DNA chain flexibility and the structure of chromatin ν -bodies

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ABSTRACT

The persistence length of high-molecular-weight, monodisperse-bihelical DNA has been evaluated from low-shear flow birefringence and viscosity data at several temperatures in 2.0 M NaCl neutral pH buffer. At these solvent conditions, both the DNA and histone components of chromatin ν -bodies have structural features similar to those in the intact nucleohistone complex at low ionic strength. The theory of Landau and Lifshitz is used to relate the experimental results to the thermodynamic functions for bending 140 nucleotide pairs of DNA into a plausible model structure: per ν -body, $\Delta G_b = 43.8 \pm 5.3$ kcal/mole, $\Delta H_b = 45.7 \pm 3.7$ kcal/mole, and $\Delta S_b = 6.2 \pm 12.4$ entropy units. This bending free energy is comparable to or less than that estimated to be required for a kinked DNA configuration and appears to be well within the range of estimated electrostatic free energies available from DNA-histone interactions in a ν -body assembly.

INTRODUCTION

There is now abundant evidence that the nucleohistone component of eukaryotic chromatin exists as a linearly repetitive array of ν -bodies (1,2) interconnected by 30 to 70 nucleotide pairs (np) of nuclease-sensitive DNA. The interconnecting region seems to be associated with very lysine-rich histones H1 and H5. The ν -body itself consists of approximately 140 np of DNA concentrated in an annular shell of roughly 110 Å outside (hydrated) diameter around an octameric inner core (3,4) of two molecules each of the histones H2a, H2b, H3, and H4. Monomeric ν -bodies have been isolated and extensively characterized using a variety of biophysical techniques (5), and further details, as well as various models for eukaryotic chromatin structure, are provided in several excellent recent reviews (6-10).

The fact that bihelical DNA is tightly packaged in ν -body structures might seem inconsistent with the large degree of chain stiffness and excluded volume typically found for this macromolecule in solution (11). This difficulty has led to the postulate

of kinks in the Watson-Crick helix designed to relieve the strain on the DNA imposed by its convoluted structure in the ν -body. As described both by Crick and Klug (12) and by Sobell *et al.* (13), the kink concept appears both sterically sound and energetically plausible. However, we are convinced by the results described below that such major dislocations in the secondary structure are not required by energetic considerations to account for the packaging of eukaryotic DNA.

The association of histones to DNA appears to occur mainly through electrostatic attractions between the basic regions of the proteins and the charged phosphate groups of DNA. Nucleohistone preparations are observed to dissociate progressively with increasing salt concentration (14), and recent studies on ν -bodies have demonstrated that DNA-histone dissociation occurs in 2 M NaCl (15,16). In addition, studies on ν -body monomers using urea as a structural perturbant have shown that simple NaCl concentrations on the order of 2 M effectively mimic the role of the opposite partner in the dissociated nucleohistone complex: thermal melting implies that a large fraction ($> 2/3$) of the DNA in the ν -body complex at low salt has essentially the same urea destabilization as naked DNA in 2.0 M NaCl (17); circular dichroism (17) and laser Raman spectroscopy (18) combined with other physical techniques (17) further demonstrate that at least the secondary structure of the inner histone "heterotypic tetramer" remains intact, including estimated α -helical content, in the transition from ν -body structure at low salt to dissociated partners at high salt. These conclusions are in agreement with the studies of Ramm *et al.* (19,20) in which it was suggested that the structures of both histones and DNA in high salt solution are similar to their respective counterparts in nucleohistone complex as determined from circular dichroism spectra, and that both high salt and the effect of the histones on DNA produce a change in helical conformation in the direction of a C-like structure.

