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Isolation of a High-Molecular-Weight High-Mobility-Group-Type Non-Histone Protein from Hen Oviduct

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An organ-specific non-histone protein, with a mol.wt. of 95000, was isolated from hen oviduct. This protein consists of approximately equal amounts of acidic and basic amino acids and has an isolectric point of 7.4. On the basis of its known characteristics, this protein is similar to the high-mobility-group proteins observed in other tissues.

Histone and non-histone proteins are the major chemical components of the eukaryotic chromatin. The structural role of these two types of proteins has been known for years. In the past decade, certain non-histone proteins were found to play a key role in the regulation of genomic activity in higher organisms (Gilmour & Paul, 1970; Teng *et al.* 1971; Baserga & Stein, 1971; Kostraba & Wang, 1975). However, the study of non-histone proteins has been hampered by their heterogeneity as well as their insolubility.

Recently, an electrophoretically high-mobility group of non-histone proteins has been isolated from various tissues of different organisms (Goodwin *et al.*, 1975; Watson *et al.*, 1977; Rabbani *et al.*, 1978; Spiker *et al.*, 1978). The high purity and good solubility of the high-mobility groups in aqueous solution have provided the opportunity for study of their biochemical nature and function. In the present paper we report the isolation of a non-histone protein from hen oviduct. This protein is tissue-specific and has a mol.wt. of 95000. Its characteristics are comparable with those of high-mobility-group proteins reported previously (Watson *et al.*, 1977; Rabbani *et al.*, 1978).

Materials and Methods

Animals and chemicals

Sexually mature White Leghorn hen (50–60 weeks old) oviduct, brain and liver were obtained from Texas Animal Specialties (Humble, TX, U.S.A.). Calf (less than 50 weeks old) thymus was collected from a local slaughterhouse. The following chemicals were obtained from the sources indicated: $CaCl_2$, $MgCl_2$ and EDTA were from Mallinckrodt (St. Louis, MO, U.S.A.); acetone, boric acid, glycerol and NaCl were from Fisher Scientific Co. (Fairlawn, NJ, U.S.A.); ultrapure sucrose and Tris base were from Schwarz/Mann Co. (Orangeburg, NY, U.S.A.); Ampholine was from LKB Laboratory (Rockville, MD, U.S.A.); CM-cellulose (CM_{52}) was from Whatman Co. (Maidstone, KY, U.S.A.); phenylmethanesulphonyl fluoride was from Pierce Chemical Co. (Rockford, IL, U.S.A.). All other chemicals were of analytical grade.

Nuclear and chromatin preparations

Fresh minced oviduct (200g) (or other tissues) were homogenized in 0.32M-sucrose containing 3mM-$CaCl_2$, 0.1mM-phenylmethanesulphonyl fluoride and 0.01% (w/v) Triton X-100 with a Polytron p-10 tissue disintegrator (Brinkman, Westburg, NY, U.S.A.) run at a rotating speed of 2750 rev./min. for 10s and 2250 rev./min. for 30s at −10°C. For liver and brain nuclei, the minced tissues were homogenized with 15 strokes in a glass/Teflon homogenizer. Nuclei were prepared from the homogenate by centrifugation through sucrose density barriers by the method of Teng *et al.* (1971). Chromatin was prepared from the nuclear preparations by the technique of Mezquita & Teng (1977).

Isolation of total high-mobility-group protein from chromatin

Chromatin was extracted with 0.35M-NaCl/0.1mM-phenylmethanesulphonyl fluoride by the method of Goodwin *et al.* (1973). The extract was fractionated by 2% (w/v) trichloroacetic acid, and the supernatant was precipitated with acetone as described by Goodwin *et al.* (1975). By applying this procedure, approx. 20mg of acetone-precipitable total high-mobility-group protein was obtained from 200g of oviduct.

CM-cellulose chromatography of non-histone protein

All procedures were carried out at 4°C and all buffers contained 0.1mM-phenylmethanesulphonyl fluoride. Two steps of column-chromatographic

purification were involved. First, the total high-mobility-group proteins were dissolved in 10mM-borate buffer, pH8.8, and dialysed against the same buffer containing 0.05M-NaCl overnight. A small amount of precipitate was spun down, and the supernatant was applied to a CM-cellulose column. A linear NaCl gradient (0.025–0.6M) in 7.5mM-borate buffer/10mM-2-mercaptoethanol was eluted through the column. The elution profile is shown in Fig. 2(*a*). The protein in peak II was precipitated with acetone.

Secondly, the protein was redissolved in 7.5mM-borate buffer/10mM-2-mercaptoethanol containing 0.05M-NaCl and fractionated through a CM-cellulose column by a stepwise elution with a graded concentration of NaCl in borate buffer (Fig. 2*b*). The protein in peak III obtained from this procedure was precipitated with acetone and the yield ranged from 1.5 to 2.0mg/200g of oviduct.

Gel electrophoresis and isoelectric focusing

The protein obtained from peak III was resolved by electrophoresis in sodium dodecyl sulphate/polyacrylamide/urea gels by the method of Laemmli (1970). This protein, which resolved as a single band in the gel [Fig. 1*a*(4)], was designated as '95K' protein. Isoelectric focusing was performed by the procedure of Teng & Teng (1975). The general procedures for the DNA and protein determinations were as previously described (Teng & Teng, 1978).

