Structure of Z-DNA in Solution. A Flow Linear Dichroism Study

Magdalena Eriksson,[a] Bengt Nordén,*[a] Per-Olof Lycksell,[b] Astrid Gräslund,[b] and Bengt Jernström[c]

[a] *Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, Sweden*
[b] *Department of Biophysics, University of Stockholm, Arrhenius Laboratory, S-106 91 Stockholm, Sweden*
[c] *Department of Toxicology, Karolinska Institutet, S-104 01 Stockholm, Sweden*

U.v. flow linear dichroism (L.D.) of the high-salt form of duplex poly(dG-dC) ('Z-DNA') shows that the reduced dichroism of Z-DNA is about twice as large as for B-DNA and the spectrum has the shape expected for Watson–Crick base-pairs oriented perpendicular to the helix axis; spectral features observed during the B→Z conversion are in qualitative agreement with a mechanism in which the base-pairs flip 180° around the glycosyl bonds.

Sequence effects on conformation and dynamics in DNA are important to study owing to their potential relations to gene regulation. Particularly, the extreme conformations adopted by certain alternating purine–pyrimidine sequences have attracted great interest.

Pohl and Jovin discovered about 15 years ago that the c.d. spectrum of poly(dG-dC) inverts in high salt solution.[1] Recent *X*-ray studies have confirmed the existence of left-hand structures for several $d(C\text{-}G)_n$ oligomers in the crystal: Z-DNA.[2—5] The high salt conformation in solution is most likely identical with Z-DNA as evidenced from several physical studies, including *X*-ray fibre diffraction,[6] n.m.r.,[7] transient electric dichroism,[8] and Raman spectroscopy.[9] However, although the structure of Z-DNA has been established in oligonucleotide crystals, the structure in solution as well as the mechanistic details of the B→Z conversion remain essentially unexplored.

We are engaged in anisotropy and chiroptical studies of nucleic acids to obtain information about their structures and interactions in solution.[10—12] We have here measured the flow linear dichroism (L.D.), defined at any wavelength by equation (1), where $A_{\parallel}$ and $A_{\perp}$ denote the absorbances of the

$$\text{L.D.} = A_{\parallel} - A_{\perp} \quad (1)$$

solution measured with light polarized parallel with and perpendicular to the direction of flow respectively.[13] A convenient, closely related quantity which we shall need for the interpretation is the reduced dichroism defined by $\text{L.D.}^{r} = \text{L.D.}/A$ where A is the absorbance of the isotropic solution.

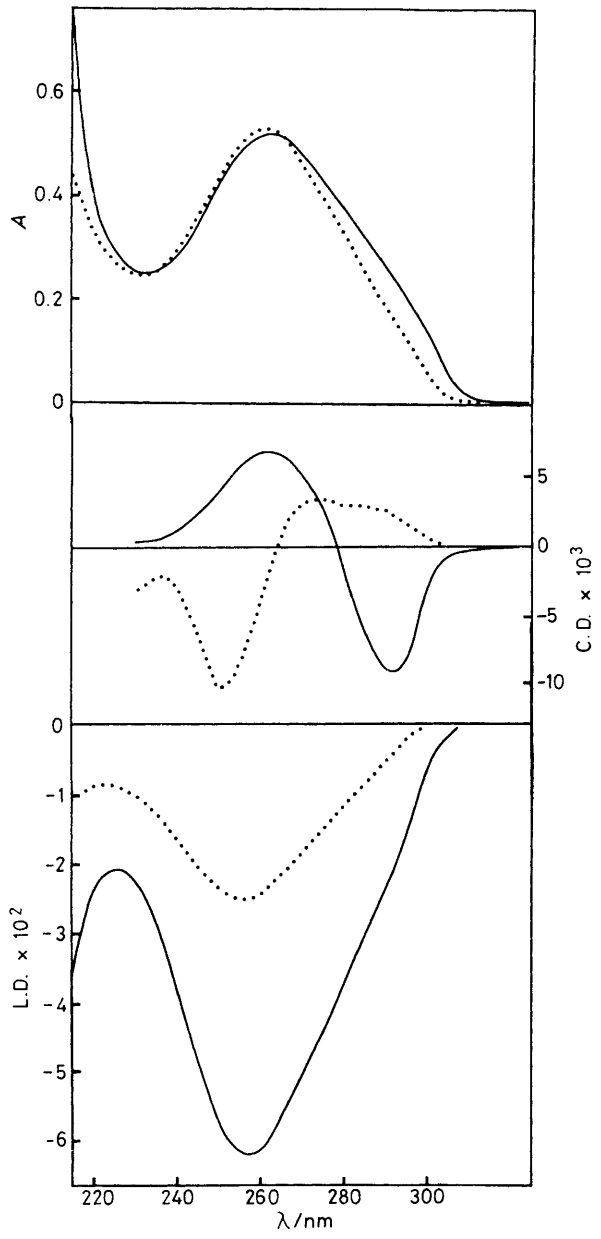

Figure 1. Absorbance (A), circular dichroism (C.D.), and flow linear dichroism (L.D.) spectra of 0.12 mM phosphate of poly-(dG-dC) · poly(dG-dC) (900 base-pairs, P.L. Biochemicals) in Z form: 2.7 M NaCl, 10 mM cacodylate buffer (——), and in B form: 10 mM cacodylate buffer (· · · · ·), at pH 7.0, 23 °C. A, C.D., and L.D. are all expressed in absorbance units and normalized to 1 cm optical path-length.

