SURVEY AND SUMMARY

Circular dichroism and conformational polymorphism of DNA

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ABSTRACT

Here we review studies that provided important information about conformational properties of DNA using circular dichroic (CD) spectroscopy. The conformational properties include the B-family of structures, A-form, Z-form, guanine quadruplexes, cytosine quadruplexes, triplexes and other less characterized structures. CD spectroscopy is extremely sensitive and relatively inexpensive. This fast and simple method can be used at low- as well as high-DNA concentrations and with short- as well as long-DNA molecules. The samples can easily be titrated with various agents to cause conformational isomerizations of DNA. The course of detected CD spectral changes makes possible to distinguish between gradual changes within a single DNA conformation and cooperative isomerizations between discrete structural states. It enables measuring kinetics of the appearance of particular conformaters and determination of their thermodynamic parameters. In careful hands, CD spectroscopy is a valuable tool for mapping conformational properties of particular DNA molecules. Due to its numerous advantages, CD spectroscopy significantly participated in all basic conformational findings on DNA.

INTRODUCTION

All known autonomous forms of life, starting from bacteria and ending with human, store their genetic information in molecules of double-stranded DNA. DNA is a giant molecule whose length is hundreds millions base pairs in man. The huge molecules of DNA are densely packed in the relatively tiny space of the cell nucleus in eukaryotes. The basic arrangement of DNA, discovered in 1953, is a right-handed double helix typical of RNA, the left-handed Z-DNA, various kinds of hairpins and slipped structures, parallel-stranded double helices, various kinds of triplexes, guanine quadruplexes, intercalated cytosine tetraplexes and other less well characterized structures (1,2). It is likely that all of the structures occur within the cell where they are stabilized by supercoiling, various proteins and low water activity. The anomalous structures play a role in DNA functions and pathology (3).

CIRCULAR DICHROISM SPECTROSCOPY

Circular dichroism (CD) is a phenomenon originating from interactions of chiral molecules with circularly polarized electromagnetic rays [for more details, see (4,5)]. Spectral studies of DNA have employed far UV (6) as well as infrared (7) light, but most analyses use ultraviolet light within 180–300 nm range (8), where bases of DNA absorb light. Absorption of right- and left-handed circularly polarized light by chiral molecules differs and the difference is called CD. The quantity used to describe this phenomenon is called ellipticity, $\Theta$, and is expressed in degrees. A more convenient characterization of CD is the difference in the molar extinction coefficients, $\Delta e = e_L - e_R$ [M$^{-1}$cm$^{-1}$].

The theoretical (i.e. quantum chemical) description of CD spectra of molecules as large as DNA is very complex, so the method is not able to provide structural information on the molecules at atomic level. For this reason, CD spectroscopy is primarily used empirically in studies of DNA. CD spectroscopy, however, has many advantages over other methods of conformational analysis. First, it is extremely sensitive, permitting work with DNA amounts as low as 25 µg. The concentration of DNA can also be very low (20 µg/ml). This is advantageous in studies of samples of low solubility (e.g. long G-rich DNA fragments) or of those that tend to aggregate under extreme solvent conditions (e.g. high salt or organic solutions, which are used to mimic interactions with proteins or other molecules in cells). Changes in the path length can

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be used to effectively alter DNA concentration by more than three orders of magnitude. Second, the studied molecules can be not only short (oligonucleotides) but also long, which is especially important with DNA. Third, the samples can easily be titrated with various agents (like salts, alcohols or acids) that induce conformational isomerizations in DNA. This makes it possible to map the whole conformational space of the studied molecule, not just a single structure. Fourth, CD spectroscopy distinguishes two-state conformational isomerizations between distinct conformers from gradual changes within arrangements characterized by a single energetic minimum. Fifth, as CD can analyse not only solutions but also films, it can be used to make correlations between infrared spectroscopy and X-ray diffraction (10). Sixth, CD measurements are fast and relatively inexpensive, which allows comparative studies of related molecules of DNA under many conditions. This represents a further source of useful information. This everything are advantages of CD spectroscopy as compared with so-called absolute methods (X-ray diffraction of crystals and NMR spectroscopy), which need crystallization (which itself is a problem), large amount of material, or long accumulation, limited experimental conditions, a single discrete structure (as opposed to a mixture of isomerizing conformers) and short molecules. These advantages are the reasons why CD data participated in practically all of the key discoveries regarding secondary structures of DNA. Further information about CD spectroscopy of nucleic acids can be found in previous review articles (11,12).

