THE SUPERHELIX DENSITY OF BACTERIOPHAGE PM2 DNA, DETERMINED BY A VISCOMETRIC METHOD

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1. Introduction

Since the discovery by Weil and Vinograd [1] that the closed circular duplex form of polyoma DNA contains right-handed superhelical turns or twists, similar superhelical turns have been found in all other closed circular duplex DNAs found in nature [2–6]. In a closed circular duplex DNA right-handed superhelical turns are introduced when the number of base pairs per helix turn increases. The current interest in the exact number of superhelical turns in closed circular duplex DNA stems from the information this may provide about the pitch of the DNA helix in vivo at the time when DNA ligase closes the last single-stranded break in the circle [7, 8].

The most popular method of determining the number of superhelical turns in DNA makes use of the fact that increasing concentrations of intercalating dyes, like ethidium, first lead to the unwinding of the right-handed superhelical turns and at higher dye concentrations to the introduction of left-handed superhelical turns [8–10]. If the degree of unwinding of the Watson–Crick helix per dye molecule intercalated is known (probably 12° in the case of ethidium), a determination of the number of dye molecules required to completely convert the twisted circle into an open circle, allows the calculation of the number of superhelical turns present in the DNA in the absence of dye [11, 12]. As first pointed out by Wang [13] this calculated number of superhelical turns is higher than the real number, because of the free energy of superhelix formation. This complication is disregarded in the remainder of this paper.

The conversion of twisted into open circles as a function of dye concentration, has been followed by sedimentation velocity and by sedimentation equilibrium analysis [8, 9]. Both methods require centrifuge runs at many different dye concentrations and are therefore laborious and expensive. A simple alternative to these analytical procedures was suggested by the observation of Opschoor et al. [14], that the intrinsic viscosity of closed circular duplex DNA is only about 40% of the intrinsic viscosity of the corresponding open circle. This should allow an accurate analysis of the unwinding of superhelical turns in a twisted circle as a function of dye concentration. In this paper we show that this is indeed the case. To test the method we have used the DNA of bacteriophage PM2 [15]. Espejo et al. [16] have demonstrated that PM2 DNA consists of twisted, closed circular duplexes with a molecular weight of 6×10^6, but the number of superhelical turns in this DNA has not been studied previously.

2. Methods and materials

2.1. *Viscosity measurements*

Intrinsic viscosities were measured in a rotating cylinder viscometer [17], manufactured by Krannich GmbH, Göttingen, West Germany, at DNA concentrations of 20–30 μg/ml and in a volume of 2.25 ml. The viscometric titration of supertwists in DNA with

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ethidium was carried out by adding a small volume of ethidium solution with a microsyringe to a stock solution of DNA. After measuring the viscosity of a sample of this mixture, the sample was added back to the stock solution and after adding a second aliquot of ethidium and thorough mixing a new sample was taken for viscometry. This procedure was repeated until the titration was completed.

2.2. *Sedimentation analyses*

Sedimentation coefficients were determined in a MSE analytical ultracentrifuge, equipped with an ultraviolet scanningsystem and multiple cell device. The boundary velocity experiments were carried out with DNA concentrations of 15–25 μg/ml and a volume of 0.6 ml per sector. Direct scans were made at a wavelength of 260 nm. In each centrifuge run 3 double sector cells were used with a pathlength of 20 mm; 5 sectors contained DNA samples, the sixth only solvent. The ethidium concentration in each sector was different and after each run it was altered by adding a minute amount of concentrated ethidium solution with a microsyringe.

2.3. *Calculation of the number of supertwists*

The number of supertwists was calculated from the Scatchard formula:

$$r = \frac{n\,c_f}{K + c_f} = \frac{c_b}{[\mathrm{DNA}]} \qquad (1)$$

and

$$c_b + c_f = c_{total} \qquad (2)$$

where r is moles of ethidium bound (c_b) per mole DNA nucleotide; c_f is the free ethidium concentration. K is the dissociation constant of the ethidium DNA complex and n is the molar ratio of intercalated dye to nucleotide at infinite dye concentration. K and n values were taken from the data of Le Pecq and Paoletti [18]. Equations (1) and (2) were combined to give a square function in c_b which was solved. The ratio r was transformed to superhelical density (the number of supertwists per 10 base pairs) as indicated in equation (3)

$$\text{superhelical density} = r \times 20 \times \frac{12}{360} = \frac{2}{3} r \qquad (3)$$

