# Condensed Form of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ as an Intermediate between the B- and Z-Type Conformations Induced by Sodium Acetate†

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ABSTRACT: Circular dichroism and laser Raman spectroscopy reveal that the synthetic DNA polymer $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ undergoes a cooperative transition induced by sodium acetate from a right-handed B-form to a left-handed Z-type conformation with a midpoint at 2.05 M. However, at concentrations only slightly higher than the end point of this transition (above approximately 2.2 M) and up to approximately 2.65 M, the Z-form is not stable in solution but aggregates to form highly condensed DNA. A manyfold increase of positive ellipticity in the range 340–250 nm is observed which is indicative of a $\psi(+)$-type structure. At even higher concentrations ($\geq$2.7 M), the Z-form is stable without condensation, and there is no change in the inverted CD spectrum. All structural transitions are reversible except that it is not possible to redissolve the highly condensed $\psi(+)$-form by further increasing the salt concentration to $\geq$2.7 M. The very high cooperativity of these transitions enables the DNA polymer to adopt three distinctly different structures (B-, Z-, and $\psi$-forms) within a narrow range of sodium acetate concentration (approximately 200 mM). The Raman spectra of the condensed form and the Z-form in very concentrated sodium acetate show that the $\psi(+)$-type state forms without substantial changes of the secondary conformation of the DNA. This indicates that the left-handed Z-helix of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ can form $\psi$-type aggregates with an ordered superstructure similar to those observed for natural right-handed DNA helices.

A variety of studies have demonstrated that $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ has unusual properties [reviewed in Wells et al. (1977) and Zimmerman (1982)] including physicochemical studies (Wells et al., 1970; Pohl & Jovin, 1972; Grant et al., 1972), its capacity to serve as a template for DNA synthesis (Grant et al., 1972), and its tendency to suffer deletions when part of a recombinant plasmid (Klysik et al., 1982, 1981). X-ray analysis of the crystallized hexamer duplex $(dC\text{-}dG)_3$ (Wang et al., 1979) and tetramer duplex $(dC\text{-}dG)_2$ (Drew et al., 1980) revealed the existence of an unusual helix conformation, the left-handed Z-DNA. Raman spectroscopic investigations on these crystals and on the polymer $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in high-salt solution (Thamann et al., 1981) showed that the crystals were, in fact, in the "high-salt" geometry as characterized by CD spectroscopy (Pohl & Jovin, 1972; Pohl, 1976; Klysik et al., 1981). This polymer undergoes a salt-induced cooperative transition from a right-handed to a left-handed helix with an inversion of the CD spectrum (Pohl & Jovin, 1972; Pohl, 1976; Klysik et al., 1981).

Several types of left-handed helices have been recognized, depending on the kind of salt and/or solvent which stabilizes the structures (Zacharias et al., 1982; van de Sande & Jovin, 1982) and on the hydration environment of the crystals (Drew et al., 1980; Wang et al., 1981). We wished to determine which anion–cation combinations are able to induce the B to Z transition and which of the chemical or physical properties of these ion pairs are responsible for any conformational differences within the family of Z structures. Therefore, we performed a systematic CD investigation, supported by laser Raman experiments, to determine the conformational changes of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in solutions with increasing amounts of inorganic monovalent salts. During this study, it became apparent that the effect of sodium acetate on the structure of this polymer was more complex than the effect of all other salts tested. This paper describes structural and kinetic properties of this system.

## Materials and Methods

*Chemicals.* All chemicals were reagent grade from Baker or Sigma.

*DNAs.* $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$, $(dT\text{-}dG)_n\cdot(dC\text{-}dA)_n$, $(dA\text{-}dT)_n$, and $(dG)_n\cdot(dC)_n$ were prepared enzymatically and characterized as described (Wells et al., 1970). *Eco*RI-linearized pVH51 plasmid DNA (Hardies et al., 1979) and $dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ (Wells et al., 1970) were prepared as described (gifts from R. G. Brennan and D. Kellogg, respectively, both of the Department of Biochemistry, University of Wisconsin at Madison).

*CD and UV Spectroscopy.* CD spectra were recorded at room temperature with a Jasco J500A spectropolarimeter which was routinely calibrated with a 0.1% solution of *d*-10-camphorsulfonic acid in water. The molar ellipticity ($\Delta E$) was calculated from the measured ellipticity $\theta$, determined in quartz cuvettes with a light path of 1 cm, according to the equation

$$\Delta E = \theta\epsilon/(33A)$$

with $\epsilon$ = molar extinction coefficient and $A$ = maximum UV absorbance. UV absorption spectra were recorded with a Varian 2300 spectrophotometer with diluted aliquots of DNA stock solutions to determine the concentration of the DNA. The values for $\epsilon$ [$\times 10^{-3}$; taken from Wells et al. (1970) and Hillen et al. (1981)] were the following: $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$, 7.1; $(dG)_n\cdot(dC)_n$, 7.4; $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$, 6.9; $(dA\text{-}dT)_n\cdot(dA\text{-}dT)_n$, 6.8; $(dT\text{-}dG)_n\cdot(dC\text{-}dA)_n$, 6.5; pVH51 DNA, 6.45.

All samples were prepared by adding an aliquot of DNA stock solution into buffer containing 10 mM sodium cacodylate, pH 7.4, to give a final absorbance between 0.7 and 0.9. The necessary amounts of sodium acetate were added with a stock solution of 4.0 M in 10 mM sodium cacodylate, pH 7.4. Final end-point CD spectra were recorded with fully equilibrated samples that did not show any changes of $\theta$ with time at selected wavelengths (292 or 275 nm). A slow scanning mode was applied with a time constant of 1 s, scanning speed of 5 nm/cm, and chart speed of 2 cm/min. To determine the

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half-times for completion ($t_{1/2}$) of a transition, the monochromator was set to 292.5 nm and the change of $\theta$ with time was recorded. The time required for addition of salt or dilution, mixing, and transferring into the cuvette was usually between 0.75 and 1.5 min. Examination of CD changes in the whole spectral region (340–240 nm) during the course of a transition were done in a fast scanning mode; the time constant was set to 0.25 s, and the scanning speed was 100 nm/min. Thus, one spectrum could be recorded in 1 min and repeated every other minute. The deviations between spectra determined in the fast and slow mode were around 5% or less.

