The isolation of two peptides from a non-histone chromosomal protein showing irregular charge distribution within the molecule

John M. Walker[†], Kenneth V. Shooter, Graham H. Goodwin & Ernest W. Johns

Chester Beatty Research Institute, Institute of Cancer Research:Royal Cancer Hospital, London SW3 6JB, England.

[†]Biology of Human Cancer Unit, Ludwig Institute of Cancer Research at the Chester Beatty Research Institute, Institute of Cancer Research.

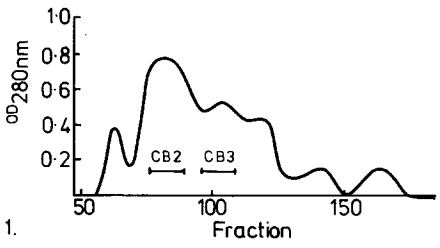
Received February 13, 1976

Summary

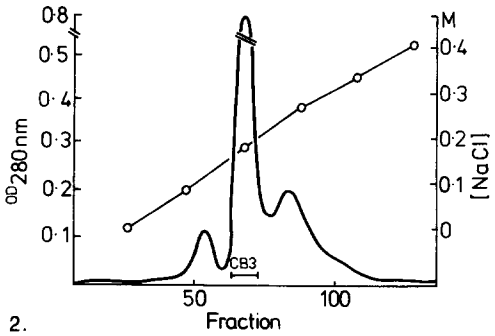
Two peptides, representing about 60% of the total molecule, have been isolated from a cyanogen bromide cleavage of the non-histone chromosomal protein HMG1. The amino acid analyses of these two peptides suggest that lysine residues are fairly evenly distributed within the molecule, whereas the aspartic and glutamic residues are irregularly distributed. One of the peptides represents the C-terminal portion of the molecule and contains a very high proportion of aspartic and glutamic residues. Unlike total HMG1, this peptide does not bind to DNA.

Introduction

One approach to the understanding of the structure and function of chromatin is to isolate and characterise its various components. The major group of proteins in chromatin, the histones, have been extensively studied and are now very well characterised. The complete amino acid sequence of four of the five main fractions is known and that of the remaining fraction (F1) is partially known for a number of different species (1). We have recently reported the isolation and characterisation of two non-histone chromosomal proteins (2,3). These new chromosomal proteins are of interest because of their unusual amino acid composition. They have 25% basic amino acids like the histones but also contain about 30% acidic amino acids and have been designated high mobility group proteins 1 and 2 (HMG1 and HMG2). Their molecular weights as determined by sedimentation equilibrium are both approx. 26000. Because of the quantities present in the nucleus (approx. 10^6 molecules) we feel that, like the histones, HMG1 and HMG2 are structural proteins and not involved in specific gene control. We have recently begun to isolate large peptides from HMG1 and HMG2 for use in conformational studies, DNA interaction studies and amino acid sequence work. The present communication describes the isolation and partial characterisation of two peptides produced by cyanogen



1.



2.

Fig.1 Separation of cyanogen bromide cleaved HMG1 on Sephadex G-50 column size 90 cm x 5 cm, flow rate 85 mls/h. The column was equilibrated in 0.01N HCl containing 1 ml/l of β-mercapto-ethanol and the sample was applied in 5 mls of the same solution

Fig.2 Ion exchange chromatography of CB3 on CM cellulose. Column size 20 cm x 1.4 cm. The column was equilibrated in 0.01M sodium acetate pH 3.3, and the flow rate was 70 mls/h. A linear NaCl gradient from 0 → 0.6M was applied in a volume of 1600 mls.

bromide cleavage of HMG1. These two peptides together represent about 60% of the total protein molecule, and provide information on the charge distribution within the molecule.

Materials and Methods

HMG1 was prepared from calf thymus chromatin as described previously (2).

Cyanogen Bromide Cleavage of HMG1

HMG1 (400 mg) was dissolved in water (24 ml) and 0.2M ammonium bicarbonate (50 ml) added with β-mercaptoethanol (1 ml). The solution was incubated at 37°C for 24 hr and then rotary evaporated to dryness. The sample was dissolved in water (25 ml) and formic acid (96%, 58 ml) added together with solid cyanogen bromide (2.5 g). The solution was then stirred at room temperature for 30 hr. After this time an equal volume of distilled water was added and the solution evaporated to dryness.

