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Studies on the High-Mobility-Group Non-Histone Proteins from Hen Oviduct

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Nuclear high-mobility-group (HMG) proteins were isolated from hen oviduct. These were proteins HMG-1, -2, -3, -14 and -17, which are equivalent to the classification of calf thymus HMG proteins. Hen oviduct proteins HMG-1 and -2 were individually isolated by $HClO_4$ extraction and CM-Sephadex chromatographic separation. Their mol.wts. were determined as 28000 and 27000, respectively. The proteins have a high content of acidic and basic amino acids. The association of proteins HMG-1 and -2 with the genome of hen oviduct nuclei was probed by a limited digestion with nucleases. Hen oviduct nuclei were incubated with deoxyribonuclease I or micrococcal nuclease until 10% of the DNA was digested. The nuclear suspension was centrifuged and the contents of proteins HMG-1 and -2 in the supernatant and sediment fractions were analysed by polyacrylamide-gel electrophoresis. HMG proteins were found to be preferentially released by micrococcal-nuclease digestion rather than by deoxyribonuclease I.

Since the discovery of nuclear HMG proteins in calf thymus by Goodwin *et al.* (1973), the research on chromatin non-histone proteins has opened up a new dimension. A series of reports has been published on their isolation, characterization, structural localization and species and tissue specificity (Goodwin & Johns, 1973, 1978; Goodwin *et al.*, 1975, 1977; Vidali *et al.*, 1977; Watson *et al.*, 1977; Levy *et al.*, 1977; Spiker *et al.*, 1978; Javaherian *et al.*, 1978; Sterner *et al.*, 1978; Rabbani *et al.*, 1978*a*). We have previously reported our observations on the HMG proteins isolated from mature hen oviduct chromatin. This class of proteins consists of an organ-specific high-molecular-weight non-histone protein with a mol.wt. of 95000 together with several low-molecular-weight HMG proteins with mol.wts. ranging from 14000 to 30000 (Teng *et al.*, 1978). In the present paper, our studies on two single proteins (HMG-1 and HMG-2) isolated from the low-molecular-weight group of HMG proteins are reported. These HMG proteins have mol.wts. of 27000–28000 and an amino acid composition comparable with that of calf thymus, spleen, liver and kidney HMG proteins (Rabbani *et al.*, 1978*a*). The association of the HMG proteins with the chromatin of hen oviduct has been probed by a limited nuclease-digestion technique.

Materials and Methods

Animals and chemicals

The mature white Leghorn hen oviduct, calf thymus and chemicals used in this study were as previously described (Teng *et al.*, 1978). The following enzymes were obtained from the sources indicated: DNAase I (bovine pancreas, activity 1410units/mg dry wt.; 1 unit causes an increase in A_{260} of 0.001/min per ml at 25°C when acting on highly polymerized DNA at pH 5.0) and micrococcal nuclease (*Staphylococcus aureus*, activity 23882units/mg of protein; 1 unit corresponds to a change in A_{260} of 1.0 at 37°C, pH 8.0, in the final assay solution) were from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.); polyamines were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); Coomassie Brilliant Blue R-250 was from Bio-Rad Laboratories (Richmond, CA, U.S.A.).

Abbreviations used: HMG protein, high-mobility-group protein; DNAase, deoxyribonuclease; SDS, sodium dodecyl sulphate.

Isolation of nuclei

The magnum portion of laying-hen oviduct was minced finely and homogenized in 0.32M-sucrose containing 3mM-$CaCl_2$, 0.5mM-phenylmethanesulphonyl fluoride and 0.01% (w/v) Triton X-100 with a Polytron P-10 tissue disintegrator (Brinkman, Westbury, NY, U.S.A.) as described by Teng *et al.* (1978). The homogenate was filtered through four layers of cheesecloth before dilution with water (at 4°C) to a final sucrose concentration of 0.25M. The diluted homogenate was layered over 0.6vol. of the original 0.32M-sucrose homogenization buffer and centrifuged at 1600*g* for 10min at 4°C in a JS-13 rotor in the Beckman J-21B centrifuge. The crude nuclear pellet was resuspended in 5 vol. (ml/g) of 2.2M-sucrose containing 10mM-Tris/HCl and 0.5mM-phenylmethanesulphonyl fluoride, pH 7.0. After homogenization (one stroke, Teflon/glass homogenizer) the suspension was centrifuged at 80000*g* for 45min at

4°C in a Ti 65 rotor in the Beckman L5-65 ultracentrifuge. The nuclear pellet was washed three times in RSB buffer (10mM-Tris/HCl, pH7.4, 10mM-NaCl, 3mM-$MgCl_2$, 0.5mM-phenylmethanesulphonyl fluoride). The purified nuclei were used immediately or frozen at −70°C in 25% (v/v) glycerol, containing 50mM-Tris/HCl, pH7.4, 0.01mM-EDTA, 0.5mM-dithiothreitol and 5mM-$MgCl_2$.

