HMG Proteins 14 and 17 Become Cross-Linked to the Globular Domain of Histone H3 near the Nucleosome Core Particle Dyad†

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ABSTRACT: HMG proteins were derivatized with the photoactivatable cross-linker *N*-succinimidyl 3-((4-azidophenyl)dithio)propionate and then allowed to associate with nucleosome core particles. Following photolysis, peptide mapping of the principal dimeric adducts was carried out. Cross-linking occurred primarily from a central location in the HMGs to a central location in H3. The positions of these cross-links, considered along with other data from the literature, show that HMG proteins 14 and 17 make important contacts to H3 near the front face of the nucleosome. This raises the possibility that HMGs 14 and 17 participate in the reported conformational transition which exposes the H3 sulfhydryls of active nucleosomes.

The chromosomal proteins, HMG14 and HMG17, are well-characterized components of transcriptionally competent chromatin (Bustin et al., 1990a,b; Brotherton et al., 1990; Graziano & Ramakrishnan, 1990). HMGs 14 and 17 are small (ca. 90–100 amino acids), nucleosome-binding proteins with a preference for nucleosomes from "active" chromatin (Weisbrod et al., 1980; Brotherton et al., 1990). Although fewer in number than the histones, these HMGs are nevertheless abundant proteins in the nucleus, being present in quantities roughly sufficient to titrate the nucleosome of the "active" regions (Weisbrod et al., 1980). Therefore, although their function remains unknown, it is assumed that their role in defining the "active" state of chromatin is a structural one.

Nucleosome structure (Richmond et al., 1984) is centered on a multimeric globular core comprised of the hydrophobic C-terminal domains of histones H2A, H2B, H3, and H4. The basic N-terminal domains of the histones are arranged on the outside of this core together with about 160 base pairs of DNA which are wrapped in two turns around the entire complex. Digestion with micrococcal nuclease trims the DNA to about 1.8 turns and a length of 146 base pairs to yield a well-defined structure termed the core particle.

Protein–DNA cross-linking experiments, carried out on reconstituted HMG–nucleosome complexes, have established that HMGs 14 and 17 are bound within the terminal 30 bp of nucleosome core DNA (Shick et al., 1985). This places the HMGs close to H2A, H2B, and H3, which also become cross-linked to the DNA within this region (Shick et al., 1985). Reference to the three-dimensional arrangement of the histones along the DNA (Bavykin et al., 1985) reveals that H4, as well, is close to the HMG-binding site.

Protein–protein cross-linking experiments have confirmed that nucleosome-bound HMGs are in close proximity to all four of the core histones. By cross-linking reconstituted HMG17–nucleosome complexes with the lysine-directed reagent 2-iminothiolane, Cook et al. (1986) showed that all four core histones became cross-linked to the HMG protein in similar amounts, with H2A being somewhat favored. Use of the shorter, photoactivatable cross-linker *N*-succinimidyl 3-[(1-(2-nitro-4-azidophenyl)-2-aminoethyl)dithio]propionate (SNAP)[1] resulted in a much more pronounced preference for H2A (Espel et al., 1985).

In order to map the regions of close approach between the HMGs and the histones, we embarked on a series of experiments using a reagent closely related to SNAP but shorter still, SADP (Figure 1). Surprisingly, rather than an even greater specificity for H2A, this cross-linker yielded a preponderance of HMG–H3 cross-links. Peptide mapping showed that cross-linking is between the central regions of the two molecules and that the HMGs are, therefore, situated near the nucleosomal dyad.

Materials and Methods

Purification of HMG Proteins 14 and 17. Our procedure was based on the methods of Goodwin and Johns (1973) and Weisbrod and Weintraub (1981) and others. Typically, 400 mL of an adult chicken blood cell suspension (cells suspended in an equal volume of Alsever's solution; Mission Labs, Rosemead, CA) was centrifuged, and the cells were washed with PBS, resuspended in 400 mL of RSB containing 0.5% Triton X-100 and 1 mM phenylmethanesulfonyl fluoride, and homogenized in a Dounce homogenizer to release the nuclei. The nuclei were recovered by centrifugation, cleaned by repeating the above procedure, and resuspended in 100 mL of RSB/1 mM phenylmethanesulfonyl fluoride. The suspension was brought to 0.4 M NaCl, sonicated, made 2% in trichloroacetic acid, and then chilled at 4 °C for 1 h to precipitate the DNA and most of the nuclear proteins. Following centrifugation (16000*g*, 10 min) the supernatant was brought to 12% in trichloroacetic acid, chilled, and centrifuged again. HMG14 and HMG17 (with some contaminating linker histone) were precipitated from this final supernatant by addition of 6 volumes of acetone (acidified with H_2SO_4) and chilling at −20 °C overnight.

The HMG proteins were collected by centrifugation, dissolved in water, dialyzed against 10 mM sodium acetate/0.1

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[1] Abbreviations: PBS, phosphate-buffered saline (140 mM NaCl, 1.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4); RSB, reticulocyte standard buffer (10 mM Tris, 3 mM $MgCl_2$, 10 mM NaCl, pH 7.4); HMG, high mobility group; SADP, *N*-succinimidyl 3-((4-azidophenyl)dithio)propionate; SNAP, *N*-succinimidyl 3-[(1-(2-nitro-4-azidophenyl)-2-aminoethyl)dithio]propionate.

