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DNA and histone H1 interact with different domains of HMG 1 and 2 proteins

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High mobility group (HMG) proteins 1 and 2 from calf thymus have been digested under structuring conditions (0.35 M NaCl, pH 7.1) with two proteases of different specificities, trypsin and V8. The two proteases give a different but restricted pattern of peptides in a time course digestion study. However, when the interactions of the peptides with DNA are studied by blotting, a closely related peptide from HMG-1 and -2 does not show any apparent binding. This peptide, from the V8 protease digestion, has been isolated by DNA-cellulose chromatography and has the amino acid composition predicted for a fragment containing the two C-terminal domains of the protein, i.e., approximately residues 74–243 for HMG-1. The same peptide shows the only interaction detectable with labelled histone H1. A separate function for the different domains of HMG proteins 1 and 2 is proposed.

Key words: chromatin/DNA interactions/HMG proteins/protein blotting

Introduction

High mobility group (HMG) proteins are the best characterized of the nonhistone proteins of chromatin (for a review, see Johns, 1982). They form a reduced set of proteins that can be grouped into two classes. HMG-1 and HMG-2 are proteins of ~26 000–29 000 daltons, they are highly homologous in sequence among themselves (Walker *et al.*, 1980) and they show spectroscopic evidence of secondary and tertiary structure (Cary *et al.*, 1976). HMG-14 and HMG-17 have mol. wts. of ~10 000–12 000 daltons; they also have homologous sequences and, in solution, they show no evidence of structure, behaving as random-coil proteins (Abercrombie *et al.*, 1978; Cary *et al.*, 1980).

HMG proteins are relatively abundant in the nucleus, 10^6 copies per cell nuclei (Goodwin *et al.*, 1978a); this fact, together with their low variability between different systems, their primary structure characteristics and their ability to interact with DNA (Shooter *et al.*, 1974) have been taken to indicate their structural role. A relationship has been reported between the presence of HMG-14 and -17 and the sensitivity of transcribable genes to DNase-I (Weisbrod *et al.*, 1980). For HMG-1 and -2 some proposals have been formulated, such as the substitution of histone H1 (Jackson *et al.*, 1979), related to their interaction *in vitro* with linker DNA (Schröter and Bode, 1982); however, the evidence is still weak. HMG-1 and -2 appear to have an unusual amino acid composition with a high proportion in both acidic (30%) and basic (25%) residues. Sequence studies (Walker *et al.*, 1980) show a striking asymmetry in the distribution of amino acids. The presence of structural domains in these proteins has been proposed (Cary *et al.*, 1976; Palau *et al.*, 1980). More recently three (Reeck *et al.*, 1982) or four (Cary *et al.*, 1983) domains have been defined in the molecule, two of them, in the N terminus, show a high degree of homology, contain the majority of hydrophobic and basic residues and adopt both secondary and tertiary structure. The C-terminal domain is in the acid-rich tail of the molecule that has a continuous stretch of >35–40 residues (Walker *et al.*, 1979).

We have examined the domain structure for HMG-1 and -2 from calf thymus following a strategy similar to that used to show the domain structure of histone H1 (Hartman *et al.*, 1977; Avilés *et al.*, 1978; Puigdomènech *et al.*, 1980). The proteins were digested with proteases and the products of the digestion analyzed. Two proteases of very different specificity, trypsin and protease V8, were used. The peptides produced were tested for their ability to interact with DNA and with histone H1 by protein blotting and some of them were identified by amino acid analysis. The results obtained suggest a different function for the domains of HMG-1 and -2 as regards their interaction with DNA and the very lysine-rich histones.

Results

Time course of proteolytic digestion of HMG proteins

HMG proteins 1 and 2 were digested separately by two proteases. Trypsin produces a preferential cleavage in basic residues while V8 protease cleaves specifically the peptide bonds of either aspartate or glutamate in phosphate buffer, pH 7.1 (Houmard and Drapeau, 1972). Digestions were carried out under conditions shown to favour the secondary and tertiary structure of HMG proteins (Cary *et al.*, 1976).

