Condensation of Supercoiled DNA Induced by $MnCl_2$

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ABSTRACT Multivalent cations condense DNA in vitro, but it had been thought that a valence of at least +3 was required in aqueous solution. We have found that Mn^{2+} can produce toroidal condensates of supercoiled plasmid DNA, but not of linearized plasmid. Mg^{2+} does not cause condensation, and neither $MgCl_2$ nor NaCl can negate the effect of $MnCl_2$, indicating that the condensation mechanism with Mn is not primarily electrostatic. Supercoiled MnDNA is more extensively digested than the linear form by S1 nuclease. Supercoiling appears to cooperate with Mn^{2+} in stabilizing helix distortions and also provides a "pressure" that enhances lateral association.

Within viruses and cells, DNA generally exists in a highly compact, condensed state. Since the discovery that DNA condensation (defined as the packing of the random coil form of DNA into small ordered structures with distinct morphology) can be produced in vitro by the naturally occurring polyamines spermidine^{3+} and spermine^{4+} (Chattoraj et al., 1978; Gosule and Schellman, 1976), the actions of multivalent cations have been intensively studied (Widom and Baldwin, 1980, 1983; Wilson and Bloomfield, 1979) for clues to the mechanism of DNA packaging in viruses and condensation in chromatin. Because multivalent cations largely neutralize the high negative charge density of the DNA, thus reducing interhelix electrostatic repulsion, it has seemed almost self-evident that cations provoke DNA condensation through an electrostatic mechanism. Wilson and Bloomfield (1979) used counterion condensation theory (Manning, 1978) to calculate that DNA condensation occurs when 89–90% of the DNA charge is neutralized. This condition can be met only by cations of charge +3 or higher in aqueous solution, although +2 ions will serve in solutions of lower dielectric constant such as water-alcohol mixtures (Bloomfield et al., 1994; Wilson and Bloomfield, 1979). Theoretical estimates of the various contributions to the free energy of condensation also implicated electrostatics as the major factor (Marquet and Houssier, 1991; Riemer and Bloomfield, 1978). However, other studies have suggested that condensing ligands may also act by crossbridging neighboring helices (Egli et al., 1991; Schellman and Parthasarathy, 1984), perturbing hydration structure (Rau and Parsegian, 1992b), or perturbing DNA helix structure (Bloomfield, 1991; Knoll et al., 1988). In this paper we show that Mn^{2+} can produce condensation of supercoiled DNA, the first time that such an effect has been observed with a divalent cation in aqueous solution at room temperature. Both the base-binding character of the metal and the supercoiled topology are required for condensation, indicating that factors other than electrostatics are dominant.

Mn^{2+}, like many other transition metals, stabilizes the conformation of DNA at low concentration, but destabilizes DNA secondary structure at higher concentration (Eichhorn and Shin, 1968; Luck and Zimmer, 1972). It interacts electrostatically with the negatively charged phosphate backbone at low concentration, but binds to bases with high affinity at higher concentration (Izatt et al., 1971), thus perturbing hydrogen bonding in base pairs. Double-stranded DNA is aggregated by divalent transition metal ions at elevated temperature (Duguid et al., 1993; Knoll et al., 1988; Yurgaitis and Lazurkin, 1981) or osmotic pressure (Rau and Parsegian, 1992a,b).

Supercoiling of DNA plays key roles in replication, transcription, and recombination. Nearly all of the DNA in living organisms is negatively supercoiled. Supercoiling facilitates secondary structure change, including the B-to-Z transition (Gruskin and Rich, 1993; Peck et al., 1986; Singleton et al., 1982), helix opening (Aboul-ela et al., 1992; Lee and Bauer, 1985), and cruciform formation (Murchie and Lilley, 1992; Panyutin et al., 1984). Cryoelectron microscopy (Adrian et al., 1990) shows that closed circular DNA is more tightly supercoiled in 10 mM $MgCl_2$ than in TE buffer (10 mM Tris-HCl, 1 mM EDTA), which might be expected to enhance the close lateral association of DNA helices required for condensation.

