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Interaction between domains in chromosomal protein HMG-1

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Peptides corresponding to the N-terminal, central and central plus C-terminal domains of high mobility group protein HMG-1 from calf thymus have been isolated after digestion in solution with protease V8 under structuring conditions (0.35 M NaCl, pH 7.1). The effect of the interaction of these peptides with DNA on the topological properties of the nucleic acid has been studied and compared with the change in superhelicity produced by the whole protein. It appears that the region responsible for this effect is the central domain of HMG-1. The isolated N-terminal and central domains of this protein maintain their secondary and tertiary structure as observed by spectroscopic techniques. However, when the central domain is covalently linked only to the acidic C-terminal part of the molecule, its secondary and tertiary structures are lost as well as its property to alter DNA superhelicity. The results are discussed in relation to the interactions occurring between the different domains and the possible functional interactions of this protein.
Key words: circular dichroism/chromatin/HMG proteins/nuclear magnetic resonance/topoisomerase I

Introduction

The structure and function of high mobility group (HMG) proteins are of increasing interest (for a review, see Johns, 1982) because of their potential effects on nucleosome structure or higher order structure of chromatin. They might take part in the packing of chromatin and/or in the regulation of gene expression. Weisbrod *et al.* (1980) have proposed that proteins HMG-14 and -17 are responsible for the maintenance of the structure that produces an increased DNase I sensitivity to transcribable genes (Garel and Axel, 1976). Functional effects on HMG-1 and -2 have been related to their ability to change the superhelicity of DNA (Javaherian *et al.*, 1978), their preferential interaction with single-stranded DNA (Isackson *et al.*, 1979) and their interaction with linker nucleosomal DNA *in vitro* (Schröter and Bode, 1982).

HMG-1 and -2 form a group of high mol. wt. HMG proteins (~29 000 daltons) whose primary structure is partially known for the calf thymus proteins (Walker *et al.*, 1980). From sequence studies a strong asymmetry appears in the distribution of amino acid residues and this has led to the proposal of a domain structure for the protein (Cary *et al.*, 1976, 1983; Palau *et al.*, 1980; Reeck *et al.*, 1982). Following the nomenclature of Reeck *et al.* (1982) three domains called A, B and C can be defined in HMG-1 and -2. Domains A and B, which correspond respectively to the N-terminal and central thirds of the molecule, have an equilibrated amino acid composition with a predominance of basic amino acids. They also show a certain degree of sequence homology among themselves (Reeck *et al.*, 1982) and display spectroscopic evidence of secondary and tertiary structure when they are isolated and purified following trypsin cleavage of the molecule (Cary *et al.*, 1983).

By digesting proteins HMG-1 and -2 with either trypsin or V8 protease a similar domain structure can be found (Carballo *et al.*, 1983). Domains A and B interact with DNA while a larger fragment containing domains B and C does not. Labelled histone H1 binds preferentially the B plus C domain peptide. From these experiments it also appeared that the central domain B requires the highest salt concentration to neutralize its interaction with DNA but when attached to the C-terminal acidic domain this interaction vanished. The differential behaviour of the central domain, when the C-terminal acidic tail is present in the larger fragment, raised the question of possible interactions between domains of the protein and/or electrostatic repulsions with the nucleic acid that could prevent the interaction with DNA through changes in the structure of the protein. Here we provide evidence that domain B is responsible for the effect of HMG-1 on the superhelicity of DNA, an effect documented for the whole protein (Javaherian *et al.*, 1978), and that the C-terminal domain has a strong interaction with the central domain, destroying its secondary and tertiary structure and its effect on DNA superhelicity when domain A is not present. A model for the structure of HMG-1 and the possible interactions occurring between domains is presented.

Results

Effects of HMG-1 peptides on DNA superhelicity

One of the characteristic features of high mol. wt. HMG proteins 1 and 2 that has been correlated with a possible function is the effect on the superhelicity of DNA (Javaherian *et al.*, 1978, 1979; Mathis *et al.*, 1980). This effect is different from the stimulation of topoisomerase activity observed by Javaherian and Liu (1983) for HMG-17. To find out whether this function could be located in a specific zone of the polypeptide chain of HMG-1, closed circular DNA from plasmid pBR322 was relaxed with calf thymus topoisomerase I, mixed with a quantity of protein or peptides and incubated in the presence of topoisomerase for 45 min at 37°C. This experiment was done with the whole protein and the three peptides that can be isolated from short V8 protease digestion in solution (Carballo *et al.*, 1983). The peptides obtained from this digestion can be separated by DNA-cellulose chromatography by elution at different ionic strengths. The ability of the peptides to bind to DNA-cellulose correlates with their ability to bind DNA, as seen by blotting (Carballo *et al.*, 1983). From the amino acid composition of the products of digestion (Table I) it is possible to assign the three main peptides to specific zones of the molecule. V1 corresponds to the

Table I. Amino acid composition and approximate number of residues of HMG-1 and peptides V1, V2 and V3 compared with the composition of fragments (74–243), (80–185) and (1–73) calculated from the sequence of the protein (Walker *et al.*, 1980).

