Paper 4: Biomolecules and Their Interactions

Module 15: DNA Supercoiling and Hyperchromicity

Introduction

Super coiling of twisted elastic bands, ropes, and electric wires is a very common phenomenon. Supercoiling of DNA is defined as over or under coiling of DNA to remove strain. It is a 'helix upon a helix' structure also known as 'tertiary structure of DNA'. Supercoiling is extremely important from biological point of view, for packaging of a DNA molecule, several feet length, to fit in to the chromatin 200 to 300 Å wide and 700 to 800 nm in length. DNA supercoiling is necessary for unfolding of the DNA molecule for DNA replication. Various physico-chemical techniques as electron microscopy, X-ray diffraction, sedimentation coefficient measurements, UV, CD and Raman spectroscopy, are used to understand DNA supercoiling.

Objectives

Objective of the present module is:

a) To introduce the reader to the existence of supercoiling in DNA,
b) To brief on mathematical expression for DNA supercolilng,
c) To discuss DNA supercoiling in nucleosome core particle,
d) To explain use of sedimentation coefficient measurement technique for estimation of DNA supercoiling,
e) To explain hyper and hypochromicity in DNA,
f) To elaborate on use of CD spectroscopy to study polymorphic structural changes in DNA,
g) To introduce to mathematical expression of DNA bending.

15.1 DNA bending and supercoiling

DNA molecule has an intrinsic ability to bend as a direct consequence of base pair distortion due to change in base composition. This was noted in single crystal X-ray diffraction studies on DNA dodecamer CGCGAATTCGCG by Dickersen's group (Dickersen and Drew 1981) (figure 15.1) (discussed in the preceding module). At the other end of complexity, is the example of circular DNA, where supercoiling has been observed (figure 15.2). If two ends of DNA are closed into a circular DNA and then allowed to retort freely, it goes into a figure of eight (DNA supercoil wiki). The two lobes appear either twisted clockwise or counter clockwise with respect to one another (figure 15.3).

Experimental studies have shown that increasing amount of Mg(II), polyamines, basic polypeptides, polyethylene oxide or some inert polymers, ethanol, polyanions, some metal ions etc. can lead to bending of DNA (Sanger, 1984).

A typical example of supercoiling is the nucleosome core particle, where DNA molecule wraps around histone core protein to compact itself in nucleus. Another example is of a small circular chromosomal DNA of SV40 (seminal virus 40). When SV40 was incubated with histone octamer, it wrapped the histone octamer in two left-handed turns. The toroidal strain could be relaxed by addition of topoisomerase- I. It had been noted that DNA does not need histone octamer to fold. In prokaryotes plectonemic supercoiling is predominant, because of circular chromosomes. In eukaryotes DNA supercoiling exists on many levels with both pletonemic and toridal or combination of both (figure 15.4).

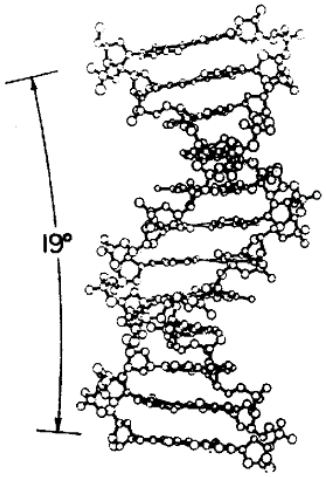


Figure 15.1 Bending of DNA dodecamer CGCGAATTCGCG (from Drew et al 1981)

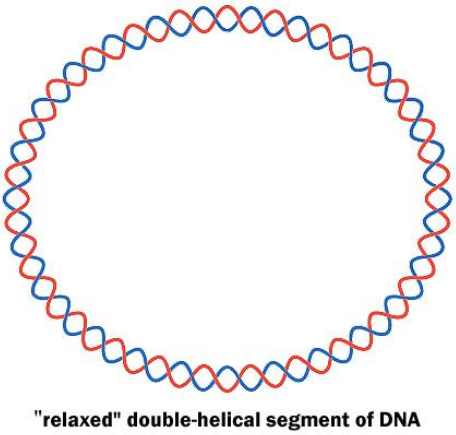


Figure 15.2 relaxed double helical DNA from en wikibooks/structural Biochemistry/DNA/supercoiling,

