Volume 8 Number 12 1980 Nucleic Acids Research

The number of charge-charge interactions stabilizing the ends of nucleosome DNA

James D.McGhee and Gary Felsenfeld

Laboratory of Molecular Biology, National Institutes of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD 20205, USA

Received 21 March 1980

ABSTRACT

It has been shown by others that the melting of DNA in the nucleosome core particle is biphasic (ref.1) and that the initial denaturation phase is due to melting of the DNA termini (refs. 1 & 2). We analyze the salt dependence of the melting temperature of this first transition and estimate that only 15% of the phosphates of the DNA termini are involved in intimate charge-charge interactions with histones. (The simplest model yields ~9%, whereas a calculated overestimate yields ~21% neutralization.) This is a surprisingly small number of interactions but we suggest that it may nonetheless be representative of *all* the core particle DNA.

INTRODUCTION

The reaction of nucleosome core particles with dimethylsulfate indicates that the major DNA groove (and probably the minor groove as well) may be completely accessible to solvent[3]. The same general conclusions are obtained from Raman spectroscopy[4] and from the demonstration that normal core particles can be reconstituted using glucosylated DNA (JDM and GF, manuscript in preparation). Earlier studies of the sites of action of different nucleases on core particle DNA had also indicated that three out of every ten phosphodiester bonds are susceptible to cleavage[5]. Taken together, the above studies suggest that, in the nucleosome core particle, the histones make contact only with the phosphodiester backbone of the DNA and that even these interactions may be relatively few in number.

In this paper, we attempt to measure the number of strong charge-charge interactions which stabilize the ends of core

© IRL Press Limited, 1 Falconberg Court, London W1V 5FG, U.K.

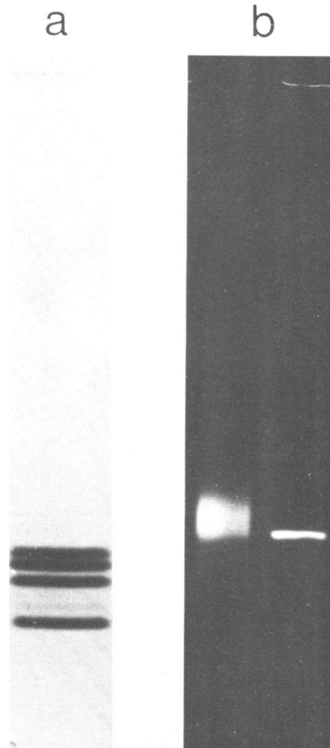
particle DNA. The starting point for this study is the thorough investigation by Weischet *et al.*[1] of the thermal denaturation of nucleosome core particles. They demonstrated that, under appropriate ionic conditions, core particle melting is biphasic, and suggested that the first phase corresponds to the denaturation of ~20 base pairs at each end of the core particle DNA. Simpson[2] has since used high temperature nuclease digestion to demonstrate this end melting directly. The initial phase of the denaturation is reversible (Weischet *et al.*[1] and confirmed in the present study) and can be analyzed by an equilibrium theory of DNA melting coupled to reversible ligand binding[6,7]. Thus the salt dependence of the Tm of this first phase of core particle denaturation can be used to estimate the number of charge-charge interactions which stabilize the DNA termini and which are sufficiently intimate to bring about counter ion release[8,9].

MATERIALS AND METHODS

Chicken erythrocyte core particles were prepared by micrococcal nuclease digestion of chromatin which had been stripped of histones H1 and H5. In more detail, chicken erythrocyte nuclei were prepared as previously described[10] and digested to 0.5-1% acid solubility with micrococcal nuclease (~5 units/ml for 30 minutes at 37°C at a DNA concentration of 5 mg/ml). The digestion was stopped by adding EGTA to a final concentration of 5 mM, the nuclei pelleted at 1000g for 5 minutes, and resuspended in 1/5 the original volume of 100 mM KCl, 50 mM Tris (pH 8.0), 1 mM EDTA. After dialysis against two changes of 0.25 mM EDTA to rupture the nuclei, membranes and debris were centrifuged out (8000g for 20 minutes) and the supernatant was dialyzed against two changes of 0.65 M NaCl, 50 mM Tris (pH 8.0), 1 mM EDTA. The chromatin was separated from histones H1 and H5 by centrifuging through 10% sucrose, containing the above 0.65 M NaCl buffer, onto a 70% sucrose cushion. The stripped chromatin peak (containing DNA of the size corresponding to 20-40 nucleosomes) was then dialyzed into the original digestion buffer (100 mM KCl, 50 mM Tris (pH 8.0), 1 mM $CaCl_2$) and redigested to 15-20 % acid solubility. Nucleosome

core particles were finally reisolated on isokinetic sucrose gradients containing 0.4 M NaCl. Figure 1a and 1b shows the core particle histone content and DNA content respectively.

