THE ISOLATION OF THE HIGH MOBILITY GROUP NON-HISTONE CHROMOSOMAL PROTEIN HMG 14

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1. Introduction

Chromatin contains a group of non-histone proteins called the High Mobility Group (HMG) proteins [1]. There are four main HMG proteins, HMG 1, 2, 14 and 17, in thymus which we have shown to be present in isolated nucleosomes [2]. Three of these proteins, HMG 1, 2 and 17, and an HMG protein from trout testis, HMG T, have been isolated in a pure form [3,4,5]. The amino acid sequence of HMG 17 has recently been determined [6]. The fourth thymus HMG protein, HMG 14, is present in chromatin in much smaller quantities than the other three and has been more difficult to isolate in a pure form. In this paper we report the large scale isolation of this protein from pig thymus.

2. Experimental

All operations in sections (2.1.). and (2.2.) below were carried out at 4°C except the acetone and ethanol precipitations which were at room temperature.

2.1. *Isolation of total HMG proteins from pig thymus*

Total HMG protein was isolated from frozen pig thymus by perchloric acid (PCA) extraction as follows [4].

Minced thymus, 1.6 kg, was divided into four portions and each blended with 600 ml 5% (w/v) PCA for 3 min using a Kenwood domestic blender. The homogenate was centrifuged at 2500 × *g* for 30 min. Each pellet was extracted twice more in the same way with 600 ml and then 300 ml 5% (w/v) PCA. The combined supernatants (6.4 litres) were filtered through surgical gauze and then through No. 4 sintered glass funnels. The filtrate was made 18% (w/v) trichloroacetic acid (TCA) by the addition of 100% (w/v) TCA. The precipitate which formed consisted of the HMG proteins and histone H1 and was collected by centrifugation at 4500 × *g* for 15 min. The precipitate was washed with acetone/conc. HCl (400 : 1, v/v) then several times with acetone before drying under vacuum. The majority of histone H1 was separated from the HMG proteins by redissolving the protein in 0.1 N HCl at a concentration of 10 mg/ml and adding 3 vol. acetone. The precipitated histone H1 was centrifuged down at 4500 × *g* for 10 min and the supernatant clarified by filtering through a No. 4 sintered glass funnel. The HMG protein in the filtrate was then precipitated by the addition of a further 3 vol. acetone. This precipitate was collected by centrifugation, washed with acetone and dried as described above.

2.2. *Isolation of protein HMG 14 from total HMG proteins*

A partial fractionation of the total HMG proteins by ethanol precipitation was carried out first, to remove about half of the HMG 1 and 2. The total HMG proteins (0.7 g) obtained as described in (2.1.) were dissolved in 0.1 N HCl at a concentration of 50 mg/ml and 12.5 vol. ethanol/conc. HCl (99 : 1, v/v) added. This precipitates HMG 14 and HMG 17 and about half the HMG 1 and 2. The protein precipitate was collected by centrifuging at 2000 × *g* for 1 h, washed with acetone and dried. The protein thus obtained was dissolved in 10 ml 7.5 mM

sodium borate buffer (pH 8.8). The pH of the solution was readjusted to 9 with 1 N NaOH and solid sodium chloride added, to bring the total sodium ion concentration to 0.1 M. The solution was clarified by centrifugation at 90 000 X *g* for 30 min followed by filtration through a small No. 4 sintered glass funnel. The protein solution was applied to a CM–Sephadex C25 column (2.5 X 22 cm) previously equilibrated with 7.5 mM sodium borate buffer (pH 8.8). 100 ml 0.1 M NaCl, 7.5 mM sodium borate (pH 8.8) was then pumped through the column at a flow rate of 1 ml/min followed by a 2 litre linear salt gradient from 0.1–0.6 M NaCl in 7.5 mM sodium borate (pH 8.8). The absorbance of the fractions at 220 nm was measured (fig.1). Protein HMG 14, eluting at about 0.25 M NaCl, was precipitated from the pooled fractions by acidifying to 0.1 N HCl and adding 6 vol. acetone. The protein precipitate was washed with acetone/0.1 N HCl (6 : 1, v/v), several times with acetone and dried. The yield of HMG 14 was 15 mg.

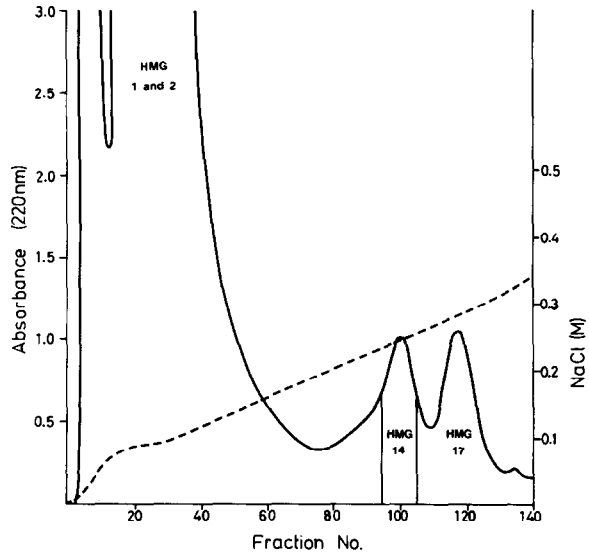

Fig.1. The elution profile of the CM–Sephadex C25 ion-exchange chromatography of the HMG proteins. 10 ml fractions were collected and the absorbance at 220 nm measured (——). The salt gradient (- - - -) was determined from the conductivity of the fractions. Fraction HMG 14 was isolated from the pooled fractions indicated by the vertical lines.

2.3. *Analytical techniques*

Polyacrylamide gel electrophoresis, amino acid analyses and N-terminal amino acid analyses were carried out as described previously [3].

