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Effect of Tyrosyl Modifications on Nucleosome Reconstitution: A Spin-Labeling Study†

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ABSTRACT: An imidazole spin-label was used to study the role of tyrosyl residues in the reassociation process for the nucleosome core particle. The nucleosome core particle, containing 145 base pairs of DNA and a histone core (two each of the four histones H2A, H2B, H3, and H4), was isolated from chicken erythrocytes. Native particles were first dissociated in 2 M NaCl and labeled with varying concentrations of imidazole spin-label. The labeled histone core and endogenous DNA were then reassociated back by salt step dialysis. Reconstituted spin-labeled complexes, purified by an isokinetic sucrose gradient, were found to have physical properties identical with those of unlabeled native particles. Spin-labeling the surface tyrosines of the histone core did not interfere with proper reassociation of the nucleosome core complex. ESR spectra of the reconstituted nucleosome core complex are not of the strongly anisotropic type, suggesting that labeled surface tyrosines in the histone core are not involved in specific DNA–histone interaction nor does wrapping of DNA on the histone core involve very close contact with the label. When labeling was carried out under denaturing conditions following exposure of the histone core to urea, additional histone tyrosine residues were spin-labeled. The resulting histone–DNA complexes that formed after reassociation had physical properties different from those of the native nucleosome core. This result suggested that some of the "buried" tyrosines are essential for specific histone–histone interactions that lead to stable histone core structures. Spin-labeling the buried tyrosines prevented the compact supercoiling of DNA into the nucleosome core particle.

The nucleosome core particle, an elementary subunit of chromatin structure, consists of 146 base pairs of core DNA and an octomeric histone core comprised of two each of the core histones (H2A, H2B, H3, and H4) [for a review, see McGhee & Felsenfeld (1980a)]. The amino acid sequences for these four histones are highly conserved throughout evolution (Isenberg, 1979). Data from studies of histone complexing (Van Holde & Isenberg, 1975; Spiker & Isenberg, 1977) and chromatin cross-linking (Kornberg, 1977; Trifonov,

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1978) all suggest that the four core histones have very specific kinds of interaction and arrangements between each other and within the histone core. Furthermore, studies from DNase I[1] digestion on nucleosomes (Lutter, 1978), histone–DNA cross-linking in chromatin (Shick et al., 1980), and the effect of DNA length on nucleosome reconstitution (Tatchell & Van Holde, 1979) all suggest that the core DNA is possibly held in a precise configuration or asymmetrically with respect to the histone core (McGhee & Felsenfeld, 1980a). What type of amino acids are involved in the DNA–histone and histone–histone interactions in the nucleosome core particle is not yet clear. It is generally suggested that electrostatic interactions between the phosphodiester backbone of the DNA and the basic residues (lysine and arginine) in the histones play an important role in DNA compaction on the histone core (Mirzabekov & Rich, 1979; McGhee & Felsenfeld, 1980b). However, it has been demonstrated that 30–40% of the basic residues, which are distributed in the N-terminal regions, could be removed by trypsin digestion, yet the chromatin still retained many of its original properties (Weintraub & Van Lente, 1974). Indeed, similar particles can be reconstituted from the trypsinized (armless) histone core, suggesting that the major DNA binding sites are probably located in those apolar central and carboxyl regions of the core histones (Whitlock & Stein, 1978).

Tyrosines have been shown to give rise to both stacking and hydrogen-bonding interactions in complex formation with DNA in other biological systems (Mayer et al., 1979; Helene & Maurizot, 1981). There are 30 tyrosines in the nucleosome core particle, and most of these tyrosines are distributed in the apolar central and carboxyl regions of the core histones (Isenberg, 1979). Laser Raman spectroscopy has suggested that 18 tyrosines are hydrogen bonded within the nucleosome (Olins, 1977; Thomas et al., 1977). Our previous ESR spin-labeling studies demonstrated that less than 4 tyrosines in the nucleosome core particle are accessible to imidazole spin-labels (tyrosine specific) while 12 tyrosines in the histone core (in 2 M NaCl) can be spin-labeled (Chan & Piette, 1980). Iodination of the tyrosines in the nucleosome has been reported by several groups (Biroc & Reeder, 1976; Weintraub et al., 1975), and in each case they demonstrated that very few tyrosines in the nucleosome can be iodinated at physiological ionic strength. Recently, Burch & Martinson (1981) studied the iodination of nucleosomes at low ionic strength and have shown a "flip-flop" in the H4 iodination pattern, corresponding to a reversible conformational change of the nucleosome at low ionic strength.

In the results reported here, we have used spin-labeling to probe the relative reactivities of the tyrosines in the nucleosome core particle under a broad range of ionic strengths (0.1 mM–2 M NaCl). We have also studied the role of tyrosines in the DNA–histone and histone–histone interactions in the nucleosome reconstitution process. The results seem to suggest that the "surface" tyrosines on the histone core are not involved or essential to histone–DNA interactions leading to the folding of DNA on the histone core. However, we present evidence suggesting that the "buried" tyrosines in the histone core are essential for the specific histone–histone interactions that lead to a stable histone core structure in the nucleosome core particle.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; DNase I, pancreatic deoxyribonuclease; *Hae*III, endonuclease R *Hae*III obtained from *Haemophilus aegyptius*; CD, circular dichroism; NMR, nuclear magnetic resonance; ESR, electron spin resonance.

