MICROHETEROGENEITY IN A NON-HISTONE CHROMOSOMAL PROTEIN

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1. Introduction

The non-histone proteins that are extracted from chromatin with 0.35 M NaCl contain a group of proteins which are characterised by their solubility in trichloroacetic acid and by their relatively low molecular weights (< 30 000) [1]. These proteins, termed the high mobility group (HMG) proteins, have been fractionated and the main components isolated in a pure form [2]. A recent detailed comparison of two of the HMG proteins which have high contents of basic and acidic amino acids (proteins HMG 1 and HMG 2) has indicated that their amino acid sequences are closely related [3]. However, the situation has been somewhat complicated by the recent finding that both proteins appear to exhibit multiple forms when analysed by isoelectric focusing. Protein HMG 1 displays a rather complex banding pattern in the pH 6–8 range which could be due to aggregation, but protein HMG 2 clearly has four main subfractions. In view of the possible importance of microheterogeneity in these non-histone proteins, reminiscent of that found with histone F1 [4], we are currently developing procedures for isolating the subfractions. In this paper a method for isolating protein HMG 2 subfractions is presented.

2. Materials and methods

2.1. *Preparation of protein HMG 2*

Total HMG protein was obtained from 2 kg of calf thymus as described previously [2] and fractionated by a modification of the chromatographic procedure described in [2]. It was found that more reproducible results were obtained by increasing the CM-Sephadex C25 ion-exchanger/protein ratio by two and by lowering the sodium chloride concentration of the loading solution from 0.2 M to 0.15 M NaCl. The modified procedure is outlined below.

The total HMG protein (approximately 2 g) dissolved in 7.5 mM sodium borate buffer (pH 8.8)–10 mM mercaptoethanol, was titrated to pH 8.8 and the solution dialysed versus the 7.5 mM borate–10 mM mercaptoethanol buffer containing 0.15 M NaCl. The solution was then loaded onto a 5 × 50 cm column of CM-Sephadex C25 equilibrated with 7.5 mM sodium borate buffer (pH 8.8)–10 mM mercaptoethanol. After pumping 400 ml of 0.15 M NaCl dissolved in the 7.5 mM borate–10 mM mercaptoethanol buffer through the column at a flow rate of 2 ml/min, a 3.2 litre linear salt gradient, 0.15–2 M NaCl dissolved in the 7.5 mM borate–10 mM mercaptoethanol, was pumped through the column at the same flow rate. Protein HMG 2, eluting at about 0.25 M NaCl (Fraction C in fig.1 of raf. [2]), was recovered by acidifying to 0.1 N HCl and adding 6 volumes of acetone.

2.2. *Fractionation of protein HMG 2*

150 mg of protein HMG 2 was dissolved in 15 ml of 7.5 mM sodium borate buffer (pH 9) and readjusted to pH 9 with 1 N NaOH. The solution was dialysed overnight versus 4 litres of the 7.5 mM borate buffer (pH 9). After centrifugation at 35 000 *g* for 1 h the solution was applied to a 2.5 × 15 cm CM-cellulose column equilibrated with 7.5 mM borate buffer (pH 9). An 800 ml linear salt gradient, 0–0.15 M NaCl dissolved in the borate buffer, was pumped through the column at a flow rate of 0.8 ml/min. 5 ml fractions were collected and the optical density at 230 nm measured. Protein fractions were recovered

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by acidifying to 0.1 N HCl and adding 6 volumes of acetone.

2.3. *Isoelectric focusing*

Isoelectric focusing was carried out using the LKB Multiphor apparatus essentially following the manufacturers' instructions (application note 75) for the pH range 3.5–9.5. Proteins were dissolved in water or 10 mM HCl at concentrations of 5–6 mg/ml and 10 μl applied onto filter paper rectangles placed on the gel. Total electrofocusing time was 2 h. The gel slab was stained with Coomassie brilliant blue [5].