The above evidence implies that 2.0 M NaCl acts as a dissociating solvent for the DNA-histone complex in a fashion analogous to the Bjerrum dissociation of strong electrolytes in polar solvents. Clearly, this analogy cannot be pushed too far however. The physical chemistry of the two systems involves many different factors: the nucleohistone complex is a relatively much more complicated system than the small ion analogues; and the evidence quoted is admittedly highly restricted and limited in scope. Nevertheless, both the DNA and histone systems appear to have features in common between their structures in intact ν -bodies and as dissociated partners in 2.0 M NaCl. Accordingly, any measurement of the energy required to bend the DNA into the highly

convoluted conformation it assumes in an intact ν -body is likely to be more realistic in 2.0 M NaCl than in lower ionic strength solvents.

In this paper we show that a useful estimate for the energy required to bend bihelical DNA into a plausible ν -body model can be obtained from low-shear flow birefringence measurements on statistically high molecular weight DNA in neutral pH, 2.0 M NaCl solutions. This energy is not large in relation to ordinary binding energies and, even including configurational free energy, is probably well under the estimated electrostatic free energy available in the ν -body system. Furthermore, this energy compares favorably with that estimated to be necessary to form the equivalent number of kinks (12).

THEORY

In solution, diffusive forces deriving from Brownian motion act upon dissolved macromolecules and cause them to seek a state of minimum configurational free energy. Due to entropic considerations, the equilibrium conformational state will, in general, involve some degree of bending in the macromolecular chain and in the high molecular weight limit will actually approach a random-flight coil subject to the constraints imposed by excluded volume and the nature and extent of intrachain interactions. A particularly successful model for treating these considerations quantitatively for DNA has been the wormlike coil model of Kratky and Porod (21) which represents the macromolecule as a continuously bending rod. The degree of bending (and hence the effective stiffness of the chain) is characterized by the persistence length, a statistical quantity which, in effect, measures the contour distance over which there occurs, on the average, a 68.4° bend. In the limit of high molecular weight, the persistence length is just $1/2$ the statistical segment length of the equivalent Kuhn random coil.

Several workers have developed theories relating the persistence length of stiff-chain macromolecules and the thermodynamics of bending (22-24). Although there are differences among these treatments, they lead generally to similar results. In the following, we utilize the theory of Landau and Lifshitz (22), to facilitate comparison of our results with other data in the literature.

Landau and Lifshitz (22) have related the total bending free energy to the persistence length for a model equivalent to the wormlike coil. The most restrictive aspects of this treatment are: (i) the assumption of a Hookean restoring force to bending, and (ii) the requirement of uniform radius of curvature throughout the contour length of

the model. The first assumption strictly limits the treatment to a weakly bending rod but nevertheless is theoretically sound (25, 26); both are plausible physically for the cases considered here.

For a coil of total contour length L , Landau and Lifshitz calculate for the bending free energy

$$\Delta G_b = \frac{f}{2} \int_0^L p^2 dl = \frac{f \phi_L^2}{2L} \quad (1)$$

where f is the restoring force constant, $d\phi$ is the angle between two tangents to the coil separated by a contour distance dl and $p = d\phi/dl$ as prescribed by assumption (ii) above. They show further that the restoring force constant bears on extremely simple and direct relation to the Kratky-Porod persistence length a :

$$a = \frac{f}{kT} \quad (2)$$

Combining equations 1 and 2 with the definition for Gibbs free energy

$\Delta G_b = \Delta H_b - T\Delta S_b$, one has finally:

$$\frac{ka}{2} = \frac{\Delta H_b L}{2\phi_L^2} - \frac{\Delta S_b L}{\phi_L^2} \quad (3)$$

where k is the Boltzmann constant.

Hence, the temperature dependence of the persistence length provides directly both the enthalpy and entropy of bending a chain of length L through a total angle ϕ_L .