In some nuclease-digestion experiments, nuclear preparation was essentially by the procedure described above, except that the bivalent cations in the isolation medium were replaced by polyamines (0.15mM-spermine and 0.5mM-spermidine) as described by Hewish & Burgoyne (1973).

Isolation of HMG proteins

Hen oviduct HMG proteins were isolated by $HClO_4$ extraction of purified nuclei by the procedures of Sanders & Johns (1974) and Rabbani *et al.* (1978*a*). Consistently, an average of 65 μg of protein/g of tissue was recovered from nuclei.

Fractionation of HMG proteins by column chromatography

HMG proteins (approx. 30mg) were dissolved in borate buffer (7.5mM-boric acid, 10mM-2-mercaptoethanol and 0.5mM-phenylmethanesulphonyl fluoride, adjusted to pH8.8 with 1M-NaOH) containing 0.05M-NaCl and fractionated through a CM-Sephadex 25 column (1cm × 8cm) by stepwise elution with graded concentrations of NaCl (from 0.05 to 0.5M) in borate buffer. The protein eluted with different concentrations of NaCl was acidified to 0.1M-HCl and precipitated by 6vol. of acetone. The HMG proteins were stored in a dry powder form at −70°C before use.

DNAase I digestion of nuclei

Nuclei were digested with DNAase I essentially as described by Weintraub & Groudine (1976). Nuclei were suspended in RSB buffer at a concentration of 2–3mg of DNA/ml. DNA content was determined by heating an appropriate sample of nuclei (25 μl/ml) in 1M-NaCl/1M-$HClO_4$ (Axel *et al.*, 1975) at 85°C for 30min. The sample was cooled for 30min at 4°C and centrifuged for 30min at 1500*g* in a Brinkman model 3200 Microfuge (Eppendorf). The supernatant was removed and the A_{260} of the solution measured. A hydrolysed solution of 1mg of DNA/ml was considered to have an A_{260} of 26 under these conditions (Schaller *et al.*, 1972). An unhydrolysed sample of nuclei was used as a control for background absorption, which represented less than 3% of the total 260nm-absorbing material. Nuclei were preincubated at 37°C for 5min before the addition of 40 μg of DNAase I (freshly dissolved)/ml. Digestion was followed by measuring the increase in 260nm-absorbing material soluble in $HClO_4$ (at 4°C). Appropriate samples (50 μl/ml) were removed and mixed with 1M-NaCl/1M-$HClO_4$. After 30min at 4°C, the samples were centrifuged as described above and the A_{260} of the supernatant was determined. All samples were corrected for background absorption (3%). Under these conditions, 10% digestion occurred in 5–10min at 37°C, and the final extent of digestion was approx. 50% in 60min. Digestion rates were determined before each experiment. The digestion was stopped by the addition of 100mM-EDTA (pH7.0) to give a final concentration of 10mM-EDTA. After 3min at 4°C the nuclei were sedimented at 4000*g* for 10min with the JS-13 rotor in the J-21B centrifuge.

Micrococcal-nuclease digestion of nuclei

Nuclei were suspended in RSB buffer plus 1mM-$CaCl_2$ at a concentration of 2–3mg of DNA/ml. DNA content and digestion rates were determined as described for the DNAase-I experiments. After preincubation at 37°C for 5min, micrococcal nuclease was added to a final concentration of 7 μg/ml. Under these conditions, 10% digestion occurred in 8–10min at 37°C. The reaction was stopped with 10mM-EDTA (pH7.0) and the nuclei were sedimented at 4000*g* for 10min at 4°C as described above .The nuclei remained relatively intact and released little chromatin into the supernatant. The pellet and supernatant from a digested sample and from a control sample incubated without enzyme were analysed for the content of HMG proteins.