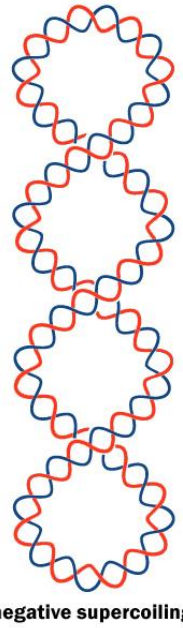


Figure 15.3Negatively supercoiled DNA

In nature DNA molecule is always present as a super helix. Positive supercoiling is noticed in DNA. Watson-Crick twist is called secondary structure. Super helix is a 'tertiary' structure. Figure 15.2 shows a relaxed or open circular Watson-Crick DNA. Figure 15.3 on the right hand side is a right handed (negative) superhelix. The relaxed structure is not normally found in nature unless DNA is nicked. Super helix is the form usually found.

Although DNA molecule is twisted lightly and has low twisting stress, a negative supercoiled knot has high twisting stress. It is more prevalent as negative supercoiling is a step towards unwinding of DNA needed for its function. Topoisomerases are enzymes that facilitate or prevent DNA supercoiling. Topoisomerase-I facilitates relaxation in negative supercoiled DNA by introducing a nick in one DNA strand and resealing it. Topoisomerase-II gives cut to both strands of DNA, unwinds it and reseals it removing two knots at a time.

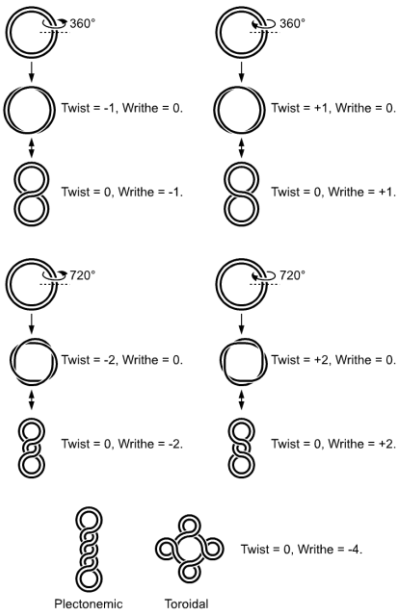


Figure 15.4 Circular DNA chromosome with a secondary helical twist only, and one containing an additional tertiary superhelical twist (Figure from Wiki)

Supercoiling allows compact packing of circular DNA (figure15.4). Supercoiling allows interaction of DNA with enzymes through 'kinks' or 'leaks' at weak points. The supercoiled strucre is described with 'twists' and 'writhes'. 'Twist' refers to number of 'turn's a DNA makes with a super helical axis. 'Writhrs' refer to circular distortion and over all non planarity.

15.2 *Mathematical expression*

Circular DNA is always isolated as super-helix with tertiary winding, right handed is a 'relaxed' or 'open circular' structure (figure 15.3 from wiki). For the purpose of computation, right handed super helix is defined as having a 'negative' number of super helical turns where as the left handed superhelix is defined as having a 'positive' number of super helical turns. In figure 15.3 both secondary and tertiary windings are right handed and the super twists are negative (-3).

DNA supercoiling can be defined as the change in linking number Lk which is the most descriptive property of the superhelices. We define a linking number Lk_0 as the number of turns in the relaxed (B-form) DNA plasmid/molecule. It is obtained

by dividing number of base pairs (bp) in the molecule by relaxed bp per turn which is 10.4-10.5.

$$Lk_0 = bp/10.4$$

Lk is the number of crosses a single strand makes over other. It is known as the Watson-Crick's 'twists' found in the circular chromosomes when it is constrained to a plane. The topology of DNA is described as by the equation

$$Lk = Tw + Wr$$

where *Tw* is the number of 'twists' (Watson-Crick's twists when the chromosome is not constrained to a plane) and *Wr* is the number of coils or 'writhes', is a number of super helical twists.

