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Accessibility of Tyrosyl Residues Altered by Formation of the Histone 2A/2B Complex†

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ABSTRACT: The availability of tyrosyl residues to surface iodination was analyzed for histone 2A (H2A), histone 2B (H2B), and the H2A/H2B complex. When H2A is free in solution (200 mM NaCl, pH 7.4) tyrosine-39 and one or both tyrosines-50 and -57 were readily iodinated. Tyrosines-83 and -121 of H2B were iodinated, both when the histone was free in solution and when it was associated with H2A, while tyrosines-37, -40, and -42 of H2B were not iodinated under either condition. When H2A and H2B were associated or covalently cross-linked, all tyrosyl residues of H2A were unavailable for iodination. We also found that the iodination of nondenatured H2A and H2B did not inhibit formation of the H2A/H2B complex. These results indicate that the amino-terminal regions of the hydrophobic portions of H2A and H2B undergo significant conformational changes upon formation of the H2A/H2B complex. These conformational shifts occur in the same region of the H2A/H2B complex that contains a contact site between H2A and H2B in the nucleosome, thus indicating an involvement of this region in chromatin assembly.

The fundamental subunit repeat of chromatin is the nucleosome [for reviews, see Felsenfeld (1978) and McGhee & Felsenfeld (1980)], composed of double-stranded, superhelical DNA wrapped around an octameric protein core. These units appear as beads on a string when viewed by electron microscopy (Olins & Olins, 1974; Oudet et al., 1975) and are capable of interacting with one another to produce highly condensed arrays (Finch & Klug, 1976; Renz et al., 1977).

The nucleosome is hypothesized to assemble by the formation of a tetrameric structure of histones 3 and 4, which interacts with the DNA (Roark et al., 1974; Daban & Cantor, 1982). The structure is completed by the addition of one H2A–H2B dimer above the plane of the DNA and one dimer below the plane (Camerini-Otero et al., 1976; Bina-Stein & Simpson, 1977; Worcel et al., 1978; Wilhelm et al., 1978; Ruiz-Carrillo et al., 1979). This H2A–H2B dimer is capable of exchanging with exogenous H2A and H2B without disrupting the nucleosome structure (Louters & Chalkley, 1984).

The hydrophobic regions of the core histones, which are involved in the formation of the H2A–H2B dimer and the

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H3–H4 tetramer (Eickbush & Moudrianakis, 1978), show an extremely high degree of primary sequence conservation between species [for a review, see Isenberg (1979)]. For H2A and H2B, this is particularly true for the tyrosyl residues, which are highly conserved throughout the whole range of eukaryotic species. The only known alterations occur in trout testis H2A, where tyrosine-57 is deleted (Bohm et al., 1980), and in a variant of sea urchin sperm H2B, where there is a substitution for tyrosine-83 (Bohm et al., 1982). In all other reported sequences the tyrosine residues are retained, and their positions relative to one another are precisely maintained. Especially intriguing are tyrosines-37, -40, and -42 of H2B, because the residues between the tyrosyl residues vary quite extensively (though in a conservative manner), but no substitutions occur at the three tyrosyl residues. This region has also been shown to be directly involved in the H2A/H2B binding site (DeLange et al., 1979; Callaway et al., 1985).

The existence of the H2A/H2B complex in chromatin has been demonstrated by cross-linking studies (Thomas & Kornberg, 1975; Martinson & McCarthy, 1976; Martinson et al., 1976, 1979; Jorcano & Ruiz-Carillo, 1979; Carter et al., 1980). This complex also exists in solution as a heterodimer, as shown by column chromatography (van der Westhuyzen & von Holt, 1971; Eickbush & Moudrianakis, 1978). Isenberg has shown that histones 2A and 2B from a wide range of species have a similarly strong affinity for one another (Isenberg, 1979), with binding constants of about 10^6 M. The involvement of tyrosyl residues in histone–histone interactions within the nucleosome core has been implicated through fluorescence anisotropy and proton magnetic resonance data (Chan & Piette, 1982; Butler & Olins, 1982; Michalski-Scrive et al., 1982) as well as by direct cross-linking (DeLange et al., 1979; Martinson et al., 1979).