To obtain CD spectra from the same highly concentrated samples used for Raman experiments, a small volume (10–15 $\mu$L) of these solutions was pressed between two cylindrical quartz plates (Spinco Model E ultracentrifuge windows) separated by a thin Teflon ring, and this set was placed into the cuvette holder of the Jasco instrument. Thus, the identical solution could be used for both CD and Raman studies, thereby avoiding any effects caused by dilution of the DNA in the concentrated samples used for Raman spectra. The spectra at both concentrations were identical.

*Raman Spectroscopy.* Raman spectra were obtained on a dual channel instrument controlled by a Tektronix 4052 computer. Samples were examined in a spinning cylindrical quartz cell with a diameter of 1.8 cm. A partition divided the cell into two compartments with usable volumes of 125–250 $\mu$L each. Together with synchronized gating electronics, this allowed spectra from two samples to be recorded simultaneously under virtually identical conditions. Frequency shifts of less than 0.1 $cm^{-1}$ have been successfully observed when similar instrumentation was used (Shelnutt et al., 1979; Laane & Kiefer, 1980). A Spex 1401 double monochromator was employed with an instrumental slit width of 3 $cm^{-1}$. Excitation was provided by a Spectra Physics Model 171 argon ion laser operating at 514.5 nm. Power levels of approximately 350 mW at the sample were used. Scattered light was detected by a cooled RCA C31034-AO2 photomultiplier tube by using digital photon counting electronics. Multiple scans were made between 400 and 900 $cm^{-1}$ under computer control, and signal averaging was used to reduce noise levels. The DNA concentration was 5–6 mg/mL.

Raman spectroscopy is a vibrational probe which is sensitive to changes in primary and secondary structure (Martin et al., 1978). Studies comparing Raman spectra of samples in solution, solid form, and crystals generally show only small differences (Thamann et al., 1981). Thus, this method can reliably discriminate between various DNA conformational families even in the presence of aggregation. Experimentally, however, particles cause additional scattering (non-Raman) which make high signal to noise ratios very difficult to obtain. Extensive signal averaging has been used to partially compensate for this effect.

## Results

*Effect of Sodium Acetate on the Equilibrium Properties of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$.* $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ can adopt a variety of different left-handed conformations depending on the stabilizing agent (Wells et al., 1977; Zimmerman, 1982; Zacharias et al., 1982; Pohl, 1976; van de Sande & Jovin, 1982). This study was undertaken to determine the chemical property of the inducing agent which was responsible for these variations within the Z family. In the course of these investigations, sodium acetate in aqueous solution was tested for its ability to induce the B to Z transition in (dG-dC) sequences. It became obvious that this salt has a more complex effect on the conformation of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ than does NaCl. In addition, it seems to be unique among a large group of monovalent salts since the effect of sodium acetate on this DNA described below has been observed neither with other sodium salts (chloride, perchlorate, bromide, fluoride, and iodide) nor with acetates of other metals (lithium and potassium) (J. E. Larson, W. Zacharias, and R. D. Wells, unpublished results).

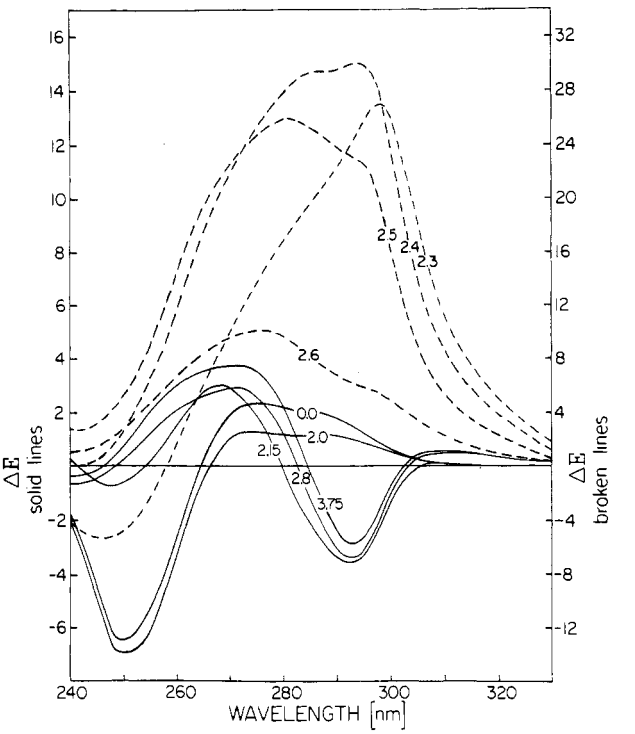



FIGURE 1: CD spectra of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in 10 mM sodium cacodylate, pH 7.5, containing various amounts of sodium acetate (M) as indicated. All curves were taken after complete equilibration of the samples at room temperature. For the samples at intermediate concentrations (dashed lines), different extents of aggregation, light scattering, and slow settling of the condensed DNA caused deviations of the amplitudes of up to 15–20%; however, the shape of these curves was reproducible.