Native DNA is usually found to be superhelical. If one goes around the superhelically twisted chromosome, counting secondary Watson-Crick twists, that number will be different from the number counted when the chromosome is constrained to lie flat. In general, the number of secondary twists in the native, supertwisted chromosome is expected to be the "normal" Watson-Crick winding number, meaning a single 10-base-pair helical twist for every 34 Å of DNA lengths. In general, biological circular DNA is untwisted, meaning thereby *Lk* is less than *Tw.*

The underwound DNA is always under a strain as a metal spring. This can be relieved by taking a negative supertwist which corrects the secondary unwinding in accordance to the topology equation above. There is one to one relationship amongst changes in Tw and Wr. If a secondary Watson-Crick twist is removed then a right handed supertwist must be removed simultaneously. Similarly if the chromosome is relaxed with no super twists, then a left handed super twist must be added.

$$\Delta Lk = Lk - Lk_0$$

If DNA is negatively supercoiled then ΔLk<0, meaning there by DNA is underwound. A standard expression irrespective of the size of molecule giving a specific linking number is

$$\sigma = \Delta Lk / Lk_0$$

The Gibb's free energy is given by

$$\Delta G / N = 10RT\sigma^2$$

The difference between the supercoiled circular DNA and uncoiled circular DNA with N>2000 base pairs (bp)

$$\Delta G / N = 700 Kcal / bp * (\Delta Lk / N)$$

16 cal/bp

Since the linking number *L* of supercoiled DNA is the number of times the two strands are intertwined (and both strands remain covalently intact), *L* cannot change. The reference state (or parameter) L_0 of a circular DNA duplex is its relaxed state. In this state, its 'writhe' *W* = 0. Since *L = T + W*, in a relaxed state *T = L*. Thus, if we have a 400 bp relaxed circular DNA duplex, *L ~ 40* (assuming ~10 bp per turn in B-DNA). Then *T ~ 40*.

Positively supercoiling:

T = 0, W = 0, then L = 0
T = +3, W = 0, then L = +3
T = +2, W = +1, then L = +3

Negatively supercoiling:

T = 0, W = 0, then L = 0
T = -3, W = 0, then L = -3
T = -2, W = -1, then L = -3

Negative supercoiling favors local unwinding of DNA. Latter is necessary for interaction of DNA polymerase

15.3 Structure of nucleosome

Nucleosome is a basic unit of DNA in eukaryotes, consisting of about 200 base pairs (bp), wound around eight histone protein core. The schematic structure of nucleosome from Stryer (1995) is shown in figure 15.5. Nucleosomes are fundamental repeating units of chromatin which pack about 2 meters of linear DNA in 10μm nucleus. These are folded through series of higher order structures to eventually form chromosomes.

Nuceosomes were first observed by Olins and Olins in 1974, in electron microscopy photographs. Three dimensional structure was proposed by Kornnberg (1974). Aron Klug provided the first evidence in 1980 that 140 bp of DNA molecule wraps around eight histone protein core as a left handed super helix in 1¾ turn with external diameter of 110Å.

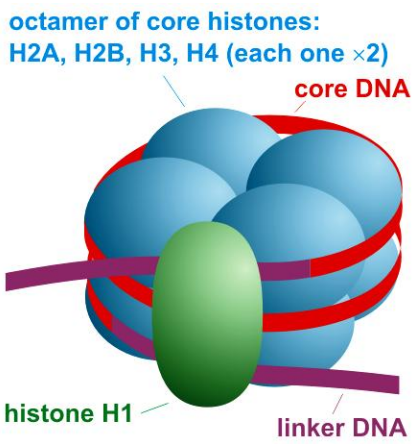


Figure 15.5 Schematic representation of nucleozome core particle, figure from Stryer 1995),

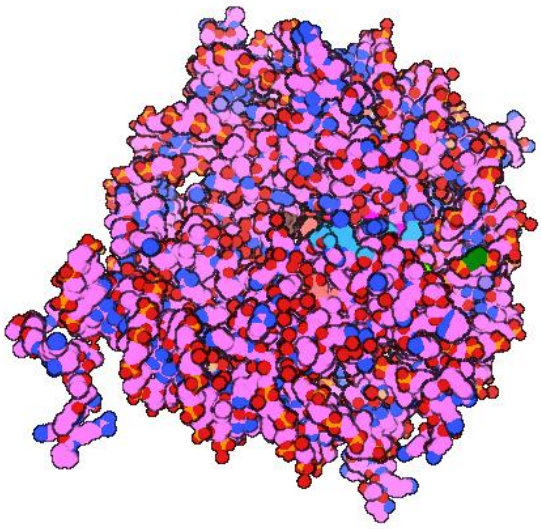
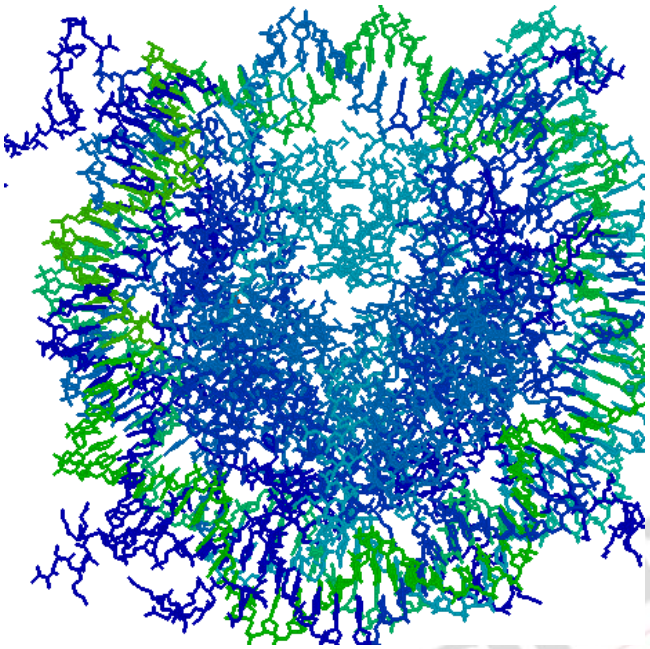


