Internal Architecture of the Core Nucleosome: Fluorescence Energy Transfer Studies at Methionine-84 of Histone H4†

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ABSTRACT: Chicken histone H4, labeled separately at Met-84 with *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid and 5-(iodoacetamido)fluorescein, was reassociated with unlabeled histones H2A, H2B, and H3 and 146 base pairs of DNA to produce fluorescently labeled nucleosomes having physical characteristics virtually the same as those of native core particles. Four types of particles were prepared containing respectively unlabeled H4, dansylated H4, fluoresceinated H4, and a mixture of the two labeled H4 molecules. Quantitative singlet–singlet energy-transfer measurements were carried out to determine changes in the distance between the two Met-84 H4 sites within the same nucleosome following conformational transitions which we have reported earlier. In the ionic strength range 0.1–100 mM NaCl, the distance between these sites is less than 2 nm except at 1 mM. Between 100 and 600 mM monovalent salt the distance separating the donor and acceptor fluors at Met-84 H4 increases to 3.8 nm. The conformational change centered around 200 mM NaCl is cooperative. Our results and those of others indicate that there is little unfolding of the histone octamer, at least around Met-84 H4, in the entire ionic strength range studied. A mechanism involving the rotation of the globular portion of H4 is proposed to account for this transition which occurs at physiological ionic strengths.

Although several crystallographic studies of the core nucleosome and the histone octamer have appeared recently (Richmond et al., 1984; Bentley et al., 1984; Burlingame et al., 1985; Uberbacher & Bunick, 1985), there is still some uncertainty about its overall shape and dimensions [see Klug et al. (1985)]. Since nucleosomes probably exhibit conformational isomerism in vivo (Weisbrod, 1982), it is possible that these discrepancies have their origin in this polymorphism. We have studied the conformation of the core nucleosome in solution by means of extrinsic fluors attached to a single benign site on histone H4 (Chung & Lewis, 1985, 1986) and found that the structure of the histone octamer undergoes only minor changes in the ionic strength range 0.1–600 mM monovalent salt. However, a significant transition occurs around 200 mM NaCl which is accompanied by the disruption of a contact region between the two histone H4 molecules as revealed by a loss of pyrene excimer fluorescence (Chung & Lewis, 1986).

In order to examine this transition in more detail, we have determined the distance between these two sites as a function of ionic strength by means of singlet–singlet fluorescence energy transfer experiments and report our results here. As in our earlier work (Chung & Lewis, 1985, 1986), we find that in the ionic strength range 0.1–100 mM monovalent salt there is little change in the interfluor separation. However, at ionic strengths greater than 100 mM monovalent salt there is a cooperative conformational transition resulting in an increase in the fluor separation to 3.8 nm. Other properties of the labeled and unlabeled nucleosomes indicate that major unfolding has not occurred. This apparent paradox can be resolved by a mechanism based on changes in the quaternary structure of the central H3/H4 tetramer which could also regulate the binding of the H2A/H2B dimers.

Materials and Methods

Preparation of Fluorescent Core Nucleosomes. Chicken core length DNA and electrophoretically homogeneous core histones were isolated as described previously (Chung & Lewis, 1985). Histone H4 was specifically modified at its single Met-84 residue by the method of Lewis (1979) using *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-IAEDANS)[1] (tritiated as well as cold) and 5-(iodoacetamido)fluorescein (5-IAF). Protein concentrations were determined by the method of Lowry et al. (1951). The extent of labeling was determined spectrophotometrically by using published extinction coefficients (Hudson & Weber, 1973; Eshaghpour et al., 1980) characterized by sedimentation velocity, denaturing, and particle gel electrophoresis, thermal denaturation, and DNase I digestion as described earlier (Chung & Lewis, 1985).

Fluorescence Spectroscopy. Corrected fluorescence excitation and emission spectra were obtained with an Aminco SPF500 corrected spectra spectrofluorometer. Slit widths of both monochromators, typically maintained at 5 nm, were decreased to 1 or 2 nm for high-resolution determinations of emission and excitation maxima. The sample compartment was strictly maintained at 22 °C, and nucleosome concentrations were in the range $(0.5–2.0) \times 10^{-7}$ M. To minimize inner filter effects, the absorbance at the excitation wavelength was kept below 0.04. Polarization, lifetime, and quantum yield determinations were performed as described previously (Chung & Lewis, 1985).

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[1] Abbreviations: *x*-AEDANS, *N*-[(acetylamino)ethyl]-*x*-naphthylamine-1-sulfonic acid where *x* = 5 or 8; AF, acetamidofluorescein; bp, base pair; EDTA, ethylenediaminetetraacetic acid; Gdn-HCl, guanidine hydrochloride; HMG, high mobility group; *x*-IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-*x*-naphthylamine-1-sulfonic acid where *x* = 5 or 8; 5-IAF, 5-(iodoacetamido)fluorescein; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; PAGE, polyacrylamide gel electrophoresis.