Figure 1 shows the electrophoretic patterns corresponding to the products of HMG-1 (A) and -2 (B) after digestion with trypsin at 0.35 M NaCl, pH 7.1 for different times. The general distribution of peptides for the two proteins is very

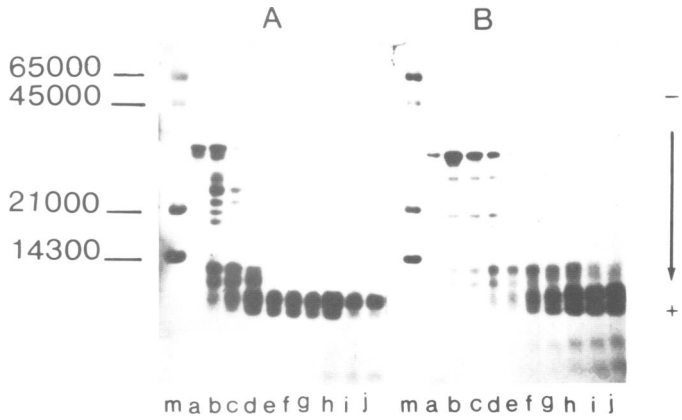

Fig. 1. SDS-polyacrylamide gel electrophoresis of the products of trypsin digestion of proteins HMG-1 **(A)** and HMG-2 **(B)** in 0.35 M NaCl, pH 7.1 for different periods of time. Protein concentration was 3 mg/ml and enzyme/protein ratio 100 μ/ml. Sample **m** corresponds to protein markers, **a**, to undigested protein and **b–j**, 2, 5, 10, 20, 30, 45, 60, 90 and 120 min of digestion.

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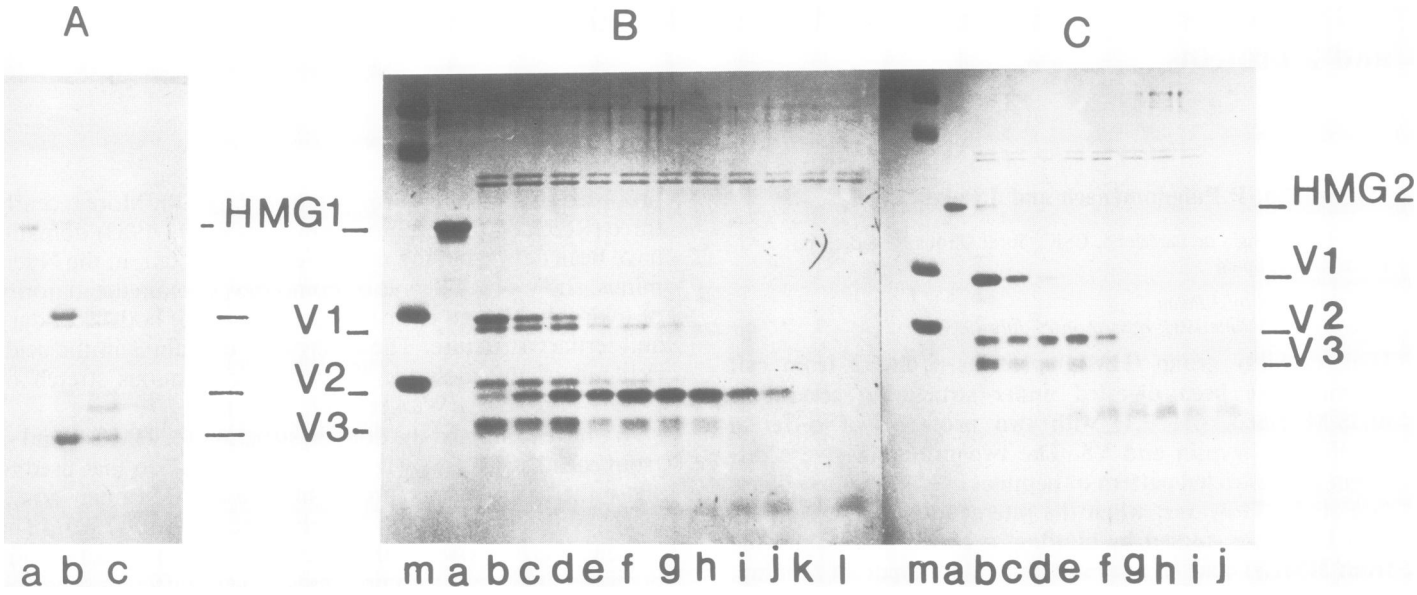

Fig. 2. SDS-polyacrylamide gel electrophoresis of HMG-1 and HMG-2 V8 protease digestion products at different periods of time. **Panel A** corresponds to the digestion products of HMG-1 (3 mg/ml) with 6.2 units of protease per ml for 0.5 **(b)** and 5 min **(c)**; **a**, is a sample of intact protein. **Panels B** and **C** show the electrophoretic patterns corresponding to the products of HMG-1 **(B)** and HMG-2 **(C)** obtained by digestion of the protein (3 mg/ml) by V8 protease at 12.4 μ/ml. Sample **m** corresponds to protein markers, **a** to indigested protein and **b**–**l** to 1, 2, 3, 5, 10, 20, 30, 45, 60, 90 and 120 min of digestion.