Materials and Methods

(A) Isolation of Nucleosome Core Particles and Histone Core. (1) Isolation of Nucleosome Core Particles. Nucleosome core particles were isolated from histone H1 and H5 depleted chromatin as described previously (Chan & Piette, 1980). The chromatin obtained after limited digestion at 37 °C with 50 units/mL micrococcal nuclease (Worthingon) was centrifuged through 5–25% isokinetic sucrose gradients in an SW 27 rotor at 27 000 rpm for 20 h. The monomer fractions were pooled and dialyzed against 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2.

(2) Isolation of Histone Cores. Histone cores were isolated from the H1- and H5-depleted chromatin according to Tatchell & Van Holde (1977). The chromatin solution was placed in 2 M NaCl with 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2 and centrifuged at 50 000 rpm for 8 h in a Ti 50.2 rotor. The supernatant was chromatographed through a Sephadex G-100 column and equilibrated with 2 M NaCl–10 mM Tris-HCl–0.2 mM EDTA at pH 7.2, and the histone core fractions were pooled, concentrated with an Amicon ultrafiltration cell, and dialyzed against 2 M NaCl–10 mM Tris-HCl–0.2 mM EDTA at pH 7.2.

(3) Electrophoresis of DNA and Histones. DNA from the nucleosome core particles was extracted according to Noll et al. (1975) and analyzed by 6% polyacrylamide slab gel electrophoresis according to the procedure of Loening (1967). The size of the core DNA was found to be 145 base pairs when phage ΦX 174 RF DNA *Hae*III digest (Biolabs) was used as a standard. Histones from the histone core and from the nucleosome core particles were analyzed by sodium dodecyl sulfate ($NaDodSO_4$)–18% polyacrylamide slab gel electrophoresis according to the method of Laemmli (1971). Equimolar amounts of histones H2A, H2B, H3, and H4 were present. Neither histones H1 and H5 nor nonhistone proteins were detected.

(B) Spin-Labeling of Nucleosome Core Particles and Histone Core. The spin-label *N*-(2,2,5,5-tetramethyl-3-carbonylpyrrolidinyl-1-oxy)imidazole was synthesized according to Adackaparayil & Smith (1977).

(1) Effects of Ionic Strength on Labeling the Nucleosome Core. Several aliquots of nucleosome core particles were first dialyzed against 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2, containing various amounts of NaCl (10^{-4}–2.0 M) and spin-labeled with an 80-fold molar excess of imidazole spin-label to total tyrosine content. A stock solution of imidazole spin-label at 5×10^{-2} M was prepared by dissolving the yellow label in dry toluene. Appropriate fractions of the spin-label stock solution were carefully pipetted into test tubes and dried with dry nitrogen. The nucleosome core particles, after dialyzed against different ionic strengths, were added at 5 OD/mL each to the dried spin-label and stirred gently with a magnetic stirrer. The reaction was allowed to proceed for 2 h at room temperature and 2 h at 4 °C. Unreacted label was removed by exhaustive dialysis against 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2.

(2) Effects of Urea Treatment on Labeling the Nucleosome Core. Aliquots of nucleosome core particles were dialyzed against 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2, containing various amounts of urea (1–8 M), and spin-labeled with a molar excess of imidazole spin-label in the same manner.

(3) Spin-Labeling the Nucleosome Core Particles in 2 M NaCl with or without Urea. For reassociation studies, large amounts of nucleosome core particles were dialyzed against 2 M NaCl–10 mM Tris-HCl–0.2 mM EDTA at pH 7.2, containing 0, 4, and 6 M urea, respectively. The dissociated

nucleosome core particles were then spin-labeled with various amounts of a molar excess of imidazole spin-label, in a manner similar to that described above. For comparison and as a control, the dissociated nucleosome core particles in 2 M NaCl with or without urea were also modified with a molar excess of non-spin-label *N*-acetylimidazole.

(*C*) *Reconstitution of Spin-Labeled Nucleosome Core Particles.* The spin-labeled, nucleosome cores in 2 M NaCl, with or without urea, were reassociated back by salt step dialysis according to Tatchell & Van Holde (1977) with minor modifications. The reconstitution was carried out at 4 °C with 4-h steps of 2.0, 1.5, 1.25, 1.0, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 M NaCl and, finally, two changes of 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2. At the end of the reconstitution, the material was first concentrated by using an Amicon ultrafiltration cell (Model 12 with a PM 10 membrane), and the concentrated materials were then centrifuged through 5–20% isokinetic sucrose gradients according to Lawrence et al. (1976) in an SW 27 rotor. Gradients were dripped from the bottom, and the absorption at 260 nm for each fraction was measured with a Beckman Model 25 spectrophotometer. The fractions were dialyzed against 10 mM Tris-HCl–0.2 mM EDTA at pH 7.2 to remove the sucrose for further biophysical studies. In some cases, at the end of each salt step dialysis, an ESR spectrum of the labeled nucleoprotein complex was recorded and the correlation time of the spin-label determined.

(*D*) *Characterization and Comparison of Spin-Labeled Native and Reconstituted Nucleosome Core Particles.* (*1*) *Sedimentation Velocity.* Sedimentation velocity experiments were performed on a Beckman Model E ultracentrifuge, equipped with a schlieren optical system, operating at 48 000 rpm with a temperature of 4 °C.

(*2*) *Circular Dichroism.* The CD spectra of native and reconstituted nucleosome complexes were recorded at room temperature with a Cary 60 CD spectrometer.

(*3*) *Thermal Denaturation.* Thermal denaturation curves were recorded on a Gilford spectrophotometer at a heating rate of 0.25 °C/min. Derivative curves ($\Delta A_{260}/\Delta T$) were calculated and normalized by a linear least-squares fit. Both native and reconstituted materials were dialyzed against 0.2 mM EDTA, pH 7.0, prior to use in the thermal denaturation experiments.

