Tewksbury, D. A. (1983) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 42*, 2724–2728.
Tewksbury, D. A., Dart, R. A., & Travis, J. (1979) *Circulation 59/60* (Suppl. II), 132 (Abstr. 513).
Tewksbury, D. A., Dart, R. A., & Travis, J. (1981) *Biochem. Biophys. Res. Commun. 99*, 1311–1315.
Tosi, M., Young, R. A., Hagenbüchle, O., & Schibler, U. (1981) *Nucleic Acids Res. 9*, 2313–2323.
Travis, J., & Salvesen, G. S. (1983) *Annu. Rev. Biochem. 52*, 655–709.
Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J., Hirose, T., & Itakura, K. (1979) *Nucleic Acids Res. 6*, 3543–3557.

Condensation of Bacteriophage ϕW14 DNA of Varying Charge Densities by Trivalent Counterions†

Jülyet Aksiyote Benbasat*

ABSTRACT: Bacteriophage ϕW14 DNA carries the hypermodified, positively charged (2+) base α-putrescinylthymine (putThy) and consequently exhibits a decreased average linear charge density compared to the conventional B-form DNA helix. Noting that the unusual physical characteristics may contribute to the collapse properties of this DNA and facilitate the exceptionally high density of packaging of its genome in ϕW14, I used total intensity light scattering to determine in vitro the critical concentrations of spermidine (Spd, 3+) required to induce the cooperative, monomolecular collapse of wild-type and mutant ϕW14 DNA samples and quasi-elastic light scattering to compare the dynamic characteristics of the compacted particles. The DNA samples carried various percentages of the modified base with average charge spacings ranging from 1.3 to 2.2 Å in comparison to T4 phage DNA (1.7 Å). The results are analyzed and discussed both from a general theoretical point of view according to the counterion condensation theory of Manning [Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179–246] and from the more specialized aspect of DNA packaging in ϕW14. In accord with theory, DNAs of lower charge density require a considerably higher critical counterion concentration (up to 118 μM Spd), whereas the outside diameter of the toroidal condensates, which they form, varies only marginally. Specific ion effects were probed by substituting hexaamminecobalt(III) (Hc, 3+) for Spd. Hc appears to be more efficient than Spd: it induces the collapse of all DNA samples at only one-sixth the critical concentration of Spd, and its condensates are 30% smaller (1072–1142 Å vs. 744–800 Å) except for wild-type ϕW14 DNA, which forms Hc-collapsed particles indistinguishable from Spd-induced condensates. Collapse occurs, again with the exception of wild-type ϕW14 DNA, when ~89% of the charges on each DNA are neutralized by territorially bound Spd. I conclude that the driving force for condensation clearly is a function of the charge density of the DNA and that the charge distribution may be an important factor in determining the degree of neutralization at which collapse becomes possible. The sample with the lowest charge density, wild-type ϕW14 DNA, does not follow the trends set by the other members of the series. The possibility is discussed that lowering the charge density by covalent modification beyond a threshold may result in the compression of the DNA double helix, thus allowing more information to be carried on a genome, the packagable length of which is determined by the encapsidation mechanism.

The polyamine-induced cooperative transition (Post & Zimm, 1979) of the DNA[1] helix from an extended conformation to a highly condensed structure in vitro (Chattoraj et al., 1978; Gosule & Schellman, 1976, 1978; Wilson & Bloomfield, 1979; Widom & Baldwin, 1980; Allison et al., 1981) is of focal interest in biology, since, in vivo, nucleic acids often occur tightly packaged and require polyamines to achieve and stabilize the compact form (Cohen, 1978, and references cited therein). When the energy requirements for such a conformational change were estimated by using the encapsidation of DNA by bacteriophage T4 as a model system (Riemer & Bloomfield, 1978), it became obvious that electrostatic repulsions vastly dominate other forces, which oppose the collapse of DNA, and that the process can, therefore, be facilitated by neutralization of the high charge density within the condensed DNA domain. According to the purely electrostatic counterion condensation theory of polyelectrolytes (Manning, 1978), neutralization of the polyelectrolyte depends on the condensation of counterions along its backbone in a process driven strictly by the charge density of the polymer and the valence of the ions. The extent of neutralization realized under specified counterion concentrations can be easily estimated from the theory, which therefore provides the means to determine the critical charge conditions beyond which the collapse of DNA becomes spontaneous (see Theory and Data Analysis). These conditions conceivably depend not only on the charge of the counterion and the extended polyelectrolyte but also on the geometry and mechanism of collapse, the balance between DNA–solvent and DNA–DNA contacts (Post

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[1] Abbreviations: DNA, deoxyribonucleic acid; Spd, spermidine; Hc, hexaamminecobalt(III) $[Co^{3+}(NH_3)_6]$; putThy, α-putrescinylthymine; hmPPUra, 5-(hydroxymethyl)uracil 5α-*O*-pyrophosphate; hmCyt, 5-(hydroxymethyl)cytosine; w.t., wild type; am37, conditional lethal mutant of bacteriophage ϕW14 defective in gene 37; am42, conditional lethal mutant of bacteriophage ϕW14 defective in gene 42; QLS, quasi-elastic light scattering.

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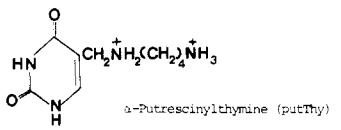
Table I: Modification and Charge Characteristics of T4 and ϕW14 Bacteriophage DNA

source of DNA	modified base[a]	z_M[f]	θ_M[g]	$\|z_p\|$[h]	b_{eff}[i] (Å/charge)
T4	glucosylated 5-hmCyt for all Cyt[b]	0	0.17	1	1.7
w.t. ϕW14	putThy for 50% Thy[b]	+2	0.12	0.76	2.24
am42 ϕW14	putThy for 21% Thy[c]	+2	0.05	0.9	1.8
am37 ϕW14	5-hmPPUra for 50% Thy[d]	−2	0.12	1.24	1.37
w.t. ϕW14 acetylated in vitro	35% Ac-putThy 63% Ac_2-putThy } for 50% Thy[e] 2% putThy	+1 0 +2	0.12	0.9475	1.9

[a] Base composition of wild-type ϕW14 DNA is 51% (G + C); 24% T. [b] From Warren (1980). [c] From Scraba et al. (1983). [d] From Maltman et al. (1981). [e] From Gerhard & Warren (1982). [f] Valence of modified portion of base. [g] Fraction of all bases modified as indicated. [h] Average fractional charge per nucleotide calculated from eq 3. [i] Effective charge spacing = 1/(average charge density), calculated from $b_{eff} = b/z_p$ assuming b = 1.7 Å/charge.

& Zimm, 1979, 1982a,b), and the possible role of the counterions in physically stabilizing the compacted form. Consequently, the critical degree of neutralization may be modulated by the specific structure of the counterion and by the charge density/distribution of the DNA. Undoubtedly, a lot of insight into the general aspects and details of electrostatic interactions that govern "DNA packaging" can be gained by studying these modulations. Specific ion effects (i.e., the effects of counterion structure) on condensation and collapse conditions have been the subject of numerous recent in vitro studies (Thomas & Bloomfield, 1983; Allison et al., 1981; Widom & Baldwin, 1980; Wilson & Bloomfield, 1979), whereas the effects of polyelectrolyte charge density have not been investigated. The present study is concerned with the compaction of a series of DNAs of varying charge density but similar length, mole percent (G + C) content, and base sequence. The series provides a range of charge densities spanning from ~1 negative charge per 1.3 Å to 1 charge per 2.2 Å compared to the conventional B-form DNA density of 1 charge (base) per 1.7 Å (reflecting a pitch of 3.4 Å per base pair).

The source of this unique DNA is bacteriophage ϕW14 of *Pseudomonas acidovorans*. Both the phage and its DNA have unusual properties, which make them attractive subjects for the study of DNA packaging and electrostatic effects. ϕW14 is structurally similar to the T-even phages, except for its short tail fibers and an isometric head comparable to the T4 petite in dimensions (Kropinski & Warren, 1970). Yet, while the petite can only harbor 68% of the full T4 genome length in its proportionately reduced head volume (Eiserling et al., 1970), ϕW14 packages a piece of DNA apparently 96% the length of T4 DNA (Scraba et al., 1983), suggesting a much higher "apparent" packing density than usually observed for phage (Earnshaw & Casjens, 1980). Scraba et al. suggest that the packing efficiency of ϕW14 may be related to the unusual physicochemical characteristics of its genome: ϕW14 DNA has a G + C content of 51 mol %, but exhibits an elevated melting temperature characteristic of a more stable helix [~73% (G + C)] and a much lower buoyant density than expected from DNA exclusively composed of the four natural bases (Kropinski et al., 1973). Both of these irregularities derive from the fact that 50% of the thymine residues in the DNA are replaced with the hypermodified pyrimidine α-putrescinylthymine (putThy) in which the diamine is covalently attached to the 5-position on the base (Kropinski et al., 1973).