THE B-FORM, DNA DENATURATION AND THE HAIRPIN

The B-form is the most frequently observed conformation of DNA. The long wavelength CD spectrum of the sequentially heterogeneous DNA is conservative (Figure 1). The base pairs in the B-form are perpendicular to the double-helix axis, which confers only a weak chirality on the molecule so that the peak intensities are relatively small (Figure 1). As (A + T) content increases, the negative band becomes deeper and conformational variability increases (compare spectra in Figure 1, the upper row). With increasing salt concentration, the long wavelength part of the CD spectrum gradually (non-cooperatively) decreases to give a continuum of CD spectra corresponding to variants of B-DNA structures differing in the number of base pairs per helix turn (13).

The CD spectra of B-forms of synthetic polydeoxynucleotides depend on the primary sequence (Figure 1). These structures constitute a family of B-DNA forms with common global features. All of them (with the exception of poly[d(IC)] (14,15), which contains a non-canonical base) are characterized by a positive long wavelength band or bands at about 260–280 nm and a negative band around 245 nm. However, the position and amplitudes of the CD bands differ markedly with sequence not only because chromophores differ, but also because of different conformational properties. Poly[d(A)]-poly[d(T)] and poly[d(G)]-poly[d(C)] adopt unusual B-forms. The first adopts so called B' heteronomous DNA-form (16,17) with a distinct geometry of dA and dT residues, the second has some distinctly A-like features (18).

CD spectroscopy can be used to monitor denaturation of DNA. Figure 2A shows CD spectra of the self-complementary duplex poly[d(AT)] measured at various temperatures. The left panel reflects non-cooperative changes in poly[d(AT)] that precede thermal melting (19); spectra in the right panel correspond to thermal melting. Melting is a cooperative (Figure 2A, insert), two-state process, which is reflected by the presence of isodichroic points of the spectra (Figure 2A, right panel). The course of melting curves enables determination of thermodynamic parameters (ΔH, ΔG or ΔS) (20–22) of the studied structure.

A hairpin is an intra-molecular variant of the B-form (3,23,24). Besides the double-helical stem it contains a single-stranded loop. Self-complementary oligonucleotides and also DNA fragments consisting of GC-rich trinucleotide repeats can adopt both a B-form duplex and a hairpin and transform between them depending on conditions. Hairpins and their sliding are considered to be a reason of the repeat expansions associated with various, mainly neurodegenerative, diseases (3,24). Because of the single-stranded loop, the duplex to hairpin transition is usually accompanied by CD amplitude reduction. Sensitivity of CD spectroscopy to the duplex–hairpin transition is dependent on the oligonucleotide primary structure. Here we show temperature-induced duplex–hairpin transition of C6G6 (Figure 2B). Electrophoretic migration (Figure 2B, insert) coincides with the CD changes.

A-FORM AND THE B–A TRANSITION

A-form is a constitutive conformation of RNA. DNA adopts the A-form in aqueous ethanol and other solutions (25). Some molecules of DNA (e.g. poly[d(A)]-poly[d(T)], Figure 1) do not adopt the A-form conformation at all. Others [e.g. (G + C) rich DNA fragments] (18,26) exhibit A-form features even in aqueous solution. The CD spectrum of poly[d(G)]-poly[d(C)] (Figure 1) is very similar to that of the true A-form DNA (Figure 3A). The CD spectrum of A-form DNA of heterogeneous primary structure is practically the same as that of the A-form of the corresponding RNA or RNA/DNA hybrid with the same primary structure. The spectrum is characterized by not only a dominant positive band at 260 nm but also a negative band at 210 nm (Figure 3A). The amplitude of the positive band is usually within 7–12 M–1 cm–1 depending on the base sequence.

