

## Graphical Analysis of Circular Dichroic Spectra Distinguishes between Two-State and Gradual Alterations in DNA Conformation

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**Abstract.** A simple graphical method is proposed to distinguish between two-state and gradual alterations in DNA conformation from circular dichroic spectra. The method is advantageous particularly to identify two-state conformational isomerizations which exhibit low degree of cooperativity. The usefulness of the method is illustrated on the examples of two synthetic DNA molecules which isomerize due to temperature changes or changes in salt concentration. The method can be used to characterize any phenomenon accompanied by alterations in optical activity.

**Key words:** Circular dichroism — DNA — Conformational alterations — Graphical analysis

### Introduction

Three basic types of double helix have so far been observed in single crystals of short DNA fragments (for review, see Dickerson et al. 1982). They were designated A, B and Z. The double helices are subject to structural constraints which are best characterized by the torsion angles  $\delta$  and  $\chi$  specifying sugar puckering and glycosidic orientation, respectively (Dickerson et al. 1982). The plots of  $\chi$  as a function of  $\delta$ , analogous to Ramachandran plots for proteins, show that only limited regions in the conformational space are accessible to the known double helices. The accessible regions are separated from each other so that DNA conformational isomerizations from one type of the double helix into another are associated with overcoming an energy barrier. The barrier carries cooperativity in the isomerization, i.e. DNA isomerizes from one conformation into another block after block. On the other hand, all the basic forms of DNA double helix can gradually change their conformations within global energetical minima through small modifications in the sugar-phosphate chain geometry and glycosidic torsion angles. In contrast to the two-state conformational isomerizations DNA exists in only one thermodynamically stable conformation in the course of gradual alterations.

Two-state and gradual changes in DNA structure have different physical properties and, most probably, a different biological relevance. Hence, it is desirable to distinguish between them experimentally. In the present paper we shall show that circular dichroic (CD) spectra can simply be used for this purpose. This work was actually motivated by the observation that CsF induced very dramatic changes in the long wavelength region of the CD spectrum of poly(dA-dT) . poly(dA-dT) (Vorlíčková et al. 1980). However we were not able to establish whether the polynucleotide undergoes a two-state process as the changes occurred over a wide range of CsF concentrations. The dependence of  $\Delta\epsilon$  (275 nm) on CsF concentration, which best reflected the changes, was far from being clearly S-shaped as it is the case with cooperative two-state isomerizations. Neither isoelliptic points, which also accompany two-state isomerizations, could be unambiguously determined because the spectrum changed little in the short wavelength region. It was only the graphical method outlined in this paper that revealed what happened with the polynucleotide. We found that at lower CsF concentrations poly(dA-dT) . poly(dA-dT) changed its conformation gradually, and that the polynucleotide only isomerized from one type of the double helix into another at CsF concentrations higher than 3 mol/l (Vorlíčková et al. 1983). The resulting double helix was termed X because its spectroscopic properties were qualitatively different from those of the known double helices A, B and Z. In this paper we present principles of the graphical method and show its applications with other synthetic polynucleotides.

## Method

DNA circular dichroism  $\Delta\epsilon_i(E)$  depends on external factors  $E$  and wavelength  $\lambda$ . An inspection of a set of spectra obtained at different values of  $E$  by no means generally indicates whether the external factor induces two-state or gradual changes in DNA conformation. An isodichroic point is usually searched for since its presence suggests a linear dependence of the spectra. However, the experimental error often makes the determination of such a point unreliable, regardless of the possibility that the spectra can be linearly dependent but need not intersect in the spectral region monitored. Yet, a linear dependence of the spectra can simply be revealed by an appropriate graphical representation. First, the wavelength  $\lambda_0$  is chosen, best at a point where circular dichroism  $\Delta\epsilon_{\lambda_0}(E)$  exhibits the largest monotonous change, and the spectra are digitized at a number of arbitrary wavelengths  $\lambda_j$ . All the digitized spectra are then plotted as dependences of  $\Delta\epsilon_{\lambda_j}(E)$  on  $\Delta\epsilon_{\lambda_0}(E)$  for various values of  $E$ . Such a diagram allows to specify at the first sight whether DNA undergoes a two-state or gradual conformational change.

