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The temperature and pH depcndence of conformational transitions of the chromatin subunit

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ABSTRACT

Hydrodynamic, spectroscopic, and chemical crosslinking studies on monomer chromatin subunits are reported as a function of ionic strength, pH, and temperature.

In earlier studies, two salt-dependent conformational transitions were described (Gordon et al., Proceedings of the National Academy of Science, 75, 660, 1978). Transition one occurred between 0.7 and 2.0 mM ionic strength and transition two occured between 5.0 and 11.0 mM ionic strength. Crosslinking at 11 mM ionic strength with formaldehyde suppressed both transitions.

In this communication we report that the second transition was characterized by changes in the circular dichroism spectra in the 260-320 nm region as well as by changes in the hydrodynamic properties. As the ionic strength was increased from 5.0 to 11.0 mM, $[\theta]_{282}$ decreased from 2000 to 1500 deg cm^2/dmole and $[\theta]_{295}$ decreased from 0 to -400 deg cm^2/dmole. Both transitions occurred in the pH range from pH 6.0 to 9.2. At pH 5.0, the two ionic strength-dependent transitions were no longer observed and the characteristic changes in the circular dichroism spectra were suppressed. The spectra of the monomer subunits at pH 5.0 showed only small changes with ionic strength and resembled the spectra of the subunits at 11 mM ionic strength above pH 6.0.

In order to characterize the transitions in thermodynamic terms an ionic strength near the midpoint of each transition was selected. Then, changes in $s_{20,w}$ and $D_{20,w}$ were measured as a function of temperature. These data allow an estimation to be made of the enthalpies and entropies of the transitions.

INTRODUCTION

Large quantities of nucleosomes have been isolated, subfractionated, and characterized following digestion with micrococcal nuclease[1]. Hydrodynamic evidence from quasielastic light scattering and from sedimentation studies have indicated the existence of two salt-dependent transitions[2]. In order to interpret the changes in the conformation of the subunit or nucleosome as a function of ionic strength, we have investigated the dependence on pH and temperature of these two salt-dependent transitions.

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MATERIALS AND METHODS

Monomer particle preparation. Nucleosomes (ν_1) from chicken erythrocyte chromatin were isolated by zonal ultracentrifugation as previously described[1]. The fraction of monomers that was soluble in 0.1 M KCl was utilized in the present studies. Prior to the hydrodynamic experiments the KCl-soluble fraction of ν_1 was dialyzed extensively against 0.2 mM EDTA (pH 7.0). These particles contain no H1 or H5, and the distribution of DNA sizes ranges between 140 and 160 base pairs.

Buffer preparation. The buffer used in the pH range of 6.5 to 7.5 was 0.2 mM EDTA. The changes in ionic strength were made by additions of 0.25 M KCl or 2.5 M KCl in 0.2 mM EDTA to the solution of nucleosomes in 0.2 mM EDTA. To lower the ionic strength, additions of 0.02 mM EDTA were made to the nucleosome solutions at higher ionic strength.

Two buffers were used in the pH range of 4-6.5; (1) sodium acetate/acetic acid and (2) 0.2 mM EDTA. Additions of sodium acetate amd acetic acid were made to obtain solutions of the desired pH and ionic strength of 50 mM[3]. Additions of NaOH and EDTA were also made to obtain solutions of the desired pH and an ionic strength of 50 mM. These solutions were then diluted to a final ionic strength of 1 mM and the pH of the diluted solutions was measured.

Sodium borate buffer was used for the pH range between 8 and 9.5. The buffer was prepared from sodium borate and boric acid.

Crosslinking with dimethylsuberimidate. The procedure used for producing a mild degree of crosslinking of nucleosomes with dimethylsuberimidate and which did not induce appreciable aggregation as measured by scattering above 300 nm was similar to that employed by Thomas and Kornberg[4]. The reaction was carried out at pH 9.2 using an ionic strength between 1 and 11 mM. The concentration of dimethylsuberimidate was 1 mg/ml, and the A_{260} of the nucleosome solution was between 1 and 4.