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M NaCl, pH 5.5, applied to a 1-mL sulfopropyl-Sephadex (Pharmacia) column, and separated by elution with a step gradient of increasing [NaCl]. HMG14 [specifically, HMG14a, the dominant HMG14 in chickens (Bustin et al., 1990b)] elutes at 0.25 M, and HMG17 elutes at 0.3 M NaCl. The HMG proteins were precipitated with acetone and dissolved in water.

Labeling of the HMG Proteins with ^{14}C. Labeling was carried out by reductive methylation (Jentoft & Dearborn, 1983). Typically, 50 μg of HMG protein was allowed to react for several hours at room temperature with 4 μCi of $H^{14}CHO$ (0.05 Ci/mmol) in 90 μL of 20 mM $NaCNBH_3$/10 mM $NiCl_2$/50 mM HEPES, pH 7.5. Unreacted HCHO was removed by dialysis. This procedure derivatized about 2 lysines per HMG (specific activity ca. 0.1 Ci/mmol).

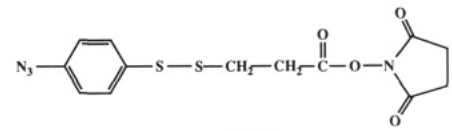
Preparation of Nucleosomes. Nuclei, prepared as above, were resuspended in RSB, pH 8, at about 4 mg of DNA/mL. $CaCl_2$ was added to 0.1 mM, and digestion with micrococcal nuclease (0.5 unit/mL; Sigma) was carried out to 15% acid solubility of the DNA and then stopped by addition of EDTA to 50 mM. The nuclei were pelleted and then resuspended in 10 mL of 10 mM Tris/10 mM EDTA, pH 8.0, and the nucleosomes were released by addition of NaCl to 0.6 M followed by homogenization. The extract was cleared by centrifugation at 16000*g* for 10 min, and the supernatant was fractionated on a Sephadex G-100 column in 1 mM Tris/10 mM EDTA/0.6 mM NaCl/0.02% sodium azide, pH 8.0. Fractions containing mostly mononucleosome, but also some dinucleosome, were pooled, concentrated using an Amicon apparatus, dialyzed against 10 mM Tris/1 mM EDTA, and stored at −20 °C.

Cross-Linking Using SADP. SADP (Figure 1), purchased from Pierce (Rockford, IL), was dissolved in dimethyl sulfoxide just prior to use and then diluted into water. HMG proteins (50 μg) were derivatized in the dark with SADP (usually 0.3 mM) in 80 μL of 50 mM HEPES, pH 7.5, for 20 min at room temperature. All unreacted SADP was quenched using 40 mM glycine, and then 350 μg (as DNA) of nucleosomes was added while the HEPES concentration was kept at 50 mM. A period of at least 2 h (still in the dark) was allowed for the association of the derivatized HMGs with the nucleosomes. Photolysis was then carried out in ice for 30 min using visible light from a 100-W Sylvania light bulb at a distance of 15 cm from the HMG–nucleosome mixture.

CNBr Digestion in Gel Slices. We followed Pepinsky (1983) with minor modifications. After a brief staining and destaining, the desired bands were excised from the first-dimension gel, soaked in water for 10 min, and then incubated in CNBr for 1 h at room temperature. To protect the SADP cross-link, no reducing agent was present. The gel slices were then soaked twice for 5 min in water and then for 15 min in unpolymerized second-dimension stacking gel mix. For experiments involving cross-link reversal, the gel slices were cut in half following the CNBr step and the two halves were incubated in parallel, one of them in gel mix supplemented with 10% 2-mercaptoethanol. The half-slices were then soaked, still in parallel, for two 10-min intervals in mercaptoethanol-free gel mix, being rinsed in running water between soakings. The slices were then laid on top of a second-dimension gel together with standards cut from the same first-dimension gel which were put through the same rinse protocol.

Two-Dimensional Polyacrylamide Gel Electrophoresis. The first dimension was an acid/urea gel (Martinson et al., 1976) supplemented with cetyltrimethylammonium bromide to the extent of 0.15% in the upper reservoir and 1% in the sample buffer (Bonner et al., 1980). Following electrophoresis, the gel was briefly stained (0.1% Coomassie blue R-250 in 45% methanol/9% acetic acid) and destained (25% methanol/7% acetic acid), and then the appropriate lane was excised, soaked at least 5 min in SDS stacking gel mix, embedded in the top of a 5-cm stacking gel, and run, for the second dimension, either on 18% acrylamide according to Bonner and Pollard (1975) or on 16% acrylamide according to Schägger and von Jagow (1987). The Bonner and Pollard gels were stained as above. The Schagger and von Jagow gels, designed to resolve small peptides, were stained by the sensitive colloidal Coomassie G-250 procedure described by Neuhoff et al. (1988) with the stain adjusted to pH 0.8 using phosphoric acid.

Amino Acid Analysis and Sequencing. This work was performed by the UCLA Protein Microsequencing Facility. Peptides were separated on a Waters μBondapak C_{18} narrow-bore column and eluted with an acetonitrile gradient containing 0.1% trifluoroacetic acid. Amino acid compositions were determined by derivatizing peptide hydrolysates (6 N HCl, 18 h, 110 °C) with phenyl isothiocyanate and separating them on a C_{18} column (acetonitrile gradient in sodium acetate). Peptides were sequenced by Edman degradation using a gas-phase sequencer.