	HMG1		V1		74–243	V2		80–185	V3		1–73
	%	N	%	N[a]	N	%	N	N	%	N[a]	N
Asx	9.6	26	14.9	25.3	22	8.9	9.3	8	8.4	6.1	5
Thr	2.4	6	2.1	3.5	3	2.7	2.8	3	2.9	2.1	2
Ser	4.4	15	4.1	7.0	6	5.2	5.4	3	12.9	9.4	9
Glx	19.0	52	27.0	45.9	43	10.5	11.0	11	14.2	10.4	9
Pro	6.0	14	7.0	11.9	11	8.6	9.0	10	4.1	3.0	3
Gly	5.4	14	5.4	9.2	9	9.4	9.8	7	9.0	6.6	5
Ala	8.6	25	8.0	13.7	15	12.1	12.7	12	8.3	6.1	6
Cys/2	0.6	2	N.D.	–	?	N.D.	–	?	N.D.	–	1
Val	2.7	5	1.2	2.0	3	2.6	2.7	3	2.2	1.6	2
Met	1.9	5	1.2	2.0	2	0.5	0.5	2	3.2	2.3	3
Ile	1.7	4	1.9	3.3	3	2.9	3.0	3	0.8	0.6	0
Leu	2.1	5	2.3	3.9	4	3.5	3.7	4	0.8	0.6	0
Tyr	3.8	9	2.6	4.4	6	3.1	3.2	5	2.1	1.5	2
Phe	4.6	11	2.2	3.8	4	4.2	4.4	4	5.9	4.3	5
Trp	N.D.	2	N.D.	–	1	N.D.	–	1	N.D.	–	1
His	2.9	3	0.65	1.1	1	1.8	1.9	1	2.2	1.6	2
Lys	20.4	52	17.5	29.6	30	20.0	21.0	23	17.1	12.5	16
Arg	3.8	9	2.1	3.5	5	3.4	3.6	4	5.5	4.0	3

[a]Carballo *et al.*, 1983.

terminal two thirds of the molecule, approximately from residue 74 to 243, that is domains B+C, following the nomenclature of Reeck *et al.* (1982), except it lacks a small fragment at the C-terminal end. V2 corresponds to the central B domain from position 80 to 185 and V3 includes the N-terminal A domain (1–73).

Agarose gel electrophoresis of the topoisomers produced by the action of topoisomerase I on the DNA-peptide complexes shows that intact HMG-1 alters the DNA superstructure, producing a number of superhelical molecules (Figure 1, tracks 3 and 4) from relaxed closed circular DNA. The three peptides obtained by V8 protease treatment were also assayed using two amounts of peptide, representing approximately one or two molecules of peptide per 2000 bp of DNA. The results shown in Figure 1 indicate that the only peptide having an effect comparable with the whole protein is V2, that is, the peptide eluted at the highest salt concentration in DNA-cellulose chromatography of the products of V8 protease digestion (Carballo *et al.*, 1983). V3, while also being able to bind DNA on blots of HMG-1 digestions and DNA-cellulose, does not have any detectable effect on DNA superhelicity. V1, that corresponds to domains B+C, therefore including peptide V2, has only a residual effect.

The effect of the different peptides can be empirically compared by using the average absolute variation in linking number produced by the topoisomerase in the presence of the different ligands calculated according to Stein (1980). Following this procedure the relative effects of the different peptides can be compared with the change in linking number produced in the presence of the whole protein. The mean value in the topoisomer distribution produced by the whole protein is 4.9 for ratio 1 and 6.3 for ratio 2, peptide V2 produces an average topoisomer of 5.3 for ratio 1 and 5.5 for ratio 2, while peptide

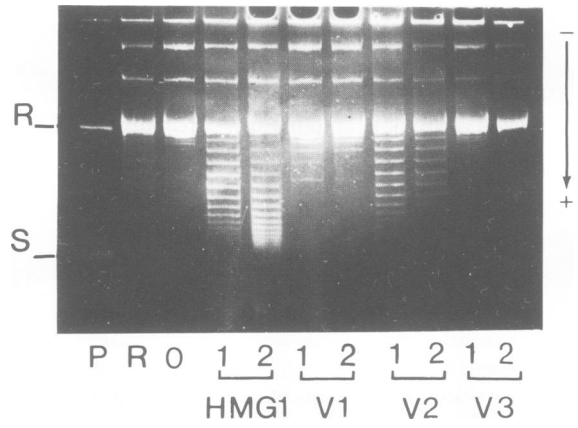

Fig. 1. Agarose gel electrophoresis of pBR322 closed circular DNA following topoisomerase I action in the presence of protein HMG-1 and its peptides. The enzyme reaction was carried out at 37°C for 60 min at 20 μg/ml relaxed DNA and 0.33 units of enzyme per μl. Two molar ratios corresponding to one or two molecules of protein per 2000 bp of DNA were used and are presented as samples 1 and 2. Sample P is the initial plasmid, R relaxed plasmid and O a sample with no peptide or protein added.

