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Polyamines and Acetylpolyamines Increase the Stability and Alter the Conformation of Nucleosome Core Particles

James E. Morgan,[†,§] James W. Blankenship,[‖] and Harry R. Matthews*[,†]

Department of Biological Chemistry, University of California, Davis, California 95616, and Department of Pharmacology, University of the Pacific, Stockton, California 95207

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ABSTRACT: The interactions of spermine (4+ charge at physiological pH), N^1-acetylspermine(3+), spermidine(3+), N^1- and N^8-acetylsperimidine(2+), putrescine(2+), hexaamminecobalt(3+), and magnesium(2+) with nucleosome core particles have been examined by using thermal denaturation and circular dichroism. Tetra- and triamines were 2–3 times more effective than diamines at stabilizing core particles against thermal denaturation. Secondary effects were also observed, with acetylpolyamines slightly less effective than unmodified polyamines of equivalent charge. Hexaamminecobalt(3+) was less effective than the triamines, while magnesium had essentially no effect. This is surprising since magnesium is more effective than diamines at stabilizing naked DNA. All the cations tested altered the circular dichroism spectra of the core particles in the DNA region (284 nm). The peak at 284 nm was suppressed by tetra- and trivalent compounds to approximately twice the extent of divalent compounds. Magnesium appears to suppress the peak by a lesser extent than the diamines. This indicates that the DNA twist and/or folding is changed by these cations. A plateau of both thermal denaturation and circular dichroism effects was observed at cation concentrations where 30–40% of the total DNA negative charges could be neutralized by the added cations. We suggest that polyamine and histone acetylation function in concert to lower the stability and change the conformation of the nucleosome core, thus facilitating replication and transcription in vivo.

Chromatin undergoes substantial structural modifications during replication and transcription (Reeves, 1984; Igo-Kemenes et al., 1982; DePamphilis & Wassarman, 1980). These modifications may be significant in the control and mechanism of chromosomal activity, but their functions are as yet poorly understood. Histone acetylation has been correlated with replication and transcription (Matthews & Waterborg, 1985; Doenecke & Gallwitz, 1982; Allfrey, 1977) and spermatogenesis (Christensen et al., 1984). Two purified histone acetyltransferases possess polyamine acetylation activity (Libby, 1978, 1980) as does a crude nuclear preparation (Blankenship & Walle, 1977, 1978). Polyamines, especially spermine (4+ charge at physiological pH) and spermidine(3+), are essential for normal cellular processes, including proliferation, replication, and transcription (Tabor & Tabor, 1984; Janne et al., 1978; Lowkvist et al., 1986; Gallo et al., 1986). Polyamines appear to be present in the nucleus in quantities sufficient to neutralize 15–30% of the DNA negative charges (McCormick, 1978). This leads to the hypothesis that histone and polyamine acetylation function together to modulate or modify chromatin structure during replication and transcription. To begin investigating this hypothesis, we previously carried out thermal denaturation studies of polyamines and magnesium with naked DNA (Morgan et al., 1986). We here report thermal denaturation and circular dichroism studies of polyamines, acetylpolyamines, hexaamminecobalt and magnesium with nucleosome core particles.

Experimental Procedures

Preparation of Nucleosome Core Particles. All procedures were carried out at 0–4 °C unless otherwise specified. Phenylmethanesulfonyl fluoride (PMSF)[1] (50 mM in 2-propanol) was added to 0.5 mM final concentration to all solutions, except final dialysis buffer, shortly before use. Nuclei were isolated from frozen (−70 °C) young steer thymus according to the procedure of Bloebel and Potter (1966), including Triton X-100 extraction, adapted to available equipment. Chromatin was isolated from the nuclei by a variation of the method of Huang and Cole (1984). Briefly, nuclei were suspended in 0.25 M sucrose, 50 mM Tris, pH 7.0, 25 mM KCl and 5 mM Mg^{2+} at OD_{260} = 50 (measured in 0.1 N NaOH). Digestion was carried out by addition of Ca^{2+} to 1 mM and micrococcal

* Address correspondence to this author.
† University of California.
§ Supported by a National Science Foundation graduate student fellowship.
‖ University of the Pacific.

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; PCA, perchloric acid; bis, *N,N'*-methylenebis(acrylamide); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; PM2, virus PM2 DNA; *Hae*III, restriction endonuclease *Hae*III; bp, base pair(s); HMG, high mobility group.