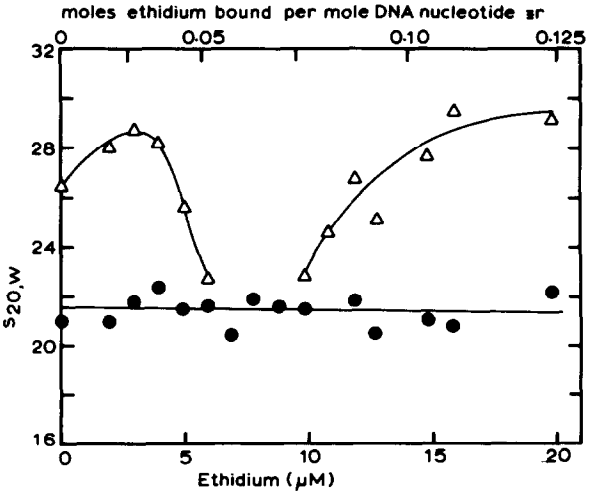

Fig. 1. Variation of the sedimentation coefficients of the twisted circular form (△ —— △) and the open circular form (● —— ●) of PM2 DNA as a function of the ethidium concentration. Sedimentation coefficients were determined by boundary sedimentation at 20,000 rpm and at 20°; PM2 DNA, 23.0 μg/ml; solvent, 0.515 M NaCl, 1.5 mM sodium citrate (pH 7.0). r values were calculated as described in Methods and materials.

by assuming that every intercalated ethidium molecule unwinds the helix by 12° [11, 12].

2.4. *Materials*

DNA from phage PM2 was isolated by the procedure of Espejo et al. [15, 16]. Ethidium was a gift from Dr. G. Woolfe of Boots Pure Drug Co. Ltd., Nottingham, England and used without further purification. Ethidium concentrations were calculated from the $A_{480\ nm}$ in suitable dilutions in 50 mM tris-HCl (pH 8.0), using a molar extinction coefficient of 5600 [19].

3. Results

Figs. 1 and 2 present a comparison of the effects of increasing concentrations of ethidium on the sedimentation coefficient and the viscosity of closed circular PM2 DNA. The two curves represent mirror images and the maximal viscosity in fig. 2 is reached at approximately the same ethidium concentrations as the minimal sedimentation coefficient in fig. 1,

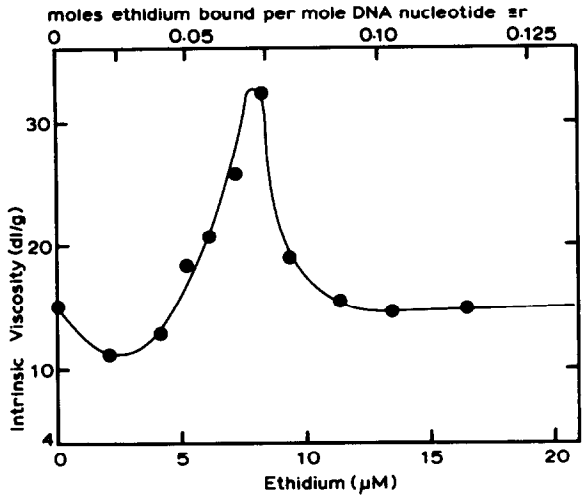

Fig. 2. Variation of the intrinsic viscosity of the twisted circular form of PM2 DNA as a function of the ethidium concentration. Intrinsic viscosities were measured at 20°; the DNA concentration was 19.5 μg/ml; solvent, 0.515 M NaCl, 1.5 mM sodium citrate (pH 7.0). *r* values were calculated as described in Methods and materials.

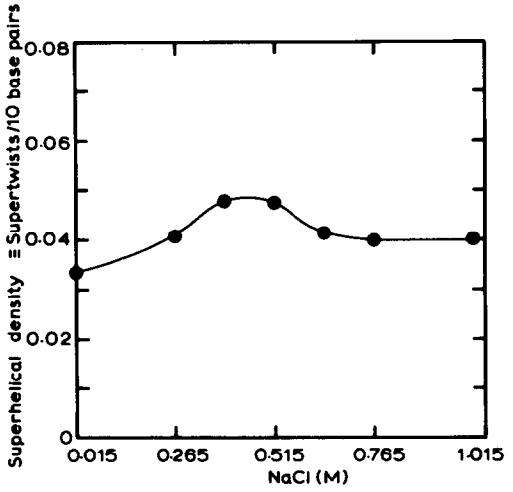

Fig. 4. The superhelical density (number of supertwists per 10 base pairs) as a function of the salt concentration. All points were determined with the viscometric method. The NaCl solutions contained 1.5 mM sodium citrate (pH 7.0). The calculations were carried out as described in Methods and materials.