Since both Mn^{2+} and supercoiling facilitate changes in DNA secondary structure, the two together should provide a good test of the hypothesis that distortion of the B-DNA helix is involved in at least some cases of DNA condensation. We used light scattering to detect condensation or aggregation, electron microscopy to characterize the morphology of the condensed particles, and S1 nuclease digestion to probe for disrupted secondary structure.

The random coil conformation of DNA scatters weakly relative to condensed DNA. When DNA molecules condense to a smaller structure, or when the average number of DNA molecules in a scattering particle increases by aggregation, the intensity of scattered light increases. The total intensity of light scattered at 90°, 1 h after addition of $MnCl_2$ to 5 μg/ml pUC 18, increases sharply with $MnCl_2$ concentration, reaching a plateau at 50 mM (Fig. 1). The intensity of scattering from linear pUC 18 also increases after addition of

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Mn^{2+}, but the increase is significantly less than that characteristic of strongly condensed DNA. Supercoiling also makes condensation easier in that less Mn is required. For example, at 2 mM Mn, the scattering intensity of linear DNA is virtually unchanged, while that of the supercoiled form has increased about threefold. Above 10 mM the shapes of the two curves are similar, although the limiting intensity from the supercoiled sample remains more than two times higher.

Electron-microscopy shows that toroids are produced upon adding Mn^{2+} to supercoiled pUC 18 solutions (Fig. 2 *A*). The toroids have an average inner radius of 123 Å and outer radius of 301 Å, corresponding to 5.6 DNA molecules/toroid, assuming hexagonal close packing of cylinders with an interhelical spacing of 28 Å (Arscott et al., 1990; Rau and Parsegian, 1992a). These values are similar to those for condensates of plasmid DNA induced by trivalent cations (Arscott et al., 1990). In contrast, linear plasmids formed thin, fibrous structures with no definite morphology (Fig. 2 *B*), which implies that the moderate increase in scattering intensity was due to aggregation rather than condensation.

To show that condensation of supercoiled DNA is a feature of the transition metal character of Mn^{2+}, and not a result of general electrostatic behavior, we first used light scattering to demonstrate that Mg^{2+} at any concentration does not condense DNA in our standard buffer (data not shown). We then tested the ability of Mg^{2+} and Na^+ to interfere with Mn^{2+}-induced condensation, using $[Mn^{2+}] = 25$ mM and 100 mM,