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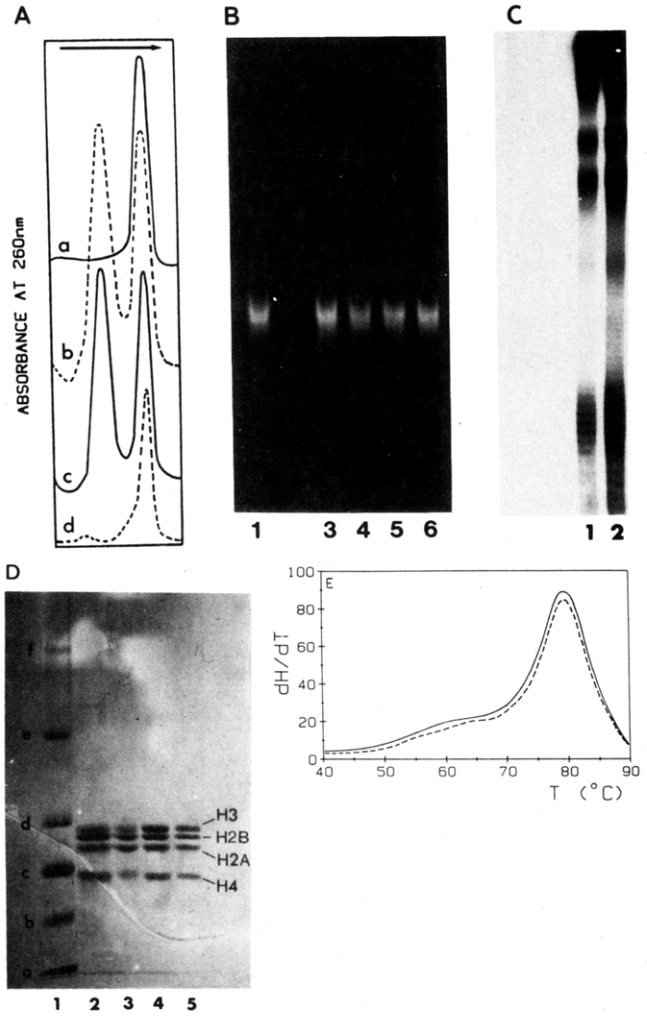

FIGURE 1: Characterization of 5-AEDANS/fluorescein doubly labeled core nucleosomes. (A) 5–20% sucrose gradient elution profiles. The core nucleosome sediments toward the bottom of the gradient. The slower sedimenting A_{260} peak is due to free DNA. 20 °C, 18 h, SW41 rotor 33K rpm, in 10 mM Tris–10 mM EDTA, pH 8. a, native core nucleosome; b, reconstitution mixture containing unmodified H4; c, reconstitution with 5-AEDANS- and fluorescein-labeled H4; d, relative fluorescence at 525 nm with excitation at 340 nm. (B) 5% TCE particle gel analysis. Nucleosomes (2.5 μg of DNA) were electrophoresed and stained with ethidium bromide. Lane 1, native nucleosome; lane 3, control reconstituted nucleosome; lane 4, dansylated nucleosome; lane 5, fluorescein-labeled nucleosome; lane 6, dansyl/fluorescein doubly labeled nucleosome. (C) DNase I digestion. Lane 1, native core nucleosome; lane 2, dansyl/fluorescein doubly labeled nucleosome (60% labeling). (D) 15% SDS–PAGE analysis. Lane 1, low molecular weight marker proteins [a, insulin (α and β chains, M_r 3000); b, bovine trypsin inhibitor (M_r 6200); c, cytochrome c (M_r 12300); d, lysozyme (M_r 14300); e, β-lactoglobulin (M_r 18400); f, α-chymotrypsinogen (M_r 25700)]; lane 2, chicken erythrocyte acid extracted histones; lane 3, native core nucleosome; lane 4, control reconstituted core nucleosome; lane 5, AEDANS/fluorescein doubly labeled core nucleosome. (E) Thermal denaturation. Native (—) and 5-AEDANS/fluorescein doubly labeled (---) nucleosomes were melted in 10 mM TCE, pH 7.4. The DNA concentration was 15 μg/mL. Hyperchromicity was monitored at 260 nm.

Energy-transfer experiments involved the preparation of core particle samples B, D, A, and DA. B contained unmodified H4, whereas D and A contained H4 labeled with 5-IAEDANS and 5-IAF, respectively. Sample DA contained modified H4 in an approximately 1:9 molar ratio of dansyl to fluorescein. Competition experiments showed that both fluorescently labeled H4s, as well as unlabeled histone H4, incorporate randomly into synthetic core particles during in vitro reconstitution. The four samples were purified on 5–20% sucrose

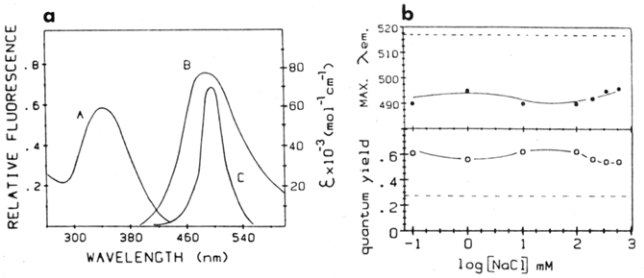

FIGURE 2: Spectral properties of 5-AEDANS and fluorescein nucleosomes. (a) (Curve A) Corrected excitation spectrum of 5-AEDANS nucleosome. Emission at 480 nm. (Curve B) Corrected emission spectrum of 5-AEDANS nucleosome. Excitation at 340 nm. (Curve C) Absorption spectrum of fluorescein nucleosomes. (b) Ionic strength dependence of maximum emission wavelength (top) and quantum yield (bottom) of 5-AEDANS nucleosomes. Excitation at 340 nm. The dashed lines show the limiting values for the two parameters when the dye is fully exposed to water.

gradients (Chung & Lewis, 1985) and dialyzed exhaustively into buffers of the appropriate salt concentration. Sample B was used for fluorescence blank subtraction. Fluorescence energy transfer was monitored by donor quenching between 430 and 480 nm according to Epe et al. (1983). This unconventional method for quantitating Förster-type energy transfer between two chromophores involves an internal comparison made before and after separation of the fluors in situ. In this case, separation was achieved by adding precisely weighed, solid Gdn-HCl to 4.5 M final concentration.

Results

Fluorophore Met-84 H4 Labeled Particles Are Similar to Native Nucleosomes. As we have shown earlier (Lewis, 1979; Chung & Lewis, 1985, 1986), Met-84 fluorophore modified histone H4 together with unmodified histones H2A, H2B, and H3 and core length DNA is easily incorporated into nucleosome like particles which can be isolated in homogeneous form from 5–20% sucrose gradients. Analysis of these labeled particles reveals that they are virtually indistinguishable from native nucleosomes on the basis of their histone composition, sedimentation velocity, gel mobility, thermal denaturation characteristics, and DNase I digestion products as shown in Figure 1. This is true for 5- and 8-AEDANS, fluorescein, and pyrene fluors even when both H4s are modified at their methionine sulfur atoms.