Sedimentation studies of the monomer subunit. Sedimentation coefficients obtained with the Beckman Model E Ultracentrifuge at speeds between 36,000 and 48,000 rpm were corrected to 20° C. The studies were performed on nucleosome solutions with A_{260} of 0.4 to 0.6 using a photoelectric scanner to follow the migrating boundaries. Previous studies[1] have shown that the sedimentation coefficient of ν_1 exhibits negligible concentration dependence below an A_{260} of 1.0. For the temperature studies, the rotor, cells and solutions were incubated at the temperature selected for the sedimentation velocity run. The nucleosomes were prepared at the desired

ionic strength and then placed at the selected run temperature for five minutes. The rotor temperature was not controlled during the run but it was monitored, and the drift in temperature during one and one-half hours was less then 0.3° C.

Diffusion studies of the monomer subunit. Diffusion coefficients were determined by quasielastic light scattering as previously described[2]. For the temperature studies the nucleosome solution was controlled with a thermostated water bath and continuously monitored with a thermister placed directly in the sample solution. As a test of the apparatus the temperature dependence of the diffusion coefficient of 1090 Å polystyrene latex spheres in aqueous suspension was measured between 7 and 35° C. The temperature correction was precise to within 1 %, and all values were adjusted to the same temperature without increasing the standard deviation of the measured diffusion coefficients.

Circular dichroism studies of the chromatin monomer subunit. The circular dichroism spectrophotometer used for these studies has been described in detail[5]. It is a single beam instrument, and the baseline must be recorded separately. Indole acetic acid, an absorbing but optically inactive sample, was used for the baseline[6]. A lock-in amplifier transmits the circular dichroism signal to a computer of average transients, and the signal is averaged to increase the signal-to-noise ratio. The averaging is performed continuously and the resultant signal observed until the desired signal-to-noise ratio is obtained.

RESULTS

Ionic strength studies. The measured sedimentation coefficients, $s_{20,w}$, as a function of the logarithm of the ionic strength are shown in Fig. 1. Some of these values were previously published in reference 2, but now we have collected about twice the initial number of points. The data are a composite of four separate studies and three different preparations of nucleosomes. Transition 1, in which the sedimentation coefficient increases from 9.4 to 10.0 S, occurs between ionic strengths of 0.7 and 2.0 mM. Transition 2, in which the sedimentation coefficient increases from 10.1 to 11.1 S, occurs between ionic strengths of 5.0 and 11.0 mM. Fig. 2 presents the results of the sedimentation equilibrium studies of the nucleosomes at 1, 6, and 11 mM ionic strength which demonstrate that the molecular weight remains essentially constant between

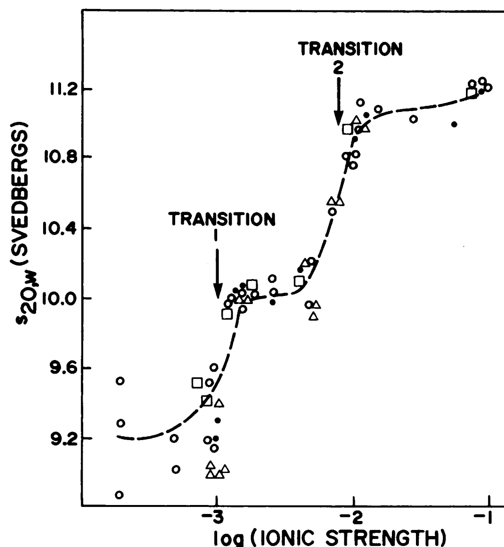

Figure 1: $s_{20,w}$ versus the logarithm of the ionic strength
Two salt-dependent transitions in $s_{20,w}$ were observed as a function of ionic strength. The midpoint of transition 1 is 1.5 mM. The midpoint of transition 2 is 7.5 mM. The data presented are a composite of four studies. The four studies were performed on two different zonal preparations and four different chromatin subunit fractionations.
(●) represents data obtained in a preliminary study. These data were previously reported[2].
(△) represents control data obtained in a pH study.
(○) represents control data obtained in a low ionic strength study.
(□) represents control data obtained in a high ionic strength study.