The buffer used in the majority of the melting experiments contained 0.4 mM NaOH, 0.44 mM cacodylic acid, 0.01 mM Na_2EDTA; final sodium concentration 0.42 mM, final pH 7.0. Prior to melting, core particles were exhaustively dialyzed into the above buffer and then diluted into the appropriate concentration of sodium chloride (or other salt) dissolved in the dialysate. At the highest salt concentrations employed (10 mM), the pH dropped



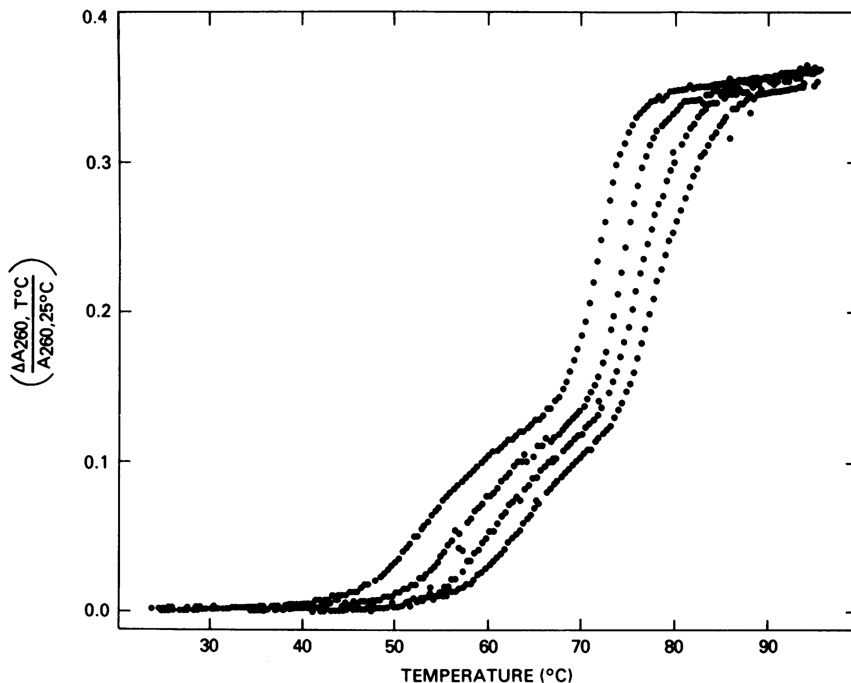
1) a) 18% polyacrylamide-SDS gel of core particle histones, stained with Coomassie Brilliant Blue;
b) 6% polyacrylamide gel of core particle DNA (on the left) stained with ethidium bromide; on the right is a restriction fragment 140 base pairs long, but with 4 nucleotide long single-strand tails.

by only 0.2 units. The initial absorbances at 260 nm were usually 0.45; however, no significant effects in Tm were observed over the absorbance range from 0.3 to 1.3. Samples were bubbled with water saturated helium gas, transferred to Teflon-stoppered cuvettes, and overlaid with spectral grade dodecane. Thermal denaturations were performed in a Beckman Acta III spectrophotometer interfaced to a Hewlett-Packard 2100A computer. The heating rate was 0.25 degrees/minute.

RESULTS

Melting Profiles of Nucleosome Core Particles

Representative denaturation profiles of nucleosome core particles, at Na^+ ion concentrations of 0.4, 1.0, 2.1 and 3.5 mM, are shown in Figure 2. As first demonstrated by Weischet *et al.*[1]