SADP

FIGURE 1: Structure of *N*-succinimidyl 3-((4-azidophenyl)dithio)-propionate, a 14-Å cross-linker. SADP couples to lysines via the imido ester moiety. The azidoaryl group can then be photoactivated at a later time to insert indiscriminately into adjacent molecules.

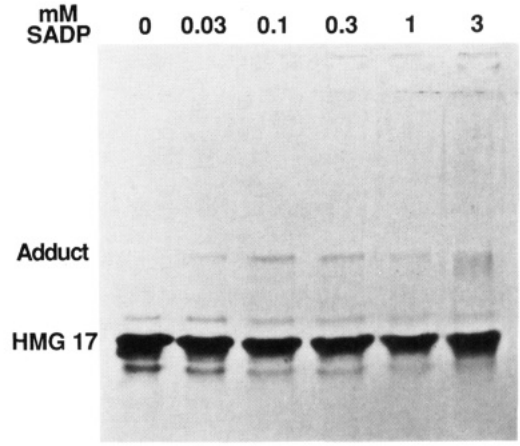


FIGURE 2: Fluorogram of products from cross-linking [^{14}C]HMGs to nucleosomes. The cross-linked material was precipitated with ethanol and resolved on an SDS gel (Bonner & Pollard, 1975), which was prepared for fluorography by soaking in Autofluor (National Diagnostics) for 15–30 min.

RESULTS

SADP-Derivatized HMG14 and HMG17 Cross-Link to Histone H3 of Nucleosomes. In order to select a suitable amount of cross-linker for routine use, various concentrations of the photoactivatable cross-linker SADP (Figure 1) were reacted with aliquots of ^{14}C-labeled HMG17. The derivatized HMGs were allowed to associate with a slight molar excess of nucleosomes (i.e., HMG:nucleosome < 1:1), and then the mixture was photolyzed to generate HMG–histone adducts. The principal adduct obtained (Figure 2) had a mobility

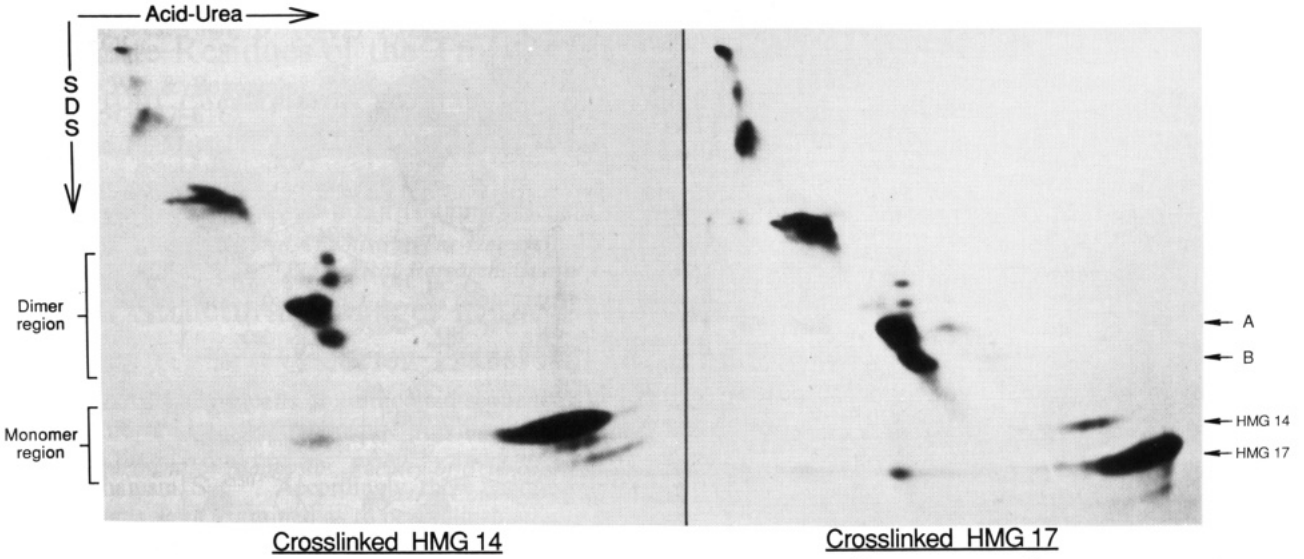


FIGURE 3: Fluorograms of the separation in two dimensions of the products of cross-linking [^{14}C]HMG14 and [^{14}C]HMG17 to nucleosomes.

slightly greater than that of an H3 dimer (calibrated in separate experiments). The optimum yield of dimer molecular weight material was obtained using HMGs derivatized with 0.1–0.3 mM SADP (Figure 2). We chose 0.3 mM SADP as the standard concentration for our subsequent work.

To identify the histone(s) to which the HMG proteins become cross-linked, HMG–histone adducts were resolved by two-dimensional polyacrylamide gel electrophoresis and recovered for further analysis. The cross-linking was carried out as above (using 0.3 mM SADP) except that, following the photolysis step, the nucleosomes were purified on a sucrose gradient (in 10 mM Tris, 50 mM NaCl, pH 7.5) prior to the polyacrylamide gel separation. The resulting fluorogram (Figure 3) shows that both the [^{14}C]HMG14 and the [^{14}C]-HMG17 yield essentially the same distribution of dimeric cross-linked products: adduct A, the major one; adduct B, of lesser yield; and several minor components. For each of the two-dimensional gels, the region containing adduct A was excised, and then adduct A was eluted, labeled with ^{125}I (to label the histone as well as the HMG component), reduced with 2-mercaptoethanol (to reverse the cross-link), and analyzed in one dimension on an SDS gel. The resulting fluorogram (Figure 4A) shows that in each case H3 is the major histone component of the adduct. Indeed, Figure 4B shows that, at least for HMG17, not only adduct A, but also adduct B, contains principally H3 as the histone component.