In this study we monitor the availability of tyrosyl residues of H2A and H2B to surface iodination in isolated monomers and in the H2A/H2B complex. The lactoperoxidase method of iodination was used because the reaction seems to occur near the surface of the enzyme (Morrison & Bayse, 1970; Bayse et al., 1972), and therefore, the reaction is thought to be preferential to tyrosyl residues exposed to the medium. We demonstrate that several tyrosyl residues of H2A and H2B go through significant conformational changes upon formation of the H2A/H2B complex.

Experimental Procedures

Materials. TFA[1] and iodogen were obtained from Pierce, ^{125}I (in 1-mCi amounts) was from Amersham, and trypsin was from Worthington. Acetonitrile (HPLC grade), water (HPLC grade), and 30% hydrogen peroxide were purchased from Mallinckrodt; lactoperoxidase and Triton X-100 were from Sigma; Cromex X-ray film was from Kodak. The HPLC system was a Beckman Model 110A, with gradient capability, and the μBondapak column was obtained from Waters Associates.

Isolation of H2A, H2B, and the H2A/H2B Complex. Whole histones were isolated by acid extraction of chromatin followed by precipitation with cold ethanol (Callaway et al., 1985). The H2A/H2B complex was isolated by chromatography of whole histone (400 mg in 4 mL of column buffer) on a Sephadex G-100 column (3.8 × 150 cm) in 200 mM NaCl/20 mM Tris, pH 7.4. A flow rate of 60 mL/h was utilized, and 10-min fractions were collected. Samples were often extracted with 5% perchloric acid to remove H1 (Johns, 1964) prior to chromatography.

H2A was isolated from the H2A/H2B complex by dissolving the sample in 6 M guanidine hydrochloride to reduce interactions, followed by chromatography on a Bio-Gel P-60 column (3.8 × 150 cm) at a flow rate of 60 mL/h in 100 mM NaCl/10 mM HCl (Bohm et al., 1973). H2B was isolated from the H2A/H2B complex or from whole histone by the same chromatography procedure described for isolation of H2A. Under these conditions, H2A elutes in fractions 60–80 while H2B elutes in fractions 95–110.

Iodination of H2A, H2B, and the H2A/H2B Complex. Samples were dissolved in 200 mM NaCl/20 mM Tris, pH 7.4, at a concentration of 0.5 mg/mL (volume of 250–500 μL), and then 20 μL of lactoperoxidase (1 mg/mL) and 15 μCi of ^{125}I were added. To initiate the reaction, 4 μL of 30% H_2O_2 diluted 1/50 000 was added, followed by five more additions at 5-min intervals to give a final concentration of 1–2 μM. Under these conditions, iodination was dependent on the presence of both lactoperoxidase and H_2O_2 and resulted in the formation of only monoiodotyrosine. The reaction was stopped at 30 min by the addition of sodium azide to a final concentration of 20 mM. Each sample was then dialyzed against the appropriate buffer for at least 24 h with three changes.

Trypsinization of Iodinated Histones. Proteolysis was done in 0.5 mL of 0.1 M NH_4HCO_3/0.6 M guanidine hydrochloride/0.1% Triton with 5% w/w trypsin for either 5 or 20 h at 37 °C with stirring. The samples were lyophilized and dissolved in 6 M guanidine hydrochloride/1% Triton X-100 and then filtered through a cellulose filter with 0.2-μm pore size by centrifugation.

Tryptic Peptide Standards. H2A/H2B complex (10 mg) was digested with 1% (w/w) trypsin for 2 h at 37 °C in 1 mL of 0.1 M NH_4HCO_3 (pH 8.0). The lyophilized digest was dissolved in 0.5% TFA and passed through a 0.2-μm cellulose filter (by centrifugation). This sample was subjected to gradient HPLC on an analytical μBondapak C_{18} column (0.4 × 30 cm) utilizing a starting buffer containing 2 g of TFA/4 L of H_2O and a second solution of 1.5 g of TFA/4 L of acetonitrile and a flow rate of 0.75 mL/min while monitoring at 280 nm. A gradient of 1%/min of the second buffer was utilized; peaks were collected by hand, concentrated, and rechromatographed under identical conditions, except that a gradient of 0.5%/min was utilized and the eluant was monitored at 220 nm. Peaks were then analyzed by amino acid analysis (Callaway et al., 1985) to identify all the tyrosine containing peptides.