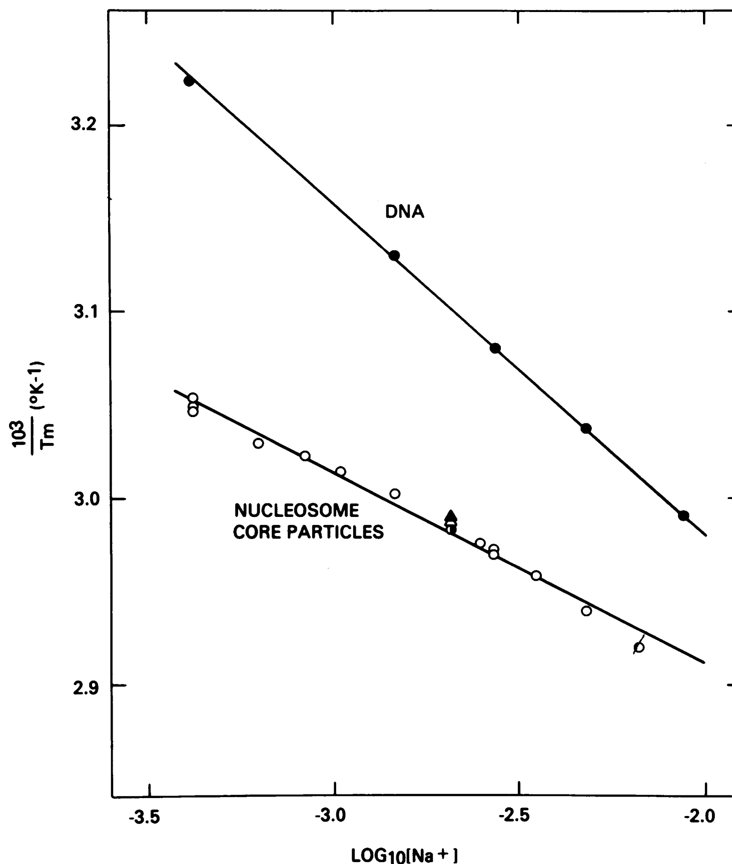


2) Thermal denaturation profiles of nucleosome core particles in 0.4, 1.0, 2.1 and 3.5 mM Na^+, from left to right.

the melting curves of core particles are distinctly biphasic at these low ionic strengths. The overall core particle hyperchromicity at 95°C (37 ± 2%) is close to that observed with the protein-free DNA controls, measured at an equivalent distance above their Tms (39 ± 2%). We also confirm that the initial phase of the transition is completely reversible; the A_{260} at 30°C is regained within 1% after heating to 62°C in 0.42 mM Na^+ and then cooling to 30°C. At NaCl concentrations above ~8 mM, the curves are no longer biphasic and aggregation occurs at high temperatures, making it difficult to interpret the data.

The extent of the first denaturation phase can be estimated reasonably accurately from the inflection points seen in Figure 2. If allowance is made for the slope of the melting profiles both before and after the transition, we estimate that 38.4 ± 0.2% (standard error) of the overall denaturation takes place in the first phase. A similar estimate (37.2%) is obtained with core particles prepared by KCl fractionation[10], (37.2%). Assuming that the melted fraction is linear in hyperchromicity (justified by the fact that the overall hyperchromicities are the same as for histone-free DNA), the present measurements indicate that 27 ± 1 base pairs are melted from each terminus of the core particle DNA at the completion of the first transition. This estimate is considerably higher than that initially reported by Weischet *et al.* [1] but is within the range determined by ^{31}P nuclear magnetic resonance[11].

Figure 3 plots (1/Tm) (in units of $10^3/°K$) vs. $Log_{10}[Na^+]$, both for core particles and for the histone-free core particle DNA; the choice of coordinates will be explained in the next section. The majority of the points in Figure 3 were obtained with NaCl as the added electrolyte. However, in order to investigate possible anion binding effects, several denaturations were done with added NaF or $NaClO_4$ instead of NaCl; the initial phases of the denaturations performed in these salts were superimposable (± 0.2°C) on the curves obtained in the same concentration of NaCl.



3) Plot of the reciprocal melting temperature ($°K^{-1}$) vs. logarithm of the sodium ion concentration. Lower line = nucleosome core particles; upper line = histone free core particle DNA. Usual electrolyte is NaCl, except for Δ=NaF, and ▲=$NaClO_4$. All solutions contained 0.44mM cacodylate buffer, pH 7.0.

Analysis of the salt dependence of the core particle melting temperatures

The following analysis draws heavily on two recent reviews of the polyelectrolyte properties of DNA[8,9]. Native double-stranded DNA (in water at 25°C) has 0.88 Na^+ ions "thermodynamically associated" with each phosphate[8,12]; of these, 0.76 Na^+ ions are held in a "condensation layer"[8,9] and 0.12 are

held by longer range interactions in the "screening layer". Analysis of the binding of oligolysines to DNA[8,13] has demonstrated that for each lysine-phosphate interaction in the complex, 0.88 Na^+ ions are indeed released into free solution. We assume this also holds true for intimate arginine-phosphate interactions. Thus, if we can determine the number of Na^+ ions released when the terminal DNA-histone bonds are melted, the analysis of Record *et al.*[8,12] can be used to estimate the initial number of lysine-phosphate or arginine-phosphate bonds holding the DNA ends to the histone core and in which the amino acid positive charges are within the ion condensation layer (i.e. ~7 A^{o} from the DNA surface[9]).

