THE ISOLATION AND IDENTIFICATION OF UBIQUITIN FROM THE HIGH MOBILITY GROUP (HMG) NON-HISTONE PROTEIN FRACTION

John M. WALKER, Graham H. GOODWIN and Ernest W. JOHNS
Chester Beatty Research Institute, Institute of Cancer Research and *Royal Cancer Hospital, Fulham Road, London SW3 6JB, England*

Received 13 April 1978

1. Introduction

Chromatin contains a group of non-histone chromosomal proteins called the high mobility group (HMG) proteins [1]. Four of the HMG proteins from calf thymus, HMG 1, 2, 14 and 17, and an HMG protein from trout testis, HMG-T, have been isolated in a pure form [2–5]. The amino acid sequence of calf thymus HMG 17 has recently been determined [6], and we have also shown that HMG proteins HMG 1, 2, 14 and 17 are present in isolated nucleosomes [7]. Although we have shown that there are 4 main HMG components in thymus [3], we have consistently noted 3 weaker bands running faster than HMG 17 on polyacrylamide gels (see gels A and B of fig.1 in [3] and this paper). These proteins have been tentatively designated HMG 18, 19 and 20, in order of increasing electrophoretic mobility. We report in this paper the isolation of protein HMG 20 from calf thymus. Chemical analysis of this protein shows it to be the same as the isolated protein ubiquitin, a protein identified as part of a non-histone chromosomal protein complex, but the function of which is as yet unknown.

2. Experimental

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

Total HMG proteins from calf thymus were obtained as described for pig thymus [3].

2.2. *Isolation of protein HMG 20 from total HMG proteins*

The total HMG proteins (0.7 g) obtained as described above were dissolved in 0.1 N HCl at 50 mg/ml and 12.5 vol. ethanol/conc. HCl (99:1, v/v) added. This precipitates HMG 14 and HMG 17, most of the HMG 2 and some HMG 1. Precipitated protein was removed by centrifugation at 2000 × *g* for 30 min. Protein remaining in the ethanol–HCl supernatant was precipitated with 6 vol. acetone, washed with acetone and dried (yield 200 mg). This material, containing mainly HMG 1 and HMG 20, was further fractionated by gel filtration. The material was divided into 2 lots of 100 mg, each dissolved in 0.5 ml 0.005 N HCl and passed separately down a Sephadex G-75 Superfine column (2.4 × 100 cm) pre-equilibrated in 0.005 N HCl. Proteins eluted were detected by their absorbance at 230 nm (fig.1). The protein was precipitated from the pooled fraction (peak A, fig.1) 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), then several times with acetone and dried. HMG 20 protein yield was 20 mg.

2.3. *0.35 M saline extraction of isolated nuclei*

In order to show the presence of proteins HMG 18, 19 and 20 in isolated nuclei the following procedure was carried out to purify calf thymus nuclei. These nuclei were then extracted with 0.35 M saline to give total calf thymus HMG proteins.

Fresh minced calf thymus, 120 g, was homogenised

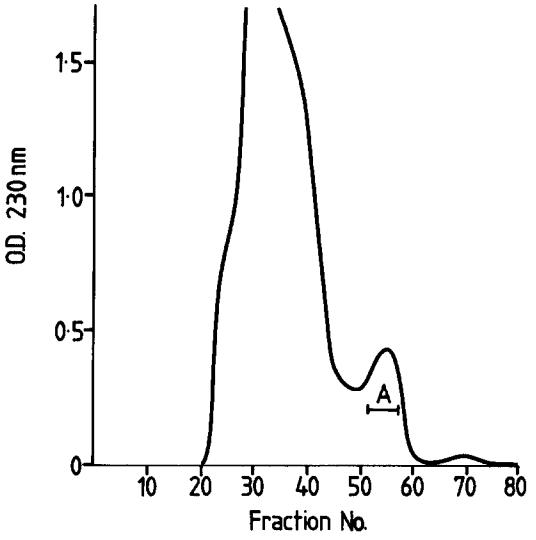

Fig.1. Sephadex G-75 Superfine gel filtration of protein recovered from the ethanol supernatant (section 2.2). Peak A was purified HMG 20 and was collected as described in section 2.2.