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nuclease (Boehringer-Mannheim) to 23 units/mL and incubation at 37 °C until 1–1.5% PCA solubility was reached (typically 6–7 min, as determined by pilot digests). Digestion was terminated by addition of EGTA to 4 mM. Chromatin was extracted from the nuclei with 1 mM EDTA and layered onto 10–28% sucrose gradients containing 60 mM NaCl, 10 mM Tris, and 1 mM EDTA, pH 7.0 (Butler & Thomas, 1980). Gradients were centrifuged for 13 h in an SW 27 rotor at 15 000 rpm. Fractions were monitored by absorbance and electrophoresis in 1.5% agarose gels in TPE-SDS buffer (described below). Fractions of high absorbance containing tetranucleosomes or longer (to eliminate nucleosomes which may have lost H2A/H2B dimer) were pooled. Histone H1 was removed by a variation of the method of Marekov and Beltchev (1981) as follows. The pooled chromatin was dialyzed into 15 mM Na_2HPO_4/0.25 mM H_4EDTA, adjusted to pH 7.0 with HCl (depletion buffer). BioRex-70 was equilibrated to depletion buffer until the pH was identical within 0.2 unit and conductance within 10% (typically five to six changes of 10 volumes, with pH adjustment of the first two changes). Twenty OD_{260} units of chromatin per milliliter of packed resin were mixed and rocked for 1 h. The supernatant (H1-depleted chromatin) was collected by centrifugation.

H1-depleted chromatin (OD_{260} = 3), in depletion buffer, was digested by adding Ca^{2+} to 0.35 mM and micrococcal nuclease to 5 units/mL and incubating at 37 °C. Optimum digestion (typically 35 min) was determined from pilot digests analyzed by electrophoresis in 5% (w/v) acrylamide/0.25% (w/v) bis gels in TPE-SDS buffer and a plateau in PCA solubility. Crude core particles were concentrated by dialysis against solid poly(ethylene glycol) and applied to a 10–28% sucrose gradient containing 15 mM Tris/15 mM Na_4EDTA, pH 7.0, and centrifuged in an SW27 rotor at 24 000 rpm for 36 h. Fractions were monitored by absorbance and gel electrophoresis. Fractions containing only core particles were pooled and dialyzed against 100 volumes of 15 mM Tris/15 mM Na_4EDTA, pH 7.0, and three changes of 1 mM NaCl/0.5 mM sodium cacodylate, pH 6.7, for 20 h each.

Purified core particles were characterized on three gel systems. Native core particles were characterized on particle gels containing 5% (w/v) polyacrylamide, 0.25% (w/v) bis, 10 mM Tris–acetic acid, 2 mM Na_2EDTA, and 10% glycerol, pH 7.8. Tank buffer was recirculated, and gels were stained with ethidium bromide [modified from Varshavsky et al. (1976)]. DNA length was characterized on gels containing 5% acrylamide, 0.25% bis, 36 mM Tris, 30 mM NaH_2PO_4, 10 mM EDTA, pH 7.8, and 0.1% SDS (TPE-SDS buffer). Gels were fixed in 50% methanol and stained with ethidium bromide (Todd & Garrard, 1977). Protein composition was characterized on a discontinuous SDS gel containing 17.5% (w/v) acrylamide/0.07% (w/v) bis (Laemmli, 1970; Mende et al., 1983) with tank buffer containing 0.05 M Tris, 0.38 M glycine, and 0.1% SDS, pH 8.8. Gels were stained with Coomassie Brilliant Blue R250. Negatives (Kodak 4147 Plus X film) of gels were scanned on a Cary 210 spectrophotometer, interfaced with a Hewlett-Packard 9845A computer, and analyzed as described (Matthews, 1984).

Cations. Polyamines and acetylspermine were purchased from Sigma as the HCl salt. Acetylspermidines were synthesized as previously described (Morgan et al., 1986). Hexaamminecobalt chloride was from Kodak (Fisher), and magnesium chloride was from Sigma.

Physical Studies. Thermal denaturation was performed as previously described (Morgan et al., 1986), with core particle OD_{259} = 0.645 (90 μM DNA phosphate), in 1.0 mM NaCl/0.5 mM sodium cacodylate, pH 6.7, with a temperature gradient of 0.25 °C/min. Preliminary circular dichroism spectra were obtained on a Jasco J-40 instrument at the University of Nevada, Reno. Main study spectra were obtained on a Jasco J-500C instrument at the University of California, Berkeley. For the main study, core particles were scanned from 340 to 240 nm at 10 nm/min, with two repeats for each sample. The initial OD_{259} was set at 1.0 for core particle samples, in buffer as above, with no added cation. Cations were added progressively to the same sample from stocks of 0.5 mM divalent, 0.333 mM trivalent, or 0.25 mM tetravalent cation to achieve the specified final concentrations. This diluted the sample to OD_{259} = 0.93 at the highest cation concentrations used. Circular dichroism values were adjusted for this dilution.