The above treatment was used by Gray and Hearst (27) to make the first quantitative estimate of the energy required for DNA packaging in a higher order biological structure. Persistence length data were obtained for DNA using the molecular weight dependence of the sedimentation coefficient (28) and the theory of the wormlike coil (29, 30) in a 0.1 M NaCl EDTA buffer over a 5 to 50° temperature range. These studies were painstakingly performed and the results carefully analyzed, but the conclusions nevertheless were subject to some uncertainty due to experimental difficulties inherent in high temperature operation of the ultracentrifuge, including the use of DNA's from different viral sources at the higher temperatures, and the fact that the persistence length values depended explicitly upon the applicability of the available theory for the wormlike coil, including its treatment of excluded volume. Nevertheless, the thermodynamic bending functions obtained by Gray and Hearst are qualitatively in

accord with our results here. They were used to rationalize the Kilkson-Maestre model for T2 bacteriophage structure (31) which entails a DNA packaging problem similar to that in eukaryotic ν -bodies.

In the present work, we have obtained persistence length values for native T2 bacteriophage DNA in 2.0 M NaCl at temperatures ranging from 5 to 45° directly from combined flow birefringence and intrinsic viscosity data at very low rates of shear. The T2 DNA molecule is bihelical and has a molecular weight of 130×10^6 daltons (28, 32, 33) with negligible polydispersity (34); this large size ensures that it meets the statistical requirements of the high molecular weight limit theory employed.

METHODS

Materials

T2 bacteriophage DNA was prepared using standard methods exactly as described in a previous publication (35). Before use, all DNA preparations were exhaustively dialyzed against the stock 2.0 M NaCl phosphate EDTA "BPES" buffer employed as solvent. The final dialyzate was retained as reference solvent. DNA concentrations were measured spectrophotometrically on a Zeiss PMQ-II using an extinction coefficient of $0.0181 \text{ cm}^2/\mu\text{gm}$ (32).

Viscosity and Flow Birefringence Measurements

Instrumentation and methods (36) and application of the technique to DNA (37) have been described previously.

As in earlier studies, flow birefringence data were obtained near the low-shear, low-concentration limits using our highly sensitive photoelectric-scanning flow birefringence apparatus with output signal time averaging. Residual instrumental effects were eliminated using the method recently described by Barrett and Harrington (38). A Teflon flow cell was used in the flow birefringence apparatus, and the usual precautions were taken (36, 37) to prevent shear breakage and to keep DNA samples from contacting materials other than glass, Teflon, and polyethylene. Blank runs with pure solvent were made at all temperatures studied and were used to correct the solution flow birefringence data for solvent effects.

At the higher temperatures, some instability in flow birefringence signal resulted, apparently due to slightly imperfect thermostating of the flow cell resulting in minute thermal gradients near the cell windows. We were unable to eliminate completely such

effects in our apparatus. The instability tended to disappear at higher velocity gradients, but was most bothersome at the critical lowest shear measurements. Where these effects tended to seriously affect experimental precision, additional data were taken to partially compensate.

Intrinsic viscosity data were obtained at the low shear limit with our cartesian diver Couette-type viscometer (35). In these measurements, also, some instability was observed at the highest temperatures, but such effects in this case could be practically eliminated by sufficiently long thermal equilibration times.

Treatment of Experimental Data

Both extinction angle and flow birefringence data were obtained from the experimental measurements. The extinction angle data were unexceptional and are not of direct interest in the present study; accordingly, we do not report them. The flow birefringence Δn vs shear rate G data for 10 shear points were least squares fitted to a cubic polynomial; the limiting slope $(\Delta n/G)_{G \rightarrow 0}$ was taken as the coefficient of the linear term. The reduced birefringence $(\Delta n/Gc)_{G, c \rightarrow 0}$ was taken as the linear coefficient of the $(\Delta n/G)_{G \rightarrow 0}$ vs concentration curves, again least squares fitted to cubic polynomials. At each temperature reported at least 25 independent concentrations were determined.

