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INTERACTION BETWEEN HISTONE H1 AND NON-HISTONE HMG14 DETECTED BY CHEMICAL CROSS-LINKING

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The interaction between histone H1 and non-histones HMG14 and HMG17 has been studied by chemical cross-linking. Cross-linking kinetics show the appearance of discrete bands which correspond to the interaction between H1 and HMG14. Interaction between H1 and HMG17 has not been detected.

Eucaryotic chromatin contains a group of non-histone proteins, the HMG group, first isolated by Johns and col.(1). There are four main proteins, named HMG1, HMG2, HMG14 and HMG17.Although their biological role in chromatin is yet unknown, two of them, HMG14 and HMG17, have been reported to be related with transcriptionally active chromatin (2-5). As described by Mardian et al. (6), HMG14 and HMG17 could be located near histone H1 in chromatin. These results have prompted us to study, by means of chemical cross-linking, the possible interaction between histone H1 and non-histones HMG14 and HMG17 in free solution. In a previous paper (7) we have made use of this technique to study the interaction between non-histone HMG1 and histone oligomers. In the present work we show cross-linking kinetics whose bands account for the existence of the interaction between histone H1 and non-histone HMG14. Our results are in accordance with the present view of H1 and HMG's in chromatin structure and function.

ABBREVIATIONS - HMG: High Mobility Group; DMS: Dimethyl Suberimidate; DTP: Dimethyl-3,3'-Dithiobispropionimidate; EDAC: 1-Ethyl-3 (3-Dimethylaminopropyl)Carbodiimide.

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METHODS

Histone H1 was obtained according to the literature(8). Non-histone proteins HMG14 and HMG17 were prepared as described by Goodwin et al.(9).Cross-linking reagents and protein standards were purchased from Sigma. Proteins to be cross-linked were dissolved in water and their concentration adjusted spectrophotometrically (ε_{230} = 3.91x10^{4}M^{-1}cm^{-1} for HMG14, ε_{230} = 2.54x10^{4}M^{-1}cm^{-1}for HMG17 and ε_{230}= 4.28x10^{4}M^{-1}cm^{-1}for H1; coefficients determined in water by a linear regression fitting after Lowry analysis). Prior to cross-linking the proteins were buffered, adjusted to the desired pH and centrifugated at 3000xg for 10 min. The concentration in total protein to be cross-linked was 1 mg/ml, the molar ratios for H1/HMG14, H1/HMG17 and H1/(HMG14+17) always being 0.5. Proteins were gently mixed and kept at rest for 10 min before addition of the cross-linker. Cross-linking reactions were carried out as described by Davies and Stark (10), at room temperature (23°C). The cross-linker/total protein ratios (r) were either 1.0 or 2.0 (w/w), as indicated in Figures. For the kinetics, aliquots were taken at different times and the reaction was quenched by addition of 0.1 volume of 2.0 M NH_4HCO_3 (11); then the sample was mixed with application buffer and loaded into polyacrylamide gels. Gels were 15% in acrylamide, prepared according to Laemmli (12), run 16 h at 20 mA, and stained by the method of Thomas and Kornberg (13). For two-dimensional electrophoresis, samples prepared as above were cross-linked using DTP at r = 1.0, in 50 mM triethanolamine buffer, pH 8.5. The mixture, quenched after 60 min reaction, was freeze-dried and redissolved in sample application buffer without 2-mercaptoethanol to preserve DTP disulfide bonds. Two-dimensional electrophoresis was performed essentially according to the diagonal method of Thomas and Kornberg (13), but using the tris buffer system to obtain a better resolution. The central strip of a sample lane from an unstained first-dimensional gel (10% acrylamide, 2 mm thick, run at 60 mA) was treated with reducing buffer containing 1.4 M 2-mercaptoethanol for 60 min, at room temperature. The strip was then assembled for a second-dimension run on a slab gel (15% acrylamide, 2 mm thick, run at 35 mA) provided with 1% agarose stacking layer. For the densitograms, after Coomasie Blue R250 staining and destaining, the cleavaged products in the second-dimension gel were scanned at 540 nm, in a Beckman DU 8B spectrophotometer.

RESULTS AND DISCUSSION

Figure 1 shows several cross-linking kinetics for the H1/(HMG14+17), H1/HMG14 and H1/HMG17 systems under different conditions.When HMG14 is present in the system tested (Figures 1A, B and C) a band appears at an approx. M_r of 5.4x10^4, as indicated by BSA and OVA markers, which is especially intense at the longest reaction times. This cross-linking band was also found to be present when the experiments were done in 10 mM borate, pH 8.5 (results not shown here), and also detected with DMS, DTP and EDAC cross-linkers. However,it seems not to be a strong interaction, as indicated by the faintness of the band. An effect of the ionic strength on the interaction between histone H1 and non-histone HMG14 was found. Figure 2 shows that this interaction appreciably decreases with increasing ionic strength, and especially above 120 mM in NaCl (plus 25 mM trietha-