α-Putrescinylthymine (putThy)

The pK_a values of putrescine covalently held at the surface of the DNA are likely to be higher than in simple aqueous solution (9.63 and 10.80 in 0.5 M KCl at 25 °C; Barbucci et al., 1970) due to the large negative local electrostatic potential (Wilson & Jones, 1981). Therefore, at pH 7 putThy is expected to have a net charge of 2+ with both the primary and secondary amines protonated. It, therefore, can effectively neutralize two adjacent negative charges on the DNA. As a result, ϕW14 DNA has an average charge density that is lower than normal B-form DNA.

In the present study I used various mutants and posttranscriptional in vitro modifications of wild-type ϕW14 DNA, which provide nucleic acids of average unit charge spacings (but not necessarily base spacings) both shorter (am37 DNA) and longer (wild-type ϕW14, acetylated ϕW14, and am42 ϕW14 DNA) than conventional B-form double helices. The pertinent characteristics of these ϕW14 DNAs are summarized in Table I in comparison to the properties of a B-form control, T4B DNA. In view of the fact that putThy is a relatively bulky residue, some justification is required in attributing the results exclusively to variations in charge density. This justification comes from studies of acetylated ϕW14 DNA. The wild-type ϕW14 DNA and its acetylated derivative have identical base sequence, composition, and frequency of putThy residues. They only differ in their linear charge, since acetylation results in considerable neutralization of the positive charge associated with putThy. Consequently, variance in the condensation characteristics of these two samples can be attributed to their difference in charge and any secondary structural effects this difference may induce.

Amber 42 is a conditional lethal mutant of ϕW14 that under nonpermissive conditions synthesizes a genome with only half the putThy residues of wild type (Miller et al., 1982, 1983). It packages a piece of DNA that has an average charge density intermediate between wild-type and acetylated ϕW14 DNA and is ~10% shorter than a complete genome (Scraba et al., 1983). In this case, too, the decisive feature of the nucleic acid is its charge density, since the minor length difference is not likely to modify the condensation behavior (Widom & Baldwin, 1980).

Thymine and putThy arise at the polynucleotide level during ϕW14 DNA biosynthesis by in situ modification of 5-hmPPUra. Under nonpermissive conditions amber mutants defective in gene 37 cannot process the pyrophosphorylated residues to form putThy (Maltman et al., 1981). The actual valence of the pyrophosphates in experimental buffer has not been determined, but if their pK_a values and the highly negative environment are considered, a charge between 1− and 2− can be expected. Consequently, am37 DNA has a higher negative charge density than a Watson–Crick double helix (if B-form is retained) while the original base sequence and contents of wild-type ϕW14 DNA are retained.

Ideally, control DNA should have been a polynucleotide of identical length, base sequence, modification, and contents as the ϕW14 genome, but carrying, like the normal B-form DNA, one negative charge per phosphate and one phosphate per base. Lacking this, T4 DNA was used for two major reasons: it is

comparable in length to ϕW14 DNA, and it carries a glucosylated 5-hmCyt group that, due to its bulkiness, may be expected to mimic the steric effects, if any, of putThy. T4 DNA is 34 mol % (G + C) compared to 51 mol % (G + C) [i.e., 49% (A + T + putThy)] in ϕW14 DNA. Yet, no major effects of G + C content in this range on condensation properties have been observed in previous studies.

I used total intensity and quasi-elastic light scattering to determine the critical concentrations of the trivalent counterion spermidine required to induce collapse of these DNA samples of varying charge density in a 1 mM sodium chloride–1 mM sodium cacodylate buffer and to compare the dynamic characteristics of the compacted particles. Specific ion effects were probed by substituting the inert, trivalent "point charge", hexaamminecobalt(III) for spermidine in similar experiments. The results are analyzed both from a general theoretical point of view according to Manning's counterion condensation theory of polyelectrolytes and from the more specialized aspect of the high-density DNA packaging in bacteriophage ϕW14.

Materials and Methods

(*a*) *Buffers and Solutions.* All buffers and solutions were prepared with distilled, deionized water and filtered through prewashed GS Millipore filters of 0.22-μm pore size. The experimental buffer was 1 mM sodium cacodylate–1 mM NaCl, pH 7.1. A single batch of buffer was prepared and used for the dialysis of the DNA samples and for all experiments throughout the study.

(*b*) *Polyamines and Hexaamminecobalt(III).* Spermidine trihydrochloride and putrescine dihydrochloride were purchased from Sigma, stored desiccated at −20 °C, and used without further purification. Recrystallized $Co(NH_3)_6 \cdot 3HCl$ (hexaamminecobalt trihydrochloride) was a gift from Dr. T. J. Thomas (University of Minnesota). A stock solution of $Co(NH_3)_6 \cdot 3HCl$ of approximate concentration (0.5 mg/mL) was prepared and quantitated by the absorbance of the solution at λ_{max} = 473 nm, ϵ = 56.2 M^{-1} cm^{-1} (Widom & Baldwin, 1980). The original stock solutions of polyamines (10 mM) and of hexaamminecobalt(III) (1.67 mM) in experimental buffer were used throughout the study to ensure internally consistent data.

(*c*) *DNA Preparation.* Various samples of the *Pseudomonas acidovorans* phage ϕW14 DNA were generously supplied by Dr. R. A. J. Warren (Department of Microbiology at the University of British Columbia); DNAs from wild-type phage and from an amber mutant in gene 42 were phenol extracted from purified, concentrated virions and purified by standard methods (Kropinski et al., 1973); intracellular DNA was extracted from host cells infected under nonpermissive conditions with a ϕW14 amber mutant in gene 37 (Maltman et al., 1981); acetylated DNA was prepared by acetylation of wild-type DNA under mild conditions and characterized by methods recently described by Gerhard & Warren (1982). All DNA samples were stored in TNE (0.01 M Tris-HCl, 0.15 M NaCl, 0.01 M EDTA, pH 7.4) over chloroform at 4 °C. Immediately before use they were dialyzed to equilibrium in four steps of 250 × volume against experimental buffer in the cold. The concentrations of all dialyzed DNA samples were determined by using an extinction coefficient ϵ_{258nm} = 6600 cm^{-1} M^{-1} nucleotides (Kropinski et al., 1973). The $\epsilon_{280}/\epsilon_{260} \simeq$ 0.510–0.535 and $\epsilon_{230}/\epsilon_{260} \simeq$ 0.425–0.445 suggested pure preparations (Marmur, 1963) of DNA.

(*d*) *Sample Preparation for Light Scattering.* All glassware was thoroughly rinsed with distilled, deionized water and prefiltered experimental buffer to exclude dust from light scattering samples.

Counterion solutions of twice the desired experimental concentration were prepared, filtered through 0.22-μm Millipore filters, degassed, and centrifuged in 12-mL tubes at 7000*g*, 20 °C, for 15 min. Aliquots (0.5 mL) from the top of the centrifuge tubes were transferred to scattering cells (7.5 mm × 10 cm disposable borosilicate test tubes).

DNA solutions were diluted into experimental buffer to twice the desired final concentration (0.75–1.5 μM DNA phosphate) and centrifuged at 3000*g*, 20 °C, for 5 min, and 0.5 mL from the top was added to the scattering cells containing the counterion solution. Mixing was by gentle tipping. This procedure avoids high local concentrations of either DNA or counterion, thus minimizing the possibility of multimolecular condensation or premature collapse (Post & Zimm, 1982a,b). The samples were allowed to equilibrate 2 h, centrifuged for 10 min at 2000*g* at 20 °C, and analyzed. Each sample at a given counterion concentration was prepared in triplicate, and each experiment was repeated at least twice. Presented data are an average of all runs. The total scattering intensities of buffer alone, of DNA in experimental buffer, and of counterion solutions at various concentrations were recorded with each set of data to provide the proper controls. The correction for light absorption by counterions was trivial even at the highest experimental concentrations of hexaamminecobalt(III), which has an absorption maximum (473 nm) very close to the laser wavelength (488 nm) used in these experiments.

(*e*) *Light Scattering.* Total intensity and quasi-elastic light scattering (QLS) measurements were performed on Dr. V. A. Bloomfield's instrument at the University of Minnesota. Details of the experimental setup have been recently reviewed (Aksiyote-Benbasat & Bloomfield, 1981; Fulmer & Bloomfield, 1982), and data analysis, with specific reference to the condensation of DNA from a free helical configuration to a compact structure, has been very lucidly described by Wilson & Bloomfield (1979). In principle, the intensity of light scattered at a given angle by a homogeneous, monodisperse solution of macromolecules is a function of their molecular weight and weight concentration. This optimal intensity is weighted by a form factor that accounts for destructive interference of radiation scattered from different parts of a molecule. While the form factor for a Gaussian random coil is very low ($\ll$1), compact molecules of the same molecular weight and concentration exhibit a form factor close to 1 and, therefore, lead to considerably higher scattering intensity. Thus, condensation of DNA by polyamines is accompanied by a surge in total scattering intensity.

Information about the dynamic characteristics of the condensed DNA can be simultaneously extracted from light scattering data, since the relative phase of radiation scattered from molecules depends on the position of the scatterers in the illuminated volume. As the molecules diffuse around with a translational diffusion constant of D_T, their relative phase changes, giving rise to time-dependent fluctuations in the intensity of the scattered light. The autocorrelation function of these intensity fluctuations around an average value can be determined by QLS and has been shown to be a single exponential with a decay rate proportional to D_T for spherical scatterers in a homogeneous, monodisperse suspension (Berne & Pecora, 1976).