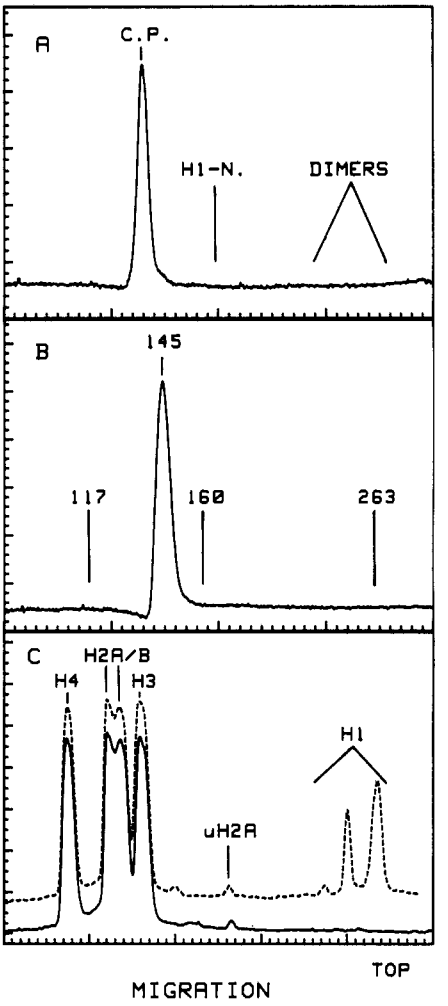

FIGURE 1: Densitometer scans of negatives (Kodak 4147 Plus-X) of gel electrophoreses. (A) Core particles on native particle gel, stained with ethidium bromide. Positions of core particles (C.P.), nucleosomes with histone H1 (H1-N.), and dinucleosomes (DIMERS) are indicated. (B) Core particle DNA on TPE-SDS DNA gel, stained with ethidium bromide. Positions of PM2/*Hae*III markers (117, 145, 160, and 263 bp) are indicated. (C) Core particle proteins (—); crude chromatin proteins (---) on an SDS protein gel (Laemmli, 1970; Mende et al., 1983). The chromatin scan has been displaced upward for clarity, and the positions of the histones (H1, uH2A, H2A/B, H3, and H4) are indicated. The gel systems are described under Experimental Procedures.

RESULTS

Purified core particles migrated on native particle gels as a single band with a small (approximately 3%) slower migrating subband, which appears to correspond to core particles containing ubiquitinated H2A (uH2A) (J. Th'ng and P. Yau,

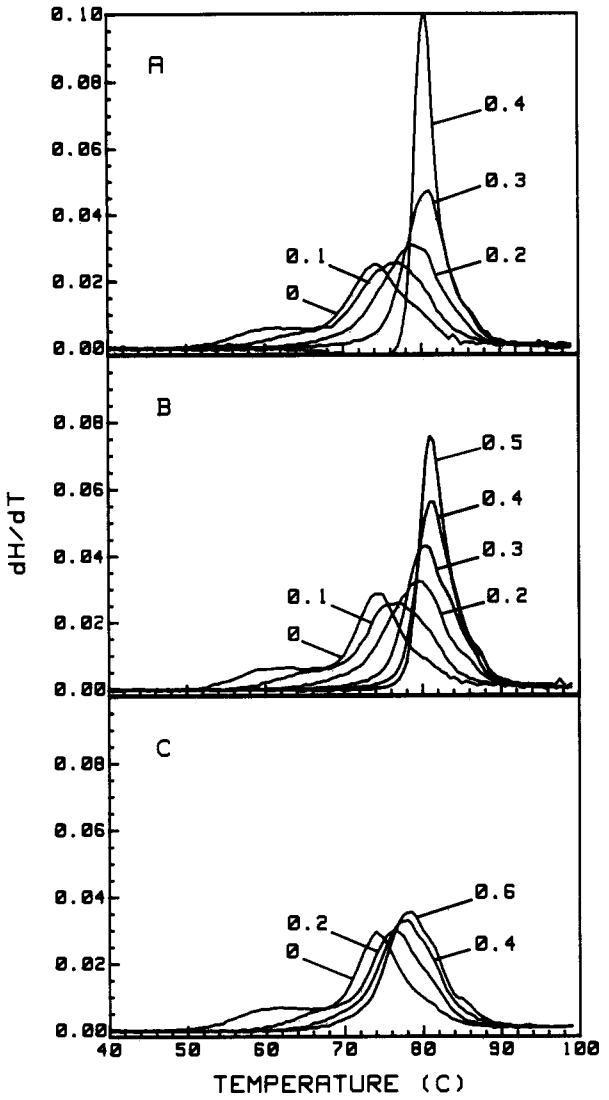

FIGURE 2: Core particle thermal denaturation profiles (shown as the first derivative of hyperchromicity with respect to temperature, dH/dT) as a function of increasing polyamine concentration. Initial OD_{259} = 0.645 in 1.0 mM NaCl/0.5 mM sodium cacodylate, pH 6.7. Concentrations of polyamines (0.1 etc.) expressed as charge ratio (+/−), defined as total polyamine positive charges divided by total DNA phosphate negative charges. (A) Spermine added; (B) spermidine added; (C) putrescine added.

