TROUT LIVER HIGH MOBILITY GROUP NON-HISTONE CHROMOSOMAL PROTEINS

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1. Introduction

The high mobility group (HMG) non-histone chromosomal proteins have been studied in detail during the past few years, 4 of these proteins, HMG1, 2, 14 and 17, having been isolated in a pure form from mammalian tissues [1–4]. Studies on these HMG proteins reveal that they are widely distributed; not only have they been isolated from a variety of mammalian tissues [1,5] but also from the transcriptionally inactive chicken erythrocyte [6,7]. Trout testis [8–11], insects [12,13], yeast [14,15] and wheatgerm [14] have also been shown to contain similar proteins.

In the case of trout testis, two HMG-like proteins have so far been described by Dixon and co-workers. These are: (a) the protein called HMG-T which is similar, but not identical, to calf thymus HMG1 and HMG2 in its amino acid analysis and N-terminal sequence [9,16]; (b) the protein H6, originally classified as a histone [10], which is similar to calf thymus HMG14 and HMG17 in amino acid analysis and N-terminal sequence [11,17,18]. The question arises as to whether these differences in the complement of HMG proteins in the two tissues (trout testis and calf thymus) are due to tissue or species specificity (or both). We have, therefore, analysed the HMG-like proteins in another trout tissue, liver, in an attempt to answer this question.

2. Materials and methods

2.1. *Preparation of trout liver HMG proteins*

The livers of rainbow trout were collected immediately after the death of the trout, and were rapidly frozen in liquid nitrogen. HMG proteins together with histone H1 were extracted from the tissue with 5% perchloric acid (PCA), followed by fractional acetone precipitation to separate HMG proteins from histone H1 as in [19]. HMG proteins were also isolated from purified nuclei as follows. All buffers contained 0.5 mM phenylmethyl sulfonylfluoride. Trout liver (50 g) was blended with 0.25 M sucrose in TKMC buffer (10 mM Tris–HCl (pH 7.5), 2.5 mM KCl, 1.5 mM $MgCl_2$ and 3 mM $CaCl_2$) containing 0.1% Triton X-100. The homogenate was filtered through surgical gauze and centrifuged for 15 min at 2000 × *g*. The pellet was suspended in 1.8 M sucrose in TKMC buffer, layered over 2.2 M sucrose, TKMC buffer, and centrifuged for 1 h at 30 000 × *g*. The nuclear pellet was washed once with the buffer and then extracted with 5% PCA as above.

2.2. *CM-Sephadex C-25 column chromatography*

Fractionation of total HMG protein obtained from the PCA extraction of trout liver tissue was carried out on a CM-Sephadex C-25 column (2.5 × 27 cm) at pH 9.0 as in [5] except that the salt gradient (700 ml) was 0.13–1.2 M NaCl.

2.3. *Preparative SDS–gel electrophoresis*

Trout liver total HMG protein (5 mg) was separated on an SDS 15% polyacrylamide gel slab [21]. The gel was stained (1 h) with Coomassie blue. After destaining, the putative HMGT band was excised and extracted (6 h, 4°C) with 66% acetic acid 20. After removing gel particles by centrifugation and filtering, the sample was dialysed against 0.3 N HCl, precipitating the protein–Coomassie complex. This was collected by centrifugation, washed out with acetone–0.1 N HCl (6:1, v/v) to remove the dye, washed with acetone and dried.

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3. Results

Figure 1 shows the SDS–gel electrophoretic analysis of PCA-extracted proteins from: (a) pig thymus tissue; (b) trout liver nuclei; (c) trout liver tissue. It can be seen that apart from histone H1 the nuclei have 4 main components labelled C, D, E and T which are present in the total tissue extract. The additional protein in the nuclear extract running just ahead of

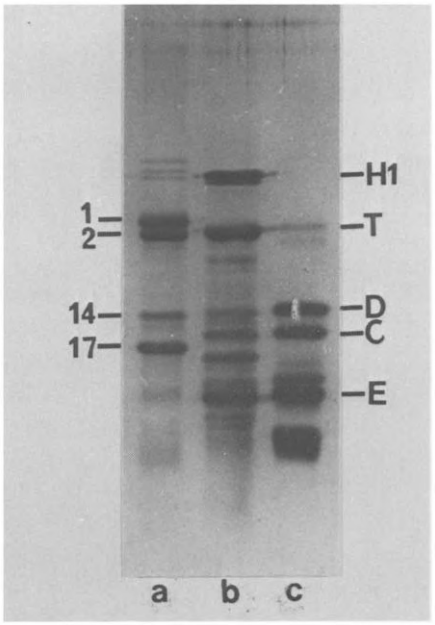

Fig.1. SDS–polyacrylamide gel electrophoresis [21] of: (a) pig thymus HMG proteins; (b) trout liver nuclear HMG proteins; (c) PCA-extracted proteins from trout liver tissue.