with 750 ml solution A (0.25 M sucrose, 3 mM $CaCl_2$, 5% isopropanol, 1 mM phenylmethyl-sulfonyl fluoride) at low speed for 3 min. The suspension thus produced was passed through a double layer of gauze and then centrifuged at 2000 × *g* for 10 min. The supernatant was discarded and the sediment gently resuspended in the same buffer (50 ml) using a Potter homogeniser. This suspension was then mixed with 125 ml solution B (2.25 M sucrose, 3 mM $CaCl_2$, 5% isopropanol, 1 mM phenylmethyl-sulfonyl fluoride), layered over 4 centrifuge tubes containing approx. 15 ml solution B and centrifuged at 30 000 × *g* for 1 h. The precipitated nuclei were then washed twice by gently blending with 50 ml 3 mM $CaCl_2$, containing 1 mM phenylmethyl-sulfonyl fluoride and 5% isopropanol, and centrifuging at 2000 × *g* for 10 min after each wash. The precipitated nuclei were then extracted 3 times with 30 ml 0.35 M NaCl containing 1 mM phenylmethyl-sulfonyl fluoride and 5% isopropanol. The total extract (90 ml) was made 2% in trichloroacetic acid (TCA) by the addition of 100% TCA, and the resultant precipitate removed by centrifugation. The supernatant containing HMG proteins was made 0.2 N HCl and protein precipitated with 6 vol. acetone. This precipitate was washed twice with acetone/0.1 N HCl (6:1, v/v) then several times with acetone and dried. Total HMG protein yield was 40 mg.

2.4. *Analytical techniques*

Polyacrylamide gel electrophoresis, amino acid analyses and N-terminal amino acid analyses were carried out as in [2]. The amino terminal sequence of HMG 20 was determined on 4 mg protein by analysis on a Beckman 890 C Protein Sequenator using the standard DMAA buffer programme. Recovered PTH amino acids were identified both qualitatively by thin-layer chromatography and quantitatively by back hydrolysis to the free amino acid with subsequent amino acid analysis, as in [8]. The molecular weight of HMG 20 was determined by sedimentation equilibrium in 0.1 M NaCl, 0.1 M HCl using the methods in [9].

3. Results and discussion

The amino acid analysis and N-terminal analysis of calf thymus HMG 20 is shown in table 1, together with the same data for ubiquitin. It can be seen that

Table 1
The total and N-terminal amino acid analyses of calf thymus HMG 20 and calf thymus ubiquitin

	HMG 20 (mol %)	Ubiquitin [10] (mol %)
Asp	9.0	8.8
Thr	8.7	8.7
Ser	4.7	4.0
Glu	16.8	16.5
Pro	5.3	4.3
Gly	6.7	6.6
Ala	4.2	3.0
Val	4.3	5.4
Cys	–	–
Met	1.2	0.8
Ile	7.3	9.2
Leu	9.9	12.0
Tyr	1.0	1.1
Phe	2.5	2.7
His	1.2	1.3
Lys	11.8	9.7
Arg	4.7	5.9
N-terminal amino acid	Met	Met

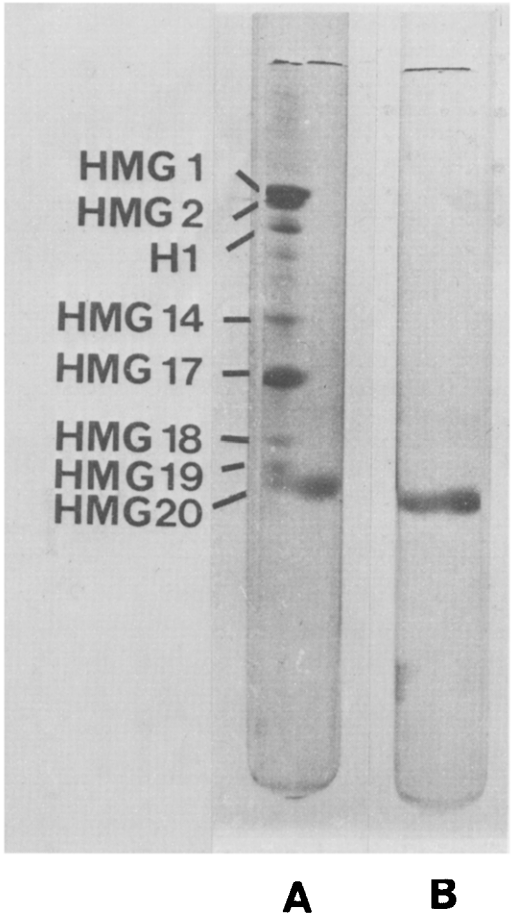

Fig.2. (Gel A) Comparative polyacrylamide gel electrophoresis of total HMG proteins (100 μg) extracted from purified nuclei against purified HMG 20 (10 μg). (Gel B) Comparative gel electrophoresis of purified HMG 20 against ubiquitin (10 μg of each protein).