Figure 15.6 CPK model using PyMOL of 1EQZ

The atomic level resolution structure was first solved by Richmond's group in 1997. The human alpha satellite pallindromic DNA was critical in achieving these results at Oak Ridge National Laboratory. The structure is remarkably conserved. Even small changes in residues could be observed in electron micrograph photographs.

The structure of nucleosomes from several organisms are now available at PDB . Figure 15.6 gives the overview of the nucleosome structure (PDB entry 1EQZ) by Harp et al (2000). The details are shown in figure 15.7 and 15.8.

In structure (1EQZ) 146 bp DNA is wrapped in 1.67 left handed super helical turn around a histone core consisting of two copies each of H2A, H2B, H3 and H4. The core particles are connected by linkers H1 and approximately 80 bp DNA. Technically, nucleosome is defined as core particle with one linker.



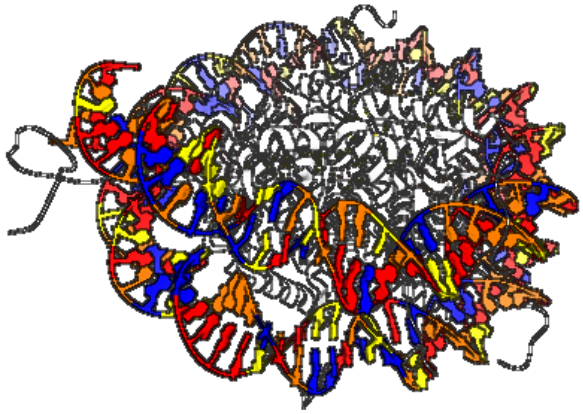


Figure 15.7 Structure of nucleosome 1EQZ by Harp et al.(2000) Figure drawn using Rasmol (left) and Pymol (right)

The structure has over 120 protein-DNA interactions unevenly spread in regions α1,α2 and L1, L2. The α1,α2 site uses two adjacent helices. L1, L2 uses loops. There are many water mediated interactions. Salt links and hydrogen bonding between protein side chains (basic and hydroxyl groups) and main chain amides, with DNA backbone phosphate form the bulk of interactions with DNA. Latter is important given ubiquitous distribution of nuclosome along genomes. Although, nucleosomes prefer some DNA sequences over other, they are practically non specific and can bind with any DNA sequence.

Non-polar interactions are made between protein side-chains and the deoxyribose groups, and an arginine side-chain intercalates into the DNA minor groove at all 14 sites where it faces the octamer surface. The distribution and strength of DNA-binding sites about the octamer surface distorts the structure of DNA within the nucleosome core.

The DNA is non-uniformly bent and also contains 'twist defects'. The twist of free B-form DNA in solution is 10.5 bp per turn. However, the overall twist of nucleosomal DNA is only 10.2 bp per turn, varying from a value of 9.4 to 10.9 bp per turn. The histone tail is flexible. The N-terminal tail of histones H3 and H2B pass through a channel formed by minor grooves of two DNA strands. The N-terminal tail of histone H4 has highly basic amino acids. These interact with H2A-

H2B dimer of other nucleosome and important for higher order structure. Linear histones such as H1 and its isomers are involved in chromatin compaction and sit at the base of the nucleosome near DNA entry and exit binding to the linker region of DNA. The non condensed nucleosomes with linkers look like 'beads and strings' in electron microscopy.