In the present experiments I have used a tunable argon laser (Lexel 95) operating at 4880 Å. The cell holder (Malvern) is filled with index-matching fluid (Dow oil 550) and is temperature equilibrated at 20 °C. The incident laser beam is focused by a lens and mirror assembly into the center of the

sample cell. Scattered radiation is collimated onto the aperature of a photon counting photomultiplier tube (ITT FW130), which is mounted on a goniometer arm for convenience in angular dependence studies. The signal is then preamplified and channeled through to (a) an Ortec 9349 pulse-counting rate meter for total intensity determinations and (b) a Ford-Langley 96-channel digital correlator for QLS measurements leading to diffusion data. Data analysis and error reduction are performed by using the cumulant method of Koppel (1972) on an interfaced Minc-23 digital computer programmed by Dr. Jason Wei of the Department of Biochemistry at the University of Minnesota.

Theory and Data Analysis

(*a*) *Theory.* The counterion condensation theory of polyelectrolytes (Manning, 1978) offers the means of interpreting experiments that probe the cause–effect relationship between the interaction of DNA with multivalent counterions and its collapse into a compact form. Manning models the nucleic acid as a linear array of negative point charges with which free counterions interact only through ordinary Debye–Hückel screening. These "territorially" bound counterions remain fully hydrated and can freely diffuse within a high-density cylindrical volume, V_p, surrounding the polyelectrolyte. This model predicts a limiting extent of "condensation" and, therefore, a specific degree of polyelectrolyte charge neutralization, r, which can be calculated in a solution containing a single type of counterion of valence z and a polynucleotide with a linear distribution of fixed univalent charge centers by

$$r = z\theta^c = 1 - (z\xi)^{-1} \quad (1)$$

where θ^c is the number of counterions associated per unit polyelectrolyte charge (1–). The reduced linear charge density

$$\xi = q^2/(\epsilon kTb) \quad (2)$$

is an inverse function of the average axial spacing between fixed charge centers, b (Å/charge center = 1.7 for B-form DNA), q is the magnitude of the electronic charge, ϵ is the bulk dielectric constant of the solution, k is the Boltzmann constant, and T is the temperature in kelvin. For the more general case where each charge center carries a valence of $|z_p|$, ξ can be substituted in the above equations by an "effective" value, $\xi_{eff} = z_p\xi$, as discussed by Record and co-workers (1976b, 1978). This is equivalent to replacing the array of z_p valent charge centers of spacing b by a regular linear array of imaginary univalent charges with an effective spacing of $b_{eff} = b/z_p$ (Å/charge). In this context b_{eff} characterizes the polyelectrolyte, and the extent of neutralization, r, appears strictly dependent on the effective charge density of the polyion and on the valence of the counterion, which together provide the driving force for the condensation of the counterions on the polyelectrolyte. As the average charge per nucleotide decreases, the r achieved by a counterion of charge z is also expected to decrease. At 25 °C a trivalent (divalent, monovalent) cation will neutralize at most 92% (88%, 76%) of the negative DNA phosphate charge if $z_p = 1$, whereas r is only 90% (85%, 70%) given a z_p of 0.8 (i.e., $b_{eff} \simeq 2.1$ Å/unit charge).

In a solution containing two species of counterion, condensation is competitive. Hence, the addition of increasing amounts of a trivalent counterion to a solution of DNA in the presence of a monovalent cation allows the neutralization potential of the system to gradually increase from 76% (for $z = 1$ alone) to a maximum of 92% (for $z = 3$ alone). This characteristic of polyelectrolyte solutions offers an experimental handle to pinpoint the critical degree of neutralization at which DNA collapses. The theoretical basis for the analysis of such "titration" data was formulated by Manning (1978) in his two-variable theory and extended by Wilson & Bloomfield (1979) to allow different domains, V_{P1} and V_{P3}, in which the two types of counterions are assumed to be territorially bound.

(*b*) *Calculation of Average Charge per Nucleotide for the Various φW14 DNAs.* In order to determine ξ_{eff} and b_{eff}, the average fractional charge associated with each nucleotide was calculated as

$$|z_p| = 1 - z_M\theta_M \quad (3)$$

where z_M is the valence of the covalently modified portion of the base (+2 for putThy, −2 for hmPPUra) and θ_M the fraction of all bases modified in this way. It is assumed that the B-form is maintained (Record et al., 1976b). Results are summarized in Table I.

(*c*) *Calculation of Extent of Charge Neutralization by Counterions.* In the present experiments each sample contains DNA of a different average charge density in the presence of an excess of counterions of valence $z_1 = +1$ and $z_3 = +3$ competing for condensation. A system in which counterions of a single type territorially interact with a polyelectrolyte whose charges are partially neutralized by covalently bound protons was treated by Manning (1981b). Combining this formalism with the extended two-variable theory, the equations required to estimate the extent of DNA charge neutralization in the presence of total counterion concentrations, c_1 and c_3, were derived:

$$1 + \ln \frac{10^3\theta_1^c}{V_{P1}^c c_1} = -2z_1\xi_{eff}(1 - z_1\theta_1^c - z_3\theta_3^c) \ln (1 - e^{-\kappa b_{eff}}) \quad (4a)$$

$$1 + \ln \frac{10^3\theta_3^c}{V_{P3}^c c_3} = -2z_3\xi_{eff}(1 - z_1\theta_1^c - z_3\theta_3^c) \ln (1 - e^{-\kappa b_{eff}}) \quad (4b)$$

The subscripts refer to the valence of the counterions and the superscript c denotes parameters on a "per unit charge" basis in contrast to a "per nucleotide" standard state. Equations 4a and 4b show the same dependence on charge density as eq 50 of Manning (1981b) and are of the same form as those derived by Wilson & Bloomfield (1979) except that ξ and b are replaced by their effective values. They are solved simultaneously by an iterative process (Wilson & Bloomfield, 1979) to generate tables of the charge fractions θ_1^c and θ_3^c (i.e., the number of cocondensed counterions per average unit polyelectrolyte charge) for polyelectrolytes of various z_p's at the constant bulk concentration of Na^+, $c_1 = 2$ mM, at which all experiments were performed, and variable c_3. The Debye screening parameter $\kappa = (8\pi N\xi_{eff}b_{eff}I \times 10^{-3})^{1/2}$ depends on the bulk ionic strength I of the solution and therefore varies slightly with c_3 at constant c_1.

V_{Pi}^c's are calculated as the volumes that will satisfy eq 4 when only one of the counterions is present in solution so that

$$V_{Pi}^c = 4\pi eNb_{eff}^3(1 + z_i)(\xi z_p - 1/z_i) \quad (5)$$

where e is the base of natural logarithm and N equals Avogadro's number.

For any combination of c_1 and c_3 the total fraction of DNA charges neutralized can now be calculated by using eq 4 and 5 and

$$r = z_1\theta_1^c + z_3\theta_3^c \quad (6)$$

which leads to the empirical critical average linear charge

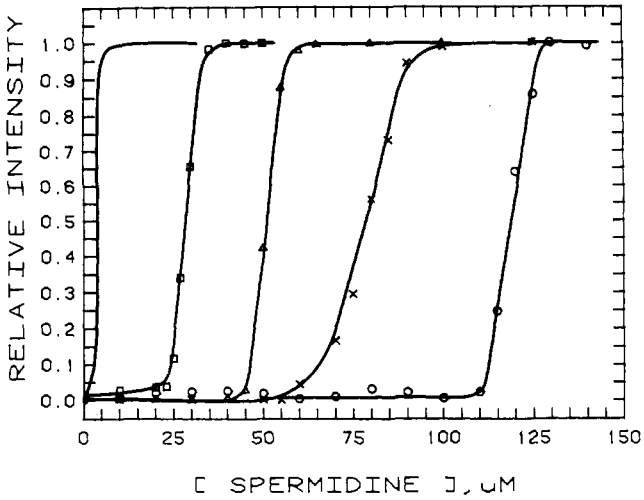


FIGURE 1: Normalized relative intensity of scattered light, $(I - I_{Bkg})/(I_{max} - I_{Bkg})$, vs. spermidine concentration at a scattering angle of 90° and a temperature of 20 °C for solutions of 1 μM DNA phosphate in 1 mM NaCl–1 mM sodium cacodylate buffer, pH 7.1: T4B DNA (○), acetylated φW14 DNA (□), am42 φW14 DNA (×), and wild-type φW14 DNA (△). (—) denotes the estimated transition curve for am37 φW14 DNA.