personal communication). No band corresponding to nucleosomes containing H1 was observed (Figure 1A). On DNA gels, a single band with an average length of 144.5 bp (based on PM2/*Hae*III standards; Kovacic & Van Holde, 1977) with a slight tail was observed (Figure 1B). The apparent width of the band was a linear function of concentration. Extrapolation of bandwidth to zero concentration and subtraction of restriction fragment bandwidth yielded an estimated standard deviation in length of ±1 bp (data not shown). Protein gels of core particles showed core histones in the same proportions as the original isolated chromatin to within the limits of measurement (approximately ±3%). A protein which comigrated with uH2A on SDS gels (Laemmli, 1970) and was not dissociable by centrifugation in sucrose gradients containing 0.6 M NaCl was also found in small amounts (2–3% of H2A) in both core particles and crude chromatin. This is tentatively indentified as uH2A. No histone H1 or HMG proteins were present in the core particles (Figure 1C).

Thermal denaturation of core particles in 1.0 mM NaCl/0.5 mM sodium cacodylate, pH 6.5 (Figures 2 and 3), resulted

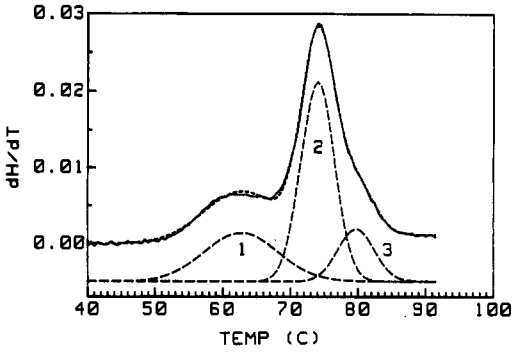

FIGURE 3: Gaussian curves fit to core particle thermal denaturation profile. Data (—); combined Gaussian curve fit to data (---); individual Gaussian curves (– –). The individual Gaussians have been displaced downward for clarity. The numbers on individual curves correspond to numbers in Figure 3 and Table I.

in a profile similar to those previously described (Weischet et al., 1978). Binding of polyamines, acetylpolyamines, or hexaamminecobalt to nucleosome core particles had significant effects upon the thermal denaturation profile. We express the concentrations of cations in this report as a "charge ratio" (+/−), defined as (total *added* cation positive charges)/(total DNA negative charges). This serves to emphasize the stoichiometry of cation binding to the core particles. Spermine (4+ charge at physiological pH), spermidine(3+), N^1-acetylspermine(3+), and hexaamminecobalt(3+) produced dramatic increases in melting temperature, suppression of the premelt region, and sharpening of the main transition (Figure 2A,B). Additionally, spermine induced turbidity, i.e., aggregation, at room temperature, and hexaamminecobalt induced turbidity during the thermal denaturation run at a charge ratio of 0.5. Turbidity was not observed with the other samples. Putrescine(2+) and N^1- and N^8-acetylspermidine(2+) moderately increased the melting temperature, moderately suppressed the premelt region, and very slightly sharpened the main transition (Figure 2C). Addition of magnesium(2+) at the same concentrations had negligible effects upon thermal denaturation (data not shown).

The thermal denaturation apparatus developed in this lab (Morgan et al., 1986) is extremely precise, for studies with chromatin, and the core particles are highly homogeneous and reproducible. We have taken advantage of this high precision to use a Gaussian curve-fitting technique (Matthews, 1985) to quantify the above qualitative observations on cation binding and allow finer analysis of the results. It was found that at least three independent curves were necessary to fit the data (Figure 3). In most cases, the original data were fit to within the noise level of the data. One curve corresponds to the premelt region, i.e., the denaturation of approximately 22 bp at each end of the DNA. The two other curves comprise the main transition, i.e., the cooperative denaturation of the entire particle (Van Holde et al., 1980). The physical significance of requiring two curves to fit the main transition is not clear, although others have made a similar observation with subnucleosome particles (Read et al., 1985). Possibly, it represents a stepwise unfolding of the particle, or the influence of heterogeneous GC vs. AT base pair composition.

Using this curve-fitting technique, it was found that the addition of cations, with the exception of magnesium, increased the T_m of the premelt region (curve 1) by a greater extent than the T_m of the main transition (curves 2 and 3) (e.g., Figure 4A,C,E). It was also found that the area of the premelt (curve 1) was reduced, while the area of the main transition (curves 2 and 3) increased, in agreement with qualitative observations above (Figure 4B,D,F). Most cations increased the area of