15.4 Use of sedimentation coefficient measurement technique for determination of DNA super coiling

Supercoiling of DNA can be ascertained by number of techniques such as: spectroscopy, low angle X-ray difftraction, electron microscopy, measurements of sedimentation coefficient *s* etc. A typical example of use of sedimentation coefficient measurement is shown in the figure 15.8

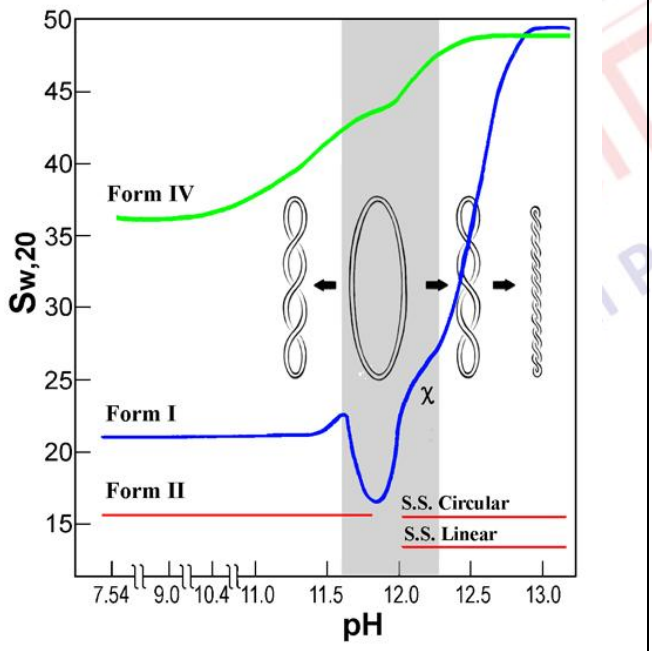


Figure 15.8 Changes in the sedimentation coefficient in the three forms (I, II and IV) of circular DNA from plasmid. Figure from wiki

"Form I" (blue curve) is the traditional native form of duplex circular DNA, from viruses and intracellular plasmids. It is covalently closed, and any plectonemic winding, if present, is therefore locked in. If one or more nicks are introduced to the form I, free rotation of one strand with respect to the other becomes

possible, and a form II (red curve) is seen. The form IV (green curve) is the product of alkali denaturization of the form I. Between pH 7 and pH 11.5, the sedimentation coefficient ***s***, for form I, is constant. Then it dips, and at a pH just below 12, reaches a minimum. With further increases in pH, ***s*** then returns to its former value. It doesn't stop there, however, but continues to increase relentlessly. By pH 13, the value of ***s*** has risen to nearly 50, two to three times its value at pH 7, indicating an extremely compact structure.

15.4 Hyperchromisity of DNA

Ultraviolet (UV) spectroscopy is one of the most important tools in understanding structural changes and interactions of DNA. Generally we measure OD (optical density) = $\log_{10}(I_0/I)$, or T (transmittance) = (I/I_0). 100. These are usually governed by Beer Lambert's law

$I = I_0$. Exp $(-\varepsilon CL)$

where ε is *molar absorptivity* (also called as *molar extinction coefficient), C* is the concentration, *L* path length. The absorbance has lots of factors built in. The UV absorption in molecules can be observed due to n-π^* and π–π^* transitions and is affected by presence of other molecules, changes in stacking interactions etc. In the presence of chemical interactions, spectral lines can change absorbance or shift. If the line shifts towards higher wavelength (red shift), it is named as 'bathochromic effect', if it moves to lower wave length (blue shift), it is known as 'hypsochromic effect'. Similarly if there is increase in absorbance it is known as 'hyperchromisity', and reduction in absorbance is 'hypochromic effect.

The UV absorption curve for DNA bases A, T, G and C is shown in the figure 15.9. The double helical B-DNA molecule has strong ultraviolet absorption at 260 nm due to n-π^* transition. This is because of ring current due to stacking interaction amongst hydrogen bonded base pairs. The average extinction coefficient for double stranded DNA this is 0.020 $(\mu g/ml)^{-1}$ cm^{-1}. For single stranded DNA it increases to 0.025$(\mu g/ml)^{-1}$ cm^{-1} (figure 15.10).