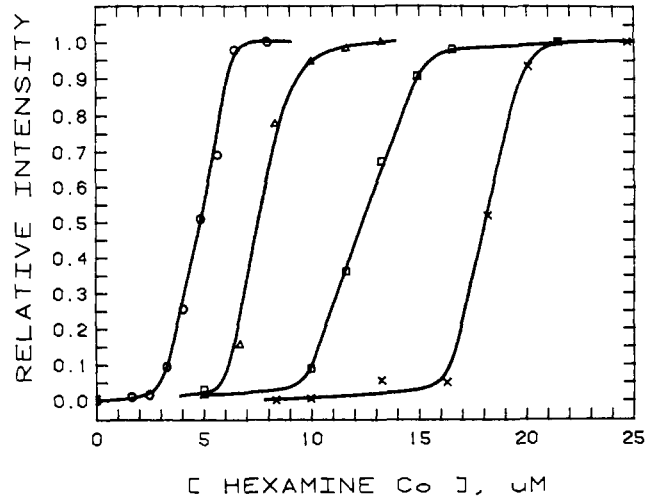


FIGURE 2: Normalized relative intensity of scattered light vs. $Co^{3+}(NH_3)_6$ concentration. Conditions as in Figure 1. Symbols: T4B DNA (○), acetylated φW14 DNA (□), am42 φW14 DNA (×), and wild-type φW14 DNA (△).

spacing b_{cr} at which intramolecular forces are balanced to allow the DNA to collapse:

$$b_{cr} = b_{eff}/(1 - r) \quad (7)$$

(d) Calculation of Shape-Dependent Hydrodynamic Dimensions of Condensed DNA. Quasi-elastic light scattering studies directly yield the diffusion coefficient, D_T, of particles in solution without imposing any perturbation on the system. The D_T of the condensed DNA particles can be interpreted in terms of hydrodynamic dimensions if their shape is known.

In the presence of either spermidine (Gosule & Schellman, 1976; Chattoraj et al., 1978; Ruben et al., 1981; among others) or hexaamminecobalt(III) (Widom & Baldwin, 1980), toroids have been the major form of collapsed B-form DNA observed by electron microscopy under a variety of conditions and methods of preparation. In my preliminary studies, collapsed acetylated and am42 φW14 DNA, as well, appeared toroidal. Since theoretically, too, this shape has been well justified (Manning, 1980, 1982), the D_T of the studied condensates can be related to the outer diameter, o, of toroids (Allison et al., 1980):

$$D_T \simeq \frac{2kT}{3\pi\eta(o + i)}\left(\frac{2h^2}{3\pi}\right)^{1/3}(0.127h + 0.908)^{-1} \quad (8)$$

where i is the inner toroidal diameter, $h = (o + i)/(o - i)$, and η is the viscosity of the solution. Equation 8 was used with the simplifying assumption that $i = 0$ ($h = 1$). The assumption is justified, given that D_T is remarkably insensitive to i (present work; Allison et al., 1981).

Results

(a) DNA Condensation Induced by Spermidine and Hexaamminecobalt(III). The effects of spermidine and hexaamminecobalt(III) concentrations on the total light intensity scattered from DNA samples of various linear charge densities at a scattering angle of 90° are shown in Figures 1 and 2, respectively. For T4B, acetylated φW14, and wild-type φW14 DNA there is a sharp increase in the intensity accompanying the cooperative collapse of the DNA into a compact conformation. The transition range of am42 DNA with either counterion is slightly wider, but background scattering and total scattering intensity after condensation are closely comparable to the other samples. This observation suggests that am42 DNA may collapse by a different mechanism, which is independent of the specific counterion. Thymine and putThy arise at the polynucleotide level in a nonrandom event, and distribution of putThy is ordered (Warren, 1981). Therefore, a clustering of the putThy toward one end of the DNA is a distinct possibility. Such a nonhomogeneous distribution of charge density could lead to varied long-range effects along the helix, which in turn could result in a widening of the transition curve. The critical concentrations of counterions which induce 50% transition in the different samples are summarized in Table II under $[X]_{cr}$. The results show that as the average charge density of the DNA decreases (i.e., the charged putThy content *increases*), higher concentrations of the polyamine are required to achieve condensation.

The critical concentration of hexaamminecobalt(III) is only one-sixth that of spermidine for all the DNA samples independently of their b_{eff}, suggesting specific ion effects and corroborating and extending the previous results obtained with T4 DNA (Thomas & Bloomfield, 1983) and various other standard B-form DNAs (Widom & Baldwin, 1980, 1983). In addition, the particles collapsed with the hexaammine appear to be more stable than spermidine-condensed DNA; i.e., they do not show the tendency to irreversibly grow into multimolecular complexes that eventually (~24–48 h) precipitate out of solution (Post & Zimm, 1982b; Widom & Baldwin, 1983).

(b) Critical Degree of Charge Neutralization of Collapsing DNA. The fractions of polyelectrolyte charge neutralized by Na^+ (θ_1^c) and by spermidine or hexaamminecobalt(III) (θ_3^c) were estimated for each DNA of charge spacing b_{eff} at the critical concentration of trivalent counterion, $c_3 = [X]_{cr}$, from the simultaneous eq 4a and 4b, and substituted into eq 6 to calculate the critical charge neutralization for collapse, r. θ_1^c, θ_3^c, and r are listed in Table II for each set of experiments, one in the presence of spermidine, the other with hexaamminecobalt(III) as the counterion. Both θ_3^c and r are clearly dependent on the counterion, whereas within each group r remains essentially independent of b_{eff} except for wild-type φW14 DNA. For spermidine, the critical $\theta_3^c \simeq 0.294$ leads to an $r \simeq 0.89$, whereas for the more efficient $Co^{3+}(NH_3)_6$, $\theta_3^c \simeq 0.284$ and $r \simeq 0.874$. Note that even though the percentage differences between the r values of 0.892 and 0.874 may seem minimal, they correspond to considerable differences in critical concentrations and should not be disregarded. The exceptional

Table II: Critical Parameters for Condensation of DNAs of Various Linear Charge Densities in 1 mM NaCl–1 mM Sodium Cacodylate Buffer, pH 7.1, Induced by Spermidine and Hexaamminecobalt(III) at 20 °C

source of DNA	b_{eff} (Å/charge)	$[X]_{cr}$ (μM)	θ_1^c	θ_3^c	r
	(A) Spermidine				
T4B	1.7	28	0.013	0.294	0.893
acetylated φW14	1.8	50	0.010	0.294	0.893
am42	1.9	77	0.009	0.294	0.892
w.t. φW14	2.24	118	0.007	0.289	0.876
w.t. φW14[a]	2.0	118	0.006	0.295	0.891
	(B) Hexaamminecobalt(III)				
T4B	1.7	4.9	0.022	0.285	0.876
acetylated φW14	1.8	7.6	0.019	0.284	0.874
am42	1.9	12.3	0.018	0.285	0.873
w.t. φW14	2.24	18.2	0.013	0.280	0.853
w.t. φW14[a]	2.0	18.2	0.012	0.287	0.873

[a] Calculations in this row are based on a shortened pitch of b = 1.5 Å/phosphate instead of the B-form b = 1.7 Å/phosphate. See Discussion for rationale.

behavior of wild-type φW14 DNA is analyzed in detail under Discussion. The counterion concentration that would yield a charge neutralization of 89% can be estimated by reversing the calculations. With b = 1.7 Å/nucleotide, b_{eff} = 2.24 Å/charge, and c_1 = 2 mM Na^+, an r of 0.892 for wild-type φW14 DNA can only be achieved in the presence of spermidine in excess of 200 μM. The discrepancy between this value and the experimentally determined $[Spd]_{cr}$ of 128 μM cannot be explained by experimental error.

(c) Direct Calculation of Spermidine Binding from Its Binding Constant. In a recent study Braunlin et al. (1982) determined the intrinsic binding constant, K, and the effective binding site size, n (in DNA phosphates per discrete site), for the interaction of spermidine with T7 DNA as a function of ionic strength. With their data, the extent x of binding of spermidine at its critical concentration to T4B DNA per phosphate (28 μM) can be directly calculated and compared to the equivalent value, θ_3^c, estimated through the polyelectrolyte theory. The McGhee–van Hippel isotherm (1974) is used for this purpose:

$$\frac{x}{L} = K(1 - nx)\left[\frac{1 - nx}{1 - (n - 1)x}\right]^{n-1} \quad (9)$$

where L is the free ligand concentration (i.e., $\sim c_3$). For spermidine binding to T4B DNA, K at the experimental buffer composition is determined from $\log K = -2.5 \log [Na^+] + 0.2$ (Braunlin et al., 1982). The estimated extent of binding varies considerably with the choice of n but is relatively insensitive to the magnitude of K if $\log K > 6.5$. In a solution containing 2 mM Na^+, $K \simeq 8.9 \times 10^6$ M^{-1}. Given the large uncertainty in the values reported for n (Braunlin et al., 1982), calculations to determine x were repeated for various n's. For n = 3.6 phosphates, the above K leads to an average of 0.260 spermidine bound per phosphate, whereas for n = 3.2, x is 0.294 in surprisingly good agreement with θ_3^c of 0.294 (Table II).

(d) Diffusion Coefficients and Hydrodynamic Dimensions of Condensed DNA. The diffusion coefficients of all the condensed DNA species were measured by quasi-elastic light scattering. The results are listed in Table III. The angular dependence of the diffusion coefficients was minimal and random in all samples, reflecting clean, monodisperse suspensions.