(*E*) *ESR Spectroscopy.* ESR spectra were recorded at room temperature with a Varian E4 ESR spectrometer operating at 9.5 GHz, as described previously (Chan & Piette, 1980). The spin concentration was quantitated by double integration of the ESR spectra with an On-line V-72 minicomputer equipped with software in Conversational Language for Spectroscopic System (CLASS). The rotational correlation time was determined from the equation given by Likhtenshtein (1976):

$$1/\tau = 3.6 \times 10^9/[[(H_0/H_{-1})^{1/2} - 1]\Delta H_0]$$

where ΔH_0 is the width of the central component in gauss and H_0 and H_{-1} are the derivative peak heights of the ESR spectral components (central peak and high-field peak). The relative peak-height ratio (H_{-1}/H_0) is taken as an empirical motional index for the spin-label; namely, the higher the ratio, the freer the motion of the spin-label.

(*F*) *Effects of Hydroxylamine on Spin-Labeled Native and Reconstituted Nucleosome Core Particles.* A 0.14 M solution of hydroxylamine at neutral pH was added to the spin-labeled, native or reconstituted, nucleosome core particles. The rate of release of the bound spin-label from the particles was followed by the ESR spectrometer. At the end of the reaction, the samples were dialyzed against H_2O to remove the released free spin-label.

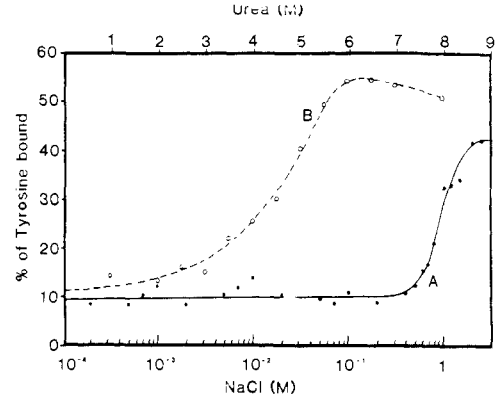

FIGURE 1: Spin-labeling of nucleosome core particles. Aliquots of core particles were dialyzed first in a buffer containing various amounts of (A) NaCl and (B) urea and were then labeled with an 80-fold molar excess of imidazole spin-labels to the total tyrosine content. After removal of unreacted labels, the concentration of the bound labels was quantitated from the ESR spectra.

Results

(*A*) *Labeling of Nucleosome Core Particles.* As shown in Figure 1A, only 8–12% of the tyrosyl residues are accessible to the imidazole spin-label when the core particle is subjected to an ionic strength of 0.1 mM–0.4 M. When the ionic strength is higher than 0.4 M, the accessibility of the tyrosines increases considerably from 12% to a maximum of 42% at 2 M NaCl. The labeling profile seems to reflect the different conformational state of the nucleosome core particle in the different ionic strengths.

Figure 1B shows the labeling profile when the core particles are labeled in the presence of varying amounts of urea. Of the tyrosyl residues 10–15% are spin-labeled when the core particle has been treated in 0–3 M urea at low NaCl concentration. The accessibility increases sharply to a maximum of 55% at 6 M urea. A higher percentage of the tyrosine can be labeled only when the core particle is exposed to both high-salt (2 M NaCl) and high-urea concentrations (data not shown).

(*B*) *Mode of Reassociation of the Spin-Labeled Histone Core with Core DNA during Reassociation.* Spin-labeled histone cores were mixed with purified core DNA in a weight ratio of 1:1 in 2 M NaCl. The mixture was reconstituted by salt step dialysis as described under Materials and Methods. At each step of the dialysis, the motional characteristic of the labeled tyrosines was examined by ESR. A typical ESR spectrum for the labeled tyrosines in the histone is given in Figure 6. The relative peak-height ratio (H_{-1}/H_0) is taken as an empirical motional index for the spin-label; namely, the higher the ratio, the freer the motion of the spin-label. Figure 2A shows a plot of the ratio (H_{-1}/H_0) vs. salt concentration during the reconstitution process. As the ionic strength is gradually decreased from 2 M NaCl, the motional freedom of the labeled tyrosines becomes correspondingly hindered. However, no sharp change is found in the range of 2–0.3 M NaCl. When the ionic strength is less than 0.3 M, the peak-height ratio decreases sharply, suggesting that the motional environment of the labeled tyrosines has become more restricted. Nevertheless, no strong immobilization has been detected at the end of complete reconstitution.

On the contrary, as shown in Figure 2B, when the ionic strength for the labeled histone core is decreased from 2 M NaCl in the absence of DNA, a relative sharp change occurs in the region of 1.2–0.8 M, suggesting a conformational de-

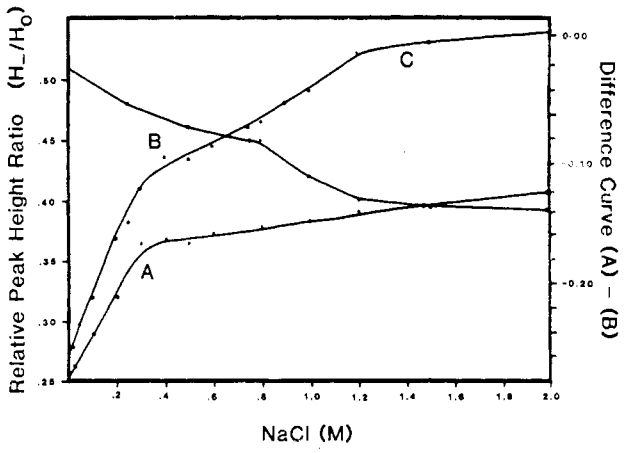

FIGURE 2: Mode of nucleosome reconstitution: a plot of the relative peak-height ratio (H_{-1}/H_0) of the ESR spectrum vs. NaCl concentration. Spin-labeled histone cores in 2 M NaCl were mixed (A) with purified core DNA in a ratio of 1:1 (w/w) and (B) without DNA. The salt concentration was decreased stepwise. At each step of the dialysis, the motional characteristics of the labeled tyrosines were examined by ESR. (C) Difference curve between (A) and (B).