Iodination of Peptides. Several samples of iodogen (10 μg each) were suspended in 50 μL of chloroform and coated on separate 12 × 75 mm test tubes. Peptides were added to the dried test tubes in 15 μL of 30% acetic acid, followed by 15 μCi of ^{125}I, and the reaction was allowed to proceed for 5 min at room temperature, after which the sample was removed from the test tube to stop the reaction.

HPLC of Iodinated Peptides. The separation of each iodinated peptide from the unmodified form was performed on an analytical μBondapak C_{18} column utilizing the solutions previously described. A 1%/min gradient (gradient I in Table I) was initiated at a flow rate of 0.75 mL/min. For resolution of all five iodinated peptides, the flow rate was reduced to 0.5 mL/min, and the acetonitrile was increased at the following rates: 4.25%/min over the first 2 min, 0.226%/min over the next 42 min, 11.75%/min over the next 4 min, and 1.6%/min over the remaining 22 min. This gradient is referred to as gradient II in Table I and is utilized in the analysis of tryptic

[1] Abbreviations: TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate.

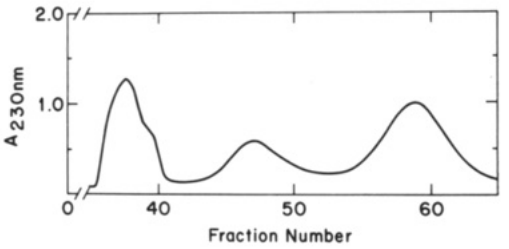

FIGURE 1: Elution profile of calf thymus histones (400 mg) on a Sephadex G-100 column (3.8 × 150 cm), equilibrated and eluted with 200 mM NaCl/20 mM Tris, pH 7.4, at a flow rate of 60 mL/h. The eluant was monitored at 230 nm, and fractions were collected at 10-min intervals.

digests of iodinated histones (Figure 3).

UV Cross-Linking of Iodinated Histones. The iodinated histones were mixed with the complementary uniodinated histone to give a 0.5 mg/mL concentration of the H2A/H2B complex. The samples (200 μL) were placed in 8 × 75 mm quartz tubes and strapped to the water jacket surrounding the medium-pressure mercury lamp (Callaway et al., 1985). The samples were irradiated (without nitrogen purging) for 30 or 180 s and analyzed by gel electrophoresis (Callaway et al., 1985).

Two-Dimensional Peptide Map. Peptides were separated by ascending thin-layer chromatography on cellulose (with a plastic support) for 3 h in butanol/acetic acid/water (200/30/75), followed by pH 1.9 electrophoresis in 8.7% formic acid/2.5% acetic acid, at 350 V for 35 min in the horizontal direction.

RESULTS

Isolation of the H2A/H2B Complex. Whole histone isolated from calf thymus was chromatographed on a Sephadex G-100 column in 200 mM NaCl/20 mM Tris, pH 7.4 (Figure 1). The first eluting peak contains H3 and H4 in an octameric or tetrameric complex. The second peak contains H1 with some A24 contamination. H1 was often removed prior to chromatography by extraction of whole histones with 5% perchloric acid (Johns, 1964). The final peak contains H2A and H2B, with minor contamination of H4. Analytical ultracentrifugation of this peak determined that the molecular weight of the eluting histone species was approximately 24 000 (data not shown), indicating that H2A and H2B are associated in a 1:1 complex (heterodimer) in this buffer. These salt and pH conditions were used in all the solution studies described in this paper, since heterodimer formation is clearly substantiated. If the solutions are stored in the refrigerator, aggregates begin to form over a period of days.