To confirm the presence of H3 in the adduct by an independent criterion, complexes of unlabeled HMG14 and nucleosomes were cross-linked, denatured under nonreducing conditions, and then labeled with [^{3}H]*N*-ethylmaleimide (NEM), a sulfhydryl-specific reagent for labeling cysteine. H3 is the only protein present which contains cysteine (Sterner & Allfrey, 1982; Bustin et al., 1990b). The adduct isolated from this mixture proved not only to be labeled but also, upon reduction, to give a pattern of radioactive CNBr peptides diagnostic of H3 (Figure 5). Moreover, by demonstrating that the sole sulfhydryl of H3 is still available to NEM in the cross-linked adduct, the data confirm that the cross-link is mediated by the internal disulfide of SADP rather than by a disulfide generated through exchange between SADP and cysteine 110 of H3.

The HMGs Cross-Link to a Central Domain in H3. The data in Figure 5 also suggest where the position of cross-linking in H3 may be. The first two lanes in Figure 5 show that the labeled H3 peptides appear only following reduction of the

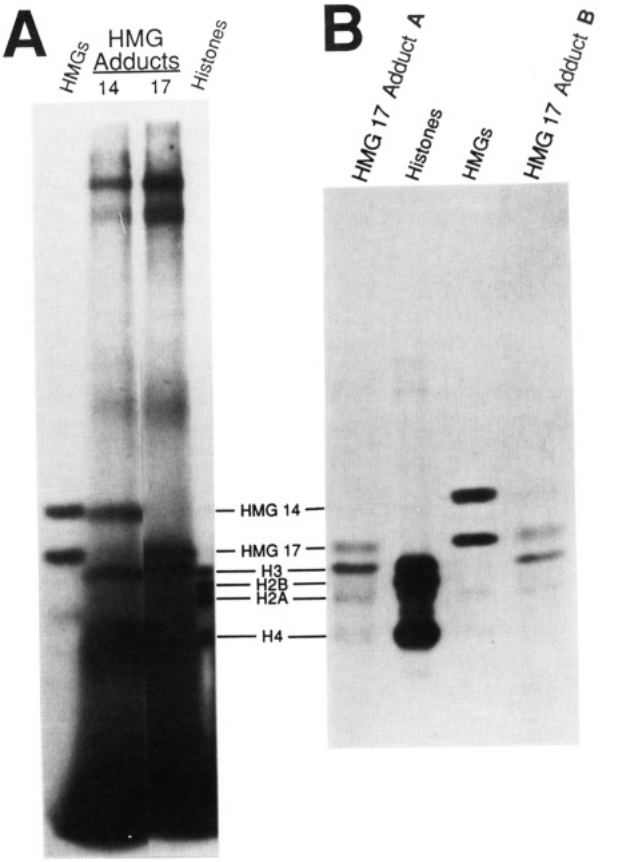


FIGURE 4: Autoradiograms of cleaved HMG–histone adducts resolved by SDS gel electrophoresis. Regions of the gels, corresponding to dimeric spots in Figure 3, were excised, and then the protein was eluted by diffusion in an SDS solution, concentrated to 40 μL in 50 mM HEPES, pH 7.5, labeled with 25 μCi of ^{125}I-labeled Bolton-Hunter reagent at room temperature for 30 min, reduced to cleave the adduct, electrophoresed (Bonner & Pollard, 1975), and autoradiographed. We do not know the source of the large amount of rapidly migrating iodine label which runs between the position of H4 and the bottom of the gel in part A.

CNBr-digested HMG–H3 adduct. Note that the material in both the reduced and the unreduced lanes of Figure 5 comes from the same gel slice which was cut in half only following the CNBr step. The failure of any labeled material to appear in the unreduced lane in Figure 5 suggests that the 30 amino acid, cysteine-containing peptide of H3 is the one that contains