Extraction of HMG proteins from nuclease-digested nuclei

The pellet and supernatant fractions from control and digested nuclei were analysed for the content of HMG proteins. HMG proteins were isolated as described by Goodwin *et al.* (1973). Nuclear pellets were resuspended in 50mM-Tris/HCl, pH7.8 (approx. 1ml/g of tissue). Solid NaCl was added to a final concentration of 0.35M. After 10min at 4°C the suspension was centrifuged at 4000*g* for 10min. The gelatinous pellet was resuspended in 0.35M-NaCl, pH7.0 (approx. 1ml/g of tissue), by homogenization by ten strokes with a loose-fitting pestle of a Dounce homogenizer. The suspension was centrifuged as above and the supernatant extracts were combined. The 0.35M-NaCl extract was then clarified of chromatin by centrifuging at 80000*g* for 30min (SW 50.1 rotor, L5-65 centrifuge) at 4°C. The supernatant fraction was made 2% (w/v) with trichloroacetic acid by the addition of 100% trichloroacetic acid. After 10min at 4°C the mixture was centrifuged at 4000*g* for 15min. The supernatant was clarified by filtering through a fibre-glass filter (Reeve Angel) and HCl was added to a final concentration of 0.2M. The proteins were precipitated with 6vol. of acetone at −20°C for 18h. The precipitate was collected by

centrifuging at 20000*g* for 15min at 4°C, washed with acetone (at 4°C) once, then dried under nitrogen. Supernatant fractions from control and digested nuclei samples were made 0.35M with NaCl, and the HMG proteins were isolated as described above. Alternatively, in some experiments HMG proteins were extracted by $HClO_4$ as described in the previous section.

Gel electrophoresis of HMG proteins

The protein obtained by column chromatography was resolved by acetic acid/urea/polyacrylamide-gel electrophoresis originally developed by Panyim & Chalkley (1969).

In some experiments, proteins were separated by tube SDS/polyacrylamide-gel electrophoresis essentially as described by Laemmli (1970) and as modified by LeStourgeon & Rusch (1973). Slab gels (1.5mm× 10.5cm) were made from a stock solution of 45% acrylamide, 1.2% *NN'*-methylenebisacrylamide, 0.375M-Tris, pH8.8, 5mM-EDTA, 0.5M-urea and 0.1% SDS. This stock solution was diluted to 18% final acrylamide concentration by the addition of buffer containing 0.375M-Tris, pH8.8, 5mM-EDTA, 0.5M-urea and 0.1% SDS. Separating gels were polymerized by the addition of 0.075% *NNN'N'*-tetramethylphenylenediamine and 0.075% ammonium persulphate. After the separating gel polymerized overnight, a 1cm stacking gel (3% acrylamide, 0.08% *NN'*-methylenebisacrylamide, 5mM-EDTA, 0.5M-urea, 0.125M-Tris, pH6.8, and 0.1% SDS) was polymerized by the addition of 0.075% *NNN'N'*-tetramethylphenylenediamine and 0.075% ammonium persulphate. Running buffer consisted of 0.025M-Tris, 0.192M-glycine and 5mM-EDTA, pH8.3.

Protein samples were dissolved directly in sample buffer (2% SDS, 0.0625M-Tris/HCl, pH6.8, 5mM-EDTA and 10% glycerol) by heating at 100°C for 2min. Protein concentration was determined in the absence of β-mercaptoethanol. Appropriate samples (30–70μg of proteins) were removed and adjusted to 100μl final volume with sample buffer. A volume of β-mercaptoethanol was added to give a final concentration of 5%. The samples were reheated at 100°C for 2min, then used immediately for electrophoresis. Samples were electrophoresed at 20mA/gel for 1h followed by 35mA/gel until the Bromophenol Blue tracking dye was 0.5–1cm from the bottom of the gel (approx. 6–8h).

Gels were stained for 1.5h in 0.03% Coomassie Blue/25% (v/v) propan-2-ol/10% (v/v) acetic acid with constant stirring, followed by 1h in 0.003% Coomassie Blue/10% (v/v) propan-2-ol/10% acetic acid and then in 0.0015% Coomassie Blue/1.5% propan-2-ol/10% acetic acid for 1h. Final destaining was in 10% acetic acid. Molecular weights were estimated by reference to the standard protein markers with known molecular weights as described in the legend of Fig. 3.

Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin (Sigma) as a standard.

Results and Discussion

The nuclei isolated from hen oviduct by a previous procedure (Teng *et al.*, 1978) gave a 40% yield. The yield of total nuclear HMG proteins from hen oviduct is approx. 65mg of protein/kg of hen oviduct. Therefore, the content of total HMG proteins per kg of hen oviduct is about 163mg. Total hen oviduct HMG proteins could be separated into several major protein fractions by CM-Sephadex chromatography. Elution with NaCl at concentrations ranging from 0.05M to 0.1M gave two protein peaks (I and II) (Fig. 1*a*). Resolutions by acetic acid/urea/polyacrylamide-gel

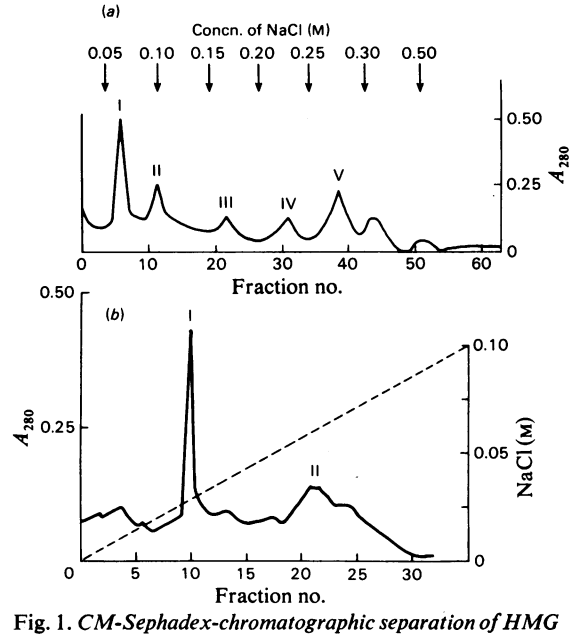

Fig. 1. *CM-Sephadex-chromatographic separation of HMG proteins*

(*a*) Approx. 30mg of total HMG protein was dissolved in 7.5mM-borate buffer with 0.05M-NaCl and applied to a column (1cm×8cm) of CM-Sephadex. The column was eluted stepwise with various concentrations of NaCl (as indicated above the arrows) in borate buffer. (*b*) The samples from peaks I and II of Fig. 1 (*a*) containing proteins HMG-1 and -2 were combined and reduced with dithiothreitol by the procedure of Ruiz-Carillo & Allfrey (1973). Approx. 15mg of the reduced sample was rechromatographed on another CM-Sephadex column by using a linear NaCl gradient (0–0.1M) in 7.5mM-borate buffer with 0.5mM-phenylmethanesulphonyl fluoride. Protein HMG-1 was eluted first, followed by protein HMG-2.