H2A and H2B Iodination. The trace labeling of H2A and H2B was performed to monitor the accessibility of tyrosyl residues in the monomer and dimer state. Figure 2 is an autoradiogram of an SDS–polyacrylamide gel following lactoperoxidase-catalyzed iodination of the histones and histone complexes. In panel I, the H2A–H2B cross-linked dimer (lane 1) incorporates ^{125}I, though to a lesser extent than H2A or H2B free in solution (lanes 2 and 3). The H2A/H2B complex, reconstituted from a lyophilized powder (lane 4) or as eluted from the Sephadex G-100 column (lane 5), is only iodinated at the tyrosyl residues of H2B. This indicates either that the tyrosines of H2A are no longer accessible following formation of the H2A/H2B complex or that the rate of H2B iodination greatly exceeds that of H2A under these conditions. We addressed this question in panel II by the addition of a 2-fold excess of H2A (lane 9) to the H2A/H2B complex (lane 10). The presence of excess H2A results in rapid iodination of both the free H2A and H2B in the H2A/H2B complex, demonstrating that the tyrosyl residues of H2A are unaccessible when it is associated with H2B.

Characterization of Iodinated Peptides. To determine the effect of complex formation on specific tyrosyl residues, we isolated all the tryptic fragments of H2A and H2B that contained tyrosines and characterized each by HPLC retention time and amino acid analysis. These purified peptides were iodinated and their retention times recorded (Table I). Heterogeneity was produced by incomplete cleavage at dibasic linkages for some of these peptides. Iodination caused a uniform increase in the retention times of the peptides on a C_{18} HPLC column in a reverse-phase system. Gradient II

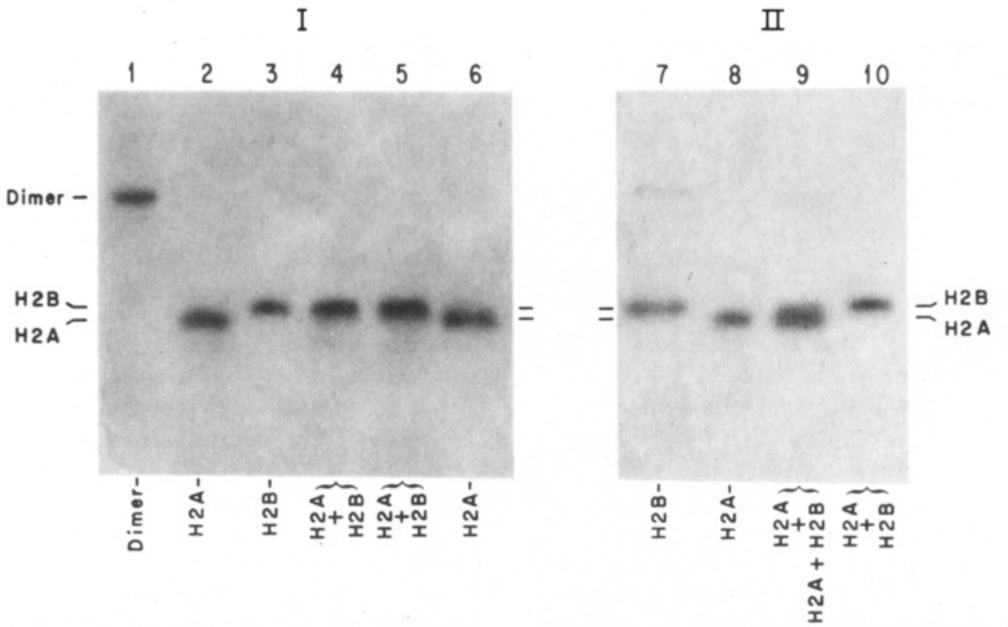

FIGURE 2: An autoradiogram of a SDS–polyacrylamide gel of histones and histone complexes iodinated in the NaCl/Tris buffer of Figure 1. Lane 1 is iodinated H2A–H2B cross-linked dimer, lanes 2, 6, and 8 are H2A iodinated free in solution, and lanes 3 and 7 are H2B iodinated free in solution. Lanes 4 and 10 represent the iodination of the H2A/H2B complex as dissolved in NaCl/Tris from a lyophilized powder. Lane 5 represents the iodination of the H2A/H2B complex as it elutes from the G-100 column (Figure 1). Lane 9 is the iodination of the H2A/H2B complex in the presence of a 2-fold excess (w/w) of H2A.