Figure 1 shows three qualitatively different groups of CD spectra of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ obtained in aqueous sodium acetate solutions in the concentration range from 0 to 3.75 M. A normal B-type conservative spectrum is observed up to 2.0 M sodium acetate. The broad long-wavelength envelope gradually decreases in intensity down to approximately 60% of its initial value in buffer alone, whereas the short-wavelength band around 250 nm shows only slight changes ($\sim$10%) with increasing salt. The group of spectra shown for 2.15, 2.80, and 3.75 M sodium acetate possesses features typical for a left-handed helix of this polymer (Pohl & Jovin, 1972; Klysik et al., 1981; Zacharias et al., 1982), which are a conservative band pattern with a negative envelope around 292 nm, a stronger positive peak at 270–275 nm, and the disappearance of the strong negative peak of the B-form at 250 nm. Surprisingly, at intermediate concentrations (dashed lines in Figure 1; 2.3–2.6 M sodium acetate), a dramatic change of shape as well as intensity of the CD spectrum occurs. The ellipticity becomes positive over the entire wavelength range (except a negative band at the short-wavelength end of the spectrum in 2.3 M) and increases in intensity up to 15- or 20-fold relative to the B-form. This nonconservative type of spectrum is always accompanied by visible aggregation of the sample and slow settling of the aggregate. Therefore, the exact amplitudes of these spectra are not completely reliable, since they vary with time and from sample to sample by up to 20–25% due to the counteracting effects of continued con-

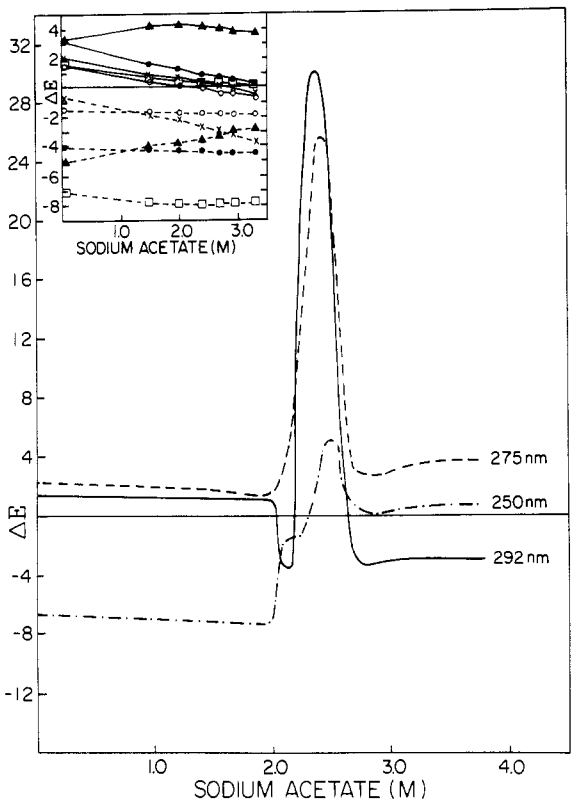



FIGURE 2: Molar ellipticity ($\Delta E$) of $(dG\text{-}dC)_n\cdot(dG\text{-}dG)_n$ for the 292-nm band (—), the 275-nm band (---), and the 250-nm band (-··) as a function of increasing sodium acetate concentration in 10 mM sodium cacodylate, pH 7.5. Each line is based on 12 points in the range ≥2.0 to ≤2.8 M, on 4 points at <2.0 M, and 8 points at >2.8 M sodium acetate. For each point, an individual sample was prepared and completely equilibrated before measurement. The insert shows the salt dependence for the 270-nm band (—) and the 250-nm band (---) of other DNA sequences for comparison: $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$ (×), $(dT\text{-}dG)_n\cdot(dC\text{-}dA)_n$ (○), $(dA\text{-}dT)_n\cdot(dA\text{-}dT)_n$ (■), $(dG)_n\cdot(dC)_n$ (▲), and *Eco*RI-linearized pVH51 (●).

densation and settling of the DNA. Yet the concentration dependence of the position of maximum ellipticity is obvious and reproducible; with increasing sodium acetate within this intermediate range, the maximum of $\Delta E$ shifts from 297 nm at 2.3 M to 276 nm at 2.6 M.

This delicately balanced dependence of $\Delta E$ on the sodium acetate concentration appears even more dramatic when $\Delta E$ at three selected wavelengths is plotted as a function of molarity of sodium acetate (Figure 2). The multiphasic nature of the conformational changes induced by this salt can be seen at each of the three wavelengths. The curves show a linear decrease of $\Delta E$ within the B family when going from 0 to 2.0 M, and there is also a slight salt dependence of the left-handed Z family above 2.7 M.

The region between 2.0 and 2.15 M can be described as a normal B to Z transition, with a sign inversion of $\Delta E$ at 292 nm, an increase at 275 nm, and a substantial loss of negative ellipticity at 250 nm. It is possible to construct a transition curve similar to the monophasic behavior of NaCl (Pohl & Jovin, 1972) by connecting the points of each line corresponding to 2.15 and 2.75 M. This would result in monophasic transition curves with a common midpoint of approximately 2.05 M and a high degree of cooperativity, indicated by a transition width of approximately 0.15 M. However, Figure 2 shows that immediately after 2.15 M the ellipticity at all wavelengths becomes positive and increases manyfold, reflecting the appearance of the nonconservative spectra from Figure 1 (dashed lines). The differences in position of the