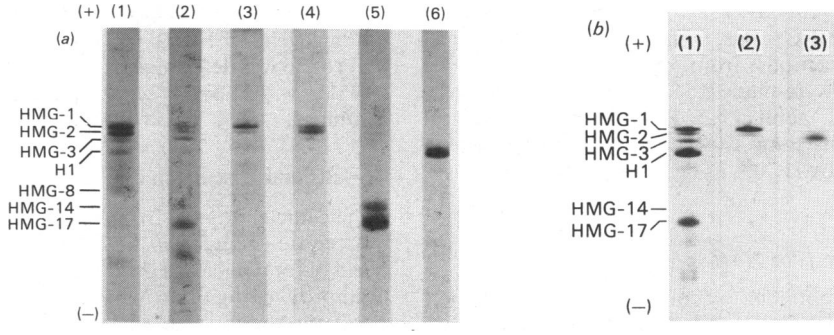

Fig. 2. *Acetic acid/urea/polyacrylamide-gel electrophoresis of HMG proteins*
Tube gels (5mm × 100mm) consisting of 15% acrylamide, 0.1% *NN'*-methylenebisacrylamide, 0.9M-acetic acid, 2.5M-urea, 0.125% *NNN'N'*-tetramethylphenylenediamine and 0.125% ammonium persulphate were polymerized overnight at 23°C. Gels were pre-run at 2mA/gel for 5h at 23°C. At the end of the pre-run, voltage was increased from 90 to 165V, after which it remained constant. Running buffer consisted of 0.9M-acetic acid. Protein samples were dissolved directly in 0.9M-acetic acid containing 15% sucrose. Appropriate samples (40–60μg of protein) were adjusted to 100μl final volume with 0.9M-acetic acid/15% sucrose, then reduced with 5% β-mercaptoethanol. Electrophoresis was at 1mA/gel (95–100V) for 5.5h at 23°C. Gels were stained for 30min with 0.5% Amido Black 10B in a solution containing 60% water, 33% methanol and 6.5% acetic acid and destained in the same solution overnight. (*a*) Gels (1) and (2) were calf thymus and hen oviduct HMG protein, respectively. Protein (peaks I, II and IV) eluted from the CM-Sephadex column (Fig. 1*a*) was individually resolved in gels 3, 4 and 5, respectively. Histone H1 isolated from the first 3 × acetone precipitation of $HClO_4$ extract was in gel (6). (*b*) Gel (1) was hen oviduct total HMG protein. The individual proteins HMG-1 and -2 obtained from linear NaCl elution of CM-Sephadex (Fig. 1*b*) were resolved in gels (2) and (3), respectively.

Table 1. *Amino acid composition of HMG proteins*
Proteins HMG-1 and -2 eluted from CM-Sephadex ion-exchange chromatography (Fig. 1*b*) were hydrolysed with acid and analysed for amino acid content on the Beckman model 121 Amino Acid Analyzer as described by Spackman *et al.* (1958). The ratios of acidic to basic amino acids (aspartic acid+glutamic acid/histidine+lysine+arginine) are 1.27 and 0.98, respectively, for proteins HMG-1 and -2.

	Content (mol/100mol of total amino acid)	
Amino acid	HMG-1	HMG-2
Asp	10.6	9.7
Thr	4.5	3.7
Ser	7.4	7.2
Glu	15.8	15.1
Pro	8.4	8.8
Gly	7.0	6.3
Ala	8.3	7.6
Val	4.3	6.7
Cys	Trace	Trace
Met	1.1	1.5
Ile	1.8	1.5
Leu	3.9	2.8
Tyr	2.8	1.4
Phe	2.9	2.4
Lys	14.3	18.2
His	1.7	2.4
Arg	4.8	4.6

electrophoresis indicated that peak I consisted of protein HMG-1 and peak II consisted of proteins HMG-1 and -2. A slight contamination with protein HMG-3 was found in peak 2 (Fig. 2*a*). When the NaCl concentration was increased to 0.2M, the other two HMG proteins (HMG-14 and -17) were eluted (Figs. 1*a* and 2*a*). This pattern of CM-Sephadex separation is like that for other HMG proteins of calf tissues and trout testis (Rabbani *et al.*, 1978*a*; Watson *et al.*, 1977; Levy *et al.*, 1977).

To obtain pure protein HMG-1 or -2 from hen oviduct, the samples from peaks I and II were combined, reduced and re-fractionated through a CM-Sephadex column with a linear salt concentration gradient from 0 to 0.1M; two distinct peaks were obtained (Fig. 1*b*). Peaks I and II were eluted at NaCl concentrations of 0.025 and 0.055M respectively. They were individually resolved by acetic acid/urea/polyacrylamide-gel electrophoresis and shown to be proteins HMG-1 and -2 respectively (Figs. 1*b* and 2*b*).

The amino acid composition of proteins HMG-1 and -2 was analysed and is presented in Table 1. The amount of acidic or basic amino acids represented approximately one-quarter of the total amino acid composition. The amounts of aromatic amino acids, i.e. tyrosine and phenylalanine, were relatively low. This characteristic amino acid composition is not distinguishable from that of other HMG proteins reported in yeast, wheat, avian erythrocyte and calf tissues (Spiker *et al.*, 1978; Vidali *et al.*, 1977; Rabbani *et al.*, 1978*b*). This general similarity of HMG proteins among various species suggested that these proteins are evolutionally conserved.