similar, as expected for proteins with a very high sequence homology and, most probably, a high degree of structural homology as well. With longer digestion times a trypsin-resistant product is observed, appearing as a double band in SDS-gel electrophoresis, a fact already observed under other ionic conditions (Palau *et al.*, 1980; Cary *et al.*, 1983). These limit peptides, as judged from their electrophoretic mobility, probably correspond to those identified by Cary *et al.* (1983) as two structured domains of HMG-1.

A different digestion pattern is observed when HMG-1 and -2 are digested with V8 protease, and again the two proteins show a very similar behaviour. Short digestion produces a limited number of predominant peptides that are progressively degraded. Figure 2 shows the electrophoretic patterns of HMG-1 (A and B) and -2 (C) proteins digested with V8 protease. At short times of digestion of HMG-1 (gel A) only two bands appear, indicating a single preferential point of attack for the protease that would produce peptides labelled as V1 and V3. When the digestion is carried out at higher concentrations of protease, an intermediate peptide (V2) appears, probably coming from the degradation of V1. For HMG-2 (gel C) three main bands appear in the gel in close agreement with HMG-1 peptides: a peptide moving with mol. wt. of 18 000 daltons (V1) and two faster peptides of 13 000 (V2) and 11 500 (V3), respectively. These peptides produced at early times of digestion are degraded and a band in the 5000-dalton region is the only one to be observed later. The overall pattern of digestion for HMG-1 (gel B) is similar to that of HMG-2 but one difference is observed. The 18 000 and the 13 000 dalton bands are split into two components (19 500 and 18 500 in one case and 14 500 and 13 000 in the other). In both cases, the final product is in the 5000-dalton region. As the results presented below show, these cleavages are probably accounted for by very close cleavage points in the sequence. This is also suggested by the pattern of peptides at initial times of digestion (see gel A) where a single band is observed. An alternative hypothesis would be the presence of a microheterogeneity in the sequence of the fragments.

DNA binding of HMG-1 and -2 proteolytic products

Two different lengths of time for the proteolytic digestion of HMG-1 and -2 proteins with trypsin and V8 protease were chosen and the peptides obtained were separated by electrophoresis and transferred to nitrocellulose filters (Bowen *et al.*, 1980). The results are shown in Figure 3 for trypsin digestion and in Figure 4 for V8 protease digestion. In both figures, panels A and B correspond to HMG-1 and panels C and D to HMG-2. Panels A and C correspond to filters stained with amido black as transfer controls of the digestion products. In all cases no qualitative difference in the transfer to the nitrocellulose filters could be observed for the different peptides or for the different gels. These filters show the digestion product of HMG-1 and -2 proteins at 2 and 60 min (samples b and c). Sample 'm' in these figures corresponds to protein markers and sample 'a' to undigested HMG-1 and HMG-2 proteins, respectively.

Nitrocellulose filters identical to those shown in panels A and C of Figures 3 and 4, were incubated with ^{32}P-labelled DNA. These results correspond to filters incubated with DNA at 50 mM NaCl; however, identical results were obtained at 25 and 150 mM NaCl while the interaction was abolished at 350 mM NaCl (not shown). The autoradiograms obtained are presented in panels B and D of Figures 3 and 4. In the autoradiogram from trypsin digestions of HMG-1 and -2 (panels B and D of Figure 3), two peptides of ~17 000 daltons do not appear, indicating that these peptides do not bind the labelled DNA. The rest of the peptides exhibit DNA binding. In the case of digestion products with V8 protease (Figure 4, B and D), the autoradiograms show a positive DNA binding with all peptides except with two major peptides in the 18 000 dalton region (peptide V1). A densitometric tracing [Figure 4 (S)] of the autoradiogram (B, lane b) compared with that of the stained filter (A, lane b) shows the differences in DNA binding by the different peptides in a more quantitative way. In all these cases, a number of peptides that are present in very low amounts, as judged by stain-