stabilization of the histone core. As the ionic strength is further decreased, the spin-labels attain their greatest freedom of motion, indicating that the histones are now in a less structured conformation, consistent with those published observations by Eickbush & Moudrianakis (1978), Isenberg (1979), and Chan & Piette (1980).

Figure 2C shows the difference curve between parts A and B of Figure 2. It clearly reflects the different conformational states of the labeled tyrosines, which can be correlated with the net interactions between the histone core and the core DNA during the reconstitution process. This curve can be divided into three regions, namely, 2–1.4, 1.4–0.5, and 0.5–0.01 M NaCl. Little or very weak interaction between the histones and DNA is detected in the region of 2–1.4 M NaCl. Considerable DNA–histone interaction takes place between 1.2 and 0.5 M NaCl. Analysis of the DNA-bound histones at 0.5 M NaCl by gel electrophoresis has shown that indeed all four histones, H2A, H2B, H3, and H4, were present in equimolar amounts (data not shown), consistent with the observation demonstrated by others (Wilhelm et al., 1978; Jorcano & Ruiz-Carrillo, 1979; Stein, 1979). As the ionic strength is further decreased from 0.5 to 0.01 M, as shown in Figure 2C, the tumbling environment for the labeled tyrosines has become more restricted, suggesting that a full compaction of the nucleosome complex takes place and its final conformation is more compact in 0.01 than in 0.6 M NaCl.

(C) Role of Tyrosines in Nucleosome Reconstitution. (1) Labeling in 2 M NaCl without Urea. Figure 3 shows the sucrose gradient profiles for the spin-labeled and reconstituted nucleosome core complexes. The native nucleosome core particles were first dissociated in 2 M NaCl without urea, labeled with varying amounts of imidazole spin-label, and reassociated by salt step dialysis as described under Materials and Methods. As shown from the gradient profiles, a major monomer peak, which sediments at a position equivalent to the native core particle ($s_{20,w}$ = 10.5 S), is found for all the reassociated samples, independent of the degree of spin-labeling. Under the specific labeling conditions (2 M NaCl without urea), only the surface tyrosines on the histone core were labeled (Chan & Piette, 1980). The percentage of the tyrosines being labeled in each case was quantitated (10–40%) and is given in the figure legend. When more than 40% of the tyrosines become labeled, a shoulder appears at a position between the monomer fraction (10.5 S) and the free core DNA

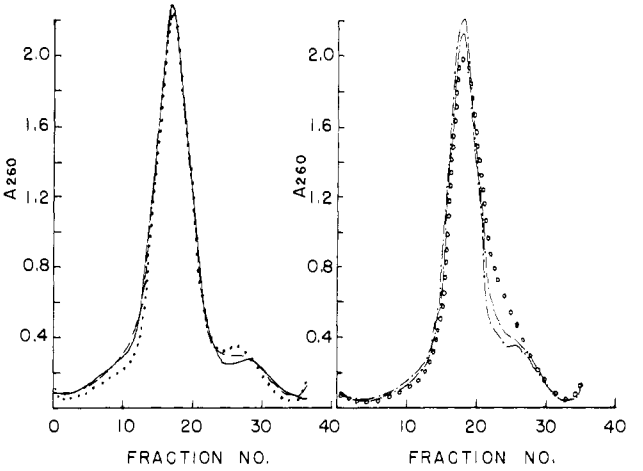

FIGURE 3: Fractionation of reconstituted nucleosome complexes by isokinetic sucrose gradient (5–25%). Nucleosome core particles were first dissociated in 2 M NaCl and spin-labeled with various amounts of a molar excess of imidazole spin-label. The mixtures were reassociated as described under Materials and Methods. At the end of reassociation, both spin-label and protein concentrations were determined. The percentages of tyrosines being labeled are (a) 0% (—), (b) 10% (- - -), (c) 20% (···), (d) 30% (- · -), (e) 37% (· · · ·), and (f) 44% (O).