Table I: HPLC Analyses of Iodinated Peptides

tryptic peptide[a]		HPLC[b] retention time (min)		
		gradient I[c]		gradient II[d]
histone	residue no.	uniodinated	iodinated[e]	iodinated
2B	121–125	11	16	16
2B	(34) 35–43[f]	11.5	16.5	18
2A	36–42	14	20	23
2B	80–85 (86)[f]	16	22	25, 27
2A	43–71	74[g]	78	70–72

[a] A total of 10 mg of H2A and H2B digested with 1% trypsin (w/w) for 2 h at 37 °C. [b] High-performance liquid chromatography on a μBondapak C_{18} column (10 μm, 0.4 × 30 cm). [c] Eluted with a 1%/min gradient of acetonitrile with 1.5 g of TFA/gal at a flow rate of 0.75 mL/min. [d] Acetonitrile increases from 8.5% at 2 min at a rate of 0.35%/min (see Figure 3 for complete gradient profile). [e] Isolated peptides iodinated with iodogen (see Experimental Procedures). [f] Incomplete cleavage of dibasic linkages. [g] A peak also elutes at 70 min; amino acid analysis indicates that this peptide contains residues 43–71 of the H2A variant, which has Met substituted for Leu at residue 51 (H2A.2).

(Table I) was designed to resolve a mixture of all five predicted iodinated peptides. It also resulted in the separation of the heterogeneous peptide containing H2B tyrosine-83 (residues 80–85 and 80–86). This gradient was used in the analysis of all the tryptic digests presented in Figure 3.

Iodinated Peptides of H2A and H2B. HPLC profiles of trypsinized histones and histone complexes that were iodinated in a 200 mM NaCl/20 mM Tris, pH 7.4, buffer are presented in Figure 3. The tryptic digest of H2B, iodinated in the monomeric state (panel A), shows four peaks of radioactivity, the first of which represents unincorporated ^{125}I that remained following dialysis. The next two peaks have retention times corresponding to the iodinated tryptic fragments of H2B containing tyrosyl residues 121 (eluting at 16 min) and 83 (eluting at 25 min), with more ^{125}I being incorporated into tyrosine-83. The last peak is partially digested H2B. The extent of H2B digestion did not affect the relative distribution of radioactivity between the two iodinated peptides. The iodination pattern of the H2A/H2B complex (panel B) is identical with that of H2B (panel A). This demonstrates that tyrosyl residues 83 and 121 of H2B do not have altered accessibility to the solution upon association with H2A and that this complex prohibits iodination of H2A residues.

The HPLC profile of tryptic fragments generated from iodinated H2A–H2B cross-linked dimer (panel C) is very similar to the profiles of iodinated H2B (panel A) and the H2A/H2B complex (panel B). The first peak again corresponds to unincorporated ^{125}I, and the second peak contains the tryptic fragment 121–125 of H2B. A small peak at 23 min is observed in some H2B iodinations and is considered to be background, although it corresponds to the position of an H2A peptide (panel D). The peaks at 25 and 27 min correspond to the H2B tryptic fragments 80–86 and 80–85, with the peak splitting as predicted by analysis of the standard peptides (Table I) and also as observed for some tryptic fragments of iodinated H2B. This profile shows that UV cross-linking does not alter accessibility of tyrosyl residues to iodination.