Thus DNA molecule shows hyperchromic effect. Absorbance of 1 corresponds to concentration of 50 (μg/ml). When the solution of DNA is heated above its melting temperature (80^o) the double stranded DNA unwinds to form single stranded DNA. There is hyperchomic effect, as the bases become unstacked and can thus absorb more light. The wavelength of maximum absorbance does not change, but the amount absorbed increases. A double strand DNA dissociating to single strands produces a sharp cooperative transition.

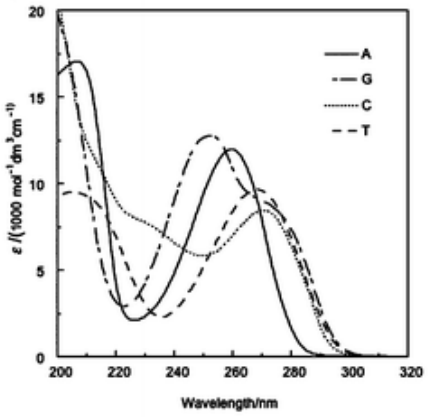


Figure 15.9 UV absorption of DNA bases, A, G, C and T. There is crowding of picks around 260nm.

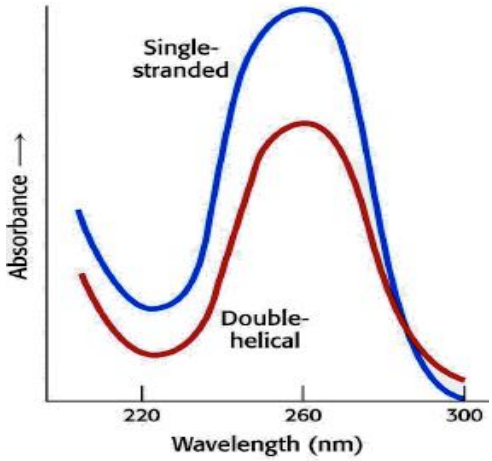


Figure 15.10 UV absorption of double helical and single stranded DNA

Hyperchromicity can be used for quantitizing DNA melting (figure 15.11). We define hyperchromicity

$A = 100 \times (Et - Ec) / Ec$

where *Et* is absorbance at temperature T and *Ec* absorbance of 100 % coil which is used to monitor helix-coil transition in DNA.

Hyperchromicity can be used to track the condition of DNA as temperature changes. The transition/melting temperature (T_m) is the temperature where the absorbance of UV light is 50% between the maximum and minimum, i.e. where 50% of the DNA is denatured. Relative absorbance is = absorbance at any temperature T/absorbance at 25°.

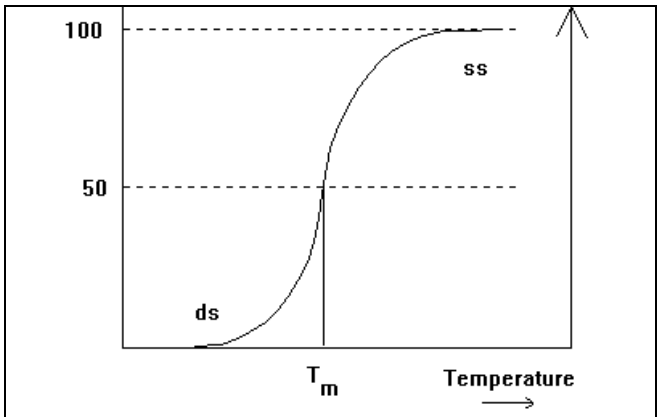


Figure 15.11 Change of absorbance during melting. Here ds and ss denote double stranded and single stranded DNA b. Change in UV absorption from 30° C to 90 C°

There is a striking increase in absorbance upon denaturization.

When DNA double helix is treated with denaturizing agents, the interaction force, holding the double helical structure is disrupted. The double helix then separates into two single strands which are in the random coiled conformation. At this time, the base-base interaction will be reduced, increasing the UV absorbance of DNA solution, because many bases are in free form and do not form hydrogen bonds with complementary bases. As a result, the absorbance for single-stranded DNA will be 37% higher than that for double stranded DNA at the same concentration. UV absorption of DNA can be used for checking DNA purity.