For T4 DNA in the presence of either spermidine or hexaamminecobalt(III), the results closely confirm previous studies by Wilson & Bloomfield (1979) and Thomas & Bloomfield (1983) at neutral pH. For variable b_{eff}, two major trends are noticeable in the data (Table III): (1) DNA condensed by $Co^{3+}(NH_3)_6$ diffuses up to 30% faster than spermidine-condensed DNA, and (2) the diffusion coefficient of compacted DNA is slightly, but reproducibly, higher for samples of higher b_{eff}. In both cases wild-type φW14 DNA is an exception to the trend: the diffusion coefficients for hexaamminecobalt(III)- and for spermidine-condensed wild-type φW14 DNA are essentially indistinguishable and very closely compare to the D_T of spermidine-condensed T4 DNA.

Table III: Hydrodynamic Properties of Condensates of DNA of Various Axial Charge Densities at 90° Scattering Angle, 20 °C, and Collapsed in the Presence of Spermidine or Hexaamminecobalt(III)

source of DNA	diffusion coeff, D_T[a] ($\times 10^{-8}$ cm^2/s)		outside diam of toroids, o (Å)	
	spermidine	Co^{3+}-$(NH_3)_6$	spermidine	Co^{3+}-$(NH_3)_6$
T4B	4.31 ± 0.16	6.15 ± 0.13	1142 ± 42	800 ± 17
acetylated φW14	4.54 ± 0.1	6.28 ± 0.19	1084 ± 24	783 ± 24
am42	4.59 ± 0.08	6.61 ± 0.29	1072 ± 19	744 ± 33
w.t. φW14	4.34 ± 0.13	4.39 ± 0.16	1134 ± 34	1121 ± 41

[a] Each D_T value represents the mean ± standard deviation from the mean of 12–18 determinations on independently collapsed triplicate samples.

Since condensed DNA appears largely toroidal in electron microscopic studies and may exist as toroids in bacteriophage heads (Marx & Ruben, 1983), I estimated the outer diameter of tori that diffuse with D_T (Table III). The diameters, calculated from eq 8, vary from 1072 to 1142 Å for spermidine-collapsed DNA of b_{eff} ranging from 1.7 to 2.2 Å/charge and from 744 to 800 Å for hexaamminecobalt(III)-induced samples, except for wild-type φW14 DNA, which is compacted to molecules of outer diameters of ~1130 Å in the presence of either counterion (Table III).

Comparison of relative sizes of condensates is only meaningful if the studied particles have the same basic shape and are monomolecular. An increase in total scattering intensity, as observed during the titration of DNA with counterions, can reflect either an increase in the concentration of collapsed particles or an increase in the size of existing condensates. The low ionic strength and low DNA concentrations used in these experiments provide conditions that minimize interchain interactions and are conducive to monomolecular collapse of the DNA (Post & Zimm, 1979, 1982a). To ensure that the observed reaction is the compaction of DNA, and not intermolecular rearrangements, the D_T of the scatterers was measured at various counterion concentrations in the transition region. In most cases, D_T slightly increased (by ~5%) as the transition progressed and leveled off at counterion concentrations at which the reaction was complete, possibly suggesting that the condensates were loosely folded at the onset of the transition and tightened up as the counterion concentration was increased. Similar changes in the hydrodynamic properties of condensates along the transition zone have very recently been reported by Widom & Baldwin (1983) as well. Samples were rejected if either D_T appeared to be lower than the norm or the quality factor (i.e., the normalized second cumulant, a measure of the square of the standard deviation of particle size) was high and/or if the total intensity fluctuated more than 10% in time. The reversibility of condensation along the transition curve by incremental additions of $MgCl_2$ provided an additional control for monomolecular collapse (Post & Zimm, 1982b; Widom & Baldwin, 1980). In one more effort to test the mode of condensation, I assumed that the T4 DNA

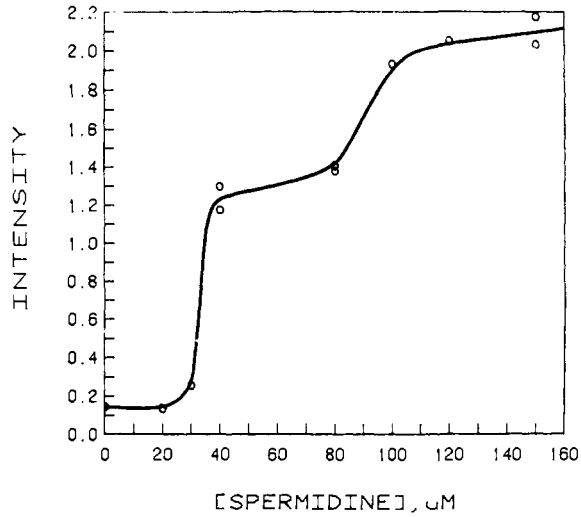


FIGURE 3: Plot of scattering intensity (I) vs. spermidine concentration for a 1:1 mixture of T4B and ϕW14 wild-type DNA, each at 1.5 μM DNA phosphate, at a scattering angle of 90° and a temperature of 20 °C in 1 mM NaCl–1 mM sodium cacodylate buffer, pH 7.1.

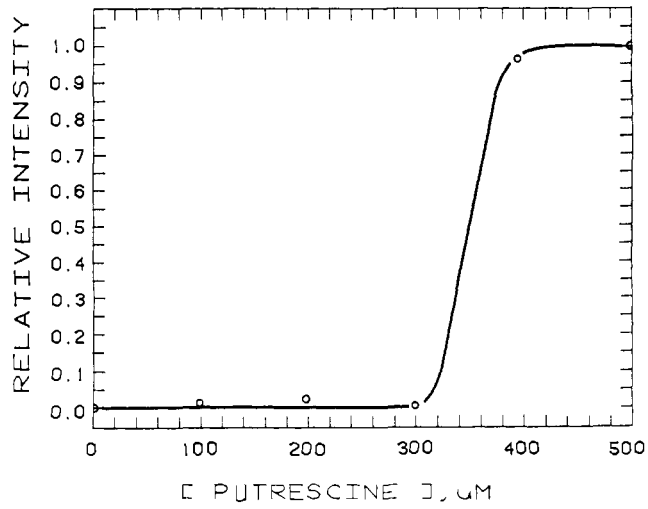


FIGURE 4: Plot of scattering intensity (I) vs. putrescine concentration for intracellular am37 DNA demonstrating the condensation of DNA of high charge density induced by a divalent cation in aqueous solution. A 4 μM DNA phosphate concentration under conditions described in Figure 1 was used.

was condensed into tori with outer diameter o = 1142 Å and inner diameter i = 350 Å [Table III and measurements from my electron micrographs in agreement with Manning's theoretical predictions (Manning, 1980, 1982) and the observations of Marx & Ruben (1983)]. A comparison of this torus volume to the total volume of a hydrated DNA helix of diameter d = 25 Å (Yamakawa & Fujii, 1973) and length L = 60 μm (Scraba et al., 1983) yields 2.9×10^{-16} and 3.0×10^{-16} cm, respectively, strongly suggesting that the observed torus can only accommodate a single T4 DNA molecule.

(*e*) *Condensation in a T4B–ϕW14 Mixed DNA System and in Intracellular DNA Preparations of ϕW14 Am37 Infected Cells (b_{eff} = 1.3 Å/charge).* The gene 37 amber mutant of bacteriophage ϕW14 will replicate its genome but cannot package it under nonpermissive conditions. Necessarily, the purification procedure involves extraction of intracellular DNA from infected host cells and subsequent separation of host DNA from phage DNA. The separation methods are only now being improved (R. A. J. Warren, personal communication); thus the present study was carried out with mixed intracellular DNA. The preparation was of low concentration (~15 μg/mL DNA), and even though the A_{260}/A_{280} and A_{230}/A_{260} ratios suggested that protein contamination was low, it was estimated from CsCl gradients that a considerable fraction of the DNA was of bacterial origin. To test if biphasic condensation can be resolved in a system containing two species of DNA of different charge densities, a 1:1 mixture of T4B DNA and wild-type ϕW14 DNA was titrated with increasing amounts of spermidine. The resulting two-step intensity profile (Figure 3) implies condensations at 38 and 96 μM spermidine separated by an intensity plateau. The higher than normal critical spermidine concentration for T4B and the lower value for ϕW14 DNA (compare Table II) may indicate competition between the two species; in spite of that, the approach does provide valuable information about the system.

Numerical calculations using eq 4 and 5 suggest that am37 DNA should condense in the presence of <2 μM spermidine (provided that collapse occurs at the empirical critical $r \simeq$ 0.89 as experienced for all other DNA species) while the host DNA has a critical spermidine concentration of 25 μM for sonicated fragments (this study). Condensation experiments with intracellular DNA and increasing concentrations of spermidine show reasonable agreement with the theory. Titration results in a surge in intensity at ~2 μM spermidine beyond which the intensity rises steadily up to a spermidine concentration of 20 μM where it levels off, suggesting a mixture of DNA, one type with a critical spermidine concentration below 2 μM and another requiring concentrations above 20 μM as expected.

