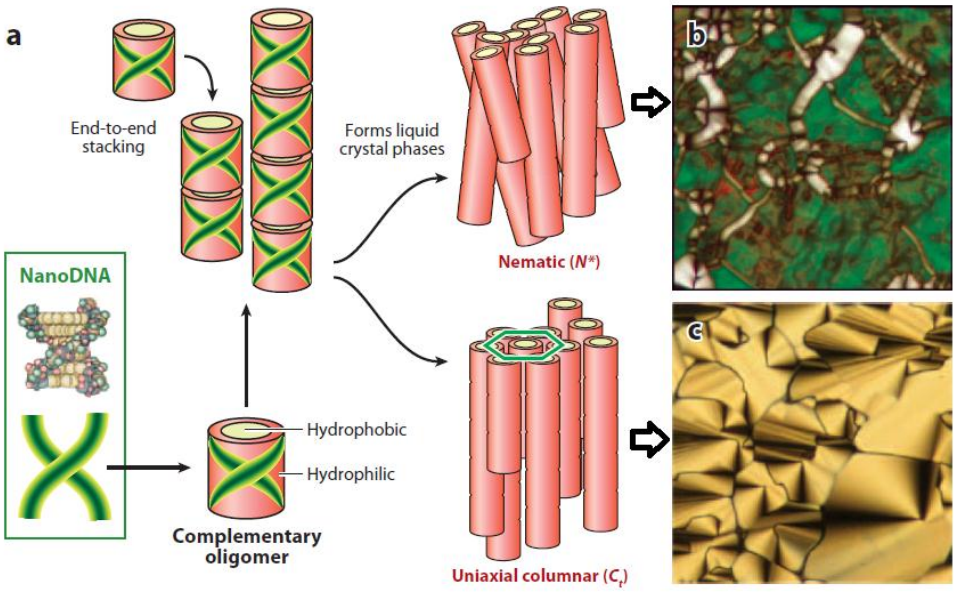

Figure 5: Nano-DNA molecules self-organize into LC phases due to the interactions between exposed terminal base pairs.[2]

5 Intermolecular forces in condensation[1,7]

Electrostatic interactions

The electrostatic interactions are long ranged and they can be moderated. For example, weak polyelectrolytes have charges that can be turned off and on by changes in the pH of the solution. Furthermore, increasing in ionic strength drecease the range of the electrostatic interactions. The effect of ionic strength is particularly pronounced when the monovalent salt is used.

High charge on the polyelectrolyte produces a strong electric field which attracts counter ions, so that they don't "wander off" on their own. In fact, the electrostatic attraction and the loss of translational and rotational entropy by counter ions due to their localization in the vicinity of polymer chain can cause counter ion condensation.

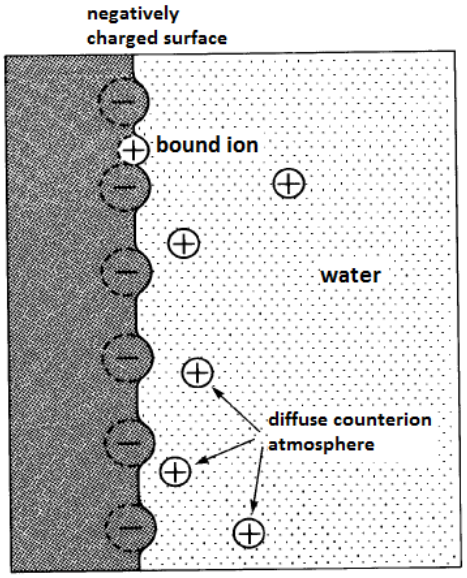

Figure 6: Schematic of counter ion distribution around polyion. Counter ions can be bound to the surface or they thermally fluctuate in a layer near the surface.[7]

Counter ion distribution around polyion, that is known as a diffuse electrical double layer, is commonly described with the Poisson-Boltzmann (PB) equation. The PB equation represents a mean-field theory since the ions in the double layer only affect each other through their average contributions to the mean-field potential.