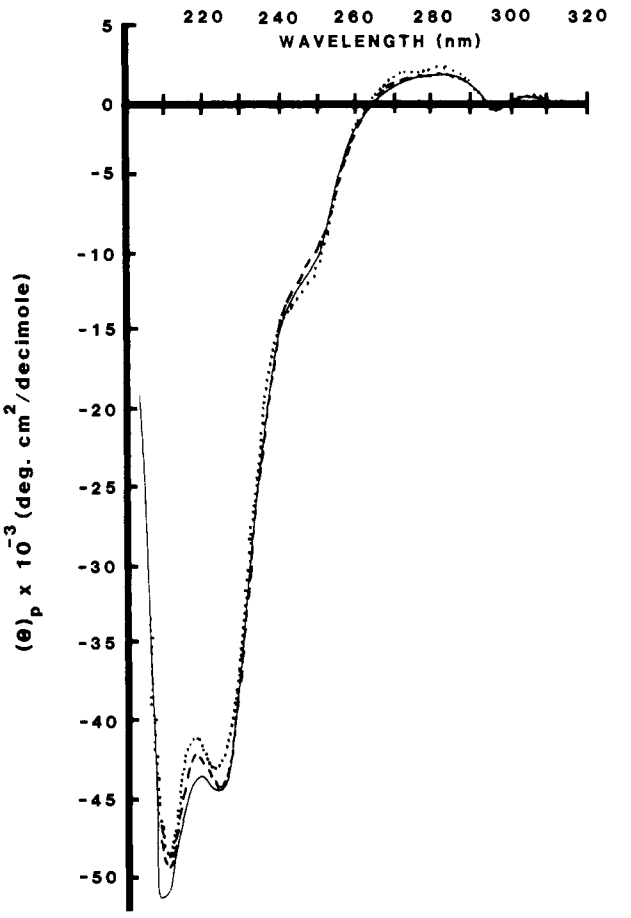

FIGURE 4: Circular dichroism spectra for nucleosome core particles: (a) native (—); (b) native and spin-labeled (- - -); (c) spin-labeled and reconstituted (···). The ellipticity is calculated on the basis of DNA concentration.

position (4.5 S), as shown in Figure 3 (curve f).

The physical properties for the labeled and reconstituted nucleosome core complexes were examined by their circular dichroism spectra and thermal denaturation profiles and were found to be nearly identical with those of the native nucleosome core particle, as shown in Figures 4 and 5, respectively. The

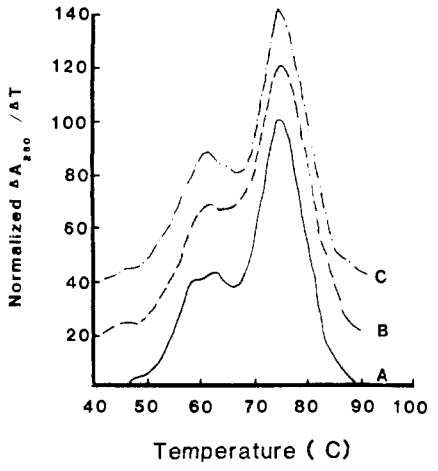

FIGURE 5: Derivative thermal denaturation profiles for nucleosome core particles: (A) native; (B) native and spin-labeled; (C) spin-labeled and reconstituted.

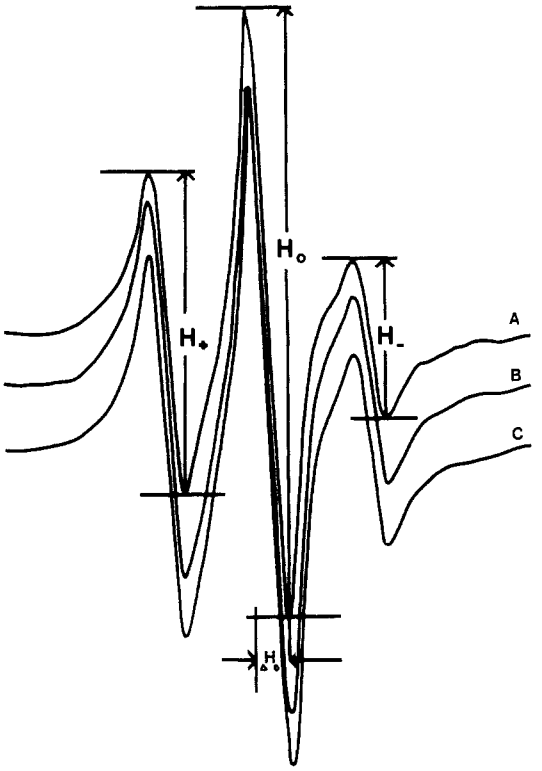

FIGURE 6: ESR spectra for the reconstituted nucleosome core particle. After reassociation, the reconstituted complexes were purified through a 5–25% isokinetic sucrose gradient as described under Materials and Methods. The monomer fractions were pooled, dialyzed, and concentrated. The percentages of tyrosines labeled in each case are (A) 6.3%, (B) 13.7%, and (C) 27.7%.

ESR spectra (Figure 6) for the reassociated complexes are also found to be very similar to the labeled native nucleosome core particle as reported earlier (Chan & Piette, 1980). The rotational correlation time for the labeled tyrosines in the reconstituted complex is found to be 1.06 ns, a value only slightly larger than that in the labeled native core particle (0.96 ns). This indicates that the wrapping of the core DNA on the labeled histone core does not strongly immobilize the motion of the spin-labels attached to those tyrosyl residues.

(*2*) *Labeling in 2 M NaCl plus 4 M Urea.* Native nucleosome core particles that are first dissociated in 2 M NaCl and partially denatured by 4 M urea and then labeled by the imidazole spin-label to varying extents and are reconstituted back by salt step dialysis without urea yield the sucrose gradient profiles for the final reconstituted complexes shown in Figure 7. Nearly complete reassociation is obtained for the non-spin-labeled complex, as shown in Figure 7 (curve a). However, when 10–30% of the tyrosines were labeled under the partial denaturation conditions (2 M NaCl plus 4 M urea), a shoulder corresponding to nonnucleosome complexes appeared in addition to the major reconstituted monomer fractions, as shown in curves b, c, and d of Figure 7, respectively. The yields of the reconstituted monomer fraction are also slightly decreased when compared to those of the nonlabeled sample. When 38 and 46% of the tyrosines were modified, the resulting complexes sedimented as a broader peak at a position equivalent to 10 and 8.7 S, as shown in curves e and f of Figure 7, respectively.