3. Results and discussion

Protein HMG 2 has a single N-terminal residue, glycine [2], and shows just one band when analysed either by SDS-polyacrylamide gel electrophoresis [2] or by electrophoresis in low pH gels provided the protein is fully reduced [3]. However, isoelectric focusing revealed that the protein has a number of subfractions [3] which are not altered by treatment with disulphide reducing agents, alkaline phosphatase or neutral hydroxylamine. To isolate these subfractions the protein was chromatographed on CM-cellulose using a shallow salt gradient. Fig.1 shows the elution profile obtained. The isoelectric focusing of the five peaks is shown in fig.2. The run-through peak (R) is a small amount of protein HMG 1 contamination; the following four peaks (A–D) are the protein HMG 2 subfractions eluting in order of increasing basicity. Peaks A, B, C and D are each composed of one prominent isoelectric focusing band but because of the high loading onto the gel (~ 50 μg protein) a small amount of cross contamination of the peaks is apparent. The amino acid analysis of a number of such preparations (table 1 gives one set of analyses) have not as yet revealed any statistically significant differences between the subfractions. The microheterogeneity observed could be due to post-synthetic modifications (although, so far, we have

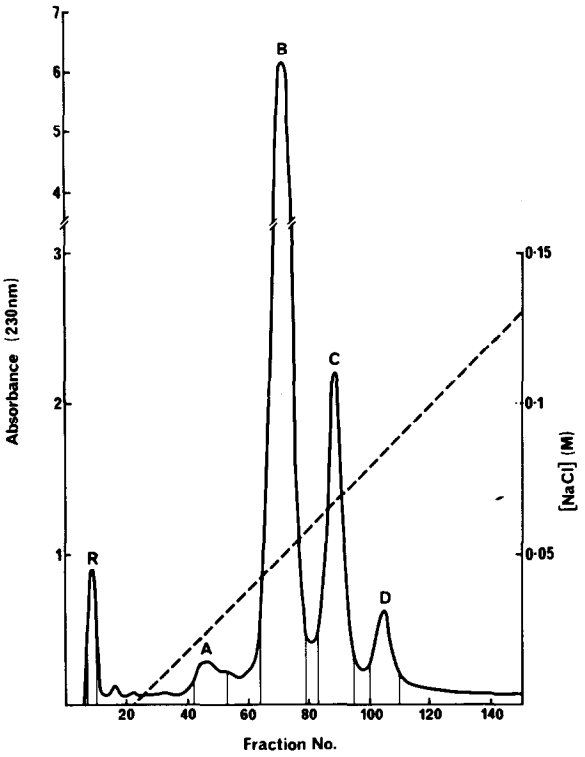

Fig.1. CM-cellulose chromatography at pH 9 of whole protein HMG 2. (———) Absorbance at 230 nm. (– – – – –) sodium chloride concentration. 5 ml fractions were collected.

Table 1
Amino acid analyses (moles %) of whole protein HMG 2 and protein HMG 2 subfractions

	Whole HMG 2	CM-cellulose fractions			
		A	B	C	D
Asp	9.3	9.3	10.0	9.7	9.0
Thr	2.7	2.1	2.0	2.0	2.5
Ser	7.4	7.2	7.6	7.5	7.8
Glu	17.5	17.3	18.4	17.6	17.3
Pro	8.9	10.6	8.3	9.4	8.3
Gly	6.5	5.9	6.6	6.2	6.8
Ala	8.1	7.0	7.7	7.7	7.9
Val	2.3	1.5	1.4	1.8	2.2
Cys	–	–	0.2	–	–
Met	0.4	0.3	0.6	0.6	0.7
Ile	1.3	1.7	1.1	1.1	1.5
Leu	2.0	2.3	1.6	1.7	2.3
Tyr	2.0	3.2	2.4	2.3	2.5
Phe	3.0	3.1	3.1	3.1	3.5
Lys	19.4	19.1	19.8	20.1	18.6
His	2.0	2.1	2.1	2.1	2.3
Arg	4.7	4.6	4.3	4.6	4.7
Yields (mg)		3	49	14	5