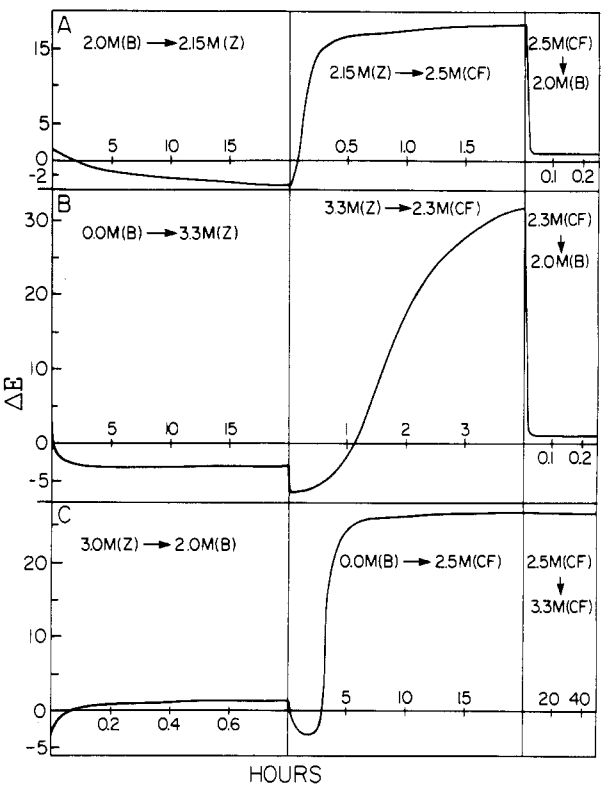



FIGURE 3: Time-dependent changes of $\Delta E$ at 292 nm for conformational transitions of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ induced by changing the sodium acetate concentration. For each box, a sample was made up in the initial salt concentration and equilibrated completely. Then, at time zero, the sodium acetate concentration was changed in one step by dilution or addition of more salt, and the development of the 292-nm CD band was monitored with time (CF = condensed form).

peaks for 292, 275, and 250 nm in Figure 2 are another demonstration of the salt dependence of the CD in the intermediate range between 2.3 and 2.6 M, as described above. This plot also clearly shows that within a very narrow concentration range of sodium acetate (approximately 100 mM), $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ can be converted from a right-handed B-form into a left-handed Z structure and subsequently into a highly condensed form, which is further described below.

The insert in Figure 2 shows the effect of sodium acetate on the $\Delta E$ of $(dI\text{-}dC)_n\cdot(dI\text{-}dC)_n$, $(dT\text{-}dG)_n\cdot(dC\text{-}dA)_n$, $(dG)_n\cdot(dC)_n$, $(dA\text{-}dT)_n\cdot(dA\text{-}dT)_n$, and pVH51 DNA. Only small and linear changes were observed for these five DNAs. Thus, both the induction of a Z-form and the multiphasic behavior with a condensed form at intermediate sodium acetate concentrations are specific for the alternating sequence (dG-dC) and were not observed with any of these control DNAs.

Also, similar studies with a 157 base pair (bp) fragment containing two blocks of (dC-dG), 32 and 26 bp in length, flanking a 95-bp *Escherichia coli lac* operator tract (Klysik et al., 1981, 1982) showed the expected B to Z transition, but no condensed form was found up to 3.5 M sodium acetate. The absence of a condensed form may be due to the short length of the (dC-dG) tracts and/or due to the presence of the natural *lac* segment.

*Kinetics of Sodium Acetate Induced Changes.* The CD properties were described above for fully equilibrated $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in aqueous sodium acetate, i.e., the end-point spectra after completion of the structural changes. To determine the rates of conversion from one type of structure shown in Figure 1 to a different form, we followed the ellipticity at 292.5 nm with time. Figure 3 shows the development of this CD band after the sodium acetate concentration was

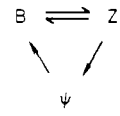
changed in one step from an initial value to a higher or lower value, as indicated in each box. Panel A (left box) shows a normal B to Z transition with a long half-time for completion ($t_{1/2}$) of 3.75 h due to the very narrow change of concentration from 2.0 to 2.15 M. After 20 h the same sample was brought up to 2.5 M sodium acetate (center box); this higher concentration initiated the condensation of the DNA with a $t_{1/2}$ value of 0.11 h. This form was diluted back to 2.0 M (right box), which in a very fast reaction ($t_{1/2} \ll 1$ min) completely redissolved the condensed DNA. The sample was optically clear again, and the CD spectrum indicated the presence of the initial B-form.

Panel B of Figure 3 shows a similar sequence with qualitatively the same features but different reaction rates due to the different concentration ranges used relative to panel A. The $t_{1/2}$ values were 0.05 h for the first step, 1.75 h for the second, and again $\ll$1 min for the third. However, when going from 3.3 to 2.3 M sodium acetate (middle box, panel B), a fast increase in negative ellipticity was observed within 1–3 min after mixing which was then followed by the gradual inversion to the very strong positive value that indicated formation of the condensed form. This approximate 2-fold intensification of the negative 292-nm band within the first few minutes after mixing was generally observed in dilution steps when the Z-form of the DNA in >2.7 M sodium acetate was brought into an intermediate concentration range between 2.3 and 2.6 M. It reflects a rapid and only transient intensification of the Z-type CD spectrum in the whole wavelength range.

Panel C (left box) of Figure 3 shows the reverse reaction from a Z-form to the B-form with a half-time of 0.03 h when going from 3.0 to 2.0 M sodium acetate in one step. With a different sample (center box) the low-salt B-form was brought up to 2.5 M sodium acetate in one step. This change of salt concentration caused the inversion of the DNA into a left-handed form ($t_{1/2}$ = 0.3 h) which, however, was not stable in this form for more than 2–2.5 h but subsequently underwent condensation as indicated by turbidity and the increase of $\Delta E$ to a strong positive value ($t_{1/2}$ = 0.9 h). Further increase of the salt concentration to 3.3 M (right box) did not redissolve the DNA precipitate, although the Z-form obtained by directly going from the low-salt B-form to ≥2.7 M sodium acetate never showed visible turbidity or precipitation (i.e., panel B, left box). In addition, the CD spectrum was unchanged over a period of more than 24 h, neglecting the loss of intensity due to the settling of the DNA aggregate.