Peptide Isolation

The peptide mixture was initially fractionated on a column of Sephadex G-50 (fig.1). The column efluent was monitored at 280 nm and peptide CB2 isolated in pure form by taking a fraction as shown on the elution profile. CB3 was further purified by ion exchange chromatography on CM cellulose (fig.2). Both peptides were recovered by precipitation with 6 vol. of acetone, centrifuged, washed three times with acetone and finally dried under vacuum. 50 mg of CB2 and 23 mg of CB3 were obtained.

Amino Acid Analysis

Total amino acids were measured using a JEOL JLC-6AH amino acid analyser. Samples were hydrolysed for 24 hr in 6N HCl at 110°C. No corrections were made for hydrolytic losses.

Polyacrylamide Gel Electrophoresis and Isoelectric Focusing

Polyacrylamide gel electrophoresis using 20% polyacrylamide gels at pH 2.4 was carried out as described by Johns (4). Analytical thin layer isoelectric focusing was performed using the LKB 2117 Multiphor apparatus essentially following the manufacturer's instructions (application note 75) for the pH range 3.5 → 9.5. Total electrofocusing time was 2 hr. All gels were stained with Coomassie Brilliant Blue.

Molecular Weight Determinations

Molecular weights were determined by the short column, low speed, sedimentation equilibrium method using a Beckman-Spinco analytical ultracentrifuge with the interference optical system. For these experiments the peptides were dissolved in 0.1M NaCl, 10mM HCl at a concentration of 4 mg/ml. The partial specific volume calculated from the amino acid composition was 0.7137 ml/g for CB2 and 0.7571 ml/g for CB3.

DNA Interaction Studies

DNA, isolated from bacteriophage T7 by the method of Lawley *et al* (5), was dissolved in 0.1M tris-HCl buffer pH 8.5 at a concentration of 100 µg/ml. To this solution was added an equal volume of a solution of peptide CB2 (2 mg/ml) in the same buffer. Sedimentation experiments were performed using the UV absorption system and the sedimentation coefficients of the DNA calculated in the usual way (6).

Results and Discussion

Peptide CB2

Peptide CB2 gave a single band on polyacrylamide gel electrophoresis and a single band on isoelectric focusing with an isoelectric point of approx. 4 (fig.3). The sample was also homogeneous as judged by sedimentation equilibrium and gave a molecular weight of 8,900, which represents one third of the total protein chain of HMG1. Since this is the only peptide fraction from the cyanogen bromide cleavage that does not contain homoserine or homoserine lactone, this peptide must represent the C-terminal portion of the molecule.

From the amino acid analysis (Table 1) we see that 47% of the peptide comprises aspartic and glutamic acid residues. This represents a total of 39 aspartic and glutamic residues out of a total of about 75 aspartic and glutamic residues in the total molecule, i.e. 50% of the total aspartic and glutamic residues of HMG1 are concentrated in this peptide. 20% of the residues in the peptides are lysine, which is approximately the same as in total HMG1.

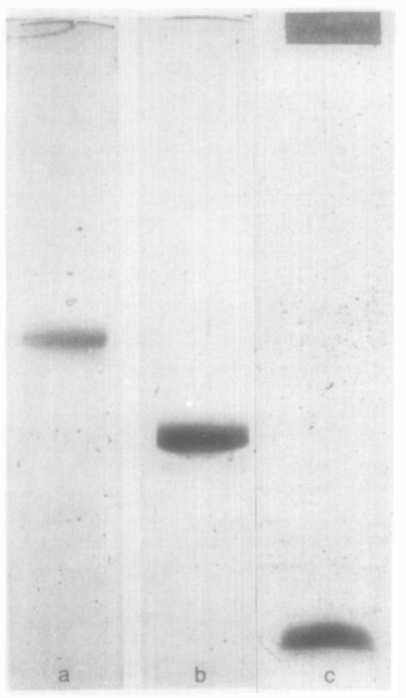


Fig.3 (a) acrylamide gel electrophoresis of CB2
(b) acrylamide gel electrophoresis of CB3
(c) isoelectric focusing of CB2

