Digestion by Micrococcal Nuclease of Mouse Submandibular-Salivary-Gland Chromatin

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Nuclei prepared from mouse submandibular salivary gland show marked fragility during isolation. However, intact nuclei relatively free from cytoplasmic contamination were obtained by homogenization in buffers containing 0.88 M-sucrose, Ca^{2+} , spermine, spermidine and the proteinase inhibitor aprotinin, followed by centrifugation through 2.2 M-sucrose. The kinetics of digestion by the micrococcal nuclease of chromatin in these nuclei are similar to those of chromatin from mouse liver nuclei. Base-pair size analysis of the solubilized DNA from both organs shows a stable high-molecular-weight species of chromatin, which is further digested to mononucleosome and subnucleosome species. With extensive digestion the chromatin becomes insoluble. The mononucleosomes produced from salivary-gland chromatin after the inhibition of endogenous proteinase activity exhibit an $s_{20,w}$ value of 11 S and contain histones H1, H2A, H2B, H3 and H4.

Currently much scientific interest is directed towards the role of chromatin in the control of gene expression and particularly the role of chromatin proteins in cellular transformation (for review see Stein *et al.*, 1978). Detailed data are available that describe the structure of the chromatin subunit, the 'mononucleosome' (Kornberg, 1977; Oudet *et al.*, 1977; Felsenfeld, 1978; Van Holde & Weischet, 1978; Thomas, 1978). More recently techniques have become available for fractionation of transcriptionally active chromatin that may elucidate the more functional aspects of eukaryotic chromatin biochemistry (Gottesfeld *et al.*, 1974; Gottesfeld, 1977; Savage & Bonner, 1978; Tata & Baker, 1978).

Studies in our laboratory are directed towards an analysis of chromatin for biochemical markers of cellular transformation in mouse submandibular salivary gland. Chromatin has been little studied in this tissue compared with liver, thymus and avian erythrocytes (Stein & Baserga, 1970; Ishida & Ahmed, 1974). This is despite the establishment of salivary gland as an epithelial-cell system *in vitro* (Stein & Baserga, 1970; Wigley & Franks, 1976; Wigley *et al.*, 1976) and its importance in studies of epithelial transformation (Wiest & Heidelberger, 1953; Knowles & Franks, 1977; Knowles, 1979). Furthermore, salivary gland has been the subject of extensive study with respect to the induction of

protein synthesis and secretion by drugs such as isoproterenol (Ekfors & Barka, 1971).

The present paper describes a method suitable for purification of nuclei from mouse submandibular gland that differs from published procedures for other tissues. These nuclei are utilized to characterize salivary-gland chromatin with respect to digestion by micrococcal nuclease and properties of the subunit mononucleosome.

Materials and Methods

Materials

The mice used in all experiments were C57BL/1crf or C57BL/1crf a^t males aged 4–6 months. Micrococcal nuclease was the *Staphylococcus aureus* enzyme (EC 3.1.31.1) purified to 8×10^3 units/mg (P–L Biochemicals, Milwaukee, WI, U.S.A.). The nuclease was stored at -80°C at a stock concentration of 5×10^3 units/ml in 0.88 M-sucrose in buffer A (60 mM-KCl/15 mM-NaCl/0.15 mM-spermine tetrahydrochloride/0.5 mM-spermidine trihydrochloride / 15 mM-2-mercaptoethanol/15 mM-Tris/HCl buffer, pH 7.4) containing 3 mM- CaCl_2 plus 20 kallikrein-inhibitor units of aprotinin/ml. Aprotinin was a solution of 23.0 trypsin-inhibitor units/ml (1 trypsin-inhibitor unit equals 90 kallikrein-inhibitor units) in 0.9% NaCl/0.9% benzyl alcohol, derived from bovine lung