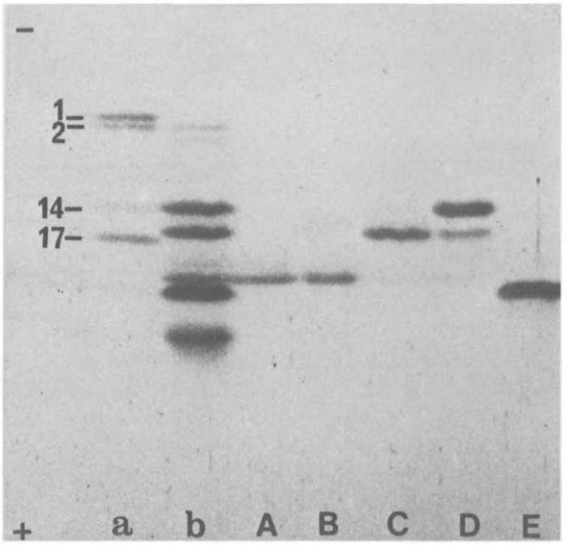

Fig.3. SDS–polyacrylamide gel electrophoretic analysis of trout liver fractions A–E obtained from the CM-Sephadex column (fig.2): (a) pig thymus HMG proteins; (b) trout liver PCA-extracted proteins; (A–E) proteins A–E of the column.

the thymus HMG17 position is an H1 degradation product. The band running ahead of E and the band running just behind E in the total tissue extract may not be nuclear proteins and are not discussed further here.

The trout liver proteins extracted from the whole tissue were fractionated by CM-Sephadex chromatography (fig.2). The proteins of interest, i.e., those of nuclear origin, elute in fractions C–E (fig.3). (Protein T elutes as a minor component in fraction B.) The

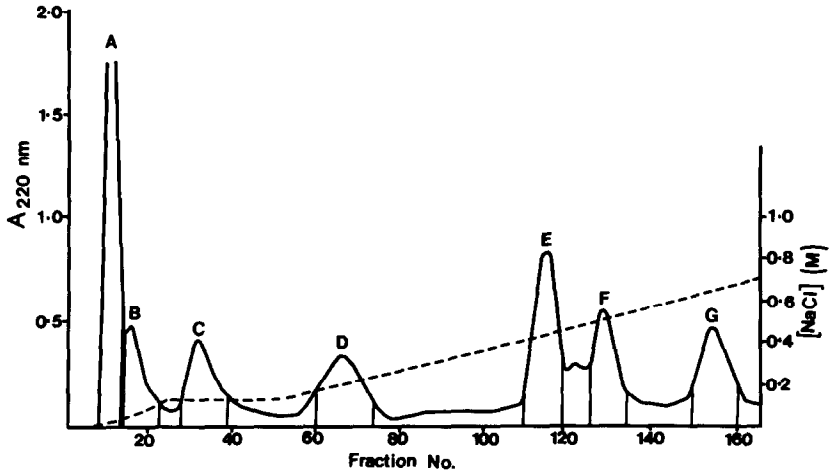

Fig.2. Cm-Sephadex ion-exchange column chromatography of trout liver HMG proteins: (——) A_{220}; (- - - - - - - - -) NaCl gradient.