The PB equation is obtained by relating the charge density $\rho_c(r)$ to the electrostatic potential $\psi(r)$ through Poisson's equation:

$$\nabla^2\psi(r) = -\frac{\rho_c(r)}{\epsilon_r\epsilon_0} \qquad (1)$$

and by assuming that ion species i with valencies z_i surround polyion according to the Boltzmann distribution. The PB theory does not take into account the characteristics of each type of ion, the dipole structure of water and ion-ion correlations. The local number density of ion i can then be written as follows:

$$n_i(r) = n_{i,0}\frac{\rho_c(r)}{\epsilon_r\epsilon_0}\mathrm{e}^{-\beta z_i e\psi(r)} \qquad (2)$$

where $n_{i,0}$ is the number density at a point where $\psi(r) = 0$.

Adding up $z_i n_i$ for all ion species gives the distribution of net charge, $\rho_c(r)$, and transforms equation (1) into the PB equation (3):

$$\nabla^2\psi(r) = -\frac{e}{\epsilon_r\epsilon_0}\sum_i z_i\, n_{i,0}\mathrm{e}^{-\beta z_i e\psi(r)} \qquad (3)$$

where ∇ is the Laplace opeartor ($\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$), r is the coordinate of a point in solution, e is the elementary charge, ϵ_r is the dielectric constant of the solution, and ϵ_0 is the permitivity of vacuum. $\beta \equiv 1/k_bT$, where k_b is Boltzmann's constant and T is the absolute temperature.

In order to solve the PB equation, a model with appropriate boundary conditions (e.g., electroneutrality of the system) has to be defined. This yields the equilibrium spatial dependence of potential $\psi(r)$ and charge density $\rho_c(r)$. In the case of DNA, a charged cylinder is usually used as a model, since DNA is relatively stiff over the contour lengths on the order of 500 Å and more or less has a cylindrical cross section with radius of about 10 Å. A cell model is also often used, particularly in the case of a finite polyelectrolyte concentration. In this case a charged cylinder and surrounding ions are enclosed in a larger cylinder with radius R, centered on the polyelectrolyte axis.

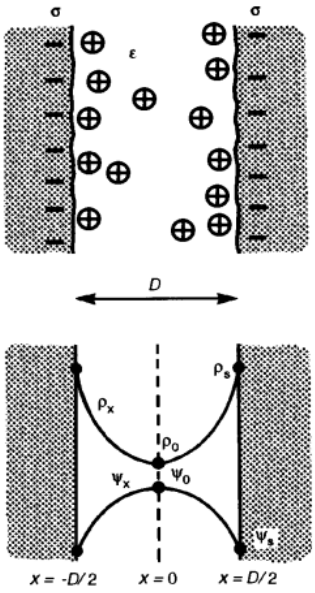

Figure 7: a) Schematic of two charged surfaces in water (salt-free case). b) The counterion density profile $\boldsymbol{\rho_c(r)}$ and electrostatic potential $\boldsymbol{\psi(r)}$.[7]

Because the PB equation is nonlinear, only very simple geometries can be solved analytically. For example, a salt-free case, where the only ions present in the solution are those that have come off the surfaces. On the other hand, in the case where charged surface is in equilibrium with a bulk salt solution, numerical solutions are required.

In addition, the Poisson-Boltzmann approach gives reliable results only in the case of low-valency ions and weakly charged objects, while in the case of high-valency ions and highly charged objects a strong coupling theory has to be used.

In the case of monovalent ions and weak electrostatic force, the PB equation may be reduced to its linearized form, Debye-Hückel equation:

$$\nabla^2\psi(r) = -\frac{e^2}{\epsilon_r\epsilon_0 k_B T}\sum_i {z_i}^2\, n_{i,0}\psi(r) = \kappa^2\psi(r) \qquad (4)$$

where $\kappa = (\beta e^2 n_0 z^2/\epsilon_r\epsilon_0)^{-1/2}$ and is known as the Debye screening length. It represents the distance at which significant charge separation can occur.