(Sigma Chemical Co., St. Louis, MO, U.S.A.). Sucrose used for all buffers was UltraPure (density-gradient grade) sucrose from Schwartz-Mann (Orangeburg, NY, U.S.A.). Proteinase K (fungal; 20 Anson units/g) was from BDH Chemicals (Poole, Dorset, U.K.) and agarose (type II, medium EEO) from Sigma Chemical Co. Acrylamide was electrophoresis-grade from Bio-Rad Laboratories (Richmond, CA, U.S.A.), bisacrylamide from Eastman (Rochester, NY, U.S.A.) and *NNN'*-tetramethylethylenediamine from Koch-Light Laboratories (Colnbrook, Bucks., U.K.). Sedimentation-coefficient markers were *Escherichia coli* β -galactosidase (Worthington, Freehold, NJ, U.S.A.), bovine liver catalase and human immunoglobulin G (both from Sigma). For enzyme assays cytochrome *c* (type VI, horse heart), UDP and *p*-nitrophenyl phosphate were from Sigma.

Purification of nuclei

Groups of ten mice were killed by cervical dislocation, and the submaxillary salivary glands and livers were removed and washed in cold 137 mM-NaCl/2 mM-KCl/8 mM- Na_2HPO_4 /1.4 mM- KH_2PO_4 buffer, pH 7.1. The wash and all subsequent procedures were performed at 0–4°C unless otherwise indicated. The glands were cleared of connective tissue, the sublingual glands and clotted blood, and were then minced to small fragments of approx. 1 mm diameter with scissors. Thorough mincing was essential in order to facilitate later homogenization. Liver was similarly washed, cleaned of connective tissue and minced. The minces were made 20% (w/v) in sucrose in buffer A containing 1.8 mM- CaCl_2 plus 20 kallikrein-inhibitor units of aprotinin/ml ('homogenizing buffer'), with sucrose concentration 0.88 M and 0.34 M for salivary gland and liver respectively. After homogenization by ten passes (five strokes) in a medium-fitting Teflon/glass homogenizer the homogenate was centrifuged at 1000 *g* for 30 min, resulting in a pellet of nuclei and cellular debris. The pellets were resuspended in 2.5 ml of the respective homogenizing buffers by gentle homogenization by hand, and this volume was mixed with 63% (w/v) sucrose in buffer A containing 1.8 mM- CaCl_2 plus 2 kallikrein-inhibitor units of aprotinin/ml, resulting in a final sucrose concentration of 59% (w/v) sucrose (2.2 M). This mixture was centrifuged at 70000 *g*_{av} for 45 min ('sucrose scrub'), and the resulting nuclear pellet was gently washed by homogenization by hand in 5 ml of sucrose in buffer A containing 3.0 mM/ CaCl_2 plus 20 kallikrein-inhibitor units of aprotinin/ml ('digestion buffer') with sucrose concentration 0.88 M and 0.34 M for salivary gland and liver respectively. This suspension was centrifuged at 1000 *g* for 30 min, and the pellet of purified nuclei was resuspended in the same 'digestion buffer' to a volume of 1.1 ml. The

number of nuclei was determined by diluting 50 μl of this suspension 10-fold with digestion buffer and counting the nuclei in an Improved Neubauer Cytometer.

Chromatin digestion

Chromatin was digested inside the intact nucleus. Micrococcal nuclease was added to purified nuclei at a concentration of 1000 units per 0.5×10^8 nuclei (approx. 1 mg of DNA) for digestion at 5°C, or at 5 and 50 units per 0.5×10^8 nuclei for digestion at 37°C. The digestion was performed with gentle shaking for the times indicated in the text. Digestion was terminated by the addition of Na_2EDTA to a final concentration of 25 mM and chilling the digest on ice. A nuclear pellet was prepared by centrifugation at 1000 *g* for 15 min, and the chromatin was liberated by resuspension of the pellet to 2.0 ml in 2 mM- Na_2EDTA , pH 8.0, vortex-mixing of the suspension and standing it in ice for 15 min. The lysed nuclei were centrifuged for 1000 *g* for 1 h, resulting in a supernatant and pellet of solubilized and insoluble chromatin respectively. DNA was assayed by the method of Burton (1956).