3. Results and discussion

The isolation of HMG 14 by the method described in this paper involves the following steps:

(i) Isolating the total HMG proteins from thymus by 5% PCA extraction.

(ii) Increasing the amount of HMG 14 relative to the other proteins by selective precipitations with 18% TCA (which removes the bulk of HMG 17), acetone (removing the majority of histone H1) and then with ethanol (removing about half the HMG 1 and 2).

(iii) The protein thus obtained, enriched in HMG 14, is finally fractionated by CM–Sephadex chromatography (fig.1). The peak eluting in between HMG 2 and HMG 17 is the purified HMG 14 as can be seen from the polyacrylamide gel electrophoretic analysis (fig.2).

In order to isolate HMG 14 it is essential to prevent proteolytic degradation during the isolation of the total HMG proteins, otherwise a number of degradation products of other nuclear proteins elute from the CM–Sephadex column together with HMG 14. Thus, instead of isolating chromatin and extracting with 0.35 M NaCl as we have done in the past when isolating other HMG proteins [1,3], the tissue is directly extracted with PCA. Since protein HMG 14 is a minor component relative to the other proteins in the PCA extract, the subsequent selective precipitation steps with TCA, acetone and ethanol are employed to obtain a protein fraction enriched as much as possible with HMG 14 prior to separation on the CM–Sephadex column.

The amino acid analysis of the HMG 14 protein prepared by this method from pig thymus is given in table 1. It can be seen that this protein is a typical HMG protein having a high content of basic and acidic amino acids. Protein HMG 14 is similar to HMG 17 in having very few aromatic residues (an amino acid analysis of pig thymus HMG 17 is included in table 1 for comparison). Also, protein

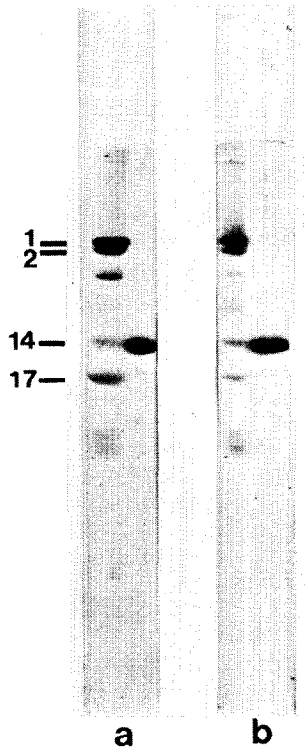

Fig.2. Comparative polyacrylamide gel electrophoresis of total HMG and the purified HMG 14 from the CM–Sephadex chromatography. On the right of each gel is the purified HMG 14. On the left of gel (a) is pig thymus total HMG protein extracted with 5% (w/v) PCA and precipitated with acetone [7]. On the left of gel (b) is total HMG extracted with 5% PCA and precipitated with 18% (w/v) TCA. (Some histone H1 is present in both total HMG protein samples and runs between HMG 2 and HMG 14.)

HMG 14 has an N-terminal proline residue the same as HMG 17 [3]. It differs markedly from HMG 17 in having a much higher content of serine and glutamic acid and a lower content of aspartic acid.

The similarity of HMG 14 and 17 raises the possibility that HMG 14 is a degradation product of HMG 17. There are, however, three lines of evidence against this.

(1) HMG 14 appears to have a higher molecular weight than HMG 17 since it has a lower mobility on sodium dodecylsulphate polyacrylamide gels (not shown).

Table 1
The total and N-terminal amino acid analyses of pig thymus HMG 14, calf kidney HMG 14 and pig thymus HMG 17

	Pig thymus HMG 14	Calf kidney HMG 14	Pig thymus HMG 17
Asp	5.6	6.8	11.7
Thr	2.8	5.2	1.4
Ser	7.3	7.8	2.3
Glu	16.3	15.4	9.4
Pro	8.3	9.3	12.1
Gly	7.2	7.6	10.8
Ala	18.2	14.2	18.3
Val	3.7	3.9	2.3
Cys	–	–	–
Met	0.4	0.3	–
Ile	–	0.6	–
Leu	1.9	2.4	1.1
Tyr	0.3	0.5	0.2
Phe	0.1	0.7	0.1
Lys	20.6	18.5	25.3
His	0.7	0.4	0.1
Arg	6.5	5.0	4.9
N-terminal amino acid	Proline	Proline	Proline

The total amino acids are given as moles % of all amino acids recovered

(2) HMG 14 is still present in PCA extracts of thymus tissue taken from animals directly after slaughter and transported to the laboratory in liquid nitrogen. In calf thymus tissue if care is not taken to prevent degradation, the quantity of HMG 14 relative to the other HMG proteins decreases (Goodwin, Walker and Johns, manuscript in preparation).

(3) From the sequence of HMG 17 [6] it does not appear possible that HMG 14 is derived from it.

It is also conceivable that HMG 14 is a degradation product produced by acid hydrolysis during PCA extraction and precipitation of HMG protein. That this is not so is demonstrated by the fact that if total HMG protein is obtained by extracting chromatin with 0.35 M NaCl and collected by liphyolisation, it still has HMG 14 present.

Finally, we have checked that HMG 14 is indeed a nuclear protein like the other three HMG proteins by isolating total HMG protein from purified nuclei from various tissues. The HMG 14 protein was found to be present in all these preparations and in table 1 is given the preliminary amino acid analysis of a partially purified HMG 14 isolated from calf kidney nuclei. It is very similar to the pig thymus protein; the higher quantity of aromatic residues in the kidney protein is probably due to contamination, as this fraction has not yet been adequately purified, but is given merely for comparison.

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