V3 has the same pattern of topoisomers as the control assay performed in the absence of any peptide, and peptide V1 has a minor effect of 2.3 for ratio 1 and 1.9 for ratio 2.

HMG proteins 1 and 2 produce a negative superhelicity in DNA, correlated with an unwinding of the double helix (Javaherian *et al.*, 1978, 1979). It was of interest to confirm this effect produced by the whole protein by comparing the electrophoretic patterns of topoisomers produced in the presence of the protein in gels containing chloroquine (Lockshon and Morris, 1983). At the same time it could be shown that the effect produced by the central domain of

HMG-1 has the same sign as the intact protein. In Figure 2, a gel similar to that presented in Figure 1, but having a higher ratio of peptide/DNA, is compared with a gel containing 6 μg/ml of chloroquine to check the sense of DNA supercoiling. These gels show that chloroquine reverses the effect of topoisomerase in the presence of either HMG-1 or its V2 peptide, indicating that they produce a negative supercoil in DNA.

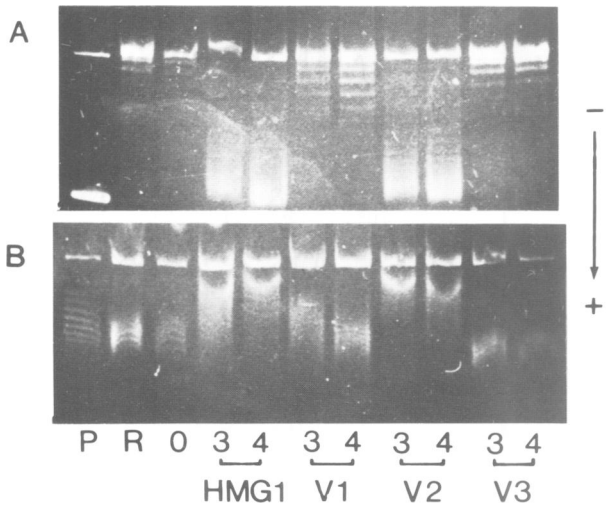

Fig. 2. Agarose gel electrophoresis of pBR322 closed circular DNA following topoisomerase I action in the presence of protein HMG-1 and its peptides. The enzyme reaction was carried out in the same conditions of Figure 1, with a ratio of 3 and 4 protein molecules/2000 bp of DNA, presented as sample 3 and 4, respectively. The electrophoresis were carried out in the same conditions as Figure 1 (**A**) and run in presence of 6 μg/ml of chloroquine diphosphate (**B**). Sample P represents the initial plasmid, R relaxed plasmid and O is a sample with no peptide or protein added.

Spectroscopic studies

As pointed out before, the central domain of protein HMG-1 loses its ability to bind DNA and to alter DNA superhelicity when it is attached to the C-terminal acidic fragment in the absence of the N-terminal domain. This could merely be the result of electrostatic repulsions between two charged macromolecules. Alternatively, such loss of properties could be due to, or could be accompanied by, structural changes in the central domain when the C-terminal end is present. Spectroscopic studies were carried out on the three peptides and on the intact HMG-1 to check these possibilities using circular dichroism (c.d.), which is sensitive to secondary structure changes, and nuclear magnetic resonance spectroscopy (n.m.r.), to give information mainly on tertiary structure of proteins. Both techniques have already been used for studies of intact HMG-1 protein (Cary *et al.*, 1976) and of peptides obtained by tryptic degradation of the molecule (Cary *et al.*, 1983). The c.d. and n.m.r. spectra of protein HMG-1 and of its peptides V1, V2 and V3 were recorded under denaturing (D_2O, pH 3) and under structuring conditions. Two types of salts were chosen for their structuring effect, either 0.15 M NaCl, pH 7.4 or 10 mM $MgCl_2$, pH 7.4. Their effect on c.d. and n.m.r. spectra is identical.