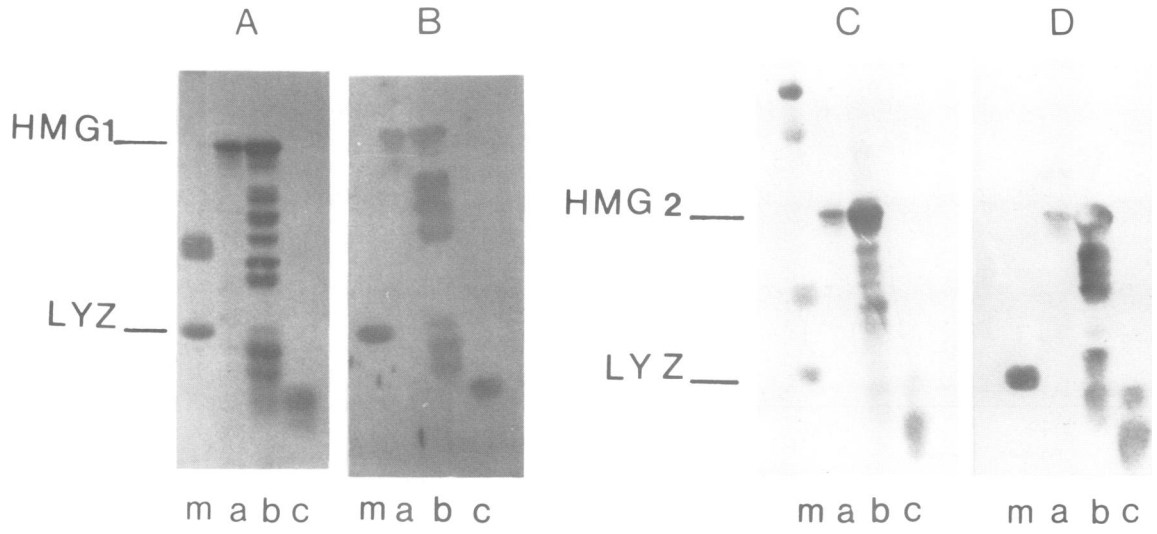

Fig. 3. DNA binding to HMG-1 and -2 trypsin digestion products. **Panels A** and **C** correspond to nitrocellulose filters where the digestion products of HMG-1 **(A)** and HMG-2 **(C)** with trypsin at different periods of time, 2 min **(b)** and 60 min **(c)**, have been transferred and stained with amido black. **Panels B** and **D** correspond to autoradiographs, obtained by incubation of duplicate nitrocellulose filters with labelled DNA at 50 mM NaCl. Tracks **m** and **a** correspond to protein markers and undigested protein, respectively. Conditions of digestion are identical to Figure 1.

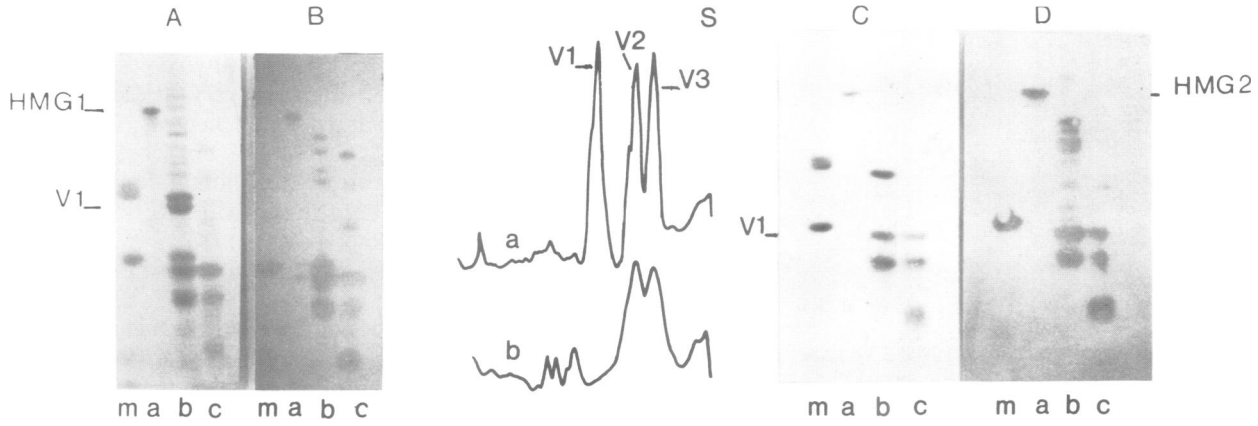

Fig. 4. DNA binding to HMG-1 and HMG-2 V8 protease digestion products, **Panels A** and **C** correspond to nitrocellulose filters stained with amido black for HMG-1 and -2, respectively. **Panels B** and **D** correspond to autoradiographs of duplicate nitrocellulose filters incubated with labelled DNA at 50 mM NaCl. **Tracks b** and **c** correspond to digestion products for 5 and 30 min, respectively, **m** to protein markers, and **a** to undigested protein. The densitometric scanning of **track b** in **panel A (profile a** in **S)** compared with **track b** of **panel B (profile b** in **S)** is also shown. Conditions of digestion are the same as in Figure 2.