15.6 Monitoring polymorphic structural changes in DNA using CD spectroscopy

Circular dichroism (CD) is a phenomenon originating from interactions of chiral molecules with circularly polarized electromagnetic rays. DNA molecule shows UV absorption within 180-300 nm, where bases of DNA (A, G, T, C) absorb light. Absorption of right- and left-handed circularly polarized light by chiral molecules differs and the difference can be measured as molar ellipticity Θ°. Generally we measure the difference in the molar extinction coefficients for left and right circularly polarized light $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ [$M^{-1}cm^{-1}$]. CD spectroscopy is a very sensitive tool and one can use samples with as low a concentration as 25 μg/ml. In DNA, formation of helical structure gives chromophores a strong asymmetric environment. We show in the figure 15.12 the CD spectra of A, B and C DNA. It is markedly different compared to the CD spectra of ZDNA (figure 15.13). Different

polymorphic forms of DNA show variation in CD spectra. Comparison for A, B and Z form is shown in figure 15.14. B-DNA has a positive band at 275 nm, negative at 240 nmand cross over at 258 nm. The A form of DNA has positive at 260 nm a small negative band at 210nm and a strong positive band at 190nm. CD spectra of C DNA was similar to A DNA but the negative band was very pronounced and positive band was practically absent. The CD spectra of Z DNA was first reported by Phol and Jovin in 1972. They measured CD spectra of a self complementary duplex poly (dG-dC).poly(dG-dC) at low and high salt (3M NaCl) at 230-300 nm. The CD spectrum approximately inverted compared to B-form of DNA (figure 15.14). There had been a negative band at 290nm, positive at 260nm and a cross over at 185 nm.

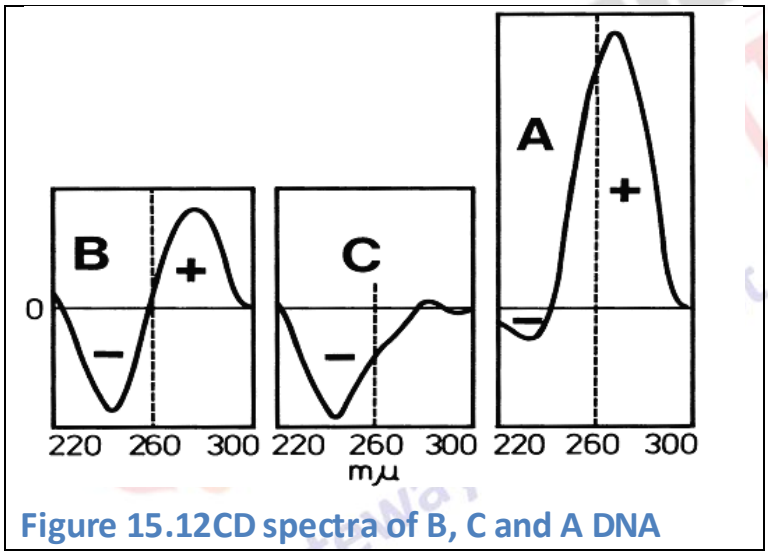


Figure 15.12CD spectra of B, C and A DNA

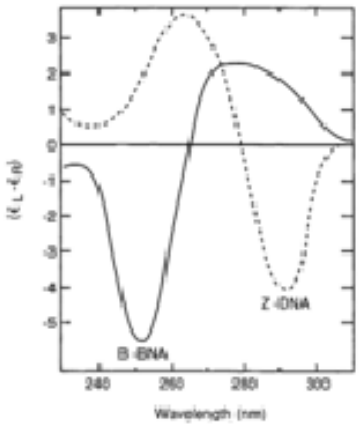


Figure15.13CD spectra BZ trasition by Phol and Jovin 1972

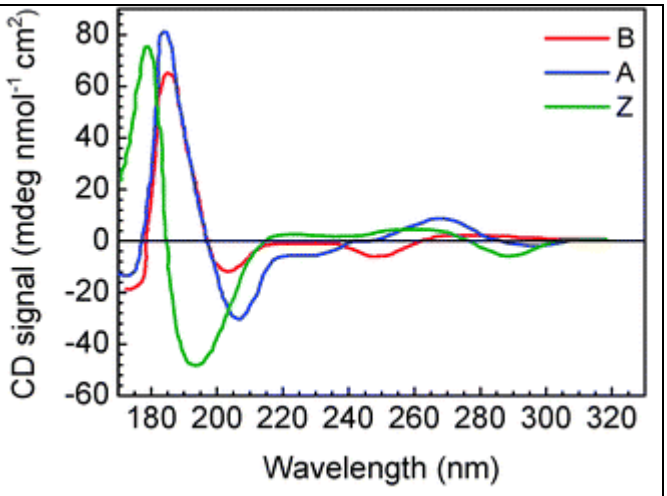


Figure 15.14 Comparison of CD spectra of A, B and Z Form

15.7 Generating a curved DNA

Several software's are now available for generation of sequence-dependent spatial trajectory of the DNA double helix and/or distribution of curvature along the DNA molecule. The nearest-neighbor wedge model is implemented to calculate overall DNA path using local helix parameters: helix twist angle, wedge (deflection) angle and direction (of deflection) angle by Sussman and Trifanov (1978). One can develop a computer program based on their algorithm.