In an earlier publication (37) we have shown that both intrinsic and form birefringence effects are determined entirely by the Kuhn statistical segment length for macromolecules of high molecular weight and kinetic chain rigidity. This result has been tested extensively, both theoretically (37,39) and experimentally (37,40) for DNA and other high polymers. The ratio of reduced flow birefringence to intrinsic viscosity is, therefore, also a direct function of persistence length in the high molecular weight limit (37):

$$\frac{(\Delta n / cG)_{c, G \rightarrow 0}}{[\eta]} = \frac{4\pi\eta_1(n_1^2 + 2)^2}{45n_1 kT} \left[(\alpha_{11} - \alpha_{\perp}) + \frac{M_m (dn/dc)^2}{2\pi N_A \bar{v}} \right] \left(\frac{2a}{D} \right) = Ca \quad (4)$$

where C is a constant for a given polymer-solvent system at a given temperature, but in general is a complicated function of temperature T , solvent refractive index n_1 and viscosity η_1 , the polarizability anisotropy $(\alpha_{11} - \alpha_{\perp})$ of the monomer unit of molecular weight M_m (e.g., a nucleotide pair in DNA), where the subscripts on the α 's are with respect to the helical axis, and the refractive index increment (dn/dc) , the partial

specific volume $\bar{v}$ and the monomer repeat distance D of the polymer.

We have previously used equation 4 to determine the absolute persistence length of T2 DNA from flow birefringence and viscosity (37). This was based upon an experimental value of $(\alpha_{11} - \alpha_{\perp}) = -12.5 \times 10^{-24} \text{ cm}^3$ from studies upon fractionated low molecular weight DNA (41). This value compares remarkably well with a theoretical estimate of $(\alpha_{11} - \alpha_{\perp}) = -14.6 \times 10^{-24} \text{ cm}^3$ obtained from a bond polarizability summation based upon the Watson-Crick B-form structure (41). At the time this work was reported, our experimental persistence length was in fair agreement with other values under similar solvent conditions obtained from analysis of light scattering, sedimentation, or intrinsic viscosity, using the theory of the wormlike coil.

More recently, Jolly and Eisenberg (42) have used photon correlation spectroscopy to redetermine the persistence length of DNA in neutral pH, 0.2 M NaCl buffer. For various reasons, we feel that the Jolly and Eisenberg result $a = 660 \pm 60 \text{ \AA}$ is the best presently available for our purposes, although it is slightly higher than other recent estimates obtained from classical light scattering (43), intrinsic viscosity (43), and sedimentation (44,45). Accordingly, we have calibrated our flow birefringence data against this value. We have made flow birefringence and intrinsic viscosity studies on T2 DNA in neutral pH phosphate EDTA buffers ranging from 0.005 to 2.0 M NaCl; this work will be published in detail elsewhere (46), but for present purposes, we note that the Jolly and Eisenberg persistence length of 660 Å combined with our result $(\Delta n/cG)_{c, G \rightarrow 0} = -6.442 \times 10^{-10}$ (in cgs unit) at 0.2 M NaCl leads to $(\alpha_{11} - \alpha_{\perp}) = -12.93 \times 10^{-24} \text{ cm}^3$ from equation 4. Values of $(dn/dc) = 0.175$ and $\bar{v} = 0.505$ used in this estimate were those determined by Cohen and Eisenberg (47); $n_1 = 1.33511$ and $\eta_1 = 0.9066 \text{ cp}$ were determined by us. This value of $(\alpha_{11} - \alpha_{\perp})$ is fairly close to our model calculation result, but we note that the estimate of a from equation 4 is sensitive to the optical anisotropy since the intrinsic and form birefringence terms are of very nearly comparable magnitude.