Hydration force[3,8]

In addition to the electrostatic repulsion, it is also necessary to consider the hydration force which is due to rearrangement of water between macromolecular surfaces. The nature of the counter ion and the charged groups on the polyion, as well as the presence of hydrophobic groups, influences the water rearrangement between the charged surfaces.

The rearrangement of water molecules around polar sites on the molecule (e.g. DNA) can be described using order parameter formalism or mean field model (MFM). Here the order parameter describes mean solvation forces. As a model for polar groups, hydration charge or its surface density is used. The surface and its periodicity (in the case of a lattice structure) are described by the structure factor $S(Q)$:

$$S(\boldsymbol{Q}) = \int d\boldsymbol{R} < \sigma_{hydr.}(\boldsymbol{r}+\boldsymbol{R})\sigma_{hydr.}(\boldsymbol{r}) > e^{-i\boldsymbol{QR}} \qquad (5)$$

where $\boldsymbol{Q}$ is an arbitraty vector, **R** runs over the surface and <> presents ensemble or $\boldsymbol{r}$-avearge over integrated surface.

From above equation, hydration pressure between two identical surfaces with zero net hydration charge can be calculated as following:

$$P_{hydr.} = \frac{\alpha}{4\pi^2} \int d\boldsymbol{Q} \frac{S(\boldsymbol{Q})}{\sin h^2[L_H(\kappa_H^{-2} + Q^2)^{1/2}]} \qquad (6)$$

where integration takes place over the all wave vectors $\boldsymbol{Q}$ and L_H is the distance between surfaces. Parameter α denotes the strength of the water molecules reorganization due to the polar groups.

The pressure between two ordered surfaces with a lattice structure of periodicity $a_{hydr.}$ can be written as follows:

$$P_{hydr.} = \frac{\alpha < \sigma_{hydr.}^2 >}{\sin h^2 \, L_H \left[\kappa_H^{-2} + (2\pi/a_{hydr.})^2\right]^{1/2}} \qquad (7)$$

where $< \sigma_{hydr.}^2 >$ is the mean square value of the surface charge density.

6 Interactions of metal ions with DNA

Chemical interactions between ions and specific binding sites on DNA can also be important, since the presence of metal ions strongly affects the function of DNA in vivo as well as its stability and structure in vitro. Many metal ions control essential biological processes of living cells and without their catalytic presence many biological reactions would not take place. While Na^+, K^+, Mg^{2+} and Ca^{2+} ions are important neurotransmitters, Zn^{2+} ions through the zinc finger play an important role in the regulation of DNA transcription and replication. On the other hand, the binding of metal ions to different sites on DNA stabilize or destabilize the secondary structure, but it can also cause a change in conformation. It is believed that many non-essential ions are even carcinogenic and mutagenic (e.g., Cd^{2+}, Hg^{2+} ions) which is a rather different consequence of metal-DNA interaction.

Positively charged metal cations are Lewis acids and they can interact either with donor/acceptor groups on nucleobases (ring-nitrogen atoms and exocyclic keto groups) or with negatively charged phosphate groups (see appendix). Binding to the hydroxyl groups of the sugars is rare. Metal ions have different affinity for different binding sites and so they are classified into two groups, soft and hard ions. The classification in the original work was mostly based on equilibrium constants for reaction of two Lewis bases competing for a Lewis acid. Soft ions are more polarizable in comparison to the hard ions and therefore the interactions between soft

ions and soft ligands have more covalent character. On contrary, interactions between hard ions and hard ligands are usually electrostatic. Examples of hard ions are Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Ba^{2+}, Zn^{2+} and Co^{3+}, whereas Cu^+, Ag^+, Hg^+, Hg^{2+}, Cd^{2+} and Pt^{2+} are classified as soft ions.[9]