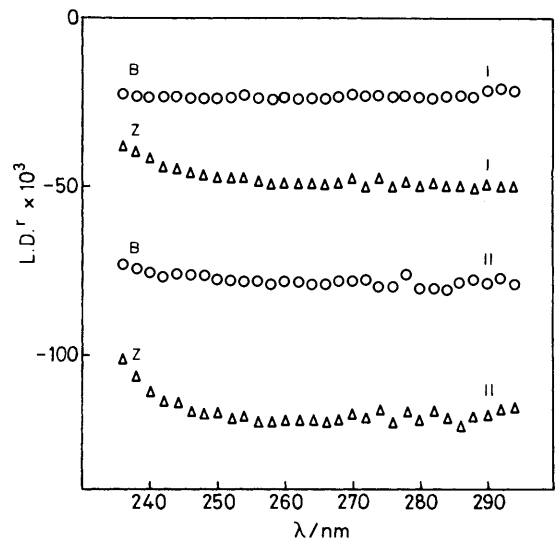

Figure 2. Reduced linear dichroism (L.D.^{r}) spectra of poly-(dG-dC) · poly(dG-dC) in B-form (○) and Z-form (△) at two flow gradients: 900 s^{-1} (I) and 6100 s^{-1} (II).

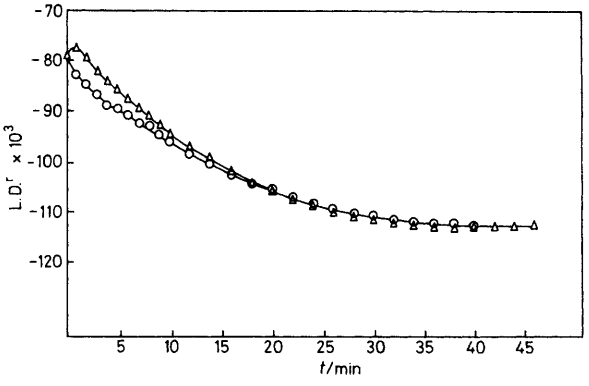

Figure 3. Kinetics of the reduced linear dichroism at 255 mm (○) and at 287 nm (△) during the high-salt induced B→Z transition of poly(dG-dC) · poly(dG-dC).

L.D.^{r} is equal to the product of an orientation factor S and an optical anisotropy factor defined by the curly bracket in equation (2)

$$\text{L.D.}^r = 3/2\, S \times \{3(-\sin\delta\sin\theta_X + \cos\delta\cos\theta_X\sin\theta_Y)^2 - 1\} \quad (2)$$

for the case of DNA,[11] where δ is an angle, in the plane of a DNA base, defining the direction of the light-absorbing π^*–π transition moment. θ_X and θ_Y are angles defining the tilt and roll of the base (or base-pair) away from its orientation perpendicular to the helix axis (tilt defined as rotation around the dyad axis, X, as a pivot axis, and roll around the orthogonal axis, Y, as a pivot axis, the Z axis being the helix axis).[11] Because the u.v. absorption of the DNA bases is due to several transitions (in G-C at least four between 220 and 300 nm), with different δ values,[11] L.D.^{r} will vary with the wavelength as soon as the bases tilt or roll. A constant L.D.^{r} is thus consistent with θ_X and θ_Y close to zero (as in the classical Watson–Crick B-DNA model) but can also arise from isotropic local mobility (*i.e.* absence of preferred pivot axes).

Figures 1 and 2 show absorbance, circular dichroism (C.D.), and flow L.D. spectra of poly(dG-dC) at low and high salt, which by the C.D. can be identified with the B and Z forms of DNA, respectively. The negative L.D. of Z-DNA, and a constant reduced dichroism L.D.^{r} (see Figure 2), are consistent with a conformation where the DNA bases are stacked in a Watson–Crick manner with their planes perpendicular to the helix axis. L.D.^{r} is essentially constant over the wavelength region 240—290 nm, even at high flow gradients showing that the local conformation is not measurably perturbed by the shear forces. The L.D.^{r} amplitude of the Z form is about twice as large as that of the B form, indicating a

better overall orientation (larger S) or on average more perpendicular orientation of the base planes.