If we wish to rotate *n* base pair segment of DNA with cylindrical polar coordinates *ri, θI, zi* and base pair rise *pr*, we can calculate the transformed coordinates: Xim, Yim and Zim as follows. We need following parameters. *H*- pitch of the large helix, α,-tilt angle, *HA*-sense of super helix (+) for right handed helix and (-) for left handed helix, Θ –rotation of the cylinder about its own axis (normally close to 0^o) and the 'kink angle' Kθ.

The radius of the super helix *R* can be easily calculated as:

R = (n*pr/ Kθ).

We calculate cylindrical polar coordinates Rim, Θim, Zim of the curved DNA using following equations:

$D = ri \times \cos(\theta i - \Theta) + R$; $E = ri \times \sin(\theta i - \Theta)$; $F = ri \times \sin(\theta i - \Theta) \times \sin\alpha$; $\tan P = F / D$,

$G = \sqrt{2\pi R^2 + H^2}$; Rim= $\sqrt{D^2 + F^2}$; $\Theta im = \pm P + (360 \times zi / G)$, and

$Zim = \pm(H \times zi / G) - E \times \cos\alpha$.

Cartesian coordinates *Xim* and *Yim* can be calculated from Rim and Θim using:

$Xim = Rim \times \cos(\Theta im)$, $Yim = Rim \times \sin(\Theta im)$.

Note: The sign (+) or (-) to be used depending on the sense of super helix. The structure generated using our program CURVE_DNA (Kothekar) is shown I figure 15.15.

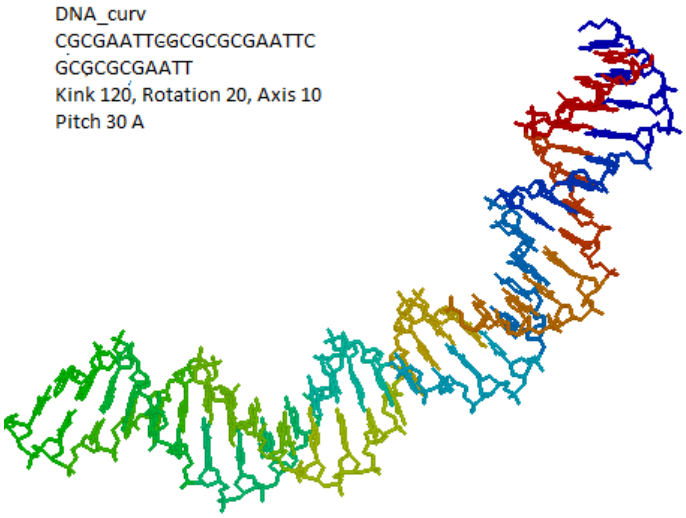


Figure 15.15 Curved DNA generated using CURVE_DNA by Kothekar

A general method for generation of base-pairs in a curved DNA structure, for any prescribed values of helical parameters—unit rise (h), unit twist (Θ), wedge roll (Θ_R), wedge tilt (Θ_T), propeller twist (Θ_P) and displacement (D) was described by Bhattacharya and Bansal (1988).

Summary

We have discussed in present module, DNA bending and supercoiling. We have given examples of supercoiled DNA in oligo nucleotides and circular DNA. Role of overwound and underwound DNA in nature is discussed. Matematical expression for quantifying DNA supercoiling is given.

We have discussed structure of nucleosome, which is a typical example of DNA supercoiling in chromosomes. We have also discussed use of sedimentation coefficient measurement for studying DNA supercoiling.

We introduced to Beer Lambert's law, hypsochromic, bathochromic, hyperchromic and hyporchromic effects in UV spectroscopy and detailed on the use of hyperchromic effect for studying DNA melting and interaction with other molecules. We have also introduced to use of CD spectroscopy for studying DNA polymorphism. Lastly we have given mathematical formalism for generating curved DNA to enable any reader to develop his/her own computer program.