Biophysical Chemistry 26 (1987) 375–383
Elsevier

BPC 01153

The B-Z conformational transition and aggregation of poly[d(G-C)] induced by moderate concentrations of $Mg(ClO_4)_2$

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Accepted 27 February 1987

Z-DNA; left-handed DNA; Kinetics; Chaotropic salt; (*M. lysodeikticus*)

$Mg(ClO_4)_2$ induces the cooperative B-to-Z transition of poly[d(G-C)]; the salt concentration at the midpoint is 0.26 M. A comparison with previous data for NaCl, $MgCl_2$ and $NaClO_4$ (F.M. Pohl and T.M. Jovin, J. Mol. Biol. 67 (1972) 375) indicates that $Mg(ClO_4)_2$ is more effective than would be anticipated from the simple additive effects of the Mg^{2+} and ClO_4^- ions (the ionic strengths of the respective transition points are: NaCl, 2.4; $MgCl_2$, 2.1; $NaClO_4$, 1.8 and $Mg(ClO_4)_2$, 0.78). These results suggest the importance of specific interactions involving ClO_4^-, particularly in the presence of Mg^{2+}. The B-Z transition of poly[d(G-C)] can be monitored spectroscopically via the large hyperchromic shift at 295 nm and the inversion in the CD spectrum. The reaction is fully reversible and can be fitted by a monoexponential function with half times varying between 8 and 150 min. The observed relaxation times are strongly dependent on the concentration of $Mg(ClO_4)_2$ with a distinct maximum at the transition point, in accordance with a concerted mechanism involving only the B and Z states. As the polymer assumes the Z conformation it progressively aggregates into a gel-like precipitate, which, however, redissolves rapidly upon lowering the salt concentration. The natural DNA from *Micrococcus lysodeikticus* which has a high GC content of 72% is also precipitated by $Mg(ClO_4)_2$ but we do not have direct spectroscopic evidence for the involvement of the Z conformation in this phenomenon. Neither calf thymus DNA (41% GC) nor poly[d(A-T)] (0% GC) aggregates under the same conditions.

1. Introduction

The synthetic DNA poly[d(G-C)] undergoes a highly cooperative transition between two helical conformations upon exposure to high salt concentrations (approx. 2.4 M NaCl, 1.5 M $NaClO_4$, 0.7 M $MgCl_2$) [1] or greater than 50% ethanol [2]. The reaction in NaCl is isoenthalpic [1,3] and has a high activation energy (> 20 kcal/mol) [1]. X-ray diffraction analyses of hexameric [4] and tetrameric [5] oligonucleotides with the same alternating sequence have demonstrated the existence of the left-handed 'Z' conformation (reviewed in ref. 6), in which the dG residues are in the *syn* conformation about the glycosidic bond and the corresponding sugar pucker is C3′-*endo as* opposed to the characteristic *anti* C2′-*endo* disposition of the dC residues (and all the residues in the case of B DNA). The postulated mechanism(s) by which increased salt potentiates this transition include (for reviews, see refs. 3 and 6–10): (i) screening of the repulsive interstrand negative charges on the phosphate groups, some of which in Z-DNA are much closer together than in B-DNA; (ii) reduction in water activity; (iii) specific cation and anion binding to the bases; and (iv) potentiation of intra- and intermolecular condensation and association. In the case of the uni-univalent chloride salts of the alkali metals, a statistical-mechanical treatment of the electrostatic and hard-sphere in-

Dedicated to Professor Manfred Eigen on the occasion of his 60th birthday.

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teractions involving the fixed charges on the B and Z helices and the mobile ions in the environment and bulk solution has reproduced the experimental dependence on salt concentration [3,8–11]. In addition to territorial binding, divalent and oligovalent cations engage in specific interactions with the DNA structure [12,13], thereby decreasing the concentration required to effect the transition, particularly if the binding is preferential for the left-handed form. Specific effects are also observed for anions such as ClO_4^- [1], but their structural basis is more obscure due to the global polyanionic nature of the DNA double helix and the attendant electrostatic repulsion.

In attempting to assess whether the B-Z and other related conformational transitions are significant in defining the structure and function of natural DNA, we have extended our search for conditions closer to those physiologically realizable under which the reaction still takes place. This report documents the finding that the Z form of poly[d(G-C)] is stabilized at moderate concentrations (0.2–0.3 M) of the chaotropic salt $Mg(ClO_4)_2$, indicating that the two constituent ions operate synergistically. A possible basis for the phenomenon is provided by the observation that the conformational transition is strongly coupled to association and aggregation of the Z-DNA.