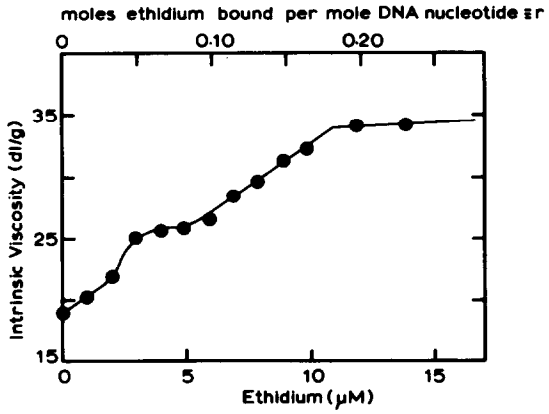

Fig. 3. The variation of the intrinsic viscosity of a mixture of twisted and open circular PM2 DNA as a function of the ethidium concentration. Intrinsic viscosities were measured at 20°; DNA concentration, 19.0 μg/ml consisting of 28% twisted and 72% open circular form as determined by band sedimentation in 3 M CsCl; solvent, 15 mM NaCl, 1.5 mM sodium citrate (pH 7.0). *r* values were calculated as described in Methods and materials.

i.e. at 0.066–0.071 mole ethidium bound per mole nucleotide. This shows that the viscometric technique can indeed be used as an alternative to sedimentation analysis. The most accurate results are obtained in the viscometer with pure closed circles; however, the titration can still be performed if a large fraction of open circles is present, as shown by the results in fig. 3.

To allow comparison of the results obtained with PM2 with those obtained for other DNAs, we have determined the number of superhelical turns in PM2 DNA at different salt concentrations. The results, presented in fig. 4, confirm the previous finding by Wang [13] that the apparent number of superhelical turns is higher at high than at low ionic strength. It does not increase linearly with ionic strength, however, but passes through a local maximum around 0.5 M NaCl. Although this may mean that the average rotation per base pair of the DNA helix does not change linearly with ionic strength more trivial explanations were not excluded. If, for instance, the binding constant of the ethidium–DNA complex is affected in a complex way by contaminating protein or divalent cations, possibly present, this could also explain the results of fig. 4.

The average intrinsic viscosity of closed circular PM2 DNA in 15 mM NaCl, 1.5 mM sodium citrate (pH 7.0) was 11 dl/g in 4 experiments (range 9–15). The intrinsic viscosity of the circle, maximally untwisted by ethidium, was on the average 2.3 times higher than that of the closed circles (14 experiments, range 1.5–3.0). This ratio is very similar to the 2.5 reported by Opschoor et al. [14] for the open and closed circular forms of phage ΦX174 replicative form DNA. Moreover, the intrinsic viscosity of theopen PM2 DNA circle of 26 dl/g is in good agreement with the value 24 dl/g, predicted by equation (8) of Opschoor et al. [14] for a non-twisted circular DNA with the size of PM2 DNA.

4. Discussion

We conclude from these results that the viscometric titration of superhelical turns in DNA can be used as an accurate and cheap alternative to the titration by sedimentation velocity experiments. As only relative viscosities are determined, the measurements can be made at relatively high shear stresses, so that the whole titration can be completed in a few hours. Only 60 μg of DNA are required and these can be recovered intact after the experiments. A significant advantage over the sedimentation analyses is, in addition, that the viscometric titration can be carried out in any solvent system, also at very low ionic strength.

The number of superhelical turns per unit length of DNA found by us in PM2 DNA is very similar to that observed for other circular DNAs, as shown by the data presented in table 1. Apparently, the intra-cellular conditions responsible for the presence of superhelical turns in circular DNAs are similar in a marine *Pseudomonas, Escherichia coli* and the nucleus and mitochondria of eukaryotes.

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Table 1
Comparison of superhelical densities of circular DNAs from various organisms.

Circular DNA from:	Mol. wt. ($\times 10^{-6}$)	Number of twists	Superhelical density	Ref.
E. coli 15 plasmid	1.45	8.5	0.039	20
Replicative form phage ΦX 174	3.4	19	0.037	20
	3.4	22	0.043	22
	3.4	12	0.024*	11
Phage $\lambda b_2 b_5 c$	26	100	0.026	20
Phage λcI857	33	136	0.027	20
Virus SV40	3.3	16	0.029	8
Polyoma virus	3.3	15	0.027	24
Bovine papilloma virus	4.9	18	0.025*	21
Chicken liver mit.	10	40	0.027*	10
Rat liver mit.	10	40	0.027	22
Sea urchin mit.	10	36	0.024	23
HeLa cell mit.	10	28	0.019	23
Phage PM2	6	38	0.043	
	6	30	0.033*	

The superhelical density (the number of supertwists per 10 base pairs) was determined in 3 M or higher CsCl concentrations with the exception of the values marked with an asterisk; these were determined in 50 mM tris-Cl or 0.015 M NaCl, 0.0015 M sodium citrate.

Note added in proof

A viscometric superhelix determination of PM2 DNA was also recently reported by Révet et al. [25].

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