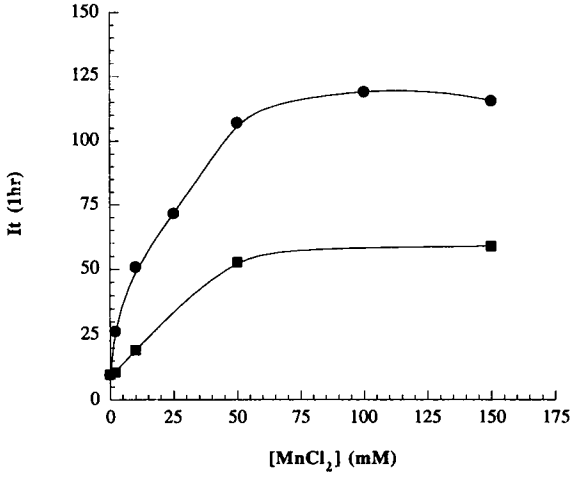

FIGURE 1 Total scattering intensity 1 h after addition of $MnCl_2$ to supercoiled (●) and linearized (■) DNA. Supercoiled pUC 18 plasmid (2686 bp) was prepared using standard procedures (Sambrook et al., 1989). Linearized pUC 18 was obtained by treating supercoiled plasmid with the single-site restriction enzyme *ScaI*, followed by phenol-chloroform extraction and ethanol precipitation. Light scattering experiments were performed with an argon-ion laser operating at 488 nm and a power output of 75 mW. Buffers were filtered through 0.22-μm GS Millipore filters before mixing. Glassware was extensively rinsed with filtered water and dried before using. DNA stock solutions were filtered through 0.45-μm GS Millipore filters before measuring the concentration by A_{260}. In this and all subsequent figures, solutions contained 5 μg/ml pUC 18, 1 mM NaCl, 1 mM cacodylate buffer (cacodylic acid, titrated with Na cacodylate to pH 7.8), and experiments were carried out at room temperature, ~25°C.

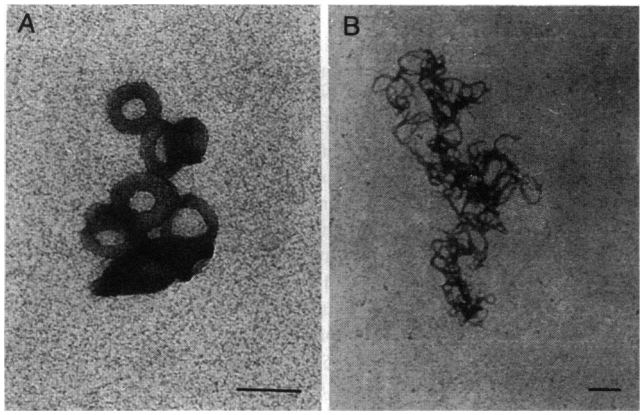

FIGURE 2 Electron micrographs of supercoiled (*A*) and linearized (*B*) plasmid pUC 18, 1 h after addition of 100 mM $MnCl_2$ (scale bar = 1000 Å). Previously published EM procedures were used (Arscott et al., 1990).

in the transition and plateau regions, respectively. Fig. 3 shows that the scattering intensity decreases only slightly with increasing NaCl concentration up to 400 mM and $MgCl_2$ concentration up to 100 mM. This is in marked contrast to DNA condensation induced by +3 or +4 ions, which is

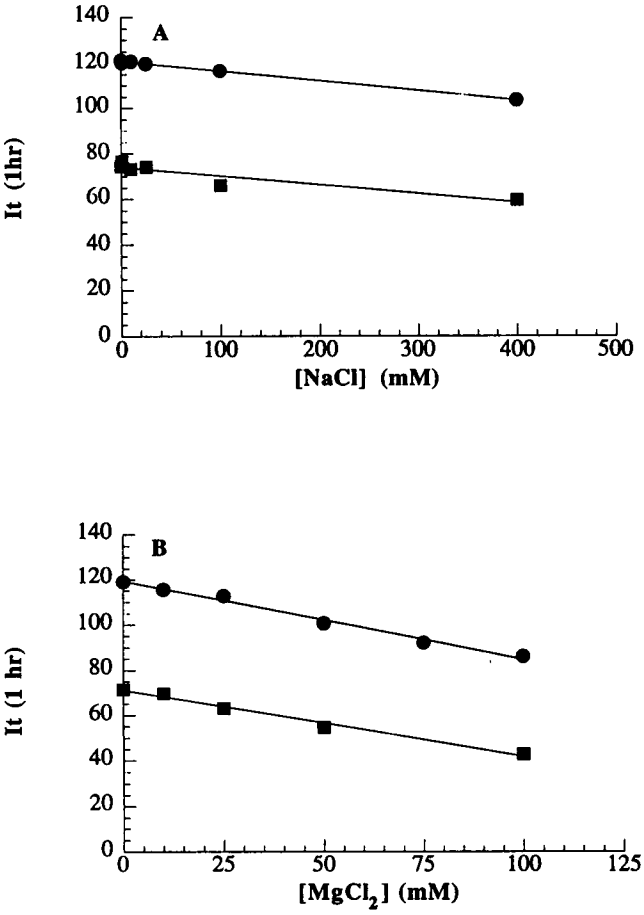

FIGURE 3 Total scattering intensity 1 h after addition of 25 mM (■) and 100 mM (●) $MnCl_2$ to a DNA solution prepared with the indicated concentration of competing cation: (*A*) NaCl; (*B*) $MgCl_2$.