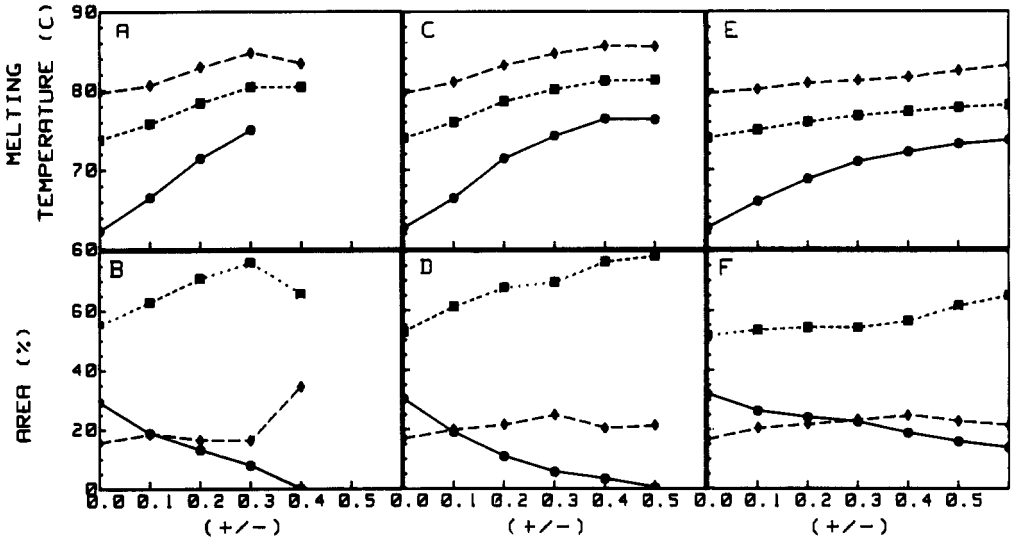

FIGURE 4: Gaussian fits to core particle thermal denaturation profiles as a function of increasing polyamine concentrations. (A, B) Spermine; (C, D) spermidine; (E, F) putrescine. (A, C, E) Melting temperature of individual Gaussians; (B, D, F) areas of individual Gaussians. Gaussian 1 (●); Gaussian 2 (■); Gaussian 3 (♦). Charge ratio (+/−) polyamine concentration is defined in Figure 2.

Table I: Summary of Thermal Denaturation and Circular Dichroism Data

ligand (charge)	thermal denaturation (Gaussian fits)[a]						circular dichroism, 284-nm peak (millideg)[b]
	T_1 (°C)	A_1 (%)	T_2 (°C)	A_2 (%)	T_3 (°C)	A_3 (%)	
none	62.7 ± 0.2	29.6 ± 1.3	74.0 ± 0.2	53.8 ± 1.4	79.8 ± 0.2	16.7 ± 1.1	1.62 ± 0.18 (n = 15)
spermine(4+)		0	80.4	65.5	83.3	34.5	0.78 ± 0.09 (n = 4)
N^1-acetylspermine(3+)	75.8	3.7	80.6	76.8	84.8	19.5	0.73 ± 0.07 (n = 3)
spermidine(3+)	76.4	3.3	81.2	76.3	85.6	20.4	0.68 ± 0.10 (n = 6)
N^1-acetylspermidine(2+)	70.3	20.5	76.7	56.3	81.2	23.2	1.06 ± 0.20 (n = 6)
N^8-acetylspermidine(2+)	70.1	21.3	76.5	56.9	81.1	21.8	1.22 ± 0.07 (n = 6)
putrescine(2+)	72.2	18.7	77.3	56.5	81.6	24.8	1.07 ± 0.09 (n = 6)
$Co(NH_3)_6$(3+)		0	79.1	76.6	82.6	23.4	0.70 ± 0.13 (n = 3)
Mg(2+)	63.0	31.1	74.0	54.0	80.0	14.9	1.35 ± 0.11 (n = 6)

[a] Thermal denaturation data at charge ratio (+/−) = 0.4. Gaussian fits as described in the text. For ligand = none, data are from four preps, n = 13; values are average ± standard deviation. [b] Circular dichroism plateau values were estimated as the average of the 284-nm peak height at charge ratios (+/−) of 0.3, 0.4, and 0.5 (except for spermine, where only 0.3 and 0.4 were used due to aggregation at 0.5). For n = 3, data were from one preparation; for other n values, data were from two preparations. Values are average ± standard deviation.

curve 2 (low T_m, main transition) slightly more than curve 3 (high T_m, main transition). However, spermine(4+), at a charge ratio of 0.4, induced an abrupt increase in the area of curve 3, at the expense of curve 2. This reflects the extreme sharpness of the melting profile seen in Figure 2A. With the exception of this abrupt change induced by spermine, the most pronounced changes in both T_m and area occur at lower cation concentrations, i.e., in the charge ratio region of 0.1–0.2, with a plateau observable at higher concentrations, i.e., above a charge ratio around 0.4 (Figure 4).