The B–A transition is a fast, two-state, and highly cooperative process. This brings isodichroic points into the CD spectra and an S-shaped dependence of CD on the concentration of the inducing agent (Figure 3A, insert), which may be ethanol (25,27), trifluorethanol (TFE) (28–30) or even methanol in the case of (G + C) rich DNA (31). Since RNA constitutively adopts the A-form, the B–A transition of DNA may precede transcription and may be instrumental for its initiation.
THE Z-FORM AND THE B–Z TRANSITION

Many studies have been devoted to the left-handed Z-form of DNA and, in the same way as the classical B- and A-forms, the Z-form has entered into textbooks (32). The base pairs in the Z-DNA double helix have an opposite orientation with respect to the backbone than the B- and the A-forms. Its CD spectrum is nearly an inversion of the CD spectrum of the B-form; it contains (Figure 3B) a negative band at about 290 nm, a positive band around 260 nm and another characteristic, extremely...
Figure 2. Temperature induced changes in CD spectra reflecting pre-melting and melting of DNA. (A) Spectra of poly[d(AT)] in 10 mM Na acetate, pH 7 at temperatures indicated. The left panel reflects changes within double-helical structure and the right panel shows a helix–coil transition. Insert: the changes in poly[d(AT)] monitored at 262 nm (triangles) and 220 nm (squares). (B) Irreversible duplex–hairpin transition of d(C6G6) in 1 mM Na phosphate, 0.3 mM EDTA, pH 7. Left: CD spectra measured at 0°C corresponding to duplex (cyan) before denaturation and hairpin (dark blue) after denaturation. Right: spectral changes induced by increasing (cyan) and decreasing (dark blue) temperatures monitored at 260 (squares) and 280 (triangles) nm. CD spectra were measured in 0.1 cm cells. Insert: polyacrylamide gel (20%) electrophoresis of the samples running in the same buffer at 2°C. The samples were exposed to the indicated temperatures prior to loading on the gel.
deep negative band at short wavelength (~205 nm) (33,34). Transitions between B-, A- and Z-forms followed by CD spectroscopy were nicely shown by Ivanov (34). As with the B-form, there are several variants of the Z-form. Their CD spectra are fairly different but transitions between them are non-cooperative (34–36) (Figure 3B, right and insert). In contrast to the B–A transition, the B–Z transition is slow. This is connected with the base pair flip that is required during the transition, which is a kinetically difficult process.

Figure 3. B–A and B–Z transitions of DNA. (A) Left panel: CD spectra reflecting trifluorethanol-induced B–A transition of d(GCGGCGACTGGTGAGTACGC) duplex. Insert: the transition monitored at 266 nm. Right panel: CD spectra of RNA of the same sequence (U instead of T) duplexed with complementary DNA strand. (B) CD spectra reflecting trifluorethanol-induced B–Z (left panel) and Z–Z' (right panel) transitions of poly[d(GC)] duplex. Insert: the transitions monitored at 291 nm. In this figure, TFE was added to the oligonucleotides dissolved in 1 mM Na phosphate, 0.3 mM EDTA, pH 7 and CD spectra were measured at 0°C.
TRIPLEXES

Triplexes of DNA do not have a characteristic CD spectrum like the A-form, Z-form or tetraplexes. Triplexes formed by various sequences provide different CD spectra (37–39). Nevertheless, in combination with gel electrophoresis, it is easy to identify CD spectrum characteristic of a studied triplex of a particular sequence; this spectrum is clearly distinct from those of its particular strands. CD spectroscopy is thus a suitable method for the study of triplex formation and for identification of triplex-forming oligonucleotides. These oligonucleotides can bind duplex DNA in a sequence-specific fashion and are thus attractive tools for manipulating gene sequence and expression (40,41).

GUANINE QUADRUPLEXES

Guanine quadruplexes are of many types but all of them are based on guanine tetrads (Figure 4, upper panels). There are two basic types of the guanine quadruplexes. The spectra of parallel quadruplexes have a dominant positive band at 260 nm, whereas the spectra of anti-parallel quadruplexes have a negative band at 260 nm and positive band at 290 nm (Figure 4A and B, respectively) (12,42–45). Both quadruplex types display an additional characteristic positive peak at 210 nm. The different CD of the parallel and anti-parallel quadruplexes originates from different stacking interactions (12) of the guanosine residues distinctly oriented around their glycosidic bonds. Intermolecular guanine quadruplexes form slowly and also transitions between different quadruplex types are slow. Quadruplex formation is induced by physiologically relevant cations namely potassium, although ethanol is a still more effective inducer (46,47). The long-wavelength CD spectrum of a parallel quadruplex is similar to the CD spectrum of the A-form. This may suggest a similar base stacking in these two conformations (48,49). The quadruplex, however, has a positive band at 210 nm, whereas the A-form displays a deep negative one (compare Figures 4A and 3A).