1 and 11 mM ionic strength. The changes observed in the circular dichroism spectra of the nucleosomes from 0.7 to 11 mM ionic strength are shown in Fig. 3.

Crosslinking studies. Crosslinking studies with formaldehyde were previously reported[2]. Dimethylsuberimidate now has been used to crosslink the monomer subunit without charge alteration. The results of these studies are presented in Table 1. When dimethylsuberimidate was used to crosslink the monomer subunit at 11 mM ionic strength, both transitions were suppressed as demonstrated by measurements of the sedimentation coefficient at 11, 5, and 1 mM ionic strength.

pH studies. The sedimentation coefficient and the circular dichroism spectra were measured at constant ionic strength as a function of pH. The sedimentation coefficients of the nucleosome at 1, 6, and 11 mM ionic

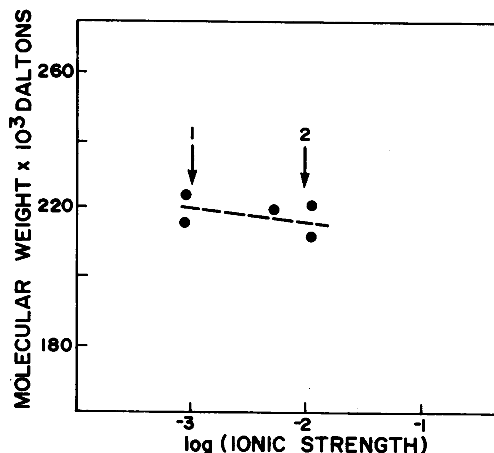

Figure 2: Molecular weight of the nucleosome as a function of the ionic strength.

Molecular weights were determined from sedimentation equilibrium measurements in the ultracentrifuge at room temperature employing the photoelectric scanner, and a sample volume of 0.12 ml. The massive AN-G rotor was used for stability. The solutions were first spun at 10,500 r.p.m. for 18 hours. In the calculation of molecular weights, a value of 0.661 ml/g was used for the partial specific volume of the nucleosome.

strength are plotted as a function of pH in Fig. 4. The observed circular dichroism spectra at 1 mM ionic strength for pH 7, pH 4.8, and pH 4.3 are shown in Fig. 5. Only data obtained with 0.2 mM EDTA as a buffer are shown from pH 4 to pH 7.5. Sedimentation coefficients obtained using the acetic acid/acetate buffer were slightly higher but showed the same increase at pH 5.

Temperature studies. The diffusion coefficient was measured as a function of temperature at several ionic strengths at pH 6.8. The $D_{20,w}$ measured for the nucleosome at 1, 7.5, and 11 mM ionic strength is plotted as a function of temperature in Fig. 6. $s_{20,w}$ measured for the nucleosome at 1, 6, and 11 mM ionic strength is plotted as a function of temperature in Fig. 7. As may be seen from these figures the diffusion and sedimentation coefficients at 1, 6, and 7.5 mM ionic strength are higher at 10-15° C than those measured at 20° C.

DISCUSSION

Additional sedimentation data have now been collected which support

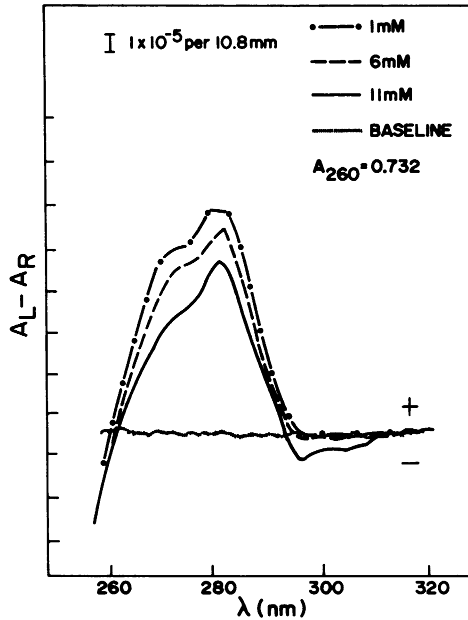

Figure 3: Changes in circular dichroism spectra as a function of ionic strength from 260-320 nm.