Taken together, Figure 3 (and other data not shown) demonstrates that the equilibrium between B-form in low sodium acetate (≤2.0 M) and Z-form in either high salt (≥2.7 M) or at approximately 2.15 ± 0.05 M is completely reversible. Experiments with varying DNA concentrations over a 5-fold range (data not shown) revealed that the reaction rate was independent of DNA concentration and confirmed earlier results which showed that the B to Z tansition is an intramolecular process (Pohl & Jovin, 1972). On the other hand, all steps in Figure 3 that lead to the formation of condensed DNA with a very high and positive CD amplitude (middle box of each panel) showed a clear dependence of the reaction rate on DNA concentration. The $t_{1/2}$ values for this condensing process increased from 0.8 h at 150 $\mu$M DNA–phosphate to 18–19 h at 30 $\mu$M, indicating that the rate-limiting step is the association of the double-stranded DNA molecules to form a compact tertiary or quaternary structure. We were not able to reverse this condensation by increasing the sodium acetate concentration in these samples to >3.0 M or diluting it to 2.15 M, which in both cases should result in an inverted Z-type spectrum. However, after dilution to 2.0 M, i.e., only 150 mM less than the latter concentration mentioned above, complete reversion to the B-form occurred in a very fast reaction within less than 1.5 min.

The observed transitions among these structures are summarized in the following scheme:




where the symbols represent families of structures characterized by either the conservative B-type CD spectrum (symbol B) and the inverted Z-type spectrum (symbol Z) or by the nonconservative $\psi(+)$-type CD (symbol $\psi$).

*Mechanistic Aspects of the Acetate-Induced Structural Changes.* To determine the presence of any intermediate conformations that were not recognized by monitoring $\Delta E$ at only one wavelength, we further investigated the processes described in Figure 3 by fast repetitive CD scans while a transition was in process. This method should reveal any non-B- or non-Z-type transient states and any changes of secondary structure preceding the condensation. Figure 4 gives examples of these sets of spectra. Panel A shows that the transition from the low-salt (≤2.0 M) B-form to the high-salt (≥2.7 M) Z-form occurs without intermediate, non-B or non-Z, states since each spectrum is the sum of different amounts of B- and Z-type curves. The same is true for the induction of the Z-form by only 2.15 M sodium acetate and for the reverse reactions (data not shown). Diluting the high-salt Z-form down to intermediate salt concentration (panel B; in this case from 3.3 to 2.5 M sodium acetate) leads to increasing amounts of positive ellipticity between 250 and 330 nm. The pattern of relative minima and maxima is maintained in the initial phase of this transition, and no spectrum with a B-type shape was observed at intermediate time points. Therefore, we believe that the condensation process is not preceded by a substantial change of the secondary structure of the DNA; this interpretation is further supported by laser Raman studies (see below).

However, when the same intermediate salt range (2.3–2.6 M) is reached starting out with the low-salt B-form (panel C; in this case from 0 to 2.5 M), a change of secondary structure is the initial step. The CD first inverts from a B-type to a Z-type spectrum and subsequently reveals the same spectral changes as demonstrated in panel B, but only after this initial transition has been completed (see also Figure 3, panel C, center box). This indicates that the B-form of the DNA is not the immediate precursor of the condensed form but that either the presence or the induction of the Z conformation is a necessary condition for the compaction of the DNA.

*Raman Spectroscopy on the Condensed Form of* $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$. Raman spectroscopy has been employed as a sensitive probe of DNA conformations including left-handed Z structures (Pohl et al., 1973; Thamann et al., 1981; Wartell et al., 1982; Martin & Wartell, 1982; Martin et al., 1978). To further study the secondary structure of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in the condensed state, we recorded laser Raman (LR) spectra of the DNA in sodium acetate concentrations where the polymer is in the high-salt Z-form and the compacted form. Figure 5 shows the two LR spectra in 2.5 and 3.3 M sodium acetate. The absence of peak intensity at 682 and 832 $cm^{-1}$ in the high-salt spectrum (3.3 M) shows that the DNA is no longer in the B-form (Wartell et al., 1982; Thamann et al., 1981). The presence of the Z-form under these conditions (or in 2.15 M salt; data not shown) is indicated by the intensity

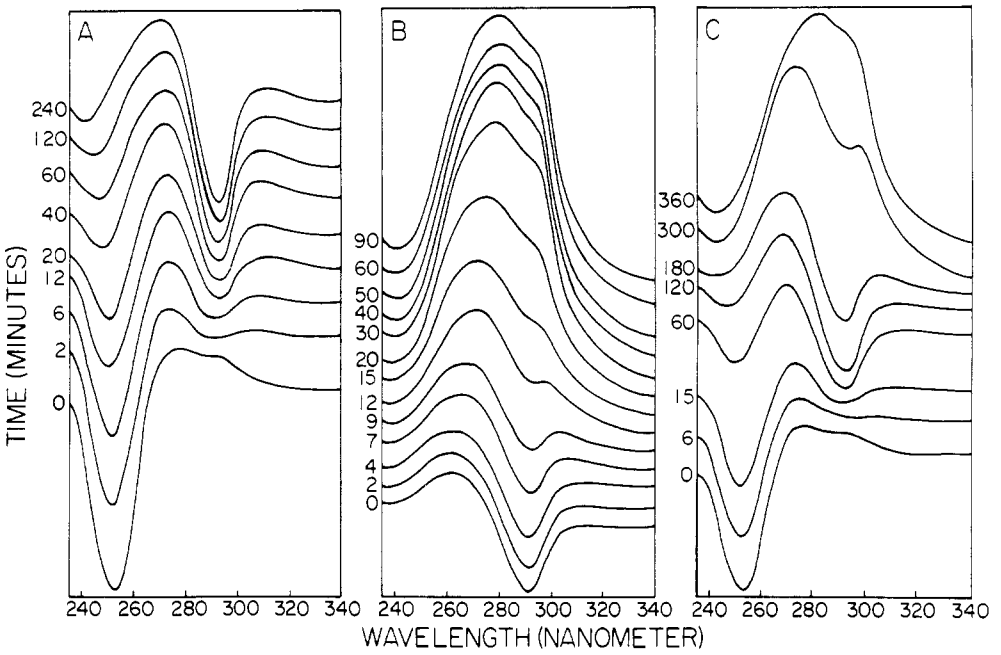



FIGURE 4: CD changes for $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ with time after the sodium acetate concentration was changed in one step as indicated. (Panel A) 2.0–3.0 M; (panel B) 3.3-2.5 M; (panel C) 0–2.5 M. All experimental readings were normalized to the same absorbance. The instrument settings for fast scanning of these spectra are described under Materials and Methods.