CYTOSINE QUADRUPLEXES

DNA strands rich in cytosine also generate quadruplexes (50,51). These consist of two parallel homoduplexes connected through hemiprotonated C-C’ pairs. The two duplexes are mutually intercalated in an anti-parallel orientation (Figure 4C). Therefore, these structures are called intercalated or i-tetraplexes. The cytosine quadruplexes provide a characteristic CD spectrum with a dominant positive band at 290 nm (52,53) (Figure 4C). Formation of cytosine quadruplexes is promoted by slightly acid pH, which is needed for C-C’ pair hemiprotonation. Interestingly, the sequences that are prone to fold into cytosine quadruplexes bind the proton to form this structure even at pH values higher than 7 (53). Similar to guanine quadruplexes, intermolecular cytosine quadruplexes are formed with slow kinetics.

DNA FRAGMENTS RICH IN GUANINE AND ADENINE

DNA fragments rich in guanine and adenine exhibit cooperatively melting conformers that differ from classical structures. Their molecular structures have not yet been solved in spite of several attempts. The first conformer is an anti-parallel homoduplex (54) containing G·A pairs. Its CD spectrum is B-like (55) (Figure 5A). This duplex is stabilized by divalent zinc cations. Low concentrations of the zinc cations induce various conformers so that the process is not of a two-state nature. This anti-parallel homoduplex is probably a part of a zinc-stabilized d(GA)n·d(GA)n·d(TC)n triplex (55).

The second conformer of the alternating GA sequence is a parallel homoduplex (56) with G·G and A·A pairs; this conformer exhibits a CD spectrum similar to that of parallel guanine quadruplexes (Figure 5B, compare with Figure 4A) but with smaller positive amplitudes. The spectral similarity indicates the presence of guanine–guanine stacking so that the adenines probably extrude from the double helix (57,58).

The third conformer is an ordered single strand containing protonated adenine (59). Its CD spectrum (Figure 5C) is nearly identical to that of the parallel homoduplex. This single strand becomes a homoduplex at higher ionic strength (60,61). The process is non-cooperative and is reflected by a gradual deepening of the negative band (62). This indicates that the guanine–guanine stacking does not change significantly and that the duplex formation is mediated by interstrand adenine–adenine interactions. It is interesting that ethanol (62) and even dimethylsulphoxide (57), both DNA denaturing agents, stabilize the single-stranded ordered d(GA)n structure as does acid pH.

ALTERNATING SEQUENCE OF ADENINE AND THYMINE

Poly[d(AT)] and e.g. d(TA)4 provide a remarkable CD spectrum at high concentrations of CsF (63) or in aqueous ethanol (64,65). In the presence of millimolar NaCl concentrations, poly[d(AT)] isomerizes into the A-form at high ethanol concentrations (27) (Figure 6). Replacement of NaCl by the same amount of CsCl results in the appearance of a spectrum with a large negative band at 280 nm and a positive one at 210 nm (Figure 6). All the spectral changes proceed with a fast kinetics. The structure in the presence of CsCl in ethanol or in CsF in aqueous solution was called X-DNA. It provides two well-separated signals in the 31P NMR spectrum like the Z-form (63). But X-DNA is not Z-DNA as the 31P signal assignments for the purine–pyrimidine and pyrimidine–purine are opposite for the two structures (63). Cooperativity of the B–X and A–X transitions (Figure 6) also excludes the possibility that X-DNA is a member of the B- or A-DNA conformational families. A structure has recently been crystallized (66) that may correspond to the anomalous X-DNA CD spectrum. It contains Hoogsteen base pairs.