There is no drastic difference between the proteins HMG-1 and -2 of hen oviduct in terms of amino acid composition (Table 1) and mol.wt. (28000 and 27000, as determined by the procedure of Weber & Osborn, 1969) (result not shown). Observations by Walker *et al.* (1976) indicated that calf thymus proteins HMG-1 and -2 are similar in their amino acid composition, in the distribution of charged amino acids along the peptide chains, and have the same *N*-terminal groups. Applying an immuno-microcomplement-fixation technique, Bustin *et al.* (1978) confirmed that calf thymus proteins HMG-1 and -2 are closely related (with only 6% difference in sequence). Hen oviduct HMG proteins have a similar range of molecular weights to those of proteins HMG-1 and -2 and HMG-T (28000, 27600 and 28700, respectively) reported in calf thymus and trout testis (Shooter *et al.*, 1974; Watson *et al.*, 1977).

In hen oviduct, protein HMG-3 is frequently observed in polyacrylamide-gel electrophoresis (Fig. 2*a*). This non-histone protein has also been observed in different calf tissues (Rabbani *et al.*, 1978*a*). Observations by Goodwin *et al.* (1973, 1978) have suggested that thymus protein HMG-3 is the breakdown product of protein HMG-1 and protein HMG-8 is a degradation product of histone H1. Bustin *et al.* (1978) were able to confirm this by providing evidence that proteins HMG-1 and -3 are immunologically indistinguishable.

We previously identified the hen oviduct chromatin HMG proteins by SDS/polyacrylamide-gel electrophoresis (Teng *et al.*, 1978). A 10% gel was used for separation, which did not provide a clear resolution for the low-molecular-weight HMG proteins. The 18% polyacrylamide gel (with 2% of SDS) used in the present work provided a satisfactory way of separating the HMG proteins 1, 2 and 95K [Fig. 3*a*, gel (2)]. Furthermore, after acetic acid/urea/polyacrylamide-gel electrophoresis, other HMG proteins, e.g. HMG-3, -14 and -17, became visible [Fig. 3*b*, gel (2)].

At present, two major isolation techniques (low-salt extraction and $HClO_4$ extraction) developed by Goodwin *et al.* (1973), Sanders & Johns (1974) and Rabbani *et al.* (1978*a*) have been tested for the isolation of hen oviduct HMG proteins. We found that both techniques could release proteins HMG-1 and -2 [Fig. 3*a*, gels (2) and (3)]. However, the $HClO_4$-extraction procedure had the advantage of separating the high-molecular-weight 95K protein from proteins