Table 1
The amino acid compositions (% mol) of trout liver proteins C, D, E and T, compared with the amino acid analyses of trout testis histone H6 and HMG-T [9,10] and calf thymus HMG14 and HMG17 [1]

Amino acids	Trout liver				Trout testis		Calf thymus	
	C	D	E	T	H6	HMG-T	HMG14	HMG17
Asp	6.0	8.0	6.1	10.1	6.7	11.3	8.1	12.0
Thr	2.6	4.1	1.6	4.6	1.6	3.0	4.2	1.2
Ser	4.4	4.3	5.1	8.8	5.6	4.5	7.8	2.3
Glu	23.8	21.9	6.6	7.3	6.1	9.1	17.1	10.5
Pro	10.9	8.4	10.0	8.2	12.3	7.6	8.5	12.9
Gly	2.8	3.3	6.2	10.3	7.4	17.4	6.5	11.2
Ala	16.0	16.6	26.5	11.3	25.4	8.3	14.5	18.4
Val	4.2	2.5	4.2	3.9	3.4	3.8	4.2	2.0
Cys	–	–	–	–	–	0.8	0.7	–
Met	0.1	0.3	–	1.6	–	1.9	–	–
Ile	0.6	–	–	1.3	–	1.5	0.5	–
Leu	0.8	1.0	1.2	2.6	1.2	2.6	2.0	1.0
Tyr	0.2	–	–	1.8	–	2.3	0.4	–
Phe	0.1	0.1	–	2.9	–	3.4	0.6	–
Lys	19.6	23.7	23.4	17.1	23.1	15.5	19.0	24.3
His	0.2	0.9	–	1.2	–	0.4	0.3	–
Arg	4.6	4.2	8.6	6.4	7.2	5.3	5.6	4.1

amino acid analyses of these 3 proteins are given in table 1 together with analyses of trout testis H6 [10], trout testis HMG-T [9], and calf thymus HMG14 and 17 [1]. It can be seen that protein E has a very similar composition to that of trout testis H6. It also has the same mobility on SDS and acetic acid gels as purified testis H6 (not shown). Thus protein E from liver is the same protein as trout testis H6 described in [10].
The amino acid compositions of proteins C, D are very similar to one another and resemble the mammalian proteins HMG14 and HMG17. Also, on SDS–polyacrylamide gels they have similar mobilities to the mammalian HMG14 and HMG17 (fig.1,3). These two proteins run closely together on acetic acid polyacrylamide gels in the thymus HMG14 position (see fig.5). Proteins C and D have not been described before in trout. To verify that they are indeed HMG proteins the amino acid sequences are currently being determined and preliminary results show an N-terminal sequence of Pro–Lys–Arg–Lys– for protein D, and Pro–Lys–Arg–Ala– for protein C. The sequences of mammalian HMG14, HMG17 (and trout testis H6) all start Pro–Lys–Arg–Lys [11,17,18]. These results show that trout proteins C, D are very similar to the mammalian HMG14 and 17 proteins.

The fourth HMG protein (T) was more difficult to isolate and was finally prepared by preparative gel electrophoresis. The electrophoretic analysis of the purified protein is shown in fig.4. The protein runs as a doublet in the thymus HMG2 position. Since trout testis HMG-T runs in this position [9] it suggests that this liver protein is HMG-T. The amino acid analysis of liver protein T (table 1) is indeed similar to the analysis of testis HMG-T in [9]. It differs somewhat in its glycine and serine contents, though.

To compare the above liver proteins with those in trout testis, HMG proteins were prepared from trout testis by PCA extraction of the tissue and analysed by electrophoresis (fig.5). It can be seen that the trout testis electrophoretic pattern of bands is very similar to that of trout liver. Thus one can see bands running in the positions of HMG-T, H6 and the liver CM-Sephadex fractions C, D. On SDS gels (not shown) trout testis HMG-T protein runs as a doublet like the liver HMG-T even after extensive reduction with mercaptoethanol.