Fluorescence Characteristics of 5-AEDANS Nucleosomes. The corrected excitation and emission spectra for 5-AEDANS nucleosomes have maxima at 340 and 490 nm as shown in Figure 2a. The emission maximum for free 5-AEDANS-labeled H4 is 505 nm. The blue shift of the nucleosome emission is probably due to the 5-AEDANS being in an apolar environment equivalent to about 60% ethanol (Hudson & Weber, 1973). The quantum yield of the 5-AEDANS-labeled nucleosomes in 10 mM NaCl is 0.62 relative to quinine sulfate (considered to have an absolute quantum yield of 0.70 in 0.1 N H_2SO_4; Scott et al., 1970) while the fluorescence lifetime is 22 ns. The wavelength of the emission maximum as well as the quantum yields for 5-AEDANS nucleosomes as a function of ionic strength is shown in Figure 2b. Even though both parameters are very sensitive indicators of the polarity of the fluor environment (Hudson & Weber, 1973), they vary only slightly in the range from 0.1 to 600 mM NaCl. For comparison, the dashed line in the top panel of Figure 2b indicates the emission wavelength maximum for a nucleosome unfolded in 4.5 M Gdn-HCl. The large red shift which is also accompanied by a 50% reduction in the quantum yield as shown by the dashed line in the bottom panel of Figure 2b

Table I: Excitation and Emission Maxima of 5-IAF Conjugates[a]

sample	max λ_{em} (nm)	max λ_{ex} (nm)
free 5-IAF dye	518	490
AF-H4	518	494
AF-H4 nucleosome	521	499

[a] The sample buffer contained 10 mM NaCl and 0.1 mM Tris–EDTA, pH 7.4. For determination of maximum emission wavelengths, the excitation was set at 490 nm. For the corresponding excitation maxima, the emission was monitored at 520 nm. Band-pass widths of both monochromators were 2 nm.

results from the complete exposure of the dye to the denaturing solvent. Even though the fluor environment remains essentially constant in the range 0.1–600 mM monovalent salt, our earlier results from collisional quenching, polarization, and pyrene excimer studies (Chung & Lewis, 1985, 1986) together with the energy-transfer data presented below clearly indicate that internal structural changes have occurred, particularly at the H4–H4 interface.

Fluorescein Emission and Complex Formation in Labeled Nucleosomes. While fluorescein emission is much less sensitive to changes in solvent polarity than that of 5- and 8-AEDANS, this fluor nonetheless behaves similarly except that apolar environments result in red rather than blue shifts. The excitation and emission properties of the free dye, AF-Met-84 H4, as well as nucleosomes containing AF-Met-84 H4 are given in Table I. When labeled H4 is incorporated into a nucleosome by reconstitution, red shifts in both the excitation and emission maxima are observed. This is indicative of a decrease in the polarity of the dye environment (Hartig et al., 1977) and parallels the emission behavior of 5-AEDANS (this work) and 8-AEDANS (Chung & Lewis, 1985) nucleosomes whose spectral properties clearly showed incorporation of the fluors into the hydrophobic interior of the histone octamer.

Cys-110 of histone H3 is in very close proximity to its twin in a nucleosome and can interact across the dyad axis (Zama et al., 1977; Camerini-Otero & Felsenfeld, 1977; Lewis & Chiu, 1980; Cantor et al., 1981). On the basis of pyrene excimer fluorescence, we have recently identified a similar homotypic interaction involving the unique Met-84 residue of histone H4 (Chung & Lewis, 1986). When two fluorescein chromophores are very near each other, they too form a complex whose absorbance at 490 nm is reduced by a factor of 2 relative to the monomeric form (Mercola et al., 1972; Bunting & Cathou, 1974). Corrected excitation spectra of fluorescein Met-84 H4 labeled nucleosomes in 10 and 100 mM NaCl are shown in Figure 3. Previous studies of 8-AEDANS- (Chung & Lewis, 1985) and pyrene- (Chung & Lewis, 1986) labeled nucleosomes show that the H4–H4 contact region around Met-84 is partially disrupted in this salt range. There is a 20% increase in the integrated excitation spectrum (Ex) of fluoresein as the monovalent salt concentration is raised from 10 to 100 mM. By 600 mM NaCl, the ratio of Ex to Ex_0 (Ex_0 = integrated spectrum in 10 mM NaCl) has increased to 1.33 (see Figure 3, inset). For comparison, the excitation spectrum area of free AF-H4 remains constant in this salt range. This result indicates that the fluorescein chromophores attached to the two H4 molecules in a nucleosome are close enough to form a complex with a decreased excitation spectrum area. As the ionic strength is raised, the fluors are no longer constrained and move apart, resulting in an enhancement of the excitation spectrum. When the extent of labeling of the H4 sample (0.47 label per H4) is taken into account, there is excellent agreement between the observed decrease in excitation spectrum area and that calculated assuming complete loss of complex by 600 mM NaCl. Below

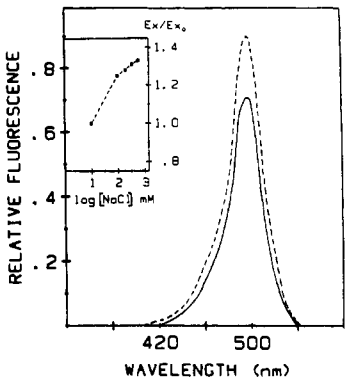

FIGURE 3: Spectral properties of fluorescein nucleosomes. Shown are corrected excitation spectra in 10 mM NaCl (—) and 100 mM NaCl (---). The emission was monitored at 520 nm. The inset shows the ratio Ex/Ex_0 as a function of the NaCl concentration. Ex_0 is the integrated excitation spectrum at 10 mM NaCl. Similarly, Ex is the spectrum at various levels of monovalent salt.

10 mM NaCl there are no changes in the excitation spectrum, indicating that the 1 mM transition does not affect the fluor complex. The significance of this result is considered later.