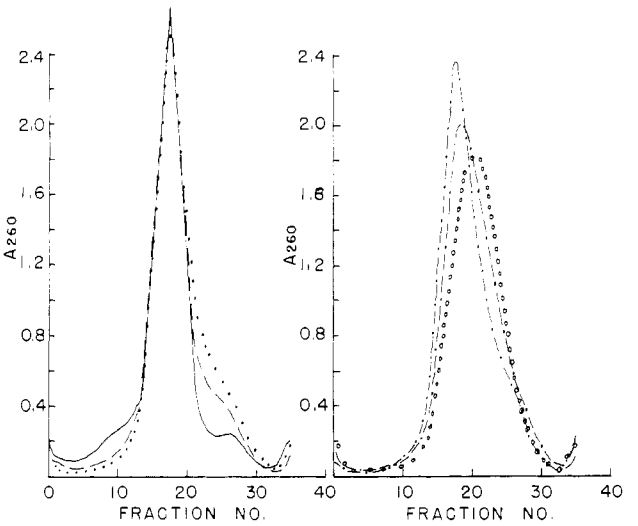

FIGURE 7: ESR spectra for reconstituted, spin-labeled nucleosomes. The native core particles were first dissociated in 2 M NaCl, partially denatured in 4 M urea, and spin-labeled with various amounts of a molar excess of imidazole spin-label. The mixtures were then reassociated and fractionated, and the A_{260} was measured. The percentages of tyrosines labeled in each case are (a) 0% (—), (b) 11% (- - -), (c) 22% (···), (d) 30% (-··-), (e) 38% (-···-), and (f) 46% (O).

The physical properties for the reconstituted monomer complexes in Figure 7 (curves a–c) are still found to be nearly identical with those of the native material. However, the complexes obtained in Figure 7 (curves d–f) have slightly different physical properties, namely, a smaller sedimentation coefficient, with an increase in DNA ellipticities and a decrease in melting temperature. Those complexes sedimenting at the shoulder region are found to be very different from the native particle. The ESR spectra for the reconstituted monomer fractions in Figure 7 (curves b and c) appeared to be very similar to those obtained previously as shown in Figure 6, but the spectra for the complexes obtained in Figure 7 (curves d–f) show faster rotational correlation time for the attached labels, suggesting a less compact structure for these complexes (data not shown).

(*3*) *Labeling in 2 M NaCl plus 6 M Urea.* It has been demonstrated previously by CD (Olins et al., 1977) and ESR spin-labeling methods (Chan & Piette, 1980) that when the histone core (in 2 M NaCl) is treated with 6 M urea, 50% denaturation occurs and the disruption of the α helix in the core histones by urea is highly cooperative. When the nucleosome core particle is dissociated in 2 M NaCl and further denatured in 6 M urea, the tertiary and secondary structures of the core histones are disrupted, thus exposing some of those buried tyrosines to the imidazole spin-labels. We have spin-labeled the denatured nucleosome core particle to different extents under these conditions and then reassociated the

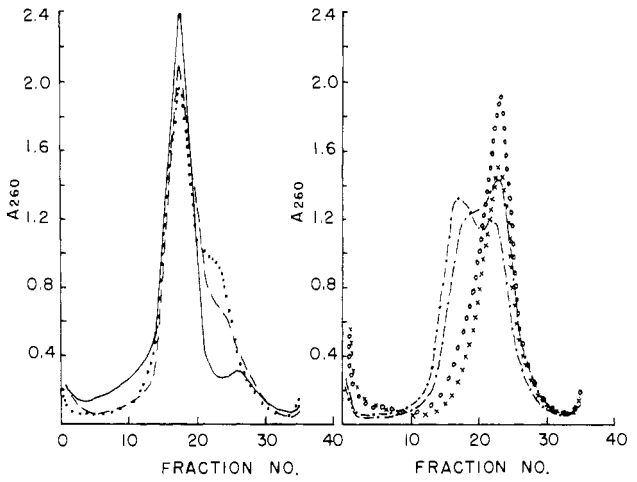

FIGURE 8: Sucrose gradient analysis of reconstituted, spin-labeled nucleosome complexes. The native particles were dissociated in 2 M NaCl plus 6 M urea, modified, and reconstituted in a similar manner. The A_{260} was measured after fractionation in a sucrose gradient. The percentages of tyrosines labeled in each case are (a) 0% (—), (b) 10% (---), (c) 23% (···), (d) 32% (-·-), (e) 40% (·---), and (f) 45% (O); (g) modified with *N*-acetylimidazole (×).

modified complexes by salt step dialysis as described under Materials and Methods. A set of sucrose gradient profiles is obtained for the reconstituted complexes as shown in Figure 8. A major monomer peak sedimenting at the 10.5S position was obtained for the non-spin-labeled complexes, as shown in Figure 8 (curve a). When only 10% of the tyrosines were labeled, a nonnucleosome complex appeared and the "shoulder" increased as more tyrosines (23–40%) in the final complexes were modified by the imidazole spin-label (Figure 8, curves b–e, respectively). At the same time, there was a decrease in the monomer fraction in each case. When more than 40% of the tyrosines were labeled, the resulting complexes sedimented at a position equivalent to 8 S as a major fraction (Figure 8, curve f). When the nucleosome core particles were modified under the same conditions with *N*-acetylimidazole instead of the spin-labeled derivative, no nucleosome-like complexes could be reconstituted, as shown in Figure 8 (curve g).