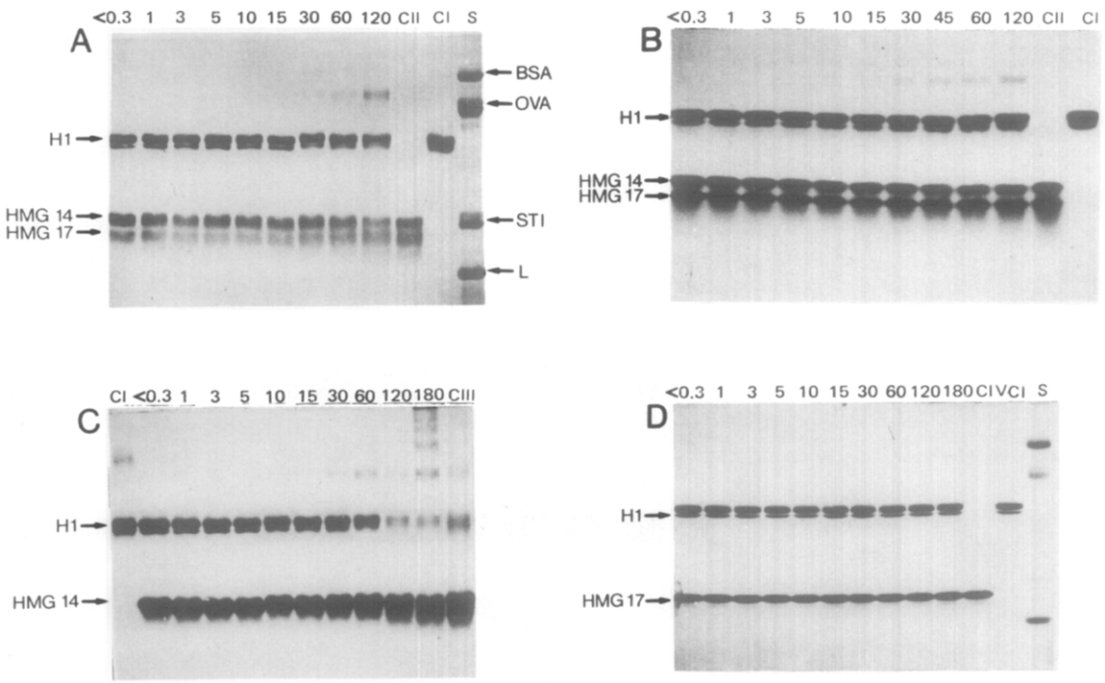

Figure 1. Cross-linking kinetics between H1 and HMG14+17, H1 and HMG14, and H1 and HMG17.
(A) H1/(HMG14+17) cross-linked with DTP at r = 1.0, in 50 mM triethanolamine buffer, pH 8.5.
(B) the same as in A but cross-linked with DMS at r=1.0.
(C) H1/HMG14 cross-linked with DTP ar r = 1.0 in 50 mM triethanolamine buffer, pH 8.5.
(D) H1/HMG17 cross-linked with EDAC at r = 1.0, in 50 mM triethanolamine buffer, pH 8.5. Numbers at the top of the Figure indicate time in minutes.
Tracks CI, CII, CIII and CIV correspond to controls of H1, HMG14+17, HMG14 and HMG17, respectively, taken at the longest times.
Track S corresponds to electrophoretic markers for apparent M_r, i.e. BSA (bovine serum albumin), OVA (ovalbumin), STI (soybean trypsin inhibitor) and L (lysozyme).

nolamine). This effect has been interpreted as indicating the existence of ionic interactions between histone and non-histone proteins. It is known that at 350 mM NaCl most non-histone proteins have dissociated from native chromatin, while as can be seen in Figure 2 the band corresponding to the H1 dimer (H1 dimer reported by Ring and Cole (14)) is present along the whole range of ionic strengths tested (histone H1 dissociates above 0.6 M NaCl). More-

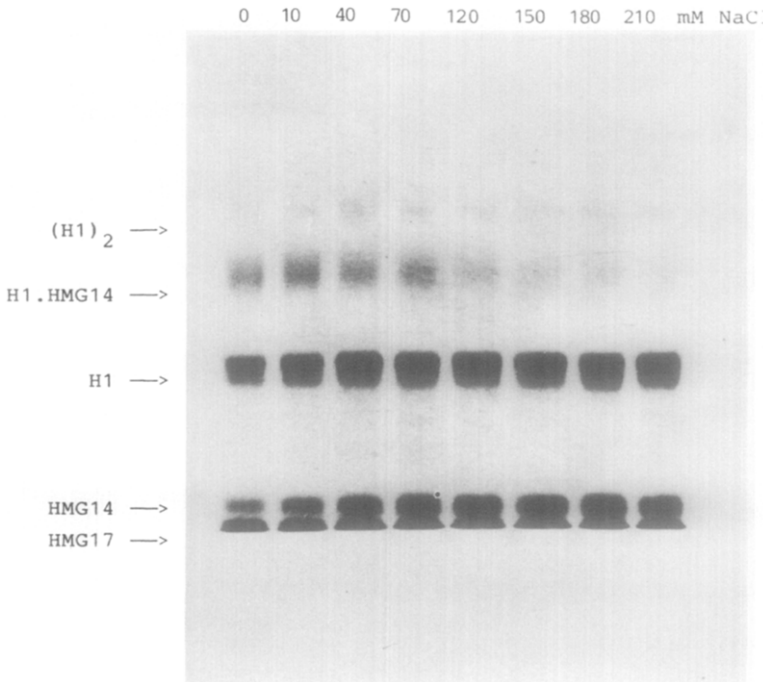

Figure 2. H1/HMG14+17 cross-linking reactions performed at different ionic strength conditions in 25 mM triethanolamine buffer, pH 8.5, plus different concentrations of NaCl, cross-linked with DTP at r = 1.0 for 120 min. NaCl molarities (mM) are indicated at the top of the Figure.