Fluorescence Energy Transfer. Ionic strength induced structural transitions in nucleosomes are accompanied by an increase in the distance separating the H3–H3 (Zama et al., 1977; Cantor et al., 1981) and H4–H4 (Chung & Lewis, 1986) contact sites within the histone octamer. The H3 studies used pyrene excimer fluorescence and singlet–singlet energy transfer methods which allowed the estimation of distances between two fluors. The latter technique is useful for separations greater than 2 nm but less than 10 nm with AEDANS and fluorescein as donor and acceptor fluors, respectively (Fairclough & Cantor, 1977). We have made similar measurements using AEDANS and fluorescein bound to Met-84 on H4 in order to characterize and quantitate the apparent expansion of the histone octamer around the H4–H4 contact region.

The distance r between donor and acceptor chromophores is related to the efficiency E of the transfer process by

$$r = R_0(1/E - 1)^{1/6} \qquad (1)$$

where R_0 is the critical distance at which $E = 50\%$. If the two chromophores are in close proximity, energy transfer occurs provided the emission spectrum of the dansyl donor overlaps the electronic excitation spectrum of the fluorescein acceptor. R_0 depends on the J_{DA} spectral overlap integral, a measure of the extent to which donor and acceptor transitions are in resonance as shown in eq 2. n is the refractive index

$$R_0 = [(8.79 \times 10^{-5})k^2n^{-4}Q_DJ_{DA}]^{1/6} \quad (\text{Å}) \qquad (2)$$

of the medium, usually assigned a value of 1.4 in biological systems (Fairclough & Cantor, 1977), Q_D is the quantum yield of the donor in the absence of the acceptor, and k^2 is a geometrical parameter that depends on the relative orientations of the two chromophores. In principle this orientation factor can vary between 0 and 4 (Fairclough & Cantor, 1972) and therefore poses the greatest uncertainty in distance determinations. In practice, further limits on its value can be set by determining the rotational freedom of the chromophores by polarization measurements as discussed in the next section. The overlap integral J_{DA} is given by

$$J_{DA} = [\sum F_D(\lambda)\, e_A(\lambda)\lambda^4\, \Delta\lambda]/[\sum F_D(\lambda)\, \Delta\lambda] \qquad (3)$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor in the absence of acceptor at wavelength λ expressed in nanometers and $e_A(\lambda)$ is the extinction coefficient of the acceptor at that

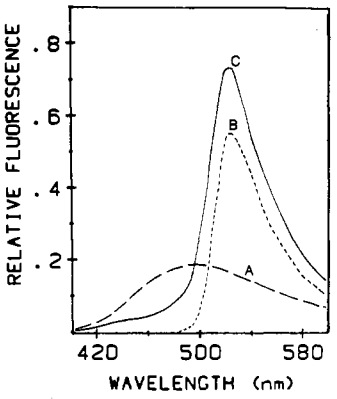

FIGURE 4: Corrected emission spectra of nucleosome samples for fluorescence energy transfer measurements. (Curve A) Emission of 5-AEDANS nucleosomes. (Curve B) Emission of fluorescein nucleosomes. (Curve C) Emission of 5-AEDANS/fluorescein nucleosomes. All spectra were recorded in 10 mM NaCl and 0.1 mM Tris, pH 7.4. Excitation at 340 nm.

wavelength. The integration of the spectral overlap was performed at 5-nm intervals, and a value of 1.50×10^{15} cm^3 M^{-1} nm^4 was obtained for nucleosomes in 10 mM NaCl. The overlap integrals were determined at different ionic strengths all containing 0.1 mM Tris, pH 7.4. The extinction coefficient of the acceptor e_A remained essentially constant at 6.8×10^4 cm^{-1} M^{-1}. These data are summarized in Table III. The extensive spectral overlap (shown in Figure 2a), in addition to the high quantum yield of 5-AEDANS and the large extinction coefficient of fluorescein makes this pair of fluorophores particularly well suited for Förster energy-transfer studies. The R_0 values calculated from eq 2 range from 4.78 to 4.92 nm again demonstrates that no major changes occur in the spectral properties of the donor and acceptor chromophores in this salt range.

Once R_0 is known, the efficiency E must be determined in order to calculate r, the donor–acceptor separation. Detection and quantitation of donor fluorescence quenching is easily accomplished because 5-AEDANS fluoresces strongly between 400 and 480 nm, whereas fluorescein exhibits no emission in the same range. Typical corrected fluorescence spectra of the various labeled core nucleosomes are shown in Figure 4. The excitation wavelength employed for energy-transfer studies (340 nm) does not fall within the excitation spectrum of the fluorescein-labeled core nucleosome (Figure 3). This ensures that, in the doubly labeled sample DA, fluorescein is accepting only donor (5-AEDANS) fluorescence rather than excitation energy at 340 nm. In a conventional energy-transfer experiment, the ratio of the quantum yields of the donor in the presence (Q_{DA}) and absence (Q_D) of the acceptor is determined directly from the fluorescence emission intensities (F_D,F_A, and F_{DA}) of the various nucleosome samples that have been corrected for background and differences in concentration:

$$Q_{DA}/Q_D = (F_{DA} - F_A)/F_D \quad (4)$$

The efficiency E of energy transfer can then be calculated by

$$E = [1 - (Q_{DA}/Q_D)]/f_A \quad (5)$$

where f_A is the fractional content of fluorescein acceptor in the doubly labeled sample. This is usually performed at 10-nm intervals between 430 and 480 nm, and the results are averaged to obtain a more accurate value for the efficiency of energy transfer (Eshaghpour et al., 1980).

Epe et al. (1983) have proposed a method of energy-transfer efficiency measurement whereby the processing of the raw data is simplified and several sources of error are eliminated (e.g., samples D and A can have an arbitrary or even unknown degree of labeling). This is accomplished by the in situ separation of fluors by enzymatic digestion or denaturation following measurements at the ionic strength of interest. We have separated the donor and acceptor chromophores by the addition of solid Gdn-HCl to 4.5 M. This dissociates the histones from the DNA and also from each other. If we denote the fluorescence emission intensities of the various samples before and after addition of denaturant by F and F′, respectively, then the efficiency is given by

$$E = [1 - (F_{DA}^*)(F'_D)/(F'_{DA}^*)(F_D)]/f_A \quad (6)$$

where (F_{DA}^*) and (F'_{DA}^*) have been corrected by subtraction of F_A or F'_A, respectively. The other quantities are defined as in eq 5. Efficiency values are determined and averaged as above.