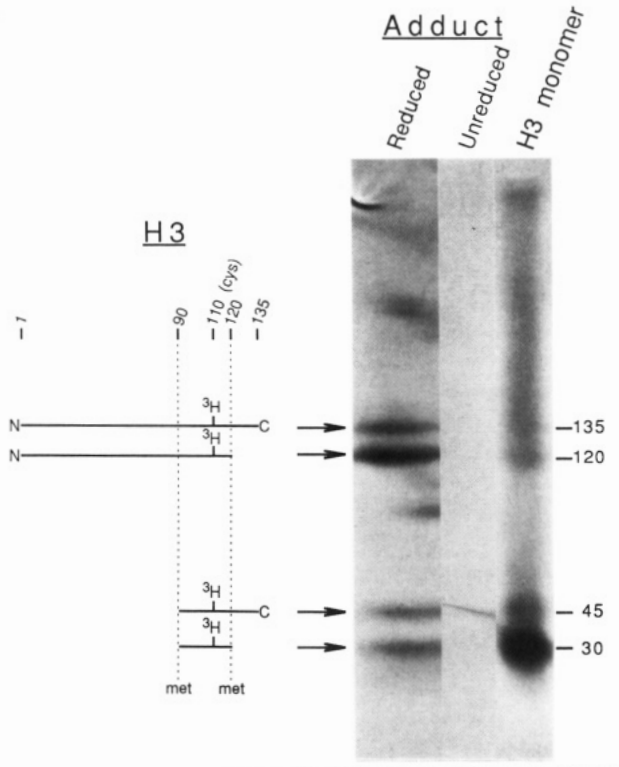


FIGURE 5: CNBr peptide map of the NEM-labeled histone component of the HMG14–histone adduct. Photolyzed nucleosomes were precipitated with ethanol, dissolved in 70 μL of 6 M guanidine hydrochloride/0.25 M Tris, pH 8.3, and then mixed and incubated with 25 μCi of [^{3}H]NEM (50 mCi/mol) for 30 min at 37 °C. Following dialysis to remove unreacted NEM, the sample was run on a first-dimension gel. The band containing the HMG–histone adduct was excised, digested with CNBr to cleave at methionines, and then cut in half. The two halves were run on a second-dimension Schagger and von Jagow (1987) gel with or without reduction as described under Materials and Methods and then fluorographed. The diagram of H3 and its peptides is drawn to scale on the basis of the sequence (Brandt & von Holt, 1974). It is apparent that the H3 monomer standard in this figure was cleaved by the CNBr more efficiently than was the H3 of the adduct. In our hands, cleavage of H3 by CNBr rarely goes to completion. The shadow bands seen below the 135 and 120 amino acid positions for the reduced adduct probably correspond to partially proteolyzed H3 (Böhm et al., 1981).

the cross-link to the HMG. The failure of this peptide, and of the partials, to appear in the gel lane without prior reduction presumably reflects their attachment to HMG14, which lacks methionine (Bustin et al., 1990b) and so is not cut by CNBr.

If it is through CNBr peptide 91–120 that H3 is cross-linked to the HMGs, then CNBr cleavage of the HMG–H3 adduct should liberate the 90 amino acid N-terminal fragment of H3 from the adduct regardless of whether the sample has undergone reduction or not. Since this large N-terminal fragment does not become labeled by NEM, an experiment like that of Figure 5 was carried out using instead the sensitive Coomassie blue staining procedure of Neuhoff et al. (1988). Figure 6 shows that, indeed, the 90 amino acid N-terminal fragment is liberated from both the reduced and the unreduced samples of CNBr-cleaved adduct for both HMG14 and HMG17. Since the 120 amino acid H3 fragment is seen only for the reduced sample, this confirms that cross-linking is to the region between 91 and 120.

In some experiments, unreduced samples did not yield even the 90 amino acid N-terminal fragment of H3 (not shown). This may reflect variation in the degree of derivatization of the HMGs from one experiment to the next. While Figures 5 and 6 demonstrate that the predominant site of H3 cross-linking lies between residues 91 and 120, some cross-linking can apparently occur upstream of residue 90.

H3 Cross-Links to a Central Domain in the HMGs. To map the position to which H3 is cross-linked in the HMGs, pepsin digestion of H3–[^{14}C]HMG adducts was carried out in gel slices, and the products of digestion were resolved by electrophoresis with or without reduction as described previously for CNBr. The results of such an experiment for the H3–HMG14 adduct are shown in Figure 7. HMG14 peptides which bear the cross-link to H3 would be expected to appear on the gel with their native mobility only after cleavage of the cross-link by reduction. Two peptides in Figure 7 fall into this category, peptides X and Z. Both are prominent in the reduced lane but do not appear in the unreduced lane of the gel. Therefore, H3 is cross-linked to peptides X and Z of HMG14.

Unfortunately, it is not possible to deduce the identity of these peptides simply by examination of the HMG14 sequence, because pepsin, unlike CNBr, has a broad cleavage specificity. Therefore, it was necessary to determine the identities of the fragments by direct characterization as described under Materials and Methods. For this purpose, a quantity of soluble HMG14 was digested by pepsin in solution, and the products of digestion were separated by high-performance liquid chromatography. The electrophoretic mobilities of peptides in individual chromatographic peaks were determined, and peptides X and Z, together with other peptides of interest, were characterized with respect to amino acid composition and sequence.

Determination of the sequence of the N-terminal 20 amino acids of peptides X and Z (as well as that of peptide Y, see legend to Figure 7) showed that these peptides have the same N-terminal sequence. Thus, peptide Z, being smaller, is wholly contained within peptide X, and we can conclude that the site of cross-linking lies within peptide Z. To determine the approximate position of the C-terminal cleavage site for peptide Z, we estimated its length on the basis of the mobilities of peptides Q and X and intact HMG14 (see legend to Figure 7). The results showed that the peptide extended from residue 23 to approximately residue 66 of HMG14. Thus, it is within this central domain that the cross-linking to H3 occurs.

When the peptides from the pepsin-digested H3–HMG14 adduct were electrophoresed in their unreduced state, the Y,Z doublet of the reduced material was replaced by a Y′,Z′ doublet which was shifted up slightly on the gel (see Figure 7). By coincidence, peptide Z′ of the unreduced material had the same mobility as that of peptide Y of the reduced material, but we know that it cannot actually be peptide Y because peptide Z is wholly contained within peptide Y and in the unreduced state peptide Y should remain cross-linked. Therefore, we surmise that the unreduced Y′ and Z′ correspond to HMG peptides Y and Z to which a small peptic fragment from H3 is linked, as diagrammed in Figure 7.