2. Materials and methods

2.1. DNA preparations

Poly[d(G-C)] used was purchased from Boehringer Mannheim and Pharmacia. Part of the Boehringer sample was deproteinized by SDS treatment and fractionated on a Sephacryl S-200 column. The peak fractions were pooled into a single sample and tested together with the original commercial product in determinations of the B-Z transition induced by $NaClO_4$. Both samples accurately reproduced the curve previously established for a locally synthesized and well-characterized poly[d(G-C)] sample with a degree of polymerization of greater than 500 [1]. Thus, the various samples were used without distinction and without further purification or characterization. The stock polymer solutions were prepared either by directly dissolving the lyophilized powder in the desired buffer or (the more general and preferable procedure) by dialyzing poly[d(G-C)] solutions. Concentrations in low salt (B form) conditions were calculated using an ϵ_p of 7100 M^{-1} cm^{-1} at 260 nm [1].

Magnesium perchlorate was obtained from Merck (Darmstadt) as $Mg(ClO_4)_2 \cdot \times H_2O$. Approx. 3 M (nearly saturated) stock solutions were made which were clarified either by centrifugation or by filtration. The exact concentration of the stock solutions was determined by titration with EDTA using Eriochrome Black T as an indicator [14] and $MgSO_4 \cdot 7H_2O$ (Merck) as a standard.

Micrococcus lysodeikticus DNA was from Miles and used without purification. Calf thymus DNA (type V) was obtained from Sigma and deproteinized with chloroform/isoamyl alcohol. Poly[d(A-T)] was from Boehringer and used without purification. Except for the 3 M $Mg(ClO_4)_2$ stock, solutions contained 10 mM sodium phosphate buffer (pH 7.2), or as indicated, 10 mM Tris-HCl (pH 7.5) or 10 mM Hepes-NaOH (pH 7.0).

2.2. Spectrophotometric and CD measurements

The ultraviolet absorption measurements were performed on three instruments: a Zeiss PM QII spectrophotometer; a Cary 118 recording spectrophotometer; and a Kontron Uvikon 820 spectrophotometer. The CD spectra were recorded on a Jobin-Yvon mark IV dichrograph. All data were collected at 25° C. The determination of the B-to-Z transition curve of poly[d(G-C)] involved two separate sets of experiments. In the first, small incremental additions of $Mg(ClO_4)_2$ were made to an (initially) 50 μM poly[d(G-C)] solution and time allowed for the A_{295} to reach its final equilibrium value. The readings were subsequently corrected for the (small) absorption of $Mg(ClO_4)_2$ and for the successive dilution of the sample. The other experiments involved continuous recording of the A_{295} of the salt-containing polymer solutions. The reactions were initiated by the rapid (i.e., within a few seconds) mixing of polymer, buffer and stock salt solutions in appropriate proportions so as to yield the desired final $Mg(ClO_4)_2$ concentration.

Either tandem quartz cells (Hellma, 2×0.437 cm path length) were used or the solutions were mixed in standard cuvettes with a Pasteur pipette or by inversion. Due to the slow precipitation of poly[d(G-C)] at higher salt concentrations the kinetic/spectroscopic method gave more reliable values for the asymptotic limit of A_{295} at high $Mg(ClO_4)_2$ concentrations (i.e., for θ values approaching 1) than the equilibrium method with incremental salt additions and lengthy equilibration periods. Thus, the limiting values in the calculation of θ were obtained from the kinetic/spectroscopic experiments. The data from the latter were fitted to a monoexponential function and the mean relaxation time (τ) of the transition process determined in the usual manner.

2.3. Determination of aggregation in DNA samples undergoing the B-Z transition

During and after all absorption measurements the cuvettes were periodically inspected for signs of precipitation (turbidity) using a strong lateral light beam from a tungsten lamp. In addition to these qualitative observations, the degree of aggregation in DNA solutions exposed to different conditions was established by the sedimentation analysis employed previously [15]. Aliquots from reaction mixtures were removed and centrifuged in Eppendorf tubes for 2 min at $12000 \times g$. The A_{295} of the supernatants was determined either directly or after dilution with buffer. The fractional aggregation was calculated from these values relative to that from a reference unreacted (soluble) DNA sample.