(D) Effects of Hydroxylamine on Spin-Labeled Tyrosines. It is well-known that hydroxylamine will cleave the ester linkage on a modified tyrosine residue (Means & Feeney, 1971); thus when the spin-labeled native nucleosome core particle or the reconstituted particle is exposed to 0.14 M hydroxylamine at pH 7.0, the spin-label is cleaved from the histones. Figure 9 shows the release of free spin-label as measured by the increase of relative ESR intensity (H_0) vs. time of reaction. The rate of cleaving of the spin-label is only slightly faster in the native nucleosome core particle than in the reconstituted particle in the first 5 min of reaction. At the end of the reaction, both samples were dialyzed extensively against 10 mM Tris-HCl–0.2 mM EDTA, pH 7.2, and less than 10% of the original spin-label concentration remained in the sample. This suggests that more than 90% of the imidazole spin-labels were previously attached to the tyrosyl residues in the core histones. The distribution and location of the labeled tyrosines in the native or reconstituted nucleosome core particles are under investigation and will be presented elsewhere.

Discussion

The specificity of the imidazole spin-label toward the tyrosyl residues of histones was demonstrated previously (Chan & Piette, 1980) and was further supported by the present observation that more than 90% of the spin-labels that were bound to the histone were cleavable by hydroxylamine (Figure 9). As can be seen from Figure 1, the accessibility of the tyrosyl residues on the nucleosome core particle to the spin-label varies under different ionic conditions. A conformational change in the nucleosome has been reported at lower ionic strengths (10^{-3} M NaCl) [references cited in McGhee & Felsenfeld (1980a)]. However, such a conformational change at lower ionic strengths was not detected in terms of the increase of tyrosine accessibility toward the imidazole spin-label, as shown in Figure 1A. This suggests that the conformational change reported at the lower ionic strength does not expose more tyrosyl residues in the core histones, since only 8–12% of the tyrosines are labeled in the region of 0.1 mM–0.4 M NaCl. When the core particle is subjected to a higher ionic strength (0.4 M NaCl), a maximum of 42% tyrosines (12 out of 30 tyrosines) becomes accessible. This observation is consistent with our previous data (Chan & Piette, 1980) for the labeling of the histone core alone (in 2 M NaCl).

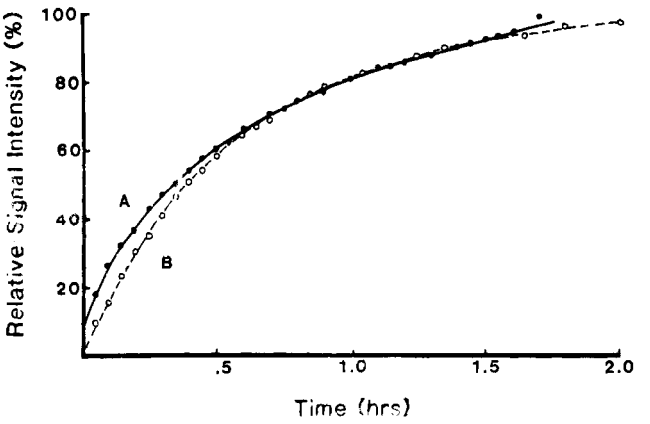

FIGURE 9: Effects of hydroxylamine on spin-labeled (A) native (●) and (B) reconstituted nucleosome core particles (O). A 0.14 M solution of hydroxylamine at pH 7.0 was added to the spin-labeled native or reconstituted nucleosome core particles. The cleavage of the spin-label from the histones was measured by the increase of the relative ESR intensity (H_0) vs. time.

The labeling profile (Figure 1B) for the nucleosome core particle as a function of urea concentration is found to be very similar to the denaturation profile for the spin-labeled core particle due to urea treatment [Figure 4b in Chan & Piette (1980)]. Only 55% of the tyrosines are labeled even though the tertiary and secondary structures of the histone core have been destroyed at above 6 M urea. Since the ionic strength and urea treatment act differently on the nucleosome structure, it is possible that some tyrosines are exposed differently to the imidazole spin-label under different denaturation conditions.

The mode of nucleosome reassociation in vitro has been studied by several investigators (Jorcano & Ruiz-Carrillo, 1979; Wilhelm et al., 1978; Stein, 1979). In most cases, the four core histones H2A, H2B, H3, and H4 and the DNA were mixed in 2 M NaCl and allowed to reassociate by a stepwise decrease in the salt concentration. At each step of the reassociation, the bound and unbound histones to the DNA were separated by centrifugation and analyzed by gel electrophoresis. It has been demonstrated that the H3 and H4 tetramer binds first to the DNA between 1.2 and 0.8 M NaCl and two H2A and H2B dimers bind progressively to that complex between 0.8 and 0.6 M NaCl. We have studied the process of reconstitution by examining the tumbling environment of the labeled tyrosyl residues in the histones at each step of the dialysis during the reassociation. Our data do not distinguish the order of preferential binding of individual histones to the

Chart I

H3:	position of conservative alterations	41, 53, 89, 90, 96, 127
	position of tyrosines	41, 54, 99
H4:	position of conservative alterations	60, 73, 77
	position of tyrosines	51, 72, 88, 98
H2A:	position of conservative alterations	10, 16, 51, 87, 99, 121, 123, 128
	position of tyrosines	39, 50, 57
H2B:	position of conservative alterations	9–10, 21–26, 32, 38, 39, 60, 75–77
	position of tyrosines	37, 40, 42, 83, 121

DNA when the ionic strength is decreased from 2 to 0.01 M NaCl. However, judging from the change in the rotational correlation times for the spin-label (Figure 2C), we concluded that the histone core binds progressively to the DNA in the range of 1.4–0.5 M NaCl. The tumbling environment of the labeled tyrosyl residues becomes more restricted when the ionic strength is decreased to 0.01 M NaCl, suggesting that a full or tighter compaction of the nucleosome complex takes place. The reconstituted complex yields a homogeneous monomer fraction in the isokinetic sucrose gradient and was found to have nearly identical physical properties with those of the native core particle. On the basis of our observation and those of others (Stein, 1979; Wilhelm et al., 1978; Jorcano & Ruiz-Carrillo, 1979), it would appear that although all histones bind to the DNA at an ionic strength around 0.6 M NaCl, the resulting complex is not as compact as it is when subjected to a lower ionic strength, that is, lower than 0.3 M NaCl.