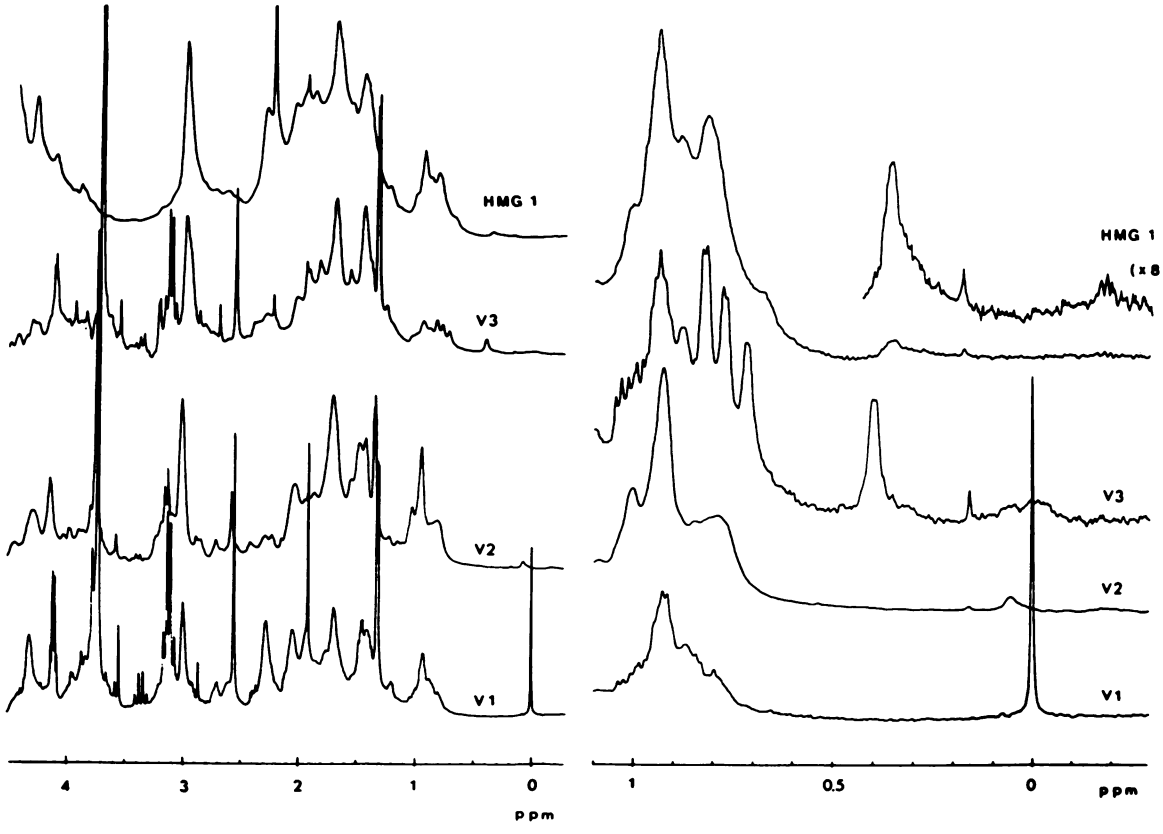

Fig. 3. High-field n.m.r. spectra at 500 MHz of protein HMG-1 and peptides V3, V2 and V1 at 0.15 M NaCl and neutral pH. The concentration of samples was between 3.5 and 4.0 mg/ml. In the right part of the figure an enlarged zone of the figure between −0.3 to 1.1 p.p.m. is shown. A detail of the whole protein (8 times magnified) is also shown.

Nuclear magnetic resonance

The high resolution and sensitivity of a 500 MHz instrument gave substantially increased information compared to similar studies carried out with HMG-1 (Cary *et al.*, 1976) and with tryptic peptides (Cary *et al.*, 1983). The spectra of the protein and of the V8 protease peptides in D_2O, pH 3 were those that could be expected for random coil polypeptides and in agreement with their amino acid composition (not shown). The high-field spectrum of HMG-1 protein under structuring conditions [Figure 3, where the zone between 1.1 and −0.3 p.p.m. is also presented in an enlarged scale and Cary *et al.* (1983)] shows a limited number of signals shifted to high field coming probably from ring-current effects. The same stands for peptide V2, while peptide V3 has four very clearly resolved peaks. The amino acid analysis of V3 peptide (see Table I) shows the absence of leucine and isoleucine. Therefore, the two methyl groups of the two valine residues can be considered as good candidates to identify these two signals. The sequence of the protein (Walker *et al.*, 1980) shows one valine in position 19 in the sequence Phe-Phe-Val and the other one in position 35 in the sequence Val-Asn-Phe. However, there are also two threonine and six alanine residues whose methyl resonances could shift to these positions as a consequence of the protein folding. Further studies are being carried out to identify clearly these resonances.

The same number of peaks shifted beyond 0.4 p.p.m. are observed in the V3 peptide and in the whole protein, especially a well resolved peak at 0.35 p.p.m. in the protein and a broad resonance at −0.18 p.p.m. (presented in the figure in an enlarged scale). The chemical shifts of these resonances do not exactly coincide in the peptides and the whole protein; in particular the resonance observed at 0.35 p.p.m. in the protein probably corresponds with the 0.40 p.p.m. resonance in peptide V3. Chemical shifts in this zone are very sensitive to slight changes in the distances between aromatic and hydrophobic residues and in the case of the 0.40 p.p.m. peak the distance change can be calculated as being between 0.1 and 0.2 nm by using the Johnson and Bovey (1958) model, if the signals are supposed to correspond to valine residues. The spectrum of peptide V1 does not show any signal that could be interpreted as due to a ring-current effect and it is identical to that observed in denaturing conditions (D_2O, pH 3, result not shown).

