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DISCRETE PROTEOLYTIC CLEAVAGE OF HIGH MOBILITY GROUP PROTEINS

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SUMMARY. Chromatographic fractionation on CM-Sephadex of a 0.35 M NaCl extract from calf thymus chromatin reveals the presence of a High Mobility Group (HMG) protein which comigrates electrophoretically with HMG-17. Further amino acid analysis and partial sequence determination suggest that this protein is a proteolytic degradation product of either HMG-1 or HMG-2 from which the acidic C-terminal region has been removed.

INTRODUCTION. Since the discovery of the high-mobility-group (HMG) proteins in the cell nucleus (1) several laboratories have been engaged in the purification and chemical characterization of proteins of the HMG class. These proteins appear to be present in all tissues so far examined (for a review see ref. 2) and they appear to be rather conserved in evolution although examples of variation from species to species and from tissue to tissue have been reported. For example, amino acid sequence analysis has revealed a trout testis specific HMG (3), while a duck red blood cell HMG (HMG-E) has been characterized by biochemical (4) and by immunological (5) techniques.

HMG proteins appear to play an important role in chromatin organization and there are several experiments indicating that they may be associated with active (or potentially active) regions of the genome. DNase I digestion of chromatin under conditions which are known to selectively digest active genes selectively removes HMG proteins (6), while the selective digestion of globin sequences by DNase I is abolished if these proteins are first extracted from erythrocyte chromatin preparations (7). Chromatin fractionations have also indicated that actively transcribing fractions are enriched in HMG proteins as compared to inactive chromatin (8).

Detailed analyses conducted on the HMG protein class have established that in calf thymus there are at least 4 proteins (HMG-1, HMG-2, HMG-14 and

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HMG-17) and that HMG-17 is not a product of proteolytic degradation of HMG-1 or HMG-2 (9).

This report shows the presence of an HMG protein which is derived from HMG-1 or HMG-2 by proteolytic cleavage of the C-terminal region and which migrates in SDS-polyacrylamide gel electrophoresis in the identical position of HMG-17. These results indicate that electrophoretic migration is not a sufficient criterion for the identification of HMG proteins and further suggest that specific enzymes which specifically cleave the acidic domains of HMG-1 and HMG-2 may exist.

MATERIALS AND METHODS.

Total HMG proteins were prepared from fresh calf thymus following procedures which have been already described (4) by extraction with 0.35 M NaCl followed by selective precipitation with TCA. Fractionation of HMG proteins was achieved by chromatography on CM-Sephadex with a NaCl gradient (0 to 0.7 M in 10 mM Na borate pH 9.0). The presence of characteristic HMGs in eluate fractions was determined by electrophoresis on SDS-polyacrylamide gradient gels (4) under conditions which we have already described. The peaks were collected, dialyzed extensively against water, and lyophilized. All the operations were conducted in the presence of 0.2 mM phenylmethylsulphonyl fluoride. Amino acid sequences were determined on a Beckman automated sequenator. Amino acid analyses were performed on a 120-B Beckman amino acid analyzer following hydrolysis of the sample in 6N HCl for 21 hours at 110° C.

RESULTS AND DISCUSSION.

When total HMG proteins are separated by chromatography on a CM-Sephadex column a complex chromatographic profile is obtained such as the one depicted in Fig. 1. We have found it convenient to screen the various

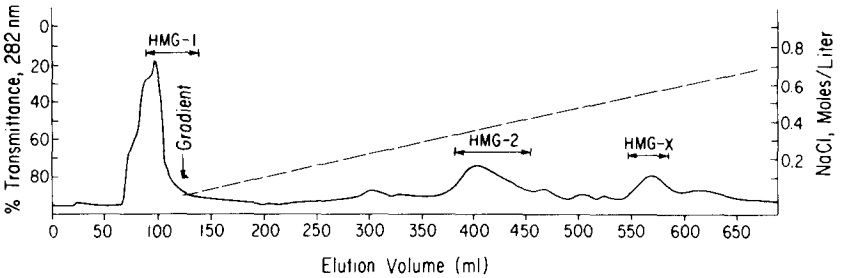

Fig. 1. CM-Sephadex C-25 chromatography of 300 mg of total calf thymus HMG proteins. The column (2.5 x 35 cm) was eluted with 10 mM Na borate, pH 9, at a flow rate of 20 ml per hr. The dashed line indicates the applied linear NaCl gradient.