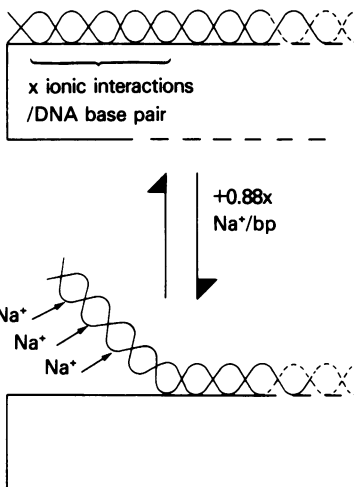
We consider the simplest model for the denaturation of the ends of core particle DNA. We assume that in the initial phase, there is no change in cation binding due to histone conformational changes; (no change is observed in protein circular dichroism[1]). The lack of differential anion binding can be demonstrated experimentally (see below). A critical assumption is that, at the completion of step 2 of Figure 4, the final state of the DNA is free single strands, with no histone attachments; this will be supported in more detail below. Finally, we assume that, on any one core particle, the melting of one end is independent of whether the other end is melted.

With these assumptions, the denaturation of these terminal 27 base pairs of DNA can be considered to occur in two (reversible) stages, as diagrammed in Figure 4.

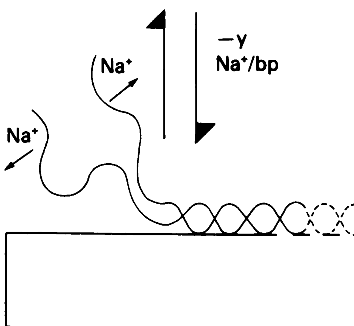
Step 1: the 27 base pair region of duplex DNA at the end of the core particle DNA is initially bound to histones by charge-charge interactions. On heating, this DNA region is released from the surface of the histones but remains helical. If there are, in the initial native complex, an average of x intimate ionic interactions between the histones and each DNA base pair (where $0<x<2$), then when these ionic interactions are disrupted, 0.88x Na^+ ions are bound per DNA base pair.

Step 2: the released section of native DNA now melts, causing the thermodynamic release of y Na^+ ions per base pair.

Step 1: Release of native DNA from histone surface, accompanied by condensation of 0.88x Na^+/bp of DNA



Step 2: Denaturation of DNA termini, accompanied by release of y Na^+/bp of DNA



4) **Schematic mechanism for melting of the terminal regions of core particle DNA; see text for detailed explanation.**

This release of counterions during the DNA helix-coil transition leads to the long established increase in DNA melting temperature with increasing ionic strength[7-9].

Thus, according to this simple model, the net ion release/base pair during the initial phase of core particle denaturation should be $0.88x + y$, where negative values correspond to ion binding and positive values to ion release; i.e. the dependence of the core particle Tm on Na^+ concentration should reflect <u>net</u> ion release, for <u>both</u> steps of Figure 4. Hence, by comparing the Na^+ ion dependence of the core particle

Tm to that for free DNA, we should, by difference, extract an estimate for x.

A quantitative expression relating the dependence of the Tm on Na^+ ion concentration (or on the concentration of other ligands as well) has been derived many times in the past[6,7], and the appropriate equations are:

for the ends of the core particle DNA,

$$d(1/Tm)/d\mathrm{Log}_{10}[Na^+] = -2.3\ (R/\Delta H)\ (0.88x+y)$$

for histone free DNA,

$$d(1/Tm)/d\mathrm{Log}_{10}[Na^+] = -2.3\ (R/\Delta H)\ (y)$$

where Tm = melting temperature in $^\circ$ K, R = gas constant, and ΔH = overall enthalpy of denaturation per base pair of DNA (including enthalpies of breaking histone-DNA bonds in the case of the core particle).

As noted earlier, the experimental data are plotted in Figure 3 according to these coordinates. Both plots are acceptably linear over the twenty-fold range of salt concentration which is available. As expected from the above analysis, the absolute value of the slope is less for core particles than for free DNA, i.e. there is less <u>net</u> ion release associated with the melting of the ends of core particle DNA than with free DNA. The numerical values of the slopes of Figure 3 are: for core particles -1.03×10^{-4} ($^\circ K^{-1}$) and for DNA -1.77×10^{-4} ($^\circ K^{-1}$). To extract values of x and y from these slopes, the enthalpies of denaturation must be known. For DNA, we take the value of ΔH = +9 kcal/mole of base pair[14]; this is, to a very good approximation, independent of salt concentration and temperature[14]. Hence, y is calculated to be 0.35 Na^+ ions thermodynamically released per base pair denatured. This is within experimental error of the value of 0.37 ions/base pair usually found for high molecular weight DNA[8].

We take the same value of +9 kcal to apply to the overall denaturation of a DNA base pair at the end of the core particle. There are several reasons for expecting this to be a completely

adequate approximation: (i) Weischet *et al.*[1] investigated this first denaturation phase calorimetrically and found essentially no enthalpy contribution above that required to melt DNA; indeed, a recent calorimetric study[29] has concluded that the overall stabilization of nucleosome DNA is almost entirely entropic in origin; (ii) in general, ionic interactions are driven by the entropy of counter-ion release and are observed to have very small enthalpies[8,9]. Furthermore, the enthalpy of dissociation of a poly-lysine DNA complex has been measured to be only +0.3 kcal/mole of interactions[15]; inclusion of this term has negligible effect on the calculated results (see below).