Characterization of chromatin

Gel electrophoresis of DNA. The DNA base-pair size distribution of nuclease digests was determined by electrophoresis in agarose gels.

A 25 μl sample of nuclease-treated chromatin was made 0.2% with respect to sodium dodecyl sulphate and 20 $\mu\text{g}/\text{ml}$ with respect to proteinase K, and was incubated at 37°C for 15 min. To this digest was added 3 μl of 50% (w/v) sucrose containing 0.2% Bromophenol Blue, and the sample was subjected to electrophoresis.

For routine determination of the nucleosome distribution of chromatin digests the sample was electrophoresed in a 10 cm $\times$ 20 cm slab gel of 2% agarose, which was poured and run in 40 mM-Tris/acetic acid / 5 mM-sodium acetate / 1 mM- Na_2EDTA buffer, pH 7.8, containing 0.05% ethidium bromide. The slab of agarose was totally immersed in the running buffer and was electrophoresed at a constant 150 V (150 mA) for 60–120 min, and the DNA bands were located by using transmitted u.v. light.

DNA base-pair size markers for electrophoresis were obtained by digesting 0.5 μg samples of either *HpaII* or *HaeIII* restriction fragments from polyoma-virus DNA with proteinase as described above. The migration of these markers during electrophoresis was directly proportional to log (DNA base-pair size of the fragment) (Griffin, 1977).

Sucrose-density-gradient centrifugation. The soluble fraction of the nuclease digest of chromatin was subjected to non-equilibrium density-gradient

centrifugation on an isokinetic sucrose gradient. A 12ml isokinetic gradient of 5–45% (w/v) sucrose was prepared, as described by Noll (1967), in buffer A containing 0.2mM- Na_2EDTA plus 2 kallikrein-inhibitor units of aprotinin/ml. A 2ml sample of solubilized chromatin was layered on to the surface of the gradient and centrifuged at $150000g_{av}$ for 16h in an MSE Super-Speed 75 centrifuge fitted with a 6×14 ml swing-out rotor. A mixture of β -galactosidase, catalase and human immunoglobulin G (each at concentrations of 10mg/ml in 0.2mM- Na_2EDTA) was centrifuged as markers having $s_{20,w}$ values of 16.0S, 11.3S and 7.0S respectively.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of chromatin proteins

Mononucleosome-associated proteins were electrophoresed by a modification of the method of Smith & Chae (1973). Samples of purified mononucleosomes from salivary gland and liver (100–300 μ g of protein) were freeze-dried and resuspended to 30 μ l in 0.125M-Tris/HCl buffer, pH 6.8, containing 4% (w/v) sodium dodecyl sulphate, 20% (w/v) glycerol, 10% (v/v) 2-mercaptoethanol and 0.001% Bromophenol Blue. The samples were heated at 100°C for 2.5min before slab-gel electrophoresis. Purified samples of mixed histones from hen erythrocytes and other proteins (2.5 μ g of protein each) were subjected to identical procedures and used as molecular-weight markers. The samples were electrophoresed in polyacrylamide gels (15% acrylamide), pH 8.8, with 3% stacking gel, pH 6.8, as described by Laemmli (1970).

Assays of enzyme activities

Acid phosphatase was assayed by using the procedure of Ignarro (1971) and succinate-cytochrome *c* reductase by the method of Tisdale (1937). Nucleoside diphosphatase was assayed with UDP as substrate by the method of Novikoff & Heus (1963) and by using the procedure of Lowry & Lopez (1946) for measurement of P_i .

All assays of enzyme activities were performed within the linear region with respect to time and enzyme concentration.

Electron microscopy

Samples of nuclei in digestion buffer were fixed as pellets in 2.5% (v/v) glutaraldehyde in 'digestion buffer' for 30min at 4°C. The samples were then washed twice in 'digestion buffer', post-fixed in 1% (w/v) osmium tetroxide for 30min at room temperature, washed in distilled water and then treated with aq. 1% (w/v) uranyl acetate for 30–60min. Samples were dehydrated with methanol (70%, 90% and then 100%, v/v), and embedded in Araldite with in-

filtration from methanol assisted by epoxypropane and thereafter by epoxypropane/Araldite (1:1 and 1:3, v/v). After staining with 25% (w/v) uranyl acetate (in ethanol) and Reynold's lead citrate, sections were carbon-coated and then viewed in a Philips 301G transmission electron microscope. All levels of each pellet were examined.