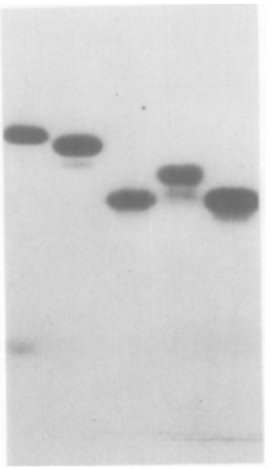

Fig. 2. SDS gradient-polyacrylamide slab gel electrophoresis of calf thymus HMG proteins. A) HMG-1, B) HMG-2, C) HMG-X, D) HMG-14, E) HMG-17.

chromatographic fractions by electrophoresis on SDS-polyacrylamide gels prior to selection of the tubes containing the various HMG proteins in order to minimize cross-contamination due to overlapping of adjacent peaks. Figure 1 indicates the elution positions of HMG-1 and HMG-2. An additional component, designated HMG-X, is eluted in a position well separated from the other HMG proteins. This protein has not been reported previously.

Electrophoretic analyses of the various HMG proteins are shown in Fig. 2. It can be seen that HMG-X is well resolved from HMG-1, HMG-2 and HMG-14, but is coincident with HMG-17 under these conditions. Despite this similarity in electrophoretic properties, amino acid analyses clearly show that HMG-X is not similar to HMG-17. In fact methionine, tyrosine, phenylalanine and histidine are found in HMG-X, but they are not present in acid hydrolyzates of HMG-17. The amino acid composition of HMG-X rather resembles that of HMG-1 or HMG-2 if either of these proteins were cleaved to remove a portion of the C-terminal region which is known to be rich in aspartic and glutamic acid residues (10).

TABLE I

Amino Acid Composition of HMG Proteins from Calf Thymus

Amino Acid	HMG-1	HMG-2	HMG-17	HMG-X
Aspartic Acid	12.9	11.9	12.4	7.5
Threonine	2.1	2.4	1.7	2.5
Serine	6.0	7.3	3.0	5.7
Glutamic Acid	18.2	17.8	10.8	10.4
Proline	5.7	7.5	10.0	6.3
Glycine	5.5	6.0	11.7	7.1
Alanine	8.3	7.9	19.6	13.1
Cysteine	1.3	1.2	n.d.*	Traces
Valine	3.4	3.2	1.9	2.6
Methionine	2.1	1.6	n.d.*	2.3
Isoleucine	1.4	1.2	0.2	2.2
Leucine	2.5	2.5	1.8	3.2
Tyrosine	2.5	2.5	n.d.*	3.5
Phenylalanine	4.3	3.5	n.d.*	4.5
Lysine	18.6	18.0	22.6	23.4
Histidine	1.5	1.4	n.d.*	1.3
Arginine	3.6	4.1	4.1	4.4

The values are expressed as mole per cent.

*n.d. = not detected

This impression is confirmed by partial sequence analyses of HMG-1, HMG-2 and HMG-X. The first 5 amino acids in the amino terminal regions of these 3 proteins are identical (Table II) and well distinct from the amino terminal sequence which has been reported for HMG-17 and HMG-14.

Our analytical results do not indicate whether HMG-X is a natural component of the HMG group, but they strongly suggest that the protein we have isolated could be derived from HMG-1 and/or HMG-2 after a discrete proteolytic digestion has occurred. The proteolytic cleavage site(s) may be very specific since a general tryptic-like activity would be expected to degrade the amino terminal portion of HMG-1 and HMG-2 where lysyl and arginyl residues are most abundantly located (10). It may be noted also that the HMG-X is rather enriched in leucyl residues as compared to the proteins from which it may be derived (Table I) suggesting that many potential chymotryptic sites persist in this fragment.

Table II

Partial Amino Acid Sequence of the Amino Terminal Region of HMG Proteins

HMG-1	GLY-LYS-GLY-ASP-PRO-
HMG-2	GLY-LYS-GLY-ASP-PRO-
HMG-X	GLY-LYS-GLY-ASP-PRO-
HMG-17*	PRO-LYS-ARG-LYS-ALA-
HMG-14*	PRO-LYS-ARG-LYS-VAL-

*These values have been obtained from J.M. Walker et al., Int. J. Peptide Protein Res. 11, 301, 1978.

The results, in any event, show that a simple gel electrophoretogram is not sufficient for the unequivocal identification of a given HMG protein (in this case HMG-17) even in a situation such as the present where a specific, intact protein (HMG-17) has been identified and sequenced (9).

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