Condensation of conventional B-form DNA in aqueous solution is not observed in the presence of di- or univalent counterions, presumably because the required extent of charge neutralization can only be achieved in the presence of higher valence counterions (eq 1). According to eq 1 and 2, DNA condensation not only depends on the valence of the counterion but also is driven by the charge density of the polyelectrolyte. To test this hypothesis and reinforce the above results, I asked whether a divalent cation can induce condensation of a DNA of higher than conventional charge density. Calculations (eq 4 and 5 for c_1 = 2 mM, $r \simeq$ 0.89, and increasing c_2) indicated that it should be possible to induce collapse of am37 DNA with divalent counterions at concentrations over 200 μM. As anticipated, an increase in scattering intensity was observed between 300 and 400 μM putrescine, and no further rise was registered up to putrescine concentrations of over 1 mM (Figure 4). The final relative scattering intensity plateaus attained in the presence of spermidine and of putrescine indicate that intracellular DNA was ~65% host and only ~35% am37.

In conclusion, preliminary results with both spermidine and putrescine clearly place the transition region for a DNA of b_{eff} <1.7 Å/charge to critical counterion concentrations below that of T4B DNA in accord with theory (Figure 1). The preparative advantage of DNA of higher than conventional charge density is obvious, since differential condensation (or precipitation in higher concentration samples) can be introduced as a highly efficient purification step.

Discussion

I have studied in comparison to bacteriophage T4 DNA the counterion-induced collapse of a series of native bacteriophage ϕW14 genomes in which thymine residues are substituted to varying degrees by the hypermodified bases putThy and hmPPUra. Since both of these bases are charged (2+ and 2–, respectively), these nucleic acids exhibit average linear charge densities that vary in the range 1.3 to 2.2 Å/charge. Qualitatively, my results are consistent with the observation of Wilson & Bloomfield (1979) that DNA spontaneously col-

lapses when a certain fraction of its backbone charge is neutralized and with the predictions of Manning's counterion condensation theory (1978) that a lowered charge density on the polyelectrolyte results in a decreased drive for the condensation of counterions. Accordingly, collapse, as evidenced by a large increase in total light scattering intensity, was observed for all DNA samples, and as a general trend, DNA of lower charge density required higher concentrations of trivalent counterions in the presence of fixed amounts of [Na^+] to drive the conformational change (Figure 1).

Data are available on the spermidine-induced in vitro condensation of conventional nucleic acids from various sources, with lengths ranging between 140 and ~200 000 base pairs (Widom & Baldwin, 1980, 1983) and G + C contents varying from 34 to 51 mol % (Thomas & Bloomfield, 1983; Allison et al., 1981; Wilson & Bloomfield, 1979; Gosule & Schellman, 1978). Studied over a wide range of DNA phosphate concentrations, these data suggest that DNA characteristics of sequence, mole percent (G + C) content, length, and concentration only minimally modify the critical collapse conditions and the dynamic characteristics of the condensed particles. Examination of Table II, on the other hand, shows that the effects of DNA charge density on the critical counterion concentration are exceptionally pronounced. An increase in original linear polyelectrolyte charge spacing from 1.7 (T4 DNA) to 2.2 Å/charge (wild-type ϕW14 DNA) prompts a 4-fold jump in the critical concentration of both spermidine and hexaamminecobalt(III), while 60 times more spermidine is required to condense wild-type ϕW14 DNA compared to am37 ϕW14 DNA (~1.3 Å/charge). Yet, a 10% decrease in average charge density of a B-form helix affects properties (such as flexibility or groove topology) that pertain to its folding and accommodation of ligands not at all or only to an extent where they do not perceptibly alter the diffusion coefficient (i.e., basic shape and size) of the stabilized condensates in the presence of spermidine. Important variations in folding behavior, which could be a reflection of changes in double helical conformation, are only observed for wild-type ϕW14 DNA, the sample of lowest charge density, especially when collapsed by hexaamminecobalt(III). These results are discussed later in connection with specific counterion effects.

Since alleviation of the charge repulsion between neighboring phosphates on the helix is required for DNA to collapse, the crucial factor to examine in the relationship between DNA and its counterions is, rather than the critical counterion concentration, the degree of DNA charge neutralization achieved at that concentration. In a trend-setting study, Wilson & Bloomfield (1979) used Manning's counterion condensation theory (Manning, 1978) to correlate the critical concentrations of various counterions to the fractional neutralization of the DNA helix at the midpoint of each transition. Comparison of a series of similar mixed-valence titration systems (e.g., Mg^{2+}–spermidine, Na^+–spermine^{4+}, Mg^{2+}–spermine, etc.) led them to conclude that, under widely different solution conditions, collapse of DNA occurs with remarkable uniformity when 89% to 90% of the phosphate charges are neutralized by counterions. This result, even though clearly empirical, does provide a point of reference within the framework of the counterion condensation theory to which experimental results can be compared. In the presence of spermidine all ϕW14 DNA samples, except for wild type, collapse when each *average unit charge* is neutralized by ~89%. Interpretation of these results depends on the assumed charge distribution of the polyelectrolytes. If the putThy affects each phosphate charge in a similar way, the DNA can be modeled as a sequence of negative unit charges spaced by b_{eff} (or charge centers of valence z_p spaced by b). Equation 7 is then applicable and leads to the conclusion that the critical charge spacing at which DNA can collapse varies depending on the original charge density of the polyelectrolyte, DNA of lower original density collapsing at larger b_{cr}. If, on the other hand, each putThy enters into direct ionic contact with two adjacent phosphates on complementary strands of the helix as suggested by space-filling models (Kropinski, 1972), a model of ϕW14 DNA emerges, in which a series of linearly positioned negative unit charges (coinciding with the phosphates) are separated by neutralized gaps at the points of putThy incorporation. For am42 DNA (or acetylated ϕW14 DNA) segments of on the average 20 (19) phosphate charge points alternate with neutral gaps of 2 (1) charge lengths. Thus, the *overall charge density* for these DNAs differs, exhibiting different driving forces and critical concentrations for counterion condensation. Yet, the *charge distribution* in each segment is similar (except gaps) to that of T4 DNA, and accordingly, each *fixed* unit phosphate charge is ultimately neutralized to the same critical extent. This "segmental" model of ϕW14 DNA seems to best explain my observations, suggesting that the drive for counterion condensation depends on the average charge density of the polyion, whereas the critical extent of neutralization at which collapse becomes possible may be enforced by the distribution of charges on the DNA. The fact that a critical ~89% of the charges need to be neutralized with spermidine, whereas a lower degree of neutralization seems to be required in the presence of hexaamminecobalt(III) (Table II), does not necessarily mean that the collapse conditions differ. As will be discussed below, the reason for the discrepancy may be a higher binding constant of hexaamminecobalt(III) to DNA with a nonelectrostatic component complementing the electrostatic interactions.

The natural polyamine spermidine is the classical trivalent counterion in the presence of which collapse of conventional B-form DNA from various sources has been observed in vitro by a multitude of means (Gosule & Schellman, 1976; Wilson & Bloomfield, 1979; Allison et al., 1980, 1981; Thomas & Bloomfield, 1983). As an isovalent alternative, hexaamminecobalt(III) was recently introduced to test the hypothesis that counterion valence rather than specific polyamine structure is instrumental in inducing DNA condensation (Widom & Baldwin, 1980, 1983; Thomas & Bloomfield, 1983). The results of these studies suggested that an inert trivalent cation with "point charge" characteristics not only could cause condensation of conventional DNA but also in this case exhibited greater efficiency: it took less than one-fifth as much hexaamminecobalt(III) as spermidine to induce DNA condensation, and the particles collapsed in the presence of hexaamminecobalt(III) had drastically smaller hydrodynamic radii. Thomas & Bloomfield (1983) attributed the differential effects of these counterions on DNA condensation to their structural differences (which are not taken into account by Manning's theory) and to the ways they bind to DNA. Present data confirm the above conclusions and suggest that they also apply to DNA of various charge densities (Tables II and III). In addition, two observations based on these data deserve further comment. First, even though the critical concentrations of both spermidine and hexaamminecobalt(III) vary according to the charge density of the DNA, their ratio $[Spd]_{cr}/[Hc]_{cr}$ remains notably constant ($\simeq$6) for each sample without exceptions. Second, hexaamminecobalt(III) does not induce more compact condensates of wild-type ϕW14 DNA even

though it remains ~6 times more efficient than spermidine in terms of its critical concentration.

The first observation can be analyzed in terms of the relative binding constants of spermidine, K_{Spd}, and hexaamminecobalt(III), K_{Hc}, to DNA. In recent literature, the possibility has been discussed and evidence reviewed that some counterions can effectively complement delocalized electrostatic binding by specific site directed interactions with the polyelectrolyte (Record, 1978; Manning, 1978, 1980; Lohman et al., 1980; Braunlin et al., 1982; Thomas & Bloomfield, 1983). Set in this context, the constancy of $[Spd]_{cr}/[Hc]_{cr}$ suggests that the component of K_{Hc} which reflects specific counterion effects is independent of the average polyelectrolyte charge density and putThy contents of the DNA. This confirms the deductions of Record et al. (1976a) that specificity originates in the nonelectrostatic component of binding. In fact, the *ratio* of critical concentrations appears to remain constant around 6 in Widom & Baldwin's (1980) studies, as well, under widely different salt composition and ionic strength conditions. Accordingly, application of the strictly electrostatic counterion condensation theory, which ignores alternate modes of binding, can be expected to underestimate the total extent of binding of counterions to DNA at any critical concentration when the binding constant includes a nonelectrostatic (specific) component. The extent of binding of spermidine to T4 phage DNA calculated directly from its binding constant, K, and the effective site size, n, compares very closely with estimates obtained through application of the counterion condensation theory [Results (c)]. The interactions of spermidine with DNA therefore appear predominantly electrostatic. Nevertheless, a certain degree of caution is advisable given the large uncertainty in the value of n and the sensitivity of the calculated extent of binding to changes in n (eq 9). In fact, $n \simeq 3$ would be as good a choice of n as 3.2 (the value used in calculations) according to Table I of Braunlin et al. (1982), in which case essentially all DNA charges would be neutralized by spermidine at its critical concentration. Thus, the possibility remains that collapse may require full charge neutralization. This possibility is compatible with the observation that DNAs of various b_{eff}'s collapse at similar empirical r's which might be underestimated by theoretical calculations to the same extent in each case.