The melting temperatures and areas of the three curves without added cations and with added cations at a charge ratio of 0.4 are summarized in Table I. Within the polyamines, the major differences in effects correlate with charge. Spermine(4+) suppressed the premelt completely, the triamines by 88–89%, and the diamines by 28–37%. Only spermine significantly shifted the area from curve 2 to curve 3, as noted above. The tetra- and triamines increase the T_m of the main transition by a greater amount than the diamines (6.4 to 7.2 °C compared to 2.5 to 3.3 °C for curve 2 and 3.5 to 5.8 °C compared to 1.3 to 1.8 °C for curve 3). Thus, at equal charge ratios, the tetra- and triamines were 2–3 times more effective than the diamines at stabilizing the core particle. At equal molar ratios, the effects are even more pronounced (Figure 7). Smaller differences in effects resulted from structural differences within the polyamines. Thus, addition of the unmodified polyamines resulted in slightly greater reduction of the premelt area and increase of the T_m (0.6–2.1 °C) than did the acetylpolyamines of equivalent charge. N^1- and N^8-acetylspermidine were identical within the resolution of the system.

Both hexaamminecobalt(3+) and magnesium(2+) effects differed markedly from those of polyamines or acetylpolyamines of equivalent charge. Hexaamminecobalt suppressed the premelt area (curve 1) completely but only increased the temperature of the main transition by 5.1 °C for curve 2 and 2.8 °C for curve 3. This is, it had less effect than the tetra- or triamines, although more than the diamines. Magnesium(2+) at these concentrations had negligible effect on either the melting temperatures or the areas of the three curves. This is a very surprising result, since magnesium has a greater effect upon the thermal denaturation of naked DNA than any of the diamines (Morgan et al., 1986).

Circular dichroism spectra of core particles (Figure 5) were similar to those previously reported (Weischet et al., 1978). Preliminary experiments showed that polyamines have a substantial effect upon the DNA region (around 284 nm) but only minor and subtle effects on the protein region (around 220 nm). Therefore, extensive investigations were undertaken only on the DNA region. The spectra had to be obtained near the limit of sensitivity of the instrument, and both short-term noise and long-term drift of base line were problems. The net result was substantial scatter in the data, which was dealt with by repetition of most cation addition runs. Standard deviations of 10–15% were typical (e.g., core particles with no added cation; Table I). Several main points stand out in the data.

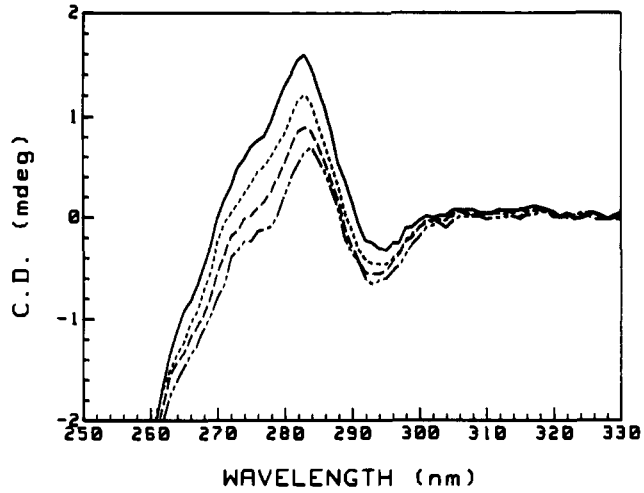

FIGURE 5: Circular dichroism spectra of core particles with added spermine. Initial OD_{259} = 1.0 in 1.0 mM NaCl/0.5 mM sodium cacodylate, pH 6.7. No added spermine (—); added spermine (+/−) = 0.1 (- - -); (+/−) = 0.2 (– –); (+/−) = 0.3 (– - – -). Charge ratio (+/−) is defined in Figure 2.

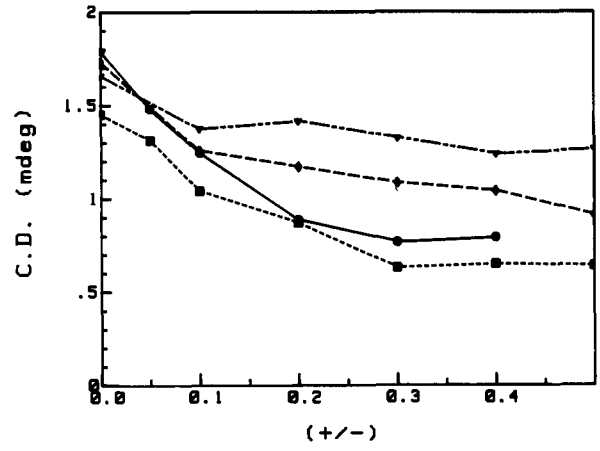

FIGURE 6: Response of core particle circular dichroism 284-nm peak to added cations. Charge ratio (+/−) as defined in Figure 2. Spermine added (●); spermidine added (■); putrescine added (♦); magnesium added (▼). Each data point is the average of two runs.