The low-field part of the spectra, corresponding to aromatic residues, presents a much larger number of resonances that can be interpreted as local influences between residues (Figure 4). In this case the spectra can be easily compared with those published at lower resolution for tryptic peptides (Cary *et al.*, 1983). The differences observed in the histidine residues between the four spectra shown cannot be attributed solely to structural effects as the pH was not adjusted to the same exact values for the different molecular species studied. From the comparison of the spectra of the peptides and of the whole protein it appears that most of the signals in the whole polypeptide come from peptide V3, that is the N-terminal domain A, however, peptide V2 (central domain B) shows well-resolved peaks shifted from their random coil position. Most of the signals observed in HMG-1 can be found in either peptide V2 or V3, the few exceptions could correspond to slight structural changes and they are marked in Figure 4 with a cross. Also, in this case, it is evident that peptide V1 shows no sign of structure observable by n.m.r. It, therefore, appears that the presence of the acidic C-terminal

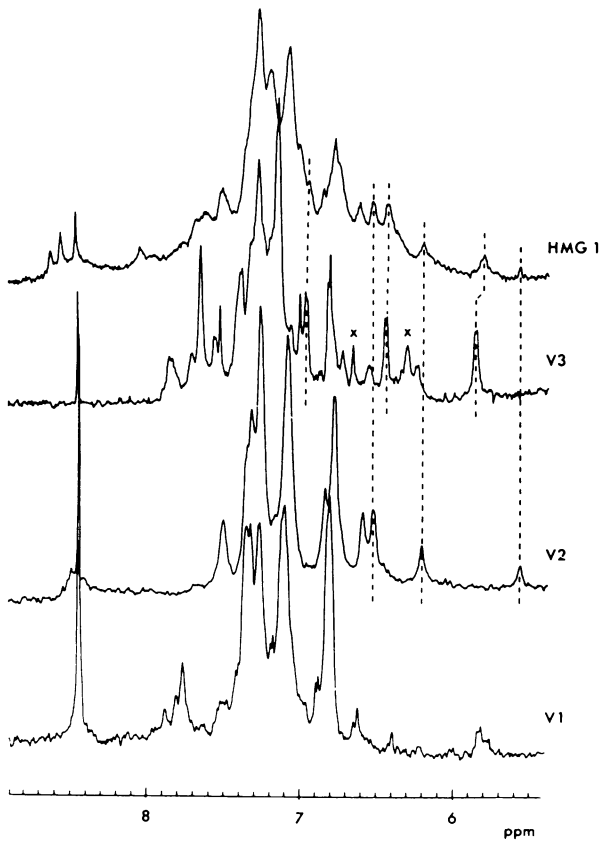

Fig. 4. Low-field aromatic n.m.r. spectra at 500 MHz of protein HMG-1 and peptides V3, V2 and V1 in the same conditions of Figure 2. The vertical lines show some of the shifted peaks in the peptides corresponding to resonances in the whole protein. Crosses mark the signals in peptide V3 not having a correspondence in the protein.

third of the molecule prevents the central domain from folding. However, when the C-terminal fragment is digested, the central domain recovers its tertiary structure.

As shown previously, the presence of the acidic C-terminal prevents the folding of the central domain of HMG-1. To study whether this is due to an unspecific electrostatic interaction between two oppositely charged polyelectrolytes, peptide V2 was mixed at low pH with a volume of a solution of poly-L-glutamic acid (average mol. wt. 11 000) corresponding to a quantity equivalent to the number of acidic residues in the C-terminal domain. The pH of the mixture was raised to a neutral value, the salt concentration was raised to 0.15 M NaCl and the spectrum was recorded. Another equivalent of poly-L-glutamic acid was then added to the sample and another spectrum was recorded. The low-field part of the spectrum of the mixture of poly-L-glutamic acid and peptide V2 compared with that of the peptide in similar ionic conditions and in denaturing conditions are shown in Figure 5. As the spectra show no change in the structure can be observed by the addition of poly-L-glutamic acid. Similar effects can be observed in the high-field part of the spectrum (not shown).

Circular dichroism

The loss of tertiary structure in the central domain of HMG-1 when it is attached to the C-terminal end in the absence of the A domain as seen by n.m.r. could be due to local changes in the structure not affecting the general conformation of the peptide. However, the same behaviour was observed with c.d.

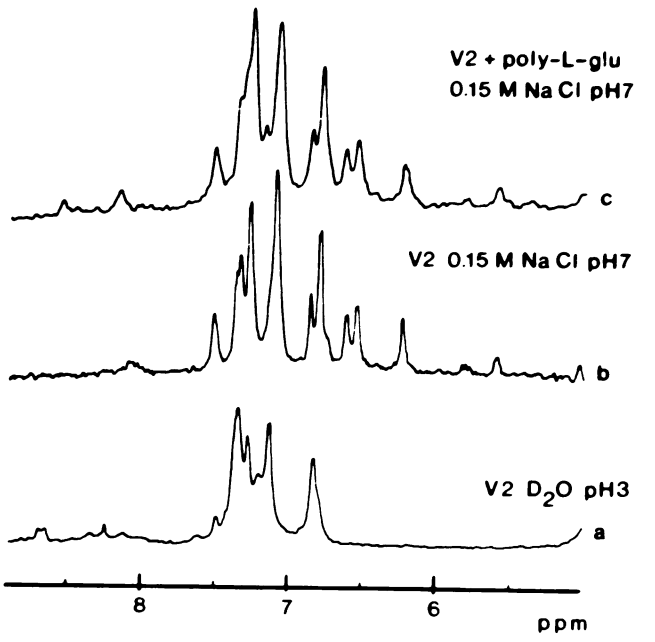

Fig. 5. Low-field n.m.r. spectra at 500 MHz of V2 peptide in different conditions. Effect of poly-L-glutamic acid. 25 μl of poly-L-glutamic acid at a concentration of 30 mg/ml in 99.99% D_2O solution were added to 400 μl of V2 peptide solution (4.75 mg/ml) in D_2O pH 3. The pH of the mixture was raised to pH 7 and the salt concentration to 0.15 M NaCl (**spectrum c**). As a comparison the spectrum of the same peptide in the same conditions (**spectrum b**) in the D_2O pH 3 (**spectrum a**) are also shown.