Let us consider a two-state conformational isomerization of DNA induced by an external factor  $E$ . The two limiting conformations involved have different circular dichroic spectra and are labeled  $I$  and  $F$ . Variations in  $E$  result in changes in the population of the conformers  $I$  and  $F$ . The circular dichroic spectra observed are then a weighted average of the spectra of the limiting conformations, i.e.

$$\Delta\epsilon_{\lambda_j}(E) = [1 - \theta(E)] \Delta\epsilon_{\lambda_j}^I + \theta(E) \Delta\epsilon_{\lambda_j}^F, \quad (1)$$

where  $\theta(E)$  is the relative population of the conformer F. As a matter of fact, equation (1) also holds for  $\lambda_j = \lambda_o$ , so that

$$\Delta\epsilon_{\lambda_o}(E) = [1 - \theta(E)] \Delta\epsilon_{\lambda_o}^I + \theta(E) \Delta\epsilon_{\lambda_o}^F \quad (2)$$

Elimination of  $\theta(E)$  from (1) and (2) gives the equation

$$\Delta\epsilon_{\lambda_j}(E) = \alpha_{j_o} \Delta\epsilon_{\lambda_o}(E) + \beta_{j_o}, \quad (3)$$

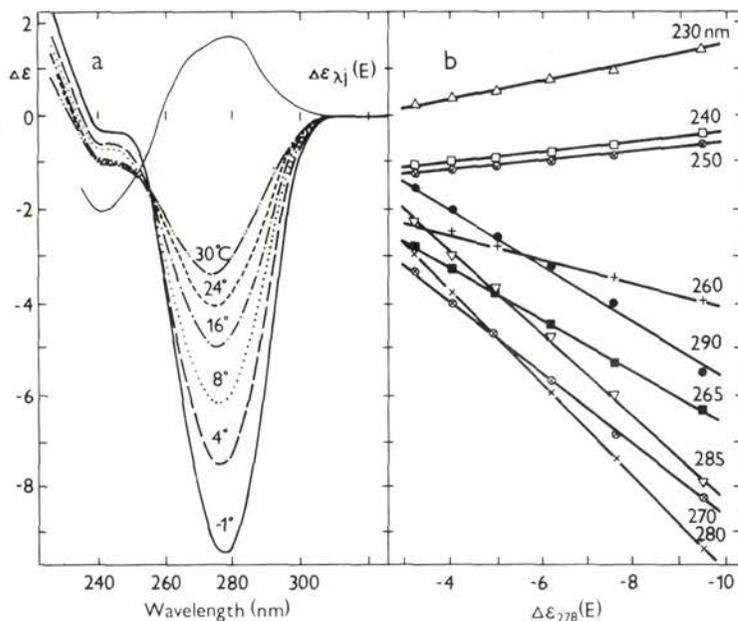
where  $\alpha_{j_o}$  and  $\beta_{j_o}$  are numbers of magnitudes depending on the ellipticities of the limiting conformations at the particular wavelengths  $\lambda_j$  and  $\lambda_o$ . Equation (3) means that the dependence of  $\Delta\epsilon_{\lambda_j}(E)$  on  $\Delta\epsilon_{\lambda_o}(E)$  for various values of  $E$  are linear for any wavelength  $\lambda_j$ , provided the external factor  $E$  induces a two-state conformational isomerization in DNA. On the other hand, linearity of the dependences at any wavelength is excluded if the conformation changes gradually, since circular dichroism of DNA and its relationship with wavelength both depend on the interactions of transition moments of bases in the double helix in a complex, non-linear manner (Charney 1979). Stereochemistry of gradual changes in the DNA double helix is another complicating factor which does not allow simplifying of the correlation between changes in DNA conformation and DNA circular dichroism to a linear relationship.

## Results

In this paragraph we will show on two examples how the graphical analysis of circular dichroic spectra can be performed in practice. The examples concern conformational transitions of DNA with a low degree of cooperativity. We will make use of this method to establish whether polynucleotides other than poly(dA-dT) . poly(dA-dT) undergo isomerization into double helix X. Prior to employing the method to analyse spectra which reflect this yet not fully understood phenomenon, we checked the correctness and reliability of the method on transitions B-A and B-Z, prototypes of two-state conformational phenomena occurring in DNA (for review, see Vorlíčková and Kypr 1985b). As expected, we obtained linear relationships at any wavelength in both cases (not shown).