The ability of cation to bind specifically to nucleic acids depends on its valence, hydration free energy and its coordination geometry. In aqueous solutions cations are surrounded by at least three hydration layers and can bind tightly to DNA as partially dehydrated or fully hydrated. Generally, there are three distinct types of interaction between cations and nucleic acids. In diffuse binding, fully hydrated cations interact with DNA via non-specific long-range electrostatic interactions. In the case of non-specific site binding, hydrated cations interact with nucleic acid structure (through hydrogen bonding of water molecules), whereas in the case of specific binding at least one cation aqua ligand is replaced by a ligand from nucleic acid structure (see figure 8).[10,11]

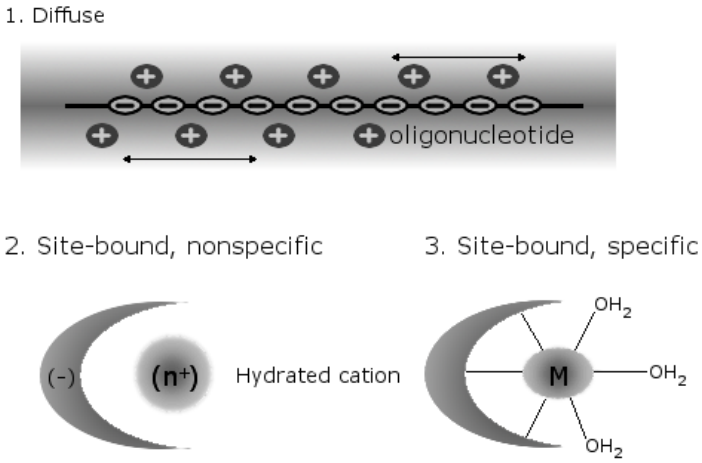

Figure 8: Three distinct types of interaction: 1. diffuse binding, 2. nonspecific site binding and 3. specific site binding.[11]

Analysis of X-ray diffraction showed that metal ions interact with DNA most commonly via water molecules in their first solvation shell, whereas direct metal ion-DNA interactions are rare. Interaction of all cations and water in the first solvation shell is a charge-dipole interaction and therefore fully hydrated ions are treated as excellent hydrogen bond donors. Due to significant degree of freedom and a large amount of solvent in the crystal structure, the localization of cations in the crystal structure is not easily determined. Some ions have rigid and stable first primary shell with tetrahedral (Li^+) or octahedral geometry (Mg^{2+}, Co^{2+}, Ni^{2+}). The special case is Zn^{2+} ion where both tetrahedral and octahedral coordination of solvent are possible.[11]

In the last fifty years many studies were focused on interactions between »non-physiological« transition metal ions and DNA. It was shown that these interactions can lead to irreversible damage of cells and finally apoptosis. In contrast to essential counter ions (K^+, Na^+, Mg^{2+}, Ca^{2+}) that bind to negatively charged phosphate groups via electrostatic interactions, transition metal ions when coordinated to nucleic acid affect base pair scheme, nucleobase acid-base equilibria and tautomeric structures.[11]

In 1993 was first published that binding of Zn^{2+} and some other divalent metal ions (Co^{2+} and Ni^{2+}) cause a formation of the M-DNA complex. J. S. Lee and others proposed a structure in which metal ions are incorporated between GC and AT base pairs (i.e., in the middle of B-DNA). NMR studies showed that the imino protons of thymine (pKa=9.9) and guanine (pKa=9,4) were not present in M-DNA explaining the requirement for an alkaline conditions (pH above 8).[12]