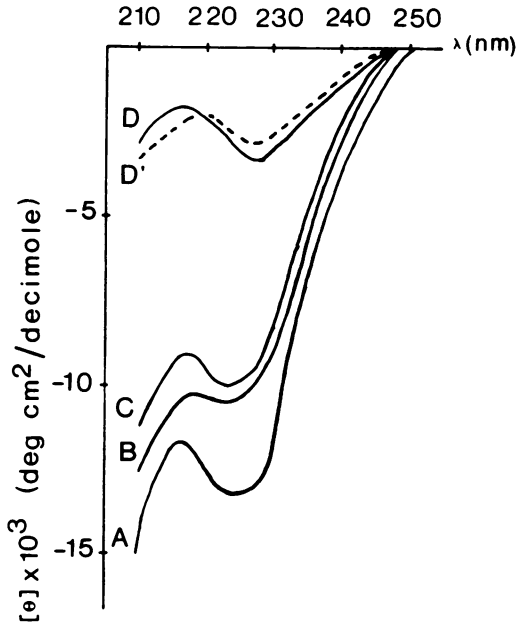

Fig. 6. C.d. spectra of HMG-1 protein (**A**), peptide V2 (**B**), peptide V3 (**C**) and peptide V1 (**D**) at 0.15 M NaCl, pH 6.5 at concentrations between 0.2 and 0.3 mg/ml. **D'** is the spectrum of peptide V1 in H_2O, pH 3.

spectroscopy that is sensitive to the formation of secondary structure. Figure 6 shows the results obtained for the three peptides studied and for the whole protein. HMG-1 has the highest ellipticity value while peptide V1 has a very low value and it does not change from acidic to neutral pH. The ellipticities of the two other peptides had intermediate values while the values expected for a random coil polypeptide were observed in distilled water and acidic pH.

The helicities for the different samples in structuring conditions were calculated using the method of Chen *et al.* (1974). For protein HMG-1 a value of 46% α-helix was found corresponding to 120 helical residues, for peptide V1, 9% (16 residues), for peptide V2, 37% (32 residues) and for peptide V3, 36% (27 residues). The values for the peptides V2 and V3, corresponding to domains B and A and for the protein agree with those measured by Cary *et al.* (1982) for similar tryptic peptides except that a lower helicity is found in our case for domain A. The reason for this difference may come from the presence in our peptide of the first 11 residues that were not present in the N-terminal tryptic peptide. Using prediction methods (Reeck *et al.*, 1982) this is expected to be a zone of very low helical content. The number of amino acids in helical configuration obtained from our results agrees with the helical prediction using the Chou and Fasman (1978) method (Reeck *et al.*, 1982). It is interesting to note that, as with tertiary structure effects observed by n.m.r., domain B when it is attached to domain C forming the V1 peptide loses its secondary structure. Finally it is worth pointing out that the sum of the helical residues in peptides V2 and V3 is less than the total helicity of the whole protein. This points to some structure existing in the acidic C-terminal domain as previously suggested (Cary *et al.*, 1983; Carballo *et al.*, 1983).

Discussion

A number of observations indicate that HMG proteins have a structural role in chromatin. They are relatively abundant (~ 10^6 copies per nuclei, Goodwin *et al.*, 1978a) and some of these fractions, HMG-14 and -17, have conformational effects on nucleosomes; these may be related to the DNase I sensitivity of specific zones of chromatin (Weisbrod *et al.*, 1980; Stein and Townsend, 1983). In the case of HMG-1 and -2 less defined hypotheses have been proposed. However, their ability to interact with DNA (Shooter *et al.*, 1974), their selectivity for single-stranded DNA (Isackson *et al.*, 1979) and their effects on DNA superhelicity (Javaherian *et al.*, 1978) point also in that direction. The results presented here indicate a highly modulable interaction of protein HMG-1 with DNA.