The positive circular dichroism signal decreased at 275 and 282 nm as the ionic strength was increased from 1 to 11 mM. The negative circular dichroism signal at 295 nm was lost as the ionic strength was decreased from 11 to 1 mM. Increasing the ionic strength caused a suppression of the circular dichroism spectra in the 260-320 nm region.

TABLE I

Crosslinking with Dimethylsuberimidate (1 mg/ml) for 90 Minutes

Ionic Strength During X-link	Ionic Strength During Study	$s_{20,w}$	Transition Investigated
10 mM	10 mM	11.2 S	control
	5 mM	11.3 S	2
	0.5 mM	11.0 S	1 & 2
5 mM	5 mM	10.3 S	control
1 mM	1 mM	10.2 S	control
	0.5 mM	10.3 S	1
0.5 mM	0.5 mM	9.5 S	control

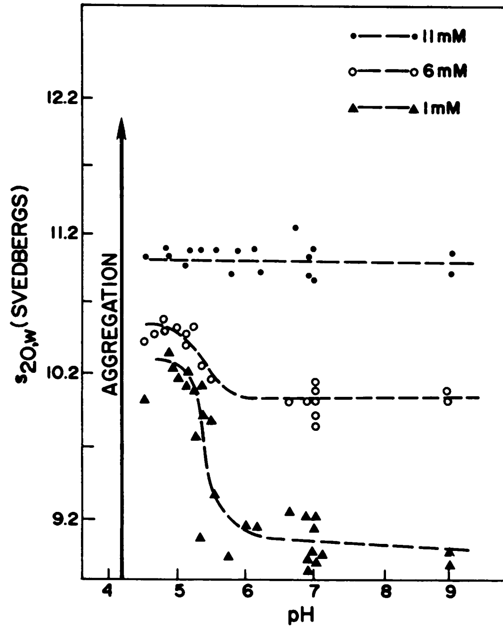

Figure 4: $s_{20,w}$ of the nucleosome at 1, 6, and 11 mM ionic strength as a function of pH

At 11 mM ionic strength the $s_{20,w}$ of the subunit was independent of pH from pH 4.5 to 9.0. At 1 and 6 mM ionic strength, $s_{20,w}$ was independent of pH from pH 5.4 to 9.2 but increased abruptly below pH 5.2. At pH 4.2 the subunits aggregated.

the existence of the two previously reported[2], reversible transitions in the hydrodynamic properties of the nucleosome with ionic strength, centered around 0.7 and 7 mM ionic strength. Sedimentation equilibrium measurements have shown that the molecular weight of the nucleosome remains constant. Therefore, the two transitions must represent changes in conformation and in the translational frictional coefficient of the nucleosomes.

Bifunctional crosslinking reagents, when employed at higher ionic strengths, resulted in a crosslinked nucleosome which did not undergo these transitions in hydrodynamic behavior when the ionic strength was lowered. Crosslinking with formaldehyde was previously reported[2]. We now have repeated these experiments using dimethylsuberimidate which served to form crosslinks between amino groups without altering the electric charge. Again the transitions in hydrodynamic behavior were inhibited by the crosslinking reagent