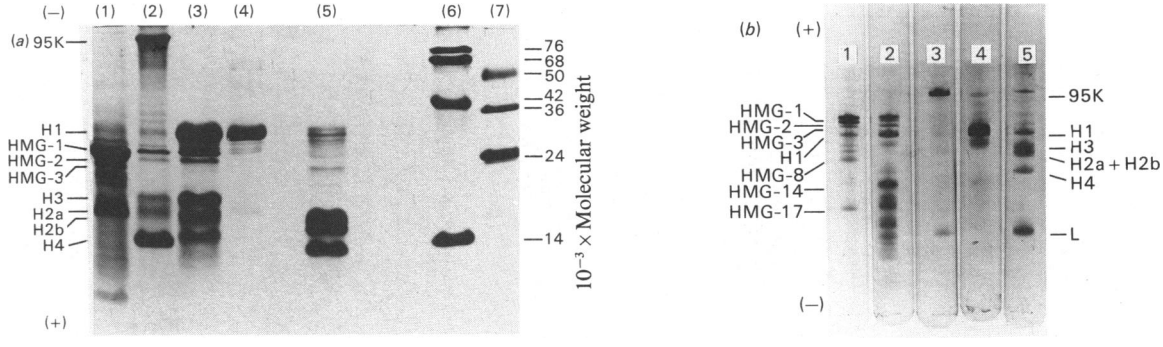

Fig. 3. *Comparison of HMG proteins from hen oviduct and calf thymus nuclei*

(*a*) Separation of HMG proteins by SDS/polyacrylamide slab gels. HMG proteins were solubilized from hen oviduct nuclei or calf thymus nuclei as described in the Materials and Methods section. Proteins (75 μg) were dissolved in 2% (w/v) SDS and separated in an 18% acrylamide gel. Hen oviduct histone H1 (20 μg) and calf thymus histones (50 μg) were simultaneously electrophoresed on the gel. Gels were stained with Coomassie Blue. Gels: (1) calf thymus total HMG proteins extracted with 0.35 M-NaCl and soluble in 2% (w/v) trichloroacetic acid; (2) hen oviduct total HMG proteins extracted by the same procedure previously described; (3) hen oviduct HMG proteins extracted with 5% (w/v) $HClO_4$ as described in the Materials and Methods section; (4) histone H1 from hen oviduct; (5) total histones (H) from calf thymus; (6) standard protein markers: lysozyme (mol.wt. 14300), ovalbumin (mol.wt. 43000), bovine serum albumin (mol.wt. 68000) and conalbumin (mol.wt. 76000); (7) chymotrypsinogen (mol.wt. 23500), alcohol dehydrogenase (mol.wt. 36000), heavy chain of γ-globulin (mol.wt. 50000). (*b*) Separation of proteins by acetic acid/urea/polyacrylamide-gel electrophoresis. Proteins were resolved as described in the Materials and Methods section. Proteins (25 μg) were dissolved in 0.9 M-acetic acid and separated by electrophoresis on a 15% acrylamide gel containing 2.5 M-urea and 0.9 M-acetic acid. Gels were stained with Amido Black 10B. Gels: (1) HMG protein from calf thymus extracted with 0.35 M-NaCl and soluble in 2% (w/v) trichloroacetic acid; (2) HMG protein extracted with 5% (v/v) $HClO_4$; (3) hen oviduct HMG 95K protein (nuclei after 5% $HClO_4$ extraction; the residual pellet was extracted with 0.35 M-NaCl and proteins soluble in 2% trichloroacetic acid were used); (4) histone H1 from hen oviduct (obtained as indicated in Fig. 2*a*); (5) total acid-soluble (in 0.2 M-H_2SO_4) hen oviduct nuclear proteins [with lysozyme (L)].

HMG-1 and -2 [Fig. 3*b*, gels (2) and (3)]. In general, the contamination by histone H1 in the $HClO_4$ extraction is greater than that by the low-salt extraction. However, the histone H1 could be removed by redissolving the proteins and precipitated by the addition of 3.5 vol. of acetone as described by Rabbani *et al.* (1978*a*).

From Fig. 3(*a*), gel (2), one could judge that 95K protein was a large proportion of the total population of HMG proteins in hen oviduct. However, no such protein was observed in calf thymus [Fig. 3*a*, gel (1)]. This is consistent with our previous observation that 95K protein is an organ-specific protein in hen oviduct (Teng *et al.*, 1978). In calf thymus, the proteins HMG-1 and -2 are the major protein fraction as compared with the other proteins. Judging by the electrophoretic pattern, the concentrations of thymus proteins HMG-1 and -2 are higher than in the hen oviduct [Fig. 3*a*, gels (1) and (2)]. In hen oviduct, the total amount of HMG proteins (after the removal of histone H1) obtained from 1 g of tissue is 0.16 mg. The DNA content is 3.3 mg/g of tissue. Therefore, total HMG proteins are 4.8% by weight of DNA. A value of 3% by weight of HMG protein from the monomeric nucleosome of calf thymus nuclei has been estimated by Goodwin *et al.* (1977).

After limited DNAase-I digestion of hen oviduct nuclei, a very small amount of proteins HMG-1 and -2 was released which could be resolved by SDS/polyacrylamide-gel electrophoresis [Fig. 4*a*, gels (3) and (4)]. However, when the same supernatant sample was run in acetic acid/urea/polyacrylamide gel, no HMG protein was visible [Fig. 4*b*, gel (4)]. A similar observation of a small amount of HMG proteins released after limited DNAase-I digestion has been reported in the thymus and liver nuclei (Goodwin & Johns, 1978). On the other hand, in comparison with the DNAase-I digestion pattern, the limited digestion of hen oviduct nuclei by micrococcal nuclease released more proteins HMG-1 and -2 [Fig. 4*a*, gels (4) and (6)]. Clear bands of proteins HMG-1 and -2 are visible in the acetic acid/urea/polyacrylamide gel [Fig. 4*b*, gel (7)]. The high-molecular-weight HMG protein (95K) was not released by limited digestion with both nucleases (Fig. 4*a*, gels (3) and (5); Fig. 4*b*, gels (3) and (6)].