Since we have calibrated our persistence length determination at 0.2 M NaCl, it is necessary to assume invariance of $(\alpha_{11} - \alpha_{\perp})$ with increasing salt in order to obtain high-salt persistence length values from our experimental data. This assumption is directly supported by the X-ray results of Wilkins et al. (48) who report B-genus DNA in nucleoprotein complexes. However, the circular dichroism of DNA is a monotonically decreasing (19,20,49,50), probably concave downward (19,20,49), function of increasing

NaCl concentration up to at least 2.0 M NaCl, and the observed changes at 260 nm are in a direction to correspond with a secondary structural transition in the B- to C-form direction (51). Additional evidence for a structural change in DNA with increasing salt or protein association is provided by flow dichroism (52) and electro-optical studies (53).

Any change in DNA secondary structure with increasing salt would affect $(\alpha_{11} - \alpha_1)$ to some degree. However, comparison of our results with available experimental data from other sources implies that any such effect is negligibly small. Persistence lengths for DNA from a wide variety of measurements have been reported at salt concentrations around 0.1 - 0.2 M NaCl. Recent estimates range from 575 Å (45) to the 660 Å value of Jolly and Eisenberg (42) used as the calibration point in the present work; the current status of the persistence length question has been extensively reviewed elsewhere (42,43,46). At higher salts, Hearst *et al.* (54) have calculated persistence lengths from the molecular weight-dependent intrinsic viscosity data of Ross and Scruggs (55), obtaining a value of 332 Å at 1 M NaCl. In a more recent communication, Rinehart and Hearst (56) have analyzed Bruner and Vinograd's (57) sedimentation data for DNA at 20° in buffers from 1 to 5 M NaCl, and obtained persistence length values of 445 to 460 Å at 2 M NaCl, depending upon whether excluded volume was held fixed or allowed to vary optimally over the range of salt concentrations investigated. Interpolation of our data in Table I gives $a \cong 455$ Å at 20°. We observe a measurable decrease in persistence length between 1 and 2 M NaCl (46); in this respect, our data are roughly comparable to their fixed excluded volume case. Comparison of these results, as well as our salt dependence data above 0.1 M NaCl (46), establishes both the salt concentration independence of $(\alpha_{11} - \alpha_1)$ and the general validity of our procedure.

This conclusion is justified also on theoretical grounds. The large negative optical anisotropy of DNA implies that the principal contribution to $(\alpha_{11} - \alpha_1)$ comes from the polarizability component normal to the helical axis, α_1 (39,58); to a first approximation, this component would be reduced in the C-form structure over the B-form by only a factor of $\cos 6^\circ = 0.995$. The effects of a B- to C-form structural transition on the axial component, α_{11} , are less clear but correspondingly less significant. In the C-form of DNA, the translation per residue along the helical axis is reduced from 3.46 Å to 3.32 Å, while the base pairs are removed roughly 1.5 Å away from the helical axis (59). This may result in some additional π -overlap and electron delocalization in the C-form structure, but the significance of this is doubtful, particularly since CNDO calculations on the sugar phosphate chain of DNA (60) have predicted considerable

electron delocalization through this structure. In any event, most of the observed circular dichroism change, and presumably secondary structural change, seems to occur above 2.0 M NaCl (19,20). Accordingly, we feel that the calibrated optical anisotropy value used here is a realistic one for DNA in 2.0 M NaCl solvent.

Several of the experimental quantities in equation 4 are temperature dependent. These are shown in Table I. Solution refractive indices, n_1 , were determined at 25° in our laboratory and were corrected for temperature dependence using handbook values for pure water. Absolute viscosities of solvent, η_1 , were determined at the respective temperatures using a Cannon-Ubbelohde capillary viscometer calibrated against pure water. Values of partial specific volume of DNA, $\bar{v}$, at the temperatures of this study were interpolated from the data of Gray and Hearst (27) by least squares curve fitting and were adjusted linearly so that the 25° value was equal to $\bar{v} = 0.537$ as interpolated from Cohen and Eisenberg's data (47) at 2.0 M NaCl.

The temperature dependence of (dn/dc) should be negligible over the range studied here; we use $(dn/dc) = 0.175$ as determined (47) in 0.2 M NaCl.