Poly[d(AT)] can also adopt the left-handed Z-form (67,68). Interestingly, despite different chromophores,
its CD spectrum is very similar to the CD spectrum of the Z-form of poly[d(GC)] (69). Methylation of cytosine at position 5 in poly[d(GC)] shifts the B–Z transition to low salt concentrations (70). As shown in Figure 7, poly[d(amo2m5C)] also undergoes a salt-induced conformational transition at low salt concentrations (71). The resulting conformer has X-form characteristics and exhibits some features of A-form (72,73). Poly[d(Gmethyl5C)] and poly[d(amo2m5AT)] differ in the placement of the amino and keto groups in the major groove (Figure 7, bottom insert): the first polynucleotide transforms highly...
Figure 5. CD spectra of d(GA)$_{20}$. (A) Spectra reflecting formation of the zinc-specific anti-parallel homoduplex. The spectra were measured in Tris–HCl buffer, pH 8.3. The yellow line corresponds to 0.7 mM ZnCl$_2$. (B) CD spectra reflecting the NaCl-induced transition of d(GA)$_{10}$ into the parallel homoduplex. To increase the salt concentration, 5 M NaCl was added to the oligonucleotide dissolved in 10 mM sodium phosphate, pH 7. (C) CD spectra of an ordered single-stranded conformer containing protonated adenine. The pH value was changed directly in the CD cell by addition of dilute HCl to the oligonucleotide in 1 mM sodium phosphate.

Figure 6. B–A, B–X and A–X transitions of poly[d(AT)]. From the left to the right: CD spectrum of the B-form measured in 1 mM sodium phosphate, 0.25 mM EDTA, pH 7 (black); ethanol-induced B–A transition measured at 10°C and monitored by $\Delta \varepsilon$ at 262 nm (96% ethanol was added to the poly[d(AT)] sample). CD spectrum of the A-form in the presence of 0.2 mM sodium phosphate and 0.05 mM EDTA and 80% ethanol (blue); ethanol induced B–X transition measured in the presence of 1.3 mM CsCl at 4°C and monitored by $\Delta \varepsilon$ at 278 nm (1.3 mM CsCl was also present in ethanol). CD spectrum of the X-form in 0.15 mM sodium phosphate, 0.04 mM EDTA, 1.3 mM CsCl and 82% ethanol (violet). CD spectra reflecting the A–X transition induced by addition of CsCl (0, 0.53, 0.59 and 1.3 mM) to the A-form at 4°C and the course of the transition monitored by $\Delta \varepsilon$ at 278 nm. This figure was adapted from Vorlickova M. et al. (J. Biomol. Struct. Dyn. 1991, 9, 571–578) with permission.
cooperatively to Z-form and the second, in a less cooperative manner, to X-form.

**DNA CONDENSATION**

DNA containing natural bases does not absorb light above 300 nm. Detection of a signal above 300 nm indicates that the DNA is condensed into particles that scatter light. This region of the spectrum must be monitored, as when this type of signal is observed, the CD changes can no longer be interpreted in terms of changes in DNA secondary structure. However, species of regularly packed, condensed DNA can be studied by CD (74,75). Infrared CD spectroscopy in combination with atomic force microscopy has proven very powerful for these analyses (76). The associates are called psi-forms or DNA wires (77,78). The psi-forms are chromosome analogues with a regular tertiary folding. These forms are characterized by either positive or negative CD signals with extremely large amplitudes (Figure 8) that are caused by chiral condensates possessing large-scale helicity. The condensation can be caused by a number of factors like high ionic strength polyethylene glycol (PEG) or other alcohols or basic proteins. These factors induce formation of short non-B (depending on nucleotide sequence) DNA segments that propagate as a perturbation along the DNA double helix and result in cooperative condensation. Condensation and decondensation processes may take place in vivo due to DNA-binding proteins such as histones and protamines, which control and modulate ionic strength and hydrophobic environment in the close vicinity of DNA molecules.

Guanine-rich DNA molecules are known to self-associate and form ordered multi-stranded arrangements, which are useful in supramolecular chemistry and nanotechnology (79). Combinations of DNA sequences that...
can isomerize between distinct arrangements represent one of the most promising nanomaterials (78). CD spectroscopy distinguishes ordered condensation from chaotic aggregation. The aggregates also scatter light but the signals in the region of DNA base chromophores absorption are low.