Using $\Delta H=+9$ kcal/base pair in the first equation above yields an overall release of 0.20 Na^+ ions per base pair for the core particle ends. Taking the value of y determined for the histone-free DNA, this corresponds to a binding of 0.15 Na^+/base pair for step 1 of Figure 4, and hence $x = 0.17$ interactions per base pair. Employing $\Delta H = +7$ kcal/bp (probably the lower limit of acceptable values for the enthalpy of DNA denaturation) lowers this estimate proportionately to 0.13 Na^+/base pair. Including a correction to the denaturation enthalpy of +0.3 kcal/interaction[15] has less than a 1% effect on the final result. A completely parallel set of experiments using core particles prepared as the KCl soluble fraction[10] yielded an estimate of 0.14 interactions per base pair (data not shown).

Limitations of the Analysis

The remainder of the paper considers limitations to the above simple analysis. In particular, we focus on factors which might have led to an underestimate of the number of ionic interactions stabilizing the core particle termini.

(1) We have assumed that, at the completion of the initial melting phase (i.e. at the end of step 2 in Figure 4), the DNA consists of *free* single strands with no remaining intimate associations with histones. Several lines of evidence support this assumption.

Both steps illustrated in Figure 4 can be distinguished during the melting of reconstituted core particles containing

poly(dAdT).poly(dAdT) [2,11]. in the first step (step 1 of Figure 4), the native DNA can apparently come loose from the core particle surface (as evidenced by a change of the DNA circular dichroism spectrum, in the absence of hyperchromicity), and protrude into solution (as evidenced by a decrease in sedimentation coefficient, even in the presence of crosslinked histones)[2].

In the next step (step 2 of Figure 4), the melting of these terminal DNA regions gives rise to free single-stranded DNA. The best evidence for this is the very sharp ^{31}P resonances, observed at the same chemical shift as melted protein-free poly(dAdT).poly(dAdT)[11]; if the melted strands were tightly bound to the nucleosome surface, a much broader resonance would have been expected. These melted terminal regions also become susceptible to a single strand specific nuclease at the same time[2]. Since the DNA melting occurs in the absence of large scale changes in protein conformation (as monitored by circular dichroism[1]) and since core particles, at the midpoint of the first transition, still maintain their toroidal shape[30], this argues that the melted DNA termini are not intimately bound to regions of the histone octamers which have maintained a rigid conformation.

A further possibility, however, is that the melted DNA single strands are bound to the histone N-terminal regions, which seem to be flexible within the isolated core particle[25]. Fortunately, this possibility can be eliminated by repeating the experiments of Figures 2 and 3 with core particles from which the N-terminal tails have been removed by trypsin. Although these experiments are complicated by some degree of heterogeneity of proteolysis and by the lack of complete reversibility of the initial phase of denaturation, the estimated number of interactions (0.20/base pair; data not shown) is close enough to that obtained above with intact core particles (0.17/base pair) to suggest that the histone tails do not bind to the denatured DNA termini. This result also implies that the histone tails are not involved, in any important way, in binding the native DNA termini to the core particle surface. Thus it seems unlikely

that the melted DNA termini are intimately associated with either a flexible or a rigid region of the histone octamer.

At the low ionic strengths of the present experiments, the melted DNA termini must still be under long range electrostatic influence of the histone positive charges. Nevertheless, the following considerations suggest that these long range DNA-histone interactions can effectively be ignored in the analysis. At the end of step 2 in Figure 4, the electrostatic potential of a melted DNA strand must be influenced not only by the histone positive charges but also by the considerably more numerous negative charges of its partner strand. Whereas the denaturation of histone-free DNA allows the partner strands to separate, either into large loops or into single strands, for the nucleosome DNA melting as diagrammed in step 2 of Figure 4, the strands can not fully separate since they remain bound to the histone core. Since we expect the DNA strand-strand interaction to more than cancel any histone-denatured DNA interactions, the use of free DNA to estimate the number of ions released during step 2 must therefore lead to an overestimate of x.

Thus we conclude that the simple picture of the melting process shown in Figure 4 is an adequate model for the present degree of experimental precision.

(2) In Figure 3, 1/Tm should rigorously have been plotted as a function of the logarithm of the _mean_ ion activity of sodium chloride, rather than the concentration of Na^+ ion. Including these corrections as well as including the dependence of activity coefficients on the salt concentration would only increase x by 10% (page 145 in reference 8).