over, the band corresponding to the H1/HMG14 interaction was found to present a sharp dependence on pH, being barely appreciable at values below 8.0 and above 9.0 (results not shown). As a control, at the longest reaction times (Figure 1C) the presence of a band at an approx. M_r of 6.3×10^4 (track CI) can be seen, which corresponds to the H1 dimer. In the case of HMG17, the results are different with respect to those presented for HMG14. In the H1/HMG17 mixtures no cross-linking band was detected with EDAC, DMS and DTP. Figure 1D shows the EDAC kinetics.

Two-dimensional analysis of an H1/(HMG14+17) cross-linked sample (see Figure 3A) also indicates the presence of a dimer located close to H1 bands. This band is too faint to be seen in the HMG14/HMG17 controls of the mono-dimensional analysis, but can be seen in the two-dimensional analysis due to the overloading of sample. This band can be attributed to HMG14 or 17-homo-or heterodimers. Two-dimensional electrophoresis of H1/HMG14 complexes (see Figure 3B)

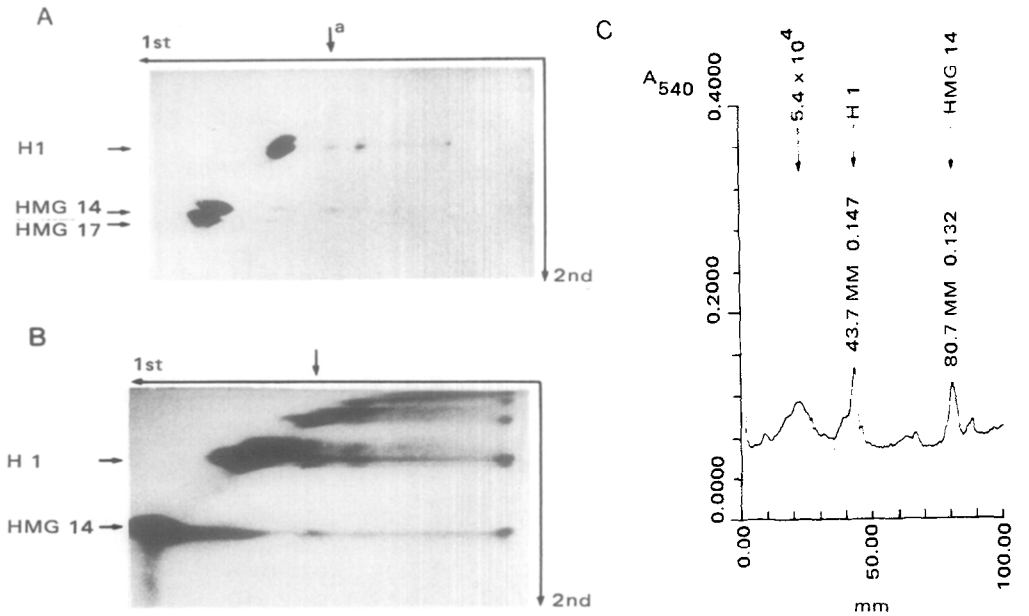

Figure 3. Two-dimensional electrophoresis of DTP cross-linked samples at r = 1.0, for 60 min in 50 mM triethanolamine buffer, pH 8.5. Arrows indicate cleavage products of H1. HMG14 complexes.
(A) H1/HMG14+17 (B) H1/HMG14 (C) densitogram of column labeled "a" from gel A.

suggests the presence of an HMG14 dimer in a position close to H1, which corresponds fairly well with the band observed in HMG14 control tracks from Figure 1C. Two-dimensional electrophoresis shows that the $5.4x10^4$ band is only composed of H1 and HMG14, its stoichiometry being difficult to establish because HMG14 stains purple while H1 stains blue (15). Nevertheless, the lack of any intermediate cleavage products and the reasonable agreement of its M_r from the $5.4x10^4$ band, with the expected one for the sum of the M_r of H1 and HMG14, suggest a 1:1 stoichiometry for the H1/HMG14 complex. From these results it is also remarkable that, in the cleavage products, the H1a/H1b subfraction areas ratio is far larger, 6.0, than that observed for the H1 sample used as a control (2.0). These results prompt the suggestion that, to some extent, HMG14 would be able to discriminate between H1 subfractions. This could be related to the results reported by Liao and Cole (16) in the sense that H1 subfractions are differently involved in the condensation of chromatin. Work is in progress to show and characterize the above described interaction in native chromatin.

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