Results and Discussion

Purification of nuclei from mouse submandibular salivary gland

Submandibular-salivary-gland nuclei are fragile and tend to rupture and spill their chromatin during homogenization (Wiest & Heidelberger, 1953). Homogenization in buffer containing 0.88M-sucrose, Ca^{2+} and the polyamines spermine and spermidine gave the highest yields of purified nuclei, approx. 20% on the basis of DNA recovery. This yield compares well with that obtained by Chauveau *et al.* (1956), who prepared highly purified nuclei from rat liver, but is lower than DNA yields from nuclei prepared by the citrate and detergent methods (for a review see Busch & Daskal, 1977).

Several variations from the 0.88M-sucrose/ Ca^{2+} procedures described above were attempted in order to increase the yield of nuclei. Decreasing the sucrose concentration of the homogenization buffer to 0.34M or washing the nuclei in 0.1–0.2% Triton X-100 before or after the sucrose scrub each diminished the yield of nuclei by some 50% compared with the standard procedure.

Spermine and spermidine markedly stabilized the salivary-gland nuclei. The removal of these components from the homogenization and scrub buffers or the use throughout of buffer systems lacking these components (Tata & Baker, 1978) resulted in a yield of purified nuclei that was some 10% of that obtained by the standard procedure. Several other authors have demonstrated a stabilization of mammalian nuclei by polyamines (Busch *et al.*, 1967; MacGregor & Mahler, 1967). Low concentrations of Ca^{2+} were included in all buffers in order to maintain nucleolar integrity (Busch & Daskal, 1977).

Aprotinin is a general proteinase inhibitor that has been demonstrated to decrease histone degradation in mouse tissues (Trauttschold *et al.*, 1967; Medvedyev *et al.*, 1978). It was included throughout the procedures in order to minimize digestion of chromatin proteins by endogenous proteinases.

Electron microscopy indicates that the salivary-gland nuclei prepared under the conditions described exhibit considerable nuclear-membrane damage. Although apparently intact double nuclear membranes may enclose some nuclei, more frequently fragments of nuclear membrane are loosely

attached to the outer surface of the nuclei and there are often bare areas. Occasional disrupted nuclei and clumps of collagen fibres are also apparent (Plate 1a). However, most of the nuclei are intact, with characteristic condensed peripheral heterochromatin (Frenster, 1974) and internally projecting masses (Plate 1b). Euchromatin strands and filaments, and discrete nucleoli, lie between these masses.

Purification of salivary-gland nuclei by the procedure described above results in nuclei that are free of cytoplasmic tags (Plate 1b). Table 1 demonstrates the purity of these nuclei as determined by the activities of contaminating microsomal (nucleoside diphosphatase), mitochondrial (succinate-cytochrome *c* reductase) and lysosomal (acid phosphatase) enzymes. The total enzyme activities in the nuclear preparation were less than 1–2% of those in the whole homogenate. The enzyme specific activities relative to protein are 10–15% of those in the whole homogenate. The slightly higher contamination by the microsomal enzyme markers may be related to the continuity between the endoplasmic reticulum and outer nuclear membrane. These purity data compare well with those of Widnell & Tata (1964) and Siebert *et al.* (1966), who prepared rat liver nuclei by using 2.4 M-sucrose/1 mM-MgCl₂ and 2.2 M-sucrose respectively.

The above data suggest that the salivary-gland nuclei prepared under these conditions, though not suitable for studies of the nuclear membrane, are sufficiently intact and free from cytoplasmic contamination as to be suitable for chromatin analysis.