(3) Oligonucleotides (and by inference the ends of polynucleotides) have a lower degree of ion condensation than polynucleotides and hence have a lower dependence of the Tm on the Na^+ concentration. Equation (6.13) of Record _et al._[8] can be used to estimate that there might be approximately 20% less ion release associated with step 2 in Figure 4, than with the melting of 27 base pairs in the interior of high molecular weight DNA. This would reduce the estimate of x to 0.09 interactions/base

pair.

(4) Alteration in the binding of anions to histones is apparently not a factor that need be considered, at least at the present low salt concentrations. Melting profiles are superimposable ($\pm$ 0.2 ^{o}C) if the core particles are denatured in equal concentrations of NaCl, NaF or $NaClO_4$ (either 2 or 8 mM). These anions span the Hofmeister series and exhibit a range of binding strengths to proteins[8,16]. If anion binding to histones were significantly perturbed by the release and denaturation of the DNA termini, the Tms would be expected to be shifted. Although there are clear examples of large anion effects on certain nucleic-acid-protein interactions[17], oligolysine-DNA binding does not show any such effect[13]. In any event, differential anion binding effects would be expected to reduce the estimate of the number of histone-phosphate interactions.

(5) We have been ascribing the number of ions released in step 1 of Figure 4 solely to the number of ionic interactions broken between the histones and the DNA termini. However, on the core particle surface, not only is the DNA bent (thereby reducing the average inter-phosphate distance) but the 27 base pairs at the DNA ends must be closely juxtaposed to the DNA duplex on the next turn of the core particle DNA (i.e. ~80 base pairs away, following the path of the supercoiled DNA). Both of these effects would lead to an increased local charge density and hence to an increased Na^+ binding in the native core particle. Thus there would be a release of Na^+ ions during step 1 of the melting process diagrammed in Figure 4, in which the neighbouring DNA duplexes separate.

To calculate the <u>maximum</u> effect which this consideration might be expected to produce, we consider the entire (2)(2)(27)=108 negative charges to be uniformly arranged on a chord joining the ends of the 27 base pair region. Hence, the counter ion condensation parameter[8,9], ξ is 9.9, and the thermodynamic ion association parameter, ψ (defined by Record <u>et al.</u>[8,11] as $\psi = 1 - 1/(2\xi)$) is 0.95, compared to 0.88 for free native DNA. In other words, because of the two above effects, each DNA phosphate is calculated to bind an additional 0.07 Na^+,

with the juxtaposition effect roughly three times as large as the bending effect. If all of these excess ions are released during step 1 of the denaturation then x = 0.49 interactions per base pair.

However, we must also include the requirement that, on the core particle surface, the two juxtaposed regions only have a net charge of ~108 (1-x/2) (thus assuming an approximately uniform density of histone interactions throughout the core particle, and automatically allowing for the reduced Na^+ binding potential of core particle DNA due to partial neutralization). At the same time, we include (i) the lower ion binding potential of the free DNA ends (using equation 6.14 of Record _et al._[8]); (ii) the lower Na^+ release which occurs during the melting of the DNA ends, as discussed earlier, and; (iii) the ~10% correction from Na^+ concentration to activity. The overall estimate of the number of ionic interactions/base pair is then x=0.42.

This last estimate of 0.42 interactions /base pair is 2.5-fold higher than the simple uncorrected estimate made initially, and should certainly be an overestimate since the increased charge density arising from the juxtaposed turns of DNA must be lower than that calculated by compressing all charges into a single linear array. As will be discussed below, the core particle melting probably takes place from the "low ionic strength conformation"[19-22]. If this conformation involves an unwinding of the nucleosome DNA supercoil, as proposed by Wu _et al._[21], then the calculated effect of juxtaposition of DNA duplexes no longer applies and the value of 0.42 interactions/base pair must be an even more severe overestimate.

(6) An obvious uncertainty in all the above analysis is how far the concepts and parameters derived from long polyelectrolytes can be applied to the detailed geometry and interactions _within_ a partially neutralized complex such as a nucleosome core particle. Undoubtedly this will be the subject of much future study. In defense of our present analysis it should be noted that, once statistical considerations are removed, a _single_ value of the association constant describes oligolysine-polynucleotide binding, from 0% up to 80% charge

neutralization[18]. This latter degree of charge neutralization is far in excess of the values applicable to a nucleosome core particle.

(7) There is as yet no clear indication of how the melting of the DNA ends, studied in this paper, is related to either the low ionic strength transition observed hydrodynamically[19-21] or to the circular dichroism transition which precedes core particle melting[1,2]. Neither of these two transitions is accompanied by any change in DNA absorbance. It seems clear, however, that at all the ionic strengths used in the present study, the core particle may be melting from the "low ionic strength" state[19]. If the 2-4 ions released/core particle in this low ionic strength transition[21,22] represent a hidden ion release in going from the native core particle to the low ionic strength form from which it melts, this would lower our estimate by ~0.1 interactions/base pair at the ends of the native core particle. Similarly, since the Tm of the circular dichroism premelt increases with ionic strength[1] and since there is no DNA melting, there must therefore be a net ion release; this could only contribute a further decrease in our estimate of the number of interactions in the native core particle.