Previously (Carballo *et al.*, 1983) it was shown that it is possible to define structural domains in HMG proteins 1 and 2 having different properties with regard to their interaction with DNA and histone H1. This effect is confirmed by our results which locate a zone in the protein that is able to change the superhelicity of DNA. It appears that the central part of the protein is responsible for this effect. While this is the domain most strongly binding to DNA it is not the only one that interacts with the nucleic acid. In fact, the N-terminal domain can also bind to DNA as seen by blotting and DNA-cellulose chromatography (Carballo *et al.*, 1983) but it does not produce any change in the topology of closed circular DNA. Thus this specific effect of the protein may be located in a well-defined zone of HMG-1; it is not destroyed by the protease treatment and it is reversibly restored in the central domain after the C-terminal tail is removed. Furthermore the sense of variation of DNA superhelicity is the same with the protein and the peptide, indicating a similar unwinding effect. The localization of specific effects of DNA-binding proteins in different structural domains has been reported in several cases such as the gene 32 DNA unwinding

protein from bacteriophage T4 (Moise and Hosada, 1976; Williams and Konigsberg, 1978). It is also important to point out that negatively supercoiled DNA is a better template for transcription than relaxed or linear DNA (for review, see Gellert, 1981). However, for HMG-1 the effect produced by the isolated central domain disappears when the C-terminal domain is attached to it.

From the n.m.r. results, and in agreement with similar observations on tryptic peptides carried out at lower n.m.r. resolution (Cary *et al.*, 1983), the N-terminal A domain shows a large number of signals due to the formation of a tertiary structure. The central B domain, although having a higher mol. wt. than domain A and a comparable number of residues with an aromatic character, shows only a limited number of signals in the n.m.r. spectrum that can be interpreted as the result of a tertiary folding, mainly in the aromatic part of the spectrum. The only structural effect that would be in agrement with the homologies between domains observed from the sequence of the protein (Reeck *et al.*, 1982) would be the similar percentage of α-helix, nevertheless the n.m.r. spectrum shows a very different pattern of resonances. In studies carried out with proteins of the histone H1 family the comparison of n.m.r. spectra showed a high degree of similarity of tertiary structures in spite of a large difference in amino acid compositions (Puigdomènech *et al.*, 1975). Slight variations in pH and local viscosity producing changes in chemical shift and line width makes difficult the comparison of the spectra of peptides with that of the protein. However, it appears that, with minor exceptions, the resonances present in the spectrum of the protein are present also in the peptides and vice versa indicating that no major structural features of the tertiary structure seem to be lost by digestion with V8 protease.

The effect of the central domain of protein HMG-1 on the superhelicity of DNA is abolished in the peptide that contains this domain attached to the C-terminal acidic part. This happens concomitantly with the disappearance of spectroscopic signals in c.d. and n.m.r. corresponding to secondary and tertiary structure. These observations indicate a strong interaction between the C-terminal and central domains of HMG-1. This interaction is not a pure electrostatic interaction between two oppositely charged electrolytes since a poly-L-glutamic acid does not have any detectable effect on the structure of the central domain. It can be concluded that at least the covalent attachment of the acidic C-terminal part is necessary to allow the interaction of these two domains. It is possible that, in the absence of the N-terminal domain, the negatively charged C-terminal tail folds over the basic DNA-interacting B domain. In this situation both the ability of the peptide to bind to DNA and the interactions between residues that stabilize the structure of the central domain are destroyed. According to these results the functions of the different domains can be defined in a more precise way. The A domain appears to be the most strongly structured part of the protein, and its presence allows the maintenance of the structure of the B domain which is responsible for the changes in the superhelicity of DNA. The C domain may be the part interacting with other proteins such as histone H1 (Carballo *et al.*, 1983) or core histones (Bernués *et al.*, 1983).

The results can be summarized in the model presented in Figure 7 where the structured and charged zones of the molecule have been represented as well as the different products of V8 protease digestion. In this model the highly structured A domain interacts with the other two domains B and

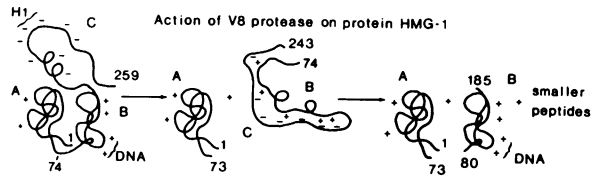

Fig. 7. Model of the structure of protein HMG-1 and the action of protease V8 on it. The charged parts of the molecule are shown as well as those interacting with DNA and histone H1. The three structural domains are also indicated.

C. Its absence would allow the C domain to fold over the DNA-binding domain B. In this situation the negative charges of the acidic tail could interact mainly with the positive charges of the central domain destroying at the same time the tertiary structure of the peptide and its ability to interact with DNA. It is important to point out that the main post-synthetic modifications of HMG-1 and -2 occur in the N-terminal end of the molecule, especially the acetylation of the lysine residues at positions 2 and 11 of calf thymus HMG-1 (Sterner *et al.*, 1979). When the protease is allowed to continue its action on the peptides, a fragment, corresponding to only the B domain, is produced that reversibly folds and retains its action on DNA. Although the results shown in the present paper correspond to *in vitro* conditions it is possible to speculate that *in vivo* conditions may exist where the C domain may fold over the DNA-interacting B domain thus having a modulating action on this function.