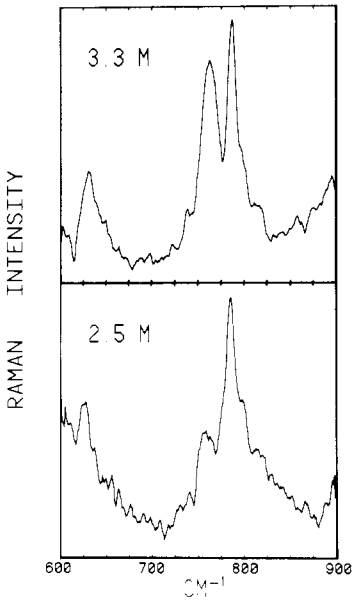



FIGURE 5: Laser Raman spectra of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in 10 mM sodium cacodylate containing 3.3 or 2.5 M sodium acetate. Both spectra are normalized to equal intensity of the cytosine peak at 785 $cm^{-1}$. The solvent spectra were subtracted from the data shown. The curves were smoothed as described (Savitzky & Golayz, 1964).

at 624 $cm^{-1}$ and two shoulders near 799 and 818 $cm^{-1}$ on the right side of the main cytosine peak at 785 $cm^{-1}$. Although the peak intensities are not identical, the peak positions correlate well with earlier LR results obtained with a 157-bp fragment containing left-handed segments under high-salt conditions (Wartell et al., 1982). It is obvious from Figure 5 that the spectrum obtained in 2.5 M sodium acetate shows the same features as the high-salt spectrum despite marked deviations in intensity at 756 or 624 $cm^{-1}$ and the shoulders near 799 and 818 $cm^{-1}$.

These studies indicate that the condensed form of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in 2.5 M sodium acetate has a left-handed structure with conformational features similar to, although not identical with, that of the Z-form in 3.3 or 2.15 M salt.

## Discussion

Circular dichroism measurements with DNA fibers at various degrees of humidity (Tunis-Schneider & Maestre, 1970; Brunner & Maestre, 1974) have provided a correlation between the A, B, and C conformations of double-stranded DNAs in solution and the shapes of their CD curves (Zimmer & Luck, 1974, 1973; Hanlon et al., 1975; Wolf & Hanlon, 1975; Hanlon et al., 1978). The comparison of crystallographic data with laser Raman results and CD spectra for $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ proves that a sign inversion of the low-salt B-form CD reflects the formation of a left-handed Z-helix (Pohl & Jovin, 1972; Pohl et al., 1973; Thamann et al., 1981). On this basis we interpret the conformation of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ observed at 2.15 ± 0.05 M and at ≥2.7 M sodium acetate as a salt-induced inversion of the B-helix into a Z-type helix. This transition is characterized by a high cooperativity (transition width approximately 120 mM) and a transition midpoint at 2.05 M. Therefore, sodium acetate appears to be slightly more effective for the B to Z transition than NaCl (midpoint at 2.5 M) but less effective than $NaClO_4$ (midpoint at 1.75 M) (Pohl & Jovin, 1972).

The slight deviations between the curves for 2.15, 2.8, and 3.75 M indicate a sensitivity of the Z-helix to ionic strength similar to that observed for the B-helix (Zimmer & Luck, 1974, 1973; Hanlon et al., 1978; Chan et al., 1979; Zacharias et al., 1982).

*ψ State of* $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$. $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in 2.3–2.6 M sodium acetate shows a 15–20-fold increase in the maximum $\Delta E$ values relative to the low-salt B-form, including long-wavelength tails in the nonabsorbing region (up to 340 nm). These general features indicate the presence of a highly condensed ψ state with an asymmetrically ordered tertiary structure. Similar structures have been observed with DNA that compact or collapse into ordered superstructures when exposed to solutions containing organic polymers (Cheng & Mohr, 1975; Lerman, 1973; Maniatis et al., 1974; Jordan et al., 1972) or ethanol and salt (Huey & Mohr, 1981; Eickbush & Moudrianakis, 1978; Potaman et al., 1981) or when complexed with certain histones (Jordan et al., 1972; Olins & Olins, 1971) or polyamines (Damaschun et al., 1978; Gosule

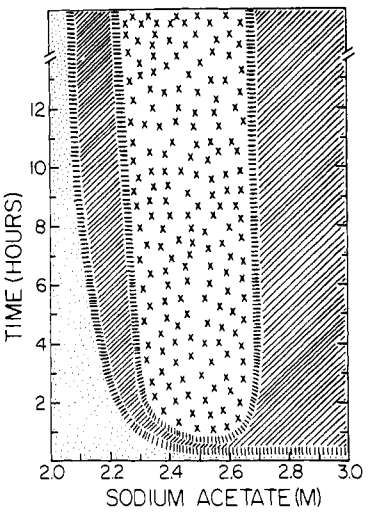



FIGURE 6: Phase diagram of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in aqueous sodium acetate. This plot describes the conformational changes with time for the polymer when a certain molarity of sodium acetate was added to its low-salt B-form. The dashed broad bands that separate the B-(dotted area), Z- (cross-hatched area), and $\psi$-form (× area) regions are positioned according to the half-times for completion of the transition under consideration. These bands should not be interpreted as sharp, well-defined lines but as an illustration of the transitional regions which form the boundaries between the predominant areas of the B-, Z-, or $\psi$-type conformational states.