RESULTS AND DISCUSSION

Persistence length values calculated from equation 4 are shown in Table I for the various temperatures investigated. It is clear that the persistence length decreases with increasing temperature, as also observed by Gray and Hearst (27) and others (43, 61), in quantitative accord with the notion that larger thermal motions effectively reduce chain

TABLE I. Experimental data and calculated persistence length values.

T °C	$1/T \times 10^3$ deg ⁻¹	η_1 (a) (cp)	n_1 (b)	$\bar{v}$ (c) (ml/gm)	$[\eta]$ (a) (dl/gm)	$\left(\frac{d[\eta]}{dc}\right)_{c,G=0}$ (cgs units) $\times 10^5$ (a)	$\left(\frac{d[\eta]}{dc}\right)_{c,G=0}$ (cgs units) $/ [\eta] \times 10^{10}$ (a)	a (Å)	$\frac{ka^2}{2} \times 10^{14}$
5.0	3.596	1.8434	1.35385	0.532	238	2.972	12.49	479	3.31
15.0	3.471	1.3841	1.35315	0.534	238	2.106	8.850	463	3.20
25.0	3.355	1.0847	1.35225	0.537	238	1.560	6.553	445	3.07
35.0	3.246	0.8769	1.35093	0.540	239	1.189	4.976	425	2.94
45.0	3.144	0.7268	1.34945	0.545	239	0.9689	4.054	420	2.90

(a) Experimentally measured at indicated temperatures.

(b) Experimentally measured at 25° and corrected for temperature dependence empirically (see text).

(c) Temperature dependence from data of Gray and Hearst (27) calibrated at 25° by the (interpolated) result of Cohen and Eisenberg (47).

stiffness at higher temperatures. Furthermore, the absolute magnitudes of our persistence length values agree well with other estimates from different physical measurements.

We observe no temperature dependence of the intrinsic viscosity within experimental error. This observation is in accord with the results of one previous investigation on high-molecular-weight DNA (55) but is at variance with another on very low-molecular-weight DNA fragments (61). The slight trend that is shown in Table I is in the wrong direction to reflect changes in chain flexibility and probably derives from effects of solution density changes on the concentrations since all concentrations were read at room temperature.

According to equation 3, a plot of $ka/2$ versus $1/T$ should be linear with slope and intercept equal respectively to the enthalpy and negative entropy of bending. Such a plot of our data is shown in Fig. 1; Gray and Hearst's values (27) are also shown for comparison. An unweighted linear least squares analysis of our results gives a slope of $9.56 \pm 0.77 \times 10^{-12}$ erg Å/rad² = 1.38 ± 0.11 kcal Å/rad² mole and an intercept of $-1.30 \pm 2.60 \times 10^{-15}$ erg Å/rad² deg = -18.7 ± 37.4 cal Å/rad² deg mole. These results are in satisfactory agreement with Gray and Hearst's earlier values: the bending free energies obtained in the two works are almost identical, and the enthalpy terms are within a factor of 2. Our small positive entropy of bending appears more plausible

Fig. 1. Persistence length ($ka/2$) vs ($1/T$)
For DNA in 2.0 M NaCl neutral pH "BPES" buffer from this study (O). Data of Gray and Hearst (27) in 0.1 M NaCl neutral pH buffer shown for comparison (●). Solid lines in each case are an unweighted linear least squares of the respective data (see text).

physically than their somewhat larger negative value, but it must be noted that both results are identical within estimated experimental error, and, indeed, the algebraic sign of the entropy of bending cannot be precisely determined by these experiments.