ing in the gels or blots, appear with a higher intensity in the autoradiogram than in the gel. This is especially the case for peptides with mobility slightly higher than that of the intact protein. This effect may be due to a high affinity of these peptides for DNA or to the saturation of the other peptides with DNA present in excess during the incubation. Undigested HMG-1 and -2 show a positive interaction with DNA. Lysozyme, used as a protein marker, also shows a positive DNA binding. The interaction of this protein in solution with DNA has been reported (Cattan and Bourgoin, 1968).

Identification of non-interacting domains of HMG proteins with DNA

To identify some of the peptides obtained in the V8 protease digestion, a chromatographic method was used. DNA cellulose was chosen to prepare the peptides that do not interact with DNA, making use of the properties already observed by blotting. The chromatographic profile, corresponding to products of a 5 min digestion of HMG-1 with V8 protease, and their electrophoretic analysis are shown in Figure 5. For HMG-2 the results are essentially identical (not shown). The material not retained in the column corresponds to the peptide that did not bind DNA in the blots (V1). Fractions V3 and V2 were obtained by passing a linear salt gradient through the column from 50 mM to 0.6 M NaCl.

These peptides were analyzed for their amino acid content to estimate their position in the parent molecule. Table I presents the amino acid composition of the peptides of HMG-1 and -2 not retained in the column (V1) and the first fraction (V3) eluted from the DNA-cellulose column, together with the number of residues according to the apparent mol. wt. of the peptides. They are compared with the composition of two peptides of HMG-1 and -2 assuming one cleavage at residue 74 that produces peptide V3 (1–74) and another at residue 243 producing the V1 peptide (75–243); this gave the best fit with the amino acid analyses obtained. The other fractions give amino acid analyses with results difficult to correlate with a defined zone in the sequence.

Histone H1 binding

The interaction of HMG proteins with histone H1 has been proposed as one of the possible functions of these proteins

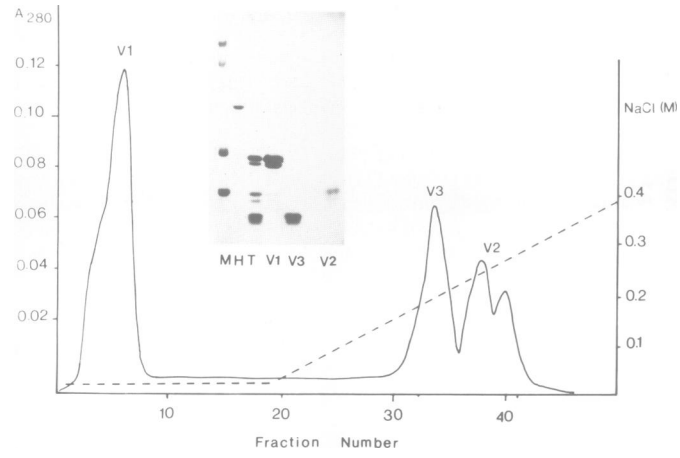

Fig. 5. DNA-cellulose chromatography of protein HMG-1 digested with V8 protease for 5 min in conditions identical to those of panel A in Figure 2. V1 is the material not retained by the column, V3 and V2 are two fractions eluted at different NaCl concentration (shown as a broken line). The gel electrophoresis of intact protein (H), the loaded sample (T), protein markers (M) and the different fractions is also shown.

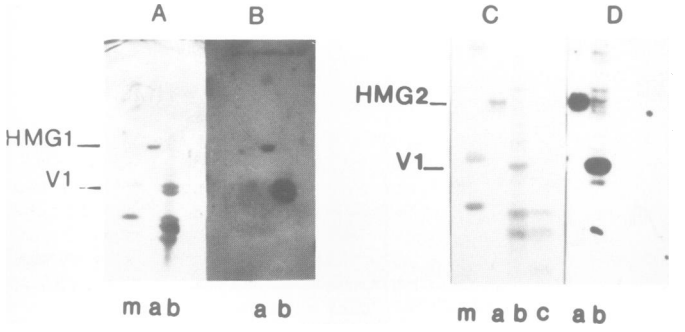

Fig. 6. Histone H1 binding to HMG-1 and HMG-2 V8 protease digestion products. **Panels A** and **C** are the amido black stained filters containing the digestion products of HMG-1 and -2, respectively, for 5 **(b)** and 30 min **(c)**. **Panels B** and **D** are the autoradiographs obtained by incubation of duplicate filters with labelled histone H1. **Tracks m** and **a** are protein markers and undigested protein, respectively.