& Schellman, 1978; Chattoraj et al., 1978) (further discussed belowed).

It should be emphasized that $\psi$ formation is not a necessary consequence of Z formation since the effect of sodium acetate on $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ appears to be delicately balanced between salt concentration and reaction time. The apparent phase diagram for this system (Figure 6) summarizes the results from Figures 1–3 and describes the conformational transitions of the polymer when exposed to different concentrations of sodium acetate for extended periods of time. The phase diagram shows that at ≤2.0 M salt the polymer remains stable in the B-form, whereas at ≥2.03 M, the B to Z transition takes place with strongly decreasing $t_{1/2}$ values when the inducing salt concentration is increased. At 2.08–2.22 M, the Z-form is stable for 4–5 days (upper limit of $y$ axis) without intermediate or subsequent formation of the $\psi$ state. In the range from 2.25 to 2.68 M, this Z-form is not stable for more than 0.5–1 h but is eventually converted into the $\psi$ state. The $t_{1/2}$ values for the sequence B → Z → $\psi$ in this concentration range strongly depend on the sodium acetate concentration present with minimal values at ≈2.55 M. At and above 2.7 M, a fast B to Z transition takes place with $t_{1/2}$ values of only a few minutes, but this transition is no longer followed by $\psi$ condensation. Obviously, the $\psi$ state is favored only within this limited salt range (2.25 and 2.68 M) and does not occur from the Z-form at lower or higher concentrations or with the B-form.

From this diagram and from Figure 3 we conclude that the Z-form is a kinetic and mechanistic intermediate for the $\psi$ formation (of this DNA and under these conditions) but that the $\psi$ state is not necessarily a time-dependent final product of the B to Z transition in general. Instead, the $\psi$ state of this polymer is a structural alternative to the monodispersely dissolved Z-form and is stabilized and thermodynamically favored only in this limited range of ionic strength.

Several models are described in the literature for the structure of tightly packed DNA that may serve as a basis to explain $\psi$-type CD spectra. The compacted DNA can be considered to consist of tightly wound supercoiled helices that form short rodlike or donut-shaped particles (Eickbush & Moudrianakis, 1978; Olins & Olins, 1971), of folded chain structures (Maniatis et al., 1974), of "skein of yarn" structures, or of twisted stacks composed of layers of parallel oriented helices (Lerman, 1973; Maestre & Reich, 1980). All these models can explain the high amplitudes of $\psi$ spectra. They are supported by experimental evidence like X-ray scattering (Damaschun et al., 1978; Maniatis et al., 1974), birefringence and polarized fluorescence (Lerman, 1973), electron microscopy (Eickbush & Moudrianakis, 1978; Olins & Olins, 1971; Chattoraj et al., 1978), or optical absorption and CD (Olins & Olins, 1971; Damaschun et al., 1978; Gosule & Schellman, 1978; Maestre & Reich, 1980) or are developed from theoretical considerations (Manning, 1980; Maestre & Reich, 1980) and from comparison with synthetic organic polymer crystals (Lerman, 1973). Our results do not permit a more detailed description of the structure of the $\psi$-form of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$. Further investigations (electron microsocpy experiments) are currently in progress.

*Secondary Structure of $\psi$ Form.* Application of liquid-crystal theory to the condensed $\psi$ state provides an explanation for the manyfold increase of the CD of this structure and assigns the $\psi(+)$ state to compact DNA possessing completely positive $\psi$ spectra (as in the case described here) and the $\psi(-)$ state to those with negative spectra (Cheng & Mohr, 1975; Reich et al., 1980; Maestre & Reich, 1980). This theory implies that the drastic CD changes accompanying the $\psi$ formation are caused mainly by ordered packaging of individual DNA helices in an asymmetric manner and not by substantial changes of the secondary structure of each duplex molecule. Of course, slight deformations of the strand geometry during compaction, caused by the decrease of the distance between the charged backbones of two neighboring helices, cannot be excluded and are very likely. Our Raman studies show that the same is true for the $\psi(+)$ form of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$. The spectrum in 3.3 M sodium acetate, when compared with results from other authors (Wartell et al., 1982; Thamann et al., 1981; Pohl et al., 1973), indicates a left-handed helix similar to, although not identical with, that of the Z conformation of this DNA in high concentration of NaCl and thereby supports our interpretation of the inverted CD spectra.The spectrum in 2.5 M sodium acetate, where the CD has $\psi(+)$-type features, shows the same band patterns and positions as the one in 3.3 M. Differences in intensities are present, which we interpret as minor deformations of the helix geometry within the Z family due to compaction and different ionic strengths in both samples. Therefore, we conclude that the polymer has a left-handed Z-like secondary structure at those salt concentrations that cause the inverted CD curves as well as in the intermediate salt range with the $\psi(+)$ spectra. Other authors have shown that the right-handed B or A conformation is also maintained in natural DNAs after compaction to $\psi(+)$ or $\psi(-)$ structures (Huey & Mohr, 1981; Potaman et al., 1981; Herbeck et al., 1976) and that there is no necessary correlation between the secondary structure of the helix and sign of the $\psi$ state.

Furthermore, the repetitive CD scans (Figure 4B) show that the $\psi(+)$ spectrum arises from the Z spectrum by gradual addition of positive ellipticity over the entire absorption range while in the intial phase maintaining the pattern of relative minima and maxima typical for the Z-form. On the other hand, Figure 4C shows that $\psi(+)$ formation from the B-form is preceded by an inversion of the CD into a Z-type spectrum, and only after completion of this event does the DNA start to aggregate and the $\psi(+)$ spectrum begin to develop (Figure

3A, C). Interestingly, the polymer $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ does not adopt a $\psi$ structure (as judged by CD) after extensive sonication of the DNA but remains in the noncondensed Z-form (data not shown). This can be explained by the dependence on molecular weight of the $\psi$ formation as described earlier (Cheng & Mohr, 1975). At the same time, this indicates that the condensed helices also possess a Z-like conformation since there is no reason to believe that the polymer adopts a different type of helix structure when disrupted to shorter chains, but still being far beyond the estimated cooperativity length of approximately 25 base pairs for the B to Z transition (Ivanov & Minyat, 1981). All these observations further support the notion that it is the left-handed Z-helix that aggregates to form a condensed $\psi(+)$ structure in this limited range of sodium acetate concentration.