The standard errors in slope and intercept of Fig. 1 derive only from the least squares analysis itself. We have observed previously that we can determine flow birefringence of high molecular weight DNA with 1 to 2% precision near the zero shear and concentration limits (36). Since the reduced values were all evaluated from 10 independent shear points for each concentration and at least 25 independent concentrations, the standard errors in the limiting slopes were in all cases small compared to the observed spread of the $(\Delta n/cG)_{c,G \rightarrow 0}$ values among themselves: The standard error of the points with respect to the line in Fig. 1 $S_n(ka/2) = 0.021 \times 10^{-14}$. Similarly, relative viscosity and concentrations (the latter determined spectrophotometrically) can be read in principle with a precision of about 0.1% although in practice somewhat greater spread is usually observed among replicate determinations. Hence, the standard errors quoted above probably represent reasonable estimates of statistical precision in these experiments.

The effect of glucosylation of the 5-hydroxymethylcytosine bases in T2 phage DNA on the reported persistence lengths cannot be determined directly from these experiments. In T2 DNA, a single glucose moiety is attached to 70% of the hydroxymethyl groups, and about 5% of them are doubly glucosylated. Unpublished experiments in our own laboratory comparing the flow birefringence and intrinsic viscosity of T2* DNA (non-glucosylated) to T2 DNA under similar solvent conditions have demonstrated only small differences which, in general, can be rationalized by the differences in molecular weight. Similar conclusions with respect to sedimentation behavior are reported by Gray and Hearst (27). Since the relationship of persistence length to experimental data given by equation 4 does not explicitly involve molecular weight, we conclude that glucosylation effects are probably unimportant in the present work.

We may now return to our original goal and relate the results of these experiments to the energetics of ν -body assembly. Olins *et al.* (10,17) have recently proposed a model for the ν -body which seems to rationalize all presently known facts. In this model 140 np of DNA are wrapped around on octameric core consisting of α -helical-rich, globular domains of histones H2a, H2b, H3, and H4 closely packed with dihedral point group symmetry (62). The globular region of each inner histone is roughly 26 Å in diameter and contains 70 to 80 amino acid residues; the remainder of the molecule is a nonglobular basic "tail" which is probably the active region in DNA binding (63),

possibly in the large groove of the Watson-Crick helix. The nucleohistone binding interactions are largely electrostatic, as evidenced by their strong dependence on solvent counterion concentration, and are probably nonspecific. The known single equivalent superhelical turn in the DNA fragment (64) may be stabilized by attachment to histones H3 and H4 at the ends (17); the DNA can now assume a configuration roughly equivalent in radius of curvature to a simple two-turn coil.

From the above model we calculate the bending energetics for a 140 np (475 Å) fragment of DNA bent through two full turns. This is equivalent to a 38 Å bending radius, a figure which is also quite consistent with the above model. Using our experimental results, we estimate $\Delta H_b = 45.7 \pm 3.7$ kcal/mole and $\Delta S_b = 6.2 \pm 12.4$ entropy units per ν -body. At 37° this corresponds to $\Delta G_b = 43.8 \pm 5.3$ kcal/mole per ν -body. This latter quantity represents the true thermodynamic work of bending 140 np of DNA into the two-turn coil described and hence is the principal quantity of interest. Although the bending mechanism and the various specific elementary processes contributing to this free energy cannot be deduced from a purely thermodynamic measurement, the numerically small entropy of bending observed both in this work and by Gray and Hearst (27) argues strongly against large solvation or ionic binding changes in the bending process such as have been postulated, for example, in the specific binding of lac-repressor to the lac-operon region of DNA (65,66).

Macromolecular binding free energies of this magnitude are not large, and represent the equivalent of only a relatively few hydrogen bonds. Furthermore, electrostatic interactions tend to be relatively strong and long range. We are not aware of studies from which experimental binding free energies of nucleohistone complexes can be derived. However, considerable evidence exists that about 50% of the phosphates in chromatin are accessible chemically in histone-bound DNA (14); presumably most of the remainder are electrostatically bound in some fashion. Thus, it seems reasonable to conclude that ample electrostatic free energy would be available in the assembly of the ν -body to overcome both the bending free energy and the configurational free energy of the DNA.