Results and Discussion

Total high-mobility-group proteins obtained from oviduct chromatin consisted of three major bands (mol.wts. of 95000, 55000 and 14000) and two minor proteins (mol.wts. 30000–40000). The proteins in the molecular-weight range of 14000–30000 are histones and high-mobility-group proteins [Fig. 1*a*(3)]. Further purification of one of the proteins was achieved by two-step CM-cellulose chromatography (Figs. 2*a* and 2*b*). The ionic strength required for eluting the protein (mol. wt. 95000) is 0.25M-NaCl at pH8.8. This is similar to the condition used for the isolation of high-mobility-group proteins 2 and 14 from calf thymus (Goodwin *et al.*, 1975).

The molecular weight of this protein was determined by sodium dodecyl sulphate/polyacrylamide/urea-gel electrophoresis. This technique avoids the possibility of non-specific aggregation of small protein molecules. As indicated in the gel picture, there is no detectable contamination of this large protein molecule by histones or by other non-

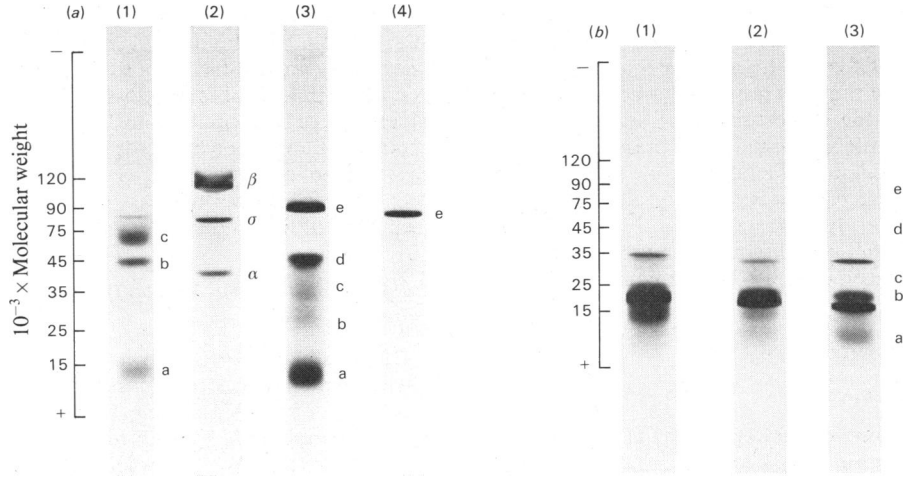

Fig. 1. *Sodium dodecyl sulphate/polyacrylamide-gel electrophorseis of chromosomal proteins*
(*a*) Gel electrophoresis was run as described by Laemmli (1970) with the following protein samples: gels (1) and (2), standard proteins: a, lysozyme; b, ovalbumin; c, bovine serum albumin; *β*, *Escherichia coli* RNA polymerase *β*-subunit; *σ*,*σ*-factor; *α*, polymerase *α*-subunit; gel (3) total high-mobility-group protein; gel (4) 95K protein (50*μ*g) obtained from peak III from CM-cellulose chromatography (Fig. 2*b*). (*b*) Comparison of high-mobility-group proteins from various tissues. Gels (1), (2) and (3) were from calf thymus, liver and brain respectively. Protein bands at the region of a–b are high-mobility-group proteins 14 and 17 and histone H1, whereas protein bands at the region of c–d are high-mobility-group proteins 1, 2 and 3. No 95K protein was observed in the e region for these tissues. The molecular weights of the proteins were determined by the method of Weber & Osborn (1969) with the following proteins (with mol.wt. indicated) as standards: *E. coli* RNA polymerase *β*-subunit (120000), *σ*-factor (89000); *α*-subunit (40000); lipoxidase (soya bean, 96000); bovine serum albumin (68000); ovalbumin 45000); lysozyme (egg white, 14000).