the analysis of both proteins is very similar and that they both share the same N-terminal amino acid. The amino terminal sequence of the first 20 residues of HMG 20 is shown in fig.3. This sequence is identical to the first 20 residues of ubiquitin isolated from calf thymus, the complete amino acid sequence of which is in [10]. Comparative gel electrophoresis of HMG 20 against ubiquitin (fig.2, gel B) shows these two proteins to have identical mobilities in the gel system used. Figure 2, gel A shows proteins HMG 18, 19 and 20 to be present in the 0.35 M saline extracts of purified nuclei, together with the previously studied

```
                              5                                  10
Met - Gln - Ile - Phe - Val - Lys - Thr - Leu - Thr - Gly -

                              15                                 20
Lys - Thr - Ile - Thr - Leu - Glu - Val - Glu - Pro - Ser.
```

Fig.3. The amino-terminal sequence of HMG 20.

high mobility group proteins 1, 2, 14 and 17. HMG 20 was determined to be mol. wt 7800 by sedimentation equilibrium. This compares favourably with ubiquitin mol. wt 8400 [10].

From the above results it is clear that the protein band designated HMG 20 in fig.2 is the same as the protein ubiquitin, and therefore the procedures outlined in section 2 offer a convenient method of preparing ubiquitin, together with the major HMG proteins, from calf thymus chromatin. The idea that ubiquitin is in some way involved in the structure of the nucleus is not without precedent. A non-histone chromosomal protein, A24, was isolated from calf thymus chromatin [11]. This protein was shown to consist of histone H2A covalently attached to a non-histone protein chain via an isopeptide linkage [12]. The amino-terminal sequence of the first 37 residues of the non-histone portion of A24 has been determined and has been shown to be identical to that of ubiquitin [13,14]. It has therefore been suggested that the N-terminal portion of non-histone protein A24 has the same sequence as ubiquitin [14]. The possible involvement of protein A24 in the regulation of gene expression and the possible manner in which ubiquitin may be released from the A24 protein complex is discussed at length in [14]. However, the exact nature of the involvement of ubiquitin in chromatin structure must, of course, await further experimental data.

Acknowledgements

The authors wish to thank Miss B. Parker for her excellent technical assistance and Dr J. R. B. Hastings for carrying out the amino acid analyses. We also wish to thank Dr G. Goldstein of the Ortho Pharmaceutical Co., NJ, for kindly providing us with a sample of ubiquitin. This work was 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.

References

[1] Johns, E. W., Goodwin, G. H., Walker, J. M. and Sanders, C. (1975) in: The Structure and Function of Chromatin, Ciba Found. Symp. 28, pp. 95–112, Elsevier Excerpta Medica, Amsterdam, New York.
[2] Goodwin, G. H., Nicolas, R. H. and Johns, E. W. (1975) Biochim. Biophys. Acta 405, 280–291.
[3] Goodwin, G. H., Rabbani, A., Nicolas, R. H. and Johns, E. W. (1977) FEBS Lett. 80, 413–416.
[4] Sanders, C. (1975) Ph. D. Thesis, London University.
[5] Watson, P. C., Peters, E. H. and Dixon, G. H. (1977) Eur. J. Biochem. 74, 53–60.
[6] Walker, J. M., Hastings, J. R. B. and Johns, E. W. (1977) Eur. J. Biochem. 76, 461–468.
[7] Goodwin, G. H., Woodhead, L. and Johns, E. W. (1977) FEBS Lett. 73, 85–88.
[8] Walker, J. M., Goodwin, G. H. and Johns, E. W. (1978) Int. J. Peptide Protein Res. in press.
[9] Walker, J. M., Shooter, K. V., Goodwin, G. H. and Johns, E. W. (1976) Biochem. Biophys. Res. Commun. 70, 88–93.
[10] Schlesinger, D. H., Goldstein, G. and Niall, H. D. (1975) Biochemistry, 14, 2214–2218.
[11] Goldknopf, I. L., Taylor, C. W., Baum, R. M., Yeoman, L. C., Olson, M. O. J., Prestayko, A. W. and Busch, H. (1975) J. Biol. Chem. 250, 7182–7187.
[12] Goldknopf, I. L. and Busch, H. (1977) Proc. Natl. Acad. Sci. USA 74, 864–868.
[13] Olson, M. O. J., Goldknopf, I. L., Gutetzow, K. A., James, G. T., Hawkins, T. C., Mays-Rothberg, C. J. and Busch, H. (1976) J. Biol. Chem. 251, 5901–5903.
[14] Hunt, L. T. and Dayhoff, M. O. (1977) Biochem. Biophys. Res. Commun. 74, 650–655.