3. Results and discussion

3.1. Spectral changes accompanying the B-Z transition of poly[d(G-C)] in $Mg(ClO_4)_2$

Upon increasing the concentration of $Mg(ClO_4)_2$ above 0.2 M, solutions of poly[d(G-C)] undergo a kinetically-resolved transition characterized by changes in ultraviolet absorbance and CD spectra. The ultraviolet absorption spectra of poly[d(G-C)] in 10 mM Hepes-NaOH buffer alone and in the presence of 0.4 M $Mg(ClO_4)_2$ are shown in fig. 1. The hyperchromism in the region of 295 nm is similar to that accompanying the B-Z transition induced by high NaCl, $MgCl_2$ and $NaClO_4$ [1]. The 295/260 nm absorbance ratio increased 3.3-fold and the peak wavelength shifted from 256 to 258 nm. Notable, however, is a vertical displacement of the high salt spectrum, undoubtedly due to aggregation (and corresponding turbidity) which develops during the reaction (see below). The CD spectrum of poly[d(G-C)] in $Mg(ClO_4)_2$ exhibited the negative peak at 295 nm characteristic of the Z form in NaCl [1] (inset to fig. 2). However, the spectrum is unusual in that at lower wavelengths the positive peak characteristic for NaCl (inset) is replaced by a negative excursion at 250 nm and a positive peak at 220 nm. The latter is not observed for either the B or Z conformations in NaCl (fig. 2, inset) and argues against a simple interpretation in terms of a partial B-to-Z reaction. Furthermore, the spectrum is not reminiscent of that measured for the aggregated B form in $MgCl_2$-EtOH [15]. We conclude tentatively that the high salt CD spectrum reflects the

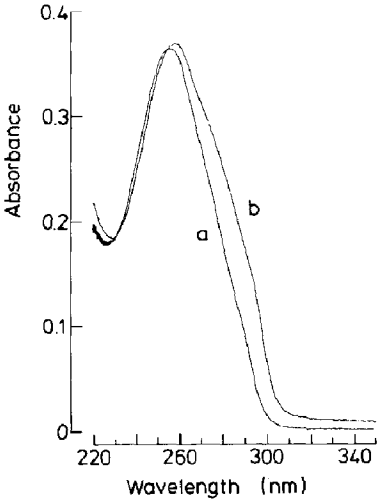

Fig. 1. Ultraviolet absorption spectra of poly[d(G-C)]. (a) B form in buffer. The positive peak is at 256 nm; (b) Z form in 0.4 M $Mg(ClO_4)_2$. The positive peak is at 258 nm. In both cases, the DNA was 50 μM in 10 mM Hepes-NaOH (pH 7.0) at 25°C. Spectrum (b) was obtained 109 min after addition of the DNA and registration of the kinetics at 295 nm.

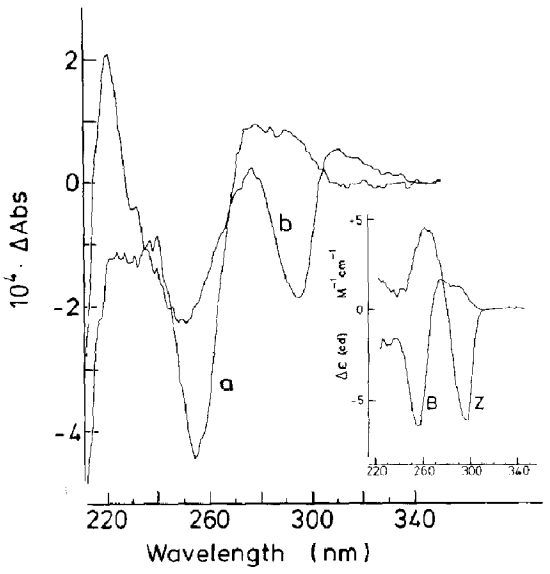

Fig. 2. CD spectra of poly[d(G-C)]. (a) B form in buffer; (b) Z form in 0.4 M $Mg(ClO_4)_2$. In both cases, the DNA was 50 μM in 10 mM Tris-HCl (pH 7.5) at 25°C. Spectrum b was obtained after addition of DNA and incubation for 2 h at 25°C and an additional 30 min at 30°C. Scans before and after the latter incubation were similar. The inset shows reference spectra in NaCl (unpublished data of McIntosh and Jovin [1]).

highly aggregated physical state of the polymer and/or real structural features specific for the Z-DNA helix in solutions of $Mg(ClO_4)_2$.

3.2. Equilibrium and kinetic characterization of the B-Z transition of poly[d(G-C)] in $Mg(ClO_4)_2$

The kinetic course of the B-Z transition in $Mg(ClO_4)_2$ (fig. 3) was substantially (although not perfectly; see inset) monoexponential. As pointed out above (and discussed further below), the slow precipitation of the polymer at and above the transition midpoint rendered the determinations by the incremental-salt addition method unreliable. However, θ values could be calculated from the kinetically observed (or sometimes extrapolated) total amplitudes of the ΔA_{295}. The combined experimental results were used to construct a curve depicting the equilibrium as a function of salt concentration, as shown in fig. 4 with the corresponding data previously reported for NaCl, $NaClO_4$ and $MgCl_2$ [1].