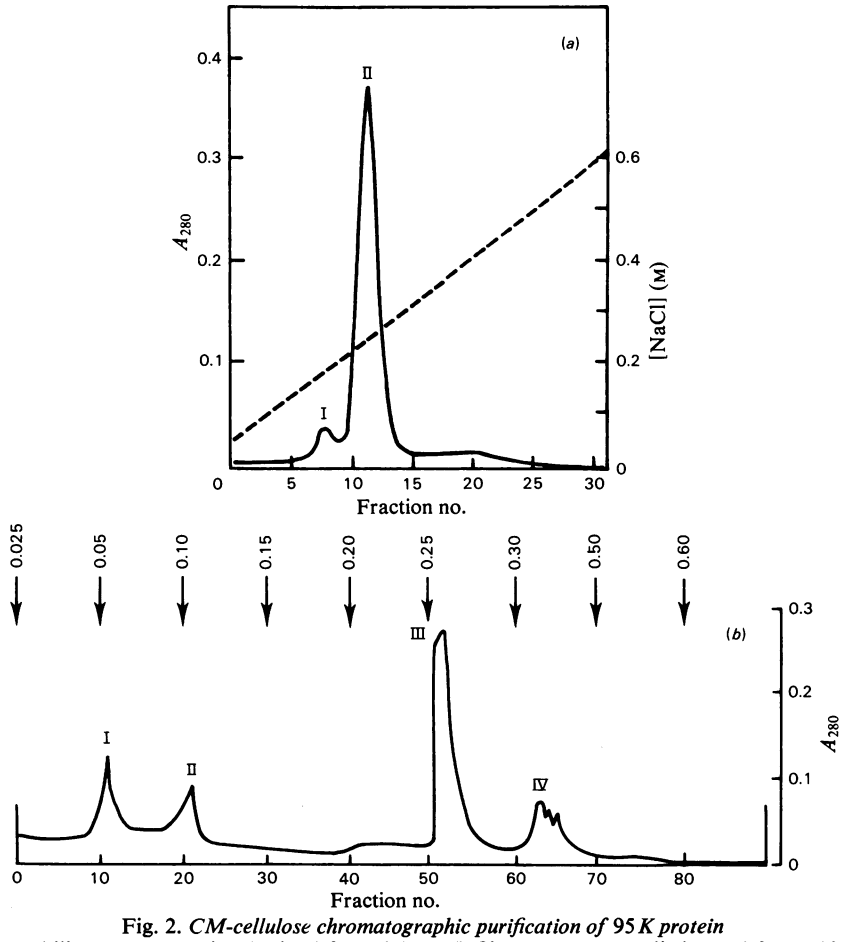

Fig. 2. *CM-cellulose chromatographic purification of 95K protein*

(*a*) Total high-mobility-group protein obtained from 0.35M-NaCl extract was applied to a 1.2cm×10cm column of CM-cellulose, that had been equilibrated with 0.025M-NaCl/borate buffer, pH 8.8 The column was eluted with a NaCl gradient. (*b*) Protein from peak II of the previous column chromatography was precipitated by acetone and dissolved in borate buffer with 0.025M-NaCl and applied to a 1.2cm×8cm column of CM-cellulose. The column was eluted stepwise with various concentrations of NaCl (as indicated above the arrows; M) in borate buffer. Protein eluted at peak III contained the 95K protein. ——, A_{280}; ----, NaCl concentration.

histone proteins [Fig. 1*a*(4)]. Thereafter we designated it as the '95K' protein. The molecular weight of this protein is 3 times that of the largest of the high-mobility-group proteins reported, i.e. high-mobility-group proteins 1, 2, 3, 14 and 17 have mol.wts. ranging from 30000 to 8500 (Goodwin *et al.*, 1975; Watson *et al.*, 1977; Vidali *et al.*, 1977). The reason for this discrepancy is unclear.

Similar techniques have been applied for high-mobility-group-protein isolation from other tissues of the chick and of calf thymus. No 95K protein has been observed in the high-mobility-group-protein fractions of liver, brain or thymus. However, the electrophoretic pattern of total high-mobility-group proteins of calf thymus in our preparation is comparable with that observed by Goodwin *et al.* (1975) (Fig. 1*b*). This observation indicated that the 95K protein is a unique organ-specific protein existing in the oviduct chromatin.

Analysis of the amino acid composition of this protein indicated that about half of the residues are either acidic or basic. The ratio of acidic amino acids to basic amino acids is 0.88 (Table 1). The isoelectric point for this protein is in the range 7.2–7.6, which indicates that it is slightly basic in nature. Compared with other amino acids, the amounts of proline, glycine and lysine are high; yet the aromatic amino acids, e.g. tyrosine and phenylalanine, are low (Table 1). This characteristic amino acid composition is similar to that reported for other

Table 1. *Amino acid compostion of the 95K protein*
The protein (125–150μg) was hydrolysed with acid and analysed for amino acid content on the Beckman Model 121 amino acid analyser as described by Spakman *et al.* (1958). Aspartic acid+glutamic acid/histidine+lysine+arginine = 0.88

Amino acid	Content (mol/100mol of total amino acids)
Aspartic acid	10.00
Threonine	7.20
Serine	5.35
Glutamic acid	9.85
Proline	12.85
Glycine	11.60
Alanine	3.55
Cystine	4.65
Valine	5.25
Methionine	1.95
Isoleucine	2.10
Leucine	2.15
Tyrosine	Trace
Phanylalanine	0.25
Histidine	1.85
Lysine	14.35
Arginine	6.35

high-mobility-group proteins isolated from thymus, erythrocytes and trout testis (Rabbani *et al.*, 1978; Watson *et al.*, 1977).

At present, the structural and functional roles of this non-histone protein are still unknown. However, in our previous publication (Teng & Teng, 1978), we have reported that a chromosomal non-histone protein (band 3 with mol.wt. of 95000) in chick embryonic oviduct increased greatly during the late stages of devlopment. The increase of this and other non-histone protein is closely correlated with the chromatin template capacity and its acceptance for oestrogen (Teng & Teng, 1978). We suspect that the 95K protein is involved in hormonal action and genomic regulation in the oviduct. These aspects need to be further investigated.

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