All the compounds tested suppressed the peak at 284 nm (Figures 5 and 6 and Table I). The initial additions of cations (charge ratio 0.1–0.2) had the greatest effect, with a plateau effect at charge ratios around 0.3 (Figure 6). Plateau values were estimated as the average of the 284-nm peak height at charge ratios of 0.3, 0.4, and 0.5 (except spermine, which produced turbidity at 0.5, as noted above) (Table I). It was found that tetra- and trivalent compounds suppressed the peak from an initial value of +1.62 mdeg to between +0.68 and +0.78 mdeg. This was substantially more than any of the diamines, which suppressed the peak to between +1.06 and +1.22 mdeg. It also appears that magnesium was slightly less effective than any of the diamines, suppressing the peak to +1.35 mdeg. However, the scatter in the data does not allow us to distinguish between the diamines and magnesium with high confidence.

Discussion

Thermal denaturation reflects the stability of double-stranded vs. single-stranded DNA and is strongly affected by cation binding (Record et al., 1978; McGhee, 1976). Polyamine–DNA interactions appear to be largely electrostatic in nature (Bloomfield & Wilson, 1981; Braunlin et al., 1982), i.e., entropy driven, and so are not expected to be strongly affected by temperature. Therefore, thermal stability or "melting temperature" should parallel stability at physiological temperatures, and we view thermal denaturation as a general assay for stability.

The data presented here show that polyamines, acetylpolyamines, and hexaamminecobalt, but not magnesium, substantially increase the stability of the nucleosome core particle. Within the polyamines, the effects roughly parallel the charge of the molecule, except that acetylpolyamines are slightly less effective than unmodified polyamines of equivalent charge. The surprising result is the lack of effect of magnesium, since magnesium is more effective than the diamines at stabilizing naked DNA (Morgan et al., 1986). Similarly, hexaamminecobalt, while having a pronounced effect upon stability, is less effective than the triamines.

Circular dichroism spectra in the 284-nm region reflect the conformation of DNA, whereas the 220-nm region reflects protein conformation in the core particle (Fasman & Cowman, 1978). The 284-nm peak due to DNA in core particles is suppressed by roughly three-fourths relative to naked DNA. This has been interpreted as reflecting either a change in the helix twist, i.e., secondary structure (MacDermott, 1985), or a change in folding, i.e., tertiary structure (Cowman & Fasman, 1978, 1980).

The data reported here show that polyamines, acetylpolyamines, hexaamminecobalt, and magnesium suppress the 284-nm peak of the circular dichroism spectrum of the core particle. The tetra- and trivalent compounds have the greatest effect, while the divalent compounds have roughly half as much effect, with magnesium apparently slightly less effective than the diamines. Thus, these compounds alter the conformation of DNA in the core particle, although it is not clear whether this involves secondary or tertiary structure or both.

Both thermal denaturation and circular dichroism effects were observed to plateau at higher cation concentrations. This was most pronounced for the tetra- and trivalent cations (Figures 4 and 6). The plateau effect is typical of a system where ligands are saturating the available binding sites (Segal, 1975). If the binding of polyamines to the core particles is of similarly high affinity to their binding to naked DNA (Dumuis-Kervabon et al., 1986), then below saturation the concentration of free ligand is a very small fraction of the total ligand. Thus, total ligand approximates bound ligand, and the ligand concentration at saturation can be used to estimate the number of binding sites (McGhee, 1976). The circular dichroism plateau near charge ratio (+/−) 0.3 and the thermal denaturation plateau near 0.4 suggest that 30–40% of the DNA phosphates are available for binding to polyamines. As a first approximation, it can be calculated that the net positive charge (i.e., total positive less total negative) on the core histones is sufficient to neutralize 57% of the DNA negative charges [see Wells (1986) and references cited therein], leaving 43% available for binding added cations. This is reasonably close to the plateau values observed with added polyamines. However, the limitations of this model are apparent from the observation that the polyamines have greater effects than the smaller cations, hexaamminecobalt and magnesium. Possibly, the separation of charges and/or the methylene groups present on the polyamines favors binding to specific sites on the core particle.

Our interest in these studies originates from the question of whether the acetylation of polyamines could have a role in the cellular processes of replication and transcription. As observed above, polyamine acetylation is linked to histone acetylation, which has been correlated with these processes.

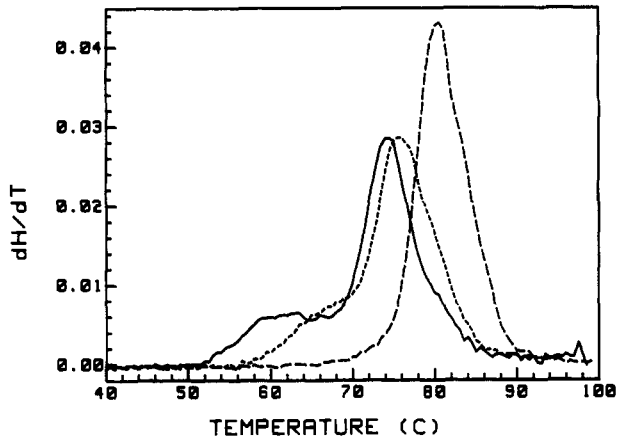

FIGURE 7: Effect of acetylation of spermidine on its ability to stabilize core particles. Core particles with no added polyamine (—); core particles with 9 μM spermidine (equivalent to charge ratio 0.3) (– –); core particles with 9 μM N^8-acetylspermidine (equivalent to charge ratio 0.2) (- - -).