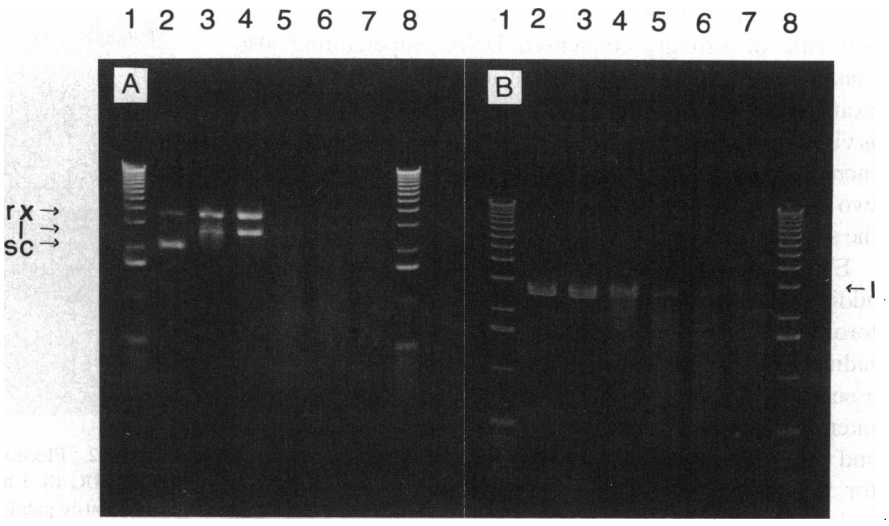

FIGURE 4 S1 nuclease digestion of supercoiled (*A*) and linear (*B*) pUC 18 with indicated concentrations of $MnCl_2$. *Lanes 1* and *8*, 1-kb ladder; *lane 2*, no $MnCl_2$; *lane 3*, 2 mM; *lane 4*, 10 mM; *lane 5*, 25 mM; *lane 6*, 50 mM; *lane 7*, 100 mM. 30 μl of DNA solution containing the given amount of $MnCl_2$ was incubated at room temperature for 1 h. S1 nuclease at 700 U/μl (GIBCO BRL) was diluted with 1X reaction buffer (50 mM NaCl, 20 mM Na acetate, 0.1 mM $ZnCl_2$) to 7 U/μl immediately before reaction, then 1 μl of diluted enzyme and 3.5 μl of 10X reaction buffer were added to each sample and incubated at 37°C for 20 min. Samples were left on ice until performance of gel electrophoresis.

rapidly reversed by addition of mono- or divalent cations (Widom and Baldwin, 1983). This implies that Mn^{2+}-induced condensation is not dominated by electrostatics, but instead depends on supercoiling and binding of Mn^{2+} to the DNA bases.

Since manganese ions and DNA supercoiling induce helix opening under certain conditions, we tested the S1 nuclease susceptibility of MnDNA for evidence of single-stranded regions or helix distortion (Fig. 4). At 2 mM Mn^{2+}, supercoiled DNA was nicked into the relaxed form and converted into the linear form. At 10 mM Mn^{2+} and higher, supercoiled molecules were completely digested. Linear pUC 18 was considerably more resistant to nuclease. It was unattacked at 2 mM Mn^{2+} and only slightly digested at 10 mM. Some linear DNA remains even at 100 mM Mn^{2+}. Although supercoiled plasmids have more secondary structural perturbations than the linear form, the UV spectra of both forms from 200 to 320 nm was indistinguishable from that of normal B-DNA even at 100 mM Mn^{2+} (data not shown). Thus we conclude that the overall secondary structure of MnDNA remains intact, but that there are occasional disruptions in the supercoiled form that somehow facilitate condensation.