The liquid-crystal theory provides an explanation for the reproducible shift of the wavelength with maximum $\Delta E$ with increasing salt concentration (from 298 nm at 2.3 M to 276 nm at 2.6 M). The theory assumes a direct proportionality according to the Bragg reflection law between the peak position of a $\psi$ spectrum and the helical or otherwise chiral periodicity of the ordered tertiary structure of the DNA (Maestre & Reich, 1980; Reich et al., 1980). Increasing salt concentration within the $\psi$ range would provide more shielding of negative charges on the helix surface and enable a closer contact between neighboring helices or layers of helices, thereby diminishing the periodicity distance and thus shifting the peak of the $\psi$ spectrum to shorter wavelengths. One might assume that the slight salt sensitivity of the Z-helix mentioned above gradually alters the geometric parameters of the Z-form when going from 2.08 to >3.0 M. It seems that only a narrow range of bond angles and charge distances within this concentration range is suitable for being packed into an ordered liquid-crystal structure whereas others are not. However, it should be pointed out that for other DNA sequences and for other solvent components, different geometrical helix parameters are feasible with and even promote compaction of the DNA (see below). This shows that the general feature of a cylindrically shaped structure itself with a certain charge distribution on its surface may be the essential requirement for any kind of linear polymer to adopt a $\psi$ structure under certain conditions.

*Cooperativity.* The B to Z (transition width $\approx$ 120 mM) conversion and the Z to $\psi$ (transition width $\approx$ 250 mM) conversion have a very high cooperativity. Thus, within a narrow range of sodium acetate concentration (200 mM) three distinct forms of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ that possess completely different physical and optical properties can be stabilized by the same agent; these are the right-handed B-form, the left-handed Z-form with monomolecular chirality, and the condensed Z-form with a chiral tertiary $\psi(+)$ structure. Similar strongly cooperative B to $\psi$ transitions for natural DNAs in solutions containing organic polymers and inorganic salts (Jordan et al., 1972) or ethanol and NaCl (Huey & Mohr, 1981) were described previously.

*Salt and Sequence Specificity.* It is surprising that the simple binary mixture of water and sodium acetate can have these substantially different effects on the structure of the dG-dC polymer. Other $\psi$-forms with natural DNA described earlier require more complex components like critical ratios of poly(ethylene oxide) to NaCl or KCl (Jordan et al., 1972; Cheng & Mohr, 1975) and ethanol to NaCl (Huey & Mohr, 1981) or certain histones (Jordan et al., 1972; Olins & Olins, 1971), polyamines (Damaschun et al., 1978; Gosule & Schellman, 1978; Chattoraj et al., 1978), or poly(amino acids) (Shin & Eichhorn, 1977). However, acetate is not only a monovalent anion of an alkali metal salt but is also the first member in a homologous series of fatty acid anions which, in general, possess ionic as well as hydrophobic properties. Thus, it is different from the other salts tested (halides, nitrates, and sulfates), and this could be the basis for its unique behavior especially in the high concentrations applied here. The sodium counterion probably partially neutralizes the phosphate charges in the DNA backbone. The high concentration of "hydrophobic tails" of the acetate ions could have an influence on the water structure and could cause dehydration of the helix. Both effects would make the helix more flexible and vulnerable to conformational variations like kinks or helix bending, thus facilitating compaction and formation of a $\psi$ structure. The base composition of the dG-dC polymer (100% GC content) could enhance these effects since it was shown that natural DNAs with higher GC content are more susceptible to $\psi$ formation than those with low GC content (Cheng & Mohr, 1975). This could also explain the inability of other control DNAs examined here to adopt a $\psi$-type structure under the same conditions as for $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$.

The high GC content of this $\psi$-form DNA could be the reason for the resistance to thermal melting of its $\psi(+)$-form in sodium acetate. The $\psi$-forms obtained from natural DNAs in poly(ethylene oxide)–NaCl solutions have a $T_m$ between 40 and 60 °C and melt in a cooperative manner similar to the melting of the double helix at higher temperature (Cheng & Mohr, 1974). This supports the liquid-crystal theory by indicating the temperature-induced breakdown of an ordered tertiary structure that is weaker than the double helix itself. The $\psi(+)$-form of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$, however, only shows a weak decrease in the CD amplitudes but not a cooperative melting behavior up to 70 °C (data not shown). Under the assumption that even in the tertiary structure there are conformational effects and helix–helix interactions that are sequence specific (Cheng & Mohr, 1975; Huey & Mohr, 1981), this temperature resistance could be a reflection of the high GC content and of the high ionic strength necessary to promote $\psi$ formation in this polymer. In addition, we were not able to convert the $\psi(+)$-form into the $\psi(-)$-form as reported by other authors with increasing salt concentration (Huey & Mohr, 1981) or addition of divalent transition metal ions (Shin & Eichhorn, 1977). This inability to obtain a $\psi(+)$ to $\psi(-)$ inversion, as found with natural DNAs of lower GC content, could be another indication of the increased stability of the $\psi(+)$-form of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ in high sodium acetate concentrations.

We wish to emphasize that this condensation of $(dG\text{-}dC)_n\cdot(dG\text{-}dC)_n$ is not related to the association of the Z-form polymer to form Z* DNA in solutions containing divalent metal ions as recently described (van de Sande & Jovin, 1982). Furthermore, we believe that this is the first description of a $\psi$-type structure originating from a left-handed Z-type conformation.

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