Materials and methods

Protein, peptide and DNA preparation

HMG-1 protein was prepared from calf thymus by extraction with 5% perchloric acid (Sanders, 1977) and fractionated by CM-Sephadex C-25 chromatography (Goodwin *et al.*, 1978b). Purity of the sample was checked by electrophoresis in 15% polyacrylamide slab gels (1.5 mm thick) according to the method of Laemmli (1970) and by amino acid analysis. Plasmid pBR322 DNA was prepared from chloramphenicol-amplified *Escherichia coli* cultures by the alkaline procedure of Birnboim and Doly (1979).

HMG-1 (1.5 ml at 3 mg/ml) in phosphate buffer pH 7.1, 2 mM dithiothreitol (DTT), 0.35 M NaCl was digested at 37°C for 45 min with 2.5 μl of V8 protease from *Staphylococcus aureus* (Miles, 685 units/mg) solution (4 mg/ml). The digestion products were fractionated by DNA-celluose (Sigma) chromatography by passing over a NaCl gradient as described by Carballo *et al.* (1983).

Topoisomerase assay of closed circular DNA

4 μg of pBR322 DNA in 220 μl of incubation buffer (50 mM Tris-HCl pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM DTT), containing 30 μg/ml of bovine serum albumin (Miles), were incubated at 37°C for 60 min with 4 μl of topoisomerase I from calf thymus (BRL, 10 units/μl) in order to completely relax the DNA. An aliquot of 20 μl was taken as a control and the reaction was stopped by the addition of 5 μl of 5% SDS. 3 μl of topoisomerase I solution were added to the rest of the pBR322 DNA solution and aliquots of 20 μl were taken and poured into four tubes containing 3 μl of HMG-1 protein and V1, V2 and V3 peptides, respectively, dissolved in the incubation buffer and at quantities corresponding to a ratio of one or two molecules of protein or peptide per 2000 bp of DNA calculated from the apparent mol. wt. of the peptides. The reaction was allowed to continue at 37°C for 60 min and it was stopped by adding 5 μl of 5% SDS to each tube, 20 μl of electrophoresis sample buffer were added and the samples were loaded onto the gel. Electrophoresis of DNA was carried out in vertical 1.6% agarose slab gels in 35 mM Tris, 30 mM phosphate, 1 mM EDTA, pH 7.9 buffer at 50 V for 15 h. Gels in the presence of chloroquine were run in the same conditions with 6 μg/ml of chloroquine diphosphate (Sigma) added in the gel and sample buffer. The gels were stained with ethidium bromide and photographed under u.v. illumination with a MP-4 Polaroid Land camera. The negatives were scanned with a Chromoscan 3 (Joyce-Loebl) densitometer. The integral of the peaks given by the instrument were used directly for the analysis of the topoisomer distribution following the method of Stein (1980).

C.d. and n.m.r. spectroscopy

C.d. spectra were obtained using a Roussel-Jouan Dichrographe II with 1 mm light path cells. Samples of HMG-1 protein and V1, V2 and V3 peptides were dissolved at concentrations of 0.2 and 0.3 mg/ml in distilled water giving pH 3 and the spectra were recorded. Sample solutions were made of 0.15 M NaCl or 10 mM $MgCl_2$ by adding the appropriate volume of 4 M NaCl or 0.1 M $MgCl_2$ solutions; the pH was adjusted to 6.5 with 1 M NaOH and the spectra were recorded. Final concentrations of all samples were checked by the method of Lowry *et al.* (1951) after recording the spectra. Molar ellipticities were calculated from spectra at different wavelengths using the mean residue weight calculated from amino acid analyses. Helicity percentage was computed from the values of molar ellipticity using the parameter equations of Chen *et al.* (1974).

500 MHz proton n.m.r. spectra were run on a Bruker WM 500 spectrometer, equipped with 40 mm bore Oxford Ins. superconducting magnet, 80 K 24 bit word length Aspect 2000 computer, CDC disk system and 16 bit ADC. All spectra were run at a probe temperature of 296°K.5 mm (528-pp Wilmad, Buena, N.J.) spinning tubes were used.

HMG-1, V1, V2 and V3 samples were prepared at a concentration of 3.5–4 mg/ml in 99.99% D_2O solution. The appropriate molarity in NaCl or $MgCl_2$ was achieved by adding measured amounts of 4 M NaCl or 0.1 M $MgCl_2$ solutions in 99.99% D_2O. Poly-L-glutamic acid sodium salt (mol. wt. ~11 000, Sigma) at 30 mg/ml concentration was added to a solution of peptide in D_2O, pH 3, and the pH and salt concentration raised as in the other samples. pH measurements were carried out using a digital pH meter (Radiometer) equipped with an Ingold combined microelectrode and pH was raised to a point between 6.5 and 7.5 by adding small amounts of 0.1 M NaOD/D_2O solution. No correction was made for deuterium isotopic effect in pH measurements. For each spectrum 500–1000 scans were accumulated in quadrature mode with typical spectral parameters of 6000 Hz sweep width, 32 K data points, 32° flip angle and an acquisition time of 2.7 s. 4 Hz line broadening was used throughout prior to transformation. HDO resonance was suppressed by the gated decoupling technique. Sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 was used as internal chemical shift reference. No degradation of protein or peptides was observed after spectra recording as checked by gel electrophoresis.

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