The HPLC profile of iodinated H2A following tryptic digestion (panel D) is distinct from the previous profiles, although the unincorporated ^{125}I peak is still present. The peak eluting at 23 min is the tryptic fragment containing tyrosine-39. Only a small protion of the iodinated H2A remained undigested (eluting at 57 min), while the hydrophobic peptide containing residues 43–71 (eluting at 70–72 min) also had

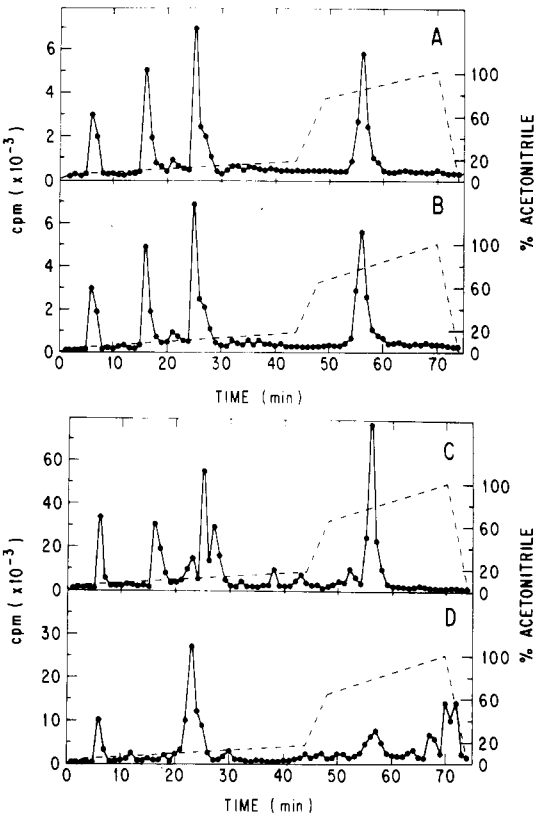

FIGURE 3: HPLC gradient elution profile of the tryptic digests of iodinated H2B (A), H2A/H2B complex (B), H2A–H2B cross-linked dimer (C), and H2A (D) on a μBondapak C_{18} column (0.4 × 30 cm) eluted with water/TFA (buffer A) and acetonitrile/TFA (buffer B) at a flow rate of 0.5 mL/min (--). The gradient is that referred to as gradient II in Table I. The eluant was collected in 1-min fractions and analyzed for the presence of ^{125}I by gamma counting.

incorporated ^{125}I. This large peptide contains tyrosines-50 and -57 and was not completely solubilized in the 6 M guanidine/1% Triton solution and was, therefore, trapped on the filters used to remove particulate matter prior to HPLC. To estimate the extent of iodination occurring at tyrosine-50 and/or -57 relative to tyrosine-39, a two-dimensional peptide map of tryptic fragments generated from iodinated H2A was chromatographed and autoradiographed (data not shown). The radioactive spots were identified by comparison to the iodinated peptides used as standards (Table I). A radioactive spot corresponding to the peptide containing residues 43–71 was more extensively labeled than the peptide containing residues 36–42. Therefore, this indicates that tyrosine-39 and probably both tyrosines-50 and -57 are iodinated when H2A is free in solution.

UV Cross-Linking of Iodinated H2A and H2B. UV cross-linking was performed to determine the influence of iodination of exposed tyrosyl residues upon the formation of the H2A/H2B complex (Figure 4). Only a fraction of the histone molecules were radiolabeled; therefore, the SDS–polyacrylamide gel stained with Coomassie Blue (panel I) would detect mainly the uniodinated molecules. An autoradiogram of that same gel monitors the fate of the radiolabeled molecules. In this manner we compared the extent of cross-linking for uniodinated H2A and H2B (panel I) to the iodinated forms (panel II). Trace-labeled H2A or H2B was exposed to UV light for 30 (lanes 1 and 2) and 180 s (lanes 6 and 7). These controls show a small amount of cross-linked dimer formation, for both the uniodinated (panel I) and the iodinated histones (panel II). For H2A, cross-linked dimer formation is probably due to a small amount of H2B con-