Characterization of chromatin

Nuclease digestion of chromatin within the intact nucleus minimizes non-specific shearing of chromatin during subsequent nuclear disruption (Noll *et al.*, 1975). Fig. 1 depicts the degree of chromatin

solubilization that occurs after the treatment of purified salivary-gland and liver nuclei with moderate concentrations of micrococcal nuclease. Digestion results in a maximal solubilization of about 50–60% of the DNA for both salivary-gland and liver chromatin (Fig. 1a). This degree of solubilization agrees with results obtained in other laboratories with rat liver chromatin (Clark & Felsenfeld, 1971). Such protection of some 40–50% of the chromatin DNA from immediate solubilization appears to reflect a close association between DNA and protein and to be independent of the type of deoxyribonuclease used (Clark & Felsenfeld, 1971).

An analysis of DNA base-pair size during digestion (Fig. 1b) indicates that DNA is digested to stable mononucleosomes of 140–200 base-pairs, which accumulate with increasing time of digestion in both tissues.

The result of chromatin digestion with higher concentrations of micrococcal nuclease is shown in Fig. 2. With extensive digestion the DNA becomes less soluble and disappears from the solution after low-speed centrifugation (Figs. 2a and 2b). Electron microscopy indicates that at these extents of digestion there is substantial nuclear disruption, with chromatin liberation and aggregation (results not shown). Such aggregation may account for the low solubility of chromatin at high extents of digestion.

Close examination of DNA base-pair profiles of salivary-gland and liver nuclei after moderate nuclease digestion reveals the presence of a high-molecular-weight DNA species that is present even in undigested controls (Fig. 1b, samples 2 and 6). Very-low-level digestion with micrococcal nuclease (0.5 unit per 0.5×10^8 nuclei), which solubilizes some 3% of chromatin, increases the concentration of this component (Fig. 3). The component is observed in chromatin samples pre-

Table 1. Marker-enzyme activities in homogenate and purified nuclei of mouse submandibular salivary gland
Mouse submandibular-salivary-gland nuclei were purified from a crude nuclear pellet by centrifugation through 2.2 M-sucrose in buffer A containing 1.8 mM-CaCl₂ plus 2 kallikrein-inhibitor units of aprotinin/ml. Samples of whole purified nuclei were then assayed for contamination by cytoplasmic enzyme activities as described in the Materials and Methods section.

Enzyme	10 ² × Total enzyme activity (μmol of product/min per ml of enzyme)		10 ² × Enzyme specific activity (μmol of product/min per mg of protein)	
	Homogenate	Nuclei	Homogenate	Nuclei
Nucleoside diphosphatase (microsomal fraction)	455.0	8.0 (1.8% of homogenate)	18.0	3.0 (16.7% of homogenate)
Succinate-cytochrome <i>c</i> reductase (mitochondria)	77.8	0.8 (1.0% of homogenate)	3.1	0.3 (9.7% of homogenate)
Acid phosphatase (lysosomes)	200.0	3.0 (1.5% of homogenate)	4.0	0.5 (12.5% of homogenate)

EXPLANATION OF PLATE 1

Electron micrographs of purified submandibular-salivary-gland nuclei

(a) A thin section of a purified nuclear pellet prepared as described in the Materials and Methods section is shown. The nuclei are discrete, with a few distorted or ruptured profiles. Nuclear membranes are loosely attached to the outer surface of the nuclei (arrows). The bar represents 5 μm . (b) A higher magnification shows the pattern of condensed (hetero-) and extended (eu-) chromatin strands and filaments. Key: n, nucleolus; nm, nuclear membrane; bare areas devoid of nuclear membrane are indicated by arrows. The bar represents 1 μm .

Fig. 1. Solubilization of chromatin from salivary-gland and liver nuclei by digestion with moderate concentrations of micrococcal nuclease

(a) Purified nuclei were incubated with micrococcal nuclease at a concentration of 5 units of enzyme/ 0.5×10^8 nuclei at 37°C . After termination of the reaction at the times indicated, the nuclei were lysed and soluble chromatin was determined as described in the Materials and Methods section. O, Salivary gland; ●, liver, (b) shows the DNA base-pair size of the soluble chromatin. The soluble chromatin resulting from the nuclease digestion was deproteinized and electrophoresed on 2% agarose gels as described in the Materials and Methods section. Samples: 1, *HpaII* restriction fragments of polyoma-virus DNA; 2–5, salivary-gland chromatin digested for zero time (2), 5 min (3), 10 min (4) and 30 min (5); 6–9, liver chromatin digested for zero time (6), 5 min (7), 10 min (8) and 30 min (9).