Like the Y,Z doublet, band X of the reduced lane also is replaced by lower mobility material in the unreduced lane. In this case, there are several bands and the mobility shifts are larger suggesting that, like fragment X itself, the cross-linked H3 fragments are less extensively degraded than are their Y′ and Z′ counterparts.

We have also analyzed the H3–HMG17 adduct (data not shown) in a manner similar to that described above for the H3–HMG14 adduct. We have obtained definitive data only for the N-terminal region of HMG17: a small peptide that behaved like peptide Q of HMG14 was found to correspond to the N-terminal 25 residues of HMG17. Thus, cross-linking in HMG17 is to a position downstream of residue 25, much as in HMG14. Since HMG17 is only 89 residues long, the

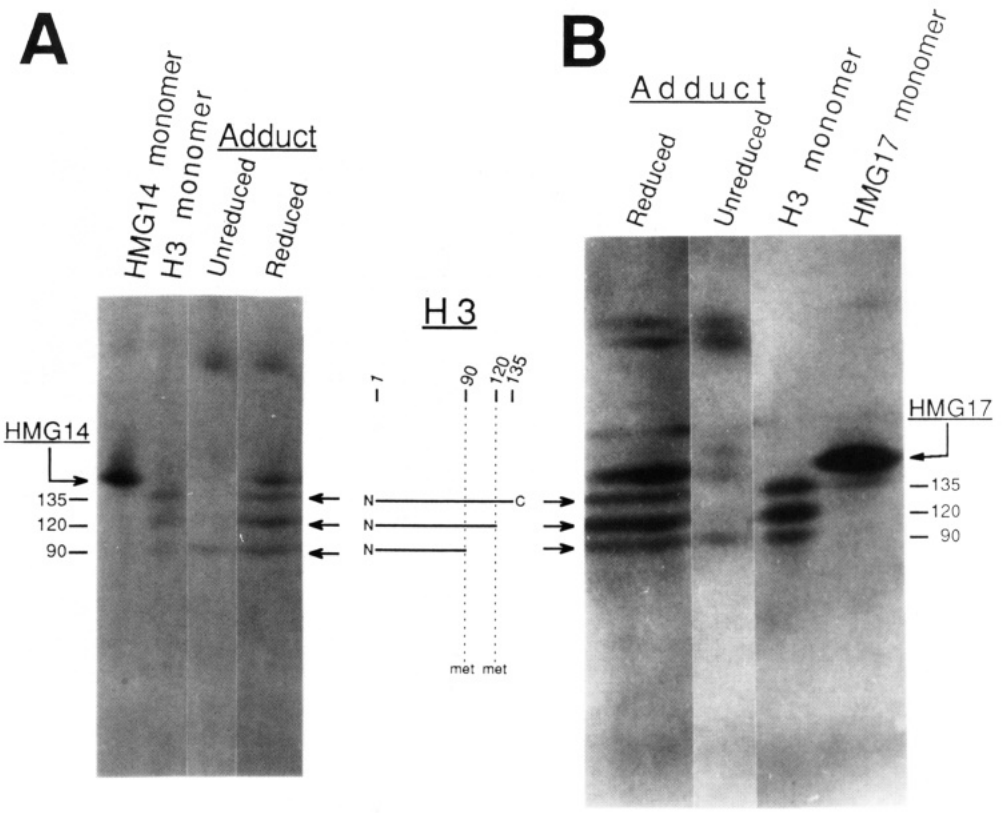


FIGURE 6: CNBr peptide maps of the H3–HMG14 (panel A) and H3–HMG17 (panel B) adducts visualized by Coomassie staining. The methods were essentially those of Figure 5 except that colloidal Coomassie staining (Neuhoff et al., 1988) rather than NEM labeling was used. Staining detects the large H3 N-terminal fragment not labeled by NEM but does not detect the smaller C-terminal fragments. Panel A is a Schagger and von Jagow (1987) gel, and panel B is a Bonner and Pollard (1975) gel. HMGs migrate faster relative to the histones in a Schagger and von Jagow gel than in a Bonner and Pollard gel, thus accounting for the apparent similarity in mobilities of the two different HMGs in the two panels. The two gel systems also differ in that the unreduced products of partial CNBr digestion of the adduct are more readily discernible on the Bonner and Pollard (1975) gel than on the Schagger and von Jagow (1987) gel.

cross-link is localized to the peptide bounded by residues 26 and 89.

Discussion

We have shown that when SADP-derivatized HMGs are reassociated with nucleosomes and cross-linked, the HMGs become attached primarily to H3. This result differs markedly, both from the finding of Espel et al. (1985) that cross-linking with SNAP yields primarily HMG–H2A adducts and from the finding of Cook et al. (1986) that 2-iminothiolane yields substantial cross-linking to all of the core histones. The latter result, however, is readily reconciled with our present report. The cross-linker, 2-iminothiolane, targets exclusively lysines, which it connects through a relatively long, 16-Å bridge. Presumably, the lysines of the relatively mobile, lysine-rich tails of all of the core histones come frequently to within this distance of a bound HMG protein.