The transition midpoints (c_t) and corresponding ionic strengths are listed in table 1 for the four salts investigated in the present and previous studies. It is evident that $Mg(ClO_4)_2$ is exceptionally effective in bringing about a B-to-Z conformation change in poly[d(G-C)]. The c_t value of 0.26 M indicates that the transition does not occur at a unique ionic strength, as might have been surmised from the NaCl, $MgCl_2$ and $NaClO_4$ data alone [1] (we do not consider here the stabilization of the Z form by cations binding selectively to the bases and sugar-phosphate backbone, e.g., the transition metals [15–18] and $Co(NH_3)_6^{3+}$ [12,13]).

Previous studies of the B-Z transition of poly[d(G-C)] induced by simple salts have established that the reaction is intramolecular, cooperative and reversible with kinetics described adequately by a simple concerted all-or-non mechanism [1,19]. The same conclusions apply to $Mg(ClO_4)_2$. For example, the salt dependence of the average relaxation time τ (fig. 5a) is qualitatively similar to that observed for the other salts [1]. The slowest conversion rate (maximal τ) corresponds very well to the c_t value derived from the equilibrium transition curve (fig. 4) and provides a good independent determination of this parameter. Using the two-state model it can be shown [1] that the forward and reverse rate constants for the B-to-Z and Z-to-B steps (k_{RL} and k_{LR}, respectively) are given by:

$$k_{RL} = \theta/\tau \text{ and } k_{LR} = (1-\theta)/\tau, \quad (1)$$

and thus, one can calculate these rate constants (fig. 5b) from the τ and θ values obtained at different $Mg(ClO_4)_2$ concentrations. Such calculations for the various salts are plotted in fig. 6, from which the following observations and conclusions can be drawn: (i) for the chloride salts, the concentration dependence of k_{LR} is greater than that for k_{RL}; the distinction largely disappears for the perchlorate salts (although the range of k_{LR} in $Mg(ClO_4)_2$ was too limited for a firm conclusion to be drawn); (ii) as c_t decreases, the minimal reaction rate also decreases, a finding which presumably reflects differential effects on

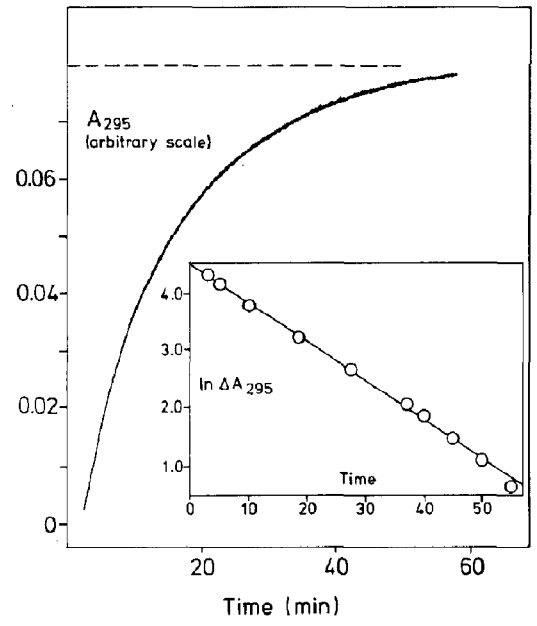

Fig. 3. Kinetics of the B-Z transition in $Mg(ClO)_2$. Time course of ΔA_{295} of a 50 μM poly[d(G-C)] solution following the rapid change in concentration of $Mg(ClO_4)_2$ from 0 to 0.38 M. The inset shows a semilogarithmic plot of $A_{final} - A(t)$.

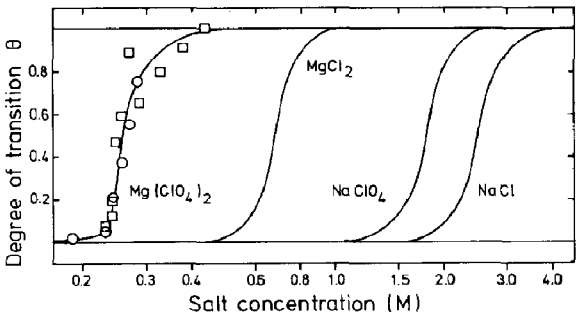

Fig. 4. B-Z equilibria of poly[d(G-C)] as a function of salt concentration. The degree of conversion (θ) was calculated from the absorbance changes at 295 nm. The $Mg(ClO_4)_2$ data are from the present work, and the other results from ref. 1. (□) Data obtained by raising the salt concentration in small increments; (□) data derived from kinetic experiments initiated by the rapid mixing of polymer solutions with appropriate volumes of a 3 M $Mg(ClO_4)_2$ stock solution.