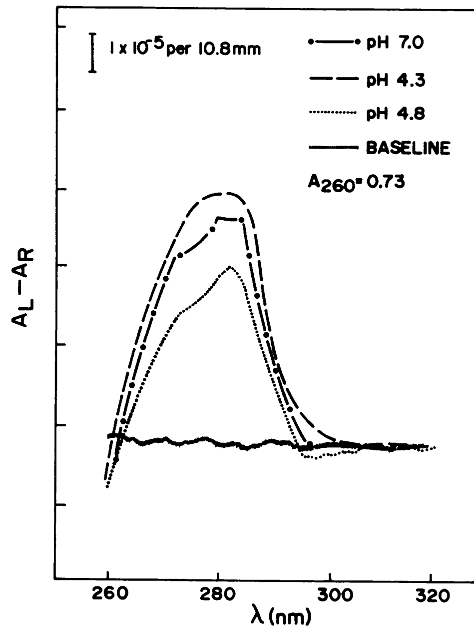

Figure 5: Changes in circular dichroism spectra at 1 mM ionic strength as a function of pH from 260-320 nm

The circular dichroism spectra of the subunit at 1 mM ionic strength were unchanged from pH 5.4 to 7.2. At pH 5.0 and 1 mM ionic strength the subunit exhibited a decreased positive circular dichroism signal at 275 and 282 nm. Below pH 4.6, the positive circular dichroism signal at 275 and 282 nm increased until aggregation occurred at pH 4.2.

even though the net charge on the nucleosome remained constant. This result also served as an additional and important control to demonstrate that the sedimentation behavior was not significantly affected by the primary or secondary charge effects over the range of ionic strengths being studied.

Changes in the circular dichroism spectrum of the nucleosome in the wavelength region between 240 to 320 nm occurred when the ionic strength was raised from 0.7 to 11 mM. We interpret these changes as being due to changes in the environment of chromaphoric groups on the DNA, since histones do not ordinarily make an appreciable contribution to the 260 to 320 nm region of the circular dichroism spectrum.

One approach to the analysis of the circular dichroism spectrum is to assume that it represents a weighted average between the B and C forms of DNA[7]. If analyzed in this manner, then we have found that as the ionic

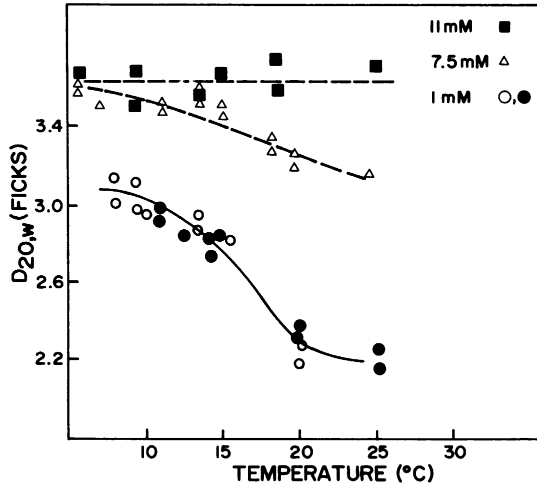

Figure 6: $D_{20,w}$ at 1, 7.5, and 11 mM ionic strength as a function of temperature (°C).

1 mM study 1 (○)
study 2 (●)
7.5 mM study 2 (△)
11 mM study 2 (■)

strength was increased across transition two, there was a concomitant decrease in the amount of B form from 25 to 18 %. This would mean that nearly one-third of the DNA in the B form is converted to the C form as

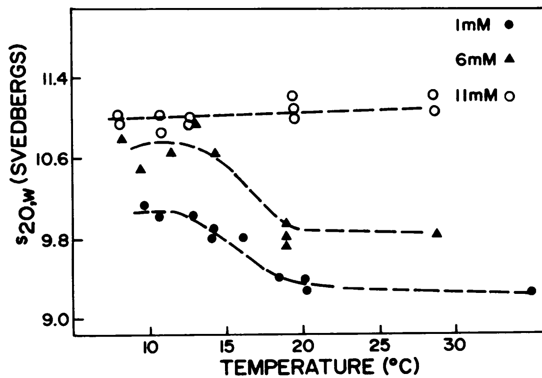

Figure 7: $s_{20,w}$ at 1, 6, and 11 mM ionic strength as a function of temperature (°C).

the nucleosome becomes more compact. Crossing the transition in the other direction, when the amount of B structure increases could be interpreted as a relaxation of the DNA when the nucleosome assumes a more extended conformation in solution.