Table II: Polarization Analysis of the Orientation Factor k^2

parameter[a]	5-AEDANS	fluorescein
emission anisotropy, EA	0.20	0.21
limiting anisotropy, A_0	0.24	0.22
dynamic depolarization, $\langle d' \rangle_d$	0.60	0.55
cone half-angle, Ψ (deg)	33	36
polarization, P	0.33	0.35
limits on k^2	0.18–0.75	(0.18–2.6)[b]
range of R_0 (nm)	3.9–5.0	(3.9–6.1)[b]

[a] Polarization measurements were carried out as described in Chung and Lewis (1985). The parameters are as defined in the text. [b] Analysis according to Dale and Eisinger (1974). The extreme limits in parentheses correspond to models with a direct in-line orientation of transition dipoles. This spatial arrangement has a very low statistical probability and was therefore excluded from consideration (Eshaghpour et al., 1980; Hillel & Wu, 1976).

Dipole–Dipole Orientation Factor. In theory the orientation factor k^2 may vary from 0 to 4 depending on the mutual disposition of the donor and acceptor transition dipoles. k^2 is usually assumed to be $^2/_3$ which is applicable in the case of chromophores having complete rotational freedom during the excited-state lifetime of the donor. Although this situation is rarely attained in practice, Stryer (1978) has estimated the error in distance measurements to be <20% on the basis of this assumption. In reality protein-bound fluors are only free to reorient about several bonds in a restricted region of space (Dale & Eisinger, 1974).

The fluors in AEDANS- and fluorescein-labeled nucleosomes have lifetimes of 22 and 5 ns, respectively, in 10 mM monovalent salt. The rotational relaxation time of the compact core nucleosome was taken to be 107 ns in calculating the limiting anisotropies of 0.24 and 0.22 for donor and acceptor chromophores (Chung & Lewis, 1985). The dynamic depolarization factor $\langle d' \rangle_d$, defined as $A_0/0.4$, was then used to determine the half-angles Ψ of the conical volume within which the chromophore has rotational freedom (Dale & Eisinger, 1974; Fairclough & Cantor, 1974). Since the rotational mobility of the fluorophore is most restricted in the compact form of the nucleosome (Chung & Lewis, 1985), the polarization data at 10 mM NaCl were used to evaluate the extreme limits on the range of k^2. The results of such an analysis are shown in Table II. In the compact form of the core nucleosome, k^2 is restricted to values between 0.18 and 0.75. This results in a range of 3.9–5 nm for the critical distance R_0 (Dale & Eisinger, 1974).

Transfer Efficiencies as a Function of Ionic Strength. The fluorescence energy transfer data are summarized in Table III. An example of donor quenching is shown in Figure 5 for a doubly labeled nucleosome at 10 and 600 mM NaCl. At the higher ionic strength there is an enhancement of the donor

Table III: Fluorescence Energy Transfer in 5-AEDANS and Fluorescein Doubly Labeled Nucleosomes

[NaCl] (mM)	Q_D	J_{DA}[a]	E_{obsd}	f_A	E_{cor}	R_0 (nm)	R (nm)[b]
0.1	0.61	1.50	0.60	0.60	1.00 ± 0.11	4.92	<2.0
1.0	0.56	1.46	0.52	0.60	0.87 ± 0.11	4.83	3.52 (2.92–3.74)
10	0.62	1.50	0.60	0.60	1.00 ± 0.08	4.92	<2.0
100	0.62	1.49	0.45	0.43	1.04 ± 0.09	4.91	<2.0
200	0.56	1.49	0.41	0.43	0.95 ± 0.11	4.83	3.01 (2.65–3.79)
350	0.54	1.47	0.52	0.62	0.84 ± 0.07	4.79	3.63 (2.96–3.79)
600	0.54	1.44	0.50	0.62	0.81 ± 0.04	4.78	3.75 (3.13–4.01)

[a] Units are $\times 10^{-15}$ cm^{-1} M^{-1} nm^4. [b] Values in parentheses correspond to limits in R obtained by the Dale and Eisinger (1974) method. See also Table II. All other parameters are as defined in text.

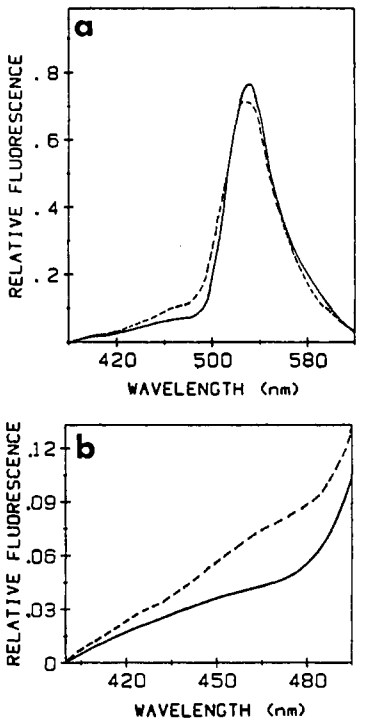

FIGURE 5: Detection of energy transfer by donor quenching. (a) Corrected spectra of 5-AEDANS/fluorescein nucleosomes in 10 (—) and 600 (---) mM NaCl. The buffer also contained 0.1 mM Tris, pH 7.4. (b) Enlarged view of the 400–500-nm region of (a). Excitation at 340 nm in all cases.

signal implying a decreased efficiency of energy transfer. The transfer efficiencies at 0.1, 10, and 100 mM NaCl are essentially 100%. This indicates that the two H4 Met-84 sites are very close together (<2 nm) within the doubly labeled nucleosome at low ionic strengths. However, the transfer efficiency at 1 mM salt is significantly less than 100%. This result is surprising as neither pyrene- (Chung & Lewis, 1986) nor fluorescein- (this work) labeled nucleosomes completely lose their excited dimer at this ionic strength. Nevertheless our earlier quenching and polarization data indicate that at 1 mM the nucleosome is less compact than at 0.1 or 10 mM salt (Chung & Lewis, 1985). Perhaps the pyrene–pyrene and fluorescein–fluorescein interactions in these nucleosomes are able to partially inhibit the 1 mM conformational transition. It is interesting to note that many other groups have also detected a distinct 1 mM transition in core nucleosomes by a variety of physical techniques [summarized by Schlessinger et al. (1982)].