Initial work on bulk nucleosomes (lacking H1) with butyrate-induced hyperacetylation showed negligible change in stability from the controls (Yau et al., 1982; Simpson, 1978). However, Bode et al. (1983) observed a change in electrophoretic mobility with more highly acetylated particles (above 10 acetyl groups per particle). Later work combined butyrate-induced hyperacetylation with magnesium solubility fractionation to produce even more highly acetylated particles (17 acetyl groups per particle) which were compared to a control non-butyrate, EDTA-soluble fraction (3.4 acetyl groups per particle) (Ausio & Van Holde, 1986). At the highest level of histone acetylation, the acetylated core particles had lower thermal stability and increased circular dichroism at 284 nm compared to control particles.

At this time, we can only speculate on the effect of acetylation on a particle initially containing both nonacetylated polyamine and histones. We suggest that the effects of the polyamine acetylation and histone acetylation would be additive. For example, consider the thermal denaturation profiles in Figure 7 of this report and Figure 6 of Ausio and Van Holde (1986). The change from spermidine to N^8-acetylspermidine increased the proportion of DNA (estimated from the hyperchromicity) in the premelt region from 5.6% to 25.6% (compared to 29.6% for controls); the change from control to very highly acetylated histones increased the proportion of the DNA in the premelt region from 27% to 46% (Ausio & Van Holde, 1986). Thus, it appears that histone and polyamine acetylation would have additive effects in increasing the amount of DNA in the premelt region, i.e., in destabilizing longer stretches of DNA at the ends of the core particle (Weischet et al., 1978; Simpson, 1979). Similarly, the circular dichroism effects may be additive [Figure 5 in this report and Figure 3 in Ausio and van Holde (1986)]. In spite of these changes, it is clear that there is no general unfolding of the particle (Ausio & Van Holde, 1986; Imai et al., 1986). Hence, we speculate that polyamine and histone acetylation work together to promote transcription or replication. The thermal denaturation pattern of nucleosome core particles in the presence of spermine or spermidine shows most or all of the DNA in a highly stable state. Acetylation of the polyamines loosens part of the DNA, presumably the DNA at the two ends (the premelt region) (Weischet et al., 1978; Simpson, 1979). Acetylation of histones apparently loosens an additional length of DNA, possibly preparing the inner DNA coil for displacement from the core (Ausio & Van Holde, 1986). Polyamine and histone acetylation would thus have complementary roles in loosening the DNA at the ends of the core particle. This model could account qualitatively for the rise in temperature of the main transition (Figure 7), but we cannot rule out possible direct effects on the inner coil of the DNA. This model suggests that these acetylation events are favorable but not sufficient conditions for opening up the nucleosome core particle. Other components, such as the lexosome-specific proteins (Prior et al., 1983), would be needed to complete the transition to a new chromatin structure.

However, polyamines and histones would be expected to behave very differently in terms of the length of time they would be associated with DNA. Acetylated polyamines would be expected to exchange with nonacetylated polyamines and diffuse out into the cytosol to be deacetylated (N^8-acetylspermidine) or oxidatively cleaved and regenerated (N^1-acetylspermidine and N^1-acetylspermine) (Seiler et al., 1981; Blankenship & Marchant, 1984). The constant activity of a polyamine acetyltransferase would be required to maintain a chromatin microenvironment in an acetylated state. The histones would be expected to be more permanently bound. This leads to a hypothesis. If an acetyltransferase evolved with both histone and polyamine as substrates, as appears to be the case (Libby, 1978, 1980), then a complementary histone deacetylation would be needed to maintain polyamine and histone acetylation in a similar equilibrium state. A very active histone deacetylase is in fact observed (Boffa et al., 1978; Hay & Candido, 1983; Waterborg & Matthews, 1982), as is a rapid turnover of histone acetate groups with half-lives of roughly 3 and 30–40 min (Jackson et al., 1975; Cousens et al., 1979), for which there is currently no other simple explanation.

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Registry No. Spermine, 71-44-3; N^1-acetylspermine, 25593-72-0; spermidine, 124-20-9; N^1-acetylspermidine, 14278-49-0; N^8-acetylspermidine, 13431-24-8; putrescine, 110-60-1; hexaamminecobalt, 14695-95-5; magnesium, 7439-95-4.

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