An alternate explanation which also does not involve the C form of the DNA may be advanced to explain the changes in the circular dichroism spectrum across transition two. It has been suggested that the circular dichroism spectrum in chromatin reflects the presence of side-by-side interactions between the loops of DNA around the histone core, which has been interpreted as a ψ-like state of the B form of DNA[8].

The transitions in hydrodynamic properties and circular dichroism spectra are independent of pH over the range between pH 6 to 9. Below pH 5, however, the transitions are greatly altered. Transition one appears to be missing altogether and transition two is much less pronounced. At pH 5, both the sedimentation coefficient and circular dichroism spectra for the entire range of ionic strengths between 0.7 and 11 mM are consistent with a more compact nucleosome conformation.

It would seem unlikely that this pH dependence of the transitions is a result of changes in the charge on the DNA. Thus, for pure DNA in 0.25 M KCl, no decrease in intrinsic viscosity occurs until pH 3.5 is reached[9]. This decrease in viscosity of pure DNA at pH 3.5 is due to the collapse of the double helix as the amino groups on the purine and pyrimidine rings involved in hydrogen-bonds, are titrated and become charged.

We propose that the observed changes in hydrodynamic and optical properties of the nucleosome at pH 5.5 are due to protonation of amino acid side chains on the histones. The observed changes in nucleosome conformation could be due either to overall charge effects or to more specific charge effects. For example, the nucleosome bears a net negative charge at pH 7 due to the ionized phosphate groups on the DNA.

As the histone histidyl and carboxyl groups are titrated between pH 6 - 4, the net charge should decrease, leading to a decrease in the electrostatic free energy of expansion. This could be sufficient to explain the pH dependence seen in the nucleosome hydrodynamic properties (Fig. 4). An alternative explanation would be that titration of specific amino acids with a pK of $\sim$ 5.5 leads to disruption of specific histone-histone interactions which allow the monomer to undergo an alteration in shape. Indeed, the histones free in solution show dramatic alterations in structure at pH 5.5 - 6. Weintraub et al.,[10] showed that the heterotypic

form of the histone complex, present at neutral pH, dissociates to a mixture of $(H3\text{-}H4)_2$ tetramer and (H2A-H2B) dimer at pH 5.5. Eikbush and Moudrianakis[11] have suggested that this dissociation is a result of the titration of histone histidine residues at pH ~ 5.5 - 6. These histidine residues are postulated to be involved in hydrogen bonding interactions which stabilize the histone octamer. If such interactions do exist, the conformational change observed in nucleosomes at pH 5.5 may also be a result of disruptions of titration of these histidines, resulting in a drastic alteration of histone-histone associations.

We further note that Eikbush and Moudrianakis[11] found that low temperature stabilized the histone octamer at neutral pH relative to $(H3\text{-}H4)_2$ and (H2A-H2B). Similarly, low temperature favors the compact form of the nucleosome (Fig. 6, 7).

The thermodynamic parameters of entropy and enthalpy change may be determined from the temperature dependence of the two transitions, if we assume a three state model in which there are three distinct conformations: open, intermediate, and compact. The most open conformation is present below 0.7 mM ionic strength, the intermediate conformation occurs between 2 to 5 mM ionic strength, and the compact conformation exists at 11 mM. Each transition is regarded as an equilibrium between adjacent states. We define two equilibrium constants

$$(1) \quad K_1 = \frac{[I]}{[O]}$$

$$(2) \quad K_2 = \frac{[C]}{[I]}$$

where [O], [I], and [C] are the concentrations of the open, intermediate, and closed forms. Under the assumptions of this model the observed sedimentation or diffusion coefficient is a linear combination of the properties of the individual forms. Thus, the equilibrium constants may be expressed in terms of the observed sedimentation or diffusion rates:

$$(3) \quad K_1 = \frac{s_{obs} - s_o}{s_i - s_{obs}}$$

$$(4) \quad K_2 = \frac{s_{obs} - s_i}{s_c - s_{obs}}$$

In equations 3 and 4, the values of all the sedimentation coefficients, s, are assumed to be corrected for the temperature dependence of the viscosity and density of water; that is, they represent values of $s_{20,w}$. Since they are measured at low concentrations, they are effectively values of $s^{o}_{20,w}$.