It has been shown that both the nucleases tested in this experiment preferentially digest the transcriptionally active region of the genome in various tissues (Weintraub & Groudine, 1976; Garel & Axel, 1976; Levy & Dixon, 1977; Garel *et al.*, 1977; Bloom & Anderson, 1978). Our experiments involving limited nuclease digestion of oviduct nuclei indicated that a portion of proteins HMG-1 and -2 is associated with this region and could be solubilized by this treatment. This association is in agreement with

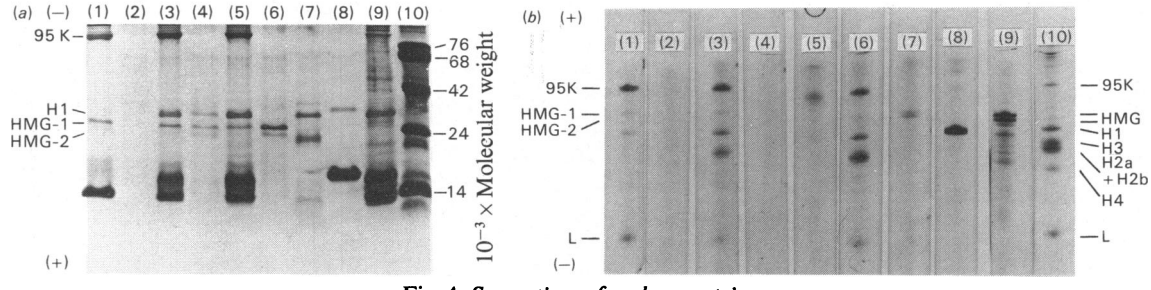

Fig. 4. *Separations of nuclear proteins*

(*a*) Separation of nuclear proteins after digestion with nucleases. Hen oviduct nuclei from 5 g of tissue were suspended in RSB buffer at a DNA concentration of 2.3 mg/ml as described in the Materials and Methods section. Then 10% of digestion of the nuclear suspension was accomplished by the addition of DNAase I (40 μg/ml for 10 min at 37°C) or micrococcal nuclease (7 μg/ml for 10 min at 37°C). Control sample was incubated without the nuclease. Protein samples were prepared and separated by gel electrophoresis as described in the Materials and Methods section. Gels: (1) control pellet proteins (60 μg); (2) control supernatant proteins (10 μg, representing 25% of the total supernatant proteins); (3) DNAase-I-digested nuclear pellet protein (60 μg); (4) DNAase-I-digested supernatant protein (20 μg, representing 25% of the total supernatant proteins); (5) micrococcal-nuclease-digested nuclear pellet protein (60 μg); (6) micrococcal-nuclease-digested supernatant protein (30 μg, representing 25% of the total supernatant proteins); (7) DNAase I enzyme alone (20 μg); (8) micrococcal nuclease enzyme alone (20 μg); (9) hen oviduct nuclear acid-soluble (0.2 M-H_2SO_4) proteins; (10) standards: lysozyme, chymotrypsinogen, ovalbumin, bovine serum albumin and conalbumin. (*b*) Separation of proteins by acetic acid/urea/polyacrylamide-gel electrophoresis. Protein samples form previous preparation (Fig. 4*a*) were resolved in this gel preparation. Gels: (1) control pellet proteins (30 μg); (2) control supernatant proteins (10 μg); (3) DNAase-I-digested pellet proteins (30 μg); (4) DNAase-I-digested supernatant proteins (20 μg); (5) DNAase I alone (20 μg); (6) micrococcal-nuclease-digested pellet proteins (30 μg); (7) micrococcal-nuclease-digested supernatant proteins (30 μg); (8) micrococcal nuclease alone (20 μg); (9) total calf thymus HMG proteins (40 μg); (10) total acid-soluble (in 0.2 M-H_2SO_4) hen oviduct nuclear proteins. Abbreviation: H, histone.

the reports of Levy *et al.* (1977), Levy & Dixon (1978) and Vidali *et al.* (1977) in calf thymus, brain and avian erythrocytes.

Observations indicated that the effect of the two nucleases tested on chromatin is different, with the micrococcal nuclease preferentially digesting the DNA in the internucleosomal spacer region and DNAase I cutting DNA strands within the nucleosomes and the DNA in the spacer region (Noll, 1974; Axel *et al.*, 1975; Burgoyne *et al.*, 1976). Therefore a preferential release of proteins HMG-1 and -2 after micrococcal-nuclease treatment may indicate that these proteins are associated with the spacer region adjacent to the core nucleosomes. This possibility needs to be further investigated.

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