Table I. Amino acid composition of HMG-1 peptides V1 and V3 (purified as in Figure 5) compared with the composition of fragments (1–73) and (74–243) calculated from the sequence of the protein (Walker *et al.*, 1980).

	V3	HMG-1 1–73	V1	HMG-1 74–243
Asp	6.1	5	25.3	22
Thr	2.1	2	3.5	3
Ser	9.4	9	7.0	6
Glu	10.4	9	45.9	43
Pro	3.0	3	11.9	11
Gly	6.6	5	9.2	9
Ala	6.1	6	13.7	15
Cys/2	N.D.	1	N.D.	–
Val	1.6	2	2.0	3
Met	2.3	3	2.0	2
Ile	0.6	0	3.3	3
Leu	0.6	0	3.9	4
Tyr	1.5	2	4.4	6
Phe	4.3	5	3.8	4
Trp	N.D.	1	N.D.	1
His	1.6	2	1.1	1
Lys	12.5	16	29.6	30
Arg	4.0	3	3.5	5

(Shooter *et al.*, 1974). To this end, calf thymus H1 was labelled with ^{32}P by protein kinase. The labelling did not change the electrophoretic mobility of the protein in SDS gels. Judging from the autoradiography of the electrophoretic gel (not shown), no specific subfraction appeared to be labelled preferentially. Figure 6 shows stained filters and autoradiograms corresponding to the incubation of histone H1, (labelled with ^{32}P as described in Materials and methods), with nitrocellulose filters containing the V8 protease digestion products of HMG-1 and -2 (digestion was for times comparable with those used for the previously presented blots). The incubation of H1 was carried out in the presence of lysozyme to avoid unspecific binding; only under these conditions was an acceptable background achieved. The results show that undigested HMG-1 and -2 proteins bind histone H1. Only the V1 peptide binds labelled histone H1 and this is the only fragment that did not bind DNA. The results for HMG-2 are comparable, as shown on panel D.

Discussion

HMG proteins 1 and 2 have a very characteristic amino acid composition. They are acidic and basic proteins, in contrast to histones which are proteins rich in amino acids of basic character. From the sequence data available (Walker *et al.*, 1980), the distribution of these two types of polar residues is clearly asymmetrical with a very acidic C-terminal tail (residues 180–260) and a more neutral N-terminal region (residues 1–180). As a result of this observation, spectroscopic studies and protease digestion, a domain structure for HMG proteins has been proposed (Cary *et al.*, 1976, 1983; Palau *et al.*, 1980; Reeck *et al.*, 1982).

We used two proteases to test *in vitro* a functional correlation with the asymmetry observed in the sequence. Trypsin has provided information about the structure of chromatin proteins, both core histones (Weintraub, 1975; Whitlock and Simpson, 1977) and histone H1 (Hartman *et al.*, 1977) and related proteins (Avilés *et al.*, 1978; Puigdomènech *et al.*, 1980). This enzyme has been used to test the domain hypothesis for HMG-1, -2 and -E (Paulau *et al.*, 1980; Reeck *et al.*, 1982; Cary *et al.*, 1983). The results for trypsin digestion presented here are in agreement with those of Cary *et al.* (1983). The differences in the digestion pattern as compared with that presented by Reeck *et al.* (1982) may be due to the different protein studied by these authors (chicken erythrocyte HMG-E instead of calf thymus HMGs), since limited changes in sequence could vary the main point of attack of the enzyme. Differences could also be due to the different extraction procedure used, 0.35 M NaCl instead of 5% perchloric acid. The first of the extraction procedures could expose primarily the B-C domains hinge (using the nomenclature of Reeck *et al.* (1982)] whilst the second method could result in a preferential exposure of the A-B hinge. A reversible refolding of the protein chain has, however, been observed for both the protein and the peptides by spectroscopic measurements (Cary *et al.*, 1976, 1983). In fact, the two models that have been proposed for a domain structure in HMG-1 and -2 do not present major discrepancies between themselves. Cary *et al.* (1983) proposed that the first 11 residues have to be considered as a domain on its own. The other three domains coincide approximately in both models, the differences probably reflecting the extent of the hinge region between domains.