We have evaluated the possibility that the tyrosyl residues are involved in the histone–DNA interactions within the nucleosome core particle. Several investigators (Mayer et al., 1979; Helene & Maurigot, 1981) have shown that tyrosines can interact with DNA by both stacking and hydrogen bonding in some protein–DNA complex formations. If the tyrosines also play a role in the DNA–histone interactions within the core particle, as the basic amino acid residues do, then the labeling of these tyrosines should either perturb the nucleosome reassociation and/or produce an anisotropic effect on the spin-label in the resulting complex. However, our data clearly show that complete reassociation can be attained even when most of the surface tyrosines on the histone core have been labeled. The ESR spectra (Figure 6) for the reassociated nucleosome complex do not show a strong anisotropic effect on the spin-label. It is interesting to notice that the DNA in the native nucleosome core particle (in 0.001–0.4 M NaCl) has greatly reduced the accessibility of the tyrosines by about 30%, when compared to the histone core alone (in 2 M NaCl); yet, the wrapping of the core DNA on the labeled core does not strongly immobilize the motion of the spin-labels attached to those tyrosyl residues. This observation possibly indicates that the labeled surface tyrosines on the histone core are not involved in the specific DNA–histone interactions and/or the wrapping of DNA on the histone core is not of a very close contact type. Since the spin-label has a diameter of rougly 5–6 Å, there is still enough space between some of the DNA–histone interfaces in the core particle to accommodate these extra bulky molecules.

The amino acid sequences for the core histones have been determined (Isenberg, 1979; Von Holt et al., 1979). Positions of amino acid variations (both inter- and intraspecies) have been detected. As pointed out by McGhee & Felsenfeld (1980a), those positions at which core histones can sustain alterations are those of the least structural consequences, either on the nucleosome surface or at points of less than intimate contact with other histones. Thus, when we compare the positions of tyrosines in the histones with the positions of the conservative substitutions (see Chart I), we find that six to eight tyrosines (underlined) are very close to those positions at which histones can sustain alterations (underlined). Thus, we are tempted to speculate that it is possible that it is these tyrosines that are being labeled in our reconstitution studies.

However, the situation is quite different in the case where the buried tyrosines have been modified. Here, we clearly see that when the buried tyrosines have been exposed by urea treatment and spin-labeled, the resulting histone–DNA complexes that formed after reassociation have physical properties different from those of the native nucleosome core particle. We have found that the effect on the improper reassociation is more enhanced when the labeling of the core histone tyrosines is carried out at 6 M urea than at 4 M urea. This disruptive effect is quite different in these two cases even though approximately the same number of tyrosines has been labeled in each case. This suggests that different buried tyrosines are exposed in 4 or 6 M urea. It has been suggested that 50% denaturation of the histone core (in 2 M NaCl) occurs at 6 M urea, and this has been interpreted as the disruption of the α helix of the histone core (Olins et al., 1977; Chan & Piette, 1980). At 4 M urea, the tertiary structure of the histone core (in 2 M NaCl) is only slightly perturbed while complete denaturation occurs at 8 M urea. Thus, our data suggest that the disruptive effect on proper nucleosome reassociation due to tryosyl modifications is dependent on the positions of the tyrosines being labeled; namely, the more buried tyrosines (which require a higher urea concentration to expose them), the more disruptive effect they have when they are modified.

Chemical modification of the histone H3 sulfhydryl residue in nucleosomes and in chromatin has been extensively investigated (Hyde & Walker, 1974; Zama et al., 1978; Dieterich et al., 1978). Recently, the effect of H3 sulfhydryl modifications on histone–histone interactions and nucleosome reconstitution has been studied (Lewis & Chiu, 1980; Wingender et al., 1981). These authors suggest that the histone core is destabilized or even structurally altered by even a minor modification at cysteine-110 while a subsequent modification of cysteine-96 in calf histone H3 has an even greater disruptive effect on nucleosome reconstitution. Our data agree with their observation that the choice of sites for the attachment of environment-sensitive probes (fluorescent probes or spin-labels) must be treated with caution.

The possibility that the disruptive effect on the reconstitution is due to the presence of the bulky nitroxide groups on the spin-labels in the histone has been examined and is eliminated by the evidence that non-spin-label *N*-acetylimidazole causes the same kind of disruptive effect on the nucleosome reassociation when the modification is carried out under the same conditions. The specific effect of tyrosyl modifications on the structure of the histone core and nucleosome folding is dependent on the positions of the tyrosines being labeled. As demonstrated by Isenberg and co-workers (Spiker & Isenberg, 1977; Isenberg, 1979), the binding surfaces between the histones are highly conserved. A small change (a single residue or more) in the region of contact between the histones may greatly change their binding energy. Thus, it is obvious that some of the internal tyrosines labeled here are vital to the proper histone–histone interactions that lead to the histone core structure.

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