Table 1

Transition salt-concentrations (c_t) and ionic strengths ($\Gamma/2$) for the B-Z conformation transition of poly [d(G-C) at 25 °C

Salt	c_t (M)	$\Gamma/2$
NaCl [a]	2.4	2.4
$MgCl_2$ [a]	0.7	2.1
$NaClO_4$ [a]	1.8	1.8
$Mg(ClO_4)_2$	0.26	0.78

[a] Data from Pohl and Jovin [1].

the nucleation and propagation phases of the overall reaction [1]; and (iii) the slope of $\log(k_{RL})$ vs. log [M] for the four salts is (fig. 6): $Mg(ClO_4)_2$, 5.4; NaCl, 0.73; $MgCl_2$, 2.0; and $NaClO_4$ (at the higher concentrations), 4.9. The similarity of the two perchlorate-salt slopes would appear to be significant, possibly reflecting a common mode of specific interaction between DNA and the perchlorate anion. The distinction is that the phe-

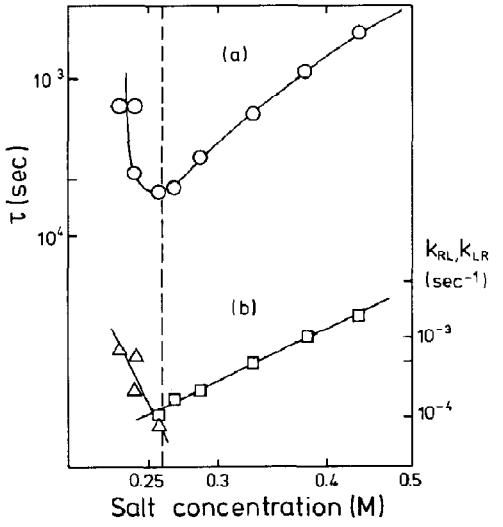

Fig. 5. Relaxation times and rate constants for the B-Z transition of poly[d(G-C) in $Mg(ClO_4)_2$. (a) Variation of the observed relaxation times with $Mg(ClO_4)_2$ concentration. The DNA was 50 μM. The broken vertical line designates the midpoint of the B-Z transition as derived from fig. 4. (b) Variation of the overall rate constants with $Mg(ClO_4)_2$ concentration. k_{RL} (□, forward direction); k_{LR} (△, reverse direction). The values were calculated not from the smoothed curve of fig. 4 but from the individual θ values.

nomenon manifests itself at much lower salt concentrations in the presence of Mg^{2+}.

Numerous factors can be identified as mediating the salt-induced B-Z transition of poly[d(G-C)] [3–13]: (i) electrostatic screening by territorially bound (mobile) counterions of the fixed negative charges on the phosphate groups, some of which are closer to each other in Z than in B-DNA [4]; (ii) steric effects, i.e., hard-sphere interactions, between the mobile ions of the supporting electrolyte and the charged groups on the DNA; (iii) alterations in the structure of bulk water due to the structure-making or structure-breaking properties of the salt(s); (iv) specific complexation by the constituent ions of the salt with distinct loci on the DNA; and (v) promotion of intramolecular and intermolecular condensation, association, and aggregation (generally favoring the Z conformation). Our results with $Mg(ClO_4)_2$ appear to support the relative importance of the last three factors. For example, NMR studies of Prestegard and Chan [20] indicate that $Mg(ClO_4)_2$ shifts the C2′-*endo*/C3′-*endo* equilibrium of the furanose pucker of deoxyribonucleotides toward the C3′-*endo* form characteristic of the purine residues in Z-DNA [4,5]. The fact that $Mg(ClO_4)_2$ is such an effective agent for promoting the aggregation and ultimate precipitation of Z-DNA also implies that there exist specific synergistic interactions involving Mg^{2+}, ClO_4^- and groups of the polynucleotide. These observations do not diminish the importance of the electrostatic and associated hard-sphere terms which account for much of the free energy balance between the B and Z forms [3,8–11]. However, the overall thermodynamic equilibrium is determined by the coupling between the various reactions: conformational transition, binding, and association/condensation [7].