Beyond 100 mM salt, the energy-transfer efficiencies decrease to 81% (corresponding to a 3.8-nm separation) at the highest salt concentration studied (600 mM), indicating a progressive separation of the two fluors with increasing ionic strength. The midpoint of this apparently cooperative transition is 200 mM.

Discussion

Conformational Changes at Low Ionic Strength. In solutions of less than 50 mM ionic strength Met-84 of histone H4 is adjacent to its twin in reconstituted nucleosomes as revealed by pyrene excimer fluorescence (Chung & Lewis, 1986), fluorescein complex formation, and AEDANS–fluorescein fluorescence energy transfer calculations (this work). In this ionic strength range the environment around a fluor attached to H4 Met-84 is quite apolar, and the fluor is probably situated on the interior of the octamer as indicated by fluorescence polarization and accessibility to quenching agents (Chung & Lewis, 1985).

While fluors bound to H4 Met-84 experience only small changes in their environment at low ionic strengths, there is nevertheless a transition centered around 1 mM monovalent salt. Our quenching accessibility and fluorescence emission intensity results (Chung & Lewis, 1985) are consistent with a less compact structure than that at 10 mM. A similar transition has been reported by many others using a wide range of physical and chemical cross-linking methods (Gordon et al., 1978; Martinson et al., 1979; Burch & Martinson, 1980, 1981; Cantor et al., 1981; Libertini & Small, 1982; Schlessinger et al., 1982; Uberbacher et al., 1983). Neutron scattering experiments (Uberbacher et al., 1983) indicate that the principal changes in this transition involve partial unwinding of the encircling DNA supercoil and that the overall shape of the protein core changes little if at all. Martinson and co-workers have shown that this transition is characterized by the disruption of some H2B–H4 intranucleosomal contacts (Martinson et al., 1980) and a change in the reactivity of certain C-terminal domain residues in H4 (Burch & Martinson, 1981) and can be blocked by the binding of histone H1 or by chemical cross-linking (Burch & Martinson, 1980). Thus, this transition probably involves the loosening of the H2A/H2B dimers from the central H3/H4 tetramer as the ends of the DNA become unbound. We note that HMG 14 and 17 bind noncooperatively at this ionic strength and appear to increase the compactness of the histone octamer (Uberbacher et al., 1982; Chung & Lewis, 1985). They may act in the same way as histone H1 at low ionic strength by securing the DNA tails (Burch & Martinson, 1980).

Conformational Changes at Moderate Ionic Strength. All of our results for fluor Met-84 H4 labeled nucleosomes indicate a cooperative transition centered around 200 mM NaCl in which the fluors at the H4–H4 interface become separated by as much as 3.8 nm at 600 mM NaCl. This transition is also accompanied by an increase in the polarity of the fluor environment as well as increased accessibility to quenching agents and fluor mobility. This conformational change is further enhanced by the cooperative binding of HMG 14/17 (Albright et al., 1980; Sandeen et al., 1980; Schroter & Bode, 1982). While there can be no doubt that a significant conformational change occurs around 200 mM NaCl, the properties of the H4-bound fluors suggest that no major unfolding

of the histone octamer is occurring. Libertini and Small (1982) report a modest transition centered around 100 mM NaCl on the basis of the pH dependence of tyrosine fluorescence anisotropy. The results of McGhee et al. (1980), Ausio et al. (1984), and Yager and van Holde (1984) from cross-linking and sedimentation analyses in this ionic strength range indicate that nucleosomes undergo at most only small changes in shape which may be attributable to partial unwinding of the DNA or minor conformational changes in the octamer.

Core and larger nucleosomes slowly dissociate into free DNA and histone octamer at ionic strengths greater than 100 mM monovalent salt (Yager & van Holde, 1984). The extent of dissociation increases with increasing temperature and ionic strength and with a reduction in the concentration of the nucleosome sample but appears to be independent of DNA length (Ausio et al., 1984). Our reconstitutions were made at high DNA concentrations, and fluorescence measurements were carried out as soon as possible following dilution from concentrated stock solutions of reconstituted labeled nucleosome. No time dependence was observed in any of our measurements. If appreciable time-dependent dissociation were occurring in our samples, then time-dependent changes should have been encountered as the fluorescence properties of the labeled free octamer are quite different from those of the labeled nucleosome. We conclude from this that the changes we have observed are not due to dissociation of the octamer from the DNA.

Model for the 200 mM Transition. The results of others mentioned above indicate that no major unfolding of the histone octamer occurs at physiological ionic strengths, and yet our results demonstrate that fluors bound to the two Met-84 H4s move 3.8 nm apart following a transition centered at 200 mM monovalent salt. To resolve this apparent paradox, we propose that at low ionic strength Met-84 is located on the exterior of the C-terminal globular H4 domain which is directly adjacent to its partner in the octamer, while at ionic strengths greater than 200 mM these domains rotate into a different orientation so that these groups are no longer close together. This is shown schematically in Figure 6. If one assumes a diameter of 2.5 nm for a single histone domain (Klug et al., 1980), then 3.8 nm falls easily within the maximum separation limit of 5 nm achievable by simple rotation of the roughly spherical domain. This model would account for the increased fluor separation while preserving the overall shape (Ausio et al., 1984; Yager & van Holde, 1984) and secondary structure of its constituents (Thomas et al., 1977; Savoie et al., 1985). Cantor and co-workers report that beyond 10 mM NaCl Cys-110 H3 fluor separation occurs only at ionic strengths greater than 500 mM NaCl and reaches a final value in excess of 7 nm (Cantor et al., 1981). However, the environment polarity and accessibility to quenching agents of fluors attached at Cys-110 H3 increases significantly in the range 100–300 mM (Cantor et al., 1981). Thus, the H4 changes appear to precede those for H3.