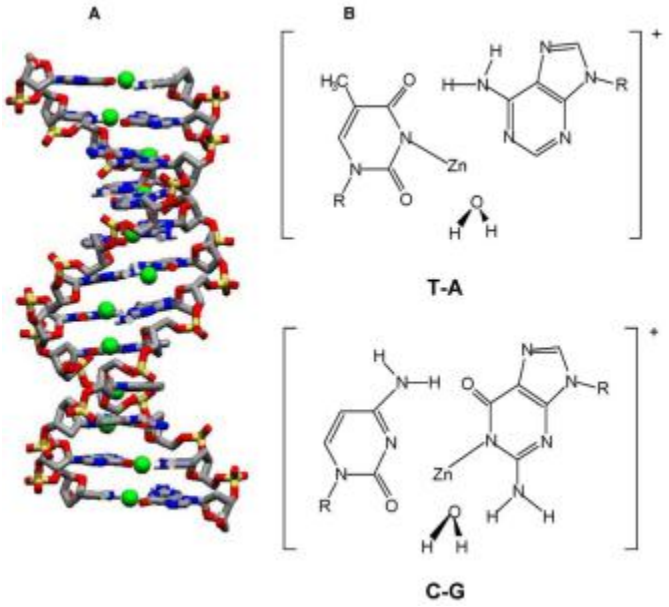

Figure 9: A: Modelled structure of M-DNA complex. B: Incorporation of Zn^{2+} ions between TA and CG base pairs.[12]

The ions which form M-DNA complex have ionic radii of 0,70 Å or less. Despite Mg^{2+} has ionic radius of about 0,65 Å, it doesn't form stable complexes with nitrogen bases since the electrostatic interactions with phosphate groups are preferred.

Binding of Zn^{2+} ions causes a distortion of double helix, since the length of N-Zn^{2+}-N bond is 4 Å in comparison to the length of Watson-Crick bond which is 3 Å. The presence of metal cations also decreases electrostatic repulsion between phosphate

groups. Consequently DNA condenses into more compact structure. With the agarose gel electrophoresis it was estimated that new structure contains at least 5% fewer base pair per turn than B-DNA.

M-DNA complex is supposed to have the properties of molecular wire.[13] It was proposed that the intercalation of Zn^{2+} ions into the double helix increases the number of free charge carriers in the conduction band of DNA. M-DNA complex and its properties have been studied by numerous techniques (NMR, EPR, CD, molecular modeling, conductivity, fluorescence, UV-VIS spectroscopy, etc.).[11] Unfortunately, all attempts to solve the crystal structure and to prove the mechanism of improved conductivity have failed. Therefore the structure and electronic properties are still controversial and remain to be proven.

Another special case is a complex between Hg^{2+} ions and DNA. Among all metal ions, Hg^{2+} shows the strongest interaction with nucleic acids. Hg^{2+} is a soft ion and therefore prefers covalent binding. Consequently, Hg^{2+} ions bind preferentially to nucleobases that was verified by UV measurements. Katz suggested the formation of Hg^{2+}-thymine complex (1:2), whereby he postulated that a chain-slippage process brings two thymine bases in the two strands together (see figure 10).[14]

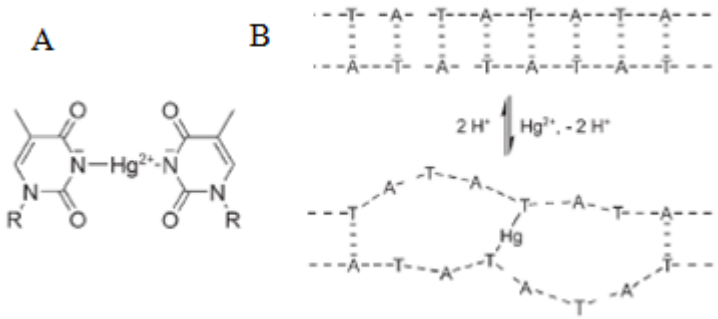

Figure 10: (A) The structure of T-Hg-T base pair (B) A chain-slippage process bringing two thymine bases together.