V8 protease cleaves HMG-1 and -2 producing peptides

whose amino acid composition are in agreement with a main cutting point at residue 74, that is at the end of domain A, just one residue away from the cutting point for trypsin found by Cary *et al.* (1983). The preservation of the acidic C-terminal tail during V8 digestion is a surprising result considering the known specificity of this protease for acidic residues. This result could be in agreement with the suggestion of Cary *et al.* (1983) that the acidic C-domain adopts a strong helical structure, since no change in helicity is observed in the protein from neutral to acidic pH. Over this range, poly-glutamic acid shows a helix-random coil transition.

The preferential point of attack in the A-B hinge is observed for both enzymes employed. The similarity of the trypsin digestion shown in Figure 1 with that reported (Cary *et al.*, 1983) and the amino acid analyses of the peptides from V8 protease indicate that the A-B hinge is the most susceptible part of the molecule probably as a result of structural constraints. The V1 peptides are the only ones that do not show binding with labelled DNA. This behaviour of the V1 peptides is not due to their binding to the nitrocellulose filter because the same lack of interaction is observed on passing the peptide mixture through a DNA-cellulose column. It is interesting to note that peptides of higher mobility than the non-DNA-interacting peptides show a positive binding for DNA. Some of these peptides probably come from the degradation of V1 peptides since they appear when this peptide is degraded. This would mean that excision of the highly acidic C-domain restores the ability of the B-domain to interact with DNA. Furthermore, the intermediate peptide V2 is the most strongly bound peptide in the DNA column. This differential behaviour of the central domain, when it is attached to the C-terminal acidic tail, could be a simple effect of electrostatic repulsion between the C-terminal tail to DNA or the result of an interaction of this domain with the central part of the protein.

The binding of histone H1 shows a behaviour complementary to that of the binding of DNA. The V1 peptides that did not interact with the nucleic acid show the only detectable interaction of the digested protein with H1. This clear-cut behaviour confirms the existence of well-defined structural domains in HMG-1 and -2 proteins. Although these experiments were carried out *in vitro*, they indicate that these domains may also have a functional role, one part (domains A and B) being responsible for the attachment of the protein to DNA while the other (domain C) is able to interact with histone H1. In this sense, HMG-1 and -2, through the interactions of the domains of its chain, could either modulate the interactions of the histone H1 family proteins with DNA or the interaction of histone H1 with HMG-1 and -2 could change the affinity of these proteins for DNA. The effects of histone H1 composition on the higher order structure of chromatin have been reported (Puigdomènech and Ruiz-Carrillo, 1982) as have the changes in chromatin structure in relation to its transcriptional activity (for a review, see Weisbrod, 1982).

Materials and methods

Protein preparation and digestion

HMG proteins 1 and 2 were prepared from calf thymus by extraction with 5% perchloric acid (Sanders, 1977) and fractionated by CM-Sephadex G-25 chromatography (Goodwin *et al.*, 1978b). Histone H1 was separated from the 5% perchloric acid extract by selective precipitation with three volumes of acetone. Purity of the samples was checked by polyacrylamide gel electrophoresis and by amino acid analysis.

For the digestion with proteases, the two fractions HMG-1 and -2 were treated in an identical way. 20 mg of HMG protein were dissolved in 5 ml of 50 mM phosphate buffer pH 7.1, 2 mM dithiothreitol (DTT), and the solution was dialyzed overnight at 4°C against the same buffer made 0.35 M in NaCl. Final protein concentration was adjusted to 1.95 units of absorption at 280 nm.

Proteolytic digestions were carried out at 37°C. 15 μl of trypsin (Sigma, 7500 units/mg) solution (1 mg/ml) or 50 μl of V8 protease from *Staphylococcus aureus* (Miles, 685 units/mg) solution (4 mg/ml) were added to aliquots (1.1 ml) of HMG protein solution in phosphate buffer/NaCl. Aliquots of the sample (100 μl) were taken at different times and poured into ice cold tubes containing 900 μl of 20% trichloroacetic acid. After allowing the sample to stand at 4°C for 15 min, the tubes were centrifuged at 3000 *g* for 10 min. The supernatants were discarded and the sediments were washed twice with a mixture of acetone-0.1 N HCl (6:1 v/v) and dried under vacuum. Samples were dissolved in the appropriate electrophoresis sample buffer and loaded onto the gel.