The collapse of DNA from a random coil to a stable compact form has routinely been analyzed as a single-step process, possibly because the methods used were designed specifically to recognize the conformational change. The observation that, for a variety of DNAs, the efficiency of a counterion is manifested in both a lowered critical counterion concentration and an increased compactness of the condensates does not imply that these two condensation characteristics are correlated. In fact, since the differential in the critical concentrations of hexaamminecobalt(III) and spermidine necessary to collapse wild-type ϕW14 DNA persists (Table II) while the size and shape distinction between the respective condensates disappears (Table III), it is conceivable that the overall reaction consists of at least two major stages, the requirements for which can be divorced: (a) an increase in the flexibility of the DNA as the DNA–DNA electrostatic repulsions vanish upon the binding of counterions and (b) stabilization of the condensate in an energy-minimizing form, the exact geometry of which may depend on the way the counterion binds to DNA. The study of Allison et al. (1981) on the collapse of B-form DNA induced by homologues of spermidine, $H_2N(CH_2)_3NH(CH_2)_nNH_2$, with n ranging from 4 to 8 can be interpreted similarly, since the critical counterion concentrations appear independent of n, whereas the condensates exhibit different stability and mode of collapse.

The finding that divalent putrescine induces toroidal structures of a nucleic acid of higher than conventional charge density (am37 DNA), whereas none of the other DNAs are affected, has interesting implications. Given the relatively high physiological intracellular concentrations of divalent salts (e.g., Mg^{2+}), DNA or portions of DNA containing hmPPUra may be present in vivo in collapsed form in contrast to the host DNA, which would under the same ionic conditions require high trivalent salt concentrations for condensation. In a recent study Krasnow & Cozzarelli (1982) showed that catenation of DNA by topoisomerases is controlled by its condensation and concluded that collapsed DNA is the proper substrate in this system, as well as in some other enzymatic systems, the reaction of which is affected by polyamines. The enzymes that modify hmPPUra to putThy may similarly be presumed to preferentially operate on collapsed or more flexible segments of DNA rather than the extended form and thus discriminate against host DNA and fully processed sections of ϕW14 DNA.

I have repeatedly noted in the above discussion that, at least quantitatively, wild-type ϕW14 DNA does not appear to follow the trends set by the T4, acetylated ϕW14, and am42 ϕW14 DNA series of decreasing charge density. The two most striking instances relate to the hydrodynamic size and shape of its condensates and the critical extent of neutralization required for collapse. Unlike the other samples, which exhibit a 30% size difference, wild-type ϕW14 DNA condensates formed in the presence of hexaamminecobalt(III) or spermidine seem to have reached a maximal degree of compactness. They cannot be distinguished from each other by either their diffusion coefficient or their appearance in the electron microscope. But they are easily differentiated from condensates of the other DNA samples under the electron microscope. My limited observations on wild-type ϕW14 DNA revealed toroids with large central holes and a body of tightly intertwined fibers unlike the loosely wound "traditional" toroids characteristic of various B-form DNAs (Chattoraj et al., 1978; Ruben et al., 1981; Widom & Baldwin, 1980; among others) as well as acetylated and am42 ϕW14 DNA. Since the partially acetylated ϕW14 DNA, in which a large fraction of the putThy is neutralized (Table I), conforms to the trends observed for T4 and am42 DNA, the charge, rather than the steric properties of the putThy, appears responsible for the exceptional reactions of the low charge density DNA. On the basis of bulkiness, there is no reason to believe that any of the ϕW14 DNA species would be in a conformation differing from that of T4 DNA, which also carries a large hypermodified base. Under high relative humidity, T4 DNA has been shown to be present in the B conformation (Mokul'skaya et al., 1975; Mokul'skii et al., 1972). At slightly lowered relative humidity, unlike unglucosylated DNA, T4 DNA passes into a highly twisted conformation (similar to ϕW14 DNA toroids?) containing 8–8.4 base pairs per turn of the helix (T-form). Space-filling Corey–Pauling–Koltun (CPK) models suggest that the putrescine side chains can bridge the major groove of the B-form double helix without apparent strain to its secondary structure. Yet, it is possible that an extensive decrease in charge density beyond a threshold may permit compression of the double helix or its restabilization in a form which may not conform to a B-form structure. In fact, as reviewed by Cantor (1981) and Zimmerman (1982), it is known that at both the theoretical and experimental levels DNA can exhibit considerable conformational flexibility even within the B-form. The suggestion that changes in average

linear charge density of DNA may induce variations in the secondary structure of the double helix is not without precedent. Chen et al. (1981) observed changes in the CD spectrum of DNA when it was covalently modified by the attachment of a positively charged *n*-butylamine to the bases in the presence of CH_2O. It is not known what type of structure these changes reflect. Whatever the exact structure, its CD spectrum indicates an increase in the duplex winding angle, and such increases correlate with a compression in the solution conformation of duplex DNA (Manning, 1981a).

Precollapse B to Z transitions that culminate in the collapse and precipitation of the Z-DNA in $MgCl_2$–ethanol solutions at critical concentrations below those expected for B-DNA have been reported by van de Sande & Jovin (1982). Similarly, it appears possible that *native* ϕW14 DNA could be in the same form as its acetylated derivative but that a conformational change could be induced in an event preceding collapse by changes in solution composition, ionic state, or degree of hydration. In vivo, this suggestion is equivalent to a conformational change of the DNA taking place during encapsidation. There is some evidence which suggests that in contrast to am42 DNA such a transition may indeed be happening in the wild-type genome and resulting in a conformation of lowered pitch. Am42 phage particles are defective because they do not contain a full-length genome (Miller et al., 1983). Comparative electron microscopic studies of wild-type and am42 DNA yield dry lengths of 59.6 and 53.1 μm, respectively (Scraba et al., 1983; Miller et al., 1983). Considering a length-guided encapsidation mechanism and assuming that the full permitted length of DNA is packaged into am42 heads, it can be estimated that the pitch of the wild-type helix should be shortened to $(53.1/59.6) \times 3.4$ Å/bp $\simeq 3.0$ Å/bp (i.e., 1.5 Å/phosphate) to allow a full genome to be present on a 53.1-μm stretch of DNA instead of its B-form length of 59.6 μm. Interestingly, if this shortened nucleotide separation of 1.5 Å/phosphate (i.e., $b_{eff} = 2.0$ Å/charge) is used in the calculations of the critical degree of charge neutralization for wild-type ϕW14 DNA, r is shifted into the range observed for the other DNA samples, 0.891 for spermidine-induced collapse and 0.873 for collapse in the presence of hexaamminecobalt(III) (see footnoted entries in Table II).

These results do not preclude the possibility that each one of my DNA samples of lower than B-form charge density may have a proportionally shortened step height in solution. The degrees of neutralization calculated from eq 4 and 5 using these shorter b_{eff}'s (with the exception of wild-type ϕW14 DNA) are very similar to my original results. In fact, unless I know more about the forces that govern the collapse of DNA and am capable of predicting under which neutralization conditions the transition should occur, it appears that the present methods cannot be used to determine accurately the average spacing of charge centers on a polyelectrolyte. Laser Raman (G. T. Thomas, Jr., personal communication) and CD studies (S. Hanlon, personal communication) that may help to clarify this issue are presently under way.