One of the main features of the model shown in Figure 6 is that different surfaces on the globular domain of H4 become available for binding following the transition. This might result in altered binding of the central H3/H4 tetramer to H2A/H2B dimers as well as to the encircling DNA. A model similar to the one proposed here has been put forward by Ellison and Pulleyblank (1984) to explain how polymorphism might arise during nucleosome assembly at physiological ionic strengths. In their scheme the unit that rotated was an H3/H4 dimer, thereby disrupting both the H3–H3 and H4–H4 contacts. Our results together with those of Cantor et al. (1981) suggest that

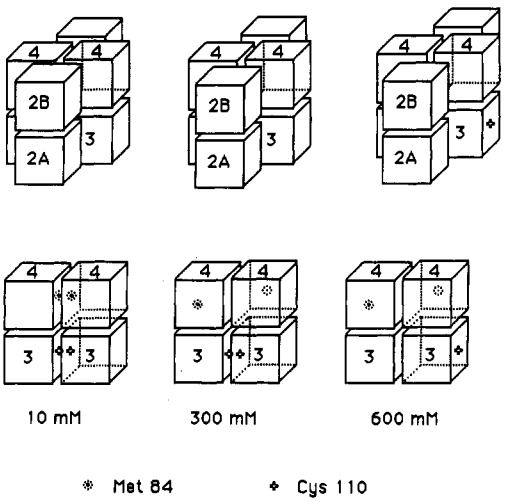

FIGURE 6: Model for the moderate and high ionic strength transitions. The globular portion of each of the eight core histones is represented for presentation purposes as a cube. They could just as easily be spheres, wedges, or other shapes. With increasing ionic strength the octamer (upper set of figures) is preserved but expands. The arrangement of H3 and H4 within H3/H4 tetramer (lower set of figures) changes with increasing ionic strength. First the H4 subunits and then the H3 subunits rotate so as to disrupt the Met-84 and the Cys-110 H3 contacts, respectively. By 300 mM monovalent salt the H4 subunits have rotated but are unexposed to the solvent. The H3 subunits remain unchanged. At 600 mM the Met-84 H4 sites in the octamer remain unexposed to the solvent, while the Cys-110 H3 sites become exposed as a result of rotation of the H3 subunits. In each case 2-fold symmetry about the vertical line through the 4–4 and 3–3 contacts is preserved. The relative histone arrangements were inferred from the electron density figures shown in Richmond et al. (1984).

these changes might involve a two-step process in which H4 rotates first followed by H3 at much higher ionic strengths. It is interesting to note that these proposed changes in the quaternary structure of the histone octamer might explain the drastic differences reported between the crystal structures of the core particle (Richmond et al., 1984) and the histone octamer (Burlingame et al., 1985). Experiments are in progress to test the central features of the model shown in Figure 6 by determining the accessibility of defined sites to chemical modification.

Dissociation of Histone H2A/H2B from Core Nucleosomes. H2A/H2B dimers can be displaced from nucleosomes at physiological ionic strengths (Baer & Rhodes, 1983; Louters & Chalkley, 1984). Read et al. (1985) have recently shown that H2A/H2B-depleted nucleosomes have physical properties very much like those of native particles. If the H2A/H2B dimer binds to both H3 and H4, then the loss of the H4 binding surface might dramatically weaken its binding even though H3 has not moved. The observation that HMG 14/17 binds cooperatively to core particles at physiological ionic strengths (Albright et al., 1980; Sandeen et al., 1980; Schroter & Bode, 1982) resulting in the increased exposure of the H4 fluor to the solvent (Chung & Lewis, 1985) might have some relevance for the role of these proteins in regulating these structural transitions. In our model, H4 would play a central role in mediating the binding of the H2A/H2B dimers to the H3/H4 tetramer. For this to be accomplished the surfaces of the globular domain of H4 must possess very distinct characteristics. The very high degree of sequence conservation of histone H4 may be a consequence of this role. This histone and H3 have the ability to specifically self-associate (Lewis et al., 1975; Morris & Lewis, 1977), complex with each other (Moss et al., 1976), and associate with histones H2A and H2B (Isenberg, 1979).

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REFERENCES

Albright, S. C., Wiseman, J. M., Lange, R. A., & Garrard, W. T. (1980) *J. Biol. Chem. 255*, 3673–3684.

Ausio, J., Seger, D., & Eisenberg, H. (1984) *J. Mol. Biol. 176*, 77–104.

Baer, B. W., & Rhodes, D. (1983) *Nature (London) 301*, 482–488.

Bentley, G. A., Lewitt-Bentley, A., Finch, J. T., Podjarny, A. D., & Roth, M. (1984) *J. Mol. Biol. 176*, 55–75.

Bunting, J. R., & Cathou, R. E. (1974) *J. Mol. Biol. 87*, 329–338.

Burch, J. B. E., & Martinson, H. G. (1980) *Nucleic Acids Res. 8*, 4969–4987.

Burch, J. B. E., & Martinson, H. G. (1981) *Nucleic Acids Res. 9*, 4367–4387.

Burlingame, R. W., Warner, E. L., Wang, B. C., Hamlin, R., Xuong, N. H., & Moudrianakis, E. N. (1985) *Science (Washington, D.C.) 228*, 546–553.

Camerini-Otero, R. D., & Felsenfeld, G. (1977) *Nucleic Acids Res. 4*, 1159–1181.

Cantor, C. R., Deiterich, A. E., & Prior, C. P. (1981) *Ann. N.Y. Acad. Sci. 366*, 246–252.