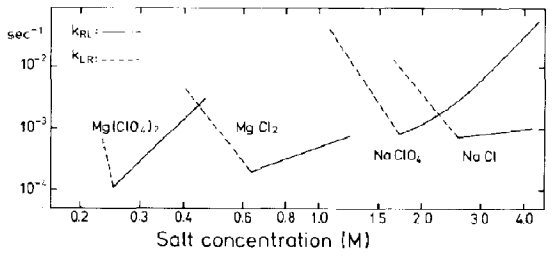

Fig. 6. Dependence of the forward (k_{RL}) and reverse (k_{LR}) rate constants for the B-Z transition of poly[d(G-C)] on the concentration of different salts. The data are presented in a log-log plot. The results for $Mg(ClO_4)_2$ are from this study and the other data are taken from ref. 1.

The distinctive properties of $Mg(ClO_4)_2$ are further demonstrated in investigations of the effect that this particular chaotropic salt exerts on the optical, hydrodynamic and morphological properties of various natural DNAs. Bauer and collaborators have demonstrated [21,22] that $Mg(ClO_4)_2$ is much more effective than $NaClO_4$ or, especially, NaCl and CsCl [23], in causing a noncooperative unwinding of closed circular duplex DNA from PM2 bacteriophage. In a study of the denaturation of various DNA samples (bacteriophages λ, T7, etc.) in $Mg(ClO_4)_2$, it was found that a sequence-specific premelting occurs followed at higher temperatures by extensive structural collapse and aggregation [24,25].

A possible structural basis for the specific stabilization by Mg^{2+} of the Z form of poly[d(G-C)] is provided by crystallographic studies in which the hydrated ion was shown to coordinate directly or through hydrogen bonds with the N7 of G and either O6 of G or a neighboring phosphate, depending on the local structure [4,13]. However, it is hazardous to assume that such complexes are stable in solution or at least constitute the dominant species. Thus, the kinetics of the B-Z transition with Mg^{2+} as the counterion are first order both in the present and previous [1] studies, as compared to the more complicated time course characteristic of the transition metals [16] or of Mg^{2+} in mixed solvents, i.e., lowered bulk dielectric [15]. The statistical-mechanical fluid physics formalism also predicts the transition midpoint for $MgCl_2$ with reasonable accuracy [8] and without the necessity for invoking specific interactions. It follows that the role of the anion is crucial. The coordination of Cl^- to amino groups of both purine and pyrimidine bases in Z-form crystals of the tetranucleotide helix [d(C-G)$_2$] has been noted [5]. Whether such interactions apply to the perchlorate ion and serve to rationalize its effectiveness in stabilizing the Z conformation of

poly[d(G-C)] (and the corresponding RNA, poly[r(G-C)] [26]), remains to be established.

3.3. Aggregation and precipitation of the Z form of poly[d(G-C)]

A gel-like fluffy precipitate slowly formed in all poly[d(G-C)] solutions which contained $Mg(ClO_4)_2$ at concentrations equal to or greater than c_t. (If left undisturbed, it accumulated as a thin layer at the bottom of the cuvette!) We confirmed the identity of the precipitate by noting the loss of ultraviolet absorbance in the overlying liquid and by direct recovery of the normal B-DNA spectrum from redissolution of the centrifuged precipitate in low salt buffer. The correlation between the time course of the B-Z transition monitored spectrophotometrically and the development of aggregation was determined quantitatively by the centrifugation assay (fig. 7). Within experimental error, both processes proceeded simultaneously, thereby suggesting that the kinetic coupling is pronounced. That is, as individual molecules of poly[d(G-C)] assume the left-handed helical conformation, they spontaneously and immediately (within the time resolution achieved in this study) engage in association and condensation reactions leading to extensive cross-linking into networks which finally precipitate. That such a circumstance would shift the overall equilibrium in favor of the Z conformation has been stressed previously [7].

The general tendency of polynucleotides in the Z conformation to aggregate (into what has been termed a 'Z*' state [15]) has been well documented [7,15–18,27]. In fact, it has been not well appreciated that this phenomenon pertains even to the Z form of poly[d(G-C)] induced by $MgCl_2$ [15] although in that case the spectroscopic manifestations are not readily evident [1], i.e., the degree of association must be greatly reduced relative to the situation we have reported here. Transition metals also lead to the efficient formation of Z*-DNA [7,16–18]. In summary, the aggregation of Z-DNA is clearly related to the degree of charge neutralization of the polyanionic helix reflecting the distribution of statistically and specifically bound counterions; the involvement of specific solvation structures and hydrophobic interactions is more difficult to assess. The latter may be of particular significance in the case of polymers carrying methyl and halogen substituents in the C5 position of the pyrimidine bases [7,12].