The function of the putThy in the genome of bacteriophage ϕW14 has been the subject of a series of recent studies (Warren, 1980; Miller et al., 1982; Gerhard & Warren, 1982; Scraba et al., 1983; R. A. J. Warren, personal communication). It has been shown that the hypermodified base contributes to the stability of the double helix (higher T_m), the crucial factor being the charge effect, since acetylated ϕW14 DNA exhibits a lowered T_m. Yet, it is unlikely that an increased T_m would be an asset to the phage, considering that ϕW14 heads are more temperature sensitive than T4 heads and disintegrate at temperatures far below the T_m of their DNA. This instability has been attributed to a higher apparent density of packaging of DNA in ϕW14 capsids. In a study comparing the intraphage DNA densities of various isometric and cylindrical phages, Earnshaw & Casjens (1980) showed that the packing of DNA within phage heads is extremely uniform: it is B-form and packed to a density of 0.5 ± 0.02 g/cm^3 (excluding associated water) almost identical with that in hexagonal single crystals of DNA. In fact, X-ray diffraction studies of deletion and insertion mutants of bacteriophage λ suggest that even though there is considerable latitude in how short a piece of DNA could be packaged, 105% of the wild-type DNA complement appears to set the upper limit (Davidson et al., 1971; Fiandt et al., 1976). This is equivalent to a maximal packaging density slightly over 0.51 g/cm^3. In comparison, assuming that ϕW14 DNA, too, is encapsidated in a B-form double-helical conformation and an arrangement similar to that of other isometric phages with an interhelix distance of $\sim$27.5 Å (Earnshaw & Casjens, 1980) and an internal capsid volume of $\sim 32 \times 10^7$ Å^3, it would have to be packaged to a density of $\sim$0.63 g/cm^3. Under the same circumstances, the 53.1-μm length of am42 DNA would yield a density of just over 0.51 g/cm^3. It is, therefore, clear that while ϕW14 capsids can accommodate am42 DNA in a B-form, the wild-type genome will only fit if it can adopt a DNA conformation (such as the A- or D-form) that allows a higher density packaging. The conclusions from the present in vitro DNA condensation studies that am42 DNA, which has a lower putThy content, remains in a B-form (possibly with minor modifications) whereas the wild-type ϕW14 DNA appears to condense in a conformation of shorter pitch as a result of its drastically lowered charge density are in complete (and encouraging) agreement with the above discussion. The fact that higher counterion concentrations are required to induce the collapse of the ϕW14 DNA is not of immediate concern since encapsidation is certainly not driven by polyamine condensation alone (Riemer & Bloomfield, 1978). Neither is it too disconcerting to note that wild-type ϕW14 DNA condensates in their observed shape and size could not be easily accommodated in capsids since, as previously observed, DNA condensed into toroids appears to have a lower density packaging (0.43 g/cm^3) than encapsidated DNA (Suwalski et al., 1969; Haynes et al., 1970).

It is well-known that intercalation or binding across either the major or minor groove of a DNA helix can profoundly influence its recognition properties as well as its physical characteristics (Berman & Young, 1981). In equilibrium binding situations recognition can be restored (or destroyed) by minor changes in environmental conditions, whereas the covalent modification of a base introduces a permanent shift in properties and annihilates any possibility of compromise. The classical example of this is the hydroxymethylation and glucosylation of the cytidine residues of T4 DNA, which allow it to escape recognition by restriction enzymes. In the present study I showed that another dimension is added when the covalent ligand is charged, since the electrostatic nature of the DNA is drastically altered in the process, resulting in conformational changes of the helix that may in turn considerably affect its specificity and response to its ionic environment.

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References

Aksiyote-Benbasat, J., & Bloomfield, V. A. (1981) *Biochemistry 20*, 5018–5025.
Allison, S. A., Easterly, R. A., & Teller, D. C. (1980) *Biopolymers 19*, 1475–1489.
Allison, S. A., Herr, J. C., & Schurr, J. M. (1981) *Biopolymers 20*, 469–488.
Barbucci, R., Paoletti, P., & Vacca, A. (1970) *J. Chem. Soc. A*, 2202–2206.
Berman, H. M., & Young, P. R. (1981) *Annu. Rev. Biophys. Bioeng. 10*, 87–114.
Berne, B. J., & Pecora, R. (1976) *Dynamic Light Scattering*, Wiley, New York.
Bloomfield, V. A., & Lim, T. K. (1978) *Methods Enzymol. 48*, 415–494.
Braunlin, W. H., Strick, T. J., & Record, M. T., Jr. (1982) *Biopolymers 21*, 1301–1314.
Cantor, C. R. (1981) *Cell (Cambridge, Mass.) 25*, 293–295.
Chattoraj, D. K., Gosule, L. C., & Schellman, J. A. (1978) *J. Mol. Biol. 121*, 327–337.
Chen, C., Kilkuskie, R., & Hanlon, S. (1981) *Biochemistry 20*, 4987–4995.
Cohen, S. S. (1978) *Adv. Polyamine Res. 1*, 1–10.
Davidson, N., & Szybalski, W. (1971) in *The Bacteriophage Lambda* (Hershey, A. D., Ed.) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Earnshaw, W. C., & Casjens, S. R. (1980) *Cell (Cambridge, Mass.) 21*, 319–331.
Eiserling, F. A., Geiduschek, E. P., Epstein, R. H., & Metter, E. J. (1970) *J. Virol. 6*, 865–876.
Fiandt, M., Gottesman, M. E., Schulman, M. J., Szybalski, E. H., Szybalski, W., & Weisberg, R. A. (1976) *Virology 72*, 6–12.
Fulmer, A. W., & Bloomfield, V. A. (1982) *Biochemistry 21*, 985–992.
Gerhard, B., & Warren, R. A. J. (1982) *Biochemistry 21*, 5458–5462.
Gosule, L. C., & Schellman, J. A. (1976) *Nature (London) 259*, 333–335.
Gosule, L. C., & Schellman, J. A. (1978) *J. Mol. Biol. 121*, 311–326.
Haynes, M., Garrett, R., & Gratzer, W. (1970) *Biochemistry 9*, 4410–4416.
Koppel, D. E. (1972) *J. Chem. Phys. 57*, 4814–4820.
Krasnow, M. A., & Cozzarelli, N. R. (1982) *J. Biol. Chem. 257*, 2687–2693.
Kropinski, A. M. B. (1972) Ph.D. Thesis, University of British Columbia.
Kropinski, A. M. B., & Warren, R. A. J. (1970) *J. Gen. Virol. 6*, 85–93.
Kropinski, A. M. B., Bose, R. J., & Warren, R. A. J. (1973) *Biochemistry 12*, 151–157.
Lohman, T. M., de Haseth, P. L., & Record, M. T., Jr. (1980) *Biochemistry 19*, 3522–3530.
Maltman, K. L., Neuhard, J., & Warren, R. A. J. (1981) *Biochemistry 20*, 3586–3591.
Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179–246.
Manning, G. S. (1980) *Biopolymers 19*, 37–61.
Manning, G. S. (1981a) *Biopolymers 20*, 2337–2350.
Manning, G. S. (1981b) *J. Phys. Chem. 85*, 870–877.
Manning, G. S. (1982) *Comments Mol. Cell Biophys. 1*, 311–323.
Marmur, J. (1963) *Methods Enzymol. 6*, 726–738.
Marx, K. A., & Ruben, G. C. (1983) *Nucleic Acids Res. 11*, 1839–1854.
McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.
Miller, P. B., Maltman, K. L., & Warren, R. A. J. (1982) *J. Virol. 43*, 67–72.
Miller, P. B., Scraba, D. G., Leyritz-Wills, M., Maltman, K. L., & Warren, R. A. J. (1983) *J. Virol. 47*, 399–405.
Mokul'skaya, T. D., Smetanina, E. P., Myshko, G. E., & Mokul'skii, M. A. (1975) *Mol. Biol. 9*, 552–555.
Mokul'skii, M. A., Kapitonova, K. A., & Mokul'skaya, T. D. (1972) *Mol. Biol. 6*, 883–901.
Post, C. B., & Zimm, B. H. (1979) *Biopolymers 18*, 1487–1501.
Post, C. B., & Zimm, B. H. (1982a) *Biopolymers 21*, 2123–2137.
Post, C. B., & Zimm, B. H. (1982b) *Biopolymers 21*, 2139–2160.
Record, M. T., Jr., Lohman, T. M., & de Haseth, P. (1976a) *J. Mol. Biol. 107*, 145–158.
Record, M. T., Jr., Woodbury, C. P., & Lohman, T. M. (1976b) *Biopolymers 15*, 893–915.
Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.
Riemer, S. C., & Bloomfield, V. A. (1978) *Biopolymers 17*, 785–794.
Ruben, G. C., Marx, K. A., & Reynolds, T. C. (1981) *Ann. Proc. Electron Microsc. Soc. Am., 39th*, 438–439.
Scraba, D. G., Bradley, R. D., Leyritz-Wills, M., & Warren, R. A. J. (1983) *Virology 124*, 152–160.
Suwalski, M., Traub, W., Shmueli, R., & Subirana, J. (1969) *J. Mol. Biol. 42*, 363–373.
Thomas, T. J., & Bloomfield, V. A. (1983) *Biopolymers 22*, 1097–1107.
van de Sande, J. H., & Jovin, T. M. (1982) *EMBO J. 1*, 115–120.
Warren, R. A. J. (1980) *Annu. Rev. Microbiol. 34*, 137–158.
Warren, R. A. J. (1981) *Curr. Microbiol. 6*, 185–188.
Widom, J., & Baldwin, R. L. (1980) *J. Mol. Biol. 144*, 431–453.
Widom, J., & Baldwin, R. L. (1983) *Biopolymers 22*, 1595–1620.
Wilson, R. W., & Bloomfield, V. A. (1979) *Biochemistry 18*, 2192–2196.
Wilson, W. D., & Jones, R. L. (1981) *Biopolymers 20*, 141–154.
Yamakawa, H., & Fujii, M. (1973) *Macromolecules 6*, 407–415.
Zimmerman, S. B. (1982) *Annu. Rev. Biochem. 51*, 395–427.