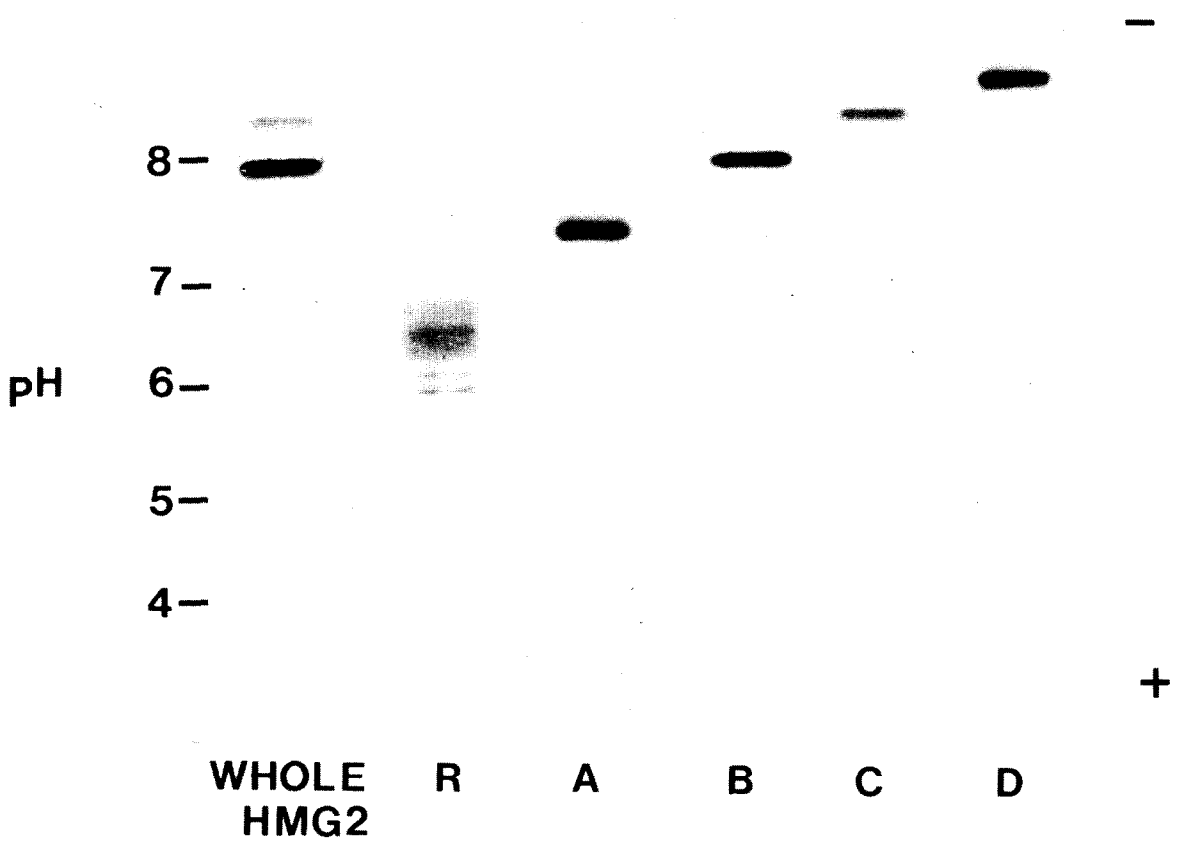

Fig.2. Isoelectric focusing of whole protein HMG 2 and fractions R, A, B, C, D obtained by CM-cellulose chromatography of whole protein HMG 2.

found that alkaline phosphatase and hydroxylamine have no effect on the isoelectric focusing pattern) or due to sequence differences. These possibilities are currently being examined in this laboratory. Finally, it is of interest to point out that the microheterogeneity in protein HMG 2 is not tissue specific since the isoelectric focusing patterns of HMG 2 from calf liver and thymus are the same (unpublished result).

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