DISCUSSION

The DNA of a nucleosome core particle has 290 phosphate groups whereas the core histone octamer has a net positive charge of ~150. Thus, overall, the negative charge of the DNA is roughly half neutralized, and there is a potential of one intimate charge-charge interaction per DNA base pair. However, at least for the 38% of the DNA lying at the core particle termini, we have estimated the actual number of such strong interactions to be much less than this, and somewhere between 0.17 and 0.42 interactions/base pair. By strong interactions, we mean interactions which penetrate the counter-ion condensation layer and lead to counter-ion release[8,9].

The low-melting ends of the nucleosome DNA need not have a density of strong ionic interactions which is lower than the

remainder of the core particle DNA, since they might be unstable for other reasons. One obvious cause of their lower stability is that it is just these terminal regions of the DNA which are close to the preceding turn of the DNA supercoil, and which are thus destabilized by the inter-duplex repulsion. At the completion of the first phase of denaturation, there is only a 4 base pair overlap left between successive supercoils; perhaps it is the disruption of the complete turn of DNA that initiates the cooperative breakdown of the remaining core-particle structure when the temperature is raised further. (This argument can obviously not be used if the low ionic strength conformation of the core particle involves unwinding of the DNA supercoil, as envisaged by Wu *et al.* [21].)

Although our data apply only to the terminal regions of the DNA, we can speculate that this same low density of strong ionic interactions might be representative of *all* the core particle DNA. Indeed, there are a number of observations which suggest that, in the nucleosome core particle, there are many positive charges which are not tightly bound to DNA. For example, Weintraub and Van Lente[23] have demonstrated that the "basic histone tails" (which contain ~30% of the net positive charge) are susceptible to proteolytic cleavage, and yet such truncated histones are still able to compact the DNA into an almost normal nucleosome[24]. Indeed, at least portions of these basic histone tails may be mobile and unattached[25].

If, as we suggest, 15% phosphate neutralization (i.e. the average of our two extreme estimates) were representative of *all* the core particle DNA, there would be an average of three strong interactions every ten base pairs and only 20% of the histone lysines and arginines would be strongly bound to DNA. Nonetheless, it can be easily calculated that this can provide a large portion, if not the entirety, of the free energy of histone-DNA binding. The free energy of binding of the DNA to the histone octamer can be estimated crudely from the melting temperatures of the main core particle transition. At 1 mM Na^+, for example, the second denaturation phase is half completed at ~25°C higher than the histone-free DNA (Figures 2 and 3); this

corresponds to an average stabilization of ~0.5 kcal/mole of base pair or 73 kcal per mole of core particle. This is a direct estimate of the free energy of DNA histone binding only if the histone core itself does not undergo a transition and if the histones, both native and denatured, do not bind to denatured DNA. Neither of these two qualifications is completely true but, for the moment, we take this value to be at least an underestimate of the binding free energy, and probably of the correct order of magnitude. We must also however, add to this estimate the free energy of the DNA bending (~13-25 kcal/mole of core particle[26,27]) and thus we estimate that the free energy of binding bent DNA to the core particle surface must be at least 85-100 kcal/mole, at 1 mM Na^+. (A recent calorimetric study has estimated this binding free energy to be ~70 kcal/mole, but the salt concentration was not stated[29].)

Neutralization of 15% of the DNA phosphates by strong interactions which penetrate the condensation layer corresponds to 44 such interactions. If we consider the association constant between DNA and the preformed histone core (K_{app}) to be unity at ~1 M NaCl, then the analysis of Record *et al.*[8,12] can be used to calculate the binding constant at 1 mM Na^+; that is, $\text{Log}_{10}(K_{app}$ at 1 mM $Na^+) = -\Delta\text{Log}_{10}[Na^+]$ (0.88) (44) =115. Hence, the standard free energy of DNA binding to the histone core (at 25°C and 1 mM Na^+) is calculated to be of the order of -157 kcals/mole. These are obviously large and uncertain numbers. Nonetheless, such calculations demonstrate that relatively few such ionic interactions per ten base pairs of DNA can impart enormous free energies for binding DNA to the nucleosome surface.

In summary, analysis of the salt dependence of the melting of the ends of core particle DNA indicates that only a small fraction of the DNA phosphates (~15± 6%)may actually be involved in strong interactions with positively charged amino acid side chains. At the moment, there is no way of knowing how this phosphate neutralization is distributed. Each phosphate may be 15% neutralized, or there may be 3 interactions in every turn of the DNA duplex; in the extreme model, there might be only one binding site, consisting of ~8 strong interactions, on the entire

27 base pair DNA terminus. Although we favor the second model, nuclease digestion studies have been taken to be more compatible with the latter model[28].