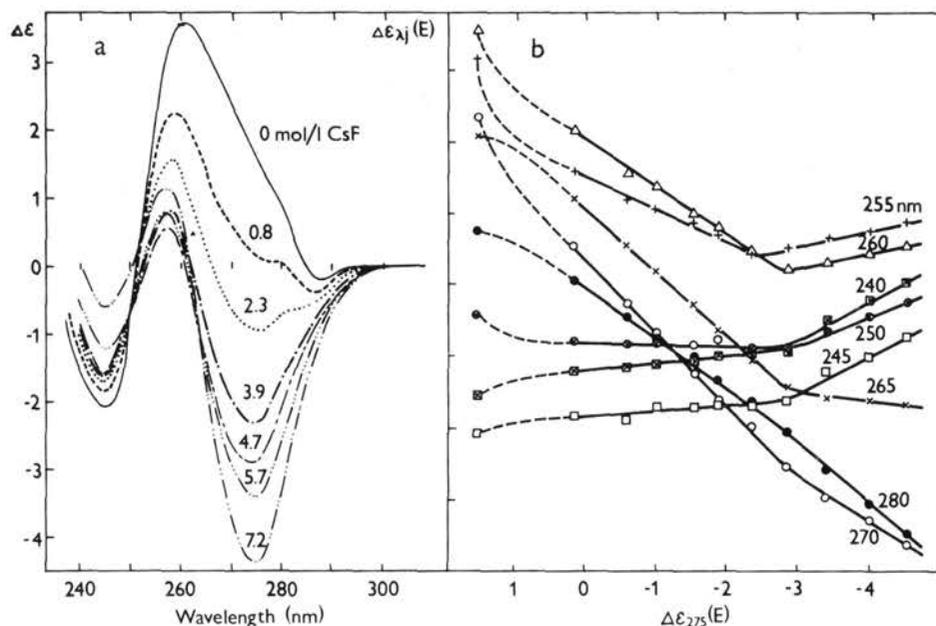
Poly(dA-dC) . poly(dG-dT) shows similar changes in circular dichroism at high CsF concentrations as does poly(dA-dT) . poly(dA-dT). However, the spectra of the former polynucleotide do not intersect at the isoelliptic point (Vorlíčková et al. 1982). Unlike poly(dA-dT) . poly(dA-dT) (Vorlíčková et al. 1983), however, decreasing temperature results in a deepening of the long wavelength CD band of poly(dA-dC) . poly(dG-dT), while the short wavelength band becomes more shallow, so that the polynucleotide spectra in concentrated CsF solution, taken at various temperatures, appear to have an isodichroic point (Fig. 1a). The graphical method does indicate that a two-state isomerization occurs because  $\Delta\epsilon_{\lambda_j}(E)$  is a linear function of  $\Delta\epsilon_{\lambda_{278}}(E)$  for any  $\lambda_j$  (Fig. 1b).

The second example is more complex and it also concerns our work aimed at a better characterization of the X form. The only difference between poly(dA-dU) . poly(dA-dU) and poly(dA-dT) . poly(dA-dT) concerns the absence of the methyl group in position 5 of the pyrimidine base of the former



**Fig. 1.** (a) CD spectra of poly(dA-dC) . poly(dG-dT) in 4.2 mol/l CsF, measured at the temperatures as indicated. (b) Graphical analysis of the spectra. The thin curve in (a) represents the spectrum of poly(dA-dC) . poly(dG-dT) in 0.05 mol/l sodium phosphate, pH 7 at 28 °C.

polynucleotide. This apparently subtle difference has significant conformational consequences (Vorlíčková and Kypr 1984; Vorlíčková and Kypr 1985a; Kypr and Vorlíčková 1985c). We observed dramatic changes in the circular dichroism of poly(dA-dU) . poly(dA-dU) in CsF solutions (Fig. 2a); however interpretation of the changes directly from the spectra was not clear. The graphical analysis (Fig. 2b) suggested that the CsF-induced changes in the conformation of poly(dA-dU) . poly(dA-dU) consisted of two consecutive two-state processes because the dependences of  $\Delta\epsilon_{\lambda_j}(E)$  on  $\Delta\epsilon_{275}(E)$  were linear at all wavelengths  $\lambda_j$ , and all of them had breaks at  $\Delta\epsilon_{275}(E) = -2.7$  corresponding to a concentration of CsF of 4.7 mol/l (Fig. 2b). Thus, the graphical method allows a discrimination between the two processes, and it defines their limits. Also, it is obvious that the spectrum obtained in the absence of CsF did not fit the linear relationship and consequently it does not represent the initial conformer which only appears in the solution after the first addition of CsF. The final conformer in the first isomerization is simultaneously the initial one in the second isomerization. The second isomerization may not be completed within the accessible range of CsF concentrations since the dependences of  $\Delta\epsilon_{\lambda_j}(E)$  on  $\Delta\epsilon_{275}(E)$  did not show any tendency to deviate from the linear course, even at the highest CsF concentrations examined