Fig. 2. Solubilization of chromatin from salivary-gland and liver nuclei by digestion with higher concentrations of micrococcal nuclease

(a) Purified nuclei were incubated with micrococcal nuclease at a concentration of 50 units of enzyme/ 0.5×10^8 nuclei at 37°C and treated as described in Fig. 1(a). O, Salivary gland; ●, liver. (b) shows the DNA base-pair size of soluble chromatin. The nuclease-solubilized chromatin was electrophoresed as described in Fig. 1(b). Samples: 1, *HaeIII* restriction fragments of polyoma-virus DNA; 2–5, salivary-gland chromatin digested for zero time (2), 5 min (3), 10 min (4) and 30 min (5); 6–9, liver chromatin digested for zero time (6), 5 min (7), 10 min (8) and 30 min (9).

pared in the presence and in the absence of exogenous spermine and spermidine (G. J. Smith, unpublished work). It is possible that this intermediate is a quasi-stable digestion product that reflects the effects of higher-order chromatin structure such as supercoiling (Felsenfeld, 1978) on the pattern of nuclease digestion of chromatin. In control preparations these species may result from

slight shearing of DNA or endogenous nuclease digestion. Recently Elgin and co-workers have described similar species after digestion of *Drosophila* chromatin with low concentrations of micrococcal nuclease (Wu *et al.*, 1979).

A characterization of mouse submandibular-salivary-gland mononucleosomes by sucrose-density-gradient sedimentation is depicted in Fig. 4. With digestion at 37°C the highly reproducible A_{260}/A_{280} profile indicates the presence of mononucleosome and subnucleosome species of $s_{20,w}$ values of approx. 11S and <7S respectively. These

Fig. 3. DNA base-pair size of chromatin solubilized from salivary-gland nuclei by digestion with low concentrations of micrococcal nuclease

Purified salivary-gland nuclei were incubated with micrococcal nuclease at a concentration of 0.5 unit of enzyme/ 0.5×10^8 nuclei at 37°C . DNA base-pair size of soluble chromatin was determined as described in Fig. 1(b). Samples: 1, *Hpa*II restriction fragments of polyoma-virus DNA; 2–5, salivary-gland chromatin digested for zero time (2), 2.5 min (3), 5.0 min (4) and 10.0 min (5).

Fig. 4. Density-gradient-centrifugation profile of salivary-gland chromatin solubilized by micrococcal nuclease

Purified nuclei were incubated with 50 units of nuclease/ 0.5×10^8 nuclei at 37°C for 5 min. Soluble chromatin was centrifuged in an isokinetic 5–45% (w/v) sucrose gradient at $150000 g_{av}$ for 16 h. Fractions of volume 0.5 ml were collected and absorbance was measured at 260 nm and 280 nm. $\blacktriangle$, A_{260} of undigested chromatin; $\bullet$, A_{260} of digested chromatin; $\circ$, A_{280} of digested chromatin.

sedimentation characteristics agree with those determined for rat liver mononucleosomes prepared from the heterochromatic region of chromatin (Gottesfeld *et al.*, 1975).