More surprising is the difference between our results and those obtained using SNAP (Espel et al., 1985). SNAP is 1 Å shorter than 2-iminothiolane, and cross-linking is mediated by photoactivation of its nitroazidophenyl group. The shorter reach of SNAP, combined with the hydrophobicity of its photoactivatable group, presumably provides the constraints which account for the greater selectivity of SNAP (Espel et al., 1985) over 2-iminothiolane (Cook et al., 1986) in HMG–nucleosome cross-linking experiments. The reagent which we have used, SADP (Figure 1), closely resembles SNAP, differing primarily in being another angstrom shorter and in lacking the nitro group on the photoactivatable azidophenyl moiety. However, despite the similarity to SNAP, SADP exhibits a markedly different cross-linking bias, being selective predominantly for H3 rather than for H2A (Figure 4).

We have confirmed the results of Espel et al. (1985) that HMG–histone cross-linking mediated by SNAP is specific predominantly for H2A (data not shown). Thus, the different results obtained with SNAP and SADP reflect differences in the chemistries of the reagents and not differences in the cross-linking methodology or in the source of the HMG proteins.

How might such minor differences in the structures of two cross-linking reagents give rise to such significant differences in protein target specificity? Clearly, the crystal structure of the nucleosome at the resolution presently available (Richmond et al., 1984) does not allow for detailed speculation. However, in general terms, the explanation is, of course, straightforward: the nucleosome-bound HMG protein is in very close proximity to both H2A and H3. The slight differences in length and geometry of the two cross-linkers apparently direct their hydrophobic, photoactivatable ends to associate with different "sticky" spots on the surface of the histone core. Apparently, one of these spots happens to be part of H2A, and the other, part of H3. Since the chemistry of attachment of both SNAP and SADP to the HMG protein lysines is identical, the implication of this conclusion is that the site of SNAP–H2A association is close to the site of SADP–H3 association on the histone core, perhaps flanking the binding surface between H2A and H3 [see Isenberg (1979)].

The proposal that a bound HMG protein may make intimate contact with the histone core at or near the region of mutual interaction between H2A and H3 is supported by data from several sources. Our present results show that HMG cross-linking is to a region in H3 close to cysteine 110 (Figure

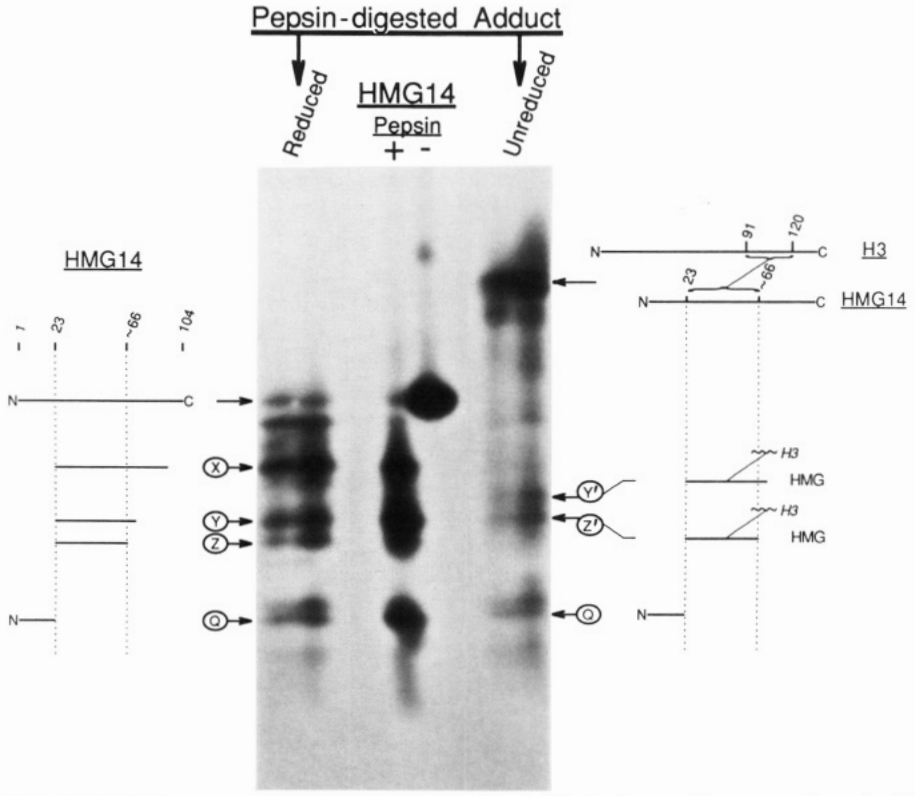


FIGURE 7: Peptide map of the HMG14 component of the H3–HMG14 adduct. Methods were the same as those for Figure 5 except that the label was provided by [^{14}C]HMG14 rather than by NEM and the digestion was with pepsin (2 mg/mL in 5% acetic acid) rather than with CNBr. The explanatory diagrams summarize the information obtained from peptide mapping of HMG14 digested with pepsin in solution (see text). High-performance liquid chromatography yielded peptide X as a separate peak, but gave peptides Y and Z as roughly equimolar constituents of a single peak. Sequencing of the Y,Z mixture gave only one major N-terminal sequence with an N-terminus corresponding to Ser 23 of HMG14. Peptide X gave the same result. Corroborating these sequence data is the amino acid composition of peptide Q, which is consistent with that for a peptide spanning residues 1–22 of HMG14. Thus, peptides X, Y, and Z constitute a family of partials differing only in their C-terminal regions. The presence of substantial amounts of an additional peptide (too small to be seen on the gel), with a sequence corresponding to the last 10 amino acids (95–104) of HMG14, suggests that the C-terminus of peptide X is Glu 94 of the HMG. The approximate locations of the Y and Z C-termini were determined by estimating the sizes of peptides Y and Z on the basis of the plotted mobilities of peptides Q and X and intact HMG14.