The subscripts obs, o, i, and c represent the observed, open, intermediate, and compact conformations, respectively.

Table 2 presents the calculated values for the enthalpy and entropy of both transitions as determined by quasielastic light scattering, and from ultracentrifugation.

The increase in entropy across both transitions as the ionic strength is lowered indicates that the system is less ordered in the open conformation than in the intermediate, and less ordered in the intermediate than the compact. This would seem to be consistent with observed changes in the circular dichroism spectra indicating an increase in the B conformation or decrease

TABLE 2
Thermodynamic Data for the Ionic Strength Transitions Exhibited by the Nucleosome

Transition	ΔH° kcal/mole*	ΔS° cal/mole deg*
Transition One		
Laser Light Scattering	-24 ± 8 †	-83 ± 18
Ultracentrifuge	-32 ± 10†	-109 ± 25
Transition Two		
Laser Light Scattering	-30 ± 10	-120 ± 20
Ultracentrifuge	-40 ± 12	-140 ± 30

* ΔH° and ΔS° were determined from

$$\Delta G^\circ = -RT\ln K$$

$$\Delta H^\circ = -R\frac{\partial \ln K}{\partial(1/T)}$$

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$$

† The uncertainties in ΔH° and ΔS° shown in Table 2 reflect both the experimental errors in the determination of the diffusion coefficient and the sedimentation coefficient and also the errors in assigning a diffusion and a sedimentation coefficient to each conformation.

In the first analysis we assumed values for s and D for each conformation which were the mean values at the ionic strengths of 1, 6, and 7.5 mM. Then ΔH° and ΔS° were calculated. ΔH° was obtained using a weighted least squares analysis of lnK versus 1/T. The assumed values of s and D for each conformation were then varied by ± 0.1 F for D and ± 0.1 S for s for each conformation. Then ΔH° and ΔS° were recalculated. The range of values calculated for ΔH° and ΔS° demonstrates the inherent limitations in this method.

in the ψ-state at low ionic strengths, which also suggests a relaxation of a distorted conformation of the DNA.

It is interesting to observe the parallel between the behavior of the nucleosome and that of the DNA-free histone complexes in solutions of different ionic strength, pH, and temperature. The formation of tetramer and octamer histone complexes has been studied primarily in solutions of 1 - 3 M NaCl. The high salt concentration shields electrostatic charges on the amino acid residues and may play a role similar to the neutralization of charged residues by the negative phosphates on the DNA in the nucleosome. Increasing the salt concentration from 2 to 3 molar or decreasing the temperature favors association of the two tetramers to form an octamer[10,12,13]. Changes with pH have been observed with histone complexes in 2 M NaCl. Thus, at pH 5 the major species found are an $(H3\text{-}H4)_2$ tetramer and an (H2A-H2B) dimer. At pH 7, the major species is the heterotypic tetramer or octamer composed of H2A, H2B, H3, and H4.

It is not at all clear that the different conformations being studied here represent different functional states of the nucleosome. Nevertheless, these changes in conformation should provide useful probes of nucleosome structure and the forces involved in holding this complex particle, the nucleosome, together.

Recently, we have completed studies on chromatin core particles which, unlike those discussed in this paper, contain almost uniform DNA lengths of 146[14] base pairs. For these particles, transition 2 is markedly depressed both in the sedimentation coefficient change and in the circular dichroism change at 7 mM. Therefore, we now believe that transition 2 may involve a loosening of the ends of DNA longer than 140 base pairs from the nucleosome, perhaps due to a stiffening of the double helix caused by increased charge-charge repulsions between neighboring phosphate groups, as the ionic strength is lowered.

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