We suggest that the same low degree of phosphate neutralization may be representative of all the core particle DNA. Such a model is very different from those proposed structures in which the basic histone tails lie in the DNA grooves, and interact with up to 50 % of the DNA phosphates. The limited contact model suggested by the results presented here, is consistent with conclusions we have reached earlier using chemical probes. We suggest that the DNA of the nucleosome core particle is to a large extent accessible to the surrounding solvent.

ACKNOWLEDGEMENTS

We should like to thank Drs. H. Eisenberg and D. Rau for helpful criticisms and suggestions. We should also like to thank Drs. P.H. Von Hippel and T. Lohman for communicating results prior to their publication.

REFERENCES

1) Weischet,W.O., Tatchell,K., Van Holde, K. E. and Klump, H. (1978) Nucleic Acids Res. 5 ,139-160.
2) Simpson, R.T. (1979) J. Biol. Chem. 254 , 10123-10127.
3) McGhee, J. D. and Felsenfeld, G. (1979) Proc. Natl. Acad. Sci., USA 76 ,2133-2137.
4) Goodwin, D. C. and Brahms, J. (1978) Nucleic Acids Res. 5 ,835-850.
5) Sollner-Webb, B. and Felsenfeld, G. (1977) Cell 10 , 537-547.
6) Crothers, D.M. (1971) Biopolymers 10 , 2147-2160.
7) Bloomfield, V.A.,Crothers, D.M. and Tinoco, I.,Jr. (1974) in Physical Chemistry of Nucleic Acids ,Harper and Row, New York.
8) Record, M.T.,Jr., Anderson, C.F. and Lohman, T.M. (1978) Quart. Rev. Biophys. 11 , 103-178.
9) Manning, G.S. (1978) Quart. Rev. Biophys. 11 ,179-246.
10) Shindo, H., McGhee, J.D. and Cohen, J.S. (1980) Biopolymers 19 , 523-537.
11) Simpson, R.T. and Shindo, H. (1979) Nucleic Acids Res. 7 , 481-492.

12) Record, M. T.,Jr., Lohman, T. M., and De Haseth, P. (1976) J. Mol. Biol. 107 ,145-158.
13) Lohman, T. M. and Record, M. T.,Jr. (1980) Biochemistry (in press).
14) Privalov, P.L., Ptitsyn, O.B., and Birshtein, T.M. (1969) 8 ,559-571.
15) Ross, P.D. and Shapiro, J.T. (1974) Biopolymers 13 ,415-416.
16) Von Hippel, P.H. and Schleich, T. (1969) in Biological Macromolecules , Vol.2 (ed.S.N.Timasheff and G.Fasman), Marcel Dekker, New York, pp.417-574.
17) Kowalczykowski, S.C., Lonberg, N., Newport, J.W. and Von Hippel, P.H. (1980) manuscript submitted.
18) McGhee, J.D and Von Hippel, P.H.(1974)J.Mol.Biol.86 ,469-489.
19) Gordon, V.C.,Knobler, C.M.,Olins, D.E.and Schumaker, V.N. (1978) Proc. Natl. Acad. Sci. USA 75 ,660-663.
20) Gordon, V.C., Schumaker, V.N., Knobler, C.M. and Horwitz, J. (1979) Nucleic Acids Res. 6 ,3845-3858.
21) Wu, H.M., Dattagupta, N., Hogan, M. and Crothers, D.M. (1979) Biochemistry 18 ,3960-3965.
22) Dieterich, A.E., Axel, R. and Cantor, C.R. (1979) J.Mol.Biol. 129 ,587-602.
23) Weintraub, H. and Van Lente, F. (1974) Proc. Natl. Acad. Sci. USA 71 ,4249-4253.
24) Whitlock, J.P.,Jr. and Stein, A. (1978) J. Biol. Chem. 253 ,3857-3861.
25) Cary, P.D., Moss, T. and Bradbury, E.M. (1978) Eur. J. Biochem. 89 ,475-482.
26) Camerini-Otero, R.D. and Felsenfeld, G. (1977) Nucleic Acids Res. 4 ,1159-1181.
27) Levitt, M. (1978) Proc. Natl. Acad. Sci. USA 75 ,640-644.
28) Finch, J.T., Lutter, L.C., Rhodes, D., Brown, R.S., Rushton, B., Levitt, M. and Klug, A. (1977) Nature 269 ,29-36.
29) Bina,M., Sturtevant,J.M. and Stein,A. (1980) Fed. Proc. 39 , abstract 1475.
30) Seligy,V.L. and Poon,N.H. (1978) Nucleic Acids Res. 5 ,2233-2252.