5). The crystal structure of Richmond et al. (1984) places cysteine 110 of H3 at the periphery of the histone core, directly beneath H2A on the front face of the nucleosome (by "front" we refer to the face surrounding position zero, where the dyad passes through the central DNA turn). For nucleosome core particles, both histone–DNA cross-linking results (Bavykin et al., 1985) and the crystal structures (Bentley et al., 1984; Richmond et al., 1984) agree that H3 and H2A simultaneously contact similar positions on the DNA on the front face of the nucleosome. Finally, the cross-linking results of Shick et al. (1985) show that core particle-bound HMG proteins share occupancy of the DNA termini with H2A and H3, and Bavykin et al. (1990) have pointed out that it is most likely the globular portions of these histones that become cross-linked to the DNA. Thus, it seems likely that the bound HMG proteins contact both H2A and H3 and that this contact is made within these two histones' globular regions, probably near the periphery of their own binding interfaces for each other (Isenberg, 1979).

Peptide mapping of the HMG14 component of the H3–HMG14 adduct localizes the SADP cross-link to a central peptide in HMG14 as shown in Figure 7. Examination of the sequence (Bustin et al., 1990b) then shows that the actual cross-linking site in HMG14 must lie between Lys 25 and Lys 66, since the initial HMG derivatization step involves the attachment of SADP to lysines. Unfortunately, we do not know whether this result points to this region as the segment of closest approach to H3 or whether it merely reflects the lysine richness of this segment, which has four times the lysine density of the rest of the protein. Either way, of course, the result does show that this segment of HMG14 approaches H3 closely. Moreover, since this H3-proximal region of HMG14 overlaps its presumptive DNA-binding region (Bustin et al., 1990b; Cook et all, 1989), our data are consistent with the results of Shick et al. (1985), who showed that one particular segment of nucleosomal DNA is contacted by both H3 and the HMGs.

Our H3–HMG cross-linking data modify previous models of HMG–nucleosome interaction (Cook et al., 1986, 1989; Bustin et al., 1990a) by emphasizing the placement of the HMGs at the front of the particle (Figure 8). Although significant cross-linking to H2A occurs when cross-linkers other than SADP are used, it appears likely that the contact with H3, which occurs near the particle dyad (see above), is the principal histone contact. This is suggested, first, by the fact that the shortest of the cross-linkers is the one specific for H3 (see above) and, second, by the results of Sandeen et al. (1980) and González and Palacián (1990), whose binding studies, taken together, imply a greater role for H3 than for H2A. Sandeen et al. (1980) showed that HMGs 14 and 17 have greater affinity for nucleosome core particles than for naked DNA, while González and Palacián (1990) showed that this high affinity for core particles was not decreased by removing H2A and H2B. Also drawing attention to the frontal location of the HMGs is the observation that the DNA segments contacted by both H3 and the HMGs are, in fact, the two ends of the core particle DNA (Shick et al., 1985). Furthermore, extension of these ends by only a few base pairs

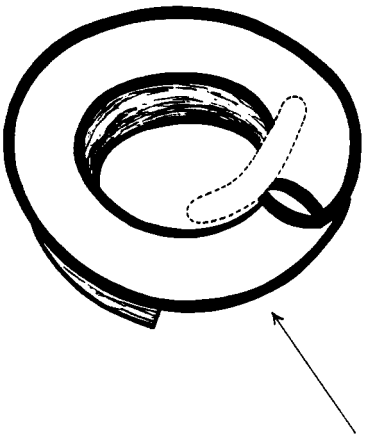


FIGURE 8: Location of HMG14/17 on the nucleosome core particle. The region occupied by an HMG extends from the inner surface of the DNA near the end of its path around the core particle (Shick et al., 1985), across H2A (Espel et al., 1985), and toward the front of the particle, to a position on H3 near the dyad axis (arrow, this work). For clarity, we show, in the view depicted, only the position of the HMG on the top of the particle and not that of the symmetrically bound HMG on the bottom or the positions of the histones inside. Of course, the boundary shown is simply the minimum required to account for the existing cross-linking data. The actual path of the HMG presumably extends beyond the area depicted.

increases dramatically the HMG–nucleosome binding affinity (Swerdlow & Varshavsky, 1983). Finally, the presence of two frontally bound HMG molecules would explain easily the formation of HMG–HMG dimers (Espel et al., 1985; Cook et al., 1986) and the existence of cooperativity between two HMGs even when binding is to core particles which have been extensively cross-linked to prevent nucleosomal conformational changes (Paton et al., 1983). Taken together these data suggest a significant HMG presence on the front face of the nucleosome.

Does the location of HMGs 14 and 17, in contact with H3 at the front of the nucleosome, present any new functional implications? Allfrey and colleagues [e.g., Chen et al. (1990)] have shown that nucleosomes in actively transcribed regions of chromatin are conformationally altered along their dyad in such a way that the H3 sulfhydryls are exposed. HMGs 14 and 17, by virtue of their interaction near this region of H3, may facilitate this conformational transition in susceptible nucleosomes.

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