Gel electrophoresis and protein blotting

SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970) in 15% polyacrylamide slab gels (1.5 mm thick). A set of protein markers were used to compare the different gels; they included bovine serum albumin (BSA, mol. wt. 65 000), ovalbumin (45 000), soybean trypsin inhibitor (21 000) and lysozyme (14 300). After electrophoresis, the samples were transferred to nitrocellulose filters (BA 85 Schleicher and Schuell). The protein transfers from polyacrylamide gels to nitrocellulose filters were made electrically in a Trans Blot device (Bio Rad) in the presence of 2 liters of 20 mM Tris, 150 mM glycine pH 8.4, 20% methanol (Renart *et al.*, 1979) and connected to 12 V for 3.5–4 h. The transfers were made in duplicate. One of the strips of nitrocellulose was stained as a transfer control according to the method of Bowen *et al.* (1980).

DNA and histone H1 binding assays

DNA from calf thymus nucleosomes (140–160 bp) obtained by micrococcal nuclease digestion of nuclei in 10 mM Tris-HCl, pH 7.4, 30 mM NaCl, 110 mM KCl, 0.2 mM $MgCl_2$, 0.4 mM phenylmethylsulphonyl fluoride, was labelled at the 5′ end to a specific activity of $\sim 10^6$ c.p.m./μg by T4 polynucleotide kinase (BRL), using [γ-^{32}P]ATP as precursor (Maxam and Gilbert, 1980). DNA was separated from unincorporated nucleotides by passage through a Sephadex G-50 column equilibrated with 10 mM Tris-HCl pH 7.1, 1 mM EDTA, 50 mM NaCl, and eluted with the same buffer.

The DNA binding reaction was carried out essentially as described by Bowen *et al.* (1980). A nitrocellulose filter strip was preincubated with 10 mM Tris-HCl pH 7.1, 1 mM EDTA, 50 mM NaCl, 0.002% Ficoll 400 (Pharmacia), 0.002% polyvinylpyrrolidone, 0.002% BSA (Fraction V, Fluka), for 30 min at room temperature and incubated with 10^5 c.p.m. of labelled DNA in the same buffer for 60 min. After incubation, the strip was washed with the same buffer for 1 h, changing the wash liquid four times. The nitrocellulose filter was dried and autoradiographed.

Histone H1 was labelled to a specific activity of 3×10^3 c.p.m./μg by protein kinase from beef heart (Sigma, 40 units/mg) using [γ-^{32}P]ATP as precursor, according to the method described by Rubin *et al.* (1974). Unincorporated nucleotides were separated from H1 by passing the reaction mixture through a column of Sephadex G-50, equilibrated and eluted with 10 mM Tris-HCl pH 7.1, 1 mM EDTA, 0.15 M NaCl.

Nitrocellulose filters containing the digestion products from HMG-1 and HMG-2 were incubated with labelled H1 in standard binding buffer (10 mM Tris-HCl pH 7.1, 1 mM EDTA, 0.15 M NaCl, 0.2% Ficoll 400, 0.2% polyvinylpyrrolidone, 0.2% BSA), lysozyme (Sigma) was added to a final concentration of 1 mg/ml. The filters were preincubated for 1 h with standard binding buffer. The incubation was for 3 h at 37°C, the filter was dried and autoradiographed with Mafe rp-x1 X-ray film in the presence of intensifying screens.

DNA-cellulose chromatography of V8 peptides

1.5 ml of HMG-1 in phosphate buffer pH 7.1, 2 mM DTT, 0.35 M NaCl were digested for 5 min by adding 25 μl of V8 protease solution (4 mg/ml). After digestion, 8.5 ml of phosphate buffer were added to the digestion mixture. The solution was passed through a column containing 1.5 g of DNA-cellulose (Sigma) corresponding to 6.5 mg of DNA, equilibrated with phosphate buffer pH 7.1, 1 mM EDTA, and washed with 15 ml of the same buffer made 50 mM in NaCl. A linear salt gradient from 50 mM to 0.6 M NaCl in phosphate buffer was passed through the column with a total volume of 100 ml. The optical transmission at 280 nm of the eluate was recorded with a LKB Uvicord II monitor. Fractions of 1.5 ml were collected, pooled and dialysed against 10 mM HCl. Finally the dialysate was lyophilized and kept for electrophoretic and amino acid analysis. Amino acid analyses were performed after 24 h hydrolyses at 110°C by 6 N HCl in a Beckman model 119 C

automatic Auto-analyzer. No corrections were made for hydrolytic losses of amino acids.

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