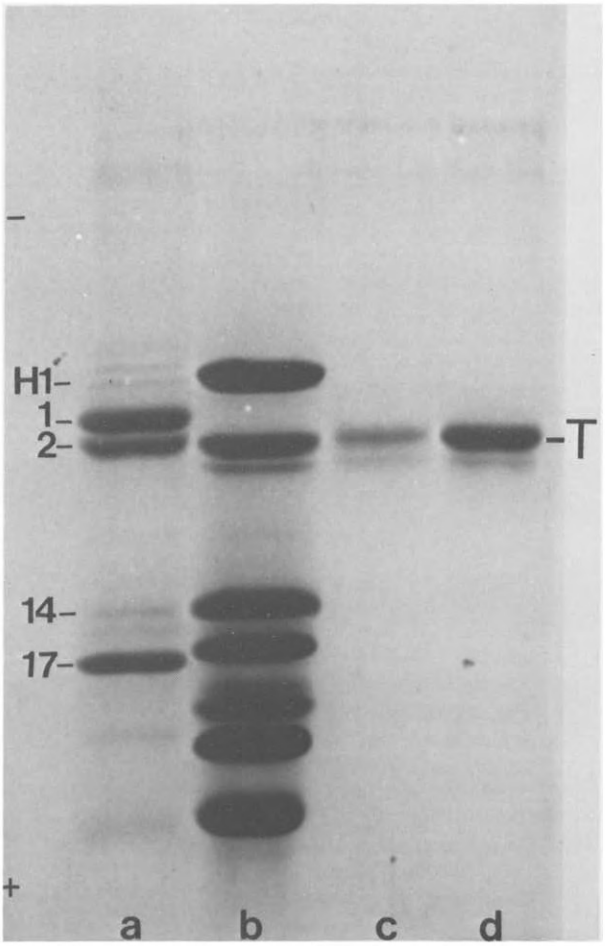

Fig.4. SDS–polyacrylamide gel electrophoresis of trout liver protein T prepared by preparative gel electrophoresis: (a) pig thymus total HMG; (b) trout liver PCA-extracted protein; (c,d) trout liver protein T from two preparations.

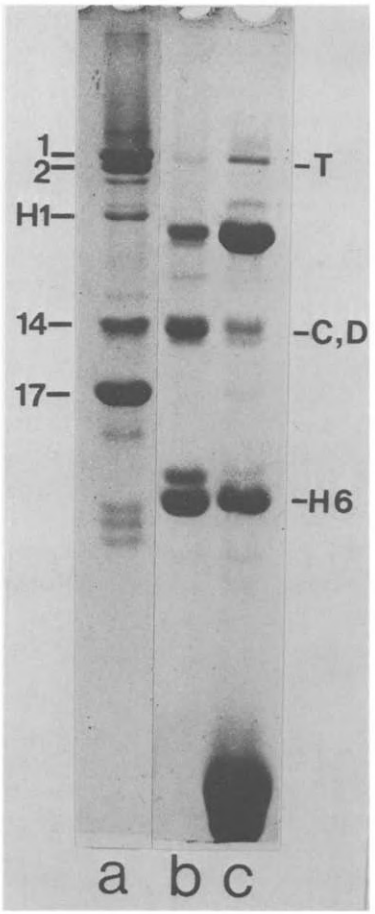

Fig.5. Acetic acid polyacrylamide gel electrophoresis [22] of: (a) pig thymus HMG proteins; (b) trout liver PCA-extracted proteins; (c) trout testis PCA-extracted proteins. (The material running at the bottom of the gel is protamine.)

4. Discussion

Proteins belonging to the HMG class have been isolated from trout liver and 4 components identified. Two of these proteins, the CM-Sephadex fraction E and the HMG protein with the lowest electrophoretic mobility prepared by preparative gel electrophoresis, appear to be similar to two of the proteins described in trout testis, proteins H6 and HMG-T, respectively [9,10]. Protein H6 from the two tissues are nearly identical as regards electrophoretic mobility and amino acid composition, but the HMG-T proteins from the two tissues may differ in their glycine and serine contents.

As far as species specificity is concerned, it has become apparent from this work and [8,10] that although calf and trout have similar HMG proteins, they do differ in number and composition (see section 1). It has been suggested (on the basis of the similarities outlined in section 1) that trout H6 is functionally analogous to HMG14 and HMG17 in calf [1]. This may not be the case in view of the findings reported here that trout liver and testis have two other proteins, the CM-Sephadex fractions C, D, with amino acid compositions and N-terminal sequences similar to HMG14 and HMG17 and with electrophoretic mobilities that are more like HMG14 and HMG17. This raises the possibility that fractions

C, D in trout correspond to mammalian HMG14/HMG17 and that H6 is truly trout-specific or present in mammals but has remained undetected. So far we have not found an H6 molecule in calf tissues. The relationship between these proteins is unclear and will remain so until the sequences of the proteins in question have all been determined, but the most likely explanation at the present is that H6 corresponds to HMG17 and proteins C, D both correspond to HMG14 (i.e., proteins C, D are HMG14 variants).

Acknowledgements

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