The importance of inhibiting the high endogenous proteinase activity of submandibular salivary gland (Ekfors *et al.*, 1972) during chromatin preparation is demonstrated in Fig. 5. Fig. 5(a) shows a sucrose-density-gradient profile that resulted after preparation of nuclei in 0.88 M-sucrose/15 mM-Tris/HCl/1.8 mM- CaCl_2 buffer, pH 7.4, and digestion

Fig. 5. Effect of proteinase inhibition on the centrifugation profile of salivary-gland chromatin solubilized by micrococcal nuclease

Purified nuclei were prepared (a) in the absence of aprotinin and (b) in the presence of aprotinin as described in the Materials and Methods section. Chromatin was solubilized by digestion with 1000 units of nuclease/ 0.5×10^8 nuclei at 5°C for 30 min and centrifuged as described in Fig. 4. $\blacktriangle$, A_{260} of undigested chromatin; $\bullet$, A_{260} of digested chromatin; $\triangle$, A_{280} of undigested chromatin; $\circ$, A_{280} of digested chromatin.

with micrococcal nuclease at 5°C. The profile demonstrates a heterogeneous distribution of A_{260}/A_{280} absorbance. DNA base-pair analysis of the peaks indicates that each contains DNA of mononucleosome length 200 base-pairs (results not shown). In contrast, preparation of nuclei and digestion of chromatin with the addition of the proteinase inhibitor aprotinin resulted in a more homogeneous profile containing mononucleosome and subnucleosome species of $s_{20,w}$ values 11S and <7S respectively (Fig. 5b). The mononucleosome peak in this profile is broader and of slightly higher $s_{20,w}$ value than for that resulting from digestion at 37°C, owing to incomplete digestion of the nucleosome linker at low temperature (Noll & Kornberg, 1977).

An analysis of the proteins associated with mononucleosome preparations that result from digestion by micrococcal nuclease of chromatin in mouse submandibular-salivary-gland and liver nuclei is shown in Fig. 6. Most of the protein from both preparations represents the 'core histones' H2A, H2B, H3 and H4 (Shaw *et al.*,

Fig. 6. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of salivary-gland mononucleosome proteins

Mononucleosomes purified by density-gradient centrifugation were extracted at 100°C for 2.5 min in 0.125 M-Tris/HCl buffer, pH 6.8, containing 4% (w/v) sodium dodecyl sulphate, 20% (w/v) glycerol, 10% (v/v) 2-mercaptoethanol and 0.001% Bromophenol Blue. Electrophoresis was performed in polyacrylamide gel (15% acrylamide) with 3% stacking gel for 16 h at 50 V. Gels were stained with Coomassie Brilliant Blue. 1, Marker proteins (bovine serum albumin, human immunoglobulin G and cytochrome c; 2, mixed histones from hen erythrocytes; 3, mononucleosome proteins from mouse salivary gland; 4, mononucleosome proteins from mouse liver. Abbreviations: BSA, bovine serum albumin; IgG(H) and IgG(L), human immunoglobulin G heavy chain and light chain respectively; CYT, cytochrome c.

1976), which co-electrophorese with those from hen erythrocytes (Fig. 7). The mononucleosomes contain lower contents of histone H1 and H1 subfractions (Medvedyev, *et al.*, 1978), owing to digestion at 37°C with release of this linker-associated histone (Varshavsky *et al.*, 1976; Noll & Kornberg, 1977). The core histones exhibit mol.wts. of 11000–15000 compared with standard marker proteins (Fig. 6). Histone H1 exhibits a high mol.wt. of about 30000–35000. Amino acid analysis suggests a mol.wt. of about 20000 for histone H1 for most species (Johns *et al.*, 1977). The apparent increase in molecular weight of histone H1 on electrophoresis appears to be an artifact of sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Weber & Osborn, 1975).

The mononucleosomes appear to contain only low contents of non-histone proteins, particularly relative to the high contents of core-histone proteins (Fig. 6).

The differences in histone H1 and H1 subfractions observed between salivary-gland and liver chromatin (Fig. 6) may reflect the quantitative differences between tissues reported elsewhere (Johns *et al.*, 1977). However, it is not possible to rule out the effects of proteolysis on certain histone species due to high activity of endogenous proteinases in salivary gland (Ekfors *et al.*, 1972). Histones H1 and H3, the concentrations of which appear to be lowered in salivary-gland mononucleosomes (Fig. 6), are reported to be particularly susceptible to proteinase activity in several tissues (Kornberg, 1977).

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