Peptide CB3

Peptide CB3 gave a single band on polyacrylamide gel electrophoresis (fig.3) but ran beyond the basic end of the isoelectric focusing gradient. The sample was homogeneous as judged by sedimentation equilibrium and gave a molecular weight of 6,500, which represents about 24% of the total molecule. Like peptide CB2, 22% of the residues are lysine. However, only 14% of the residues are aspartic or glutamic acid. This is a total of only 8 aspartic and glutamic residues in a total of 59 amino acids. This portion of the molecule is therefore basic, and has an isoelectric point $>$ pH 8.5.

From the above results we can conclude that the aspartic and glutamic residues of HMG1 are irregularly distributed along the protein chain, and are, at least partly, concentrated in the C-terminal portion of the molecule. The C-terminal portion of the molecule is acidic and highly charged, 55 of the last 82 residues being either aspartic acid, glutamic acid or lysine. In both peptides lysine is present in the same ratio as found in total HMG1 which suggests that the lysine residues are fairly evenly

Table 1 Amino acid composition of protein HMG1 and peptides CB2 and CB3

Amino acid	HMG1	CB2 mol/100 mol	CB3
Aspartic acid	11.1	16.9	5.8
Threonine	2.9	2.4	3.0
Serine	5.6	1.9	5.1
Glutamic acid	19.4	30.1	8.3
Proline	7.1	4.0	12.0
Glycine	5.7	3.3	7.5
Alanine	9.1	12.2	5.3
Valine	2.4	1.5	1.7
Cysteine	trace	-	0.5
Methionine	1.4	-	-
Isoleucine	1.8	2.0	6.4
Leucine	2.0	1.8	6.1
Tyrosine	0.8	0.4	2.4
Phenylalanine	4.2	0.3	6.7
Lysine	19.3	20.2	21.9
Histidine	1.5	0.2	2.0
Arginine	3.9	1.6	3.9
Homoserine and Homoserine lactone	-	none	$\sim$0.5

Table 2 Interactions of CB2 and HMG1 with DNA in 0.1M tris-HCl buffer pH 8.5

Sample	$S_{20,w}$
T7-DNA	17.9 (SD = 0.2)
T7-DNA + CB2 (1/10, w/w)	18.7 (SD = 0.2)
T7-DNA + HMG1 (1:10, w/w)	80 (SD = 0.6)

distributed within the molecule. We have previously shown by sedimentation equilibrium studies that HMG1 binds to DNA (7). Under identical conditions no significant binding of CB2 to DNA could be observed (Table 2). Neither can interactions with DNA be observed by n.m.r. studies (P.D. Cary and E.M. Bradbury, personal communication). This is not surprising in view of the highly acidic nature of the peptide, and we may therefore conclude that the C-terminal portion of HMG1 is not involved in binding to DNA.

Acknowledgements

This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research:Royal Cancer Hospital) from the Medical Research Council and the Cancer Research Campaign. One of us (J.M.W.) acknowledges the Ludwig Institute of Cancer Research for a Fellowship.

References

1. De Lange, R.J. and Smith, E.L. (1975) Ciba Found.Symp. 28, pp.59-70, Elsevier Scientific Publishing Company, Amsterdam.
2. Goodwin, G.H., Nicolas, R.N. and Johns, E.W. (1975) Biochem.Biophys. Acta, 405, 280-291
3. Walker, J.M., Goodwin, G.N. and Johns, E.W. (1976) Eur.J.Biochem. In Press.
4. Johns, E.W. (1967) Biochem.J. 104, 78-82.
5. Lawley, P.D., Lethbridge, J.M., Edwards, P.A. and Shooter, K.V. (1969) J.Mol.Biol. 39, 181-198
6. Shooter, K.V. and Butler, J.A.V. (1956) Trans.Faraday Soc. 52, 734-742
7. Shooter, K.V., Goodwin, G.H. and Johns, E.W. (1974) Eur.J.Biochem. 47, 263-270.