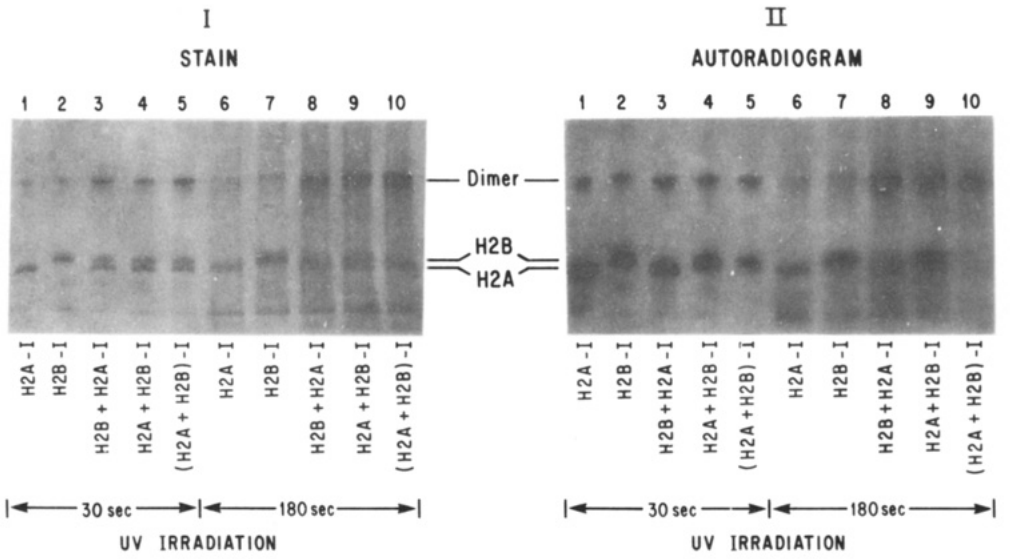

FIGURE 4: SDS–polyacrylamide gel (stained with Coomassie Blue, panel I, and autoradiographed, panel II) of iodinated histones and histone complexes, UV irradiated for 30 (lanes 1–5) or 180 s (Lanes 6–10). Iodinated H2A (I-H2A) was irradiated in the absence (lanes 1 and 6) or presence (lanes 3 and 8) of exogenous H2B. Iodinated H2B (I-H2B) was irradiated in the absence (lanes 2 and 7) or presence (lanes 4 and 9) of exogenous H2A. The H2A/H2B complex was iodinated as an associated pair (lanes 5 and 10) and subsequently irradiated.

tamination (detected on SDS gels), while for H2B the dimer has a slightly slower mobility upon SDS gel electrophoresis, which indicates that it is probably the H2B–H2B cross-linked homodimer. H2A can form the H2A–H2B cross-linked dimer when uniodinated (panel I, lanes 3 and 8) or when iodinated (panel II, lanes 3 and 8) at the previously identified residues. The iodination of H2B when free in solution (lanes 4 and 9) or associated with H2A (lanes 5 and 10) does not inhibit the formation of the H2A–H2B cross-linked dimer. This latter result was expected since H2B iodinates at the same positions before or after association with H2A.

Discussion

To investigate the involvement of tyrosyl residues in the H2A/H2B complex, trace labeling of tyrosines using the lactoperoxidase system was employed. Under the conditions used, only a small percentage of each tyrosine at the surface of the histones or histone complexes would be labeled. In this manner we could monitor the accessibility of tyrosyl residues when H2A and H2B exist free in solution or as an associated heterodimer complex.

When H2A is free in solution, tyrosine-39 and one of or both tyrosines-50 and -57 are available for iodination. These tyrosines become unavailable for modification upon formation of the H2A/H2B complex, but iodination of these residues does not prohibit the formation of the cross-linked H2A–H2B dimer by UV irradiation. When H2B is free in solution or associated with H2A, tyrosines-83 and -121 are both readily iodinated. As expected, the iodination of free H2B did not inhibit dimer formation, since the iodinated residues are exposed to the medium. Tyrosines-37, -40, and -42 are unavailable for iodination when H2B is bound to H2A, probably due to the direct involvement of some of these tyrosyl residues (at least one) in the contact site. They are also unavailable in free H2B, implying that residues 37–42 of H2B are buried within its own tertiary structure prior to association with H2A. These results indicate that significant conformational changes occur in both H2A and H2B upon association. H2A may bury tyrosyl residues within its own tertiary structure or in the H2A/H2B interface, while H2B opens a hydrophobic pocket to allow juxtaposition of the tyrosine at residue 40 and proline-26 of H2A.