These conclusions appear to be qualitatively supported by the results of studies of lac repressor protein binding to DNA. Revzin and co-workers (67) have studied the nonspecific binding of the lac repressor to bacteriophage λ DNA at several temperatures and under various solvent conditions. Using boundary sedimentation techniques in the analytical ultracentrifuge, these workers were able to measure absolute binding constants

from which a standard free energy of association $\Delta G^\circ = -7.43$ kcal/mole at 20° is obtained. From an analysis of ionic strength dependence, it was furthermore established that this binding free energy corresponds to the association of about 11 ± 1 basic residue-DNA phosphate ion pairs per (tetramer) repressor molecular (67) while circular dichroism studies (68) show that the total site size for binding encompasses about 12 DNA base pairs and involves a change in DNA secondary structure upon association. The association free energy given by von Hippel and co-workers is less than one-half that obtained by Riggs *et al.* (65,66) for the specific binding of lac repressor to the lac operator region of *E. coli* DNA, but comparison to the present case is probably much more appropriate.

If binding in the lac repressor-phage λ DNA system is equivalent on a nucleotide pair basis with the ν -body system, assuming 50% base pair-histone associations in the latter, almost 50 kcal/mole of binding free energy would become available in the assembly of a ν -body. However, we note that the estimate of 50% base pair-histone interactions is based upon analysis of total accessible phosphates (14) and as such is certainly an upper limit figure. It is entirely possible that a fraction of inaccessible phosphates may be shielded in the nucleohistone complex but do not necessarily interact electrostatically with a basic histone residue. If the true number of basic residue-DNA phosphate interactions in nucleohistone is estimated by analogy to the lac repressor-phage λ DNA system and taken as 11/12 the number of covered sites, the binding free energy might be reduced to a value comparable to our estimated bending free energy for a smoothly bent DNA ν -body model.

It will also be useful to compare the thermodynamic bending energy obtained here with that estimated for the equivalent kinked model. Crick and Klug (12) have proposed a model for nucleohistone DNA based upon 1 kink through a 95 to 100° angle every 20 nucleotide pairs; this model is functionally very nearly equivalent to the two-turn DNA coil upon which our own calculations are based. Using their estimate of 4 to 6 kcal/mole per kink, which is in line with known base stacking energies (69), the total bending free energy for the DNA in the kinked configuration would be comparable to our estimate above. On the other hand, if kinking should occur as frequently as 10 nucleotide pair intervals, as postulated in the model of Sobell *et al.* (13), the total bending free energy per ν -body might be considerably higher for a kinked structure than for the equivalent structure involving smooth bends.

Crick and Klug (12) have noted a number of specific advantages that nature might derive from kinked DNA as well as the structural problem of a Watson-Crick helix bent

through a small radius as suggested here. On the other hand, kinks have not been observed in electron micrographs of either naked DNA or of lac-DNA bound to lac-repressor (70). Nor, to the best of our knowledge, have they been implicated in the solution properties of DNA: The persistence length of DNA seems to be independent of chain length over a wide range of molecular weight (42–45), whereas if the free energy of kinking is 5 kcal/mole, thermal fluctuations should introduce, on the average, about 1 kink per 3×10^6 daltons molecular weight. Finally, an appreciable activation energy barrier between kinked and straight conformations of DNA, if it exists, might impose kinetic restrictions to ν -body assembly and disassembly.

On the basis of present evidence, we do not believe that a definitive choice can yet be made between smoothly bent and kinked DNA in eukaryotic ν -bodies and other biological structures involving high DNA packing ratios. Our results, along with Gray and Hearst's earlier data (27), suggest that smooth bending of DNA is at least comparable to kinking from a thermodynamic standpoint. Furthermore, our studies support this conclusion in a 2.0 M NaCl buffer, a solvent condition under which reversible dissociation of structurally relatively unperturbed DNA and histone tetramer may occur.

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