RELATIONSHIPS BETWEEN SOLUTION AND CRYSTAL CONFORMATIONS OF DNA

Conformations of DNA in aqueous solutions and crystals differ. CD spectroscopy contributed to the elucidation of the relationship between DNA conformations in these two environments. It was shown that the DNA structures observed in crystals are adopted in aqueous ethanol or trifluorethanol solutions (Figure 9) (30). This observation has been made for DNA duplexes and also guanine quadruplexes (44,46,80).

DISCUSSION

The purpose of this short review was to summarize the characteristics of CD spectra of important conformations of DNA and to demonstrate that CD spectroscopy is an extremely useful method to study the wide range of DNA conformational properties. CD is complementary to NMR and X-ray crystallographic studies as it can reveal aspects inaccessible to these absolute methods: unlike the latter methods, CD can be used to study long DNA molecules, which DNA, in reality, is. It can be used to efficiently and rapidly evaluate the effect of changing environment on DNA behaviour and to follow conformational transitions. DNA isomerizations between various conformers and the conditions controlling these isomerizations are important as they probably serve as the basis for regulation of gene expressions (3).

CD spectroscopy is a powerful method for studying DNA-conformational properties but the structural conclusions must be extracted with caution. For an accurate interpretation of spectral changes, it is important to trace the whole spectral region. Following only a single band (e.g. the band at 260 nm) and ignoring the remaining regions of the spectrum can lead to incorrect conclusions. One must study CD changes induced by solution
conditions (temperature, pH, salts) that give rise to particular DNA arrangement rather than make structural interpretations based on a single spectrum taken under one condition. Following the course of appearance and denaturation of a structure aids in its characterization. These thorough CD spectroscopy procedures have been critical in discover of important properties of DNA. Historically, CD has been a pioneering method in the study of novel DNA structures. For example, CD spectroscopy was used to show that various arrangements found in DNA fibres (B, C, D, T) all belong into the wide B-family of structures (13). Left-handed Z-DNA was detected by CD spectroscopy (33) almost a decade before it was identified by X-ray crystallography (81). CD spectroscopy was used to find the unusual X-form (63) whose molecular structure is still a matter of debate. The method is useful for mapping and analysis of anomalous arrangements in selected regions of genomic DNA known to be important from a biological or medical point of view. CD, and other techniques for structural analysis reveal secondary and tertiary DNA structures in the linear primary genomic sequence; these structures are proving to be critical to the normal and pathological functioning of genomic DNA.

Many recent structure–function DNA studies have been devoted to quadruplexes. Particular genomic regions, especially in gene promoters, have a potential to form quadruplexes (82,83), which are believed to form in vivo and affect cellular processes (79). CD spectroscopy is one of the most suitable methods to study quadruplex formation in various DNA motifs. Quadruplex formation in telomeric DNA is thought to be related to senescence and cancer. Thus, the telomeric G-quadruplex is an attractive target for cancer therapy (84–86). An understanding of the quadruplex structure and the ability to monitor formation or dissociation of this structure is important for the rational drug design. In this respect, CD spectroscopy has contributed significantly (44,45,87–91) to demonstration of the polymorphism (85,92–95) of the human telomere DNA structure (Figure 9). CD spectroscopy was one of the first methods used to show (44) that the parallel quadruplex of the human telomere DNA fragments observed in crystals (93) is not formed in vitro in K+ containing aqueous solution. CD was employed to demonstrate that the topology of the quadruplex folding depends on the number of human telomere DNA repeats and proposed the so-called (3 + 1) quadruplex structure (44) that was subsequently demonstrated by NMR (94,95). CD spectroscopy also provided the basis for the suggestion that a long telomeric sequence has a structure of beads on a string, like nucleosomes (44). This concept, which may contribute to the explanation of the gradual shortening of telomeric DNA, has generally been accepted (86,89,96) though it has not yet been confirmed by another method.

Last but not least, an important task of CD spectroscopy is the search for specific ligands that recognize DNA sequences and structures with high selectivity (97). These compounds may be used to target and regulate functional elements important in gene expression, including viral genes and oncogenes. The ability to selectively modulate the activity of genes is a long-standing goal in molecular medicine.

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