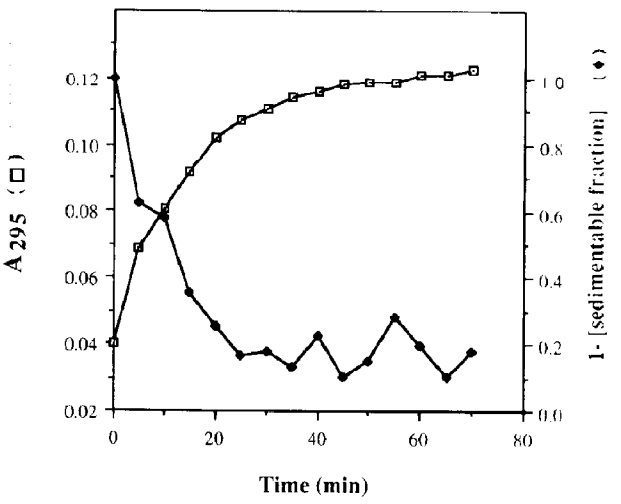

Fig. 7. Correlation between the B-Z transition kinetics of poly[d(G-C)] in $Mg(ClO_4)_2$ monitored by A_{295} and by determination of the fractional sedimentability. The reaction was initiated by the addition of DNA (final concentration 50 μM) to a 0.4 M $Mg(ClO_4)_2$ solution containing 10 mM Hepes-NaOH (pH 7.0). The kinetics were recorded continuously at 295 nm. Aliquots were removed from the 3.8 ml reaction volume at 5-min intervals and centrifuged. The supernatants were diluted 1:4 with buffer and the A_{260} recorded. The fractional sedimentability was calculated by normalization to the absorbance calculated from the dilution of the stock DNA solution (the uncertainty in this procedure is about 10%). Due to the finite time of transfer and centrifugation, the values for the initial phase of rapid reaction are systematically skewed from their nominal positions (by approx. 1 min).

3.4. Aggregation and precipitation of M. lysodeikticus DNA in $Mg(ClO_4)_2$

The GC content of the DNA of the microorganism *M. lysodeikticus* is 72% [28] and we tested the possibility that a detectable fraction of the DNA would assume the Z form in the presence of $Mg(ClO_4)_2$. *M. lysodeikticus* DNA was rapidly mixed with $Mg(ClO_4)_2$ to a final salt con-

centration of 1.1 M and an A_{260} of 0.5. We recorded the ultraviolet absorbance spectrum before mixing, monitored A_{295} at high instrument sensitivity after mixing, and finally recorded the spectrum again several hours later. By both criteria, there was less than 0.3% conversion of the DNA sample to the Z form. However, at salt concentration above 1.1 M, *M. lysodeikticus* DNA solutions showed signs of aggregation, i.e., light scattering.

We pursued this observation by preparing an *M. lysodeikticus* DNA sample ($A_{260} = 2.3$) in 2.2 M $Mg(ClO_4)_2$ in parallel with similar solutions of calf thymus DNA and poly[d(A-T)]. After 2 days, there were definite signs of precipitation in the *M. lysodeikticus* DNA solution but not in the case of the other two DNAs. The aggregation of the DNA with 72% GC (but not of DNA with 41 or 0% GC content) at high concentrations of $Mg(ClO_4)_2$ appears to indicate that in spite of the inability of this salt to bring about a spectroscopically detectable B-to-Z transition in the linear natural sequences, the particular influences of the constituent ions of $Mg(ClO_4)_2$ on G · C base-pairs are being expressed. It is worth noting that neither the poly[d(G-C)] nor the *M. lysodeikticus* DNA precipitates could be redissolved upon further increasing the $Mg(ClO_4)_2$ concentration to near the solubility limit of approx. 3 M.

Zimmer and Luck [29] investigated the effects of salt addition on the CD spectra of various DNAs, one of the combinations being a DNA sample of 72% GC content and $NaClO_4$ [29]. As the salt concentration was increased from 0 to 7.2 M changes were observed in the CD spectrum, but they did not resemble the characteristic inversion during the B-to-Z transition of poly[d(G-C)] [1] (fig. 2). Our conclusions concerning the lack of a perceptible Z structure in the *M. lysodeikticus* DNA/$Mg(ClO_4)_2$ system are in agreement with these observations.