The inability of the H2A tyrosyl residues 39, 50, and 57 to be iodinated in the H2A/H2B complex, while rapidly iodinating when H2A is free in solution, does not necessitate that these residues exist in the H2A/H2B interface. The increase in α-helicity that accompanies association of H2A and H2B (D'Anna & Isenberg, 1974) indicates that conformational changes may be occurring in regions topologically removed from the H2A/H2B interface. Such conformational changes could result in the inaccessibility of previously exposed tyrosyl residues or slight alteration in the microenvironment surrounding the tyrosines. These small perturbations could also alter the pK or hydrogen-bonding pattern of the phenolic group, rendering it less reactive to iodination (Morrison & Schombaum, 1976). However, the lactoperoxidase method of iodination has been used successfully to identify exposed tyrosyl residues in cytochrome c (Osheroff et al., 1977) and fibrinogen (York & Blomback, 1979).

The relative rates of iodination of the various accessible tyrosine residues were determined for each histone monomer and H2A/H2B dimer. The iodination reaction was terminated at either 4 or 9 min (10%–30% of the incorporation observed at 30 min), and tryptic peptides were generated and analyzed for ^{125}I content by the HPLC protocol described above. At these early time points (data not shown) the relative distribution of ^{125}I in the various tyrosines was indistinguishable from that found at 30 min (Figure 3). This indicates that during this time period of the reaction the observed differences in iodination patterns of the monomers compared to the dimers reflect true changes in accessibility and rates of iodination. At 30 min we calculate that 0.4% of the total available tyrosine in these proteins has been iodinated, and therefore, we are examining only initial rates of reaction, as we intended.

The results indicate that the interaction of H2A with H2B is responsible for the inaccessibility of H2A tyrosyl residues and could account for the observation that H2A is not iodinated in intact chromatin (Weintraub et al., 1975; Biroc & Reeder, 1976; Griffiths & Huang, 1979; Burch & Martinson, 1981). The tyrosyl residues of H2A are located in the hydrophobic region of the protein, and it is surprising to find this region available to iodination when the histone is free in solution. Gel filtration chromatography showed that in the NaCl/Tris buffer used in the iodination experiments H2A was

not aggregated but exists in a monomer or homodimer state (data not shown). Tyrosine-39 is predicted to be in a β-bend (Moss et al., 1976); this secondary structure might secure the side chain on the exterior of the protein and account for its accessibility in the unassociated state.

Tyrosine-121 of H2B is near the COOH terminus, and this region is predicted to possess little secondary structure; therefore, it is expected to readily iodinate in solution or when bound to H2A. It is unclear why tyrosine-83 iodinates to a greater extent than tyrosine-121 both in solution and in the H2A/H2B complex, since tyrosine-121 should be readily available for modification. This may best be explained by the prediction that in free H2B tyrosine-83 should exist in a β-bend (Moss et al., 1976) and could be held in a rigid conformation exposed to the medium. Since the distribution of ^{125}I is unperturbed by interaction of H2B with H2A, this predicted secondary structure appears to be unaltered in this region of H2B following complex formation. Also, UV cross-linking of the H2A/H2B complex did not alter the iodination pattern of the complex, supporting the assumption that the cross-link does not greatly alter the conformation of the complex (Callaway et al., 1985).

The observation that the iodination of H2A did not inhibit the formation of the H2A–H2B cross-linked dimer is in agreement with a paper by Kleinschmidt & Martinson (1984). They demonstrated that iodinated H2A can replace H2A in the nucleosome and that this nucleosome is slightly more stable to salt denaturation. The iodination of the tyrosyl residues causes an increase in hydrophobicity of the modified amino acid, as demonstrated by the increased retention times of the modified tryptic peptides following reverse-phase HPLC on the μBondapak C_{18} column (Table I). These results suggest that increasing the hydrophobic nature of the tyrosyl residues of H2A does not prohibit subsequent conformational changes that occur in the region of these residues upon formation of the H2A/H2B complex.

Registry No. L-Tyrosine, 60-18-4.

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