Chung, D. G., & Lewis, P. N. (1985) *Biochemistry 24*, 8028–8036.

Chung, D. G., & Lewis, P. N. (1986) *Biochemistry 25*, 2048–2054.

Daban, J., & Cantor, C. (1982) *J. Mol. Biol. 156*, 771–789.

Dale, R. E., & Eisinger, J. (1974) *Biopolymers 13*, 1573–1605.

Dieterich, A., Axel, R., & Cantor, C. (1977) *Cold Spring Harbor Symp. Quant. Biol. 42*, 199–206.

Dieterich, A., Axel, R., & Cantor, C. (1979) *J. Mol. Biol. 129*, 587–602.

Eisenberg, H., & Felsenfeld, G. (1981) *J. Mol. Biol. 150*, 537–555.

Ellison, M. J., & Pulleyblank, D. E. (1983) *J. Biol. Chem. 258*, 13321–13327.

Epe, B., Steinhauser, K. G., & Wooley, P. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2579–2583.

Eshaghpour, H., Dieterich, A., Cantor, C., & Crothers, D. (1980) *Biochemistry 19*, 1797–1805.

Fairclough, R. H., & Cantor, C. R. (1978) *Methods Enzymol. 48*, 347–379.

Gordon, V. C., Knobler, C. M., Olins, D. E., & Shumaker, V. N. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 660–663.

Hartig, P. R., Bertrand, N. J., & Sauer, K. (1977) *Biochemistry 16*, 4275–4282.

Hillel, Z., & Wu, C. W. (1976) *Biochemistry 15*, 2105–2113.

Hudson, E., & Weber, G. (1973) *Biochemistry 12*, 4154–4161.

Igo-Kemenes, T., Horz, W., & Zachau, H. G. (1982) *Annu. Rev. Biochem. 51*, 89–121.

Isenberg, I. (1979) *Annu. Rev. Biochem. 48*, 159–191.

Klug, A., Rhodes, D., Smith, J., Finch, J. T., & Thomas, J. O. (1980) *Nature (London) 287*, 509–516.

Klug, A., Finch, J. T., & Richmond, T. J. (1985) *Science (Washington, D.C.) 229*, 1109–1110.

Lewis, P. (1979) *Eur. J. Biochem. 99*, 315–322.

Lewis, P., & Chiu, S. (1980) *Eur. J. Biochem. 109*, 369–376.

Lewis, P., Bradbury, E. M., & Crane-Robinson, C. (1975) *Biochemistry 14*, 3391–3400.

Libertini, L. J., & Small, E. W. (1982) *Biochemistry 21*, 3327–3334.

Louters, L., & Chalkley, R. (1984) *Biochemistry 23*, 547–552.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Martinson, H. G., True, R. J., & Burch, J. B. E. (1979) *Biochemistry 18*, 1082–1089.

McGhee, J. D., Felsenfeld, G., & Eisenberg, H. (1980) *Biophys. J. 10*, 261–270.

Mercola, D. A., Morris, J. W. S., & Arquilla, E. R. (1972) *Biochemistry 11*, 3860–3874.

Morris, G., & Lewis, P. N. (1977) *Eur. J. Biochem. 77*, 471–477.

Moss, T., Cary, P. D., Crane-Robinson, C., & Bradbury, E. M. (1976) *Biochemistry 15*, 2261–2267.

Prior, C. P., Cantor, C. R., Johnson, E. M., Littau, V. C., & Allfrey, V. G. (1983) *Cell (Cambridge, Mass.) 34*, 1033–1042.

Read, C. M., Baldwin, J. P., & Crane-Robinson, C. (1985) *Biochemistry 24*, 4435–4450.

Reeves, R. (1984) *Biochim. Biophys. Acta 782*, 343–393.

Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., & Klug, A. (1984) *Nature (London) 311*, 532–537.

Sandeen, G., Wood, W. I., & Felsenfeld, G. (1980) *Nucleic Acids Res. 8*, 3757–3778.

Savoie, R., Jutier, J. J., Alex, S., Nadeau, P., & Lewis, P. N. (1985) *Biophys. J. 47*, 451–459.

Schlessinger, F. B., Dattagupta, N., & Crothers, D. M. (1982) *Biochemistry 21*, 664–669.

Schroter, H., & Bode, J. (1982) *Eur. J. Biochem. 127*, 429–436.

Scott, T. G., Spencer, R., Leonard, N., & Weber, G. (1970) *J. Am. Chem. Soc. 92*, 687–695.

Stockley, P. G., & Thomas, J. O. (1979) *FEBS Lett. 99*, 129–135.

Stryer, L. (1978) *Annu. Rev. Biochem. 47*, 819–846.

Thomas, G. J., Prescott, B., & Olins, D. E. (1977) *Science (Washington, D.C.) 197*, 385–388.

Uberbacher, E. C., & Bunick, G. J. (1985) *J. Biomol. Struct. Dyn. 2*, 1033–1055.

Uberbacher, E. C., Mardian, J. K. W., Rossi, R. M., Olins, D. E., & Bunick, G. J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5258–5262.

Uberbacher, E. C., Ramakrishnan, V., Olins, D. E., & Bunick, G. J. (1983) *Biochemistry 22*, 4916–4923.

Weintraub, H., Worcel, A., & Alberts, B. (1976) *Cell (Cambridge, Mass.) 9*, 409–417.

Weisbrod, S. (1982) *Nature (London) 297*, 289–295.

Weischet, W. O., Tatchell, K., van Holde, K. E., & Klump, H. (1978) *Nucleic Acids Res. 5*, 139–160.

Wingender, E., Maas, K., & Bode, J. (1981) *Int. J. Biol. Macromol. 3*, 114–120.

Yager, T. D., & van Holde, K. E. (1984) *J. Biol. Chem. 259*, 4212–4222.

Zama, M., Bryan, P. N., Harrington, R. E., Olins, A. L., & Olins, D. E. (1977) *Cold Spring Harbor Symp. Quant. Biol. 42*, 31–41.