4. Concluding remarks

DNA is demonstrably a very polymorphic macromolecule [30] and the free energy differences which mediate the complex conformational equilibria are determined by subtle interplays of multiple structural and environmental factors [3,9,10], as emphasized by the phenomena reported here and elsewhere. In the case of natural DNA in vivo, the intervention of multiple ligands and global-local topological features will determine the expression of the Z conformation in susceptible DNA sequences. In particular, it can be anticipated that specific proteins will be involved [6].

Acknowledgements

The authors thank Mrs. M. van der Ploeg for some of the experimental work and Dr. Robert Clegg for valuable discussions regarding several aspects of this study.

References

1 F.M. Pohl and T.M. Jovin, J. Mol. Biol. 67 (1972) 375.
2 F.M. Pohl, Nature 260 (1976) 365.
3 D.M. Soumpasis and T.M. Jovin, in: Nucleic acids and molecular biology, vol. 1, eds. F. Eckstein and D.M. Lilley (Springer Verlag, Berlin, 1987) in the press.
4 A.H.-J. Wang, G.J. Quigley, F.J. Kolpak, G. van der Marel, J.H. van Boom and A. Rich, Science 211 (1981) 171.
5 H. Drew, T. Takano, S. Tanaka, K. Itakura and R.E. Dickerson, Nature 286 (1980) 567.
6 A. Rich, A. Nordheim and A.H.-J. Wang, Annu. Rev. Biochem. 53 (1984) 791.
7 T.M. Jovin, L.P. McIntosh, D.J. Arndt-Jovin, D.A. Zarling, M. Robert-Nicoud, J.H. van de Sande, K.F. Jorgenson and F. Eckstein, J. Biomol. Struct. 1 (1983) 21.
8 D.M. Soumpasis, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 5116.
9 D.M. Soumpasis, in: Proc. 4th Conversation Biomolecular Stereodynamics, eds. R.H. Sarma and M.H. Sarma (Adenine Press, New York, 1986) p. 47.
10 D.M. Soumpasis, J. Wiechen and T.M. Jovin, J. Biomol. Struct. Dynam. 4 (1987) 535.
11 D.M. Soumpasis, M. Robert-Nicoud and T.M. Jovin, FEBS Lett. 213 (1987) 341.
12 M. Behe and G. Felsenfeld, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 1619.
13 R.V. Gessner, G.J. Quigley, A.H.-J. Wang, G.A. van der Marel, J.H. van Boom and A. Rich, Biochemistry 24 (1985) 237.
14 K. Kodama, Methods of quantitative inorganic analysis (Wiley, New York, 1963) p. 398.
15 J.H. van de Sande and T.M. Jovin, EMBO J. 1 (1982) 115.
16 J.H. van de Sande and T.M. Jovin, EMBO J. 1 (1982) 777.
17 T.M. Jovin, J.H. van de Sande, D.A. Zarling, D.J. Arndt-Jovin, F. Eckstein, H.H. Füldner, C. Greider, I. Grieger, E.

Hamori, B. Kalisch, L.P. McIntosh and M. Robert-Nicoud, Cold Spring Harbor Symp. Quant. Biol. 47 (1983) 143.
18 W. Zacharias, J.E. Larson and J. Klysik, J. Biol. Chem. 257 (1982) 2775.
19 F.M. Pohl, Cold Spring Harbor Symp. Quant. Biol. 47 (1983) 113.
20 J.H. Prestegard, and S.J. Chan, J. Am. Chem. Soc. 91 (1969) 2843.
21 W.R. Bauer, Biochemistry 11 (1972) 2915.
22 W.R. Bauer, J. Mol. Biol. 67 (1972) 183.
23 J.C. Wang, J. Mol. Biol. 43 (1969) 24.
24 G.S. Ott, D. Bastia and W. Bauer, Biochim. Biophys. Acta 518 (1978) 216.
25 G.S. Ott, R. Zeigler and W.R. Bauer, Biochemistry 14 (1975) 3431.
26 K. Hall, P. Cruz, I. Tinoco, Jr, T.M. Jovin and J.H. van de Sande, Nature 311 (1984) 584.
27 T.J. Thomas and V.A. Bloomfield, Biochemistry 24 (1985) 713.
28 H.A. Sober, Handbook of biochemistry, selected data for molecular biology, 2nd edn. (Chemical Rubber Co., Cleveland, 1970).
29 C. Zimmer and G. Luck, Biochim. Biophys. Acta 361 (1974) 11.
30 S.B. Zimmerman, Annu. Rev. Biochem. 51 (1982) 395.