A strong evidence for the binding effects and the conformational changes in the double-helical structure of DNA upon interaction with metal ions can be obtained by circular dichroism. It was shown that, Hg^{2+} ions when interact with DNA, cause an inversion of CD spectrum, which indicates a B-DNA* to Z-DNA transition.[15,16]

* B-DNA is a right-handed double helix and Z-DNA is a left-handed double helix.

7 Conclusion

DNA is an interesting supramolecular structure and is responsible for development and functioning of almost all living organisms. Inorganic cations and charged organic molecules compensate the negative charge and thereby facilitate DNA packaging into a cell or a viral capsid. While in vivo DNA packaging requires a substantial metabolic energy, in vitro DNA condensation occur spontaneously in the presence of multivalent ions. DNA condensation arises from a complex interplay of interactions, including strong electrostatic forces, the loss of DNA configurational entropy and the tight bending of the stiff helix. In addition, chemical interactions between ions and specific binding sites on DNA are also important.

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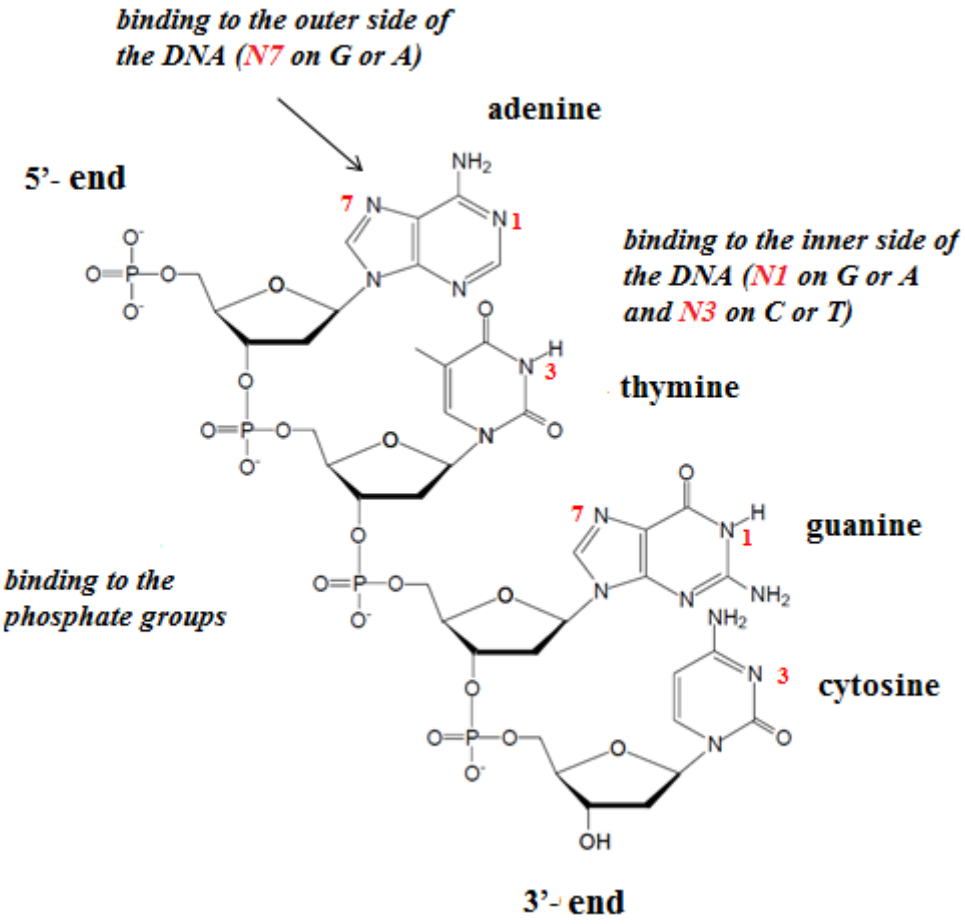
Appendix



Tetranucleotide dATGC. Presented are different modes for binding of ions to DNA.