L.D.^{r} *vs.* shear plots on short DNA (~500 base-pairs) were correlated against the Peterlin–Stuart theory for hydrodynamic orientation of rigid rods, yielding rotational diffusion constants and optical anisotropy factors for the DNA.[14,15] Rod lengths obtained from the rotational diffusion constants through the Perrin–Broersma relation[16] were in fair agreement with the sample size and Z-DNA being ~10% longer than B-DNA. The optical factors were much smaller than expected for a rigid rod with perpendicular base-pairs. This suggests a considerable flexibility of the helix, probably in combination with a local mobility of the base-pairs. The observed constancy of L.D.^{r} with wavelength requires that the mobility is essentially isotropic.

L.D.^{r} measured as a function of time during the salt-induced B→Z conversion contains some evidence for a deviation from the more perpendicular base-plane orientations of the equilibrium conformations. Figure 3 shows how L.D.^{r} at 287 and 255 nm changes differently with time, with a significant variation of the ratio $L.D._{287}^r/L.D._{255}^r$, implying that L.D.^{r} of DNA during the conversion is no longer wavelength-independent. Qualitatively, the variation points to a less negative L.D.^{r} at 287 compared to 255 nm. This behaviour is, for example, opposite to the wavelength dependence observed in the L.D.^{r} spectrum of the A form DNA,[12] in which the base-pairs have a tilt by some 20° in θ_X. The present results suggest that we have instead a roll in θ_Y during the conversion. This can also be realized by insertion of the relevant δ values for the transitions of guanine and cytosine[11] into equation (2).

The L.D.^{r} variation observed during the conversion is thus in qualitative agreement with what would be expected if base-pairs are turned around a pivot axis approximately parallel to the hydrogen bonds. A rolling around this axis is identical with the base-pair flipping, which from topological arguments has been proposed as an essential mechanistic step of the B→Z conversion.[17]

The further quantitative extension of these results is reserved for a subsequent paper. We stress two points now. First, the flow linear dichroism spectrum of high-salt poly(dG-dC) in solution, here measured for the first time, displays a sign, a magnitude, and a shape which are consistent with the length and base-pair orientation of Z-DNA as determined in the crystal. Secondly, the L.D.^{r} variation during the process of B–Z interconversion can indicate that a fraction of the base-pairs are in a state of rolling around a pivot axis parallel to the hydrogen bonds. The latter observation may suggest L.D. as a tool for further exploration of the mechanism of conformational interconversion.

This study was supported by grants from the Swedish Natural Science Research Council and Magn. Bergwalls Stiftelse. We thank Professor Anders Ehrenberg for stimulating discussions.

Received, 14th May 1985; Com. 662

References

1 F. Pohl and T. Jovin, *J. Mol. Biol.*, 1972, **67**, 375.
2 A. Wang, G. Quigley, F. Kolpak, J. Crawford, J. H. van Boom, G. van der Marel, and R. Rich, *Nature (London)*, 1979, **282**, 680.
3 H. R. Drew, T. Takamo, S. Tanaka, A. Itakura, and R. E. Dickerson, *Nature (London)*, 1980, **286**, 567.
4 A. H. Wang, G. J. Quigley, F. J. Kolpak, G. van der Marel, J. H. van Boom, and A. Rich, *Science*, 1981, **211**, 171.
5 R. Wing, H. Drew, T. Takano, C. Broka, S. Tanaka, K. Itakura, and R. Dickerson, *Nature (London)*, 1980, **287**, 755.
6 M. Behe, S. Zimmerman, and G. Felsenfeld, *Nature (London)*, 1981, **293**, 233.
7 D. J. Patel, S. Q. Kozlowski, A. Nordheim, and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 1413.
8 H. M. Wu, N. Dattagupta, and D. M. Crothers, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 6808.
9 T. J. Thamann, R. C. Lord, A. H. J. Wang, and A. Rich, *Nucleic Acids Res.*, 1981, **9**, 5443.
10 B. Nordén and F. Tjerneld, *Biopolymers*, 1982, **21**, 1713.
11 Y. Matsuoka and B. Nordén, *Biopolymers*, 1982, **21**, 2433.
12 Y. Matsuoka and B. Nordén, *Biopolymers*, 1982, **22**, 1731.
13 For a review on L.D. applications see for example B. Nordén, *Appl. Spectrosc. Rev.*, 1978, **14**, 157.
14 A. Peterlin and H. A. Stuart, *Z. Physik*, 1939, **112**, 129.
15 A. Wada, *Appl. Spectrosc. Rev.*, 1973, 1.
16 S. Broersma, *J. Chem. Phys.*, 1960, **32**, 1626.
17 S. C. Harvey, *Nucleic Acids Res.*, 1983, **11**, 4867.