It is interesting that the rate of S1 nuclease attack on the initially supercoiled sample does not decelerate to that of the linearized sample, even when it must be nicked at an early stage. One possible reason for the continued higher rate of digestion of the supercoiled form is that Mn^{2+} binding produces several distorted regions, and that dissociation of Mn^{2+} from regions other than the first-nicked site (which would allow relaxation of these regions to an undistorted, S1-resistant form) is slow compared with the rate of S1 attack. We have no evidence to support this hypothesis or to distinguish it from other possibilities, however.

Mn^{2+}-induced DNA condensation occurs at relatively high Mn concentrations, with a transition midpoint of 25 mM (Fig. 1). This contrasts with the much lower concentration of cations required in the more standard type of condensation (Widom and Baldwin, 1980; Wilson and Bloomfield, 1979). For example, Mg^{2+}-induced condensation in a 50% methanol-water mixture (dielectric constant, 63) occurs at 0.8 mM (Wilson and Bloomfield, 1979). This high concentration is consistent with a mass action effect in which Mn^{2+} binds to the bases, thereby perturbing normal base pairing. Raman spectroscopy of calf thymus DNA with 100 mM $MnCl_2$ shows that the metal binds to purine N7 and pyrimidine N3, induces partial disordering of the B-form backbone, and destabilizes base stacking and base pairing (Duguid et al., 1993). Therefore, it is reasonable to assume that secondary structure perturbation is responsible for, or at least connected with, Mn^{2+}-induced DNA condensation.

Negative supercoiling of a plasmid may facilitate condensation in two ways. First, it stabilizes distortions that unwind the DNA helix. Second, it brings into close proximity, with roughly parallel orientation, long stretches of DNA (Boles et al., 1990). In that way, it may be viewed to produce a "pressure" pushing DNA helices together, akin to the osmotic stress produced by high concentrations of neutral polymer. Rau and Parsegian (1992a) found that osmotic stress causes DNA to assemble into hexagonal arrays of parallel rods. Net attraction was measured with Mn^{2+} at slightly elevated temperatures as the DNA helices were pushed together, an effect explained by reorganization of the water layers surrounding the DNA to produce attractive hydration forces.

Electron microscopy (Adrian et al., 1990; Bednar et al., 1994) shows that divalent ions facilitate the close approach of two supercoiled DNA segments. Increasing salt reduces the effective diameter of DNA (Stigter, 1977), permitting the close approach of DNA segments. Analysis of the probability of knot formation shows that Mg^{2+} not only shields the negative charged DNA molecules, but may also induce an attractive potential between DNA segments at a concentration of ~50 mM (Shaw and Wang, 1993). In our case, supercoiling of DNA which holds the two helices together in the presence of Mn^{2+} may stabilize a nucleus that enables other DNA segments to join an ordered condensed structure (Bloomfield, 1991). Neither nicked circular nor linear DNA can hold extensive stretches in parallel juxtaposition in this

way, so we surmise that a crucial contribution to nucleation of DNA condensation is missing in these forms.

The mechanism of condensation of supercoiled DNA by Mn^{2+} appears to be quite different from that believed to underlie the more extensively studied condensation induced by higher valence cations. Supercoiling brings long stretches of the DNA into close, nearly parallel juxtaposition; Mn^{2+}, like any cation, facilitates this close approach by reducing the coulombic repulsion between DNA segments. Mn^{2+} and supercoiling stress cooperate in producing and stabilizing localized distortion of the double helix that somehow produce, or augment, an attractive force between helices. It cannot be ruled out that such distortions occur even in DNA condensation produced by tri- and tetravalent cations (Bloomfield and Wilson, 1981; Thomas and Bloomfield, 1985). Whether such helical distortions are directly involved in crossbridging between helices, or whether they modulate electrostatic or hydration forces, remains to be determined.

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