**Fig. 2.** (a) CD spectra of poly(dA-dU) . poly(dA-dU) in 0.01 mol/l sodium acetate, pH 7 and at CsF concentrations as indicated. (b) Graphical analysis of the spectra.

(Fig. 2b). The implications of the peculiar conformational variability of poly(dA-dU) . poly(dA-dU) in CsF solutions have been discussed elsewhere (Vorlíčková and Kyr 1984).

The graphical analysis is also helpful in determining isodichroic points which often accompany two-state conformational isomerizations. Isodichroic points are intersections of spectra of the limiting conformers so that spectra of their conformational blends obtained in the course of their transition should also pass through them. The independence of  $\Delta\epsilon_{\lambda_j}(E)$  of the inducing factor  $E$  is reflected by horizontal lines in relationships of the type shown (Fig. 2b). In practice, the position of isodichroic points is marked by wavelengths at which the slopes of the dependences of  $\Delta\epsilon_{\lambda_j}(E)$  on  $\Delta\epsilon_{\lambda_0}(E)$  change their sign. This occurs between 245 nm and 250 nm and between 260 nm and 265 nm with the first and second conformational isomerization of poly(dA-dU) . poly(dA-dU), respectively (Fig. 2a). The exact positions of the respective isodichroic points are at 249 nm and 264 nm. This information could have hardly been derived directly from Fig. 2a.

The spectra of the limiting conformers may occasionally not intersect in the spectral region monitored, and the absence of an isodichroic point is thus insufficient to suggest that the changes observed do not reflect a two-state

conformational isomerization. Even in such a case it is the graphical analysis that provides a quite reliable information. We encountered such a situation in studying poly(dI-dC) . poly(dI-dC) (Vorlíčková and Kypr 1985a).

## Discussion

Two-state conformational isomerizations are of great importance in biology. Enzymes or proteins regulating the activity of genes can serve as typical examples of complex biomacromolecules which are in the "active" or "inactive" state in dependence on their conformation. The biological role of conformational isomerizations of DNA has so far been a subject of speculations only. A classical example of conformational isomerization of DNA is the B-A transition which can conveniently be monitored by CD (Ivanov et al. 1974). Recently much attention has been paid to B-Z isomerization as it involves an alteration in the sense of the double helix winding (Pohl and Jovin 1972; Wang et al. 1979). Both the B-A and B-Z transitions are highly cooperative and their two-state nature is thus immediately obvious from the S-shaped nature of the dependence of ellipticity at a selected wavelength on the concentration of the inducing agent.

It nevertheless appears that DNA is also capable of undergoing two-state conformational isomerizations with a low degree of cooperativity. The isomerization of poly(dA-dT) . poly(dA-dT) into the double helix X is an example (Vorlíčková et al. 1983). Similar isomerizations have also been observed with other alternating purine-pyrimidine DNAs in which GC base-pairs did not dominate (Kypr and Vorlíčková 1985b; Vorlíčková and Kypr 1985a). In addition, recent data indicate that double-stranded RNA (Preisler et al. 1984) and DNA (Vorlíčková and Kypr, unpublished data) molecules undergo two-state isomerizations, though not between as different conformations as in high-salt solutions, even under conditions close to physiological. Double helices of nucleic acids thus seem to exist in a number of discrete conformational states which are more or less populated in dependence on the base sequence and environment.

The idea of digitizing CD spectra of DNA and treating them as collections of vectors is not new (Marck et al. 1978 and references therein). Previous methods, however, qualitatively differed from our method as for the determination of the number of independent spectra or vectors. Marck et al. (1978) used the Schmidt orthogonalization algorithm for this purpose, which requires the use of a computer and is difficult to employ when the experimental error of the measurements is taken into account. By contrast, our method works without computer and inherently considers the experimental error.

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